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SYMPOSIUM ON THE ECOLOGY AND BEHAVIOR OF WILD AND FERAL EQUIDS

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Androgens in Feral Stallions

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Abstract

Seasonal and diurnal patterns of 17 beta-hydroxyandrogens and testosterone in feral stallions are reviewed, and new data for two-year-old stallions with descended and undescended testes are presented. Previous work has demonstrated that mature feral stallions (three years or older) have a seasonal pattern of testosterone and total androgen secretion with peak levels occurring in May and a nadir from late September through December. As the blood levels of total androgens increased, the percent which was testosterone also increased. The seasonal pattern was similar to that of Quarter horses. Diurnal patterns indicated peak levels of testosterone at 0800 hrs and the nadir at 2300 hrs. An eight-year-old bachelor stallion which had demonstrated a consistent lack of libido had significantly lower testosterone levels ($P < 0.01$) than harem stallions, suggesting a relationship between plasma testosterone levels and reproductive behavior in feral stallions. The day-time blood levels of testosterone and total 17 beta-hydroxyandrogens were evaluated in two two-year-old stallions which had descended testes and had recently been driven from their respective bands. The same steroids were examined in 19 two-year-old stallions with undescended testes, all of which were permitted to remain with their harem bands. The two-year-old males with descended testes had testosterone concentrations of 1.9 and 4.8 ng/ml with a mean of 3.35 ng/ml, while the 19 two-year-old males with undescended testes had a mean testosterone concentration of 1.14 ± 0.23 ng/ml.

Presently there are an estimated 50,000 to 70,000 feral horses inhabiting the western United States. The herd size increases annually due to the protection afforded by the Wild and Free Roaming Horse and Burro Act of 1971 (P.L. 92-195) and inadequate means of population control. Reversible chemical fertility control, focused upon the stallion, is currently a possibility. The stallion is the target animal, rather than the mare, because of the harem band social structure. Such an approach requires a background study of basic reproductive physiology of the feral stallion.

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This paper reviews previous work regarding the plasma levels of 17-beta-hydroxyandrogens and testosterone in feral stallions, and the diurnal variations and seasonal changes of these steroids. Additionally, new data regarding plasma testosterone in feral stallions with descended and undescended testes are presented.

Methods and Materials

Diurnal Study: Four stallions from the Pryor Mountain Wild Horse Range in Montana were captured and confined to a corral several days before blood was collected. On the day of collection, the horses were restrained by halter ropes, to posts on opposite sides of each horse. This permitted the animals some movement but without danger of injuring themselves. The four animals were identified by name: Crooked Creek (CC); Welfare (W); Black (B); and Sorrel (S). These stallions were nine, eight, eight, and six years old respectively, and all were in good physical condition.

Beginning at 1600, 10 ml blood samples were collected hourly, by jugular venipuncture, in heparinized tubes, centrifuged, and the plasma immediately frozen on dry ice.

Seasonal Study: Thirty-four sexually mature (three years of older) stallions were captured on their home range in the Pryor Mountains, during September, October, December, March, May, and July. The animals were brought down by heading and heeling and blood samples were collected by jugular venipuncture. The heparinized blood samples were packed in ice and centrifuged within 24 hrs. of collection.

Immature Stallion Study: Nineteen two-year-old stallions with undescended testes were captured on the Pryor Mountain range. These sexually immature stallions were all permitted, by their respective harem stallions, to remain with their respective harem groups. Two additional two-year-old stallions were captured, which had descended testes and which had been recently driven from their harem groups. Blood samples were collected from all 21 animals as previously described.

Testosterone Assay: Total 17 beta-hydroxyandrogens were extracted from duplicate 0.5 ml plasma samples with three washes of diethyl ether, totalling 10 ml. The ether extracts were washed with two volumes of 0.1 N NaOH to remove estrogens. Testosterone (4-androsten-17 beta-ol-3-one) was separated from other 17 beta-hydroxyandrogens by Sephadex LH-20 column chromatography. The column was 6 mm (I.D.) X 31 cm and the solvent system was reagent grade heptane:chloroform: ethanol:water (50:50:1:saturation). Columns were prepared daily from a solvent-Sephadex slurry and eluted with 100 mls of solvent before use. The testosterone elution profile and percent recovery were established with ^{3}H-testosterone. One ml fractions 31 through 40 yielded 95 ± 2.8 percent (N=10) of the ^{3}H-testosterone.

Ether extracts of the plasma were dried and 1.0 ml of column solvent was added to each tube. Testosterone was collected in elutions 31 through 40, eluates were dried and 2 mls of ether were added and dried. Thin-layer chromatography was used to confirm results obtained with column chromatography. TLC was carried out with precoated silica gel plates (Van Waters Rogers) and petroleum ether:toluene (1:1 by volume) and 85 percent methanol. Identification was made with p-methoxybenzaldehyde (Kodak Chemicals).

Quantitation of plasma testosterone was accomplished by competitive-protein-binding assay described by Ganjam and Amann (1973). The protein-tracer-solution (PTS) consisted of 99.5 mls distilled water, 0.5 mls of third trimester human pregnancy plasma and (1,2,6,7-^{3}H) testosterone (New England Nuclear). The PTS was prepared fresh daily and stored at 5°C for at least one hour before use. The testosterone standard (Steraloids, Pawling, N.Y.) was recrystallized once and stored at 5°C in ethanol at a concentration of 10 ng/ml. Unbound steroid was removed by the addition of 40 mg Florisil (60/100 mesh) and shaking for two minutes. After 15 additional minutes of refrigeration, 0.5 mls of the PTS-testosterone complex was pipetted into 5 mls of toluene base scintillation cocktail (Liquiflor, New England Nuclear). This two-phase system was counted 24 hrs later in a Nuclear Chicago Model 720 LSC. Counting efficiency for tritium was 22 percent.

a) Recovery. Recovery of testosterone after extraction and column chromatography was 91.2 ± 3.7 percent (N=10) as determined by internal standard.
b) Precision. The coefficient of variation was 10.4 percent for 22 duplicate determinations carried out on different days. The inter-assay and intra-assay variation was 12.1 percent and 8.2 percent respectively.
c) Accuracy. Known amounts of testosterone standard (N=25) were assayed. Analysis of variance yielded a correlation coefficient of $r = 0.932$.
d) Specificity. The specificity of this assay is dependent upon the separation of testosterone from other 17 beta-hydroxyandrogens and estrogens (Vermeulin and Verdonck, 1970) and was evaluated by examining the constancy of testosterone standards after chromatography.
e) Sensitivity. Ten distilled water blanks gave an average value of 0.12 ± 0.09 ng/ml. The lowest value for measurable testosterone standards which differed from the blank value ($P < 0.05$) was 0.28 ng/ml.

Statistical Methods: Data were analyzed by analysis of variance and tested for significance with Duncans New Multiple Range Test (Alder and Roessler, 1972).

Results

Diurnal Variation: One of the horses, (S), never adjusted to the restraint and handling procedures. After the tenth hour of sampling this animal was released in order to prevent increased stress and probable death. As a result no data have been included for this horse. None of the remaining three horses showed undue stress; however, (W) remained somewhat aggressive throughout the study.

A definite diurnal variation in plasma testosterone was exhibited by all three stallions. The mean testosterone concentrations reached a nadir of 1.37 ±0.75 ng/ml plasma at 2300 and a peak of 3.48 ± 0.12 ng/ml plasma at 0800. The mean low concentration at 2300 differed significantly ($P < 0.01$) from those at 2300 to midnight and through 0400. The general pattern of testosterone levels indicated an increase beginning in the early morning hours. The testosterone concentrations more or less maintained a plateau during the daylight hours between 0800 and 1800.

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Of the three animals, the stud stallion Welfare had the highest mean testosterone concentration for the 24 hrs (3.2 ± 0.7 ng/ml). The stud stallion Crooked Creek had a 24-hr mean testosterone concentration of 2.7 ± 0.6 ng/ml. The bachelor stallion Black had a 24-hr mean testosterone concentration of 1.5 ± 0.9 ng/ml and differed significantly ($P < 0.01$) from both of the stud stallions. At both midnight and 0400 the bachelor stallion's testosterone concentrations were nondetectable (Kirkpatrick et al., 1976).

Seasonal Variation: A clear seasonal variation existed for plasma 17-beta-hydroxyandrogen and testosterone concentrations.

Since only two stallions were captured in September, these data did not lend themselves to statistical analysis and are therefore not considered here. The 17-beta-hydroxyandrogen nadir occurred in December (2.11 ± 0.39 ng/ml). Disregarding the two stallions in September, the testosterone nadir also occurred in December (1.55 ± 0.34 ng/ml) and the peak in May (3.04 ± 0.36 ng/ml).

When seasonal testosterone concentrations were examined as a percentage of the total androgens, the results were as follows: September=58%, October=67%, December=73%, March=81%, May=85%, and July=80%. As total 17-beta - hydroxyandrogen concentrations increased, the proportion which constituted testosterone increased dramatically (Kirkpatrick et al., 1977).

Immature Stallion Androgens: The testosterone levels for the twenty-one two-year-old stallions are shown in Figure I. The 19 two-year-old males with undescended testes had a mean testosterone concentration of 1.14 ± 0.23 ng/ml of plasma. The two two-year-olds with descended testes had concentrations of 1.9 and 4.8 ng/ml with a mean of 3.35 ng/ml.

Discussion

It is clear that in the three wild stallions examined a definite diurnal variation exists with regard to plasma testosterone. Diurnal testosterone variations have also been demonstrated in man (Faiman and Winter, 1971; Barberia and Cortes-Gallegos, 1973; Leymarie et al., 1974); the rat (Kinson and Liu, 1973); and the rhesus monkey (Goodman et al., 1974). Increased testosterone levels have been shown to occur in rams (Wetteman and Desjardins, 1973) during the dark hours. While not as clearly evident as in other species, the data of Katongole et al. (1971), imply a diurnal testosterone variation in bulls. In domestic horses, several investigators have noted diurnal variations of plasma testosterone (Ganjam and Kenney, 1974; Cox and Williams, 1975; Sharma, 1976).

The pattern of changes of plasma testosterone in the wild stallions is qualitatively similar to those seen in rats and man, with the nadir occurring between 1900 and midnight and the highest concentrations occurring between 0600 and noon, and differs from that of the rhesus monkey where highest concentrations occur at 2100. In several breeds of domestic horses, Ganjam and Kenney (1975) noted a diurnal variation of plasma testosterone, ranging from 4.2 ± 0.4 ng/ml at 0600 to 3.0 ± 0.3 ng/ml at 1800. These values are quantitatively similar to those found in the wild stallions. Cox and Williams (1975) examined diurnal variations of plasma testosterone in Welsh Pony stallions during April and June and noted irregular patterns of secretion.

The 24-hr mean testosterone concentration for the bachelor stallion (B) was significantly lower ($P < 0.01$) from the mean testosterone concentrations in the two stud stallions. Both the stud stallions have been successful in recent years, in gathering and maintaining a harem and both are assumed to have sired colts. The bachelor stallion, however, has not demonstrated even an effort to gather a harem. This is highly unusual for an eight-year-old wild stallion (Hall, 1972). Whether the significantly lower testosterone concentration represents a cause or an effect of such sexual disinterest is unanswered by this study.

When the mean testosterone concentrations of the wild stallions are compared with those of rats (Bartke et al., 1973); man (Faiman and Winter, 1971); the rhesus monkey (Goodman et al., 1974); the ram (Wetteman and Desjardins, 1973); and the bull (Katongole et al., 1971) they appear to be quite low. The wild stallion study reported here was conducted in late October, during the non-breeding season. It has been shown that in both wild stallions (Kirkpatrick et al., 1977) and domestic stallions (Berndtson et al., 1974; Raeside, 1978) testosterone concentrations fluctuate seasonally and are relatively low in October.

The 34 wild stallions examined in the seasonal study showed androgen and testosterone concentrations below 2 ng/ml during the three non-breeding months and above 2.38 ng/ml during the breeding months. Peak breeding activity occurred at the same time as peak androgen/testosterone concentrations. Increased aggressiveness between males also coincided with the higher concentrations. Berndston, Pickett,and Nett (1974) reported seasonal changes in testosterone in Quarter Horse stallions. These variations were similar to those in the 34 wild horses, with the nadir occurring in October and the peak in May. Although it is not reported in their study, it is assumed that nutritional levels remained fairly constant throughout the year. If that is the case, then nutrition must not be the predominant stimulus for androgen/testosterone fluctuations in the Quarter Horse. Nutrition is very poor during the winter months for the wild horse and the annual 'green-up' occurred at the same time androgen levels began to increase. A more recent study (Thompson et al., 1977) failed to confirm this seasonal pattern of androgens in domestic stallions, yet Raeside (1978) demonstrated seasonal testosterone patterns in domestic stallions which were similar to those seen in feral stallions. Whether nutrition plays any significant role in regulating androgen levels remains unanswered at present, but from the comparison of events in the wild and domestic horse, it appears improbable. The marked seasonal change in androgen/testosterone ratio indicates that testosterone becomes the predominant androgenic steroid during the breeding season. Exactly what the large proportion of non-testosterone androgens consists of during the non-breeding season is unknown and remains to be examined. All samples were collected between 800 and 1600 hrs. The diurnal study indicates fluctuations are very small during this period of the day, and it is improbable that diurnal variations caused erroneous results in the seasonal data.

Although the sample size of two horses, in the immature stallion study, precludes definite conclusions, there appears to be a dramatic increase in plasma testosterone near the time young stallions are ejected from their bands. This increase in testosterone results in plasma concentrations similar to those in mature harem stallions. Since we

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have previously seen correlations between aggressive behavior and plasma testosterone concentrations, as well as seasonal behaviors associated with reproduction, it is reasonable to hypothesize that testosterone-induced behavioral changes, or testosterone-based pheromonal messages emanating from young stallions are ultimately responsible for their ejection from harem bands.

Acknowledgements

This study was funded in part by the Eastern Montana College Foundation and Bureau of Land Management contract YA-512-CT8-21. We gratefully acknowledge and thank Mr. Lowell Brown, Mr. Gene Nunn, and Mr. Lynn Taylor, of the Bureau of Land Management, for the expert capture and handling of the horses.

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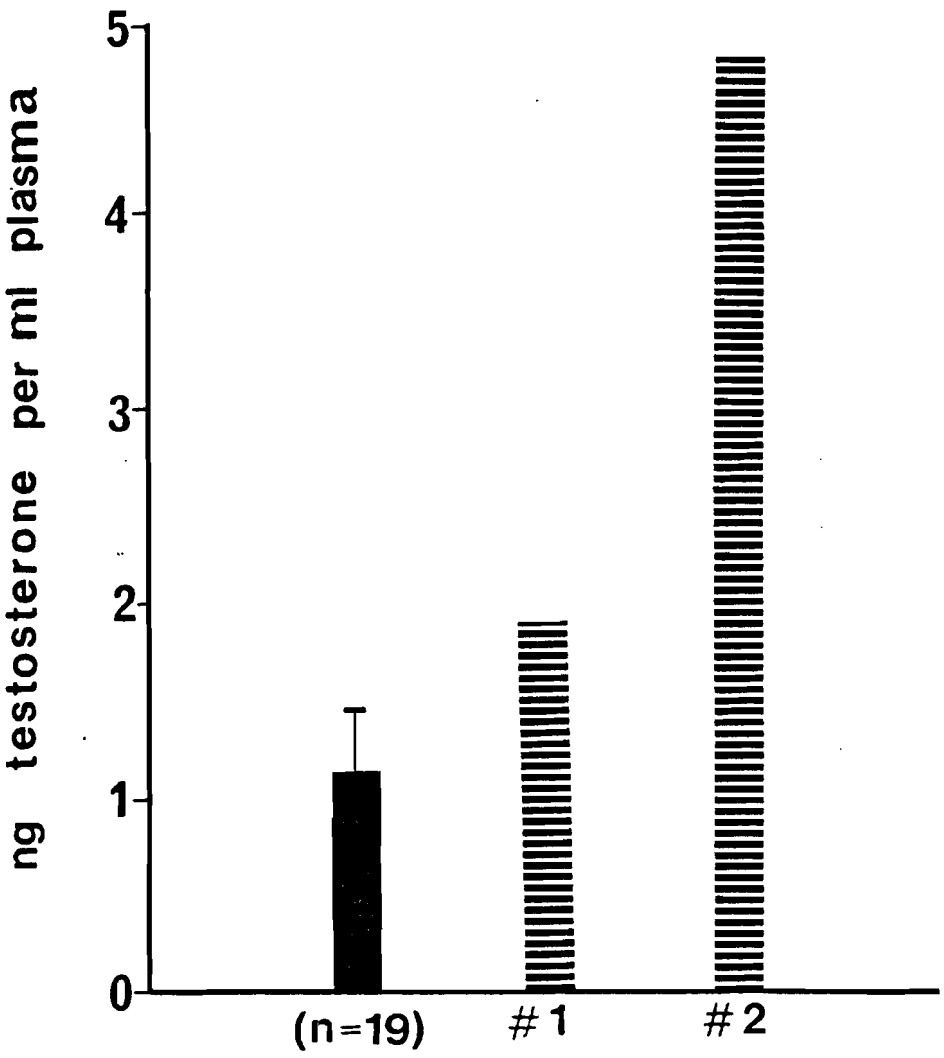

Fig. 1. Testosterone levels in 19 two-year-old males with undescended testes (solid bar) and two two-year-old males with descended testes (hatched bars).

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Hormones and Behavior

Volume 13, Issue 1, August 1979, Pages 49-65

Sociosexual behavior and the ovulatory cycle of ponies (*Equus caballus*) observed in harem groups

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Abstract

Observations of sociosexual behavior of adult ponies, made on two harem groups (each comprised of one vasectomized male and three females), were correlated with follicular development and ovulation for a total of 15 cycles (minimum of 2 cycles per female). Mean cycle length (interovulatory interval) was found to be 19.7 days, with behavioral estrus lasting 7–8 days (5.5 days preovulatory; 2.3 days postovulatory). Estrous females typically showed increased frequencies of approaching and following the stallion, urinating, presenting, clitoral winking, and tail raising. Approaching and following the stallion appeared earlier and persisted longer than other estrous responses. Deviations from the modal estrous pattern included cycles with subestrus, continual estrus, behavioral estrus in the absence of ovulation, and displays of female mounting. Dominance tests revealed that a mare's status was unaffected by the phases of the estrous cycle. The presence of more than one estrous female affected the copulatory performance of both stallions, most notably in reduced latencies to first mount, intromission, and ejaculation, in spite of differences between the stallions in sexual vigor. Each stallion usually selected the dominant mare for copulation when there were multiple estrous females present, but mounts were not displayed exclusively to one female per test. The social testing situation made apparent the importance of use of space in sociosexual communication in this species, particularly in avoidance of the stallion by diestrous mares and standing alone or in proximity to him by estrous mares.

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Hormones and Behavior

Volume 14, Issue 1, March 1980, Pages 55-64

Dexamethasone suppression of sexual behavior in the ovariectomized mare

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Abstract

The influence of steroids of adrenal cortical origin on estrous behavior in the ovariectomized mare was evaluated by adrenal suppression via dexamethasone (DEX) administration in two experiments. In Experiment I, 12 mares (six DEX, six control) were tested for sexual behavior in harem groups (two DEX and two control mares plus one stallion per group) for 9 consecutive days. In Experiment II, estradiol (E_2) was given to a group of DEX-treated mares as an additional control. Twelve mares (four DEX, four DEX + E_2, and four control) were tested in harem groups (one DEX, one DEX + E_2, and one control mare plus one stallion per group) for 10 days. All DEX mares showed a clear suppression of sexual response compared to control or DEX + E_2 mares, indicating that the estrous behavior seen in ovariectomized mares may be due to steroids from the adrenal cortex. The control and DEX + E_2 mares were similar in all measures of proceptivity. Despite being more receptive, as indicated by fewer negative responses, the DEX + E_2 mares received fewer intromissions and ejaculations than did the control animals. The ability of estradiol to induce estrous behavior in the dexamethasone-suppressed mare notwithstanding, other adrenal steroids, e.g., androgens, may be involved in estrous behavior in the untreated, ovariectomized mare.

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HORMONES AND BEHAVIOR 14, 46–54 (1980)

Sexual Behavior in Ovariectomized and Seasonally Anovulatory Pony Mares (*Equus caballus*)

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Ten ovariectomized (OVEX) and ten intact, but seasonally anovulatory (ANOV), pony mares were observed for sexual activity with five stallions, using a "harem group" social testing paradigm (two OVEX and two ANOV mares plus one stallion per group) for 15 consecutive daily tests lasting 20 min each. All mares in both conditions showed proceptive behavior in at least one test, all mares but one were mounted, and 14 of 20 mares received ejaculations. No statistical differences were found between the two conditions for any measure of proceptivity, copulatory activity, or days in estrus. The quality of estrus was judged to be equivalent to that displayed by periovulatory mares during their initial and terminal days of estrus, but less intense than that seen near ovulation. Mares in both groups were in estrus during approximately 60–70% of the tests and only 3 of the 20 mares were sexually refractory for more than five consecutive tests. Thus, the typical 2-week phase of sexual refractoriness seen in intact diestrous mares was absent in OVEX and ANOV mares, suggesting that the ovary plays a major role in actively suppressing estrous responses during the luteal phase of the cycle.

Steroids of gonadal origin have long been assumed necessary for the induction of estrous behavior in female mammals. Although Old World primates, such as the majority of rhesus and stumptail macaques, continue to show some copulatory behavior following ovariectomy (Goldfoot, Wiegand, and Scheffler, 1978; Baum, Everitt, Herbert, and Keverne, 1977), as is apparently also the case with a small percentage of rabbits (Yaschine, Mena, and Beyer, 1967), gonadectomized females of most mammalian species do not solicit coitus nor tolerate mount attempts unless administered exogenous steroids (Young, 1961; Goy and Goldfoot, 1975).

In this laboratory, estrous behavior has been noted in long-term ovariectomized mares (Garcia, published in Ginther, 1979). Estrous response has also been reported in mares whose ovaries were involuted during the nonbreeding season (Van Niekerk, 1967; Ginther, 1974; Webel, Franklin, Harland, and Dzuik, 1977). The present study was undertaken

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to investigate in more detail the sexual response of mares in which ovarian hormones are absent (ovariectomized mares) or minimal (seasonally anovulatory mares).

METHODS

Subjects

Five stallions, 10 ovariectomized (OVEX) mares, and 10 seasonally anovulatory (ANOV) mares were used. The ponies were of apparent mixed breeding and weighed between 160 and 250 kg. The stallions ranged in age from approximately 3 to 20 years and the mares from 5 to 15 years. Four of the stallions were intact and one was vasectomized. The sexual experience of the mares before ovariectomy was not known. Gonadectomy was performed 6 months to 2 years before the present project. Rectal palpation revealed no evidence of ovarian fragments in any of the OVEX mares. After the completion of behavioral testing, an OVEX mare which had shown consistently high levels of estrous behavior was sacrificed and the entire reproductive tract removed after gross inspection of the body cavity. The tract was examined and the ovarian pedicles excised for histology.

In a previous study of intact, ovulating mares (Asa, Goldfoot, and Ginther, 1979), estrous behavior was never observed in mares with follicles of less than 15 mm diameter. Day (1940) reported that diestrus was accompanied by 10- to 15-mm follicles in pony mares. Therefore, the 10 ANOV mares were assigned to the project only after rectal palpation revealed small ovaries with follicles of no more than 10 mm diameter.

Mares were pastured together outdoors and provisioned with hay. Stallions were stabled separately and fed hay and grain.

Behavioral Testing

The study was conducted for 15 consecutive days from January 9 to 23, 1978. Behavior tests were given for 2 days before the study began to familiarize the animals with the testing situation. Five harem groups, each consisting of two OVEX mares, two ANOV mares, and one stallion, were assembled daily. Assignment of animals to harems was random each day with the exception that stallions were sometimes chosen to insure that each mare was tested with each stallion at least one time. Each harem group was observed daily for 20-min periods in the early afternoon. The outdoor paddock used for testing was approximately 6 by 9.6 m, with a straw-covered concrete base.

Behavioral data were taken on an SSR event-recording keyboard (Stephenson and Roberts, 1977) which allowed rapid notation of the incidence, latency, duration, and sequence of behaviors onto magnetic tape for subsequent computer analysis. Behaviors reported included approach, follow, urination, tail raise near stallion (TRNS), walk away,

mount, intromission, and ejaculation, which are defined elsewhere (Asa, Goldfoot, and Ginther, 1979). Kick, which was not defined previously, was scored only when both hind feet were used to strike out forcefully.

A behavioral index of estrus was developed in an earlier study in which all observations were made for 1-hr periods (Asa *et al.*, 1979). That index has been modified here for 20-min tests and expanded to allow quantitative analysis of the behavior of mares in conditions less vigorous than full estrus. For this study mares were considered in full estrus when they had more than two occurrences of urination and ≥20 sec of tail raise near the stallion (TRNS) during the 20-min test. The category of weak estrus included mares with one of the following: one occurrence of urination plus ≥20 sec TRNS, two or more urinations but no TRNS, or no urination but ≥60 sec TRNS per 20-min test.

Statistical Analysis

Comparison of the OVEX and ANOV groups for the display of various proceptive and receptive measures of the mares as well as for copulatory activity of the stallions was made by *t* tests for independent samples. A paired *t* test was used to evaluate the effect of weak or full estrous conditions on the frequency of kicks or walk-aways per mount.

To investigate the possibility of changes in the expression of estrus as the study progressed, the 15 days were divided into three 5-day segments. Analysis of variance for repeated measures (Winer, 1962) was used, with means for each mare for each 5-day period used as the three repetitions. The relationship between frequencies of urination (an indicator of proceptivity) and kicks and walk-aways per mount (indicators of nonreceptivity) was calculated using the Pearson product-moment correlation and the *t* test for significance of a correlation.

RESULTS

All mares in both groups showed proceptive estrous signs on at least one of the test days, although the intensity of estrus was sometimes low. Estrus determinations for the OVEX group ranged from one mare that was only in weak estrus in one test to another that was in full estrus for all 15 tests. The OVEX group means, as shown in Table 1, were 7.7 (± 1.75 SEM) tests with full estrus and 2.9 (± 0.74 SEM) tests with weak estrus for the 15 tests. The ANOV group means were 4.8 (± 1.45 SEM) tests with full estrus and 3.9 (± 0.98 SEM) tests with weak estrus. The ANOV group had one mare that was only in weak estrus for one test, but another that was in full estrus for 14 tests.

That mares in both groups were attractive and receptive to the stallions was demonstrated by male approaches and by precopulatory investigation and stimulation similar to that displayed to periovulatory estrous mares (Asa *et al.*, 1979), as well as by the complete male ejaculatory pattern. All

TABLE 1
Comparison of Ovariectomized and Seasonally Anovulatory Mares for Mean Number of Tests with Estrus and Copulatory Activity

	Meal number of tests[a] per mare with					
	Weak estrus	Full estrus	Total	Mounts received	Intromissions received	Ejaculations received
Ovariectomized mares (n = 10)	2.9 ±0.74[b]	7.7 ±1.7	10.6 ±1.5	7.2 ±1.1	2.3 ± 0.52	1.8 ±0.47
Seasonally anovulatory mares (n = 10)	3.9 ±0.98	4.8 ±1.4	8.7 ±1.3	5.8 ±1.1	2.7 ±1.0	2.0 ±0.84

[a] Total number of tests = 15.
[b] $\bar{x}$ ± SEM.

mares, except one in the ANOV group, were mounted at least once and all but four ANOV and two OVEX mares received ejaculations. Most of the ejaculations (94.8%) occurred with full estrous mares, 5.1% with mares in weak estrus, and none with mares judged not to be in estrus.

No differences were found between the two groups of mares on any of the 15 behavioral endpoints (Tables 1, 2). The means for the two groups for incidence of estrus and selected measures of proceptivity, attractiveness, and copulatory activity are shown in Tables 1 and 2. The mean frequencies for two forms of negative behavior, walking away during a mount (OVEX 1.2 ± 0.1, ANOV 1.2 ± 0.2) or kicking at any time during a test in which a mount occurred (OVEX 3.6 ± 1.8, ANOV 3.1 ± 0.5) were not different for the two groups of mares. The proportion of kicks and

TABLE 2
Comparison of Ovariectomized and Seasonally Anovulatory Mares for Mean Frequency or Duration of Selected Measures of Proceptivity and Copulation

	Mean frequency or duration per mare per test						
	Tail raise near stallion (sec)	Female urination	Female approach plus follow	Male approach	Mount	Intromission	Ejaculation
Ovariectomized mares (n = 10)	278 ±51[a]	2.3 ±0.49	5.5 ±0.24	2.5 ±0.22	1.2 ±0.28	.26 ±0.07	.11 ±0.03
Seasonally anovulatory mares (n = 10)	209 ±57	1.7 ±0.62	3.7 ±0.52	2.4 ±0.21	1.3 ±0.34	.21 ±0.08	.13 ±0.13

[a] $\bar{x}$ ± SEM.

walk-aways was no higher for weak estrus than for full estrus in either group. The mean number of kicks, but not walk-aways, per mount was negatively correlated with the mean frequency of urination, a measure of estrous intensity, for both groups (OVEX: $r = -0.359$, $P < 0.005$; ANOV: $r = -0.423$, $P < 0.005$).

Only one mare was consistently in estrus throughout the 15 days of the study. The others displayed an irregular pattern of full, weak, or no estrus from day to day (Fig. 1). Comparison of means for estrus and copulatory activity among the first, second, and third 5-day segments of the study yielded no differences. There was a mean of 1.58 (± 0.07) mares in full estrus and 1.18 (± 0.03) in weak estrus per harem group per test (out of four mares/harem group), with medians of two in full and one in weak estrus per test. The identity of those females in estrus in each test varied from day to day.

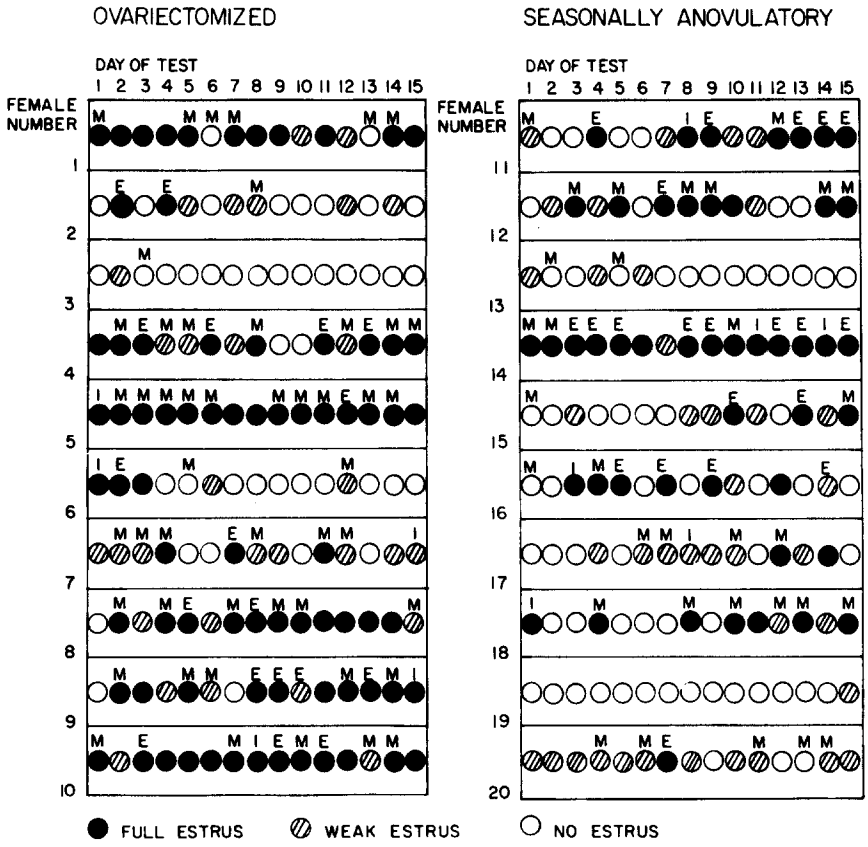

FIG. 1. Pattern of incidence of full, weak, or no estrus for 10 OVEX and 10 ANOV mares during 15 consecutive days of testing in harem groups. E, ejaculation; I, intromission; M, mount.

The five stallions displayed wide variability in copulatory performance, although mean number of mares in estrus per test was not different among stallions. In 15 days of testing, stallion 400 achieved only four ejaculations while stallion 100 had 12. The ratio of mounts per ejaculation was 3.25 for stallion 100, but 31.5 for the younger, less experienced stallion 200. The amount of time spent in precopulatory activity was reflected by latencies to first mount. These times ranged from a mean of 0.3 min for stallion 200 to 9.7 min for stallion 400. Stallion 100, the oldest, had the highest total number of ejaculations and the shortest latency to ejaculation while stallion 200, the youngest, had the most mounts and the longest ejaculation latency. For ejaculatory mounts, however, the mean interval from mount to ejaculation was not different among stallions.

Gross inspection of the body cavity and the reproductive tract of the sacrificed OVEX mare revealed no signs of ovarian-like tissue. Likewise, microscopic examination of the sectioned and stained ovarian pedicles disclosed no ovarian remnants or regrowth.

DISCUSSION

In this study both seasonally anovulatory mares and ovariectomized mares solicited copulation from stallions, were sexually attractive to them, and tolerated mounts, intromissions, and ejaculations. Such behavior in the pony under reduced hormone conditions is striking in light of the widely held belief that hormones of ovarian origin are exclusively responsible for displays of estrus in infraprimate mammals (Goy and Goldfoot, 1975; Leshner, 1978; Young, 1961). Nonetheless, it would be misleading to conclude that the pony is "gonadally emancipated," since the ovary is clearly involved in the regulation of estrous behavior during normal ovulatory cycles. Intact mares display very clear patterns of estrus for approximately 1 week out of every 3 throughout the spring and summer (Ginther, 1979). The week of behavioral estrus, with ovulation on about the fifth day, is followed by a period of marked sexual refractoriness during the 2-week diestrous phase, when mares respond to a stallion's approach and investigations with avoidance or threats (Asa *et al.*, 1979).

Comparisons of mares from this study to intact estrous and diestrous mares of a previous study suggest that the removal of the ovaries or the natural suppression of ovarian function during the winter months results in a chronic condition of moderate behavioral estrus, in which behavioral measures of attractivity, proceptivity, and receptivity are found with probabilities of at least 50% on any given test day. In contrast, estrus is seen on only about 33% of the days for intact mares during the ovulatory season (Asa *et al.*, 1979).

Proceptive components of estrus, such as the frequency of urinations, of following, or of standing near the stallion with the tail elevated were displayed with rates similar to those of intact mares during the initial and

terminal days of ovulatory estrus. Receptive elements of the response pattern were slightly reduced, however, in that proceptive OVEX and ANOV mares seemed to show more hard kicking and avoidance responses than intact estrous mares.

The intensity of estrus in ANOV and OVEX mares fluctuated in irregular patterns throughout the course of this study, perhaps due to the specific social composition of the groups formed on a given day, including individual preferences by mares for certain stallions, and dominance/subordination relationships among mares. Alternatively, these daily changes might have been related to changes of nonovarian-source steroids which could have been contributing to the phenomenon.

Only 3 of the 20 mares in this study displayed periods of sexual refractoriness for longer than 5 consecutive days, whereas half of the ponies never had more than a 2-day period in which estrous responses were not displayed. Thus, the absence of *cyclic* periods of nonreceptivity and nonproceptivity most accurately characterizes the behavioral changes following ovariectomy or seasonal ovarian quiescence in the mare. In contrast, a continual absence of estrous responses is typical of other ovariectomized or anovulatory infraprimate species.

These several observations and comparisons suggest the following three hypotheses. First, the luteal ovary appears to play a controlling role in the inhibition of estrous behavior, raising the possibility that progestational secretions of the corpus luteum provide the mechanism for periodic sexual inhibition during diestrus.

Second, although a nonhormonal mechanism might be responsible for estrous activity after ovariectomy, we hypothesize that the estrous behavior seen in this study was most likely supported by sex steroids, probably from the adrenals. Adrenal cortical mediation of sexual activity has been extensively considered in primate species (Everitt and Herbert, 1971; Everitt, Herbert, and Hamer, 1972; Goldfoot *et al.*, 1978; Baum, Slob, de Jong, and Westbroek, 1978). Hay and forage, which may contain phytoestrogens, present another potential source of steroids.

Third, since periovulatory estrus is more vigorous than that observed in OVEX and ANOV females, it is hypothesized that additional steroidal stimulation for these behavioral tendencies is supplied by the ovarian follicles in the days just prior to ovulation. Thus, it is likely that the follicular ovary of the mare, as in other species, increases the probability that copulation will occur during fertile phases of the cycle. However, the luteal ovary of the mare appears necessary to suppress estrus during the infertile phase of the cycle and therefore might be exhibiting a more potent inhibition of estrus than that thought to exist in most other infraprimate species (Feder, 1977).

The steroid condition resulting from ovariectomy represents an experimental manipulation which mares would normally never experience.

However, winter anestrus does occur naturally (Ginther, 1979), and 40–50% of mares in a typical free-ranging herd fail to deliver live young in a given season (Nelson, in press; Feist & McCullough, 1975). Thus, estrus might be displayed by some percentage of the nonpregnant, free-ranging mares during winter months.

The display of estrous behavior by mares at times during which conception is impossible might have functional significance to this species. The most commonly reported social unit of ponies and horses is of the harem type, with a stallion, several mares and their offspring (Feist and McCullough, 1976). Sexual activity outside of the breeding season, made possible by the absence of complete control of steroids from the ovaries over estrous behavior, could contribute to the maintenance of social bonds between the harem stallion and his mares. Further work directed to this possibility as well as toward the question of whether sex steroids from adrenal sources mediate these events is currently being investigated.

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The effect of estradiol and progesterone on the sexual behavior of ovariectomized mares

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Abstract

Daily treatment (5 days) with estradiol resulted in increased levels ($p<0.05$) of proceptive behavior in ovariectomized as compared to control mares (N=8 per treatment) within 4 hr of injection and for the 4 subsequent days. Ejaculations occurred more often ($p<0.05$) in estrogen-treated mares on days 2–5, but the number of precopulatory investigations by the stallions was not altered. Progesterone treatment resulted in an absence of sexual behavior except in one mare on Day 1. Control mares exhibited varying levels of sexual interest. The concurrent administration of estradiol and progesterone produced a biphasic effect on proximity-related behaviors. Proximity behaviors were initially (Day 1) greater and subsequently less in the group treated with both hormones than in the group treated with estradiol alone. Injections of free estradiol resulted in a shorter latency to effect for two measures of proceptivity than did injections of estradiol benzoate. A dose response test for progesterone showed no effect with 0, 1, or 10 mg, but 100 mg was inhibitory ($p<0.05$). These results demonstrated that within 4 hr estradiol stimulated, while progesterone inhibited estrous behavior in ovariectomized mares and that concurrent administration of estradiol and progesterone produced a biphasic effect, first enhancing, then suppressing some aspects of the estrous response. The relatively short latency to action of all treatments and the biphasic effect of concurrent estradiol and progesterone may be attributable to low level, endogenous hormones.

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Keywords

Estradiol; Progesterone; Mares; Ovariectomy; Sexual behavior; Estrous behavior; Biphasic effect

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Effect of Stress Induced by Gathers and Removals on Reproductive Success of Feral Horses
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Effect of stress induced by gathers and removals on reproductive success of feral horses

Michael C. Ashley and Dale W. Holcombe

Abstract Successful management of many species often relies on actions that involve intensive handling of individuals. Knowledge of how such handling may affect reproduction of a particular species is important and may be applicable to managing other species. We used pregnancy testing, field observation, Bureau of Land Management (BLM) records, and adopter surveys to determine effects of stress induced by gather and removal management practices on the reproductive success of feral horses in the Garfield Flat (GF) Herd Management Area (HMA) in Nevada. We analyzed pre-release confinement effect data from gathers conducted in August 1993 and January 1997 and data on the additional effect of removal in 1997. We used data for an ungathered population in the Granite Range HMA (GR) in Nevada as controls both years. Data from ungathered GR horses also were used as controls for habitat effects on reproduction. Granite Range and ungathered GF mares in 1997 had similar reproductive success rates (P=0.42). Pregnant removed mares in 1997 had less reproductive success than ungathered mares at GR (P=0.003) and GF (P=0.005). Gathered and released GF mares had less reproductive success than ungathered GF mares (P=0.05). The results suggest that minimizing time that mares are held prior to release will reduce fetal loss.

Key words abortion, horses, management, reproductive success, stress

In several studies, fetal loss in feral and domestic horses has been attributed to stress, often associated with frequent and extensive handling of pregnant mares (Mitchell 1971, Osborne 1975, Roberts 1986). Berger's (1983) study of feral horses in the wild found that forced copulation and social environment change after band takeovers in the Granite Range of Nevada caused abortions.

Most feral horse herds in the United States are managed intensively by the Bureau of Land Management (BLM, Boyles 1986). The Public Rangelands Improvement Act of 1978 authorized excess feral horse removal to preserve the quality of range habitat. Gathers and removals are the principal means to reduce populations to acceptable management levels (AML) for individual herd management areas (HMA; Boyles 1986). Each HMA is assessed by BLM range-condition specialists to determine stocking levels for livestock, feral horses, and game species. A 3- to 5-year interval between gathers and removals is necessary as many horse herds increase ≥20%/year (Wolfe 1980, Eberhardt et al. 1982, Berger 1986, Garrott and Taylor 1990). The removal magnitude depends on number of excess horses and the desired ending population size relative to the AML.

Gathers and removals may induce stress in several ways and for varying periods of time. Large-scale BLM gathers involve helicopter use to chase horses into a funnel trap leading to a corral. Trapped horses are individually restrained, aged, examined for physical

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condition, and segregated for removal or release as adult and juvenile males, lactating mares and foals, and nonlactating mares. *Ad libitum* food and water is provided in corrals to minimize nutritional stress.

Horses released after a gather typically have been held for 2 to 4 days at the capture site. Horses removed for adoption face longer periods of stress. From the capture site horses are trucked to a processing facility, often hundreds of km away. Here they are again individually restrained for blood sampling, aging, and freeze-branding. Some adoption occurs locally; however, many more horses are transported to satellite adoption centers across the country. Horses can spend months in confinement before adoption. We investigated the role that stress plays in reproductive failure following gathers and removals. We feel that the results of this study are valuable to BLM management and are applicable to managing large-bodied ungulates in general, when translocations involve extensive handling or long periods of confinement.

Study sites

For >20 years, BLM has actively managed feral horses at the Garfield Flat (GF) and Granite Range (GR) HMAs in the Great Basin by conducting periodic gathers and removals to control population size. Both areas are subject to cattle grazing. The vegetation at both study areas is predominantly mixed grass and shrubs at lower elevations. At higher elevations, pinyon pine (*Pinus monophylla*) and western juniper (*Juniperus occidentalis*) predominate at GF. Western juniper also occurs at higher elevations at GR with stands of quaking aspen (*Populus tremuloides*) at some springs. The 607-km^2 GF HMA, located in Mineral County in west-central Nevada, is bordered on the east, north, and west by the Garfield Hills and on the south by the Excelsior Mountains. The playa at GF is 1,700 m above sea level (asl), with the Garfield Hills and Excelsior Mountains each rising above 2,400 m asl (United States Department of Interior 1985). Water is available year-round from 4 springs and ephemerally from 4 small catchments, ≤100 m^2 each (Figure 1). The 411-km^2 GR HMA is located in Washoe and Lander counties in northwest Nevada in the Granite

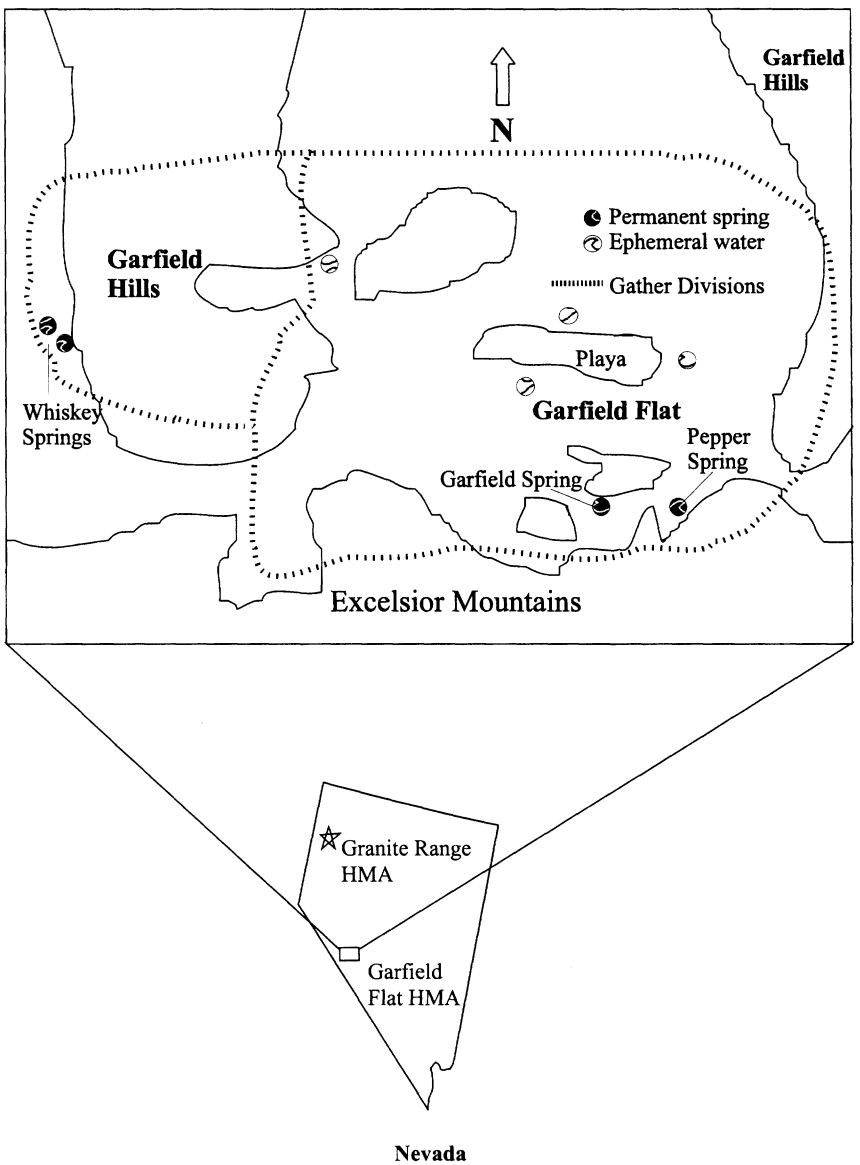


Figure 1. Locations of study sites and representation of the Garfield Flat Herd Management Area, including locations of available water and division of the HMA for the 1993 gather.

A mare being herded toward a squeeze chute for tissue collection and freeze-branding at the 1993 gather.

Range Mountains. Elevations range from 1,200 m asl in the surrounding lowlands to 2,735 m asl at Granite Peak (United States Department of Interior 1981). At this site, water is available year-round from numerous permanent springs and ephemeral ponds ranging from 50 to >400 m^2 in size.

Materials and methods

Gathers and removals

The first gather at GF was conducted in August 1993. The second occurred in January 1997. For both, a helicopter, drift fence, and funnel trap were used to capture horses. In the 1993 gather, resident horses in the eastern portion of the HMA were captured the first day and processed and released the second day. This procedure was repeated for horses in the western portion of the HMA on the following 2 days (Figure 1). Eighty-eight horses from the eastern area and 81 horses from the western area were captured, accounting for about 95% of the total HMA population. All horses gathered in 1993 were released except for 4 foals that were removed and one foal that was destroyed because of injuries. In 1997, horses were captured from the entire HMA over a 2-day period. Processing started immediately and continued for 2 days post-capture, culminating with the release of horses not shipped for adoption. The released horses were held 3 or 4 days, depending on capture order. Horses removed for adoption were trucked on days 3 and 4 to the Palomino Valley Center (PVC), a BLM adoption and processing center 246 km from the study site. Horses from the 1997 gather were examined and freeze-branded at PVC 2 weeks after the conclusion of the gather.

Horses appeared to be stressed at gathers, exhibiting agitated behavior. Confinement kept horses from different bands, groups of ≥1 adult female and any offspring attended by ≥1 adult male, at much closer quarters than we observed on the range. Mares too close to an unrelated foal were often threatened or kicked by the foal's mother. In contrast, we found that intra-band female–female aggression in the field was infrequent and limited to mild threats. Band spacing in the field generally precludes aggressive behavior. Inter-band aggression was usually observed between band stallions that often traveled >100 m to interact. Some bands were permanently disrupted after the release.

Tissue collection

In January 1997, we collected jugular blood samples at the gather site and at PVC in evacuated tubes from horses that would be ≥2 years old the following spring. We separated the serum component on site with a portable centrifuge (5,000 × g for 5 minutes) and stored the samples on ice for transport. We found that centrifugation at greater gravity than applied by Wolfe et al. (1989) efficiently separated the serum in less time. At the lab, we pipetted ≥1 ml serum/sample into individually labeled 1.5-ml microcentrifuge tubes and stored them at −80°C until samples could be assayed. Serum progesterone was determined using a solid phase radioimmunoassay kit (Coat-a-Count, kit #TKP6, Diagnostic Products Corporation, Los Angeles, Calif.; Wolfe et al. 1989). The coefficients of variation for progesterone were 8.1% for intra-assay and 9.7% for inter-assay results. Because nonpregnant mares generally do not cycle during the winter, we classified mares that had titers of ≥2 ng/ml serum progesterone as pregnant (Plotka et al. 1988).

Field methods

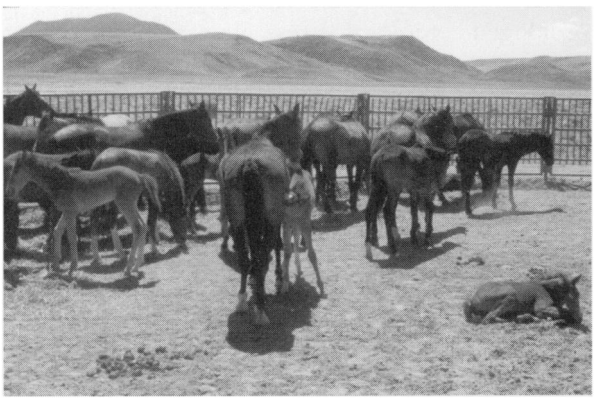
All released horses from both gathers at Garfield Flat were photographed and left-hip freeze-branded with unique numbers. Age (American Association of Equine Practitioners 1981), sex, and distinguishing marks were noted to aid in field identification. In February 1993 and November 1995 the BLM conducted gathers and removals at the GR HMA, releasing 359 and 123 freeze-branded horses, respectively. Identifying marks also were recorded for many of these horses.

We conducted intensive ground surveys of the GF and GR horses from March through August of

1994 and 1997 to assess reproductive success. Using 80-mm, 20X60-power spotting scopes, we identified horses by number and, when necessary, unique markings. To have reasonable confidence of accurately assessing reproductive success or failure, we only used data on mares that were sighted prior to, during, and after the prime foaling season, April to July. These sightings allowed us to assess whether an animal was visibly pregnant and whether a viable foal was produced. Mares classified as pregnant that were not accompanied by a foal by the end of August were scored as reproductive failures. We verified the foaling success or failure of all GF mares released after the 1997 gather. All successful pregnancies at both study sites were detected pre-partum in that surveyed mares seen with foals had been classified correctly as pregnant prior to foaling. Some of these mares at both sites were classified pregnant but were unaccompanied by foals at the end of the season. These reproductive failures of visibly pregnant mares may have included losses to mountain lions (*Puma concolor*) or coyotes (*Canis latrans*), even though we found no direct evidence of predation.

We assessed reproductive success for GR horses in 1994 and 1997 to serve as unmanipulated controls for the GF horses. The GR horses were gathered February 1993 and November 1995, early enough that there should have been no negative impacts on foaling in 1994 and 1997.

We collected GF removed mare reproductive success data in 1997 from BLM adoption records, adopter questionnaire, and telephone interviews with adopters. Unable to separate early foal death from fetal abortion in the field population, we included one stillborn, one death during birth, and the death after 1 day of a premature foal as evidence of reproductive failure for removed horses. In this tally, we also included 3 cases of pregnant removed mares that died prior to adoption. The BLM determined that the cause of these deaths was "poor condition" that could have been influenced by prolonged stress.



Segregated bands of lactating mares and foals at the 1993 gather.

Data analysis

Foaling success is a dichotomous event suitable for 2-by-2 contingency table analysis for pairwise comparisons. We used the 1-tailed Conditional Binomial Exact Test (CBET, Rice 1988) for these analyses, expecting that increased periods of stress would result in lesser foaling rates. This test is robust with moderate to small sample sizes and occasional small cell values.

The tests of differences in 1997 foaling success between gathered Garfield Flat mares and ungathered GR and GF mares are not equal comparisons, as the last 2 classes of mares were not tested for pregnancy. It is unlikely that all of the ungathered GR and GF mares assessed for foaling success were pregnant. Accordingly, this makes the comparisons of foaling success conservative tests.

Results

At GF, 45 (67.2%) of 67 marked mares foaled in 1994. These data represented all but 2 of the mares released in 1993 that would be ≥2 years old the following year. At GR, 20 (71.4%) of the 28 marked mares we surveyed foaled during the 1994 field season. Gathered and released GF mares had a foaling rate little different from GR mares (CBET: $P=0.43$). The minimum age of marked GR mares in 1994 was 4. Garfield Flat mares ≥4 years old ($n=34$) foaled at a greater rate (77.4%) than GR but did not differ significantly (CBET: $P=0.30$).

At the 1997 gather, released horses were held somewhat longer than in 1993, 3 to 4 days depending upon capture order. Removed horses were held ≥21 days before adoption. During the 14-day period prior to blood sampling at PVC, there were 15 to 18 spontaneous miscarriages as evidenced by shed fetuses (Sharon Kipping, PVC Manager, personal communication). One mare died that had a well-developed fetus *in utero*. Two pregnant mares died at interim locations before being adopted.

We pregnancy-tested 21 mares that were released at GF in 1997 and found 18 (85.7%) pregnant. Ten of these mares (55.6%) foaled. Of the mares removed in 1997 (66 tested, 1 autopsied), 43 (64.2%)

were pregnant. We verified the reproduction of 37 pregnant mares with only 17 (45.9%) producing viable foals. We also collected resighting data to determine reproductive success of 17 ungathered mares at GF in 1997. Fourteen (82.4%) produced foals. That same season we identified 54 mares at GR and found that 40 (74.1%) produced foals.

Two bands in the field, summer 1996.

At GF in 1997, stress duration for the 3 classes of mares was 0 days for ungathered ($n = 17$), 3.5 days for gathered and released ($n = 18$), and 21 days for removed mares ($n = 37$). Pairwise comparisons of the 3 classes of mares showed that pregnant mares exposed to longer periods of stress had lesser reproductive success. The greatest difference in reproductive success was between ungathered and removed mares (CBET: $P = 0.005$). There also was a significant difference between ungathered and released mares (CBET: $P = 0.05$). There was, however, no significant difference between released and removed mares (CBET: $P = 0.26$).

The reproductive rates of GR and the ungathered GF horses did not differ (CBET: $P = 0.42$). Granite Range horses had a greater reproductive rate than the pregnant released mares at GF, but the difference was not significant (CBET: $P = 0.08$). As expected, GR horses had greater reproductive success than pregnant mares removed from GF (CBET: $P = 0.003$).

Discussion

The effect of gather-and-removal-induced stress on pregnant feral horses is reduction in foaling success, sensitive to the duration of the stressor. The GF horses that were removed for adoption were transported long distances and subjected to repeated handling. Long-distance transportation has been linked to abortions in 2-year-old domestic mares (Roberts 1986). Mitchell (1971) and Osborne (1975) associated abortions in yearling and 2-year-old domestic mares with extensive handling. Generally, high pregnancy rates for yearlings and 2-year-olds followed by fetal losses have been reported for domestic (Mitchell and Allen 1975) and feral horses (Lucas et al. 1991). The reproductive losses for GF mares, however, were spread across age classes.

Stress, whether acute or chronic, stimulates the release of cortisol and other adrenocorticoids in mammals (Yates and Urquhart 1962). For example, in studies of gray squirrels (*Sciurus carolinensis*, Guthrie et al. 1967) and snowshoe hares (*Lepus americanus*, Boonstra et al.1998), trapping stress elevated serum corticosteroids. Snowshoe hares produced smaller litters during the decline phase of their 10-year cycles because of chronic stress imposed by elevated predation (Boonstra et al. 1998). Decline in serum progesterone has been linked to acute stress (van Niekerk and Morgenthal 1982). Serum progesterone is critical to pregnancy maintenance (Ball 1993). It likely is that severe and chronic reduction of serum progesterone due to prolonged removal stress was responsible for spontaneous abortions at PVC and overall decrease in reproductive success. An examination of serum cortisol and progesterone levels in response to gathers and removals would provide a definitive causal relationship between stress and reduced reproductive success.

Band takeover is another possible source of stress for feral mares. Berger (1983) reported instances of harassment and forced copulation in the Granite Range by stallions that had taken over bands. The stress of forced copulation coupled with changes in social structure caused induced abortions in mares

less than 6 months pregnant (Berger 1983). During our 6 seasons of field work, we observed many cases of harassment but only one forced copulation. The sole forced copulation was performed by a resident band stallion upon an unreceptive yearling mare. Most harassment incidents, however, were performed by bachelor males directed at harem mares. We saw no cases of harassment or forced copulation by stallions that acquired new bands. Further study is necessary to better evaluate the potential effects of forced copulation, harassment, and change in social structure on reproduction.

Removals from managed herds reduce the number of young horses ≤ 5 years old. Interestingly, this has the effect of temporarily increasing the proportion of the more reproductively successful horses in the population (Garrott et al. 1991). Seemingly, this works at cross purposes to desired population sizes at or below AML. To counter this effect, horses of all ages or a greater proportion of prime-age horses should be removed. Additionally, timing of removals can be exploited to extend the period of reduced population size. Gathers conducted in September or October, after the foaling season (Garrott et al. 1991), would reduce the next year's reproduction by interrupting early-stage pregnancies at a time when successful re-impregnation is unlikely. Feral horses are protected federally, and the BLM is charged with maintaining the general health and well-being of those horses under its jurisdiction (Boyles 1986). Such pregnancy disruptions would achieve a temporary reduction in population growth without large impacts on mare condition due to the small resource investment in early-stage fetuses.

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Variation in the Short-Term Changes in Bone Cell Activity in Three Regions of the Distal Femur Immediately Following Ovariectomy

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ABSTRACT

The effect of ovariectomy (OVX) on cancellous bone in the rat is not uniform at all sites of the skeleton. We report variation in the short-term effects of adult OVX in three regions of the distal femur: the diaphysis (DIA), the metaphysis (META), and the epiphysis (EPI). Cancellous bone parameters were estimated in the three separate zones of the femora and compared with changes in bone cell activity, as estimated by osteoclast surface (Oc.S) and bone formation rate (BFR). Changes were studied for 30 days in a series of rats either sham-operated (Sham) or ovariectomized (OVX) at 7 months of age. Oc.S and BFR were elevated following OVX in all regions. The time course for the OVX-induced changes differed between regions: DIA, both Oc.S and BFR were elevated at day 9; META, Oc.S was also elevated at day 9, while the rise in BFR was delayed until day 21; EPI, Oc.S remained stable but increased relative to ovary-intact rats by day 18 due to reduced levels in the latter, but BFR did not rise until day 28. These changes in bone cell activity following OVX produced a 71% reduction of cancellous bone in the DIA and a 35% reduction in the META. In contrast, no OVX-induced bone loss was observed in the EPI. This study shows that bone cell activity increases in each region of the distal femur within the first 30 days following OVX, independent of bone loss. However, the time course of increased bone cell activity is not uniform. These data highlight the role of local factors in the response to ovarian hormone deficiency. (J Bone Miner Res 1998;13:1451–1457)

INTRODUCTION

Bone loss resulting from estrogen deficiency in the rat consists predominantly of cancellous bone loss[1] through a mechanism that involves elevated turnover.[2,3] In the rat distal femoral diaphysis (DIA) and proximal tibia, bone resorption increases at ~6 days postovariectomy (post-OVX), while formation is not increased until 9–12 days, and cancellous perforation is detected by 12–15 days postoperation.[2,3] The bone loss at these sites does not result from a generalized thinning of all trabeculae but by perforation and removal of the entire trabeculae.

A similar mechanism of perforation is proposed for both postmenopausal osteoporosis[4] and OVX-induced bone loss.[5,6] However, the rate and magnitude of bone loss varies within the skeleton in all species studied. The factors responsible for such selective loss are not understood. Possibly mechanical strain, exerted locally within each individual trabecula, may play a role in determining survival, with the lesser strained units being removed first.[7] This theory is supported by the preferential loss of horizontal trabeculae in human vertebral bodies[8] and the disruption of the pattern of OVX-induced bone loss in the tibia of the rat when performed in concert with hemicordectomy.[9]

It has been reported that the extent of bone loss varies between neighboring regions within a single bone. Bone mineral density of the femoral epiphysis (EPI) in the rat did not decrease in a 26-week period following OVX at 4, 10, or

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52 weeks of age, despite significant decline in the distal metaphysis (META) during this period in all three groups.[10] Westerlind et al.[11] reported that OVX in the rat decreased the cancellous bone volume (BV/TV) in the femoral META by 75% after 11.5 months, while the EPI remained unchanged, despite a 220% increase in eroded perimeter and a 440% increase in the fluorochrome-labeled perimeter in the latter region.[11] This heterogeneity has also been alluded to by others[12–14] and suggests the existence of large variations in the susceptibility of cancellous bone to ovarian hormone deficiency. However, it is unknown whether activation of bone cells with ovarian hormone deficiency occurs simultaneously in all regions or whether there is a delay in those regions that are apparently resistant to ovarian hormone-deficient bone loss.

We now report the cellular and morphologic events occurring in the EPI during a period of accelerated turnover and bone loss in the DIA of the distal femur. The study samples three separate regions of the rat distal femur for 30 days following OVX. Static and dynamic histomorphometric techniques are used to examine the effect of estrogen deficiency on the morphology of the cancellous bone as well as resorption and formation variables.

MATERIALS AND METHODS

Animals

Ninety female 6-month-old Sprague-Dawley rats were obtained from the Gilles Plains Animal Resource Center (Gilles Plains, South Australia). Rats were housed at 22°C on a 12 h light/dark cycle in standard cages housing groups of six animals and were supplied with commercial rat chow containing 0.76% calcium, 0.46% available phosphorus, and 2000 IU/kg of vitamin D_3 (Milling Industries, Murray Bridge, South Australia) and tap water ad libitum. All procedures involving the animals were approved by the Institute of Medical and Veterinary Science Animal Ethics Committee.

Surgery

At 7 months of age, six animals were killed as a baseline group and the remainder allocated to either OVX or sham operation (Sham), both performed under halothane anesthesia. All rats received demeclocycline (Lederle, Sydney, Australia) 30 mg/kg of body weight and calcein (Sigma Chemical Co., St. Louis, MO, U.S.A.) 20 mg/kg of body weight by intraperitoneal injection, 6 and 2 days, respectively, prior to killing. At 9, 15, 18, 21, 24, 28, and 30 days postoperation, six OVX and six Sham rats were weighed and killed by cervical dislocation under halothane anesthesia, and the right femora were excised.

Tissue preparation

The distal 20 mm of each femur was bisected in the sagittal plane. Cutting and trimming were performed using a low-speed saw (Buehler Ltd., Evanston, IL, U.S.A.), with a diamond-tipped cutting blade. Bone samples were fixed in 10% neutral buffered formalin for 4 h, dehydrated in graded ethanol (70%, 90%, 2× 100%) for 1 h each, then transferred into two changes of methyl methacrylate (BDH, Poole, U.K.) containing 8% v/v K-Plast Plasticizer (Medim, Giessen, Germany) for 3 days. All processing was conducted at 4°C. The samples were polymerized in a 37°C waterbath in sealed polypropylene tubes using 4 ml of methyl methacrylate containing 8% v/v K-Plast Plasticizer and 0.9% w/v K-Plast initiator for 16 h. Five-micrometer-thick longitudinal sections were cut using a Jüng K motorized microtome (Reichert, Heidelberg, Germany).

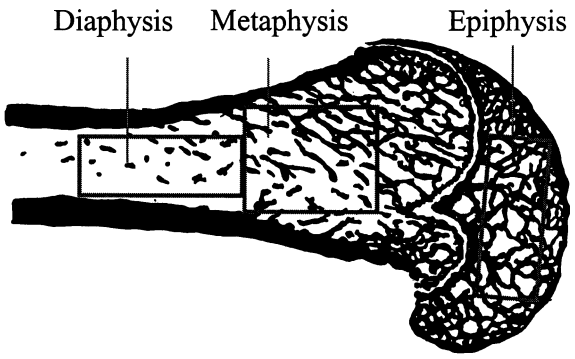

FIG. 1. Longitudinal section of distal femur showing margins for sampling.

Histomorphometry

The percentage of marrow cavity occupied by mineralized bone and its surface length were measured in 5 μm sections stained by a modified von Kossa silver technique.[15] Sections were partitioned into three regions (Fig. 1): the epiphyseal region below the growth plate; the metaphyseal region extending from 3 mm above the most distal point of the growth plate, excluding primary spongiosa from analysis, to 7 mm above the growth plate; and the diaphyseal region from 7–12 mm above the growth plate. The mean sampling area (mean ± SEM) was 14.4 ± 0.3 mm^2 in the DIA, 9.2 ± 0.2 mm^2 in the META, and 10.3 ± 02 mm^2 in the EPI. An Olympus BH-2 microscope (Olympus, Tokyo, Japan) interfaced with a Quantimet 520 Image Analysis System (Cambridge Instruments, Cambridge, U.K.) was used for all measurements. BV/TV, trabecular number (Tb.N), and trabecular thickness (Tb.Th) were calculated from the percentage area and surface data.[16]

Dynamic markers of bone turnover were measured in all three regions. Extent of the double fluorochrome labeled surface as a percentage of total surface (dLS) was determined manually on unstained 5-μm-thick sections using a Zeiss fluorescence microscope (Zeiss, Jena, Germany) with an ocular mounted Weibel II graticule (Graticules Ltd., Tonbridge, U.K.) at ×250 magnification. The interlabel distance was measured at multiple sites along the labeled surface using an ocular mounted micrometer at ×400 magnification. The mineral apposition rate (MAR) was calculated by dividing the interlabel distance by the time interval

Table 1. Diaphyseal Morphometry

	Days postoperation							
Variable	*0*	*9*	*15*	*18*	*21*	*24*	*28*	*30*
BV/TV (%)								
sham*	8.23 (1.8)	5.68 (1.3)	2.00 (0.8)	3.44 (0.79)	3.97 (0.75)	3.27 (0.67)	3.74 (0.78)	5.01 (1.58)
OVX*†		3.28 (1.1)	0.53 (0.4)	1.96 (0.54)	1.80 (0.46)	1.19 (0.50)	1.58 (0.35)	1.44 (0.35)‡
Tb.Th (μm)								
sham*	63.84 (6.03)	54.91 (4.58)	36.63 (2.51)	45.86 (2.12)	44.75 (3.71)	43.33 (6.92)	53.12 (4.74)	55.30 (5.19)
OVX*†		48.48 (6.43)	26.23 (2.62)	43.33 (4.75)	39.55 (4.33)	33.16 (3.64)	37.75 (1.27)‡	36.11 (2.95)‡
Tb.N (/mm)								
sham*	1.24 (0.17)	0.98 (0.14)	0.54 (0.10)	0.72 (0.14)	0.89 (0.16)	0.76 (0.16)	0.67 (0.09)	0.81 (0.22)
OVX*		0.60 (0.15)‡	0.20 (0.03)	0.42 (0.10)	0.44 (0.11)‡	0.32 (0.09)‡	0.41 (0.08)‡	0.41 (0.11)‡
Oc.S (%)								
sham	22.28 (1.6)	16.78 (1.9)	26.87 (2.4)	21.28 (1.4)	11.21 (5.2)	17.41 (7.2)	17.5 (4.0)	16.35 (1.6)
OVX*†		27.58 (1.5)‡	43.86 (2.3)‡	32.92 (0.9)‡	31.71 (2.5)‡	23.00 (3.9)	28.90 (3.1)‡	31.48 (2.6)‡
BFR ($\mu m^3/\mu m^2$/day)								
sham	9.91 (2.7)	14.92 (6.6)	23.37 (1.3)	5.97 (3.4)	15.22 (5.2)	41.04 (7.0)	20.69 (1.6)	16.63 (5.2)
OVX*†		54.59 (9.5)‡	39.00 (5.1)	40.55 (11.3)	51.74 (12.0)‡	57.46 (5.7)‡	58.08 (14.5)‡	64.83 (12.5)‡

Values are mean (SEM) for cancellous bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), osteoclast surface (Oc.S), and bone formation rate (BFR) measured in the diaphyseal region of the distal femur in nonoperated rats (0 days), sham-operated (sham), and ovariectomized rats (OVX) at 9, 15, 18, 21, 24, 28, and 30 days postoperation at 7 months of age.

* Significant change with time ($p < 0.01$). † Significantly different to sham with time ($p < 0.05$). ‡ Significantly different to sham ($p < 0.05$).

between labels (4 days). The percentage of cancellous surface covered by osteoclast-like cells was estimated manually using an ocular mounted Weibel II graticule for 5-μm sections stained with a modified von Kossa silver technique followed by a hematoxylin and eosin counterstain (VK/HE) at $\times$200 magnification. Osteoclast surface (Oc.S) was estimated using the VK/HE sections. Osteoclasts were identified as cells with multinucleate, round speckled nuclei, and foamy eosinophilic cytoplasm adjacent to cancellous bone surfaces, except those covered with osteoid. In both dLS and osteoclast estimates, contiguous fields were counted to include all cancellous bone in each region. Single sections were examined for each parameter, and analytical variance was not assessed.

Calculations and statistics

The bone formation rate (BFR; tissue level, total surface referent) was calculated as the product of MAR and dLS. Intraregion analysis was conducted using repeated measures analysis of variance with contrast to compare the mean values for each variable for the two operative groups at each time point. Change through time was estimated by regression analysis assessed for each group and compared with the corresponding operative group in that region. Inter-region analysis was conducted by analysis of variance with contrast on the slopes of the regression equations of time on the difference between the individual OVX values and the mean of the Sham value for each time point. Due to the difference in baseline values between regions, structural cancellous bone variables were normalized by expressing the resultant individual OVX values as a percentage of the mean Sham value for that time point and region. This design of the statistical examination included a correction for multiple analyses. Statistics were performed using PC-SAS (SAS Institute, Cary, NC, U.S.A.) with $p < 0.05$ considered significant.

RESULTS

Completeness of OVX was confirmed at death in all OVX rats by the absence of ovarian tissue and atrophied uterine horns. Body weight (mean $\pm$ SEM) was not greater in OVX animals compared with ovary-intact animals, 324 $\pm$ 4.5 g and 318 $\pm$ 2.9 g, respectively.

Intraregion comparison

In the DIA (Table 1), BV/TV fell in both groups (Sham, $p < 0.005$; OVX, $p < 0.0001$), with a greater loss in the OVX group ($p < 0.05$), decreasing to 29% of the Sham level at day 30. There was a decrease in both Tb.N and Tb.Th in both groups, which was greatest at day 15. Tb.N was decreased in the OVX group compared with Sham by day 21, and Tb.Th was decreased by day 28. Oc.S was not altered in the Sham group but there was an increase in the OVX group ($p < 0.005$), which was detectable at the first time point, day 9. BFR did not change in the Sham group throughout the period. In the OVX group, it was increased compared with Sham at day 9 ($p < 0.0001$).

In the META (Table 2), BV/TV also fell in both groups (Sham, $p < 0.01$; OVX, $p < 0.0001$). Again the bone loss was greater in the OVX group with the OVX value 65% of

Table 2. Metaphyseal Morphometry

	Days postoperation							
Variable	*0*	*9*	*15*	*18*	*21*	*24*	*28*	*30*
BV/TV (%)								
sham*	25.91 (2.38)	19.24 (1.31)	16.57 (1.60)	17.62 (1.53)	18.43 (1.68)	15.58 (0.98)	23.46 (2.34)	17.38 (1.80)
OVX*†		15.23 (1.83)	9.18 (1.04)‡	11.98 (1.52)‡	13.22 (1.50)‡	9.79 (1.37)‡	11.35 (1.21)‡	11.29 (0.78)‡
Tb.Th (μm)								
sham*	89.88 (5.30)	79.32 (4.27)	63.77 (4.60)	64.72 (2.67)	67.60 (4.01)	60.02 (2.22)	81.24 (6.36)	66.52 (4.90)
OVX*		72.21 (5.67)	50.39 (3.07)	64.73 (5.79)	59.58 (4.80)	56.61 (4.40)	65.64 (8.09)‡	59.28 (2.40)
Tb.N (/mm)								
sham	2.85 (0.14)	2.42 (0.05)	2.58 (0.07)	2.72 (0.20)	2.72 (0.18)	2.60 (0.12)	2.87 (0.15)	2.60 (0.17)
OVX*†		2.08 (0.12)	1.79 (0.11)‡	1.84 (0.13)‡	2.20 (0.11)‡	1.70 (0.14)‡	1.75 (0.10)‡	1.91 (0.13)‡
Oc.S (%)								
sham*	16.60 (1.4)	14.50 (0.9)	17.21 (0.46)	12.14 (0.5)	9.81 (0.8)	8.37 (1.4)	12.57 (1.2)	11.68 (1.3)
OVX*†		23.27 (2.4)‡	25.79 (1.9)‡	17.81 (0.8)‡	18.88 (1.8)‡	22.81 (2.5)‡	25.89 (2.9)‡	27.5 (2.8)‡
BFR ($\mu m^3/\mu m^2$/day)								
sham*	15.88 (2.2)	9.36 (2.2)	21.08 (2.7)	25.40 (3.6)	20.70 (4.5)	42.90 (5.9)	31.09 (4.9)	21.84 (4.9)
OVX*†		15.50 (4.2)	17.68 (1.7)	30.16 (7.6)	44.01 (7.3)‡	68.50 (8.13)‡	58.70 (7.1)‡	59.4 (4.6)‡

Values are mean (SEM) for cancellous bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), osteoclast surface (Oc.S), and bone formation rate (BFR) measured in the metaphyseal region of the distal femur in nonoperated rats (0 days), sham-operated (Sham), and ovariectomized rats (OVX) at 9, 15, 18, 21, 24, 28, and 30 days postoperation at 7 months of age. *Significant change with time ($p < 0.01$). † Significantly different to sham with time ($p < 0.0001$). ‡ Significantly different to sham ($p < 0.05$).

the Sham values at day 15. BV/TV fell as a result of a decrease in Tb.Th in the Sham group, whereas in the OVX group it was due to both decreased Tb.Th and Tb.N. Tb.N was decreased in the OVX group compared with Sham by day 15 and reduced only at day 28. Oc.S increased in the OVX group while it decreased in the Sham group ($p < 0.005$), resulting in significantly greater levels in the OVX group ($p < 0.0001$) at the first time point, day 9. BFR increased in both groups (Sham, $p < 0.005$; OVX, $p < 0.0001$). The rise was greater in the OVX group ($p < 0.0001$) by day 21.

In the EPI (Table 3), BV/TV also fell in both groups (Sham, $p < 0.001$; OVX, $p < 0.0001$) to 85% of baseline values at 30 days. However, there was no difference between operative groups. The reduction in BV/TV was due solely to a reduction in Tb.Th. Tb.N was stable in the Sham group and tended to increase in the OVX group ($p < 0.01$). There was no OVX-induced rise in Oc.S in this region. However, a significant decrease in the Sham group ($p < 0.001$) resulted in relatively greater Oc.S in the OVX group ($p < 0.0001$) delayed until day 18. BFR increased in both groups (Sham, $p < 0.005$; OVX, $p < 0.0001$), and the rise was greater in the OVX group ($p < 0.005$) delayed until day 28. Changes in BFR were primarily due to changes in dLS, because MAR rose only in the META (Sham, $p < 0.05$; OVX, $p < 0.001$) and Sham EPI ($p < 0.05$) (data not shown).

Inter-region comparison

The OVX-induced decrease in BV/TV in the DIA was significantly greater than in the EPI ($F = 7.21$, $p < 0.01$). There was also a trend for bone loss to be greater in the META compared with the EPI ($F = 3.12$, $p < 0.08$). There was no difference between loss of BV/TV in the DIA and the META. Tb.N was significantly reduced in the DIA ($F = 5.81$, $p < 0.05$) and META ($F = 5.96$, $p < 0.05$) compared with the EPI. There was also a trend for a greater reduction in the DIA compared with the META ($F = 3.16$, $p < 0.08$). Tb.Th was significantly reduced in the DIA compared with the META ($F = 4.15$, $p < 0.05$). The time for the response of Oc.S to OVX in the DIA was less than for both the META ($F = 7.09$, $p < 0.01$) and the EPI ($F = 4.63$, $p < 0.05$). There was no significant difference between the META and EPI (Fig. 2A). The time for the response of BFR to OVX was significantly less in the DIA than for both the META ($F = 6.02$, $p < 0.05$) and the EPI ($F = 5.93$, $p < 0.05$), while the META was significantly less than for the EPI ($F = 5.55$, $p < 0.05$) (Fig. 2B).

DISCUSSION

This study has identified variations in the short-term response to systemic ovarian hormone deficiency in three regions of the rat distal femur, and the extent of variation in response to ovarian hormone deficiency has been quantitated. Thirty days of ovarian hormone deficiency produced a reduced BV/TV compared with ovary-intact animals of 71% in the DIA, but only 35% in the META, while the EPI showed no decline. Bone cell activity increased in all regions, although the time course of this increase was not uniform between regions and was independent of bone loss. The response of appendicular cancellous bone to the sys-

Table 3. Epiphyseal Morphometry

	Days postoperation							
Variable	*0*	*9*	*15*	*18*	*21*	*24*	*28*	*30*
BV/TV (%)								
sham*	44.83 (1.72)	38.22 (2.20)	37.83 (1.58)	39.14 (1.32)	33.45 (2.59)	34.49 (1.75)	32.87 (1.35)	37.19 (2.64)
OVX*		43.59 (2.03)	37.66 (2.58)	40.58 (3.28)	31.20 (2.18)	33.38 (1.60)	34.29 (1.82)	37.97 (4.28)
Tb.Th (μm)								
sham*	159.08 (5.87)	152.04 (9.95)	140.50 (6.66)	137.87 (5.60)	130.37 (13.06)	127.44 (8.33)	124.06 (5.15)	126.39 (15.46)
OVX*		156.19 (5.79)	139.74 (5.87)	152.52 (9.57)	118.45 (3.67)	117.81 (3.95)	130.24 (8.70)	107.55 (5.70)
Tb.N (/mm)								
sham	2.82 (0.08)	2.52 (0.06)	2.70 (0.09)	2.85 (0.09)	2.59 (0.08)	2.72 (0.10)	2.67 (0.16)	3.05 (0.20)
OVX*		2.80 (0.14)	2.70 (0.16)	2.69 (0.24)	2.62 (0.13)	2.85 (0.18)	2.66 (0.14)	3.49 (0.26)†
Oc.S (%)								
sham*	13.72 (1.7)	12.79 (1.6)	13.80 (1.0)	8.53 (0.9)	8.58 (1.1)	5.99 (0.6)	8.99 (2.0)	8.61 (2.2)
OVX*‡		14.74 (1.9)	17.82 (1.7)	13.78 (0.5)†	16.71 (2.3)†	18.70 (2.9)†	15.54 (0.8)†	15.83 (1.3)†
BFR ($\mu m^3/\mu m^2$/day)								
sham	12.22 (1.62)	10.86 (3.0)	16.41 (4.7)	18.79 (7.44)	29.08 (2.1)	29.90 (7.7)	23.72 (4.2)	20.69 (6.6)
OVX*†		17.80 (4.7)	16.54 (2.9)	15.69 (2.9)	25.94 (4.4)	32.82 (4.6)	41.92 (6.6)†	44.72 (3.4)†

Values are mean (SEM) for cancellous bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), osteoclast surface (Oc.S), and bone formation rate (BFR) measured in the epiphyseal region of the distal femur in nonoperated rats (0 days), sham-operated (sham), and ovariectomized rats (OVX) at 9, 15, 18, 21, 24, 28, and 30 days postoperation at 7 months of age. * Significant change with time ($p < 0.005$). † Significantly different to sham ($p < 0.05$). ‡ Significantly different to sham with time ($p < 0.0001$).

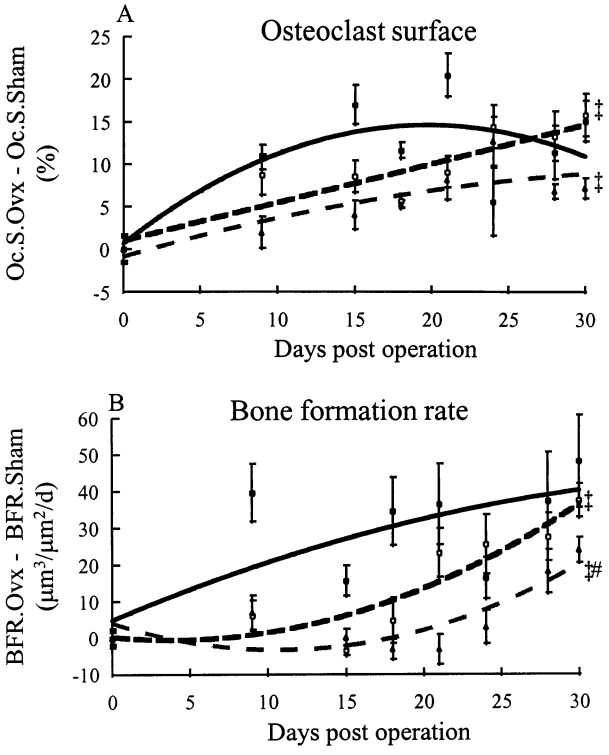

FIG. 2. OVX-induced changes from ovary-intact groups in Oc.S (A) and BFR (B) in the DIA (■), META (□), and EPI (▲) of the distal femur in the rat, expressed as mean ± SEM. ‡Significantly different to DIA with time ($p < 0.05$). #Significantly different to META with time ($p < 0.05$).

temic loss of estrogen has generally been studied using single sample areas only or regions from disparate parts of the skeleton.[13,14] Such variations within a single bone suggest the importance of local factors in the mediation of this process.

In a study of the short-term effects following OVX, the impact of a remodeling transient must be considered.[17] Estrogen deficiency triggers a change in activation frequency and thus there is a period when resorption exceeds formation purely due to increased recruitment of osteoclasts. The true steady-state alteration to bone balance cannot be assessed until at least the end of the first remodeling period. This has been estimated in rat caudal vertebrae to last for 38 days.[18] The rapid nature of bone loss in this study highlights the impact of a remodeling transient in the META and DIA, and further suggests that bone cell activity increased prior to the initial time point 9 days after operation. This finding is in keeping with previous studies.[2,3]

Following OVX, Tb.N was reduced in the META by 25% and in the DIA by 50%, displaying the pattern typical of estrogen-deficient bone loss reported previously.[2,3,9,12–14,19–21] These reductions were significant when compared with the EPI, which showed no reduction in Tb.N. The absence of a reduction in Tb.N in the epiphyseal region of OVX groups is atypical for cancellous bone, and the mechanism behind the immunity of epiphyseal trabeculae to dissolution remains elusive. The most compelling explanation is the influence of mechanical strain.[3,12] External mechanical loading has been shown to inhibit resorption,[22] a finding consistent with the lack of an absolute increase in resorption in the EPI following OVX. Other explanations include baseline levels of turnover[14] and osteoprogenitor cell populations as possible factors.[12] However, structural differences between the regions may also be involved. In a steady state, increases in bone turnover are unable to produce permanent alterations to BV/TV.[23,24] Computer modeling has shown that a doubling of activation frequency causes transient, mainly reversible, bone loss, but when accompanied by an increase in resorption depth, the irreversible component is more pronounced.[25–27] Epiphyseal trabeculae are thicker than in the other regions, making perforation less likely.

Following OVX, resorption was elevated in all regions. The time course for these changes, however, was not uniform. In the DIA and META, Oc.S was increased at day 9 post-OVX, while in the EPI the Oc.S did not rise at all but showed a relative increase by day 18. The increase in Oc.S following OVX relative to ovary-intact groups was greater in the DIA than in the other two regions. The BFR was increased in the DIA at day 9. In the META, the increase was delayed with BFR not exceeding ovary-intact levels until day 21. In the EPI, the increase was further delayed until day 28. These differences in response were significant among all three regions. Such findings are in keeping with differences in the time course of increased turnover between the proximal tibial META[21] and the first lumbar vertebrae,[14] with a more rapid response occurring in the tibia. Thus, factors local to particular regions must alter the time course of alterations to bone turnover subsequent to estrogen deficiency. This is the first time that variation in the time course of response to estrogen deficiency has been reported in neighboring anatomical regions within a single bone.

In contrast to previous studies,[2,3] a decrease in Tb.Th in each area, independent of estrogen status, was observed associated with decreased Oc.S and increased BFR over the 30 days. In 7-month-old rats, the longitudinal growth rate is reduced to 0.12 mm/month or 0.3% of the total femoral length.[28] Age-related loss of BV/TV is limited to ~1.2% per month in the DIA and 0.3% per month in the META and EPI. Thus, it is possible that the decrease in Tb.Th is the result of a temporary disturbance due to the operations. The long-term study of these areas would determine whether these changes are sustained.

The data presented here display the extent to which cancellous bone loss due to estrogen deficiency is modulated by local factors. The time course of increased bone turnover was different in all regions, being more rapid proximally. The exact mechanism involved in the epiphyseal immunity to bone loss is uncertain. However, the speed at which estrogen deficiency induces bone loss suggests that increased resorption depth is likely to be an important factor.

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An Economic Analysis of Alternative Fertility Control and Associated Management Techniques for the Pryor Mountain Wild Horse Herd

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Executive Summary

Contemporary cost projections were computed for several alternative strategies that could be used by BLM to manage the well-studied Pryor Mountain wild horse population. The alternatives included (1) existing contraceptive, gather and selective removal methods; (2) different contraceptive techniques offering effectiveness of greater duration; (3) manipulation of herd sex ratio; and (4) a gather and removal-only scenario. Costs were projected for a 20-year economic life using the Jenkins wild horse population model and cost estimates from BLM that reflect this herd's specific removal, adoption, and contraceptive application expenses. Important findings include:

- The Pryor Mountain herd has a low intrinsic growth rate that tends to moderate wide inter-annual population fluctuations and greatly minimizes cost differences among contraceptive scenarios.
- All contraceptive scenarios have roughly equal costs compared with exclusive gather and removal management without contraceptive application.
- Treatment with contraceptives under the existing application protocol is predicted to be approximately 50% less costly than gather and removal management alone.
- Annual application via darting is cost-effective on this herd compared with more "conventional" contraceptive applications every 4 years even if the contraceptives have a long (3-year) duration. However, the longer-acting contraceptives reduce the expected inter-annual variability in average expenses.
- The Alternative Baseline scenario is predicted to be the least expensive option, but the consistent treatment of all older aged mares called for in this scenario is unlikely to be sustainable over a 20-year period without reducing the population below desired AML levels.
- Average annual costs would decline slightly if the herd's sex ratio were adjusted to leave more males on the range, but care must be used not to remove too many mares of prime foaling age lest the population decline below desired levels.
- In general, cost estimates are most sensitive to annual monitoring expenses, not to direct contraceptive or removal/adoption costs.
- The 4-year gather cycle is appropriate for this herd, though costs are not very sensitive to the gather frequency.

Introduction and Objective

This report is an addendum to a previous economic analysis conducted by USGS for BLM that examined contraceptive and management options for three wild horse populations (Bartholow 2004). The sole focus of this current report is the Pryor Mountain wild horse herd, which has previously been subject to contraceptive treatment. Management flexibility is, however, important, and treatment regimes may change through time. The current report examines some potential management alternatives in a manner that is generally parallel to, but not identical with, Bartholow (2004).

The BLM's goal for the Pryor Mountain herd is management at a clearly established appropriate management level (AML) at minimum cost. Alternative means to achieve this goal are explored in this report and include: (1) the status quo of annual contraceptive application, selective removal and adoption; (2) the frequency of gathers and how efficient they are in rounding up animals; (3) status quo plus several alternative contraceptive application scenarios, specifically the duration of the contraceptive agent and using contraceptives annually or in concert with a 4-year gather cycle; and (4) other potential management techniques, such as sex ratio manipulation through age- and sex-specific removal decisions.

Study Area

The Pryor Mountain Wild Horse Range was established in 1968 and occupies 38,000 acres of multi-jurisdictional lands along the border of Montana and Wyoming, about 13 miles north of Lovell, Wyoming. This mostly arid Range supports about 100-200 wild horses in a largely free-roaming condition. Harsh winters and abundant predators may help limit the number of older animals (with few >16 years old) and contribute to variable, and intermittently high, foal mortality. Conflicting management perspectives stimulated research on several issues germane to proper management of this population, including vegetation dynamics and population genetics (Singer and Schoenecker 2000).

Fertility control research began on the Pryor Mountain herd in 2001 and continued in 2002 and 2003. Contraceptives have been applied using remote darting techniques not necessarily associated with regular gathers. Darting uses an initial primer and annual booster, and can take place over a period of time, reaching 100% of the mares targeted for the contraceptive program. BLM currently envisions giving the contraceptive agent porcine zona pellucida (PZP) vaccinations to all yearling and 2-year-old mares and those 14 years and older, allowing younger mares to mature in a healthy state prior to the rigors of pregnancy and allowing older mares to live their senior years in better overall health. This protocol, termed “compassionate-use” fertility control, is envisioned to improve mare health, reduce foal loss, and help prevent orphaned foals.

Additional information concerning this herd is available from BLM (USBLM 2004).

Methods

Step 1. Define Baseline and Specific Alternatives

This analysis examines the following scenarios:

- Baseline Scenario – Existing "baseline" conditions as reflected in current management policy (USBLM 2004). This is a regular 3-4 year gather with age-specific removal rates, specifically 50% for age classes 1, 2 and 3, and 25% of 4-year olds. It is assumed that 100% of horses removed are targeted for the adoption pool, and are held an average of 30 days prior to adoption. Removals are assumed never to end up in long-term holding facilities.

  "Compassionate-use" fertility control involves 100% treatment for yearling and 2-year old mares, and also for mares 14 years and older. Under the baseline scenario, contraceptives are applied via annual darting and do not require gathering.

  Unfortunately, existing management of the Pryor Mountain herd can only be approximated by the current Jenkins model. The limitation arises because the model does not allow for dual management (contraceptives and removals) on two different frequencies (annual and 4-year) at two different efficiencies (100% for contraceptive darting and only 70% for gathers). Further, the cost estimation program does not allow differing costs for contraceptive application that might occur in conjunction with a 4-year gather as opposed to annual darting. In order to approximate existing management, I ran the Jenkins model on a 1-year gather cycle, but removed animals at one-fourth the removal rate that would have occurred if it had been a 4-year gather Actually, I used a rate of 0.175 [0.7 x 0.25] to account for the normal gather efficiency of 70% (Table 1). This technique is not a completely faithful representation for all the reasons mentioned, but it should reasonably approximate baseline costs.

  Table 1. Comparison of removal rates for annual darting scenarios and conventional 4-year gather cycle. Rates for annual darting scenario are 0.175 times the rates for the regular gather scenarios, but must be rounded to the nearest whole percentage for the Jenkins model.

Age class	Regular 4-year gather scenarios	Annual darting scenarios
0	0	0
1-3	50	9
4	25	4

  One other model adaptation concerned handling removal rates for 14-year old horses. The Jenkins model lumps older animals into ages 10-14, 15-19, and 20+. I approximated removal of older animals by spreading applicable rates among the age classes, with values derived from Linda Coates-Markle.

  Efficacy rates for dart-delivered contraceptives are shown in Table 2, along with rates for more conventional contraceptives, discussed below.

- Alternative Baseline – Modification of the Baseline Scenario being considered for the Pryor Mountain herd. This alternative is identical to the Baseline scenario except that it also treats 50% of all other age classes (3-13) with contraceptives.

- Conventional Contraceptive Scenarios – These scenarios are meant to represent contraceptive strategies being used, or anticipated, on many other BLM-managed wild horse herds (USBLM 2002), and are similar to those investigated by Bartholow (2004). Instead of the annual darting, contraceptives would be applied solely in conjunction with a regular 4-year gather cycle, and nominally represent either 2- or 3-year duration, defined more precisely with percent effectiveness in the first and subsequent years as shown in Table 2.

  Contraceptives are assumed to be applied to all mares returned to the range. Note that it is considered to be the case, and the Jenkins model assumes, that if the vaccine does not produce infertility in the first year for a given mare, it would never be effective in subsequent years until re-treatment. The 1-year scenario values also given in Table 2 represent values found with annual darting on the Pryor Mountain herd and used in conjunction with the Baseline scenarios, while the 2-year scenario values represents values from a recent assessment of a different herd.

  Table 2. Annual effectiveness of existing and potential contraceptive treatments. These are gross efficacy rates that are further tempered by age-specific fertility rates in the Jenkins model. Values for 1- and 2-year treatments confirmed by Linda Coates-Markle. Values for 3-year agent were liberally extrapolated from 2-year efficacy rates. One-year contraceptives represent the effectiveness achieved through darting (primer and booster).

Nominal Duration	Effectiveness Year 1 (%)	Effectiveness Year 2 (%)	Effectiveness Year 3 (%)	Effectiveness Year 4 (%)	Effectiveness Year 5 (%)
1-year	90	0	0	0	0
2-year	94	82	68	0	0
3-year	94	82	68	33[1]	0

[1] Since running these simulations, newly collected data are available for the Clan Alpine herd (J.W. Turner, Jr., technical notes on Clan Alpine HMA Wild Horse Survey, June 2004, supplied by Linda Coates-Markle). These notes do not support 4th year effectiveness. Therefore, the cost estimates for scenarios involving the 3-year contraceptive will, of necessity, be too low.

- Gather Frequency Scenarios – represent regular gather interval in years (e.g., 2, 4, 6, or 8 years).

- Sex Ratio Scenarios – represent long-term changes to the herd sex structure by favoring removal of horses of one sex over another during the normal selective removal process (e.g., 60% male to 40% female). Note that sex ratios will usually not match among the alternatives. This is because the sex ratio is generated by long-term changes to sex- and age-class specific removal rates and could not be precisely predicted by specifying

population model inputs. In other words, a sex ratio resulting from a given simulation might or might not have been what I was trying to achieve.

- As appropriate, combinations of the above scenarios have been considered. For example, 3-year Contraceptive/Sex ratio-55 would mean the combination of a 3-year contraceptive duration and 55 male:45 female sex ratio. All unspecified parameters are the same as the baseline case, unless otherwise stated.

- Removal-only – One removal-only (no contraceptive application) scenario was included solely for cost comparison.

Step 2. Organize Jenkins Model Input Data and Parameters

Data representative of the Pryor Mountain Herd Management Area (HMA) were compiled and organized in a fashion suitable for the Jenkins wild horse population model (Jenkins 2002). Much of the vital background and operational philosophy for the Jenkins model has already been supplied by Bartholow (2004) and will not be repeated here. Pryor Mountain HMA data and modeling parameters were taken from a Jenkins' model data set and other information supplied by Linda Coates-Markle in early 2004, greatly simplifying this effort. I assumed that the data accurately described the Pryor Mountain population's demographics. A summary of important demographic parameters for this herd is given in Table 3 and a complete listing of the baseline data set for the Pryor Mountain herd is given in Appendix A.

Table 3. Key demographic elements and information concerning compassionate-use fertility control for the Pryor Mountain HMA considered in this analysis.

	Pryor Mountain
Initial population sex ratio (% male)	53
Sex ratio at birth (% male)	51
Age 0-9 female survival (geometric mean %)	89
Age 0-9 male survival (geometric mean %)	87
Average foaling rate age 2-9 (%)	62
Gather trigger (# of horses)	150
Gather efficiency (%)	70
AML (# of horses)	100
AML includes foals?	No
Released mares treated for contraceptive alternatives by age	Foals: 0% 1 & 2 year: 100% 3-13 year: 0% 14+: 100%

Step 3. Exercise the Jenkins Model for Each Scenario

Each scenario was run as a separate simulation using model input parameters to describe the various management actions that might be taken, contraceptive effectiveness, and so on. I have assumed that the Jenkins model provides a reasonably accurate portrayal of population dynamics and that model results can then be used in evaluating a variety of cost-minimization strategies.

Values or settings for the Jenkins' WinEquus model used in Pryor Mountain simulations were:

- Simulations were run for 20 years (producing 21 years of simulation output) with 100 trials each (100 trials is the default)
- Gathering for removal occurred at regular 4-year intervals
- When fertility control was used:
    - Gathers for fertility control occurred regardless of population size
    - Gathers continued after removals to treat additional females to be released (default if the above condition is true). Note, however, that the percentage of females actually treated by age class depends on other model input.
- Scaling factors for annual variation, which interestingly come from Garrott and Taylor (1990) and therefore specifically represent the Pryor Mountain herd:
    - survival probabilities = 1.00 (default)
    - foaling rates = 1.00 (default)
- Correlation between annual variation in survival probabilities and foaling rates = 0.00 (default). According to Linda Coates-Markle (May 2004), there may be some evidence supporting a non-zero correlation for the Pryor Mountain herd due to density-dependent phenomena. However, since these scenarios are explicitly meant to stabilize the population within rather narrow density limits, I left the correlation at zero.
- Initial population size is exact and unsmoothed [different from previous simulations (Bartholow 2004)]
- Foal survival is not density dependent (default)
- Minimum age of sanctuary-bound horses: Not applicable (default)

Step 4. Estimate Dollar Value for Each Management Cost Component

Dollar values were estimated for the main gathering, treating, and selective removal expenditures, along with associated costs related to wild horse management. Dollar figures were taken from Bartholow (2004) and supplemented with information provided by Linda Coates-Markle (Table 4). These costs represent FY 2004 values, but are assumed to increase 3% annually regardless of geographic area to parallel the inflation rate BLM uses for planning. Removal costs include all expenses of gathering and transport to adoption facilities, averaged across all removed horses. Preparation and holding costs include freeze branding and required vaccinations. Adoption costs are largely administrative and include follow-up compliance checks (site visits to adopted horses).

Table 4. Variable cost estimates for the Pryor Mountain wild horse population. Cost estimates were supplied by Linda Coates-Markle, BLM/MT (5/24/2004) and differ from those listed for Montana in Bartholow (2004) because they include labor as a variable cost.

Removal Cost (/horse)	Prep & Holding Cost (/horse/day)	Adoption Cost (/horse)	Compliance Check (/horse)
$800	$40	$1100	$225

Costs used in this analysis for multi-year contraceptives are given in Table 5. Several other potential costs were also considered in the analysis. It was assumed that the minimum gather cost was $15,000, whereas a value of $10,000 was used previously by Bartholow (2004). This comes into play only if the number of animals removed times the appropriate per horse removal cost would be below $15,000. However, for the Baseline and Alternative Baseline scenarios, I used a minimum cost of $3,750 ($15,000/4) to allow gathering and contraceptive application every year in the model, as discussed previously. Though this modification may underestimate costs when real 4-year gathers round up few horses, it represents the best approximation available.

A $5,000 per year HMA census cost was applied for non-gather years to assess contraceptive treatment effectiveness and routine monitoring per the recommendation of Ron Hall (2003). Though the Pryor Mountain herd does not employ census flights due to intensive on-the-ground monitoring, labor costs for this monitoring are roughly equivalent to Hall's cost recommendation and are treated identically in this analysis. However, census costs are problematic for the Baseline and Alternative Baseline scenarios because gathers occur every year in the simulation, though not in reality, and the cost estimation program only tallies a census expense if no horses are gathered. To make up for these "missing" census costs, I added back the $5,000 expense for the 15 years the census would have occurred, averaged over the 20-year period. In other words, I added $3,750 to the average annual cost estimated for the Baseline and Alternative Baseline scenarios to properly account for underestimating census costs (5000*15/20).

Table 5. Estimated per horse costs for contraceptive application. Costs for the 1-year agent represent $20 for the primer and $20 for the booster, plus an additional $66 for labor ($11 per hour times 6 hours per mare, on average) to find and apply the darts. Costs for the 3-year agent are composed of the total cost of a 2-year agent plus additional 12-month time-release pellets. Estimates derived from Linda Coates-Markle (BLM/MT) 9/30/2003 and 5/26/2004.

Contraceptive Duration	Estimated Cost per Horse	Comment
1 year	$106	Remotely applied by darting
2 years	$214	Applied with gather
3 years	$309	Applied with gather

Step 5. Estimate Dollar Costs from Simulated Scenarios

The results of the Jenkins model simulations were summarized and converted to dollar expenses over a 20-year planning horizon. Tallying the total expenditures required all cost estimates previously described, including which ages were eligible for adoption and how long adoptable horses are held. Note that the Pryor Mountain population never contributes unadoptable animals because only young age classes are removed. Results were summarized by software that computed the mean number of horses gathered, removed, and treated by sex and age class for each year of the 20-year simulations, along with average annual costs. In addition, the cost summarization step computed the likely annual variation in costs that would be expected as a result of the variability inherent in the Jenkins model.

Step 6. Conduct Sensitivity Analysis

The Jenkins simulation model captures environmental and demographic variability, but the uncertainty in cost estimates for the various management options remained to be explored. To accomplish this, a sensitivity analysis was performed for the Pryor Mountain population to see where opportunities for cost cutting might lie and which factors contribute most to the bottom line.

Results

Results for the Pryor Mountain HMA are given in Table 6 and Figures 1-4. The results confirm that a four-year gather cycle is a good management decision, though there is not much difference among the alternatives (Figure 1), presumably because the herd is so closely controlled no matter which scenario is chosen – an artifact of this population's low growth rate. Interestingly, a 6-year gather cycle is predicted to be more costly than an 8-year cycle. Annual costs are insensitive to gather efficiency (Figure 2), but are somewhat responsive to slight changes in sex ratio (Figure 3). Even eliminating male removal all together, simulations suggest that the sex ratio would not rise much beyond 53-54% males. In an attempt to further elevate the sex ratio, I increased the female removal rates by 50% (i.e., from 50% on age class 1 to 75%), but this had virtually no affect on the long-term sex ratio and increased the average annual management costs. Results from these experiments are not shown in Table 6.

Average annual cost estimates (Figure 4) are relatively uniform across many of the alternatives, with the exception of the removals-only case. The Alternative Baseline scenario is predicted to be the least expensive, but also has a significant negative effect on the herd's growth rate over the long term. Inspection of the results across the alternatives suggests that growth rates below minus 1.0% are likely too aggressive and cannot be sustained over the 20-year period without significantly increasing the probability of falling below the 100-horse AML. This is also true with scenarios designed to leave more males on the range. Care must be taken to not remove too many mares of prime foaling age lest the population decline below desired population levels.

There is a noteworthy difference reflected in the coefficient of variation between the darting scenarios and the more conventional 2- or 3-year contraceptive scenarios (Table 6). This is

presumably due to the lack of longevity of the 1-year contraceptive used in darting versus the persistent effects of the 2- and 3-year contraceptives.

It is important for the reader to understand that running the same parameters through the Jenkins model repeatedly can produce very different sets of results due to the random nature of the simulations. This randomness can produce variations on the order of 5% or more. Because Table 6 records the mean annual cost from just one set of 100 trials for each scenario, one must use caution when interpreting the results. In short, predicted mean costs for many of the contraceptive scenarios given in Table 6 may or may not be significantly different from one another in a statistical sense. Statistical differences were not assessed in this analysis because one could always increase the number of trials, thus assuring significant differences without any true justification.

Table 6. Summary of results for scenarios of the Pryor Mountain HMA.

Scenario	Average Annual Cost ($)	Percent of Baseline Cost (%)	Median Annual Growth Rate (%)	CV (%)
Baseline	11019[1]	100	-0.7	154.3
Alternative Baseline	7717[1]	70	-4.3	146.7
2-year contraceptive	12877	117	+0.2	82.7
3-year contraceptive	12283	111	0.0	86.3
2-year contraceptive/ Sex ratio-53	10941	99	-0.8	71.0
2-year contraceptive/ Sex ratio-54 (No male removal)	9585	87	-1.3	64.1
3-year contraceptive/ Sex ratio-52	10700	97	-0.2	67.2
3-year contraceptive/ Sex ratio-53 (No male removal)	10157	92	-1.2	62.3
3-year contraceptive/ 2-year gather cycle	12376	112	-1.4	126.0
3-year contraceptive/ 6-year gather cycle	14022	127	-0.1	82.7
3-year contraceptive/ 8-year gather cycle	13206	120	+1.2	59.8
3-year contraceptive / -10% gather efficiency	12294	112	+0.2	77.5
3-year contraceptive / +10% gather efficiency	12141	110	-0.7	91.9
Removals only	16049	146	+0.9	80.0

[1] Includes $3,750 to make up for "missing" non-gather year census costs, but is likely an underestimate due to inability to accurately reflect the $15,000 minimum gather costs when removals are low.

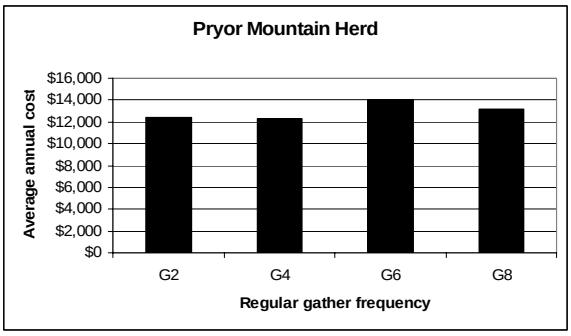

Figure 1. Annualized cost over a 20-year period for four gather frequencies for the Pryor Mountain HMA (G-2, 4, 6, and 8 years, respectively).

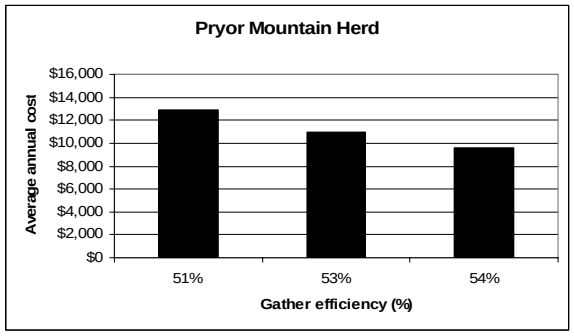

Figure 3. Annualized cost over a 20-year period for three resulting sex ratios associated with 2-year contraceptives for the Pryor Mountain HMA (51, 53, and 54% male, respectively).

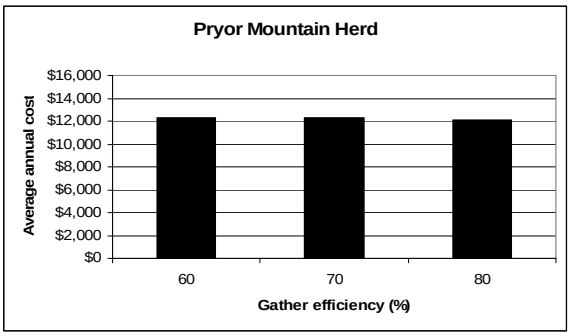

Figure 2. Annualized cost over a 20-year period for three gather efficiencies for the Pryor Mountain HMA (60, 70, and 80%, respectively).

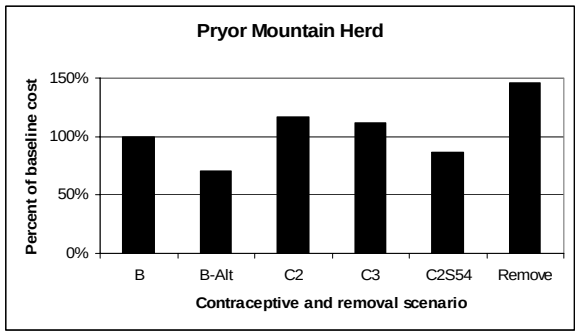

Figure 4. Percent of Baseline cost over a 20-year period for six scenarios.

Sensitivity Analysis for Cost Components and Related Factors

A basic sensitivity analysis was completed for the various elements that contribute to the cost estimate for the Pryor Mountain herd. This analysis tests how sensitive bottom line costs are to small changes in each contributing factor. Figure 5 was generated by changing each cost and management factor ±10% and taking the ratio of the resulting cost fluctuation to the base cost of the Baseline and Alternative Baseline Scenarios for the Pryor Mountain herd. I did not test the sensitivity of varying age thresholds because these are essentially fixed for this herd, though additional scenarios could explore some of these options.

The results indicate that annual monitoring is the cost component that would benefit the bottom line total expenses if it could be reduced regardless of the scenario. Then, for the Baseline Scenario, the next most sensitive cost factors are the costs of adoption, average per day holding costs (the somewhat mislabeled *$/Unadoptable/day* in this case), and number of days held until adoption. Costs related to contraceptive treatment and gathering fall in line next for the Baseline scenario, followed by the minimum gather cost, which contributes little to the sensitivity, indicating that that cost is trivial compared with other management expenses.

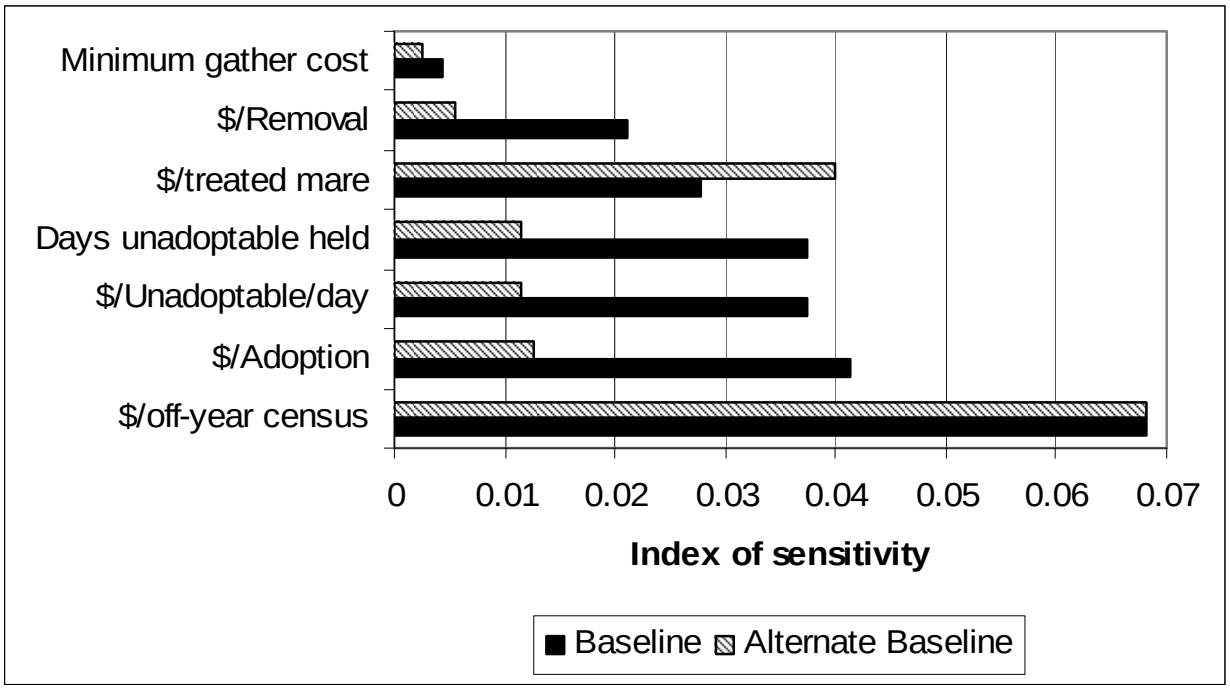

Figure 5. First order sensitivity analysis for management costs and other attributes for the Pryor Mountain herd.

Results for the Alternative Baseline scenario (Figure 5) were considerably different after the monitoring costs. Most factors were far less important for this scenario, relative to those monitoring costs, except for the contraceptive treatment costs. In other words, because this alternative treats far more age classes of adult mares, these additional costs add up significantly. This is not to say that these additional treatment expenses may not be cost-effective, because

they are (Table 6). Rather, it simply says that if these treatment costs could be reduced, the savings would be substantial.

Conclusions and Discussion

The Pryor Mountain herd does not appear to have the same "vitality" as the three populations previously studied (Bartholow 2004). Based on the best, most recently collected data, male and female survival rates for the Pryor Mountain population are estimated to be less than 90% per year, in contrast to above 90% for the other three herds, and moderate foaling rates in the low 60% range are well below the average for the other three populations (72%). Collectively, these differences mean that the intrinsic growth for this herd is far lower than that for other populations, with median annual growth rates near 1% for the Removal-only scenario compared to about 17% for the three populations studied previously. BLM personnel confirm that the current Pryor Mountain growth rate is near zero and has not been above 10% for the last decade (Linda Coates-Markle personal communication). This implies that contraceptive treatment and removals for the Pryor Mountain herd can be far less intensive than for many herds yet still achieve cost savings. It also implies that small changes in management emphasis can result in undesirable negative growth rates.

The Pryor Mountain herd exhibits a higher expected annual variation than any of the herds examined previously. In part, this is a misleading conclusion because the high coefficient of variation can also result if there are no horses ever going into long-term holding facilities, thus creating large differences between gather and non-gather years. However, the Jenkins model simulations also suggest that the population fluctuates more widely *around* the 100-horse AML level rather than generally being *above* that level more often than not, as seen in simulations for other populations. For example, a few of the randomly-generated simulation traces resulted in fewer than 50 horses under the Baseline scenario (Figure 6). This phenomenon points to an additional weakness inherent in how the Jenkins model was applied here that was not discussed by Bartholow (2004): management actions such as contraceptive application would not continue unchanged for 20 years if the result of that application were perceived as jeopardizing the population's persistence. Adaptive assessment and management would prevail.

Caveats discussed in the initial report (Bartholow 2004) apply to this analysis. Several other concerns or imperfections with the Pryor Mountain modeling, particularly with trying to approximate the Baseline scenarios, have also been noted in this report. One additional caveat is worthy of mention. Recall that one goal of compassionate-use fertility control for the Pryor Mountain herd is to help reduce mortality of older mares and help prevent orphaned foals. Simulations conducted here did not include these potential feedback mechanisms and there is no way to conveniently do so. Research must continue to determine whether compassionate-use fertility control is successful in reducing these mortality sources.

Bartholow (2004) made some recommendations for potential modifications to the Jenkins model. Application of the WinEquus model to this specific herd was not a perfect fit for a variety of reasons. If there are other BLM-managed herds similar to the Pryor Mountain population that intermix annual darting and gathering, BLM might consider developing a model that could be

tailored to these sorts of conditions. If this is the only herd, software modifications would not likely be worth the effort.

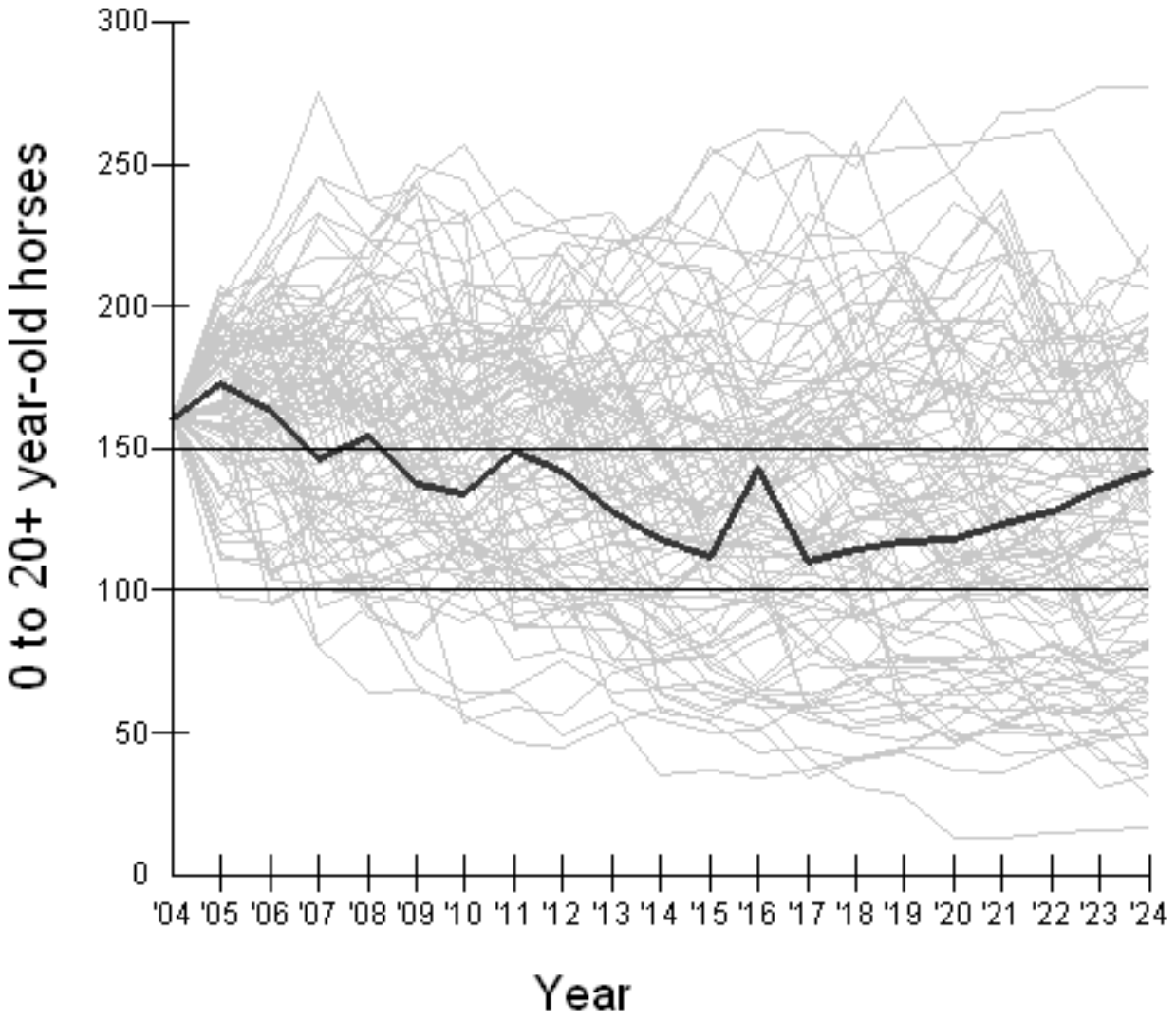

Figure 6. The "Most Typical Trial" simulated by the Jenkins model for the Pryor Mountain Baseline scenario.

Finally, as mentioned in the footnote added to Table 2, since running these simulations, newly collected data are available for the Clan Alpine herd (J.W. Turner, Jr., technical notes on Clan Alpine HMA Wild Horse Survey, June 2004, supplied by Linda Coates-Markle). These notes do not support 4th year effectiveness. Therefore, the cost estimates for scenarios involving the 3-year contraceptive will, of necessity, be too low.

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Appendix A. Listings of Jenkins Model Parameters for the Pryor Mountain HMA

The following table provides the basic parameters used in Jenkins' model simulations for the Pryor Mountain herd. Notes imbedded in the files used state that: (1) the initial age structure represented conditions in the fall of 2003 and was considered very accurate; (2) survival estimates represented 1996-2000 conditions, but assumed male survivals were about 97% that of females, as recorded for earlier (1976-1986) data; and (3) foaling rates represented 1996-2003 data, though no mares were ≥ 20 years old. In addition, Linda Coates-Markle confirmed that the initial population numbers by sex and age class should be considered exact counts and not a 90% value as was the case for previous simulations on other herds (Bartholow 2004).

Table A.1. Pryor Mountain Jenkins' model log file for Baseline Scenario. Foaling rates were entered with three digits of precision but only appear with two in this view. Also, log files incorrectly report that gathers do not continue after removals to treat additional females (Steve Jenkins, personal communication). This error has been corrected in this table.

Age Class	Initial Base Population		Survival Probabilities		Foaling Rates	Percentages for Removals		Percentages for Fertility Treatment
	Females	Males	Females	Males		Females	Males	
foal	10	12	0.700	0.700	0.00	0%	0%	0%
1	4	8	0.800	0.800	0.07	9%	9%	100%
2	8	8	0.931	0.902	0.48	9%	9%	100%
3	6	1	0.931	0.902	0.47	9%	9%	0%
4	4	7	0.930	0.901	0.64	4%	4%	0%
5	7	3	0.929	0.901	0.63	0%	0%	0%
6	6	6	0.929	0.900	0.64	0%	0%	0%
7	5	8	0.927	0.899	0.62	0%	0%	0%
8	4	3	0.925	0.897	0.71	0%	0%	0%
9	3	2	0.923	0.895	0.79	0%	0%	0%
10-14	16	17	0.907	0.879	0.58	0%	0%	50%
15-19	3	8	0.816	0.791	0.08	0%	0%	100%
20+	0	2	0.207	0.207	0.00	0%	0%	100%

Sex ratio at birth: 51% males
Scaling factors for annual variation: survival probabilities = 1.00, foaling rates = 1.00
Correlation between annual variation in survival probabilities and foaling rates = 0.00

Management by removals and fertility control
Starting year is 2004
Gathering occurs at regular interval of 1 years
Initial gather year is 2004
Gathers for fertility treatment occur regardless of population size.
Gathers continue after removals to treat additional females.
Threshold population size for gathers is 150.
Target population size following removals is 100.
Foals are excluded from AML.
Percent of population that can be gathered = 70%.
Percent effectiveness of fertility control: year 1 is 90%, year 2 is 0%, year 3 is 0%, year 4 is 0%, year 5 is 0%.

Table A.2. Pryor Mountain Jenkins' model log file for the more conventional 2-year contraceptive scenarios.

Age Class	Initial Base Population		Survival Probabilities		Foaling Rates	Percentages for Removals		Percentages for Fertility Treatment
	Females	Males	Females	Males		Females	Males	
foal	10	12	0.700	0.700	0.00	0%	0%	0%
1	4	8	0.800	0.800	0.07	50%	50%	100%
2	8	8	0.931	0.902	0.48	50%	50%	100%
3	6	1	0.931	0.902	0.47	50%	50%	0%
4	4	7	0.930	0.901	0.64	25%	25%	0%
5	7	3	0.929	0.901	0.63	0%	0%	0%
6	6	6	0.929	0.900	0.64	0%	0%	0%
7	5	8	0.927	0.899	0.62	0%	0%	0%
8	4	3	0.925	0.897	0.71	0%	0%	0%
9	3	2	0.923	0.895	0.79	0%	0%	0%
10-14	16	17	0.907	0.879	0.58	0%	0%	50%
15-19	3	8	0.816	0.791	0.08	0%	0%	100%
20+	0	2	0.207	0.207	0.00	0%	0%	100%

Sex ratio at birth: 51% males
Scaling factors for annual variation: survival probabilities = 1.00, foaling rates = 1.00
Correlation between annual variation in survival probabilities and foaling rates = 0.00

Management by removals and fertility control
Starting year is 2004
Gathering occurs at regular interval of 4 years
Initial gather year is 2004
Gathers for fertility treatment occur regardless of population size.
Gathers continue after removals to treat additional females.
Threshold population size for gathers is 150.
Target population size following removals is 100.
Foals are excluded from AML.
Percent of population that can be gathered = 70%.
Percent effectiveness of fertility control: year 1 is 94%, year 2 is 82%, year 3 is 68%, year 4 is 0%, year 5 is 0%.

Appendix B. Program to Estimate Economic Costs from WinEquus Simulation Results

As mentioned in Appendix A, the Jenkins model was used to simulate the population's alternative futures and the simulation results were written to a text file. A Microsoft VisualBasic program was constructed to read these results and calculate average yearly costs as well as overall average costs for a 20-year period. User-specified input to this program (Figure B.1) includes estimates for the individual components of the variable costs for each state.

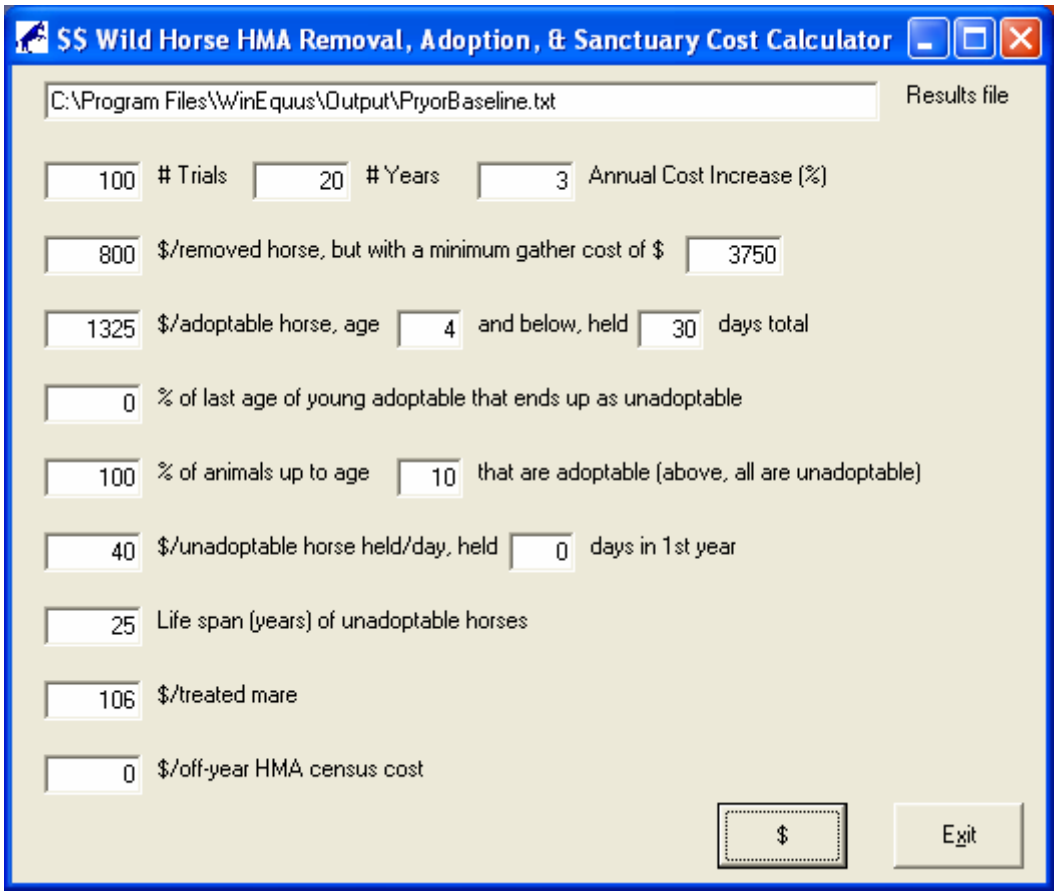

Figure B.1. Input parameters for companion program to estimate costs of each specific simulation run with the Jenkins modeling software. The image pictured is for the Baseline case and is considerably different from the more conventional scenarios. Note that since the Pryor Mountain herd produces only adoptable horses, cost values for that category will never apply. However, adoptable horses still accrue daily holding costs.

The number of *trials* and number of *years* are set to match parameters in the Jenkins model set-up. The number of trials and number of years capture both the variability inherent in the stochastic simulation model and any population adjustments in age and sex structure that occur over about one horse life span. The *annual cost increase* adjusts all future expenditures for the rate of inflation. The *$/removed horse* reflects the cost of gathering and removal averaged across all removed horses. *Minimum gather cost* is just what it says: i.e., even if the number of gathered horses is small, there would be a minimum cost just to have a gather. The *$/adoptable horse* reflects the combined cost of adoption and compliance checks (Table 3). All horses up to the

first age listed are assumed to be adoptable, except for the *% of last age of young adoptable that ends up as unadoptable*. In other words, a certain percentage of the oldest age class of adoptable animals is considered unadoptable. [Note that since the Pryor Mountain herd does not produce unadoptable horses, this value is set to zero and therefore cost values for "unadoptable" horses will never apply.] Adoptable animals are held for the first number of *days* listed prior to adoption. A *% of animals up to age* xx are also considered adoptable. Unadoptable animals accrue a cost of *$/unadoptable horse held/day*, are held *days in 1st year*, and 365 days thereafter through their *life span*. Note that adoptable horses also accrue the same holding cost for the days they are held prior to adoption. Contraceptive application is reflected in the *$/treated mare* cost estimate. *$/off-year HMA census cost* reflects any additional costs involved with a contraceptive program in non-gather years (typically years 2 through 4), such as flight costs to assess treatment effectiveness and perform other routine monitoring (Hall 2003). This last item would be zero except for scenarios involving contraceptive treatment.

The program reads the simulation results, averaging the costs for each year over the number of trials for which the software was run, and then summarizes the results across all simulation years. The output from this program looks like that shown in Table B.1.

The economic model output contains the name of the Jenkins model simulation results file and echoes the input values used. *Expenses* are inflation-adjusted values and *CV* is the coefficient of variation: i.e., the percent that expenses might be expected to vary annually given the variability reflected in the stochastic population model. The CV value is calculated as one standard deviation from the mean value for the year divided by that mean value. The remaining values listed (population size, sex ratio, number gathered, number treated, number removed, number adopted, number unadoptable, number held, and number dying) also represent annual averages, rounded to the nearest animal. The *mean* values listed near the bottom are averages across the number of years, except for those associated solely with gathering (population size, sex ratio, number gathered, number treated, number removed, number adopted, number unadoptable), which are averaged across the number of gathers. Finally, the program provides the percentage of the mean annual expense attributable to the total cost of adoptions, long-term holding, and contraceptive treatment.

Scanning the values listed in Table B.1, one can usually see how the population is adjusting through time to the management strategy implemented in the population modeling software. It is also a useful way to assess whether the selective removal rates specified in the Jenkins model have been effective in reaching the specified herd-specific AML – if foals are included in the AML.

Table B.1. Example output from cost estimator program for Pryor Mountain Baseline Scenario.

C:\Program Files\WinEquus\Output\PryorBaseline.prn 6/17/2004 9:01:26 AM
Trials = 100 Years = 20 Inflation % = 3
$/Removed horse = 800 , with minimum gather cost = 3750
$/Adoption = 1325 up to age 4 and held 30 days
0 % of last 'fully' adoptable age diverted to unadoptable
100 % of ages up to 10 that are adoptable
$/Unadoptable/Day = 40 held 0 days the 1st year
Life span (yrs) = 25 $/Treated mare = 106
$/Off-year census = 0 (Include for treatment scenarios only!)

Year	Expense	±_CV	PopSize	SexRat	Gather	Treat	Remove	Adopt	UnAdopt	Held	Die
1	$939	19.2%	161	0.528	97	9	0	0	0	0	0
2	$2,982	180.5%	168	0.513	105	8	1	1	0	0	0
3	$9,403	108.6%	168	0.507	106	13	2	2	0	0	0
4	$8,592	119.5%	163	0.505	102	14	2	2	0	0	0
5	$9,583	124.5%	160	0.502	100	12	2	2	0	0	0
6	$8,479	138.5%	158	0.504	99	12	2	2	0	0	0
7	$7,825	143.9%	152	0.502	96	12	2	2	0	0	0
8	$7,216	152.2%	151	0.504	98	11	1	1	0	0	0
9	$7,797	145.8%	148	0.506	96	11	1	1	0	0	0
10	$7,192	156.5%	143	0.503	93	11	1	1	0	0	0
11	$6,885	162.4%	139	0.502	90	11	1	1	0	0	0
12	$6,652	174.6%	136	0.501	89	10	1	1	0	0	0
13	$5,741	193.6%	134	0.502	87	10	1	1	0	0	0
14	$6,984	176.3%	136	0.502	89	10	1	1	0	0	0
15	$7,345	180.0%	134	0.507	88	11	1	1	0	0	0
16	$7,312	162.4%	133	0.509	86	10	1	1	0	0	0
17	$8,924	156.4%	132	0.509	86	10	1	1	0	0	0
18	$9,180	164.7%	131	0.510	85	10	1	1	0	0	0
19	$8,603	180.6%	127	0.506	83	10	1	1	0	0	0
20	$7,752	178.1%	123	0.505	80	9	1	1	0	0	0
Mean	$7,269	154.3%	145	0.506	98	11	1	1	0	0	0

79.1% for Adoptions
0.0% for Holdings
20.9% for Treatment

Metabolism
Clinical and Experimental

Effect of ovariectomy and estradiol replacement on skeletal muscle enzyme activity in female rats☆

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PlumX Metrics

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Abstract

Abstract

In female rats, ovariectomy (OVX) is associated with increased body fat and insulin resistance, and estradiol replacement prevents these alterations. These metabolic changes related to the estrogen-deficient state might be due, in part, to alterations in skeletal muscle substrate metabolism. We tested the hypothesis that estradiol affects the regulation of enzymes involved in substrate oxidation and storage within skeletal muscle. Specifically, we examined enzymes involved in the regulation of glycogen synthesis (glycogen synthase [GS]), glycolysis (phosphofructokinase [PFK]), tricarboxylic acid cycle activity (citrate synthase [CS]), and [beta]-oxidation ([beta]-hydroxyacyl-CoA dehydrogenase [[beta]-HADH]). Twenty-two, female Sprague-Dawley rats (7 to 8 weeks old) were separated into 3 groups: OVX + placebo (P; n = 8), OVX + estradiol (E_2; n = 8), and sham-operated (S; n = 6). Rats from E_2 and P groups were pair-fed to the S group to control for OVX-induced changes in food intake. After 16 days, activities of GS, PFK, CS, and [beta]-HADH were measured in vastus medialis muscle. GS fractional velocity was significantly lower (*P* [lt] .05) in P (mean [plusmn] SE; 39.7% [plusmn] 6.2%) compared with both S (61.9% [plusmn] 8.8%) and E_2 (65.8% [plusmn] 8.4%) rats. In addition, E_2 rats (41.4 [plusmn] 2.0) had significantly higher (*P* [lt] .05) CS activity than P (34.9 [plusmn] 2.0) and S (33.9 [plusmn] 1.4 [mu]mol/min/g) groups. There was no effect of OVX or estradiol replacement on [beta]-HADH or PFK. Our results suggest that, independent of alterations in food intake, estradiol availability affects the regulation of enzymes involved in nonoxidative glucose disposal (GS) and oxidative metabolism (CS) in skeletal muscle.

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Impact of ovariohysterectomy and food intake on body composition, physical activity, and adipose gene expression in cats[1]

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ABSTRACT: The mechanisms contributing to BW gain following ovariohysterectomy in domestic cats are poorly understood. Moreover, the effects of food restriction to maintain BW following spaying have been poorly studied. Thus, our primary objective was to determine the effects of spaying and food restriction to maintain BW on adipose and skeletal muscle mRNA abundance and activity levels in cats. After a 4-wk baseline period (wk 0), 8 adult (approximately 1.5 yr old) domestic shorthair cats were spayed and fed to maintain BW for 12 wk. After 12 wk, cats were fed ad libitum for an additional 12 wk. Body composition was determined, activity levels were measured, and adipose and muscle biopsies were collected at wk 0, 12, and 24. Fasting blood samples were collected at wk 0, 6, 12, 18, and 24. To maintain BW post-spay, food intake was decreased ($P < 0.05$) by 30%. During this phase, mRNA abundance of adipose tissue lipoprotein lipase and leptin was decreased ($P < 0.05$), representing only 52 and 23% of baseline expression, respectively. Interleukin-6 mRNA, however, was increased ($P < 0.05$) 2-fold. Physical activity was decreased ($P < 0.05$) by wk 12, most dramatically during the dark period (approximately 20% of baseline activity). During ad libitum feeding (wk 12 to 24), food intake, BW, body fat percentage, and total fat mass were greatly increased ($P < 0.05$). Compared with wk 0, circulating leptin concentrations tended to increase ($P < 0.10$) by wk 18 and 24 (4.45 vs. 10.02 and 9.14 ng/mL, respectively), whereas glucose (91 vs. 162 mg/dL) and triacylglyceride (30 vs. 48 mg/dL) concentrations were increased ($P < 0.05$) by wk 24. Adipose tissue lipoprotein lipase, hormone sensitive lipase, and adiponectin mRNA were decreased ($P < 0.05$) at wk 24. Adipose interleukin-6 mRNA was increased ($P < 0.05$) at 24 wk. Physical activity was further decreased ($P < 0.05$) by wk 24, during the light (60% of baseline) and dark (33% of baseline) periods. In summary, spaying and food restriction affect physical activity levels and several genes associated with lipid metabolism (decreased lipoprotein lipase), food intake (decreased leptin expression), and insulin insensitivity (increased interleukin-6). By identifying these changes, targets for nutritional intervention or lifestyle management have been identified that may curb the risk of obesity and related disorders in spayed cats.

Key words: adipose tissue, body composition, cat, gene expression, ovariohysterectomy, physical activity

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INTRODUCTION

Feline obesity has rapidly increased over the past few decades, with 30 to 40% of cats in the United States being considered overweight or obese (Scarlett et al., 1994; Lund et al., 2005). Although gonadectomy is an effective strategy for controlling overpopulation, it is a risk factor for feline obesity. The effects of estrogen on metabolic rate and disease risk are evident but poorly understood. Estrogen not only has receptors in the hypothalamus, but has also been shown to interact with leptin and increase its action, suggesting a role in appetite regulation (Cooke and Naaz, 2004). Estrogen has also been associated with increased voluntary exercise, with ambulatory activity decreasing in ovariectomized rats (Shimomura et al., 1990). Although these interactions have been demonstrated, the mechanisms contributing to increased food intake and BW gain in spayed cats are unknown.

Because altered glucose and lipid metabolism accompany BW gain, identifying differences in skeletal muscle and adipose tissue after spaying is of interest. Skeletal muscle plays an important role in glucose metabolism and accounts for approximately 80% of insulin-mediated glucose uptake (Defronzo et al., 1981), a response that is diminished in obese individuals. In addition to

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its role in energy storage, adipose tissue is now accepted as an active endocrine organ intricately involved in energy homeostasis and metabolism. The metabolic dysfunction that occurs in muscle after spaying, with BW gain, or both, is not entirely understood, but may be partly attributed to low-grade inflammation. It is now well accepted that much of this inflammation is derived from inflammatory mediators produced by immune cells present in adipose tissue. Because estrogen receptors are expressed in several cell types in adipose tissue including preadipocytes and macrophages (Gulshan et al., 1990; Venkov et al., 1996), studying the effects of spaying on adipose tissue function is justified.

Recent feline genome sequencing initiatives have allowed the measurement of gene transcripts that may aid in our understanding of spaying and its effect on adipose and muscle metabolism. The objectives of this experiment were 2-fold. Because the effects of food restriction to maintain BW following spaying have been poorly studied, we first aimed to evaluate the effects of spaying and 12 wk of food restriction on BW, body composition, activity levels, blood metabolites, and mRNA abundance of genes associated with lipid metabolism and inflammation in subcutaneous adipose and skeletal muscle tissues of adult cats. Second, we aimed to measure these same outcomes in cats fed ad libitum for an additional 12 wk.

MATERIALS AND METHODS

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before experimentation.

Animals and Diet

Eight adult (mean age = 1.6 ± 0.13 yr old; mean BW = 3.52 ± 0.72 kg) female domestic shorthair cats (Liberty Research Inc., Waverly, NY) were used. Cats were individually housed in stainless steel cages measuring 0.61 × 0.61 × 0.61 m in the animal facility of the Edward R. Madigan Laboratory at the University of Illinois. Room dimensions were 7.32 m wide × 9 m long × 3.96 m high. The room was climate-controlled (22°C) with a 16 h light:8 h dark cycle. Cats were fed a commercially available, nutritionally complete dry cat food (Iams Original Formula Cat Food, Procter and Gamble, Dayton, OH) throughout the experiment and were given ad libitum access to water. The primary ingredients in the diet included chicken, chicken by-product meal, corn grits, corn meal, chicken fat, fish meal, and dried beet pulp. Analyzed dietary chemical composition is listed in Table 1.

Table 1. Analyzed chemical composition of diet fed to spayed cats

Item	%, as fed
DM	93.47
OM, % of DM	93.19
Acid hydrolyzed fat, % of DM	22.35
CP, % of DM	36.13
Total dietary fiber, % of DM	5.71
GE, kJ/g	22.97

Experimental Design

Experimental design is presented in Figure 1. Four weeks before the experiment, cats were acclimated to the diet and fed at a level to maintain BW. During this time, cats were also acclimated to a daily activity schedule that allowed for group interaction but individual housing and feeding. This period also allowed for an accurate estimate of the caloric intake required to maintain a stable BW before treatment. Cats were housed in pens individually for 7 h/d (0800 to 0900 h; 1000 to 1200 h; 1500 to 1900 h) and group housed for 17 h/d in the room, including the dark period. After the baseline period (wk 0), all activity and body composition measurements were performed and blood and tissue samples were collected. Cats were then spayed and studied for 24 additional wk. At wk 0 (pre-spay), 6, 12, 18, and 24, fasted blood samples were collected for glucose, leptin, ghrelin, triacylglyceride (**TG**), NEFA, fructosamine, and insulin analysis. Subcutaneous abdominal adipose and LM biopsies also were collected at wk 0, 12, and 24.

Daily food intake and twice weekly BW were measured throughout the study. In the 12 wk after spaying, food intake was adjusted so that BW was maintained within 200 g of baseline BW. After wk 12 measurements, cats were fed ad libitum for an additional 12 wk. Periods of 12 wk were chosen based on previous experiments demonstrating that caloric intake reaches a stable plateau by approximately wk 12 after spaying (Flynn et al., 1996).

Body Composition

Lean mass, fat mass, and bone mineral mass were determined using dual-energy x-ray absorptiometry, which has been previously validated in cats (Speakman et al., 2001). Determinations were conducted after ovariohysterectomy and tissue biopsy, while still under sedation. Cats were placed in ventral recumbency and body composition was analyzed using a Hologic model QDR-4500 Fan Beam x-ray Bone Densitometer and software (Hologic Inc., Waltham, MA).

Activity Level Assessment

Voluntary activity levels was evaluated using Actical activity collars (Mini Mitter, Bend, OR), which were worn around the neck for 9 consecutive days before wk 0, 12, and 24. The collars contained omnidirectional sensors capable of accurately incorporating both intensity and duration of movements. Once the collars were

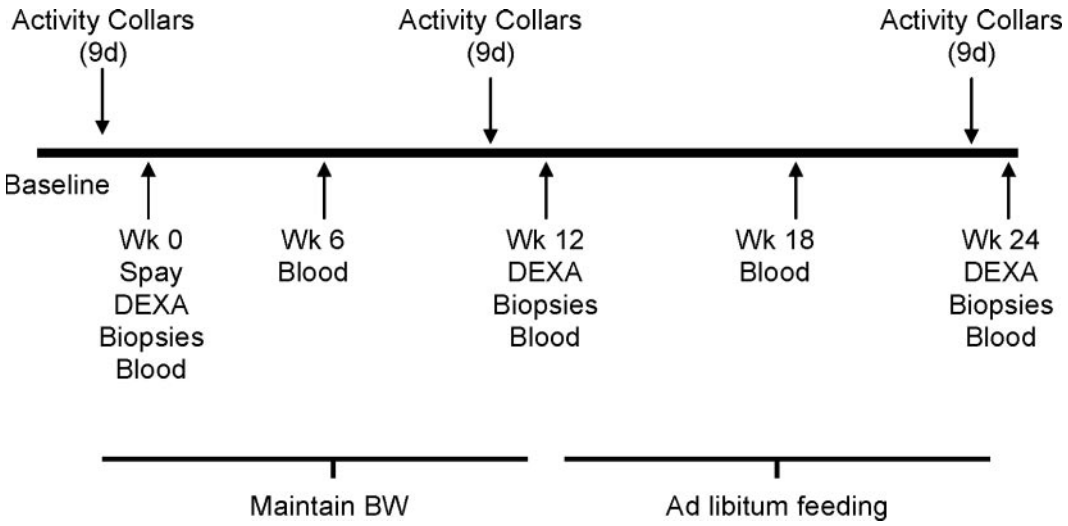

Figure 1. Timeline and data collection time points of experiment. DEXA = dual-energy x-ray absorptiometry.

removed, the Actical software analyzed the data compiled by the collar and converted it into arbitrary numbers referred to as activity counts. In the current data set, average activity was presented in activity counts per epoch (epoch length = 0.25 min). Actical software allowed for the determination of average activity counts per epoch during the light and dark periods without human interference.

Ovariohysterectomy

Food was withheld for at least 8 h before ovariohysterectomy. Cats were given free access to water. Cats were premedicated with ketamine and equal parts valium (11 mg/kg intramuscular), and anesthesia was induced and maintained with isoflurane in 100% oxygen. Briefly, a ventral medial incision was made and ovariohysterectomy was performed by T. K. Graves and T. Keel using standard techniques. Postsurgery, butorphanol (0.22 mg/kg) was administered to alleviate discomfort. All cats were completely recovered within 2 wk postsurgery, returning to their normal food intake and activity levels.

Sample Collection and Analysis

Representative diet samples were collected and ground using a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen and dry ice in preparation for chemical analyses. Diet samples were analyzed for DM and OM according to AOAC (1984). Crude protein was determined according to AOAC (1995) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, MI). Fat concentrations were measured by acid hydrolysis (AACC, 1983) followed by ether extraction (Budde, 1952). Gross energy was measured by use of a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL). Total dietary fiber concentration was determined according to Prosky et al. (1992).

Fasting (8 h) blood samples (3 and 8 mL) were collected at wk 0, 6, 12, 18, and 24 via jugular venipuncture. One sample (3 mL) was collected into BD Vacutainer tubes (#367835, 5.4 mg of K_2 EDTA, Becton, Dickinson, and Company, Franklin Lakes, NJ) for plasma. The second sample (8 mL) was first used for glucose measurement (1 drop needed) using the glucose oxidase method by utilizing a Precision G Blood Glucose Testing System (Medisense, Bedford, MA). The remainder of the second blood sample was transferred to the appropriate serum separator tubes (#367985, Becton, Dickinson, and Company). All tubes were centrifuged (13,000 $\times$ g for 15 min at 4°C) within 1 h of collection. Following centrifugation, supernatant was collected, separated into respective cryovials, and stored at −80°C until further analysis.

Serum fructosamine, TG, and NEFA were analyzed by the University of Illinois Clinical Diagnostic Laboratory (Urbana). Plasma leptin concentrations were determined using a commercially available RIA kit (Multispecies Leptin RIA Kit, Linco Research, St. Louis, MO) previously validated in cats (Backus et al., 2000). Serum insulin concentrations were determined using a commercial RIA kit (Double Antibody RIA, Diagnostic Systems Laboratories Inc., Webster, TX) validated in the cat by the manufacturer. Serum total ghrelin concentrations were determined using a commercially available enzyme immunoassay kit (Ghrelin Rat EIA Kit, Phoenix Pharmaceuticals Inc., Belmont, CA) following a 10-fold dilution of serum. Insulin, leptin, and ghrelin data were analyzed using GraphPad Software (Prism Software, San Diego, CA) before statistical analyses were performed.

At wk 0, 12, and 24, approximately 1 g of subcutaneous abdominal adipose tissue and 1 g of LM was collected following an overnight fast. Cats were sedated using atipamazole (0.02 mg/kg), atropine (0.04 mg/kg), and medetomadine (0.02 mg/kg). Samples were immediately flash frozen in liquid nitrogen and stored at −80°C until further analyses.

Total RNA Extraction and Quantitative Reverse Transcriptase-PCR

Total cellular RNA was isolated from adipose tissue and skeletal muscle using Trizol (Invitrogen, Carlsbad, CA). The concentration and purity of RNA was determined using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). Conversion of RNA to complementary (**c**)DNA was carried out using methods described by the ABI cDNA Archive kit (Applied Biosystems, Foster City, CA). Isolated cDNA was amplified using quantitative reverse transcriptase-PCR on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Gene-specific primers for adiponectin, IL-6, IL-1, uncoupling protein 2, lipoprotein lipase (**LPL**), vascular endothelial growth factor, chemokine receptor 5, estrogen receptor 1, hormone-sensitive lipase (**HSL**), leptin, toll-like receptor 4, tumor necrosis factor α (**TNF-α**), and glyceraldehyde-3-phosphate dehydrogenase, were designed using Primer Express 2.0 Software (Perkin Elmer, Boston, MA; Table 2). The mRNA abundance for all of the genes was tested in adipose tissue, whereas only estrogen receptor 1, uncoupling protein 2, LPL, and HSL were measured in skeletal muscle.

The PCR mixture contained 1 ng/μL and 10 ng/μL of cDNA from adipose and skeletal muscle tissue, respectively, 15 pmol of each primer, and 5 μL of SYBR Green (Applied Biosystems). The final volume was adjusted to 10 μL using sterile deionized water. The 18S ribosomal RNA gene was used as an internal standard. Originally, glyceraldehyde-3-phosphate dehydrogenase was attempted as an internal standard. However, expression levels were affected over time and were determined to be inappropriate for our data set. Data analyses were carried out with sequence detection system software (Applied BioSystems).

Statistical Analyses

The experimental design consisted of a single factor (week) experiment with repeated measures. Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Cat and week were tested as fixed effects. Least squares means for food intake and BW data were compared with wk 0, and least squares means for all other data were compared between week by using the least significant difference method when the main effects were significant. Results are presented as means ± SEM. The correlation between leptin and body fat mass was analyzed using the Pearson correlation in SAS. Relative calculated levels of mRNA in fat and skeletal muscle tissues were analyzed using MIXED procedure of SAS. Again, cat and week were tested as fixed effects. A probability of $P < 0.05$ was considered significant and $P < 0.10$ was considered to be a trend.

RESULTS

Food Intake and BW

To maintain ideal BW during the first 12 wk after spaying, food intake was decreased, with decreases ($P < 0.05$) occurring by wk 7 (30.6% by wk 12; Figure 2). After 12 wk, cats were fed ad libitum. As expected, a large increase in food intake resulted from wk 12 to 13, followed by a gradual decline that was still greater ($P < 0.05$) at wk 23 than at baseline (40.5% increase from wk 12 to 24). Food intake at wk 24 was not different from baseline.

Body Composition

Total fat mass and bone mineral content were greater ($P < 0.05$) at wk 24 compared with wk 0 and 12 (Table 3). Lean mass also was greater at wk 24 compared with wk 0 and tended ($P < 0.10$) to be greater than at wk 12. Body fat percentage did not differ from wk 0 to 12, but was greater ($P < 0.001$) at wk 24 compared with wk 0 and 12. Although lean mass (g) increased from wk 0 to 24, the dramatic change in fat mass resulted in decreased ($P < 0.05$) lean mass percentage at wk 24 compared with wk 0 and 12. Bone mineral content percentage increased ($P < 0.05$) from wk 0 to 24, which

Table 2. Forward (FOR) and reverse (REV) primers used for quantitative reverse transcriptase-PCR analysis

Gene	Primer	Sequence
18S ribosomal RNA	FOR	5′-GCC GCT AGA GGT GAA ATT CTT G-3′
	REV	5′-CAT TCT TGG CAA ATG CTT TCG-3′
Adiponectin	FOR	5′-TTG AAG GTC CCC GAG GTT T-3′
	REV	5′-TAC GTA GGC ACT TTC TCC AGG TT-3′
IL-6	FOR	5′-CTC AGG GCT GTT CGG ATA ATG-3′
	REV	5′-GAG AAA GGA ATG CCC GTG AA-3′
Lipoprotein lipase	FOR	5′-TCT GCG GGA TAC ACC AAG CT-3′
	REV	5′-CCT CCG CCA TCC AGT TGA T-3′
Hormone sensitive lipase	FOR	5′-GCC ACC AGA GGC CTT TGA A-3′
	REV	5′-TGA GAT GGT GAC CGT GAG CTT-3′
Leptin	FOR	5′-AGA GTC GCT GGT CTT GAC TTC AT-3′
	REV	5′-GCC AAT GTC TGG TCC ATC TTG-3′
Tumor necrosis factor	FOR	5′-TAG CAA ACC CCG AAG CTG AA-3′
	REV	5′-ATT GGC CAG GAG GGC ATT-3′

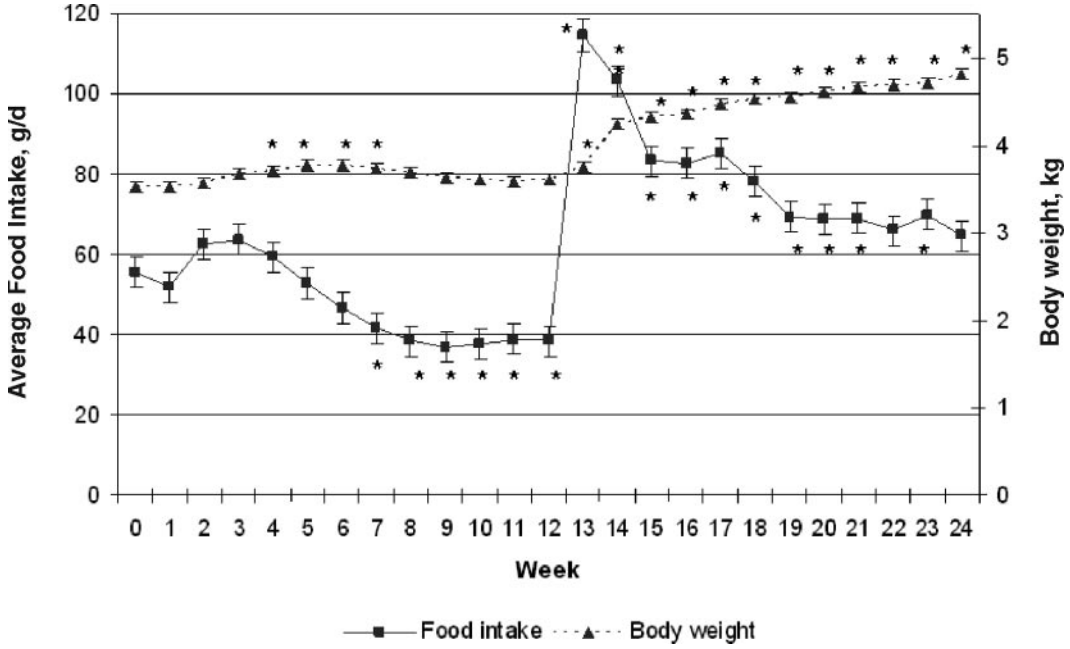

Figure 2. Average food intake and BW of spayed cats. Cats were food restricted following ovariohysterectomy (wk 0) to maintain baseline BW for 12 wk and then fed ad libitum for another 12 wk. *Means that differ from wk 0 ($P < 0.05$), n = 8. Food intake SEM = 3.80; BW SEM = 0.06.

was likely the result of an increased need for skeletal support of a greater BW.

Physical Activity and Intensity

From wk 0 to 24, total daily activity decreased by 52%, decreasing ($P < 0.001$) from 30.0 to 14.3 ± 1.82 mean activity counts per epoch. In the first 12 wk, activity during light hours did not change, but activity during the dark period decreased ($P < 0.001$) dramatically (Figure 3). From wk 12 to 24, total activity decreased ($P < 0.05$) further, primarily because of the decreased ($P < 0.05$) activity during light hours.

Blood Metabolites

By wk 24, cats had increased ($P < 0.001$) serum glucose and TG concentrations compared with wk 0, 6, 12, and 18 (Table 4). Circulating leptin also tended to be greater ($P < 0.10$) at wk 18 and 24 compared with wk 0.

Adipose and Skeletal Muscle Tissue mRNA Abundance

Adipose LPL mRNA abundance was decreased ($P < 0.05$) at wk 12 and 24 compared with wk 0 (Table 5). Similarly, HSL mRNA abundance was decreased ($P < 0.05$) at wk 24 compared with wk 0 and 12. In this study, TNF-α mRNA tended to decrease ($P < 0.10$) by wk 12 compared with wk 0, but was not different at wk 24 (Table 5). Adipose IL-6 mRNA expression was increased ($P < 0.05$) at wk 12 and 24 compared with wk 0 (Table 5). Adipose leptin mRNA expression levels decreased ($P < 0.05$) at wk 12, but were not different at wk 24 compared with wk 0 (Table 5). Adipose adiponectin mRNA was not different between wk 0 and 12, but was decreased ($P < 0.05$) at wk 24 (Table 5). Skeletal muscle mRNA abundance was not changed ($P > 0.05$) throughout the experiment (data not shown).

DISCUSSION

Soon after spaying, most cats have a significant increase in food intake, leading to BW gain that is pri-

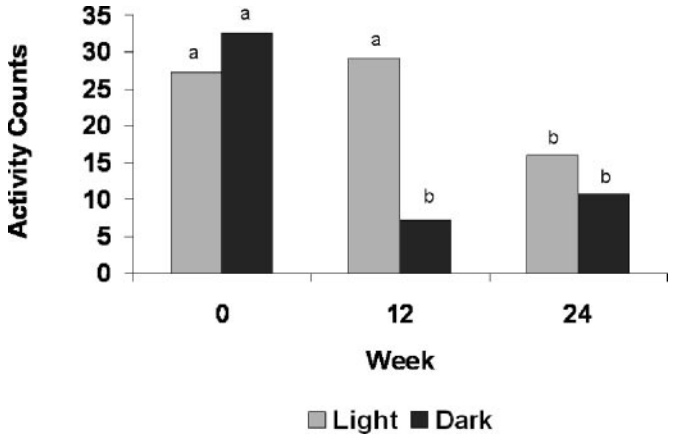

Figure 3. Average activity levels of spayed cats during dark and light periods. Data compiled by activity collars were converted into arbitrary numbers referred to as activity counts by Actical software (Mini Mitter, Bend, OR). Average activity is presented in activity counts per epoch (epoch length = 0.25 min). [a,b]Within an activity period [when lights were on (light) vs. when lights were off (dark)], means lacking a common letter differ ($P < 0.05$); n = 8; light SEM = 2.18; dark SEM = 2.10.

Table 3. Body composition of cats during wk 0, 12, and 24 of the study[1]

Item	Wk 0	Wk 12	Wk 24	SEM
Total fat, g	750.78[a]	706.99[a]	1,644.96[b]	90.17
Total lean, g	2,742.26[a]	2,862.97[ab]	3,086.67[b]	40.72
Total BMC, g	40.72[a]	43.79[a]	64.60[b]	2.51
Fat, %	20.55[a]	18.58[a]	33.50[b]	0.99
Lean, %	78.29[a]	80.18[a]	65.15[b]	0.98
BMC, %	1.16[a]	1.24[ab]	1.35[b]	0.05

[a,b]Least squares means within a row lacking a common superscript differ ($P < 0.05$), n = 8.

[1]Body composition was measured following a baseline period (wk 0), 12 wk after spay and food restriction to maintain BW (wk 12), and another 12 wk of ad libitum feeding and consequent BW gain (wk 24). BMC = bone mineral content.

marily in the form of fat mass. Obesity-related insulin resistance and alteration in blood lipids soon follow, increasing the risk of diabetes mellitus and other disorders. Although these physiologic responses are commonly reported, few studies have been designed to identify contributing mechanisms. Moreover, the effects of food restriction to maintain BW following spaying have been poorly studied to date. Therefore, our primary objective was to evaluate the effects of spaying and 12 wk of food restriction on activity levels and mRNA abundance of genes associated with lipid metabolism and inflammation in subcutaneous adipose and skeletal muscle tissues of adult cats. Our secondary objective was to measure these responses when cats were fed ad libitum for an additional 12 wk.

Similar to what has been reported by other research groups, our data suggest that caloric restriction of approximately 30% is required to avoid BW gain following ovariohysterectomy in cats. Flynn et al. (1996) and Hoenig et al. (2002) reported a reduced caloric intake of 37 and 13%, respectively, to maintain baseline BW in spayed cats. Because cats were fed to maintain BW for the first 12 wk, body composition and blood metabolite concentrations were not altered during this period. This allowed for the measurement of activity and gene expression changes due to spaying and food restriction without the bias of BW gain.

Effects of Spaying and Food Restriction on Physical Activity

Reduced physical activity has been suggested as a contributing factor to increased BW gain following ovariohysterectomy but has not been quantitatively measured in cats. Flynn et al. (1996) subjectively measured activity in cats following ovariohysterectomy, reporting no significant differences from pre-spay. However, in that study, cats were only observed for 5 to 10 min/d over the course of a few weeks. The use of activity collars in this experiment allowed for continuous monitoring over several days during dark and light periods and without human interference. Our results demonstrated a dramatic decrease in physical activity after gonadectomy and food restriction, especially during the dark period. To our knowledge, these data are the first of its kind to be reported in spayed cats. Given this interesting outcome, further research is needed to distinguish cause-and-effect relationships between physical activity and the physiological and gene expression changes observed in this experiment.

Effects of Spaying and Food Restriction on Adipose mRNA Abundance

Lipid metabolism is commonly altered following ovariohysterectomy, BW gain, or both (Hoenig et al.,

Table 4. Fasting blood glucose, insulin, triacylglyceride (TG), NEFA, leptin, and ghrelin concentrations in spayed cats[1]

Item	Wk 0	Wk 6	Wk 12	Wk 18	Wk 24	SEM
Glucose, mg/dL	90.88[a]	106.50[a]	77.00[a]	109.75[a]	162.00[b]	10.88
Insulin, μIU/mL	3.86	3.72	6.26	4.12	3.32	0.50
TG, mg/dL	30.25[a]	35.38[a]	24.50[a]	33.50[a]	48.13[b]	3.91
NEFA, mEq/L	0.49	1.58	0.54	0.53	0.59	0.24
Leptin, ng/mL	4.45[y]	4.12[xy]	3.65[x]	10.02[z]	9.14[z]	1.32
Ghrelin, ng/mL	2.38	3.97	3.73	3.90	3.54	1.24

[a,b]Least squares means within a row lacking a common superscript differ ($P < 0.05$); n = 8, except wk 6 NEFA (n = 5) and wk 18 leptin (n = 7).

[x–z]Least squares means within a row lacking a common superscript differ ($P < 0.10$).

[1]Blood samples were collected following a baseline period (wk 0), 6 and 12 wk after spay and food restriction to maintain BW (wk 6 and 12), and another 6 and 12 wk of ad libitum feeding and consequent BW gain (wk 18 and 24).

Table 5. Relative mRNA expression in adipose tissue in spayed cats[1]

mRNA	Wk 0	Wk 12	Wk 24
Lipoprotein lipase	$968^{a} \pm 159$	$504^{b} \pm 110$	$543^{b} \pm 50$
Hormone sensitive lipase	$702^{a} \pm 120$	$513^{ab} \pm 98$	$436^{b} \pm 49$
Tumor necrosis factor	$4{,}911^{x} \pm 957$	$2{,}110^{y} \pm 620$	$3{,}779^{x} \pm 1409$
IL-6	$210^{a} \pm 18$	$533^{b} \pm 53$	$463^{b} \pm 71$
Leptin	$388^{a} \pm 92$	$90^{b} \pm 18$	$259^{a} \pm 39$
Adiponectin	$1{,}070^{a} \pm 335$	$425^{ab} \pm 143$	$460^{b} \pm 124$

[a,b]Within a gene, means lacking a common superscript differ ($P < 0.05$); n = 8.
[x,y]Within a gene, means lacking a common superscript differ ($P < 0.10$); n = 8.
[1]Results are expressed as arbitrary units ± SEM; mRNA abundance in adipose tissue was measured following a baseline period (wk 0), 12 wk after spay and food restriction to maintain BW (wk 12), and another 12 wk of ad libitum feeding and consequent BW gain (wk 24).

2006), but is rarely tested in spayed cats that are food-restricted. Lipoprotein lipase and HSL are key regulators of lipid storage and affect blood lipid concentrations. The effects of estrogen on LPL activity remain controversial. For example, Palin et al. (2003) reported decreased LPL protein expression at their greatest dose ($E_2\,10^{-7}$ mol/L) of estrogen, but increased expression at lesser doses ($E_2\,10^{-12}$ mol/L). Other research conducted in mice has shown that estrogen results in decreased LPL activity (Venkov et al., 1996). In the current experiment, wk 12 adipose mRNA abundance of HSL was unchanged, but that of LPL was decreased. However, blood lipid concentrations at 12 wk were not changed. Thus, because mRNA abundance and enzyme activities may not always agree with one another, further research is required to determine whether the decreased LPL mRNA is physiologically relevant.

One mechanism by which LPL is thought to be affected is through inhibition by proinflammatory cytokines such as TNF-α and IL-6 that are known to increase with obesity (Greenberg et al., 1992; Kern et al., 1995). Cytokine response to spaying with food restriction has not been well studied. To our knowledge, IL-6 mRNA expression has not been reported in feline adipose tissue. Elevated proinflammatory cytokine concentrations following reduced estrogen production have been reported in some human studies (Cheleuitte et al., 1998), but not others (Rogers and Eastell, 1998). Increased IL-6 expression at wk 12 in the current study suggests that estrogen withdrawal may increase proinflammatory cytokine production and is independent of BW gain. Increased IL-6 also correlates with the decreased LPL expression, supporting its inhibitory role. Tumor necrosis factor-α mRNA, however, tended to be decreased in adipose tissue at wk 12. More research is needed to determine mechanisms by which estrogen directly or indirectly affects cytokine mRNA and protein abundance.

Gonadal steroids modulate central nervous system effectors of energy homeostasis that are targets of leptin action. Whereas androgens are anabolic in nature, estrogens are catabolic and decrease food intake and BW (reviewed by Mystkowski and Schwartz, 2000). Mechanisms by which these effects occur, however, are poorly defined. Because estrogen receptors are present in adipose tissue (Pedersen et al., 1996), estrogen is thought to have a direct effect on leptin biosynthesis. Studies using rat adipocytes in culture have supported this hypothesis, demonstrating increased leptin secretion with the administration of estrogenic compounds (Machinal et al., 1999). Although BW and fat mass did not change during food restriction and spaying in the current experiment, leptin mRNA abundance was greatly decreased (approximately 23% of baseline expression) in adipose tissue. These data suggest that estrogen withdrawal and food restriction to maintain BW result in decreased leptin secretion. Because circulating leptin was not different, further study is warranted.

Effect of Ad Libitum Feeding on Body Composition and Blood Metabolites

As expected, ad libitum feeding resulted in dramatic increases in BW, body composition, and blood metabolite concentrations. Over the 12-wk period, total fat mass increased by approximately 120% compared with about 13% of lean mass. Harper et al. (2001) reported similar data (40% increase in fat mass and 10% in lean mass), suggesting that most BW gained following gonadectomy is in the form of fat. Concentrations of blood glucose, TG, and leptin were increased with BW gain. Because cats may display stress-induced hyperglycemia (Rand et al., 2002), an indicator of blood glucose concentrations over a 2- to 3-wk period (fructosamine) was measured. Although fructosamine concentrations increased with BW gain (data not shown), all values were within the normal range for cats (Bennett, 2002). Hypertriglyceridemia, which is often present in obese and type-2 diabetic cats (Hoenig et al., 2003), was also noted by wk 24. Finally, leptin concentrations tended to increase with BW gain during ad libitum feeding. Despite the lack of large changes in blood leptin concentrations, its relationship with body fat mass was highly correlated (r = 0.80). Therefore, ad libitum feeding resulted in the desired physiologic outcomes for comparison with mRNA abundance and physical activity.

Effect of Ad Libitum Feeding and BW Gain on Adipose mRNA Abundance

Altered adipose and skeletal muscle gene expression levels were expected to accompany the dramatic changes in body composition from wk 12 to 24. Decreased adipose tissue LPL mRNA abundance agrees with the results of Hoenig et al. (2006), who reported decreased LPL mRNA and enzyme activity levels in obese versus lean cats. Those researchers proposed that decreased adipose tissue LPL mRNA expression is compensated for by increased LPL expression in skeletal muscle, potentially increasing lipid deposition in skeletal muscle and contributing to insulin resistance. Adipose HSL mRNA was decreased with BW gain, which contradicts obese feline data reported by Hoenig et al. (2006), but is in agreement with Large et al. (2000) who noted decreased HSL mRNA, protein levels, and activity in obese humans. Decreased adipose HSL and LPL mRNA expression suggest a reduced activity of adipocytes with BW gain, yet the signals for such changes remain unknown.

Adiponectin mRNA abundance was decreased with BW gain, a result reported in other species, including humans (Weyer et al., 2001). Circulating adiponectin concentrations have been positively correlated with insulin sensitivity in humans and cats (Weyer et al., 2001; Hoenig et al., 2007). Although circulating adiponectin was not measured in this experiment, tissue mRNA levels suggest the potential for insulin resistance with sustained obesity. Although blood leptin tended to increase with BW gain, as reported in previous feline studies (Appleton et al., 2000), the lack of change in leptin mRNA was surprising. This discrepancy indicates that adipose tissue mRNA expression may not be indicative of plasma leptin concentrations.

Does Spaying Alter "Set Point"?

Many of the observed changes in this experiment suggest an altered "set-point" BW and metabolic activity within tissues with spaying. A "sliding set point" has been reported in seasonal animals that is dependent on photoperiod (reviewed by Rousseau et al., 2003), but has not been suggested with estrogen withdrawal. Several outcomes of this experiment, however, suggest the possibility of such a response. Briefly, adipose leptin mRNA was dramatically decreased after spaying and food restriction. Even though circulating leptin concentration was not different after 12 wk, this tissue-specific response justifies further study. Adiponectin and HSL mRNA concentrations were numerically decreased as well. Decreased adipose LPL mRNA and increased IL-6 mRNA without change in BW suggest a regulatory capacity of estrogen in these systems as well. Further support comes from the lack of expected responses to extreme BW gain during ad libitum feeding. Despite doubling fat mass from wk 12 to 24, leptin and TNF-α mRNA levels were not different from baseline as has been demonstrated in previous feline studies (Hoenig et al., 2006), and mRNA of LPL and HSL continued to be significantly decreased. Given the number of unexpected outcomes and the inability to measure cause-and-effect with such an experiment, further research is necessary to clearly identify the role of estrogen in these biological pathways and determine whether dietary intervention (e.g., those containing estrogenic compounds) may successfully minimize these changes and avoid BW gain following ovariohysterectomy.

We have presented several novel findings in food-restricted cats to maintain BW following ovariohysterectomy. During this period, voluntary physical activity was dramatically reduced, especially during the dark period. Moreover, our data suggest that estrogen withdrawal and food restriction affect the expression of several genes associated with lipid metabolism and inflammation, namely LPL, leptin, and IL-6, that may contribute to changes in appetite, blood lipid concentrations, and insulin sensitivity. Although further research is required for validation, these changes suggest that the set point of a cat may be adjusted by gonadectomy, leading to increased food intake and the physiologic outcomes that follow. By identifying changes in gene expression and physical activity of estrogen withdrawal and food restriction, potential targets for nutritional intervention or lifestyle management have been identified.

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WILD HORSES OF THE GREAT BASIN

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Abstract

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Short-term effect of ovariectomy on measures of insulin sensitivity and response to dexamethasone administration in horses

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Objective—To evaluate the effect of ovariectomy on insulin sensitivity in horses and determine whether the effects of suppression of the hypothalamo-pituitary-adrenal axis differ before and after ovariectomy.

Animals—6 healthy mares.

Procedures—The horses underwent an IV glucose tolerance test (IVGTT), an insulin sensitivity test, and a dexamethasone suppression test before and 5 weeks after ovariectomy. Body weight, serum cortisol and plasma ACTH concentrations, serum insulin-to-blood glucose concentration ratios, and changes in blood glucose concentration with time after injection of glucose or insulin were compared before and after ovariectomy.

Results—The dexamethasone injection resulted in a decrease in serum cortisol concentration before and after ovariectomy. In all horses, baseline plasma ACTH concentrations were within the reference range before and after ovariectomy. For each mare, results of an IVGTT before and after ovariectomy were considered normal. No significant differences in basal blood glucose concentration or time to reach baseline glucose concentration after an IVGTT were observed. Basal serum insulin concentration and serum insulin-to-blood glucose concentration ratios were not significantly different before or after ovariectomy, nor was the mean time to attain a 50% decrease in blood glucose concentration after insulin injection.

Conclusions and Clinical Relevance—Results indicated that ovariectomy does not appear to modify dexamethasone response in horses and that it does not modify short-term measures of insulin sensitivity. Findings suggested that horses undergoing ovariectomy are not at higher risk of developing equine metabolic syndrome or hypothalamo-pituitary-adrenal axis dysfunction and associated morbidity.

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Transcervical hysteroscopic sterilization using cyanoacrylate: A long-term experimental study on sheep

Sergio Bigolin, Djalma José Fagundes, Henri Chaplin Rivoire, Anna Tereza Negrini Fagundes, Anna Luiza Negrini Fagundes

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Abstract

Aim: We investigated the transcervical hysteroscopy application of n-butyl-2-cyanoacrylate in the uterine tube lumen of a non-rodent animals (sheep) with fallopian tube dimensions similar those in humans.

Methods: Standard hysteroscopic procedures were performed on female Texel sheep (n = 26). The right and left ostia were identified. For each ewe, a urethral catheter (5Fr) was used for the delivery of 0.5 mL of saline or an equal volume of n-butyl-2-cyanoacrylate into the uterine tube. Following the procedure, ewes were housed with males of proven fertility for 90 days (equivalent to 5.5 estrous cycles). Postmortem (dye and burst pressure) and *in vivo* (hysterosalpingogram) testing for tube patency were both performed 90 days and 180 days following the procedure.

Results: All animals receiving the saline treatment became pregnant. Gross inspection of uterine tubes following n-butyl-2-cyanoacrylate treatment revealed no visceral adhesions or fibrosis. However, postmortem testing revealed total obstruction within the fallopian tubes. This was confirmed by hysterosalpingogram, in that iodine contrast did not escape into the abdominal cavity.

Conclusion: The cyanoacrylate promoted a reliable fallopian tube obstruction without fibrosis in an animal model exhibiting a similar tube diameter to that found in women. The technique can be evaluated for efficacy *in vivo* using hysterosalpingography.

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Effects of Neodymium:Yttrium Aluminum Garnet Laser Irradiation on Endometrium and on Endometrial Cysts in Six Mares

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Effects of neodymium:yttrium aluminum garnet (Nd:YAG) laser irradiation on equine endometrium were evaluated *in vitro* and in six mares with endometrial cysts. The Nd:YAG laser was applied to six endometrial sites, in each of five uterine specimens, with power densities of 5659 to 33,954 J/cm^2. Depth of tissue ablation was measured and graded on histologic sections of the tissue lesions. Power density had a significant effect on the depth of tissue ablation ($p < .001$). Grade 3 lesions (full-thickness ablation of the endometrium) were created with energy densities of 16,977 to 33,954 J/cm^2. Six mares had endometrial cysts treated by photoablation. Two of the four mares that were reproductively sound but barren, despite appropriate breeding, produced foals after treatment. One mare remained reproductively unsound after treatment, and another mare that was treated postpartum was bred successfully.

ENDOMETRIAL CYSTS CAN cause conception failure and abortion in broodmares by interfering with implantation or movement of the conceptus.[1-5] There are two forms of endometrial cysts: glandular and lymphatic.[1-6] Glandular cysts are small (several mm in diameter)[1-3,5] and may be associated with periglandular fibrosis. Glandular cysts are normal during pregnancy.[1,5,7] Lymphatic cysts are larger (1 to 20 cm in diameter)[2] and are usually found in multiparous mares older than 10 years of age.[1,4,5] Lymphatic cysts may be associated with a normal uterus or with chronic inflammation of the uterus.[1,4,5] Diagnosis of uterine cysts requires ultrasonography or endoscopy, although large cysts can be palpated per rectum.[1-5] Reported methods of treatment include electrocoagulation,[1] puncture by biopsy instrument during endoscopy,[2,4] intrauterine surgery,[1,4] and intrauterine saline infusion.[1] Endometrial cysts often recur after treatment because of failure to remove the entire cyst.[1] Complete cyst removal can be achieved by electrocoagulative and surgical techniques. These techniques are invasive, however, and technically difficult.[1] Laser treatment of equine endometrial cysts has not been reported.

The objectives of this study were: to determine the gross and histopathologic effects of neodymium:yttrium aluminum garnet (Nd:YAG) laser irradiation on uterine tissues *in vitro,* to determine the depth of tissue ablation that occurs with various power densities, and to report the use of Nd:YAG laser for the treatment of endometrial cysts in mares.

Materials and Methods

Laser

A Nd:YAG laser with a 4 m long, 2.1 mm diameter coaxial transmitting fiber was used.* The laser beam was transmitted through a 600 μm diameter fiber made of glass that was surrounded by a polyethylene tube. Carbon dioxide flowed continuously through the polyethylene tube to cool the glass fiber during activation of the laser.

Five uteri were obtained, refrigerated, and used within 24 hours. Incisions were made through the dorsal wall of

* Fiberlase 100 medical laser system, Living Technology Inc., Warminster, Pennsylvania.

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the cervix, the uterine body, and the uterine horns to expose the endometrial surface. Six sites, each separated by 1.5 cm, were selected in either the left or right uterine horn and the uterine body. In each uterus, the laser fiber was positioned perpendicular and 1 cm from the endometrial surface, giving an effective spot size of 1.5 mm diameter. A power setting of 100 watts (W) in a continuous mode was used. Exposure times, in 1 second increments, ranging from 1 to 6 seconds, were randomly selected to yield a range of power outputs from 100 to 600 joules (J). The power density (J/cm^2) applied to each site was calculated.

Each irradiated site was dissected from the uterus, pinned to a wooden tongue depressor, and fixed in 10% neutral buffered formalin. The lesion was visible grossly as a focal depigmented region or as a focal area of tissue ablation (laser crater). Fixed tissues then were sectioned with a razor blade through the center of the lesion and parallel to the plane of laser application. Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Thermal injury, determined by evidence of protein coagulation, charring, cavitation, and crater formation on light microscopy, was recorded. Lesions were graded as follows: grade 1, no ablation of the endometrial tissue but evidence of thermal injury to the endometrium; grade 2, ablation of the endometrial tissue (crater formation evident within the endometrium) with evidence of thermal injury to the myometrium; grade 3, ablation of endometrial and myometrial tissue (crater formation evident within the endometrium and myometrium) with evidence of thermal injury extending no further than the myometrium; grade 4, as for grade 3 except that thermal injury extended to the serosal surface.

The depth of the crater, when present, was measured using a micrometer and 4× objective on a microscope. Measurements were made from the edge of the crater to its deepest level, perpendicular to the endometrial surface. Two-way analysis of variance was used to compare the depth of tissue ablation (crater) at various energy densities and between uteri. Differences were considered significant at $p < .05$. Linear regression analysis of the depth of tissue ablation on power density was performed, and the correlation coefficient was calculated. The correlation coefficient was considered significant at $p < .05$.

Clinical cases. The medical records of mares presented for laser treatment of endometrial cysts between 1983 and 1991 were retrieved. The signalment, breeding history, method of detection of the uterine cysts (ultrasonographic or endoscopic examination), and the number, size, and location of cysts were recorded. Follow-up information on postoperative complications and reproductive performance was obtained from the owner or referring veterinarian.

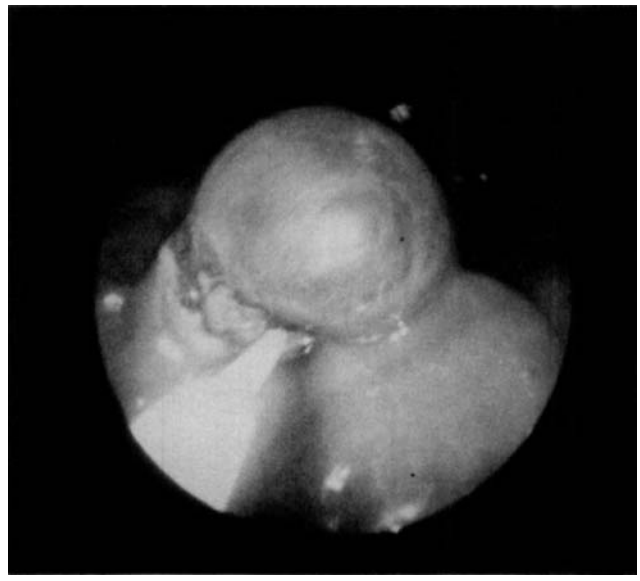

Fig. 1. Endoscopic appearance of an endometrial cyst. Nd:YAG laser (lower left) is being applied to the base of the cyst during initial blanching of the cyst membrane.

Surgical Technique

Each mare was positioned in a stocks and administered xylazine (0.3 to 0.5 mg/kg, intravenously [IV]) and butorphanol (0.02 mg/kg, IV) for sedation and analgesia. The vulva was prepared with a surgical scrub.† A 1 m flexible endoscope‡ with 2 mm and 3.4 mm biopsy channels was selected for passage of the laser fiber. Cold sterilization§ of the endoscope was performed before each use. A video camera and color monitor were coupled to the endoscope, which then was inserted into the uterus. The laser fiber was passed through the 2 mm biopsy channel until 1 to 2 cm of the fiber was visible on the monitor. The uterus then was inflated with CO_2 through the polyethylene tubing surrounding the laser fiber.

Photoablation of endometrial cysts was performed in two stages, with the laser fiber positioned 1 cm from the cyst surface. Initially, the power was set at 50 W in a continuous mode. Cysts were irradiated until the entire visible surface of the cyst was blanched (Fig. 1). Then the laser was set at 100 W in a continuous mode, and the cyst membranes were punctured. After evacuation of cyst fluid, the remaining endometrial cyst membranes were photoablated. Smoke was evacuated with a motorized, mechanical filtering device‖ attached to the 3.4 mm biopsy channel of the endoscope. Power expended for treatment

† Betadine Scrub, Purdue Frederick Co., Norwalk, Connecticut.

‡ GIF 2T10, Olympus Corp., Medical Instrument Division, Lake Success, New York.

§ Cidex, Surgikos Inc., Arlington, Texas.

‖ Biovac-LFA, Stackhouse Associates, Manhattan Beach, California.

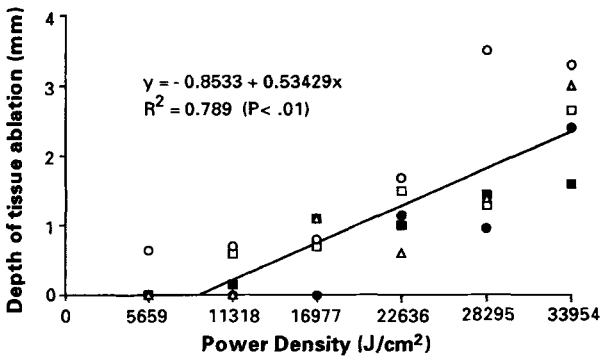

Fig. 2. Graphic representation of the regression analysis of depth of tissue ablation (mm) on power density (J/cm^2). Each uterus is represented by a different symbol.

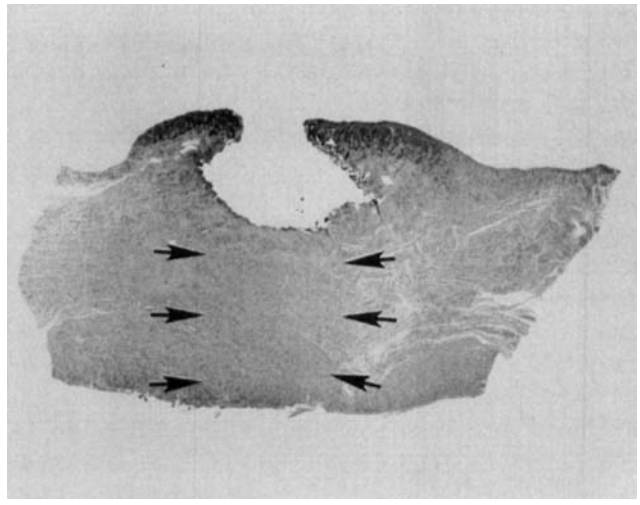

Fig. 3. Histologic appearance of the lesion created in uterus 5 with an Nd:YAG energy density of 33954 J/cm^2. Laser irradiation has created a crater in the endometrium and myometrium with thermal damage (arrows) extending through the myometrium. (Grade 4 lesion) (H&E, ×4).

of each mare's uterus was recorded in joules, and power density was calculated. After operation, each mare was treated with procaine penicillin G (22,000 IU/kg, intramuscularly, every 12 hours) for 3 days and flunixin meglumine (1.1 mg/kg, IV) once.

Results

In Vitro Laser Application

The relationship between depth of penetration and power density is shown (Fig. 2). The grades of laser lesions produced by different power densities (J/cm^2) are recorded in Table 1. Two-way analysis of variance showed that power density had a significant effect on depth of tissue ablation ($p < .001$). In addition, there was a significant difference between the five uteri in the depth of tissue ablation obtained at the different power densities ($p < .05$).

TABLE 1. *Relationship Between Power Density (J/cm^2) and the Histologic Grade of the Lesion Produced in Five Equine Uterine Specimens*

	Energy Density (J/cm^2)					
Uterus	5659	11318	16977	22636	28295	33954
1	1	1	1	2	2	3
2	2	2	2	3	4	4
3	1	2	3	3	3	4
4	1	2	3	3	3	4
5	1	1	3	3	3	4

Lesion grade: 1 = no ablation of the endometrial tissue, but thermal injury to the endometrium; 2 = ablation of the endometrial tissue, with thermal injury to the myometrium; 3 = ablation of endometrial and myometrial tissue, with thermal injury extending no further than the myometrium; 4 = as for grade 3, except that thermal injury extended to the serosal surface.

Microscopic examination of uterine tissues showed mild autolysis, as evidenced by epithelial sloughing. Laser irradiation caused extensive thermal injury at all power densities, including tissue sections with no evidence of tissue ablation. In uterine tissues exposed to energy densities of ≥5659 J/cm^2, a laser crater was identified (Fig. 3) with evidence of thermal injury at the periphery of the crater (Fig. 4).

Clinical Cases Six mares (Table 2) had endometrial cysts treated by Nd:YAG photoablation. Based on the

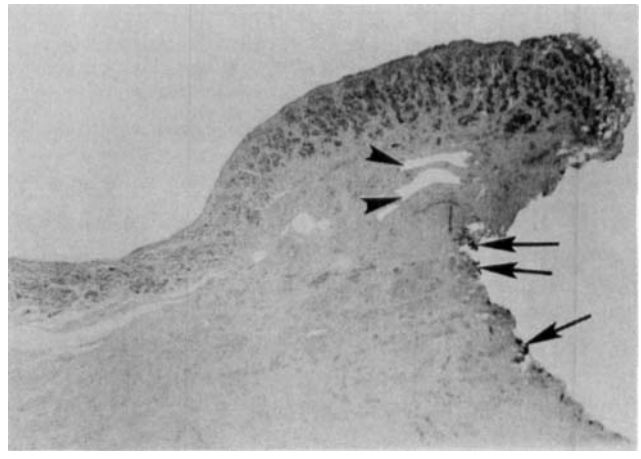

Fig. 4. Histologic appearance of the left margin of the crater in Fig. 3. Carbonization (black arrows) and cavitation (black arrowheads) are evident at the periphery of the laser crater. Tissue detail is indistinct because of protein coagulation. (H&E, ×100).

size of the cysts (0.5 to 5.5 cm diameter), and the age of the mares, the cysts were most likely lymphatic in origin.[4,5] Mares 1 through 4 were admitted for treatment of uterine cysts because of reproductive failure, despite normal cyclic activity, a uterine biopsy of grade 2 or less (Kenney system),[7] and no evidence of endometritis. For mares 1 and 2, successful conception had been determined by ultrasound examination by the referring veterinarian. Abortion or resorption of the conceptus was assumed when the mares returned to estrus. In mare 1, implantation of the conceptus adjacent to a uterine cyst was detected on ultrasound on several occasions. Mare 5 had a grade 3 uterine biopsy and chronic endometritis. *Streptococcus* spp. and fungal organisms were cultured from uterine swabs. Endometrial curettage was performed twice in an attempt to reduce infection and remove the endometrial cysts. Uterine cysts were diagnosed in mare 6 during the previous breeding season. These cysts were observed again on a postpartum ultrasound examination and had increased in size. Prophylactic removal of the endometrial cysts was recommended before breeding.

In mares 1 through 4, all uterine cysts were completely ablated. Mare 5 required two laser treatments. On the first treatment, three cysts were photoablated, but the remaining cysts could not be seem because of uterine fluid present at the time of examination. During the second treatment, three months later, small scars from the previous surgery were noted on endoscopic examination, and the remaining cysts were photoablated. In mare 6, the two largest cysts were photoablated, but the uterus could not be completely examined because of uterine fluid present at the time of examination. Two small cysts (< 1 cm) detected with ultrasonography on a subsequent examination were left untreated. No postoperative complications occurred in any of these mares.

Mare 1 produced a live foal the next season and was in foal at the time of follow-up. Mare 2 was bred during the next season, but continued to abort or resorb the conceptus, and was eventually euthanatized because of reproductive failure. Mare 3 was not bred until the second breeding season after treatment, and during the subsequent 3 years, she produced three live foals and was in foal at the time of follow-up. Mare 4 was examined endoscopically at 3 and 6 weeks after laser treatment. On both occasions, a small scar was evident at the site of the largest cyst. Despite multiple attempts, this mare did not produce a foal. Mare 5 continued to have endometritis and was used as an embryo donor for 2 years, after which she was euthanatized because of chronic laminitis. Mare 6 was bred one month after laser treatment, and produced a live foal but was not rebred.

Discussion

The Nd:YAG laser has a wavelength of 1.06 μm (near infrared spectrum) that is primarily absorbed by protein.[8] When applied to tissues, extensive penetration, reflection, and scattering of the radiation occurs, resulting in latent

TABLE 2. *Clinical Details, Date of Admission, Laser Power Used, and Outcome of Six Mares Admitted for Laser Treatment of Endometrial Cysts*

Mare	Date of Admission	Age (Years)	Breed	History	Cyst Location	No. of Cysts	Size (cm)	Laser Power (J)	Power Density (J/cm^2)	Outcome
1	12/89	16	TB	Foaled 1989, bred successfully 1989, aborted or resorbed conceptus several times	R & L uterine horns	12	0.5–4	14,500 (all cysts)	820532	Produced one foal (1991); in foal
2	12/89	24	TB	Foaled 1988, bred successfully 1989, aborted or resorbed conceptus twice	Uterine body	1	4	8,247 (all cysts)	466685	Unable to produce foal, euthanatized
3	12/89	12	Arabian	Foaled 1986, unable to conceive	R & L uterine horns, body	7	0.5–5	NR		Produced three foals (1989-91); in foal
4	1/86	19	QH	Foaled 1984, unable to conceive	L uterine horn	Many	0.5–3.5	4,000 (all cysts)	226354	Unable to produce a foal
5	9/86 and 12/86	24	Arabian	Foaled 1984, chronic endometritis, grade-3 uterine biopsy, endometrial curettage performed	R & L uterine horns, body, cervical os	5	1–5.5	NR (all cysts)		Embryo donor (1987–89), euthanatized
6	3/91	15	QH	Foaled 1991, cysts larger on postpartum examination than in 1990	R uterine horn	4	1–4	2,670 (2 cysts)	151091	In foal

TB = Thoroughbred; QH = Quarterhorse; R = right; L = left; NR = not recorded.

thermal necrosis; thus the Nd:YAG laser is excellent for tissue photoablation. This effect is maximized with high power (up to 100 W), bare fiber applications.[8-10] The Nd:YAG laser beam is conducted through a flexible fiber that can be passed through an endoscope biopsy channel. This allows use of the laser within cavities that can be examined by an endoscope such as the upper respiratory tract and the reproductive tract.[8-11] A bare fiber, high power ND:YAG laser was chosen for the current study because of its ease of use in the reproductive tract in standing horses and its ability to photoablate tissues.

In vitro laser application demonstrated that increasing power density resulted in increased depth of uterine tissue ablation. One hundred watts was chosen as the power setting, because uterine cysts had been successfully ablated at 100 W in clinical cases. A grade 3 lesion was created in each uterus with power densities of 16,977 to 33,954 J/cm^2 (300 to 600 J). Because uterine cysts are superficial endometrial structures,[3-5] ablation of endometrium and myometrium (a grade 3 lesion) would be excessive. A grade 4 lesion was created in four uteri with a power density of 28,295 to 33,954 J/cm^2 (500 to 600 J). *In vivo,* a grade 4 lesion would likely result in uterine perforation because of full-thickness thermal necrosis.[8-11] These experimental observations suggest that during photoablation of endometrial cyst membranes at a power setting of 100 W, power densities of less than 16,977 J/cm^2 should be used during a single application. Cooling of tissue between applications should be allowed to prevent excessive thermal injury. During blanching of the cyst membrane, minimal Nd:YAG laser irradiation reaches the underlying endometrial tissue. After puncture and fluid evacuation of the cyst, however, laser irradiation is absorbed by the endometrium. Our inability to determine the extent of thermal injury during cyst membrane ablation prompted us to perform the *in vitro* study.

Analysis of variance showed a significant difference between depth of tissue ablation for the various laser power densities in different uteri. This suggests that different laser–tissue interactions can be expected for each horse. Reasons for this might include the age and breed of the horse, the stage of the reproductive cycle,[12] and the degree of uterine pathology, for example, the degree of fibrosis. There also may be different laser–tissue interactions between refrigerated uterine tissue and uterine tissue in living horses. Absorption of Nd:YAG laser irradiation in living tissue would likely be less, because the blood-filled vessels would tend to insulate tissue because Nd:YAG laser irradiation is poorly absorbed by water.

Clinically, the Nd:YAG laser has been shown to be beneficial for treatment of pharyngeal cysts in horses.[8,10] Initial blanching of cysts at a power setting of 50 W ensures absorption of radiation by protein within the cystic fluid without rupturing the cyst. This results in thermal damage to the inner lining, and decreases recurrence.[8,10] The cyst then is punctured to evacuate the fluid, and the cyst lining tissue is then photoablated at 100 W. In the current study, endometrial cysts were photoablated successfully whenever they were adequately visualized. In two mares, some of the cysts were not seen because of intrauterine fluid. Based on these experiences, the authors recommend evacuation of uterine fluid before starting laser treatment. Follow-up endoscopic examination of mares 5 and 6 showed that sites of laser cyst ablation remained as small scars.

Recurrence of endometrial cysts has occurred with techniques that incompletely remove the cystic structure, including puncture by biopsy instrument, needle aspiration, and endometrial curettage.[1,2,4] In mare 5, endometrial curettage had failed to remove the endometrial cysts. Complete removal of cysts has been achieved with intrauterine surgery and electrocoagulative surgery.[1] Previously reported intrauterine surgical techniques required general anesthesia and laparotomy, and electrocoagulative surgery required use of instrumentation that was difficult to manipulate.[1] In six mares where electrocoagulative surgery was used, an 8 cm diameter cyst was too large to remove by this technique.[1] Laser ablation of cysts compares favorably to these techniques because complete cyst destruction is possible, and the technique is easy to perform. Although the largest cyst that was ablated in the current study was 5.5 cm in diameter, there is no indication based on our clinical experience that larger endometrial cysts could not be treated with the Nd:YAG laser. Based on reports of laser treatment of pharyngeal cysts, cyst recurrence is unlikely.[8,10]

Mares 1 through 4 met previous criteria for reproductive failure associated with endometrial cysts.[1] These criteria were failure to conceive despite appropriate breeding, an absence of endometritis, a uterine biopsy grade not higher than grade 2, and the presence of endometrial cysts.[1] In the current report, mare 1 had ultrasonographic evidence of interference between the conceptus and an endometrial cyst. Of these four mares, mares 1 and 3 produced foals, suggesting that photoablation of endometrial cysts can result in successful conception.

Mare 5 had a poor prognosis for future reproductive performance because of evidence of a fungal endometritis and a grade 3 uterine biopsy. This mare was referred specifically for laser treatment of endometrial cysts in the hope that cyst ablation, particularly at the internal cervical os, might enable successful treatment of the endometritis. Mare 6 was treated prophylactically. This mare may have been bred successfully without treatment of cysts. Others have reported that some mares with endometrial cysts can carry pregnancies to term.[1] Therefore, mares probably should meet the criteria described earlier for reproductive failure associated with endometrial cysts before treatment

is considered. Laser treatment of mare 6 did not have a detrimental effect on reproductive performance, and breeding as early as one month after treatment is apparently feasible. Treatment during anestrus is suggested, however, so that lased tissue has time to heal before the breeding season. In addition, inflammation associated with laser treatment should be allowed to subside before breeding.

Laser ablation of endometrial cysts should be performed with a series of low-power density applications ($\leq$11,318 J/cm^2) to prevent excessive ablation of uterine tissue. With appropriate application and case selection, photoablation with a Nd:YAG laser is an effective method of treating endometrial cysts and may result in successful conception. Further studies on Nd:YAG laser interactions with uterine tissues *in vivo* are warranted. Treatment of a large number of mares, with long-term follow-up, is also needed to fully evaluate the efficacy of laser photoablation of endometrial cysts.

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BLM

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NATIONAL SYSTEM OF PUBLIC LANDS

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Cover Photographs – Wild horses in Utah and burros in Nevada.

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ACRONYMS

- ❒ AML – Appropriate Management Level
- ❒ APHIS – Animal Plant Health Inspection Service (Department of Agriculture)
- ❒ AUM – Animal Unit Month
- ❒ AU – Animal Unit
- ❒ BLM – Bureau of Land Management (Department of Interior)
- ❒ CFR – Code of Federal Regulations
- ❒ DNA – Determination of NEPA Adequacy
- ❒ DR – Decision Record
- ❒ EA – Environmental Assessment
- ❒ EIS – Environmental Impact Statement
- ❒ ESI – Ecological Site Inventory
- ❒ FLPMA – Federal Land Policy and Management Act of 1976
- ❒ FONSI – Finding of No Significant Impact
- ❒ HA – Herd Area
- ❒ HMA – Herd Management Area
- ❒ HMAP – Herd Management Area Plan
- ❒ IBLA – Interior Board of Land Appeals
- ❒ IUD – Intrauterine Device
- ❒ LUP – Land Use Plan
- ❒ MFP – Management Framework Plan
- ❒ MLRA – Major Land Resource Area (NRCS Ecological Site Descriptions)
- ❒ MUD – Multiple Use Decision
- ❒ NEPA – National Environmental Policy Act of 1969
- ❒ NPO – National Program Office (Reno, Nevada)
- ❒ NRCS – Natural Resources Conservation Service
- ❒ PZP – Porcine Zona Pellucida
- ❒ RMP – Resource Management Plan
- ❒ ROD – Record of Decision
- ❒ SOPs – Standard Operating Procedures
- ❒ TES – Threatened, Endangered or Sensitive Species
- ❒ TNEB – Thriving Natural Ecological Balance
- ❒ WFRHBA – Wild Free-Roaming Horses and Burros Act of 1971 (as amended)
- ❒ WH&B – Wild Free-Roaming Horses and Burros
- ❒ WHT – U.S. Forest Service Wild Horse Territory
- ❒ WO – Washington Office (Headquarters)
- ❒ WSA – Wilderness Study Area
- ❒ USC – United States Code
- ❒ USFS – United States Forest Service (Department of Agriculture)
- ❒ USGS – United States Geological Service (Department of Interior)

CHAPTER 1—INTRODUCTION

GENERAL

The Bureau of Land Management (BLM) is responsible for the protection, management and control of wild free-roaming horses and burros (WH&B). Under the Wild Free-Roaming Horses and Burros Act (WFRHBA), WH&B are considered an integral part of the national system of public lands in the areas where they were found in 1971. The BLM's goal is to manage healthy WH&B populations on healthy rangelands. To achieve this goal, the BLM designates Herd Management Areas (HMAs) for the long-term maintenance of WH&B herds and collects data about the animals and their habitat. The BLM also prescribes management to assure WH&B populations are in balance with other uses of the public lands and that a thriving natural ecological balance (TNEB) is achieved and maintained. Activities are carried out with the objective of maintaining free-roaming behavior and at the minimum feasible level of management necessary to attain the objectives identified in approved land use plans (LUPs) and Herd Management Area Plans (HMAPs).

1.1 PURPOSE

This handbook describes the authorities, objectives, policies and procedures that guide the management of WH&B on the public lands administered by the BLM.

1.2 OBJECTIVE

The objective of this handbook is to provide guidance for the protection, management and control of WH&B in accordance with the 1971 WFRHBA, as amended, and the implementing regulations in 43 Code of Federal Regulations (CFR) 4700.

CHAPTER 2—LAND USE PLANNING

GENERAL

Section 202(a) of the 1976 Federal Land Policy and Management Act (FLPMA) requires the Secretary to develop, maintain, and when appropriate revise LUPs that provide (by tracts or areas) for the use of the public lands. The responsible BLM official shall follow the established LUP procedures in 43 CFR 1600, associated BLM manual sections and policy for fulfilling the planning requirements prescribed in the statute.

LUPs are the basis for every on-the-ground management decision that the BLM makes. LUPs establish goals and objectives (desired outcomes), identify the management actions needed to achieve the desired outcomes, and identify allowable uses of the public lands.

2.1 LAND USE PLAN DECISIONS

LUP planning requirements for the WH&B program are applicable to all BLM LUP documents, including Management Framework Plans (MFPs) and Resource Management Plans (RMPs).

2.1.1 Comparability Consideration

Under 43 CFR 4700.0-6(b), WH&B shall be considered comparably with other resource values in the formulation of LUPs. This means WH&B are to be considered in the same manner as other resource values (e.g., cultural, historic, scenic, rangelands, timber, and minerals). WH&B are a resource value, as opposed to a land use (e.g., livestock grazing or timber harvest).

2.1.2 Herd Areas

Herd areas (HAs) are limited to areas of the public lands identified as habitat used by WH&B at the time that the WFRHBA passed (December 15, 1971). When preparing a LUP, identify the HAs (in whole or in part) which will not be managed as HMAs and explain the reasons they will not be managed for WH&B.

HA boundaries can be adjusted through a LUP when the current boundary does not correctly portray where WH&B were found in 1971 based on well-documented historical data. Existing herd areas where all animals were privately-owned (claimed during the claiming period) shall be dropped from HA status in a LUP.

Under 43 CFR 4710.2 and to assure transparency regarding the disposition of HAs, the authorized officer shall permanently maintain a record regarding the location of all HA boundaries and explanations of any changes in field office (FO) files.

2.1.3 Herd Management Areas

HMAs shall be designated in those HAs within which WH&B can be maintained over the long term in LUPs. For each HMA designated within the planning area, the LUP should identify the following:

- The HA(s) that contain the HMA.

- The initial and estimated herd size that could be managed while still preserving and maintaining a TNEB and multiple-use relationships for that area.

- The guidelines and criteria for adjusting herd size.

LUPs should also identify:

- The HMAs to be managed for non-reproducing wild horses to aid in controlling on the range population numbers and the criteria for their selection (16 United States Code (USC) § 1333(b)(1)). See Chapter 4 (Population Management). Examples of criteria that could be used to select HMAs for management of non-reproducing wild horses include: no special or unique herd characteristics, low ecologic condition, limited public land water, and reliance on private water.

- The management tools to control population size within AML and to extend (reduce) gather frequency.

2.1.4 Herd Areas Not Designated as HMAs

Where appropriate, the LUP may include decisions not to manage WH&B in all or a part of an HA. An example is intermingled and unfenced private lands within HAs where the landowners are unwilling to make them available for WH&B use, or the animals present at that time were later found to be claimed domestic horses (or burros). Another example would be where essential habitat components (forage, water, cover and space) are unavailable or insufficient to sustain healthy WH&B and healthy rangelands over the long term.

2.1.5 Changes to HA or HMA Boundaries

Decisions to change HA boundaries, to designate HMAs for the maintenance of WH&B, or to remove all or a portion of an area's designation as an HMA must be made through a LUP amendment, revision or new RMP (43 CFR 4710.1 and H-1601-1: Land Use Planning Handbook).

HMA boundaries may be changed within HAs through the LUP process to facilitate WH&B management or mitigate unacceptable impacts to other resources.

An area may lose its designation as an HMA when WH&B cause unacceptable impacts to other resource values, or conditions change and one or more of the four essential habitat components are not present in sufficient quantities to sustain WH&B use over the long term. Similarly, if conditions change, all or part of an HA may be reconsidered for designation as an HMA through LUP. See Chapter 3 (Habitat Management).

2.1.6 Wild Horse and/or Burro Ranges

An HMA may be considered for designation as a WH&B Range to be managed principally, but not necessarily exclusively, for WH&B. Criteria for consideration as a WH&B Range should include the presence of one or more of the following: unique herd characteristics, outstanding viewing opportunities, unique landscape, or significant historical or cultural features (H-1601-1: Appendix D). The authorized officer, currently only the Director or Assistant Director (AD) (refer to BLM Manual 1203: Delegation of Authority), may establish a WH&B Range after a full assessment of the impact on other resources through the LUP process.

2.2 GRAZING AUTHORIZATIONS WITHIN HMAs

Domestic horses and burros may not graze under permits or leases within HMA boundaries (43 CFR 4710.5(b)). If necessary to provide habitat for WH&B, to implement herd management actions, or to protect WH&B from disease, harassment or injury, the authorized officer may close areas of the public lands to grazing use by all or a particular kind of livestock (43 CFR 4710.5(a)).

Closure to livestock grazing may be temporary or permanent (43 CFR 4710.5(c)). Prior to issuing a final decision to permanently close an area to livestock grazing use, a LUP amendment should be completed. Completion of site-specific environmental analysis and issuance of a proposed and final decision pursuant to 43 CFR 4160 (2005) is also required.

2.3 OTHER PERMITTED ACTIVITIES

2.3.1 Commercial Recreation Use

Proposals for commercial recreational use of the public lands should be evaluated through the appropriate BLM permitting process. Permits should stipulate the safeguards necessary to protect the health and welfare of WH&B, particularly before, during and immediately following the peak foaling period (e.g., generally March – June). See Chapter 4 (4.4.4).

Proposals for motor vehicle racing, air racing, or other potentially intrusive activities have potential to harass or harm WH&B. Permit authorizations should contain provisions to minimize impacts to WH&B from these activities. Should adequate safeguards prove impractical, permits should not be approved (refer to BLM Manual Section 2930 and H-2930-1: Recreation Permit Administration).

2.3.2 Energy and Minerals Exploration and Development

The health and welfare of WH&B should be considered during project planning for hard-rock mining or oil and gas exploration and development; non-mineral sales such as gravel; or wind or solar energy development proposals. Specific mitigation measures will be identified through a site-specific environmental analysis and decision process in accordance with the 1969 National Environmental Policy Act (NEPA). Mitigation measures will be implemented through applicable permits.

2.4 LAND USE PLAN GOALS AND OBJECTIVES

LUPs normally contain general habitat and population management goals and objectives. LUPs may also include the management requirements or actions necessary to make progress toward attainment of Land Health Standards and to preserve and maintain a TNEB and multiple-use relationship on the public lands.

2.4.1 Habitat and Population Management

Habitat or population management and monitoring objectives regarding the management of a specific HMA or complex of HMAs are normally identified in a Herd Management Area Plan (HMAP) rather than a LUP.

2.5 IMPLEMENTATION DECISIONS

Implementation decisions make progress toward achieving LUP goals and objectives and may include:

- Establishing or adjusting appropriate management levels (AMLs) based on monitoring and evaluation, including the population range within which the herd size will be allowed to fluctuate.
- Identifying and setting objectives for herd composition, animal characteristics and habitat development needs (e.g., HMAPs).
- Specific habitat improvement projects (e.g., construction, modification, or removal of fences; water development construction/reconstruction or removal; or re-vegetation projects).
- Site-specific population management actions (e.g., decisions to gather/remove excess WH&B, apply fertility control, or adjust age or sex ratios).

2.5.1 Appropriate Management Level (AML) Adjustments

AML may be adjusted (either up or down) through a site-specific environmental analysis and decision process (NEPA). An analysis under NEPA is also required to establish a population range (upper and lower limit) for AMLs initially established as a single number. Development of a LUP amendment or revision is not generally required.

2.5.1.1 AMLs Established in LUPs

- When AML is established in a LUP, follow the process outlined in the LUP to adjust AML.

- When the LUP does not outline a process for AML adjustment, the LUP may need to be amended or revised to adjust AML.

2.5.1.2 AMLs Established through Implementation Decisions

See Chapter 4 (4.2.2).

2.5.2 Herd Management Area Plans

Herd Management Area Plans (HMAPs) are prepared under 43 CFR 4710.3-1. HMAPs establish short- and long-term management and monitoring objectives for a specific WH&B herd and its habitat. HMAPs also identify the actions to be taken to accomplish herd and habitat management objectives. An HMAP assists the authorized officer in tracking progress toward achieving LUP goals.

HMAPs tier to and must be in conformance with the applicable LUP. If the proposed management strategy is not consistent with the LUP, then the LUP should be amended, or the proposal should be modified or rejected.

HMAPs may be prepared for a single HMA or a complex of adjacent HMAs where animal interchange occurs. When two or more jurisdictions have management responsibility for portions of a single HMA or an HMA complex, BLM will designate one field office with the lead responsibility for development of the HMAP and management of the HMA or complex. When a WH&B herd is dependent on the habitat within a BLM HMA and an adjacent U.S. Forest Service (USFS) Wild Horse Territory (WHT), the agencies should jointly determine lead responsibility for development of the HMAP and management of the herd and habitat.

2.6 NOTICES, CONSULTATIONS AND HEARINGS

2.6.1 Agency Consultation and Public Involvement

See BLM Manual Section 4710.25.

2.6.2 Hearings

See BLM Manual Section 4740.3.

CHAPTER 3—HABITAT MANAGEMENT

GENERAL

Habitat for WH&B is composed of four essential components: forage, water, cover, and space. These components must be present within the HMA in sufficient amounts to sustain healthy WH&B populations and healthy rangelands over the long term. If they are not present in sufficient amounts, the authorized officer should consider amending or revising the LUP to remove the area's designation as an HMA. If the decision is made to return a designated HMA to HA status, the total population of WH&B should then be gathered and removed. See BLM Manual Section 4710.3.

A recurring pattern of WH&B movement out of the HMA to access forage, water, or thermal or hiding cover is an indication that year-long WH&B use cannot be sustained. If one or more of the key habitat components is missing, the HMA should be considered as unsuitable for year-long use. In these situations, the authorized officer should consider removing the area's designation as an HMA through LUP. An exception would be two or more HMAs which adjoin and are managed as a complex of HMAs, or HMAs which adjoin USFS WHTs that can be managed as a complex.

3.1 FORAGE (VEGETATION)

Forage (vegetation) is one of the essential components of WH&B habitat. The authorized officer should determine whether vegetation provides sustainable forage (and cover) for the animals. Vegetation should be managed within each HMA in a manner that achieves and maintains a TNEB and assures significant progress is made toward achieving the Standards for Land Health and other site-specific or landscape-level objectives.

3.2 WATER

An adequate year-round quantity and quality of water must be present in the HMA to sustain WH&B numbers within AML. If baseline information concerning access to and availability of water does not exist, then a public land water inventory should be conducted.

If privately-owned water is essential to sustaining WH&B populations within HMAs, cooperative agreements with the owners or acquisition of water rights should be considered. If agreement cannot be achieved with private water owners, or if public land water cannot be developed and maintained, one of the following should occur:

- AML should be adjusted based on the available public land water within the HMA boundary.

- The authorized officer should amend or revise the LUP to remove the area's designation as an HMA.

3.3 COVER (VEGETATION) AND SPACE

The terrain and vegetation are needed to provide WH&B with escape (hiding) cover and shelter from the prevailing weather. (Vegetation also provides sustainable forage. See 3.1 above). WH&B require sufficient space to allow the herd to move freely between water and forage within seasonal habitats. Cover and space are interrelated. If the HMA has barriers preventing free movement of WH&B throughout the HMA or between forage and water, it would not have sufficient cover and space. Barriers can be natural (e.g., rock rims, rivers) or human-induced (e.g., fences, highways).

3.4 HABITAT IMPROVEMENT PROJECTS

3.4.1 Nonstructural Improvements

Nonstructural improvement projects such as seeding, prescribed fire, emergency fire rehabilitation or shrub and tree removal have potential to increase the forage available for WH&B use and to improve habitat conditions. They may also cause grazing animals to concentrate their use within the project area. As a result, it may be necessary to close these areas to grazing until vegetation management objectives are achieved.

Before making the decision to exclude an area from grazing use, the authorized officer should first take a hard look at alternatives to fencing. These alternatives could include:

- Reduce WH&B populations to the AML lower limit.
- Reduce WH&B to a number below the AML lower limit (based on the available forage and water).
- One of the above, in combination with closing the area to domestic livestock use (in whole or in part).
- Use of mineral supplements to modify distribution.
- Restrict or eliminate access to selected water sources.

If the authorized officer determines fence construction is necessary, the fences should be designed to maintain WH&B access to critical water sources. Fences should then be removed once objectives have been achieved.

3.4.2 Structural Improvements

Construct and maintain structural improvement projects (e.g., fences, cattle guards, or water developments) in a manner that protects the wild, free-roaming nature of WH&B and provides for normal herd distribution and movement as well as genetic interchange.

While some projects might increase the forage, cover, or water available for WH&B, others might negatively impact individual animals or entire populations:

- Fences and cattle guards might restrict seasonal WH&B movement or use of critical escape (hiding) or thermal cover, key spring-summer-fall-winter use areas, or critical water sources.

- Water development projects might encourage use outside the HMA or concentrate use in sensitive areas.

Existing projects that negatively impact WH&B should be considered for removal or modification through coordinated activity plans. Proposed projects should be analyzed and mitigation proposed to minimize adverse impacts to WH&B where possible.

3.4.2.1 Existing Fences and Cattle Guards

Existing fences and cattle guards with negative impacts to WH&B should be reviewed to determine:

- If the project is still needed; or
- Whether the project can be modified to minimize impacts to WH&B.

Possible fence or cattle guard modifications could include:

- Make fences open-ended allowing WH&B movement around the ends.
- Construct large gates or sections of "let-down" fence, or consider the use of electric take-down fence where feasible, to allow movement when fences are not needed for livestock control.
- Weld rebar strips between cattle guard grates; or
- Replace the cattle guard with a gate, if possible.

3.4.2.2 Proposed Fences and Cattle Guards

Fencing within an HMA should be done only after the impacts are carefully analyzed through the NEPA process. If fences and cattle guards are essential for proper resource management, they should be constructed with particular attention to location and design. Avoid locating fences across migration routes, to prevent WH&B (or livestock) concentration, or the unintended trapping or death of WH&B.

3.4.1.3 Existing Water Developments

Consistent with resource management objectives, existing projects may be modified to provide WH&B with access to water through one or more of the following methods:

- Piping water to a trough away from the source.
- Piping water to a trough outside an exclosure.
- Retaining a portion of the source outside the exclosure.

3.4.1.4 Proposed Water Developments

Water sources may be excluded from use by WH&B to protect the water source, the associated riparian area, and to maintain or improve the quality and quantity of water. Where possible, projects should be designed to provide WH&B with access to water as described in 3.4.1.3 above.

Fences constructed in proximity to major WH&B water sources and smaller riparian pastures or exclosures should be constructed with a top rail composed of pipe or wooden poles. The top rails provide a visual barrier to prevent WH&B from entering the exclosure and becoming trapped. Project design may also consider the use of specialized gates (e.g., finger gates) to allow any WH&B entering an exclosure to exit safely.

Reservoirs and similar water sources should be designed and maintained with gently sloping (rather than steep) sides to avoid trapping foals.

Where WH&B are, or may become, dependent upon mechanically provided water, the authorized officer shall assure that alternative sources of known and accessible water are available to WH&B in the event of a system failure of a well, storage tank, pipeline, trough, or float device.

Actions to assure water is available to WH&B may include:

- Entering into Cooperative Range Improvement Agreements (Form 4120-6) whose terms and conditions require water to be made available to WH&B and wildlife.

- Using WH&B program funding (in whole or part) for construction and/or maintenance of a well, spring development, catchment, pond, or other permanent water improvement providing WH&B with access to water on public land.

- Acquiring the necessary water rights in order to provide and maintain access to water sources. Refer to BLM Manual 7250 (Water Rights).

CHAPTER 4—POPULATION MANAGEMENT

GENERAL

Under the 1971 WFRHBA, WH&B are to be managed in a manner designed to achieve and maintain a thriving natural ecological balance and protect the range from the deterioration associated with WH&B overpopulation.

4.1 MANAGEMENT REQUIREMENTS

4.1.1 Self-Sustaining

WH&B shall be managed as self-sustaining populations of healthy animals in balance with other uses and the productive capacity of their habitat. Some selected HMAs may be managed for non-reproducing wild horses to aid in controlling on-the-range population numbers (see 4.5.4).

4.1.2 Free-Roaming Behavior

In accordance with 43 CFR 4700.0-6(c), management activities affecting WH&B shall be undertaken with the goal of maintaining free-roaming behavior.

4.1.3 Constraints on Management

Management of WH&B on the public lands is limited to herd areas (HAs), consistent with the WFRHBA (16 USC § 1339) which states: “Nothing in this Act shall be construed to authorize the Secretary to relocate wild free-roaming horses or burros to areas of the public lands where they do not presently exist.”

Consistent with 43 CFR 4710.3-1, herd management areas (HMAs) shall be established for the maintenance of WH&B herds. In delineating each HMA, the authorized officer shall consider the appropriate management level for the herd, the habitat requirements of the animals, and the relationships with other uses of the public and adjacent private lands, and the constraints contained in § 4710.4.

4.1.4 Minimum Feasible Level of Management

As required in 43 CFR 4710.4, management shall be at the minimum level necessary to attain the objectives identified in approved LUPs and HMAPs.

1. Limit population management actions by:

    a. Establishing AML as a population range within which herd size will be allowed to fluctuate.

    b. Evaluating HMAs that require frequent emergency or nuisance removals due to inadequate habitat (e.g., limited availability of forage or water) for the possible removal of the area’s designation as an HMA through LUP.

2. It is not consistent with management at the minimal level to provide supplemental feed or rely on water developments that require frequent maintenance. It may, however, be appropriate to provide water in temporary emergency situations.

4.1.5 Thriving Natural Ecological Balance (TNEB)

Consistent with 43 CFR 4700.0-6, WH&B shall be managed in balance with other uses and the productive capacity of their habitat (i.e., WH&B will be managed to achieve and maintain a thriving natural ecological balance (TNEB) and multiple use relationships on the public lands).

The WFRHBA requires the BLM to manage horses in a manner that is designed to achieve and maintain a thriving natural ecological balance on the public lands (16 USC § 1333(a)). *See also Animal Protection Institute of America*, 109 IBLA 112, 115 (1989) ("…the 'benchmark test' for determining the suitable number of wild horses on the public range is 'thriving natural ecological balance'…") (*Dahl v. Clark*, 600 F. Supp. 585, 594 (D. Nev. 1984)).

To achieve a TNEB on the public lands, WH&B should be managed in a manner that assures significant progress is made toward achieving the Land Health Standards for upland vegetation and riparian plant communities, watershed function, and habitat quality for animal populations, as well as other site-specific or landscape-level objectives, including those necessary to protect and manage Threatened, Endangered, and Sensitive Species (TES). WH&B herd health is promoted by achieving and maintaining TNEB.

4.2 ESTABLISHING THE APPROPRIATE MANAGEMENT LEVEL

4.2.1 AML Definition

The appropriate management level (AML) shall be expressed as a population range within which WH&B can be managed for the long term. AMLs previously established as a single number will be modified to include an upper and lower limit. For reporting purposes, the upper limit of the AML range will be used.

AML applies to the number of adult wild horses or burros to be managed within the population and does not include current year's foals. All WH&B one year of age and older are considered adults (a foal is considered one year of age on January 1 of the year following its birth).

The AML upper limit shall be established as the maximum number of WH&B which results in a TNEB and avoids a deterioration of the range. This number should be below the number that would cause rangeland damage (refer to *Animal Protection Institute of America v. Nevada BLM*, 118 IBLA 63, 75, (1991)).

The AML lower limit shall normally be established at a number that allows the population to grow (at the annual population growth rate) to the upper limit over a 4-5 year period, without any interim gathers to remove excess WH&B. Some HMAs may require more frequent removals to maintain population size within AML. For HMAs that require more frequent gathers, the authorized officer should consider management options which would either extend the gather

cycle or broaden the AML range; amend or revise the LUP to remove the area's designation as an HMA; or manage the HMA for non-reproducing wild horses.

4.2.2 Establishing or Adjusting AML

An interdisciplinary and site-specific environmental analysis and decision process (NEPA) with public involvement is required to establish or adjust AML.

4.2.2.1 Establishing AML

When establishing AML, the analysis shall include an in-depth evaluation of intensive monitoring data or land health assessment. Intensive monitoring data shall include studies of grazing utilization, range ecological condition and trend, actual use, and climate (weather) data. Population inventory, use patterns and animal distribution should also be considered. A minimum of three to five years of data is preferred. Progress toward attainment of other site-specific and landscape-level management objectives should also be considered. See Appendix 3 (AML Establishment and Adjustment).

4.2.2.2 Evaluation and Adjustment of AML

In-depth AML evaluations should be completed when review of resource monitoring and population inventory data indicates the AML may no longer be appropriate. The following should be considered when evaluating AML:

- Changes in environmental conditions which may have occurred since the AML was established. Changing environmental conditions could include drought, wildfires, noxious weed infestations, effect of varying numbers of WH&B on forage utilization or range ecological condition/trend, an increase or decrease in the available forage, changes in livestock management, etc.

- The presence of any newly listed Threatened, Endangered or Sensitive Species (TES).

- Any additional resource monitoring, population inventory or other relevant data collected since AML was established.

For additional information refer to Appendix 3 (AML Establishment and Adjustment) and BLM Manual Section 4720.3.

4.2.3 WH&B Animal Unit and Animal Unit Month

Wild horses, one year of age or older, count as one (1) Animal Unit (AU) and burros one year of age and older count as 0.5 AU. One Animal Unit Month (AUM) is the amount of forage necessary to sustain one adult horse or two adult burros for one month (or approximately 800 pounds of air dried forage).

4.2.4 Forage Allocations for WHB

The amount of forage available to allocate to WH&B shall be determined through in-depth evaluation of resource monitoring data and following a site-specific environmental analysis and decision process. Forage for WH&B (AUMs) is allocated based on the AML upper limit.

4.3 DETERMINATION OF EXCESS

Before issuing a decision to gather and remove animals, the authorized officer shall first determine whether excess WH&B are present and require immediate removal. In making this determination, the authorized officer shall analyze grazing utilization and distribution, trend in range ecological condition, actual use, climate (weather) data, current population inventory, wild horses and burros located outside the HMA in areas not designated for their long-term maintenance and other factors such as the results of land health assessments which demonstrate removal is needed to restore or maintain the range in a TNEB.

The term "excess animals" is defined as those animals which must be removed from an area in order to preserve and maintain a thriving natural ecological balance and multiple-use relationship in that area (16 USC § 1332(f)(2)). This definition underscores the need to remove excess animals before damage to the range begins to occur.

4.4 PARAMETERS FOR POPULATION MANAGEMENT

4.4.1 Age Structure and Sex Ratio

Age structure and sex ratio influence herd health, social interactions, and population growth rates of reproducing WH&B herds. A herd's age structure and sex ratio may have been altered by previous selective removal policies or natural mortality and should be estimated from the best available data (e.g., capture records, preparation records, and final gather reports). Most populations will have representatives from each age class, and rapidly growing populations will have a greater proportion of younger WH&B. WH&B populations will produce roughly equal numbers of males and females over time.

In order to maintain the desired age structure and sex ratios, consider retaining male and female animals from each age group (0-4, 5-9, 10-15, 15+ years of age) following a removal operation. If specific age structure and sex ratio objectives are not established for an HMA or complex of HMAs, a desired post-gather age structure and sex ratio should be established during the pre-gather planning and analysis process. These objectives should be consistent with the selective removal criteria outlined in BLM Manual Section 4720.33 and the AML lower limit.

The authorized officer should consider alternatives which would manage WH&B herds for a sex ratio with a female component of less than or equal to 50 percent, to reduce population growth rates and extend the gather cycle. Opportunities to manage sex ratios as a tool to slow population growth rates are discussed later in this chapter.

4.4.2 Selective Removal

Selective removal and release of animals back to the range should be conducted to achieve AML, and the age structure and sex ratio objectives established for the specific HMA or complex of HMAs. In the absence of specific age structure or sex ratio objectives for the HMA or complex, see BLM Manual Section 4720.33.

4.4.3 Population Growth Rates

Population growth rates represent the net difference between births and deaths over time. Population growth rates can be highly variable and influenced by habitat quality, forage production, water availability and other factors. Data from recent gathers and population estimates derived from aerial surveys are used to determine population growth rates. The annual growth rate from Year 1 to Year 2 is calculated as follows:

Population on 2/28 (Year 2) – Population on 2/28 (Year 1)
÷ Population on 2/28 (Year 1) ×100 = Percent Annual Growth Rate

The same formula can be used to calculate growth over a period of several years (e.g., between aerial population surveys which are normally conducted at 3-4 year intervals).

Significant variation from historical growth rates may indicate that either immigration into the HMA or migration out of the HMA is occurring.

4.4.4 Foaling Period

Foaling period can be documented for each population (individual HMA or HMA complex) through direct observation. The capture of wild horses by using a helicopter to herd the animals is prohibited during the foaling period, which is defined as six weeks on either side of the peak of foaling to assure that young foals are mature enough to be able to remain with their band during gather activities. This period is generally March 1 to June 30 for most wild horse herds. Helicopters may be used year-round in the removal of burros. See BLM Manual Section 4740.1.

The capture of wild horses and burros by using bait (e.g., food, water, salt or sexual attraction) to lure animals into a trap may be used year-round.

Population surveys or distribution flights involving fixed-wing aircraft may be conducted during the foaling period. The use of helicopters for these activities during this time should be evaluated on a case-by-case basis.

4.4.5 Gathers and Gather Cycles

The gather cycle is the interval between gathers. Gather frequency is a function of the AML range, population growth rates and other environmental factors. Once AML is achieved, gathers to remove excess WH&B should be planned to occur at intervals of approximately 4 to 5 years to maintain population size within AML.

4.4.6 Genetic Diversity

Reproducing WH&B herd health is dependent, in part, on maintaining desirable genetic diversity (avoiding inbreeding depression).

4.4.6.1 Baseline Genetic Diversity

Baseline genetic diversity will be determined for all WH&B herds. Once a baseline is established, additional samples will be collected to reassess genetic diversity every other gather (e.g., every 6-10 years). If testing indicates diversity is less than desired, the herd should be reassessed more frequently (e.g., every gather).

In the past, genetic diversity was assessed by evaluating blood samples from the herd. Hair samples are now used to assess genetic diversity. Procedures for collecting and processing WH&B hair samples are described in Appendix 1 (Genetics Data and Hair Sample Collection Instructions). A report assessing genetic diversity is developed for each set of samples from an HMA.

Baseline data can be compared between adjacent HMAs to determine if the WH&B herd is isolated, or if genetic material is being exchanged between reproducing herds as part of a larger population of WH&B. Movement of WH&B from one HMA to another may enhance genetic diversity.

4.4.6.2 Interpreting Genetics Data

Measures of individual animal and population-wide genetic diversity are based on the number of individuals sampled within each HMA or complex. The most important measure of genetic diversity is Observed Heterozygosity (Ho). The observed heterozygosity is a measure of how much diversity is found, on average, within individual animals in a WH&B herd and is insensitive to sample size, although the larger the sample, the more robust the estimate.

Population-wide measures for the presence of genetic variants and the effective number of alleles provide information about changes in a herd's diversity, now and in the future. Other measures provide a comparison of the herd's genetic similarity to domestic horse breed types. All measures are compared to values derived from domestic horse breeds and to WH&B herds which have already been sampled.

Values below the mean for feral populations are an indication that the WH&B herd may have diversity issues. Herds with observed heterozygosity values that are one standard deviation below the mean are considered at critical risk. For DNA-based (hair) samples this value is 0.66, and the value is 0.31 for blood samples.

Few herds sampled to date show values below the feral mean. Those with values below the feral mean tend to be herds with small population size, which are the most at-risk for inbreeding. There is no specific diversity value that is a clear indicator of imminent inbreeding risk. The lower the genetic variation, the greater the risks for inbreeding-related impacts to the WH&B

herd. Mitigation to address or resolve genetic diversity concerns should be considered using the recommendations in the genetics report as a starting point.

4.4.6.3 Herd Size

A minimum population size of 50 effective breeding animals (i.e., a total population size of about 150-200 animals) is currently recommended to maintain an acceptable level of genetic diversity within reproducing WH&B populations (Cothran, 2009). This number is required to keep the rate of loss of genetic variation at 1 percent per generation. Animal interchange between adjacent HMAs with smaller population sizes may reduce the need for maintaining populations of this size within each individual HMA. Research has not yet established a recommended minimum breeding herd size for burros.

4.4.6.4 Management Actions

If the recommended minimum wild horse herd size cannot be maintained due to habitat limitations (e.g., insufficient forage, water, cover and/or space) or other resource management considerations (e.g., T&E species), a number of options may be considered as part of an appropriate site-specific NEPA analysis to mitigate genetic concerns:

- Maximize the number of breeding age wild horses (6-10 years) within the herd.
- Adjust the sex ratio in favor of males to increase the number of harems and effective breeding males.
- Introduce 1-2 young mares every generation (about 10 years), from other herds living in similar environments.

If wild horse herd size in small, isolated HMAs is so low that mitigation is not feasible, consideration should be given to managing the HMA for non-reproducing wild horses or to removing the area's designation as an HMA through LUP.

4.4.7 Herd Social Structure

Some management actions (e.g., sex ratio adjustments to favor stallions) may affect social structure and herd interactions (e.g., band size).

4.4.7.1 Wild Horses

The social structure of most wild horse herds consists of breeding and bachelor bands. Breeding bands or harems usually consist of a dominant stallion, lead or dominant mare(s), a group of breeding mares, and associated foals and yearlings. Most wild horses breed and foal in the spring of the year. Bachelor bands consist of various aged males that either have not yet established their own harem or have lost their mares. The composition of bachelor bands varies considerably throughout the year. In most breeding bands, male foals leave the band at 1 or 2 years of age and generally join bachelor bands.

Although wild horse bands tend to use the same habitat areas (home ranges) from year to year, they are not territorial and do not defend preferred habitat areas. As a result, bands often graze

and water near each other, and there may be movement of mares between bands. This, in combination with the periodic displacement of the dominant stallion and removal or death of other horses, results in a very fluid social structure in most herds. These factors are beneficial in enhancing genetic diversity.

4.4.7.2 Wild Burros

Wild burros do not form breeding bands. There are no strong individual bonds other than jenny-foal relationships. Wild burros present themselves as single animals, all-male groups, all-female groups, jenny-foal groups, or mixed groups. All of the groups are variable and their composition may change at any time. This loose social structure, where all animals are potential breeding partners, maximizes genetic diversity in small or dispersed burro populations.

Some of the older jacks establish a breeding territory but do not prevent other males from entering this area unless there is an estrous female present. It is common for males to roam freely throughout their habitat and breed upon encountering an estrous female. Large male groups may form in the vicinity of an estrous female, and it is normal for the jenny to have multiple breeding partners.

In dispersed populations in a desert environment, breeding efficiency increases as the population densities increase. As daily temperatures increase and water availability decreases, more and more animals will gather around the remaining available water sources. These areas become important areas for maximizing breeding efficiency. This temporary or seasonal increase in population density increases the chance for males to encounter estrous females. Thus, although breeding occurs year-round, increased breeding and foaling may occur during this period of time.

4.5 POPULATION CONTROLS

The authorized officer may consider a number of population control methods to achieve and maintain WH&B population size within AML.

4.5.1 Natural Population Controls

The rate at which WH&B herd sizes naturally increase or decrease is affected by a number of factors, including the nutritional value of the forage consumed, weather, disease, and predation. Experience gained in managing WH&B and other large herbivores indicates that soil, vegetation, and water resources are almost always severely damaged before these factors negatively affect population growth rates. Human intervention may be necessary when these factors, acting alone or in combination, do not exert sufficient influence to maintain WH&B population size within AML.

4.5.2 Gather and Removal

When the authorized officer has determined that excess WH&B exist, gathers to capture and remove the animals immediately or as soon as possible are required. For additional information, refer to BLM Manual Section 4720 (Removal) and 43 CFR 4720.1, 4740.1 and 2.

4.5.3 Reduce Population Growth Rates

During gather or herd management area planning, the authorized officer should consider a range of alternatives to reduce population growth rates and extend the gather cycle for all wild horse herds with annual growth rates greater than or equal to 5 percent. Alternatives may include but are not limited to: use of fertility control, adjustments in the sex ratio in favor of males, a combination of fertility control and sex ratio adjustment, and management of selected HMAs for non-reproducing wild horses. Additional management alternatives (tools) may be considered in the future, pending further research (see Chapter 8).

4.5.3.1 Use of Fertility Control as a Tool to Slow Population Growth Rates

a. Use of Porcine Zona Pellucida (PZP). There are two forms of the conventional PZP agent:

- The 1-year agent, delivered as a liquid primer injection and follow-up booster one month later; additional boosters must be injected annually by hand or by darting to continue treatment.

- The 22-month agent that includes the same primer shot as the one-year agent as well as a second injection of three time-release pellets (1-, 3- and 12-month pellets) to booster the vaccine over a 12-month period of time.

Foaling rates of 6 percent in Year One, 14 percent in Year Two and 32 percent in Year Three following treatment compared with 54 percent foaling in untreated controls have been reported for PZP-22 (Turner, 2007). Maximum effectiveness is not achieved unless mares are treated during a 3-4 month window prior to foaling. Research has shown that the best time to apply PZP is during the winter gather season (i.e., November-February).

The use of PZP, under an investigational exemption held by the Humane Society of the United States, requires treated mares to be physically marked (freeze branded) or readily identifiable in order to be compliant with FDA requirements. Application is limited to individuals specifically trained to handle, mix, and administer the product. Post-treatment monitoring in accordance with the Standard Operating Procedures (SOPs) is required.

As part of an appropriate environmental analysis, the authorized officer will analyze the use of the 22-month PZP vaccine in all wild horse herds, particularly those where: (1) the annual herd growth rate is greater than 5 percent and (2) the post-gather herd size is 50 animals or greater. Fertility control will be most effective when treatment of 50-90 percent of all breeding-age mares within the herd is possible using application in conjunction with gathers or remote delivery (darting). Our current understanding is that to maximize treatment effects, at least 90 percent of all mares should be treated.

The authorized officer should apply the 22-month PZP vaccine to all release mares when the NEPA analysis supports its use. In herds where sex ratio adjustments are made, fertility control may be implemented in combination with sex ratio adjustments to further reduce population growth rates.

b. Increased Use of PZP. One option to slow population growth rates (and reduce the number of excess wild horses removed) would be to gather selected HMAs and apply PZP-22 every 2 years. Because PZP does not totally eliminate reproduction, some excess horses may need to be removed from treated herds over time. Implementation should generally be limited to HMAs that have had high gather efficiencies (i.e., a high percentage of the actual population is captured). Because 70-90 percent of the breeding-age mares may need to be treated to effectively reduce population growth rates, 80-100 percent of the actual population may need to be captured.

c. Remote Application of PZP. Remote application of the 1-year formulation of PZP is problematic, as it is very difficult to approach most wild horses closely enough to allow darting (i.e., follow-up treatment). Remote application of PZP-22 is not possible at the present time since the pellets must be administered by hand injection.

   Remote application of the 1-year PZP agent may be considered for herds where individual horses can be identified (consistent with FDA requirements) and are approachable. Remote application is limited to individuals specifically trained to administer the product by darting.

4.5.3.2 Adjust Male/Female Sex Ratios

The authorized officer should consider alternatives which would manage WH&B herds for a sex ratio with a female component of less than or equal to 50 percent, as this reduces the population growth rate and extends the gather cycle. See Chapter 4 (4.4.1).

Adjusting sex ratios to favor males is another possible management tool which should be considered when the suppression of herd growth rate is desired. This management option should be considered in HMAs and complexes where the low end of AML is greater than 150 animals. Implementation of sex ratio adjustments is most feasible during maintenance gathers (4-5 years after AML is achieved). Sex ratio adjustments may be accomplished by shifting the overall sex ratio to favor males by (1) releasing greater numbers of stallions post-gather or (2) releasing geldings back to their home range following castration. Adjusting the sex ratio so that males comprise 60-70 percent of the adult herd could be considered.

Herd dynamics may change somewhat with adjustments in sex ratios. An increase in the proportion of stallions may have a greater impact when water resources are limited and bands are more concentrated.

Though additional information is needed, geldings are likely to have fewer impacts on the herd's social structure than would an increase in the proportion of stallions. Based on anecdotal observations, geldings released back to their home range: (1) tend to remain near where they were released (with adequate forage and water), (2) form small bachelor groups rather than join

with a reproducing band, (3) maintain better body condition than the herd average because they are sexually inactive, (4) live longer in comparison to sexually active horses, and (5) were easy to recapture (many have been recaptured and released several times).

Post-treatment monitoring should be conducted to observe behavior of individual animals and the herd during the first breeding season following treatment. Monitoring should be designed to determine whether bachelor stallions or geldings interfere with breeding harems, and whether there is increased competition for forage or water. Monitoring should also be designed to determine if the bachelor geldings form bands or intermix with the breeding population and whether band size changes with a greater proportion of stallions. This monitoring information will be used to determine if sex ratio adjustment is an effective population management technique that should be continued.

If post-treatment monitoring indicates that initial introductions of geldings or a greater proportion of stallions have resulted in negative impacts to breeding harems, mitigation could include removal of the additional stallions or geldings in whole or in part, no further introductions of stallions or geldings during future gathers, or release of a larger proportion of mares during the next gather. The need for post-treatment monitoring will decrease as the effectiveness of sex ratio adjustments as a tool to slow population growth rates is determined.

4.5.4 Manage Selected HMAs for Non-Reproducing Wild Horses

Under the WFRHBA (16 USC § 1333(b)(1)), the authorized officer may determine whether AML should be achieved by removal of excess animals, or if options such as sterilization or natural population controls should be implemented. Consistent with this authority, some selected HMAs may be managed for non-reproducing wild horses to aid in controlling on the range population numbers.

4.5.4.1 Manage HMAs for Non-Reproducing Wild Horses

LUPs should identify the HMAs to be managed for non-reproducing wild horses and the criteria for their selection. Completion of additional site-specific environmental analysis, issuance of a decision, and providing opportunity for administrative review under 43 CFR Part 4.21 may also be necessary.

Actual on-the-ground implementation would be influenced by gather efficiency and it may take several gathers to work toward an HMA with non-reproducing wild horses. Animals would be gathered to the extent possible and sterilized for return to the range, or removed. A safe, effective and humane means to sterilize stallions is castration, but a safe, effective and humane means to sterilize females has not yet been perfected. Therefore, initial efforts should focus on returning sterilized males to the HMA of origin. Sterilized males from HMAs with similar environments may be added as long as population size remains within AML. Care should be taken to ensure they are introduced and located near other animals in areas with good water and forage.

4.5.4.2 Manage Reproducing and Non-Reproducing Wild Horses in Selected HMAs

In selected HMAs with large AMLs, management of both reproducing and non-reproducing wild horses could be considered. As an example, non-reproducing wild horses could be geldings released back to their home range HMA following castration. Post-treatment monitoring and/or mitigation would be conducted as discussed in 4.5.3.2 above.

4.6 PRIORITIES FOR GATHER AND REMOVAL

When removal of excess WH&B from the range is necessary, gathers shall be prioritized in the following order:

- Emergencies
- Court Orders
- Nuisance animals (public health and safety)
- Impacts to threatened, endangered, or sensitive species (TES)
- WH&B located outside the HMA in areas not designated for their long-term maintenance
- Landowner request
- To achieve and maintain population size within AML
- Coordinate gathers across State, District and/ or Field Office boundaries and conduct gathers jointly whenever possible to improve gather efficiency and implementation of other population control measures such as application of fertility control and sex ratio adjustments.

4.7 NATIONAL GATHER SCHEDULE

A National Gather Schedule is developed annually based on the need to remove excess WH&B to achieve or maintain AML and the available funding and facility space. Situations that may require adjustments to the National Gather Schedule are: (1) Emergencies and (2) Escalating Problems.

4.7.1 Escalating Problems

Escalating problems are defined as conditions that deteriorate over time. The key indicator is a decline in the amount of forage or water available for WH&B use, which result in negative impacts to animal condition and rangeland health. Causal factors are normally drought or animal numbers in excess of AML. These situations can be detected in advance and are managed through the normal planning process. See BLM Manual Section 4720.21.

4.7.2 Emergencies

Emergencies generally are unexpected events that threaten the health and welfare of a WH&B population and/or their habitat. Examples of emergencies include fire, insect infestation, disease, or other events of a catastrophic and unanticipated nature. Immediate action is normally required. For some emergency actions, it may be appropriate to use alternative means or

procedures to comply with NEPA (see BLM NEPA Handbook, H-1790-1: National Environmental Policy Act Handbook, Chapter 2.3 Emergency Actions). If timeframes permit, a gather plan environmental assessment should be prepared prior to WH&B removal. If this is not possible, emergency actions should be documented and a report prepared after resolution of the problem. Public notification can be addressed through a press release. See BLM Manual Section 4720.22.

4.8 POPULATION MODELING

Version 3.2 of the Winn Equus population model developed by Dr. Steven Jenkins will be used during gather or herd management area planning to analyze and compare the effects of proposed wild horse management. The model is not designed for use on burros.

The model's primary purpose is to analyze and compare the effects of the proposed action (and alternatives) on population size, average population growth rate, and average removal number. Possible management alternatives or strategies may include, but are not limited to:

- Proposals to gather/remove excess animals, with or without fertility control.
- Proposals involving various gather cycles.
- Proposals to make adjustments in sex ratios.
- Proposals to manage all or a portion of a herd as a non-reproducing population.
- Proposals for gate-cut or selective removal gather.
- No Action.

Another objective of the modeling is to identify whether any of the alternatives would be likely to "crash" the population based on a number of stochastic factors (varying environmental conditions).

Use of the Win Equus population model requires the following:

- **Gather Planning:** Run the model for 10 years and 100 trials; select and display the most typical trial.
- **Herd Management Area Planning:** Run the model for 20 years and 100 trials; select and display the most typical trial.
- **Adjustment of Sex Ratios:** Select 100 percent gather for mares and adjust (decrease) the gather percentage for studs to achieve the desired post-gather sex ratio.
- **Results Analysis:** Drop the highest trial and the lowest trial and analyze and compare the remaining trials.

4.9 HUMANE DESTRUCTION

The BLM authorized officer will euthanize or authorize the euthanasia of a wild horse or burro when any of the following conditions exist:

1. Displays a hopeless prognosis for life.

2. Is affected by a chronic or incurable disease, injury, lameness or serious physical defect (includes severe tooth loss or wear, club feet, and other severe acquired or congenital abnormalities).

3. Would require continuous treatment for the relief of pain and suffering in a domestic setting.

4. Is incapable of maintaining a Henneke body condition score greater than or equal to 3, in its present environment.

5. Has an acute or chronic illness, injury, physical condition or lameness that would not allow the animal to live and interact with other horses, keep up with its peers or maintain an acceptable quality of life consistently or for the foreseeable future.

6. A State or Federal animal health official orders the humane destruction of the animal(s) as a disease control measure.

7. Exhibits dangerous characteristics beyond those inherently associated with the wild characteristics of wild horses and burros.

    a. Dangerous animals are defined as those horses or burros that are unusually aggressive and pose an unacceptable risk of injury to humans or other animals.

    b. Unusually dangerous horses or burros possess characteristics that are not desirable to breed into the wild herd.

    c. It is also reasonable to conclude that an average adopter could not humanely care for the animal.

CHAPTER 5—INVENTORY AND MONITORING

GENERAL

The authorized officer is required to identify, plan, collect and analyze the resource monitoring data necessary to prepare resource management plans, plan amendments, gather plans, herd management area plans or other associated environmental documents through which WH&B management decisions are made.

Inventory (monitoring) shall be completed in order to determine:

- If an overpopulation of WH&B exists and action is needed to remove the excess animals.
- WH&B AMLs; and,
- If AMLs should be achieved by removal of the excess animals or other appropriate means.

Monitoring data is needed to support AML establishment and decisions to remove excess WH&B. Various rulings from the Interior Board of Land Appeals (IBLA) underscore the need to base WH&B management decisions on the results of monitoring.

5.1 HABITAT MONITORING

The primary purpose of habitat monitoring is to collect the resource data necessary to:

- Make a determination of excess animals (i.e., support the need to gather and remove excess wild horses or burros).
- Establish or adjust AML.
- Develop or revise HMAPs.
- Evaluate conformance with Land Health Standards, LUP goals and objectives, or other site-specific or landscape-level objectives.

Collection of habitat monitoring data should be coordinated with other resource programs (e.g., range, watershed, wildlife) to maximize efficiency and minimize duplication.

Appendix 2 (Habitat Monitoring References) provides a list of BLM Technical References that can assist in planning for, and collection and evaluation of monitoring data. BLM Technical References can be found at: http://www.blm.gov/nstc/library/techref.htm

5.1.1 Annual Monitoring

Annual monitoring objectives include:

- Determine whether or not forage and water is adequate to support the animals in a healthy condition through the remainder of the year.
- Assess and evaluate animal condition.

To achieve these objectives, monitoring efforts should focus on the following:

- Evaluate current year's forage production and water flows.
- Evaluate/measure use, map patterns of use and monitor seasonal distribution/movement.
- Evaluate WH&B body condition.

Data collection to accomplish the above could include:

1. Photographs and Field Inspection Notes. Document observations on current year's growing conditions (average, below average or above average precipitation and soil moisture); plant phenology (are plants maturing earlier or later than normal); and forage production and water flows (average, below normal, above normal). When taking photographs, label each photograph with the date and the location to facilitate re-photographing the area in the future.

2. Use Mapping. Map utilization of current year's growth. Where possible, document forage utilization by WH&B in rest pastures or prior to livestock use. Where separation of use by type of animal is not possible, map total utilization at the end of the season. Use the information collected to identify and establish key areas, determine distribution, and seasonal use areas. If mapping utilization of the HMA on an annual basis isn't possible, focus monitoring on measuring utilization at key areas used by WH&B within the HMA on an annual and continuing basis.

3. Utilization. Estimate the proportion of annual forage production used by herbivores (WH&B, domestic livestock, wildlife, and insects).

4. Residual Vegetation or Stubble Height, Woody Species Use or Streambank Alteration. Measuring stubble height, use on woody species, and the degree of streambank utilization occurring annually (especially in rest pastures or prior to livestock use) may also be helpful in documenting resource impacts associated with WH&B use.

5. Grazing Use Records. Summarize the actual grazing use (animal unit months of forage) by livestock, WH&B, and wildlife by unit or pasture for the year.

6. Weather Data. Document information on temperature, precipitation, and growing conditions.

7. Animal Condition. Using the Henneke Body Condition Scoring (BCS) system, document the number of animals seen, where they were observed, and their BCS class. Document average group size (e.g., animals are concentrating in large groups or scattered, small groups, evidence of lameness, or other possible animal health concerns). This information may be used to assist in the evaluation of TNEB within the HMA.

When collected, data should be filed in the 4710-Herd Management case file and cross-referenced to the applicable rangeland management or other resource monitoring files.

5.1.2 Long-Term Monitoring

The objective of long-term monitoring is to determine whether management is resulting in significant progress toward attaining Land Health Standards, other applicable site-specific or landscape-level objectives, or changes in range ecological condition and trend (up, stable or downward). Data are typically collected by an interdisciplinary team of resource specialists in preparation for a formal and detailed evaluation of current management and its effect on resource conditions.

5.1.2.1 Ecological Sites

Rangeland landscapes are divided into ecological sites for the purposes of inventory, evaluation and management. Each site is defined and described by the Natural Resources Conservation Service (NRCS) based on climate, geology, soils, vegetation and other environmental factors. An ecological site is also described based on its ability to produce and support a characteristic plant community (i.e., kind, amount, and proportion of natural vegetation). Ecological site descriptions can be used to evaluate current management and to identify the potential effects in range ecological conditions or trends that could be expected from proposed changes in WH&B management. Ecological site descriptions for each Major Land Resource Area (MLRA) are available from NRCS. If an area has not yet been inventoried by NRCS, an interdisciplinary team of resource specialists will use the best available data to identify the ecological sites in the HMA.

5.1.2.2 Riparian Areas

Properly functioning riparian areas produce abundant forage, important habitats for fish and wildlife, and improve water quality and quantity. Functional-at-risk riparian areas have one or more attributes which place them at risk of deterioration, while nonfunctional riparian areas are unable to capture sediment, enhance infiltration, recharge aquifers or dissipate high energy flows. Assessments of proper functioning condition can be used to identify issues, establish objectives, and propose and evaluate changes in WH&B management.

5.1.2.3 Key Areas

Key areas are selected to monitor changes in range ecological condition or trend across representative areas within the HMA or complex which may result from current WH&B management. Key areas may be selected to represent a particular plant community or a specific ecological site. They should also be selected to represent ecological conditions and trends, utilization or use patterns, seasonal distribution, and resource production within the HMA or complex.

Long-term monitoring within key areas may include measurements of frequency, production, and cover (canopy cover, foliar cover, ground cover and basal cover). Plots are generally read at intervals of 5-10 to 15-20 years.

5.1.2.4 Key Species

Key plant species are selected for forage utilization studies. Key species are important forage species within plant communities that can indicate changes in resource conditions resulting from WH&B management. More than one key species may be selected, depending on management objectives.

Key forage species are used to indicate the allowable degree of forage utilization on a key area which promotes attainment of attaining vegetation management objectives. They may be identified during land use planning, or result from habitat management objectives established during site-specific planning efforts such as herd management area planning.

5.1.3 Integrated (Interdisciplinary) Resource Monitoring Plans

Development of integrated (interdisciplinary) resource monitoring plans can help assure that the data needed to support WH&B management decisions is collected in an effective and efficient manner. Resource monitoring plans should identify the following:

- **Who** (The resource specialty responsible for data collection, i.e., WH&B specialist, range specialist, etc.).
- **What** (Data to be collected).
- **When** (Time of year data should be collected and how often).
- **Where** (Location(s) for data collection, i.e., location and documentation of key areas).
- **Why (**The specific monitoring objective).
- **How** (Methods to be used).

Also refer to Appendix 4 (Table 4.2).

5.2 POPULATION MONITORING

An accurate and current assessment of WH&B health and condition, as well as population size, growth rate, and distribution, is needed for proper WH&B management. At a minimum, population surveys should be conducted every 2 years whenever possible, and within 6-12 months prior to establishing the need to gather and remove excess WH&B.

5.2.1 Population Estimation

WH&B population size should be estimated primarily by aerial survey. See BLM Manual Section 4710.45.

1. Aerial surveys will be conducted and documented using standardized sampling methods and procedures. Survey methods (types of aircraft and techniques) should utilize the most effective and cost-efficient techniques for the specific HMA or complex.

2. To permit comparison with prior data and detect population trends, aerial surveys should be conducted during the same time of the year, using the same or similar type of equipment, methodology, and personnel.

3. The following data should be should be recorded during aerial surveys:

   - HMA name and number
   - State/District or Field Office
   - Survey date
   - Observer(s) name
   - Weather conditions
   - Type of aircraft used
   - Altitude
   - Flight time
   - Time of day
   - Survey method (direct count, simultaneous double-count, mark-resight, etc.)
   - Flight path, number and location of animals seen in GPS/GIS data format
   - Number of adults, number of foals
   - Basic herd health and condition information
   - Basic information about range, forage or water conditions, wildlife, or livestock use, etc.

4. In areas where gather efficiencies have been historically low or WH&B range widely, a post-gather survey should be conducted in the 6-12 months following gather completion to obtain a more accurate estimate of the number of WH&B left on the range.

5. Monitoring WH&B distribution is critical in identifying seasonal use areas, determining movement patterns and identifying areas for habitat monitoring. While seasonal distribution may vary over the years, movement is normally related to the abundance and quality of the forage, climate, weather patterns, and availability of water.

6. The HMA monitoring file shall contain a permanent record of data collected during the aerial surveys and the results should be entered into the Wild Horse and Burro Program System (WHBPS) and the local GIS data base.

5.2.2 Herd Condition and Health Monitoring During Gathers

The condition class of individual horses should be evaluated and documented during gathers using the Henneke Body Condition Scoring System (refer to H-4760-1: Compliance Handbook).

1. Data should also be collected on age structure and sex ratio. This information can be used to evaluate the effects of the management actions on herd health and condition.

2. Estimate the number of WH&B left on the range (number remaining ungathered) following each gather.

3. Other data that may be collected during gather operations include: parasite load, disease (from blood samples), percentage of pregnant mares (through analysis of blood or fecal samples), and/or the effects of fertility control.

4. The incidence of albinism, parrot mouth, club feet, severely crooked legs or other physical deformities should also be documented and the subject animals removed from the population when possible. The incidence of these traits should be minimized within individual herds over time.

CHAPTER 6—HERD MANAGEMENT AREA PLANNING

GENERAL

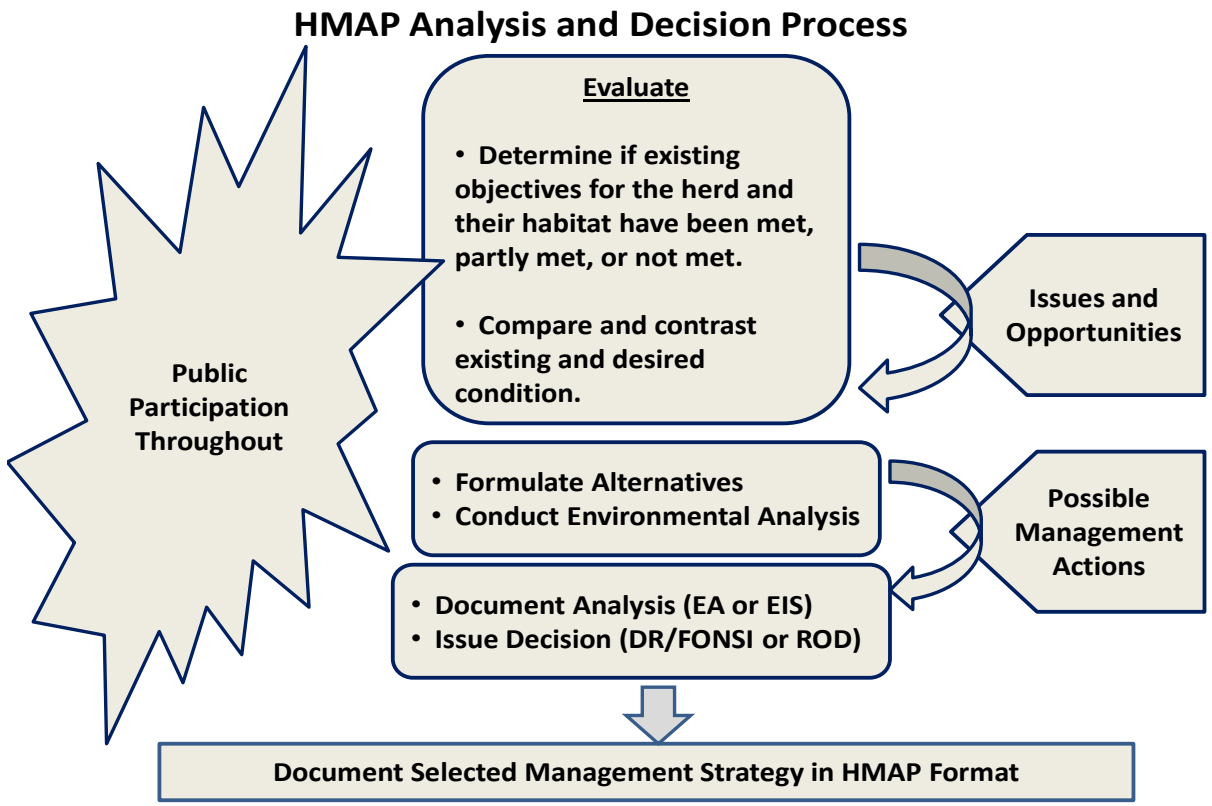
Herd Management Area Plans (HMAPs) identify and set objectives for WH&B herds and their habitat. HMAPs are prepared with public involvement through a site-specific environmental analysis and decision process (NEPA). During the NEPA process, the environmental impacts associated with a range of alternative management strategies for the WH&B herd and its habitat is analyzed.

The approved HMAP outlines the selected management actions, together with the management and monitoring objectives which, when implemented, would make progress toward achieving land health standards, LUP goals/objectives, and other relevant objectives.

6.1 HMAP ANALYSIS AND DECISION PROCESS

An overview of the HMAP analysis process is provided in Figure 6.1. Also refer to Appendix 4 (Herd Management Area Planning) for additional information.

Figure 6.1
Overview of the HMAP Analysis and Decision Process



6.1.1 Evaluate Current Management

The first step in the HMAP analysis process should generally be to evaluate existing management. At the conclusion of the management evaluation, a report will be prepared and made available to the public for a 30-day review and comment period (public scoping). To facilitate public review, the document will be posted on the state, district or field office web page.[1] The management evaluation report is not a decision subject to administrative appeal.[2] Rather, the report documents the key issues identified as a result of the management evaluation. A range of the possible management alternatives (Proposed Action and Alternatives) that would address or resolve the identified issues may also be identified. The range of alternative may be expanded as a result of scoping comments.

Key steps in the management evaluation process include:

1. Review existing goals and objectives for the herd and its habitat and determine whether these have been met, partly met, or not met. Incorporate a summary of the rationale supporting BLM's determination in the evaluation report.

2. Compare and contrast the existing and desired condition for the herd and its habitat. Refer to the information in Chapter 3 (Habitat Management) and Chapter 4 (Population Management) as a starting point for the analysis.

3. Document the gaps (or differences) between the current and desired conditions. These differences represent the issues to be addressed and resolved (i.e., the purpose and need for action).

4. Identify the possible management actions that could be implemented in response to the identified issues.

5. Formulate a range of alternative management strategies for the herd and its habitat from the list of possible management actions (Proposed Action and Alternatives). Each alternative considered should meet the purpose and need for action and respond to the identified issues in whole or in part.

6. Finalize the management evaluation report and provide it to the public for a 30-day review and comment period (i.e., public scoping).

6.1.2 Conduct and Document the Site-Specific Environmental Analysis

As a next step, conduct and document the interdisciplinary, site-specific analysis of potential impacts that could result from implementation of the No Action (generally defined as "Continue

[1] If an Environmental Impact Statement (EIS) is prepared, scoping will be conducted following issuance of a Federal Register Notice.

[2] Once the HMAP decision is made, the Evaluation Report is part of the administrative record for that decision.

Existing Management" for HMAP development and analysis), the Proposed Action, and other action alternatives. Refer to H-1790-1: National Environmental Policy Act Handbook.

Though it may be appropriate in some instances to prepare an Environmental Impact Statement (EIS), normally an environmental assessment (EA) is prepared to document and analyze an HMAP decision. The section below provides guidance about how an HMAP EA may be structured.

6.1.2.1 Preparing the HMAP Environmental Assessment (EA)

1. Introduction. Limit the information included in the "Introduction" section of the EA to the minimum needed to allow the reader to understand the existing situation. Include a brief description of the HMA and its location, and the AML (and when/how it was established). Describe the purpose and need for the Proposed HMAP. Identify the relevant issues, summarize the results of public scoping, and document how the BLM used the comments in preparing the preliminary environmental assessment. Tier the analysis to applicable decisions in the LUP.

2. Proposed Action and Alternatives. Each alternative considered in detail in the EA shall meet the purpose and need for action and respond to the identified issues to varying degrees. The range of alternatives considered will include a No Action Alternative (i.e., Continue Existing Management) and the Proposed Action (Proposed HMAP). Other alternatives should be developed in response to the purpose and need and the identified issues, as appropriate.

   Each alternative will describe the strategy to be used to manage the herd and its habitat over the long-term, together with the associated management, monitoring and implementation objectives. In formulating the alternative management strategies, various management tools may be considered to protect, control and manage WH&B populations and their habitat. For example, consider differing gather seasons or methods, various tools to slow population growth, or various habitat improvement tools, projects or techniques.

3. Management Objectives and Actions. Management objectives and actions may differ by alternative. This section of the EA would summarize the objectives and actions specific to each alternative.

   For each alternative specify: (a) the proposed habitat and population management objectives, (b) the associated management actions (e.g. implementation objectives), and (c) the proposed monitoring objectives. These objectives and actions should conform to and be consistent with LUP goals.

   a. SMART Objectives. Well-written objectives should be specific, measurable, achievable/attainable, reasonable/relevant and trackable within a specified timeframe (SMART):

1) Specific: Specifies what and where.
2) Measurable: Identifies the amount of change expected; desired change can be measured or observed.
3) Achievable/Attainable: Realistic for the specific site conditions.
4) Reasonable/Relevant: Limited in scope, within the BLM's control and influence, measures results (not activities).
5) Trackable/Timeframe: Identifies where the activity will occur, short- and long-range targets, as well as interim steps and a plan to monitor progress.

b. Habitat Objectives. Habitat objectives may involve vegetation, trend or key area objectives for upland vegetation or riparian plant communities within the HMA or complex or objectives to maintain or improve the wild, free-roaming behavior of the population. Site-specific objectives to improve habitat conditions (e.g., forage or water) may also be established.

Examples of SMART Objective(s)

- ❒ Improve riparian condition from functioning at-risk (FAR) in 1998 to properly functioning condition (PFC) by decreasing utilization from heavy to moderate within a ½ mile circumference of Bird Spring.
- ❒ Adjust sex ratios from 40 percent studs and 60 percent mares in 2010 to 60 percent studs and 40 percent mares by 2014.

c. Population Objectives. These objectives would establish a framework for management of the WH&B herd over the longer term. Objectives could include when and how AML would be adjusted in the future, or when and how the population within the HMA would be gathered. Among other population management parameters, objectives may be identified for desired age structure and sex ratio, animal condition, phenotype, genetic diversity, population growth rate, or selective removal criteria. These objectives may be based on historical attributes or other management considerations.

d. Monitoring Objectives. As necessary, identify a monitoring objective that would measure the progress made toward achieving proposed habitat or population management objectives.

e. Implementation Objectives (Management Actions). Implementation objectives are management actions that, when implemented, are expected to make progress toward attaining the proposed habitat or population management objectives. These could include application of fertility control, adjustment of sex ratios to favor males, or other actions to slow population growth, management actions to assure genetic diversity or achieve the desired sex ratio or age structure for the WH&B herd, or range improvement projects.

4. Description of the Affected Environment and Environmental Impacts

   a. General Description of the Affected Environment. This section of the EA should include a concise general description of the HMA or HMA complex. Voluminous material may be included in the EA appendix. Maps, tables, charts or graphs may also be useful in summarizing or displaying relevant information. Information could include:

      1) General information about the HMA or complex's size, location, acres of public land or other land ownership, general description of topography, elevation, climate, and dominant vegetation.

      2) A summary of the WH&B herd's history using best available data. This could include the probable origin of the WH&B population, identification of the general type of WH&B historically occupying the HMA, any interchange between this herd and adjacent HMAs, known genetic diversity (observed heterozygosity or Ho) and any introductions of WH&B from another HMA.

      3) A brief discussion of any historic adjustments that have been made in the HA/HMA boundary, and the name and date of the decision document(s) used to initiate those adjustments. Maps or descriptions of HA/HMA boundaries may also be included.

      4) Initial forage allocations together with the existing AML range. The name and date of any decision document(s) used to establish or adjust AML along with a brief description of the causal factors leading to the change.

      5) Any population and habitat management actions or events that have impacted herd size including scheduled, emergency or nuisance animal gathers, application of fertility control, and the occurrence of catastrophic events such as extended drought or wildfire.

      6) A concise list of applicable LUP decisions that affect the HA/HMA, including any constraints on WH&B distribution and management (voluminous information can be included in an appendix to the EA).

      7) A list of applicable Land Health Standards (these may be included in an appendix to the EA or an internet address can be provided if they are available on the internet).

      8) Brief descriptions of other resource values and uses relative to the management of WH&B, as appropriate. This could include: authorized and/or actual livestock use, TES species and their habitat, Wilderness, Wilderness Study Areas (WSAs), etc.

b. Description of the Affected Environment and Environmental Impacts. For each element of the human environment that has the potential to be affected by the Proposed Action or alternatives, describe the existing situation (affected environment). Also describe the direct and indirect impacts to these resources that could result from implementation of the Proposed Action and Alternatives.

5. Cumulative Impacts. The National Environmental Policy Act (NEPA) regulations define cumulative impacts as impacts on the environment that result from the incremental impact of the Proposed Action when added to other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions (40 CFR 1508.7). Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time.

   In assessing the cumulative impacts associated with preparation of a proposed HMAP, first define the area of potential effect—cumulative impacts will be evaluated within the area of potential effect.

   Next define the issues and resource values identified during scoping that are of major importance (in accordance with the 1994 BLM *Guidelines for Assessing and Documenting Cumulative Impacts*, the cumulative impacts analysis should be focused on only those issues and resource values identified during scoping that are of major importance).

6. Public Review and Comment. The HMAP environmental assessment shall be made available to the public for a 30-day review and comment period by posting on the state/district or field office web page. See H-1790-1 for instructions if an EIS has been prepared.

7. Finalize the HMAP EA. Soliciting public comments obligates reviewers to give fair consideration to the input received. Summarize the results of public review and comment and document how the BLM used the comments in finalizing the environmental assessment.

6.1.2.2 Prepare the Decision Record and Finding of No Significant Impact (FONSI) or an EIS and Record of Decision (ROD)

Based on the analysis of potential environmental consequences, the authorized officer will determine whether to prepare an EIS or issue a Decision Record (DR) and "Finding of No Significant Impact" (FONSI). A FONSI documents why implementation of the selected alternative will not result in environmental impacts that significantly affect the quality of the human environment. The DR identifies the authorized officer's final decision and is a separate and subsequent document to the FONSI. See H-1790-1 for additional information.

If an EIS has been prepared, due to the potential for significant environmental impacts, the authorized officer's final decision will be documented in a Record of Decision (ROD).

6.1.2.3 Prepare the HMAP

As a final step and to facilitate on-the-ground implementation, document the selected management strategy, together with the habitat and population management, monitoring and implementation objectives in HMAP format. The HMAP shall be included as an attachment to the authorized officer's final decision.

The approved HMAP should include the following components:

- **Introduction.** Briefly introduce or summarize the relevant background information (repetition of detailed information incorporated in the NEPA document is not required in the approved HMAP).

- **Management Strategy.** Summarize the selected management strategy as outlined in the EA together with the site-specific habitat and population objectives and management actions established to accomplish the objectives.

- **Monitoring and Evaluation Plan** (refer to 6.1.2.3.1).

- **Tracking Log/Project Implementation Schedule** (refer to 6.1.2.3.2).

6.1.2.3.1 Resource Monitoring Plan/Schedule

The HMAP shall include a resource monitoring plan and schedule that will be used to facilitate the timely and effective completion of habitat and population monitoring. The plan should identify the specific monitoring item, the methodology to be used, the resource specialty needed to complete the monitoring (e.g., field office WH&B specialist, rangeland management specialist, wildlife biologist, etc.), where and when monitoring should occur, as well as any actions to be taken to adjust or adapt management as needed and appropriate pending monitoring results. The plan should include both habitat and population monitoring components. See Appendix 4 (Table 4.2).

6.1.2.3.2 Tracking Log/Project Implementation Schedule

The HMAP will include a tracking log and project implementation schedule to facilitate and monitor plan implementation. The tracking log may be developed in a tabular format, and will list the selected habitat and population management actions (what), the entity responsible for implementation (e.g. who -- BLM, USFS, volunteer groups, etc), and define where, when, and how often the action is to be completed. Columns to document the date the action was completed and/or implemented and for any remarks (e.g. project numbers from BLM's Rangeland Improvement Project system, etc) should also be included. See Appendix 4 (Table 4.3).

6.2 HMAP MONITORING AND EVALUATION

Resource (habitat and population) monitoring data should be analyzed periodically to determine whether minor adjustments in management are needed (adaptive management). Periodic evaluations are conducted to assure HMAP implementation is on track and to gauge progress toward achieving the selected habitat and population management and other relevant objectives. Refer to the process outlined in 6.1.1.

The goals for HMAP monitoring and evaluation are twofold: (1) to track implementation of the management actions/decisions outlined in the HMAP (implementation monitoring); and (2) to collect the data/information necessary to evaluate the effectiveness of those decisions (effectiveness monitoring).

1. Implementation Monitoring. Implementation monitoring tracks implementation of the selected management actions. It answers the question "Did we do it?" To assure selected management actions are being implemented in a timely manner, annual review is recommended.

2. Effectiveness Monitoring/HMAP Evaluation. Plan evaluation is the process of periodically reviewing the HMAP to determine if it is effective in making progress toward or accomplishing the approved habitat and population management objectives (plan evaluation typically occurs at intervals of 5, 10 or 15-20 years). Effectiveness monitoring answers the question "Did it work?"

   The HMAP shall also be evaluated to determine: (1) whether management goals and objectives are still appropriate or need to be revised, and (2) whether progress is being made toward achieving the goals and objectives, or additional management actions are needed (adaptive management). Evaluation of the HMAP could also be completed in conjunction with land health assessment.

   Evaluations that highlight the need for substantial changes may require consultation with interested public as well as appropriate NEPA analysis and documentation prior to implementation.

6.3 4710 CASE FILE

A 4710-Herd Management Area file shall be established for individual HMAs or complexes. The case file should include (but is not limited to) the following:

- A copy of the HMAP.
- HMA and HA maps.
- The associated NEPA and BLM decision documents (or a cross-reference to those documents).
- Any correspondence pertaining to HMA management.
- Aerial survey results.

- Field inspection reports and monitoring data (or a cross-reference to the applicable rangeland management or other case files).
- Information on habitat improvement projects (or a cross-reference to the applicable rangeland management or other case files).
- Final gather reports.
- Fertility control reports, if applicable.
- Results from periodic HMAP evaluations.

Additional 4710 case files may be created if the number and type of monitoring studies is large and varied (e.g. 4710-1, 4710-2, etc).

Relevant data and information should also be entered in a timely manner into WHBPS, as appropriate.

HA/HMA maps and a record of the HA/HMA history should be included in the 4710 HMA file. However, the HA and HMA boundaries delineated in the State GIS data base and provided to the National Coordinator for upload into the National Data Set serve as the official record. Any future changes to HA or HMA boundaries should be documented in the State GIS data base as well as in the HMAP file.

CHAPTER 7—DECISIONS AND APPEALS

7.1 DECISIONS

Implementation decisions form the basis for approval of on-the-ground management actions. When implemented, these actions further the incremental progress toward achieving LUP goals and objectives, Standards for Land Health, or other site-specific habitat or population management objectives. If proposed management is not in conformance with the LUP, the proposal shall be modified or rejected, or the LUP shall be amended or revised.

WH&B implementation decisions can include:

- Establishing or adjusting AML
- Developing HMAPs
- Population management actions
- Habitat improvement projects

Program-specific procedures must be followed when issuing a decision. WH&B implementation decisions can be made in a variety of ways and administrative review (appeal) requirements may vary based on the decisions made (Figure 7.1).

Figure 7.1
Decisions and Appeals Process

An Overview

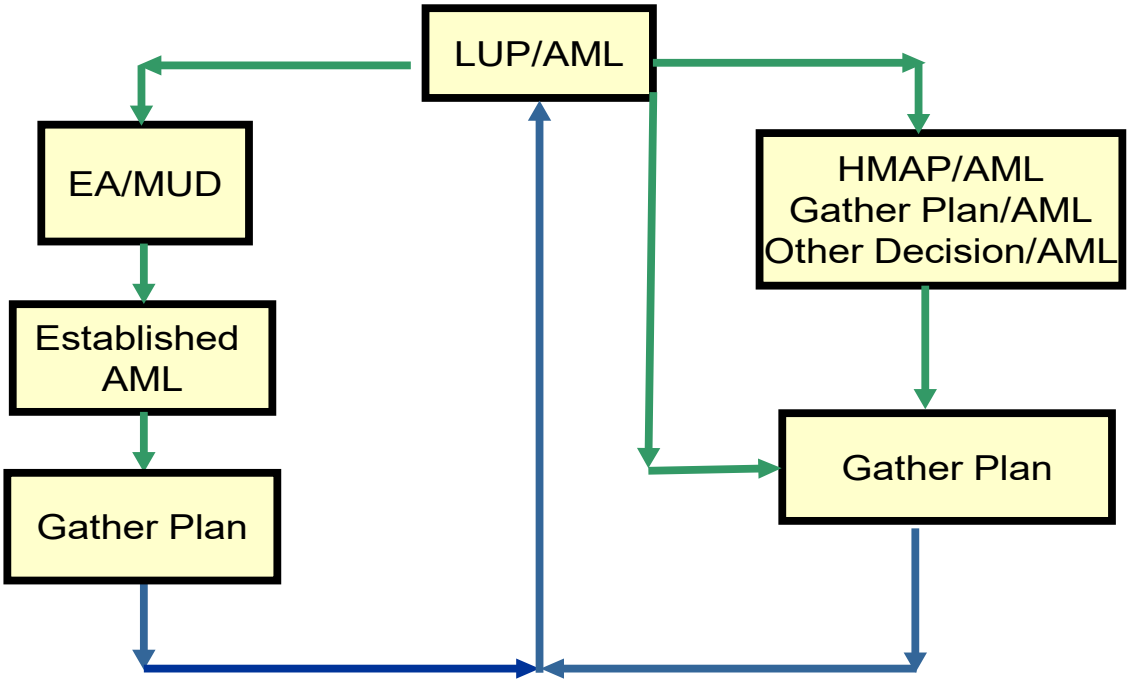
Authority	43 CFR 4770.3(c)	43 CFR Part 4.21
Decision Type	Decisions to Gather/Remove Excess WH&B	AML, HMAP or Project Decisions
Effective Date	• *Emergency Removal Decisions:* make effective upon issuance. • *TNEB Removal Decisions:* make effective on a date specified in the decision *(*e.g., 31-76 days prior to proposed gather start).	Effective the day after the appeal period expires (i.e., 31 days) unless a petition for stay is filed.
Administrative Review Timeframe	Appeals and petitions for stay must be filed within 30 days of the date of the decision.	
Implementation Timeframe	Unless a petition for stay is granted, the gather and removal may proceed as outlined in the authorized officer's decision.	The authorized officer's decision may be implemented immediately if the Appeals Board either denies the petition for stay or fails to act on it within 45 calendar days of the expiration of the time for filing a notice of appeal.

* AML decisions issued as MUDs are heard by a OHA/ALJ and are not implementable until OHA has ruled.

7.1.1 AML Decisions

AML decisions determine the maximum number of WH&B to be managed in the HMA that results in a TNEB and avoids a deterioration of the range. Decisions to establish or adjust AML or establish a population range if the AML was initially set as a single number, can be made in a number of ways (refer to Figure 7.2).

Figure 7.2
AML Decisions



AML decisions are generally implementation decisions as compared to LUP decisions. All AML decisions must meet the following criteria:

1. AML is set based on in-depth analysis of site-specific resource monitoring data including grazing utilization, trend in range condition, actual use and climatic factors (i.e., AML is not set based on administrative convenience or single surveys); and

2. Guidelines and criteria for adjusting herd size are included. Refer to *Dahl v. Clark*, 600 F. Supp. 585 (D. Nev. 1984); *Animal Protection Institute*, 117 IBLA, 208, 219 (1990); and *Animal Protection Institute*, 109 IBLA 112, 118 (1989).

AML decisions may also be made through:

- Issuance of WH&B (single-use) decisions.

- Issuance of multiple-use decisions (MUDs, which allocate forage for domestic livestock, wildlife and WH&B).
- As a part of HMAP decisions.
- As part of gather/removal plan decisions.

AML is not generally established or adjusted as part of the gather planning (NEPA) process due to the in-depth and complex nature of the analysis required. Complexity also increases if allocation of the available forage for use by wildlife, livestock and WH&B will be made concurrently (e.g., MUDs).

If the authorized officer elects to formally review AML as part of the same environmental document which evaluates the proposed removal, the AML decision should be separated from the gather/removal decision. This may be accomplished by issuing separate Decision Records:

1. Gather/removal decision.
2. AML decision.

Separation of the two decisions is desirable because there are different rules governing the timing and appeals process for those decisions:

- Under 43 CFR 4770.3(c), the authorized officer can make decisions to remove wild horses or burros from public or private lands effective upon issuance or on a date specified in the decision, where removal is needed to preserve or maintain a thriving ecological balance and maintain a multiple use relationship.

- The authorized officer does not have similar authority with respect to issuing AML decisions, which, if no petition for stay is filed, become effective on the day after the expiration of the appeals period. See 43 CFR § 4.21(a)(2).

7.1.2 Gather/Removal Decisions

Prior to removing WH&B from public lands, the authorized officer must make a determination, based on current information, that excess animals are present and their removal is necessary to restore a TNEB and multiple-use relationship. BLM's authority to remove excess animals from public lands is found in 16 USC § 1333(b)(2) of the WFRHBA (as amended). This provision requires the BLM to immediately remove the animals upon determination that excess WH&B exist, so as to achieve appropriate management levels, restore a thriving natural ecological balance, and protect the range from the deterioration associated with the overpopulation.

In making the determination that excess WH&B are present and require immediate removal, the authorized officer will analyze current information including grazing utilization and distribution, trend in range ecological condition, actual use, climate (weather) data, current population inventory, WH&B located outside the HMA in areas not designated for their long-term maintenance and other factors which demonstrate removal is needed to restore or maintain the range. Justifying a removal based on nothing more than the established AML is not acceptable.

7.1.2.1 Environmental Analysis (Gather Plans)

The following tools can be used to focus the discussion during the site-specific environmental analysis conducted for Gather Plans:

1. Limit the analysis to that needed to determine the potential impacts associated with the Proposed Action and Alternatives, including No Action. Refer to H-1790-1 for a detailed discussion of the appropriate components of a NEPA analysis.

2. Tier the analysis to LUPs, HMAPs, or other relevant decision documents, as appropriate.

3. Incorporate previous analysis or decisions by reference (e.g., reference to previous AML decisions, HA/HMA boundary decisions, etc), as appropriate.

These tools allow reference to the information without the need to either include the documents in whole or in part, or to restate the information in voluminous detail.

7.1.2.2 Decision Record (Gather Plan)

Unless an emergency situation exists, gather/removal decisions shall be issued 31-76 days prior to the proposed gather start to provide an opportunity for administrative review of the authorized officer's decision to be completed. The Decision Record (DR) for Gather Plans that are documented in an environmental assessment should:

1. Summarize the substantive comments received and describe how BLM used these comments to finalize the environmental assessment. See 7.2 for additional information.

2. In accordance with 43 CFR 4770.3(c):

   - The authorized officer will issue gather decisions effective upon a date established in the decision in situations where removal is required by applicable law, or is necessary to preserve or maintain a thriving natural ecological balance and multiple use relationship.

   - When an emergency situation exists, and timeframes permit, a Gather Plan EA should be prepared and the decision should be made effective upon issuance. See BLM Manual Section 4720.36.

3. Cite the regulatory authority upon which the decision is based.

4. Explain the timeframe and procedures for filing an appeal.

5. Describe the procedures for requesting a stay of the action under 43 CFR 4.21.

7.1.3 Other Management Decisions

Other management decisions may include:

- Approval of HMAPs that establish site-specific population or habitat objectives or actions.
- Project-specific decisions (i.e., water development construction or reconstruction, etc.).

These decisions are made following site-specific environmental analysis (NEPA) with public involvement and are subject to administrative review (appeal) under 43 CFR 4.21.

7.1.4 Actions Excluded from Further NEPA Documentation

Certain actions may be categorically excluded from NEPA analysis (refer to H-1790-1, Chapter 4, Categorical Exclusions). Other actions may have already been adequately analyzed through NEPA and a Determination of NEPA Adequacy (DNA) may be prepared.

Before conducting a new NEPA analysis for a proposed Gather Plan, existing NEPA documentation should be reviewed to determine if it is adequate (i.e., review previous Gather Plan EAs together with the associated Decision Records and Findings of No Significant Impact). Changes in numbers of WH&B since the previous gather that result in changes in forage utilization, use patterns, and/or ecological conditions and trends, or changing environmental conditions such as drought, wildfire, noxious weed infestations, and others, may require that a new NEPA analysis be conducted.

If the existing NEPA documentation appears to be adequate, consult with the Office of the Solicitor to determine whether the issuance of a DNA may be appropriate.

7.2 PUBLIC INVOLVEMENT

The authorized officer may conduct public scoping. See H-1790-1, Chapter 6.3.

The authorized officer will provide the public 30 days to review and comment on the NEPA document, typically an Environmental Assessment that documents and analyzes the environmental effects of the BLM's Proposed Action.

The authorized officer shall make Gather Plan EAs and DNAs available to interested individuals, groups, and agencies for a 30-day review and comment period, except when an emergency situation exists. The NEPA document(s) identified in the DNA (e.g., the EA and Finding of No Significant Impact (FONSI)) will also be made available to the public for information.

The authorized officer should consider substantive comments and summarize how they were addressed in the NEPA document or DNA for the Gather Plan. This summary should be presented in the NEPA document, the DNA, or the decision document. See H-1790-1, Chapter 6.9 for additional information.

Possible options for responding to substantive comments include:

- Modifying one or more of the alternatives.
- Developing and evaluating additional alternatives.
- Supplementing, improving, or modifying the analysis.
- Making factual corrections.
- Explaining why the comments do not warrant further agency response, citing cases, authorities or reasons to support the BLM's position.

When an EIS has been prepared, follow the guidance provided in H-1790-1, Chapter 9.

7.3 APPEALS (ADMINISTRATIVE REVIEW)

Any party who is adversely affected by a final decision of the authorized officer may file an appeal and request a stay of the action. WH&B decisions are subject to administrative review (appeal) and remedies under the provisions of 43 CFR 4.21, 4.410, and 4770.3.

As a general matter, WH&B decisions will not become effective during the time in which a person adversely affected may file a notice of appeal. Unless a petition for a stay pending appeal is filed together with a timely notice of appeal, these decisions will become effective on the day after the expiration of the time during which a person adversely affected may file a notice of appeal (generally 31 days from the decision date). If a petition for stay pending appeal is filed together with a timely notice of appeal, a decision will become effective immediately if:

- The stay request is denied; or,
- The Interior Board of Land Appeals fails to act on the petition for a stay within 45 days from the date of the decision.

Under certain circumstances, however, the authorized officer may make decisions to gather/remove excess wild horses or burros effective upon issuance or on a date specified in the decision. See 43 CFR 4770.3(c).

Under the authority provided in 43 CFR 4770.3 (c), the authorized officer may make decisions to gather/remove excess wild horses or burros effective upon issuance or on a date specified in the decision.

When issued under this authority, the gather may be implemented unless a stay is granted or a Federal Court enjoins the gather.

7.3.1 AML Decisions

AML decisions are subject to administrative review:

1. When administrative review of multiple-use decisions (MUD) is requested, BLM may ask to combine the wild horse decision with the grazing decision for review by an administrative law judge (ALJ) in the Office of Hearings and Appeals (OHA).

2. Wild horse decisions are subject to administrative review by the Interior Board of Land Appeals (IBLA) in accordance with 43 CFR 4.21 and 4.410.

7.3.2 Gather/Removal Decisions

The authorized officer has the authority to make decisions to gather and remove excess WH&B effective upon issuance or on a date specified in the decision when removal is court ordered or is needed to preserve a thriving natural ecological balance and multiple use relationship.

Any party who is adversely affected by the authorized officer's final decision may file an appeal and/or request a stay of the action with the Interior Board of Land Appeals (IBLA). The appeal and/or request for stay must be filed within 30 days from the date of the decision and in accordance with the regulations found at 43 CFR 4.411(a).

7.3.3 HMAP or Other Management Decisions

AML, HMAP, or other management decisions are subject to administrative review through the IBLA under provisions of 43 CFR 4.21 and 4.410. Under this authority, decisions **cannot** be implemented effective upon issuance or on a date specified in the decision.

A notice of appeal must be filed in the office of the authorized officer within 30 days from the date of the decision. See 43 CFR 4.411.

CHAPTER 8—RESEARCH

GENERAL

Direction to conduct research is contained in the WFRHBA, 16 U.S.C. § 1333(b)(2)(C)(3), and the Strategic Plan for Management of Wild Horses and Burros on Public Lands (1992). In late 2000, the Midcontinent Ecological Science Center of the U. S. Geological Survey (USGS) was charged with developing a strategic research plan for the management of WH&B. The purpose of the strategic planning process was to:

- Review past progress and identify problems that could be addressed with research.
- Set broad research goals central to the BLM's mission for WH&B management.
- Establish specific, time-bound, measurable research goals, and strategies to achieve them.
- Evaluate the progress towards those goals at set time periods, and to readjust the planning as needed.

There had been no previous prioritization of the BLM's WH&B research and management needs, and no effort to develop a strategy for fulfilling those needs within a specified time period. The strategic planning process was designed to fill this void.

8.1 STRATEGIC RESEARCH PLAN

The Strategic Research Plan for Wild Horse and Burro Management identified research priorities to include health and handling, fertility control, population estimation and modeling, genetic conservation, habitat assessment and setting population goals. The components of fertility control and population estimation have been implemented and research is being conducted. Genetic diversity of WH&B herds is being evaluated. Other research projects may be initiated as needed to support the management of WH&B. Research results will be used to improve management practices within the WH&B program.

8.1.1 Development

The Strategic Research Plan was a joint effort between the USGS, the BLM, the Animal and Plant Health Inspection Service (APHIS), and the U.S. Department of Agriculture (USDA). The plan was developed over a period of 2 years with input from 39 subject area experts representing 11 universities, 3 Federal agencies (BLM, USGS, APHIS), and two State wildlife agencies.

The USGS took the lead role in planning and coordinating meetings of the expert committees and in drafting the Strategic Plan based on committee and agency input. Assisting in this effort were the BLM's National WH&B Research Coordinator and equine health experts from APHIS.

Principal input from the USGS was focused on topics of contraception, aerial population estimation, population modeling, and genetics. Principal input from APHIS was focused on topics of disease and animal health monitoring and surveillance.

Advice and input has also come from BLM including managers and specialists assigned to the WH&B program, the National WH&B Advisory Board, and WH&B staff in the Washington Office. Advice and input was also provided by the BLM Director's Science Advisory Committee, and from seven topic-specific advisory panels that were convened by the USGS.

8.2 RESEARCH REQUESTS

A National Research Advisory Team exists to review research proposals, monitor research project progress, update the Strategy as necessary, and provide recommendations to the WH&B Division Chief. The Research Advisory Team has been formed with equal participation by the BLM (BLM's National WH&B Research Coordinator), the USGS, and APHIS.

All requests for research and/or research proposals pertaining to the Wild Horse and Burro program, whether generated from the field or through field-related contacts, are to be directed to the Research Advisory Team for review. This Team is also available to assist field offices with the development of research proposals. Proposal format and submission should be coordinated through the National WH&B Research Coordinator. Proposals will be reviewed on an as-needed basis and recommendations for funding will be made directly to the WH&B Division Chief.

8.3 RESEARCH PROJECTS

8.3.1 Fertility Control

An effective, safe, and cost-effective fertility control agent is an essential tool in the management of wild horses. A Fertility Control Field Trial Plan has been developed and field research is underway for two components of the Plan: (1) Individual-Based Trials and (2) Population-Based Trials. Over the last decade, BLM research efforts in fertility control have been focused on PZP (Porcine zona pellucida) and the development of time-release pellets to extend the effectiveness of PZP. PZP has proven effective in reducing foaling rates, the population impacts of which are presently being determined.

8.3.2 Other Possible Fertility Control Tools

Other possible fertility control tools that could potentially be considered in the future include:

- Spaying mares.
- Vasectomizing studs.
- Use of IUDs.
- Use of GonaCon™.
- Use of SpayVac™.
- Use of other fertility control agents or sterilants.

8.3.2.1 Spaying (Mares)

Spaying mares involves major abdominal surgery, is risky, and requires good post-operative care. Spaying mares could be considered in the future if safe, effective and humane surgical methods and post-operative care procedures can be perfected for use on wild horses.

8.3.2.2 Vasectomies (Stallions)

Performing vasectomies on stallions is not a widely practiced procedure within veterinary medicine. Post-vasectomy, it is expected that studs would retain their stud-like behavior. By contrast, gelding studs (castration) is a routine veterinary procedure in both domestic and wild horses. However, geldings lose their stud-like behavior after a few months.

Vasectomized studs would be expected to continue to cover mares and keep them in a harem, but eventually most mares would be bred by an intact stallion. If this continued over several estrous cycles it could lead to extended foaling seasons and potentially an increase in foal mortality. Research shows that at 15-33 percent of foals are sired by non-harem stallions, making it unlikely that fertility control focused on males would be effective in slowing population growth (Bowling and Touchberry 1990, Kaseda and Kahlil 1996, Asa 1999). Further research is needed to perfect a safe technique for performing vasectomies in stallions and demonstrate whether this approach will reduce population growth rates.

8.3.2.3 Use of IUDs

Pilot studies using coil-type intrauterine devices (IUDs) and glass balls or marbles as IUDs have failed to demonstrate a long-lasting effect on conception in mares. In both instances, mares "slipped" the devices and became pregnant soon thereafter. The application of IUDs is further complicated by the difficulties associated with identifying a time window for application when mares are not pregnant.

8.3.2.4 Use of GonaCon™

GonaCon™ is an experimental fertility control vaccine that is being developed for potential use as a management tool for deer. Tests of the GnRH vaccine are ongoing in several States and countries, involving a wide range of wildlife and feral species, including horses. A research study conducted with estray horses in Nevada suggests GonaCon™ will reduce foaling rates for 1 year following treatment. At present, the effectiveness of GonaCon™ as a fertility control agent beyond one year appears similar to or less than that of PZP-22, suggesting limited potential for use of the product to reduce population growth rates over a longer period of time.

8.3.2.5 Use of SpayVac™

SpayVac™ is an experimental fertility control vaccine using PZP antigens and a novel liposome technology. It is easy to handle and administer. A single vaccination with SpayVac™ has maintained a high level of contraception throughout a 4-year study with estray horses in Nevada. However, there is currently no regulatory approval for the management or investigational use of

SpayVac™ through the EPA or FDA, and the product is not currently commercially available. SpayVac™ may have potential for use as an effective, longer-lasting fertility control agent in the future and could offer an alternative to spaying mares. However, additional research would be needed before it could be used on a population-management basis.

8.3.3 Population Estimation and Setting Population Goals

Aerial surveys rarely detect all WH&B within an HMA. As a result, population estimates must be developed using correction factors to account for WH&B not identified during the census. At present, research is being conducted on two techniques (simultaneous double-count and photo mark-resight) that will allow use of such correction factors.

8.3.4 Other Research

In the future, research may be conducted in other areas identified as research priorities, depending on need and funding. When other research needs are identified or research projects are proposed, they will be reviewed and evaluated by the National WH&B Research Advisory Team and recommendations will be made to the WH&B Division Chief.

GLOSSARY OF TERMS

Activity Plan – Examples of activity plans include Herd Management Area Plans (HMAPs) and Allotment Management Plans (AMPs). These plans lead to implementation decisions that usually describe multiple projects or management actions and apply best management practices to make progress toward attainment of Land Use Plan (LUP), Land Health Standards, or other resource objectives.

Adaptive Management – WH&B management is adjusted as indicated based on the results of monitoring and evaluation.

Adult Wild Horse – WH&B 1 year of age and older are considered adults. A foal is considered 1 year of age on January 1 of the year following its birth.

Adult Breeding Population - Wild horses within a population that are 1 year of age and older.

Aerial Survey – A method of counting WH&B using an aircraft. Aerial survey allows the BLM to obtain estimates of WH&B population size with associated confidence intervals.

Animal Unit (AU) - Wild horses 1 year of age and older count as 1 AU and burros 1 year of age and older count as 0.5 AU.

Animal Unit Month (AUM) – The amount of forage (approximately 800 pounds of air dried forage) necessary to sustain one adult horse or two burros for one month.

Appropriate Management Level (AML) - The number of adult horses or burros (expressed as a range with an upper and lower limit) to be managed within an HMA. Forage for WH&B (AUMs) is allocated based on the AML upper limit.

AML Range – The number of adult WH&B within which herd size will be allowed to fluctuate.

AML Upper Limit – The maximum number of WH&B that results in a thriving natural ecological balance (TNEB) and avoids a deterioration of the range. This number should be somewhere below the number that would cause damage to the range (refer to 118 IBLA 75).

AML Lower Limit – The number that allows the population to grow to the AML upper limit over 4-5 years, without the need for gathers to remove excess WH&B in the interim.

Authorized Officer – Any employee of the BLM to whom authority has been delegated to perform the duties described in this Handbook (refer to BLM Manual 1203 for a further explanation of delegation of authority).

Census – A complete count of the total WH&B population within an area. Because aerial surveys do not usually detect all WH&B within an HMA, a true census is usually not possible. Better population estimates are developed using correction factors to account for WH&B not seen during the survey (i.e., sightability correction factors).

Considered Comparably - During the LUP process, WH&B are considered in the same manner as other resource values (e.g., cultural, historic, scenic, rangelands, timber, and minerals).

Emergency – An unexpected event that threatens the health and welfare of a WH&B population and/or its habitat. Examples include fire, insect infestation, disease or other events of a catastrophic and unanticipated nature.

Escalating Problems - Conditions that deteriorate over time. The key indicators are a decline in the amount of forage or water available for WH&B use, with negative impacts to rangeland health and, ultimately, animal condition. Causal factors are normally drought and/or animal numbers in excess of AML. These situations can be detected in advance and are managed through the normal planning process.

Evaluation (LUP Evaluation) – The process of reviewing the land use plan and periodic plan monitoring reports to determine whether the decisions and NEPA analysis are still valid and whether the plan is being implemented.

Evaluation (HMAP Evaluation) – Periodic review of herd management area plans (HMAPs) to determine if the selected management practices were implemented as planned (answers the question "Did we do it?") and whether or not the plan is effective in making progress toward established habitat and population management objectives (answers the question "Did it work?").

Excess Animals – Wild, free-roaming horses or burros which have been removed or which must be removed from in order to preserve and maintain a thriving natural ecological balance and multiple-use relationship in an area.

Fertility Control – A tool to decrease fertility and which, when implemented, reduces (slows) population growth rates and extends the gather cycle.

Free-Roaming – WH&B are able to move without restriction by fences or other barriers within a HMA.

Goal – A broad statement of a desired outcome.

Genetic Diversity – The absence of inbreeding depression as monitored through an established baseline and periodic reassessment. This represents an expression of the genetic health of an individual animal or the population.

Herd (Reproducing) – One or more stallions and their mares and foals.

Herd Area (HA) - Geographic areas of the public lands identified as habitat used by WH&B at the time the WFRHBA was enacted (12/15/1971).

Herd Management Area (HMA) – May be established in those HAs within which WH&B can be managed for the long term. HMAs are designated through the LUP process for the

maintenance of WH&B herds. In delineating each HMA, the authorized officer shall consider the appropriate management level (AML) for the herd, the habitat requirements of the animals, the relationships with other uses of the public and adjacent private lands, and the constraints contained in 43 CFR 4710.4.

Herd Management Area Plan (HMAP) – An HMAP establishes management actions and short- and long-term management and monitoring objectives for a specific WH&B herd and its habitat. HMAPs assist the authorized officer in tracking progress toward achieving LUP goals. They are prepared under authority of 43 CFR 4710.3-1. An HMAP can be prepared for an individual HMA or a complex of HMAs.

Implementation Decision – Decisions that make progress or take action toward achieving LUP goals and objectives.

Implementation Plan – Refer to Activity Plan above.

Land Use Plan (LUP) – LUPs provide (by tracts or areas) for the use of public lands. LUPs are prepared in accordance with established land use planning procedures in 43 CFR 1600 and pursuant to FLPMA. They establish goals and objectives (desired outcomes), identify the management actions needed to achieve the desired outcomes, and identify the allowable uses of the public lands.

Manage Principally but not Exclusively – An HMA may be considered for designation as a wild horse or burro range when there is significant public value present, such as unique characteristics in a herd or an outstanding opportunity for public viewing. These HMAs may be managed principally, but not exclusively, for wild horse or burro herds.

Minimal Feasible Level of Management - The minimum number of habitat or population management tools or actions necessary to attain the objectives identified in approved LUPs and HMAPs for a HMA or HMA complex.

Monitoring – The process of collecting and analyzing the data necessary to evaluate existing management and determine whether progress is being made toward attaining established habitat and population management goals and objectives.

Non-Reproducing Wild Horses – An HMA composed, in whole or in part, of sterilized wild horses (either stallions or mares) to aid in controlling on the range population numbers.

Objective(s) – A description of the desired outcome of a management decision. Objectives are specific, measurable, achievable/attainable, reasonable/relevant, and progress can be quantified within established timeframes for achievement (SMART).

Plan Monitoring – The process of tracking land use plan implementation, including collecting and assessing the data or information necessary to evaluate the effectiveness of land use planning decisions.

Population Growth Rates – Represent the net effect of births and deaths in any given year. The BLM reports annual population numbers as of February 28th.

Range – The amount of land necessary to sustain an existing herd or herds of wild free-roaming horses and burros, which does not exceed their known territorial limits.

Resource Management Plan – see LUP above.

Self-Sustaining – The ability of reproducing herds of wild horses and burros to maintain themselves in a healthy condition and to produce healthy foals.

Standards for Land Health – Standards are statements of physical and biological condition or degree of function required for healthy sustainable rangelands. Achieving or making significant progress towards these functions and conditions is required of all uses of public lands. Historical data, when available, should be utilized when assessing standards.

Standard Operating Procedures – The Bureau follows standardized methods of operation which have been developed over time to assure the safe, effective, and humane handling and treatment of WH&B.

Thriving Natural Ecological Balance (TNEB) – WH&B are managed in a manner that assures significant progress is made toward achieving the Land Health Standards for upland vegetation and riparian plant communities, watershed function, and habitat quality for animal populations, as well as other site-specific or landscape-level objectives, including those necessary to protect and manage Threatened, Endangered, and Sensitive Species.

Utilization – The portion of annual forage production that has been consumed by herbivores (WH&B, domestic livestock, wildlife, and insects). The term is also used to refer to the pattern of such use.

WH&B Range – A HMA which meets the special criteria defined in H-1601-1, Land Use Planning Handbook. These include unique herd characteristics, viewing opportunities, unique landscape, significant public demand or other reasons. WH&B Ranges are managed principally, but not necessarily exclusively, for WH&B.

Wild Free-Roaming Horses and Burros – All unbranded and unclaimed horses and burros that use public lands within 10 contiguous Western States as all or part of their habitat, or that have been removed from these lands by the authorized officer, or have been born of wild horses or burros in authorized BLM facilities, but have not lost their status under the WFRHBA (16 USC § 1332 (f)).

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Kaseda, Y., and A.M. Khalil. 1996. Harem size and reproductive success of stallions in Misaki feral horses. Applied Animal Behaviour Science 47:163–173.

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Turner, J.W., Jr., I.K.M. Liu, D.R. Flanagan, A.T. Rutberg, and J.K. Kirkpatrick. 2007. Immunocontraception in wild horses: one inoculation provides two years of infertility. Journal of Wildlife Management 71(2):662–667.

APPENDIX 1 - Genetics Data and Hair Sample Collection Instructions

Analysis of DNA to determine genetic diversity of wild horse and burro (WH&B) herds is currently being done using hair samples rather than blood samples. Unless there is a previously recognized concern regarding low genetic diversity in a particular herd, it is not necessary to collect genetic information at every gather. Typical herds should be sampled every other gather. A new baseline does not need to be established through hair analysis if blood analysis has already been completed. Please follow the instructions below for collecting hair samples. Call BLM's National WH&B Research Coordinator if you have any questions.

While it is preferable to sample release horses, you may sample removed horses if necessary. In complexes or HMAs where separate breeding populations are suspected, WH&B in each distinct population should be sampled separately. Do not mix samples from different horses or different breeding populations. The minimum sample size is 25 animals or 25 percent of the post-gather population, not to exceed 100 animals per population. Samples should be collected from males and females in the same approximate ratio as the population. Animals of any age class may be sampled. Burros should be sampled in the same manner as horses.

1. You will need one plain white paper envelope (a white #10 business envelope works best) for each horse. DO NOT use plastic or zip-lock bags and DO NOT use plastic coated envelopes or envelopes with windows in them.

2. Hair samples must be obtained by pulling the hair out by the root NOT cutting or shaving it off the horse. This is because the DNA is in the root follicle not the hair itself. Mane hair will work, but for foals or young horses you may need to obtain tail hair. Please submit about 30 hairs per animal. A bundle of 30 hairs is about the diameter of a pencil.

The easiest way to pull a good sample is to grasp a bundle of hair and wrap it around a clean mane comb or hoof pick. Holding the bundle close to the neck, pull **straight out** firmly. Foal hair is more brittle and tends to break off. If you are having trouble getting hair with the root attached try obtaining a tail hair sample instead.

(James Sturdivant – The Jockey Club, modified with permission)

3. Check that you have the hair roots or hair bulbs attached to the hair at the base. They look, and feel like little bumps on the end of each hair.

Keep the hair in a loose bundle pointed in one direction or twist it together and place it in an envelope. You can cut off excess hair and leave only a few inches with the hair root attached to put in the envelope if that is easier.

4. Seal the envelope and **write the sample number on the envelope.**

Also write the sample number, color, sex and age of the horse on the data collection sheet. If animals cannot be aged in years, at a minimum record adult, yearling or foal. Be sure to keep stray hairs out of the comb and off your clothes so they don't contaminate the next sample.

Please NOTE: it is best to sample when the hair is dry. If you need to sample when it is raining or the horses are wet, then DO use zip-lock bags for each sample AND keep the samples cool not frozen (refrigerate, then ship the samples with cold packs).

Please fill out the top of the form completely, including the HMA name, HMA number and the date the sample was collected.

Within 3 days after hair collection, send a copy of the data collection form to:

Attn: Research Coordinator, National Program Office-Reno

FEDEX the samples, to arrive on a weekday not the weekend **with the Data Collection Sheet to Dr. Gus Cothran, address below:**

Dr. E. Gus Cothran
VIBS, CVM
TAMU4458
Texas A&M University
College Station, TX 77843
Phone (979) 845-0229

For questions or comments, contact: **National Program Office @ 775-861-6583**.

HAIR SAMPLE GENETICS DATA COLLECTION SHEET
WILD HORSE AND BURRO HERD GENETICS EVALUATION

PAGE _____ OF _____

HMA: _____ HMA #: _____

DATE: _____

TRAP/SUBGROUP: _____

Field Office:_____ STATE:_____

BLM CONTACT PERSON: _____

PHONE: _____

ADDRESS:
_____

EMAIL: _____

SAMPLED HORSES WERE (circle) RELEASED or REMOVED

	SAMPLE #	COLOR	SEX	AGE	COMMENTS
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

APPENDIX 2 - Habitat Monitoring References

BLM Technical References can be found at http://www.blm.gov/nstc/library/techref.htm
A list of references which may be helpful follows:

1. Biological Soil Crusts: Ecology and Management, TR-1730-2, Interagency, 2001.

2. Inventory and Monitoring, Ecological Site Inventory, TR-1737-7, BLM, 2001.

3. Measuring and Monitoring Plant Populations, TR-1730-1, 1998

4. National Range Handbook, Handbook, H-4410-01, 1990.

5. Rangeland Inventory and Monitoring: Selected Bibliography of remote Sensing Applications, TR-4400-9, BLM, 1986)

6. Rangeland Health Standards, Handbook 4180, BLM, 2001.

7. Interpreting Indicators for Rangeland Health, Version 4, TR 1734-6, Interagency, 2005.

8. Rangeland Monitoring and Evaluation, TR-4400-1, BLM 1988.

9. Rangeland Monitoring and Evaluation Handbook, BLM Handbook H-4400-01, 1990.

10. Rangeland Monitoring: Actual Use Studies, TR-4400-2, BLM, 1984).

11. Rangeland Inventory and Monitoring: Supplemental Studies, TR-4400-5, BLM, 1992.

12. Rangeland Monitoring: Analysis, Interpretation, and Evaluation, TR-4400-7, BLM, 1984.

13. Riparian Area Management, Grazing Management for Riparian Areas, TR-1337-4, BLM, 1989.

14. Riparian Area Management, Management Techniques for Riparian Areas, TR-1737-6, BLM, 1992

15. Riparian Area Management, Process for Assessing Proper Functioning Condition, TR-1737-9, Interagency, 1990.

16. Riparian Area Management, Process for Assessing Proper Functioning Condition for Lentic Riparian-Wetland Areas, TR-1737-11, Interagency, 1990.

17. Riparian Area Management, Grazing for Riparian-Wetland Areas, TR-1737-14, BLM, 1997.

18. Riparian Area management, A Guide to Managing, Restoring, and Conserving Springs in the Western Unites States, TR-1737-17, BLM, 2001.

19. Riparian Area Management, Riparian Wetland Soils, TR-1737-19, Interagency, 2003.

20. Statistical Considerations in Rangeland Monitoring. (See TR 4700-8, 1986)

21. Sampling Vegetation Attributes, TR-1734-4, 1996.

22. Utilization Studies and Residual Measurements, TR-1734-3, Interagency, 1996.

APPENDIX 3 - AML Establishment and Adjustment Process

AML decisions determine the number of WH&B to be managed within an HMA or complex of HMAs. AML is expressed as a population range with an upper and lower limit. The AML upper limit is the number of WH&B which results in a TNEB and avoids a deterioration of the range. The AML lower limit is normally set at a number that allows the population to grow to the upper limit over a 4-5 year period, without any interim gathers to remove excess WH&B. See Chapter 4.

A multi-tiered analysis process should be used to establish and adjust the appropriate management level (AML) of WH&B.

- ❒ **Tier One**: determine whether the four essential habitat components (forage, water, cover and space) are present in sufficient amounts to sustain healthy WH&B populations and healthy rangelands over the long-term.

- ❒ **Tier Two**: determine the amount of sustainable forage available for WH&B use.

- ❒ **Tier Three**: determine whether or not the projected WH&B herd size is sufficient to maintain genetically diverse WH&B populations (i.e., avoid inbreeding depression).

Should the Tier One analysis determine that one or more of the essential habitat components is insufficient to maintain a healthy WH&B population and healthy rangelands; the authorized officer should consider amending or revising the LUP to remove the area's designation as an HMA.

Tier 1

Determine if the four essential habitat components (forage, water, cover, and space) are present in sufficient amounts to sustain healthy WH&B populations and healthy rangelands over the long-term. In making this determination, the most limiting factor(s) within the HMA should be considered. In some HMAs, the most limiting factor may be:

- The water available for WH&B use.
- The limited habitat available for WH&B use on either the summer or winter range, in HMAs with seasonal ranges.
- The naturally occurring, low productive capability of the dominant ecological sites.
- The low ecological status of key WH&B use areas.
- Low annual rainfall or extended periods of drought.

The essential habitat components must be located on public lands within the HMA boundary. If forage or water located on private lands within the HMA is needed to maintain healthy WH&B populations, a written agreement with the private landowner allowing use by WH&B is required. In the absence of private landowner agreement, the forage and water on private lands is not available for use by WH&B and may not be included when establishing or adjusting AML.

Forage

The amount of forage available for WH&B use is expressed in animal unit months (AUMs). One AUM is defined as the amount of forage necessary to sustain one adult horse or two adult burros for one month (or approximately 800 pounds of air dry forage). Twelve AUMs of forage are needed to support one wild horse or two adult burros for one year.

As a **starting point**, the amount of sustainable forage available for WH&B use may be determined based on the results of utilization monitoring and use pattern mapping for the years evaluated. A determination of forage availability should not be based on the results of only one year's monitoring data. A minimum of three to five years of monitoring data is preferred. This is because forage production can vary substantially from year to year based on the timing and amount of precipitation received, among other factors. Under the same level of grazing, use pattern mapping may indicate light to moderate utilization during above normal precipitation years, yet indicate heavy or severe utilization during below normal precipitation years.

The nature of the forage (i.e., perennial vs. annual) should also be considered. While annual forage may be available to support WH&B use during years with normal or above normal precipitation, it may be greatly reduced during below average precipitation years. As a result, annual forage is not typically used to support or justify WH&B numbers within a HMA. Within perennial ranges, the production of perennial species may also be greatly reduced during drought, and the production of annual grasses and forbs may be largely absent.

The flexibility to remove WH&B in below average forage years, or to move the animals to another area, can be constrained by funding, contractor capability, facility space, or animal behavior. To prevent range damage or adverse impacts to animal health, the upper limit of AML for WH&B should be established in consideration of the most limiting forage (or water) production years.

Other Considerations

1. Situations in which the Land Health Standards are achieved but population inventory indicates more WH&B are present than expected. These situations may indicate additional forage is present to support use by higher numbers of WH&B, wildlife, or livestock, consistent with LUP guidance.

2. Situations in which deteriorated land health conditions exist but population inventory indicates the number of WH&B is the same or lower than expected. These situations may indicate there is less forage available to support the existing use by WH&B, wildlife, or livestock, and that a reduction in the allowable use may be needed, consistent with LUP guidance.

The number of WH&B which can be sustained based on the available forage is determined in **Tier Two.** To determine if there is sufficient forage to sustain long-term WH&B:

- Analyze utilization data, use pattern mapping, and/or production, ecological site condition (ESI), trend, frequency, precipitation (weather), and indicators of land health.

- Determine the AUMs of actual use by WH&B for each of the evaluation years. (Calculate the AUMs of actual WH&B use based on population estimates derived from aerial surveys. You can also project actual use by WH&B using previous population estimates together with herd's average annual population growth rate.)
- Identify key WH&B use areas (distribution). Calculate carrying capacity on the basis of the key use areas (primary range).

Water

The amount of water available for WH&B use is generally based on public, natural waters (i.e., water occurring on private lands is not considered unless a written agreement with the private landowner is obtained). Water availability during drought conditions is also considered. Sufficient water for WH&B must be available during drought to achieve and maintain a thriving natural ecological balance and multiple use relationship on the public lands.

In determining the amount of water available for WH&B use, a thorough inventory of the available public, natural water resources is needed. Developed and man-made waters should also be inventoried if they are available for WH&B use (i.e., BLM holds the necessary water rights and WH&B access to the water can be depended upon over the long-term). The water resources inventory should include the name, location, and flow (in gallons per minute or cubic feet per second). Wild horses require a minimum of 10 gallons of available water per animal per day (15 gallons per day is not unusual during the hot, dry summer months) while burros require about 5 gallons available per animal per day. The analysis of available water should also be based on the most limiting season of the year (i.e., generally summer when flows are reduced).

Cover and Space

A recurring pattern of WH&B movement out of the HMA to access forage, water, or thermal or hiding cover is an indication the HMA cannot sustain year-long WH&B use. If one or more of the key habitat components is missing, the HMA should be considered as unsuitable for year-long use by WH&B. In these situations, the authorized officer should consider amending or revising the LUP to remove the area's designation as an HMA. An exception would be two or more HMAs which adjoin and are managed as a complex of HMAs, or HMAs which adjoin Forest Service wild horse territories (WHT) that can be managed as a complex.

The results of the **Tier One** analysis may be summarized in a simple table (see example Table 3.1 below). The table should be followed by a detailed description of the analysis and the rationale used in making the determination that a key habitat component either is (or is not) sufficient to support healthy WH&B populations and healthy rangelands over the long-term.

Table 3.1
Sufficient Forage, Water, Cover and Space

HMA	Forage		Water		Cover		Space	
	Sufficient	Insufficient	Sufficient	Insufficient	Sufficient	Insufficient	Sufficient	Insufficient
HMA 1	X			X	X		X	
HMA 2		X		X	X		X	
HMA 3	X			X	X		X	

Tier 2

Determine the AUMs of sustainable forage available for WH&B use. Based on the desired level of utilization, propose an AML for WH&B (expressed as a range with an upper and lower limit).

Forage availability should be determined based on in-depth analysis of rangeland monitoring data. As a first step, monitoring data should be reviewed to determine if Land Health Standards or other site-specific vegetation management objectives are being met. If standards and objectives are being met, WH&B population estimates are then examined to determine the range in number of WH&B using the HMA during the evaluation years. The upper values are used to establish AML when no land health issues occur.

In HMAs where Land Health Standards or other objectives are not being achieved, WH&B use should be examined to determine if this level of use has contributed to or is the causal factor for the standards or objectives not being met (or only partly met). When standards or objectives are not being met and WH&B use is a contributing (or causal) factor, AML is proposed based on the estimated number of WH&B present relative to the level of forage utilization that is occurring (i.e., AML would be established at a number below that which has contributed to the standards or objectives not being met). The need for frequent emergency removals of WH&B due to lack of forage and/or water or the emigration of WH&B out of the HMA due to population size or concentration levels may also be a consideration in proposing AML for WH&B.

The sustainable forage (carrying capacity) available for WH&B use within a HMA is determined pending detailed analysis of utilization data and use pattern mapping for all users. For each evaluation year, determine the following: (1) weighted average utilization, (2) potential carrying capacity, and (3) the proposed carrying capacity. An example for the Mojave Desert ecosystem follows.

Example – Determining Sustainable Forage Available for WH&B Use (Mojave Desert Ecosystem)

The following example can be used to assist with establishing and/or adjusting AML for WH&B use. To determine if there is sufficient forage to sustain long-term WH&B use, analysis of utilization data, use pattern mapping, and/or production, ecological site condition, trend, frequency or other indicators of rangeland health is necessary.

Selection of Allowable Use Criteria

The recommended allowable utilization by all herbivores under year-round use within the Mojave Desert ecosystem is 25-35 percent of the current year's forage production.

WH&B use will be limited to 25 percent of current year's production on key vegetation species as measured at seed dissemination for these species.

Rationale: Within the Mojave Desert ecosystem, allowable use levels of 25 percent are recommended for areas in poor ecological condition or for areas grazed during the growing season. Allowable use levels of 35 percent are recommended for areas in good ecological condition or for areas grazed during the dormant season. Allowable use is established at 25 percent for this HMA due to year-round grazing by WH&B as well as a number of areas in poor ecological condition.

Determine Weighted Average Forage Utilization

Deriving a weighted average utilization from use pattern maps generally provides a more representative picture of the forage use that has occurred throughout the HMA and leads to a more accurate estimate of carrying capacity. To derive a weighted average utilization for the HMA, determine the number of acres within the HMA which received moderate, heavy or severe utilization for each evaluation year.[3] Refer to BLM Technical Reference 4400-7 for additional information.

The weighted average use is calculated by multiplying the number of acres by the mid-point of the utilization category (example: moderate use is classified as 41-60 percent, therefore, 50 percent is the mid-point used in the calculation).

Data used in the example carrying capacity analysis outlined below includes: utilization data, use pattern mapping data, actual livestock use information, and WH&B population estimates.

[3] To protect rangeland health over the long-term, consider WH&B use only on the primary range when deriving a weighted average utilization. Primary range is defined as the areas which animals (WH&B) prefer to use when management is limited (refer to Glossary of Terms Used in Range Management, 4th Edition, Published by Society for Range Management, 1998). Secondary range is defined as range which is not ordinarily used until the primary range has been overused. Therefore, areas receiving slight or light use or that are unused should not be considered when deriving a weighted average utilization.

Weighted Average Utilization =

(Moderate use acres*50 percent) + (Heavy use acres*70 percent) + (Severe use acres*90 percent)

Analyze the total acres of moderate, heavy, and severe use.

1995 $\frac{(19639*50\text{ percent}) + (34351*70\text{ percent})}{53{,}990}$ = 63 percent

1996 $\frac{(8023*50\text{ percent}) + (48957*70\text{ percent}) + (9885*90\text{ percent})}{66{,}865}$ = 71 percent

1997 $\frac{(3081*50\text{ percent}) + (22888*70\text{ percent})}{25{,}969}$ = 68 percent

1999 $\frac{(5627*50\text{ percent}) + (3696*70\text{ percent})}{9{,}323}$ = 58 percent

2000 $\frac{(4344*50\text{ percent}) + (4386*70\text{ percent})}{8{,}730}$ = 60 percent

2002 $\frac{(5627*50\text{ percent}) + (3696*70\text{ percent})}{9{,}323}$ = 58 percent

2004 $\frac{(4395*50\text{ percent}) + (7933*70\text{ percent})}{12{,}328}$ = 63 percent

The example below demonstrates the impact of variable precipitation (and forage production) on actual forage utilization and the desired carrying capacity.

Potential Carrying Capacity =

Average use in AUMs (of cattle or horse use)*25 percent Allowable Use Objective
Weighted Average Utilization

Year	Average Use (AUMs)		Effective Precipitation
1995	3000 AUMs*25 percent / 63 percent	= 1190 AUMs	Above Average
1996	600 AUMs*25 percent / 71 percent	= 211 AUMs	Severe Drought
1997	444 AUMs*25 percent / 68 percent	= 163 AUMs	Severe Drought
1999	600 AUMs*25 percent / 58 percent	= 259 AUMs	Severe Drought
2000	1440 AUMs*25 percent / 60 percent	= 600 AUMs	Slightly Below Average
2002	1632 AUMs*25 percent / 58 percent	= 703 AUMs	Average
2004	3576 AUMs*25 percent / 63 percent	= 1419 AUMs	Well Above Average

Proposed Carrying Capacity =

In the example above, the carrying capacity would be set in consideration of the driest years in order to prevent damage to the range or adverse impacts to animal health.

211 (1996) +163 (1997) +259 (1999) +600 (2000) +703AUMs (2002) = 387 AUMs
5 (# years of data)

Proposed AML =

387 AUMs (Desired Carrying Capacity) = 32 cattle or wild horses
12 months

Or:

$$\frac{\text{387 AUMs (Desired Carrying Capacity)}}{\text{6 months}} = 65 \text{ burros}$$

***Discussion*:**

- Allocation of the forage between WH&B, wildlife and domestic livestock would be made after site-specific NEPA analysis is completed.

- In the example above, the upper limit of the AML range for wild horses was calculated by dividing the proposed carrying capacity for the HMA (expressed in AUMs) by 12 months.

- The upper limit of the AML range for burros was calculated by dividing the proposed carrying capacity for the HMA by 6 months (an adult burro is equivalent to only 0.5 AU).

- The lower limit of the AML range would be determined based on the herd's average annual growth rate (i.e., the lower limit will normally be set at a number that allows the herd to grow to the upper limit over a 4-5 year period without the need for gathers to remove excess WH&B in the interim).

Tier 3

Determine whether or not the WH&B herd size proposed in Tier Two is sufficient to maintain genetically diverse WH&B populations (i.e., avoid inbreeding depression). To avoid inbreeding depression in wild horse populations, a minimum herd size of 50 effective breeding animals (a total population size of about 150-200 animals) is recommended. An effective breeding size for burro herds has not yet been determined.

If the herd size proposed in Tier Two is not sufficient to maintain genetically diverse WH&B populations, determine if there is WH&B interchange between the HMA and other adjacent HMAs or WHTs and whether this interchange would be sufficient to maintain genetic diversity (avoid inbreeding depression). Genetic diversity baseline or monitoring information can be used to evaluate whether WH&B interchange between HMAs is occurring.

If the proposed herd size is less than 150 animals and the HMA is isolated with limited potential for WH&B egress/ingress, possible management actions which could be considered as part of an appropriate site-specific NEPA analysis could include:

1. Removing the area's designation as an HMA through LUP and returning it to HA status.
2. Maximizing the number of breeding age WH&B in the herd (animals age 6-10 years).
3. Adjusting the sex ratio to favor males to encourage formation of additional breeding harems.
4. Introducing 1-2 young mares from outside the HMA every generation (about every 10 years). Introduced animals should come from herds living in similar environmental conditions.

Documentation

The results of the multi-tiered analysis should be documented in an HMA (AML) Evaluation Report. The completed report should be provided to the affected and interested public for a 30-day review and comment period (e.g., public scoping). The management evaluation report is not a decision subject to administrative appeal. However, once the decision is made, the Evaluation Report is part of the administrative record for that decision.

Following public scoping, a site-specific environmental analysis should be completed to analyze the environmental impacts associated with the Proposed AML and any alternatives. As part of the environmental analysis, summarize the results of public scoping and document how BLM used the comments in preparing the environmental assessment (or EIS).

Based on the analysis of potential environmental impacts, the authorized officer will determine whether to prepare an Environmental Impact Statement (EIS) or issue a Decision Record and Finding of No Significant Impact (FONSI). A FONSI documents why implementation of the selected AML will not result in environmental impacts that significantly affect the quality of the human environment. Or, if an EIS has been prepared, the authorized officer's decision will be documented in a Record of Decision.

Administrative Review

AML decisions are subject to administrative review (appeal) pursuant to 43 CFR Part 4.

APPENDIX 4 - Herd Management Area Planning

Herd management area plans (HMAPs) establish the management actions and the short and long-term management and monitoring objectives for WH&B herds and their habitat. Once established with public involvement through site-specific NEPA analysis and decision, the objectives guide management of the HMA or complex over the life of the plan.

Management Evaluation

Typically, the first step in the HMAP analysis process should be to evaluate existing management. The existing vs. the desired condition of the both the herd and its habitat are examined during the evaluation. Refer to Figures 4.1 and 4.2.

Figure 4.1
Habitat Management Evaluation

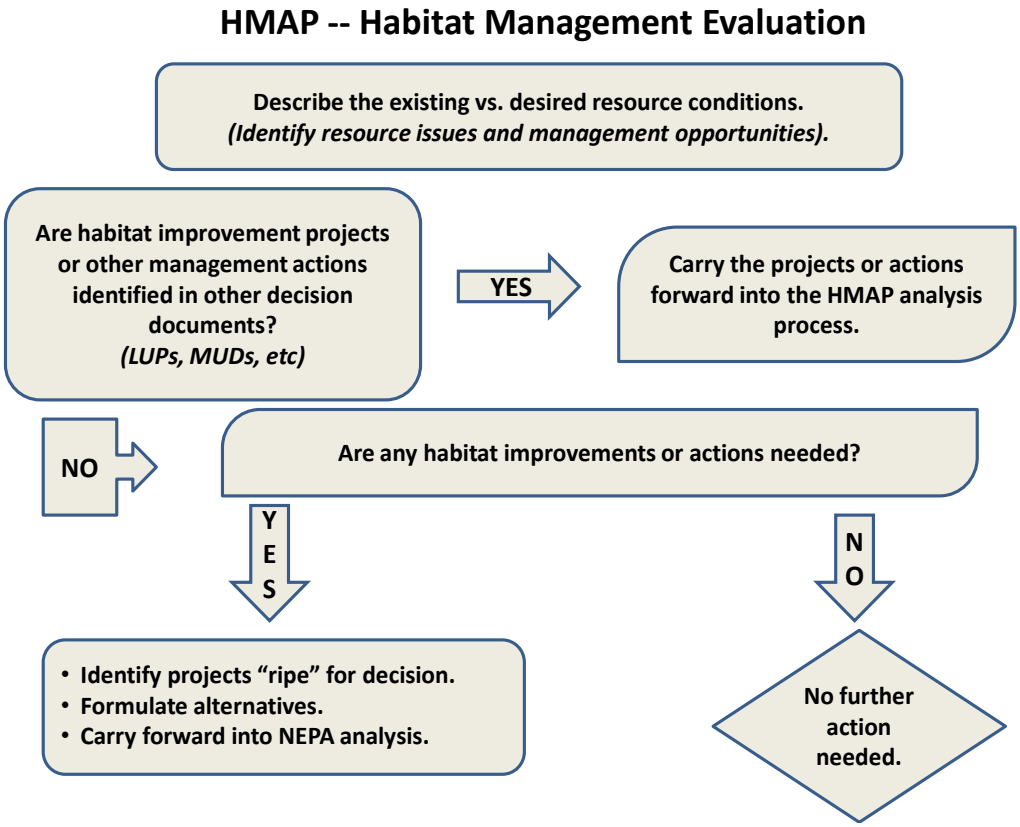
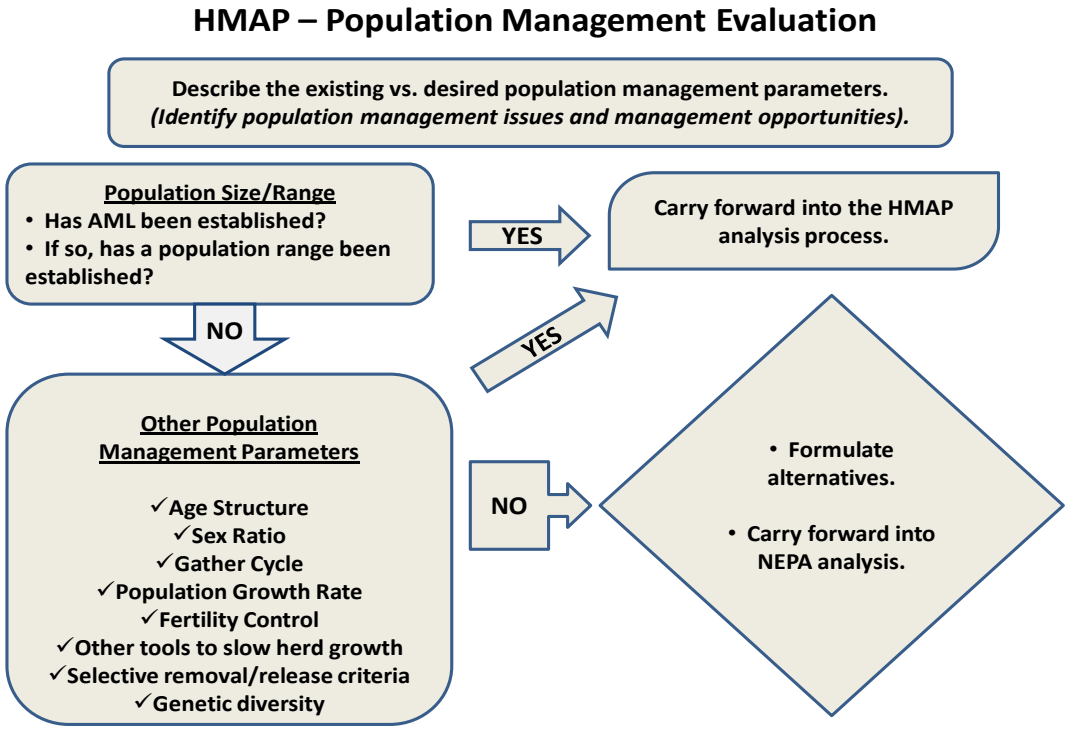


Figure 4.2
Population Management Evaluation



At the conclusion of the management evaluation, a report is prepared and provided to the public for a 30-day review and comment period (public scoping). The management evaluation report is not a decision subject to administrative appeal. Rather, the report documents the key issues identified as a result of the management evaluation. A range of the possible management alternatives (Proposed Action and Alternatives) which could address/resolve the identified issues may also be identified.

NEPA Analysis and Decision
The next step in the HMAP analysis process would be to analyze the potential environmental consequences which could result from implementation of the Proposed Action or Alternatives through completion of a site-specific environmental analysis (NEPA).

To prepare a concise environmental assessment (EA), site-specific management, monitoring and implementation objectives can be displayed in a tabular format within the environmental assessment. Refer to the example which follows.

Example – Documentation of the No Action Alternative in the HMAP EA

Alternative One: No Action Alternative, Continue Existing Management
Under Alternative 1 (No Action), the HMA would continue to be managed as a range of 150-250 wild horses as follows:

- The sex ratio of the animals released back to the range following future gathers would be about 34 percent males and 64 percent females.
- Existing monitoring, including utilization, forage condition, water availability, animal health and periodic population estimates and sampling for genetic diversity (inbreeding) would occur.
- AML would be adjusted, as needed, based on in-depth analysis of resource monitoring data.
- Fertility control would be applied to mares released back to the range following future gathers.

Table 4.1
No Action Alternative (Continue Existing Management) in HMAP Format

Management Objective(s)	Monitoring Objective(s)	Implementation Objective(s)
A. Control Population Numbers Manage the wild horse population within the established AML range to protect the range from the deterioration associated with overpopulation.	Aerially estimate populations a minimum of once every 3-4 years. Determine population number and average annual herd growth rate.	Schedule gathers to remove excess wild horses when (1) the population reaches or exceeds the AML upper limit (about every 3 years), or (2) when forage utilization exceeds 50 percent of current year's growth in ½-2/3 of the key areas; or (3) when animal health/condition is at risk.
B. Assure Land Health Limit utilization by all herbivores to 50 percent of the current year's above ground primary production for key grasses and 45 percent for key shrubs and forbs.	• Locate key monitoring areas within the HMA. • Establish baseline trend studies using the frequency sampling procedures outlined in the (cite applicable BLM Technical Reference or Handbook). • Measure utilization at key areas and complete use pattern mapping annually.	Re-adjust AML or identify management actions to address resolve land health issues, as needed/appropriate, based on results of land health monitoring.

Based on the analysis of potential environmental consequences, the authorized officer will determine whether to prepare an EIS or issue a Decision Record and FONSI.

HMAP
As a final step and to facilitate on-the-ground implementation, the selected management strategy (selected alternative), together with the habitat and population management, monitoring and implementation objectives are documented in HMAP format. The HMAP is included as an attachment to the authorized officer's final decision.

HMAP Format

1. Introduction. Briefly summarize the relevant background information.

2. Selected Management Alternative. Briefly summarize the selected management strategy/alternative as outlined in the EA, together with the site-specific habitat and population management objectives and management actions.

3. Monitoring and Evaluation Plan.

4. Project Tracking and Implementation Schedule.

The Monitoring Plan and Tracking Log/Project Implementation Schedule may also be prepared in a tabular format. See the examples which follow.

Table 4.2
Example -- Monitoring Plan

Item	How	Who	When	Actions to Take (Adaptive Management)
Population Management Monitoring				
Manage wild horse populations within the established AML range.	Aerial survey flights following established protocols. Simultaneous double count method or other approved protocol pending population estimation research results & recommendation.	FO WH&B Specialist	Aerial population estimates a minimum of every 3 years, i.e., 2010, 2013, etc.	Schedule gathers to remove excess wild horses when (1) the population reaches or exceeds the AML upper limit (about every 3 years), or (2) when forage utilization exceeds 50 percent of current year's growth in ½-2/3 of the key areas; or (3) when animal health/condition is at risk.
Habitat Management Monitoring				
Limit utilization by all herbivores to 50 percent of the current year's above ground primary production for key grasses and 45 percent for key shrubs and forbs.	Measure utilization at key areas and complete use pattern mapping annually.	FO WH&B Specialist	Annually (October)	Re-adjust AML or identify management actions to address resolve rangeland health issues, as needed/appropriate, based on results of rangeland health monitoring.

Table 4.3
Example – Tracking Log/Project Implementation Schedule

Description	Who	Where	When	Completed	Remarks
Population Management Actions					
Schedule gathers to remove excess wild horses when the population reaches/exceeds AML, utilization exceeds allowable limits, or animal health/condition is at risk.	BLM	(Name) HMA	About every 3 years (2010, 2013, etc)		
Habitat Management Actions					
Reconstruct Cactus Spring water development.	Friends of Spring Snails	T. 44 N., R 12 W, SE,SE Section 2	2009	2010	RIP No. XXXXXX

BLM

U.S. Department of the Interior
Bureau of Land Management

Final Environmental Assessment

Barren Valley Complex
Wild Horse Gather Plan

U.S. Department of the Interior
Bureau of Land Management
Vale District/Jordan Field Office
100 Oregon St., Vale, OR

NATIONAL SYSTEM OF PUBLIC LANDS

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1.0 Purpose of and Need for Action

1.1 Background

This Environmental Assessment (EA) is a site-specific analysis of the potential impacts that could result with the implementation of the Proposed Action or alternatives to the Proposed Action. Preparation of an EA assists the BLM authorized officer to determine whether to prepare an Environmental Impact Statement (EIS) if significant impacts could result, or a Finding of No Significant Impact (FONSI) if no significant impacts are expected.

The Barren Valley Complex comprises three Herd Management Areas (HMAs) in southeastern Oregon. Historically, the HMAs have been gathered both simultaneously and separately. Gathering the HMAs separately was not always successful due to wild horses crossing into adjoining HMAs during gather operations, therefore, the HMAs have been designated as a complex to be managed complete gather operations simultaneously.

Coyote Lake/Alvord-Tule Springs HMA comprises about 559,400 acres of public land. The HMA is located in Malheur and Harney Counties, west of Burns Junction, Oregon and east of Fields, Oregon (Map 1). The AML for wild horses within the HMA of 198-390 wild horses was reaffirmed in the Southeast Oregon Resource Management Plan Record of Decision (SEORMP/ROD, 2002). The HMA was last gathered in November 2008. The BLM is proposing to gather about 220 wild horses and remove approximately 50 excess wild horses from within and outside the Coyote Lake/Alvord-Tule Springs HMA beginning in about September 2011. One hundred ninety-eight wild horses would remain in the HMA after the gather; of these, about 80 would be mares treated with fertility control and about 120 would be studs or geldings to adjust the sex ratio and slow population growth.

The Sheepshead/Heath Creek HMA comprises about 204,500 acres of public land. The HMA is located in Malheur and Harney Counties, immediately northwest of Burns Junction, Oregon (Map 1). The AML for wild horses within the HMA is 161-302 wild horses. The AML was reaffirmed in the Andrews Management Unit Record of Decision and Resource Management Plan (AMURMP/ROD, 2005). The HMA was last gathered in November 2008. The BLM is proposing to gather about 350 wild horses and remove approximately 200 excess wild horses from within and outside the Sheepshead/Heath Creek HMA beginning in about September 2011. One hundred sixty-one wild horses would remain in the HMA after the gather; of these, about 60 would be mares treated with fertility control and about 100 would be studs or geldings to adjust the sex ratio and slow population growth.

The Sand Springs HMA comprises about 192,524 acres of public land. The HMA is located in Malheur County, immediately northeast of Burns Junction, Oregon and east of Highway 78 (Map 1). The AML for wild horses within the HMA is 100-200 wild horses. The AML was established in Southern Malheur Management Framework Plan (MFP) (1975) and was reaffirmed in the SEORMP/ROD (2002). The HMA was last gathered in September 2006. Currently, the numbers of wild horses residing in Sand Springs HMA is slightly below the low end of AML, therefore, the BLM is proposing to gather about 80 wild horses and remove only those animals that cross into Sand Springs HMA from Sheepshead/Heath Creek HMA during gather operations beginning in September 2011. There have also been approximately 25 wild horses observed outside of the Sand Springs HMA on public lands not designated as a herd area. Any wild horses residing outside the Sand Springs HMA would be gathered and removed. The current population of approximately 90 wild horses would remain in the HMA after the gather; of these, about 40 would be mares treated with fertility control and about 60 would be studs or geldings to adjust the sex ratio and slow population growth.

Since the previous gather in 2008, wild horses have concentrated in the Sheepshead/Heath Creek HMA and in Red Mountain North Pasture of the Coyote Lake/Alvord-Tule Springs HMA. There has also been movement into the Sand Springs HMA do to overcrowding in the Sheepshead/Heath Creek HMA. Based upon all information available at this time, the BLM has determined that approximately 250 excess wild horses exist within the Complex and need to be removed. In addition, approximately 25 wild horses exist south of the Sand Springs HMA that need to be gathered and removed. This assessment is based on the following factors including, but not limited to:

- An estimated population of 700 wild horses exists in the Barren Valley Complex in 2011 with approximately 200 horses in excess of the AML lower limits in Sheepshead/Heath Creek HMA and approximately 50 horses in excess of the AML lower limits in Coyote Lake/Alvord-Tule Springs HMA.
- Use by the approximately 25 wild horses residing south of the Sand Springs HMA is not within a herd area and does not comply with existing land use plans.
- Use by wild horses in Sheepshead/Heath Creek HMA is exceeding the maximum forage allocated to their use by approximately 10 - 30% in 2011.
- Riparian monitoring completed in 2008 and 2010 documents severe utilization of forage within riparian habitats, and extensive trampling and trailing damage by wild horses in the entire complex, but especially in Sheepshead/Heath Creek HMA and Red Mountain North Pasture of the Coyote Lake/Alvord-Tule Springs HMA.

1.2 Purpose of and Need for Action

The purpose and need for the proposed action is to meet the established objectives and goals of the SEORMP/ROD and AMURMP/ROD by maintaining the AML for the HMAs in the Barren Valley Complex (SEORMP/ROD, 2002, 55-57 and AMURMP/ROD, 2005 50-53). The purpose of the action is to gather and remove excess wild horses from the Barren Valley Complex. This action is necessary to maintain a thriving natural ecological balance which protects public land resources from deterioration. During the most recent field monitoring in 2010 and 2011, the wild horse population in the complex is concentrated in the Sheepshead/Heath Creek HMA and Red Mountain North Pasture of Coyote Lake/Alvord-Tule Springs HMA. This has created heavy to severe utilization of riparian and upland vegetation in and adjacent to perennial streams, springs, and reservoirs. Resource damage is occurring in some areas of the HMA due to the current overpopulation of wild horses, and is likely to continue to occur as well as increase without immediate action.

This action is needed in order to achieve and maintain a population size within the established AML, protect rangeland resources from further deterioration associated with the current overpopulation, and restore a thriving natural ecological balance and multiple use relationship on public lands in the area consistent with the provisions of Section 3(b)(2) of the Wild Free-Roaming Horses and Burros Act (WFRHBA) of 1971.

1.3 Land Use Plan Conformance

The Action Alternatives are tiered to the SEORMP/FEIS (2001, Chapter 3 242-246) and AMURMP/FEIS (2004, Chapter 4 171-183) and are in conformance with decisions made in the SEORMP/ROD (2002, 55-57) and AMURMP/ROD (2005, 50-53). Objectives identified for wild horse herds in these documents include (1) maintaining and managing HMAs at AMLs to ensure a thriving natural ecological balance between wild horse populations, wildlife, livestock, vegetation resources, and other resource values, and (2) enhancing and perpetuating special and unique characteristics that distinguish the herd.

1.4 Relationship to Laws, Regulations, and Other Plans

Statutes and Regulations

This action is governed by the WFRHBA of 1971 (Public Law (PL) 92-195 as amended) and Title 43 Code of Federal Regulations (CFR) part 4700. Gathering and disposal of the wild horses would be in accordance with PL 92-195 as amended by PL 94-579 (Federal Land Policy and Management Act (FLPMA)) and PL 95-514 (Public Rangelands Improvement Act (PRIA)). Included are:

- ❒ **43 CFR 4710.3-1 Herd management areas.**
  Herd management areas shall be established for the maintenance of wild horse and burro herds. In delineating each herd management area, the authorized officer shall consider the appropriate management level for the herd, the habitat requirements of the animals, the relationships with other uses of the public and adjacent private lands, and the constraints contained in 4710.4. The authorized officer shall prepare a herd management area plan, which may cover one or more herd management areas.
- ❒ **43 CFR 4710.4 Constraints on management.**
  Management of wild horses and burros shall be undertaken with limiting the animals' distribution to herd areas. Management shall be at the minimum feasible level necessary to attain the objectives identified in approved land use plans and herd management area plans.
- ❒ **43 CFR 4720.1 Removal of excess animals from public lands.**
  Upon examination of current information and a determination by the authorized officer that an excess of wild horses or burros exists, the authorized officer shall remove the excess animals immediately.
- ❒ **43 CFR 4740.1 Use of motor vehicles or aircraft.**
  (a) Motor vehicles and aircraft may be used by the authorized officer in all phases of the administration of the Act, except that no motor vehicle or aircraft, other than helicopters, shall be used for the purpose of herding or chasing wild horses or burros for capture or destruction. All such use shall be conducted in a humane manner.
  (b) Before using helicopters or motor vehicles in the management of wild horses or burros, the authorized officer shall conduct a public hearing in the area where such use is to be made.

1.5 Conformance with Rangeland Health Standards and Guidelines

As stated in 43 CFR 4180.2(b) - "Standards and guidelines must provide for conformance with the fundamentals of 43 CFR 4180.1." The Standards and Guidelines for Grazing Management for public lands have been reviewed by the Departmental Review Team who found that they comply with the requirements of the regulations. Gathering excess horses conforms to the standards and guides which were developed with full public participation and in consultation with Oregon/Washington's resource advisory councils and are in conformance with appropriate land use plans.

1.6 Decision to be Made

The authorized officer would determine whether to implement the proposed population control measures in order to achieve and maintain population size within the established AML and prevent the further deterioration of rangeland and riparian resources resulting from the current wild horse overpopulation. The authorized officer's decision would not set or adjust AML nor would it adjust livestock use, as these were set through previous decisions.

2.0 Proposed Action and Alternatives

2.1 Introduction

This section of the EA describes the Proposed Action and alternatives, including any that were considered but eliminated from detailed analysis. Five alternatives are considered in detail:

- Alternative 1: Proposed Action – Capture wild horses in order to remove approximately 250 excess wild horses, remove approximately 25 outside horses, apply PZP-22 fertility control vaccine to released mares, and establish a 60% male sex ratio.
- Alternative 2: – Capture wild horses in order to remove approximately 250 excess wild horses and remove approximately 25 outside horses (no fertility control or sex ratio adjustment).
- Alternative 3: Capture wild horses in order to remove approximately 250 excess wild horses, remove approximately 25 outside horses, and apply PZP-22 fertility control vaccine to released mares.
- Alternative 4: Capture wild horses in order to remove approximately 250 excess wild horses, remove approximately 25 outside horses, and establish a 60% male sex ratio.
- Alternative 5: No Action — Defer gather and removal.

The Proposed Action, Alternative 2, Alternative 3 and Alternative 4 were developed to respond to the identified resource issues and the Purpose and Need to differing degrees. The No Action Alternative would not achieve the identified Purpose and Need. However, it is analyzed in this EA to provide a basis for comparison with the other action alternatives, and to assess the effects of not conducting a gather at this time. The No Action Alternative is in violation of the WFRHBA which requires the BLM to immediately remove excess wild horses.

2.2 Description of Alternatives Considered in Detail

2.2.1 Management Actions Common to Alternatives 1-4

- ❑ The gather would begin about September 2011 and take about 20 days to complete. Several factors such as animal condition, herd health, weather conditions, or other considerations could result in adjustments in the schedule.
- ❑ Gather operations would be conducted in accordance with the Standard Operating Procedures (SOPs) described in the National Wild Horse and Burro Gather Contract (Appendix A). The primary gather (capture) methods would be the helicopter drive method with occasional helicopter assisted roping (from horseback).
- ❑ Trap sites and temporary holding facilities will be located in previously used sites or other disturbed areas whenever possible. Undisturbed areas identified as potential trap sites or holding facilities would be inventoried for cultural resources. If cultural resources are encountered, these locations would not be utilized unless they could be modified to avoid impacts to cultural resources.
- ❑ An Animal and Plant Inspection Service (APHIS) or other veterinarian may be on-site during the gather, as needed, to examine animals and make recommendations to BLM for care and treatment of wild horses.
- ❑ Decisions to humanely euthanize animals in field situations will be made in conformance with BLM policy (Washington Office Instruction Memorandum 2009-041). Current policy reference: http://www.blm.gov/wo/st/en/info/regulations/Instruction_Memos_and_Bulletins/national_instruction/2009/IM_2009-041.html
- ❑ Data including sex and age distribution, condition class information (using the Henneke rating system), color, size and other information may also be recorded, along with the disposition of that animal (removed or released).
- ❑ Hair samples would be collected on about 50-100 animals to assess the genetic diversity of the herd. Samples would also be collected during future gathers as needed to determine whether BLM's management is maintaining acceptable genetic diversity (avoiding inbreeding depression).
- ❑ Excess animals would be transported to the Burns BLM corral facility where they will be prepared (freeze-marked, vaccinated and de-wormed) for adoption, sale (with limitations) or long-term holding.
- ❑ Temporary closure of roads within the HMA during gather operations may be instituted as necessary to allow for safe and effective operations to proceed.

2.2.2 Alternative 1. Proposed Action

The Proposed Action would gather about 650 and remove approximately 275 excess wild horses from within and outside the Barren Valley Complex beginning about September 2011. Animals would be removed using a selective removal strategy. Selective removal criteria for the HMA include: (1) First Priority: Age Class – Four Years and Younger; (2) Second Priority: Age Class – Eleven to Nineteen Years (3) Third Priority: Age Class Five to Ten Years 4) Fourth Priority: Age Class Twenty Years and Older should not be removed from the HMA unless specific exceptions prevent them from being turned back to the range. Irrespective of their age class, all animals residing outside the HMA would be removed. Up to 559 of the captured wild horses would be released; of these, about 223 would be mares of which most would be treated with fertility control and about 336 would be studs (or geldings) as follows:

- ❒ Mares would be treated with a two-year Porcine Zona Pellucida (PZP-22) or similar vaccine and released back to the range. Fertility control treatment would be conducted in accordance with the approved standard operating and post-treatment monitoring procedures (SOPs, Appendix B). Mares would be selected to maintain a diverse age structure, herd characteristics and conformation (body type).
- ❒ Studs and geldings would be selected for release with the objective of establishing a 60% male sex ratio. Studs and geldings would be selected to maintain a diverse age structure, herd characteristics and body type (conformation).
- ❒ Post-gather, every effort would be made to return released horses to the same general area from which they were gathered.

2.2.3 Alternative 2: Removal Only

Alternative 2 would gather about 650 and remove approximately 275 excess wild horses from within and outside the Barren Valley Complex beginning about September 2011. Fertility control would not be applied and no changes to the herd's existing sex ratio would be made.

2.2.4 Alternative 3: Removal with Fertility Control

Alternative 3 would gather about 650 and remove approximately 275 excess wild horses from within and outside the Barren Valley Complex beginning about September 2011. Fertility control would be applied, but no changes to the herd's existing sex ratio would be made.

2.2.5 Alternative 4: Removal with Sex Ratio Adjustment

Alternative 4 would gather about 650 and remove approximately 275 excess wild horses from within and outside the Barren Valley Complex beginning about September 2011. The herd's existing sex ratio would be adjusted to establish a 60% male sex ratio. Fertility control would not be applied.

2.2.6 Alternative 5: No Action

Under the No Action Alternative, no gather would occur and no additional management actions would be undertaken to control the size of the wild horse population at this time.

2.3 Summary Comparison of Alternatives

Table 1: Summary Comparison of the Alternatives

Item	Alternative 1: Proposed Action	Alternative 2: Removal Only	Alternative 3: Removal and Fertility Control only	Alternative 4: Removal and Sex Ratio only	Alternative 5: No Action
Impacts to Wild Horses					
• Gather Number	650	650	650	650	0
• Removal Number	275	275	275	275	0
• Fertility Control - # Mares	200	0	200	0	0
• Post-Gather Sex Ratio	60/40	50/50	50/50	60/40	0
• Post-Gather Population Size by HMA	CLAT 198 SHHC 161 SS 90	CLAT 198 SHHC 161 SS 90	CLAT 198 SHHC 161 SS 90	CLAT 198 SHHC 161 SS 90	CLAT 255 SHHC 350 SS 90

2.4 Alternatives Considered but Dismissed from Detailed Analysis

2.4.1 Use of Bait and/or Water Trapping

It would not be timely, cost-effective or practical to use bait and/or water trapping as the primary gather method because the number of water sources on both private and public lands within and outside the HMA would make it almost impossible to restrict wild horse access to the selected water trap sites. When water sources become limited, accessibility to these water sources is extremely remote, thereby, becoming an unpractical and economically unfeasible method of gathering horses. As a result, this alternative was dismissed from detailed analysis.

2.4.2 Remove or Reduce Livestock within the HMA

This alternative was not brought forward for detailed analysis because it is outside the scope of the analysis and it is contrary to previous decisions which allocated forage for livestock use. Such an action would not be in conformance with the existing land use plan, would be contrary to the BLM's multiple-use mission as outlined in the 1976 Federal Land Policy and Management Act (FLPMA), and would also be inconsistent with the WFRHBA which directs the Secretary to immediately remove excess wild horses.

2.4.3 Gather the HMA to the AML Upper Limit

This alternative was dismissed from detailed study because AML would be exceeded the foaling season following the gather in 2011. This would result in the need to follow up with another gather within one year, and increased stress to individual wild horses and the herd and continuing resource damage due to wild horse overpopulation in the interim. Nor would this alternative be consistent with the WFRHBA, which upon determination excess wild horses are present, requires their immediate removal.

2.4.4 Fertility Control Treatment Only (No Removal)

Population modeling was completed to analyze the potential impacts associated with conducting gathers about every 2-3 years over the next 20 year period to treat captured mares with fertility control. Under this alternative, no excess wild horses would be removed. While the average population growth would be reduced for the next couple of years, AML would not be achieved and the damage to the range associated with wild horse overpopulation would continue. This alternative would not meet the Purpose and Need for the Action, and would be contrary to the WFRHBA, and was dismissed from further study.

3.0 Affected Environment

3.1 General Description of the Affected Environment

The Barren Valley Complex encompasses approximately 956,000 acres of public land within Malheur and Harney Counties, Oregon (Map 1). The complex is made up of three HMAs; Coyote Lake/Alvord-Tule Springs, Sheepshead/Heath Creek, and Sand Springs HMAs. The complex is partially located in the Owyhee uplands with the Owyhee River the east boundary of the Sand Springs HMA. Highway 78 is the boundary between the Sheepshead/Heath Creek and Sand Springs HMAs. The rest of this complex is located in the Alvord Desert and the eastern foothills of the Steens Mountains.

Precipitation in the Barren Valley Complex averages 6 inches in the Steens Mountain rainshadow to 12 inches on the top of the Sheepshead Mountains. Most of this precipitation comes during the winter and spring months in the form of snow, supplemented by localized thunderstorms during the summer months. The primary native vegetation varies throughout the allotment from salt desert vegetation communities to shrub-steppe communities. There is a variety of big sagebrush, low sagebrush, and perennial grasses.

3.2 Description of Affected Resources/Issues

Table 2 lists the elements of the human environment subject to requirements in statute, regulation, or executive order which must be considered.

Table 2: Supplemental Authorities and Other Elements

Supplemental Authorities	Present	Affected	Rationale
ACECs	**YES**	**NO**	Saddle Butte and Palomino Playa ACECs in Sand Springs HMA on the Vale District. Alvord Desert, Mickey Hot Springs, Mickey Basin, Serrano Point, and Borax Lake ACECs in Coyote Lake/Alvord-Tule Springs HMA on the Burns District. To prevent any impacts to ACECs, trap sites and temporary holding facilities would be located in previously disturbed areas. Use of trap sites or holding facilities outside existing areas of disturbance would not be located in areas with existing ACECs.
Air Quality	**YES**	**NO**	The planning area is outside a non-attainment area. Implementation of the Proposed Action would result in small and temporary areas of disturbance.
Cultural Resources	**YES**	**NO**	To prevent any impacts to cultural resources, trap sites and temporary holding facilities would be located in previously disturbed areas. Cultural resource surveys would be conducted prior to using trap sites or holding facilities outside existing areas of disturbance.
Environmental Justice	**NO**	**NO**	Not present.
Fish Habitat	**NO**	**NO**	Not present.
Floodplains	**NO**	**NO**	Not present.
Forest and Rangelands	**YES**	**YES**	Discussed below.
Human Safety	**YES**	**NO**	Implementing the road closures identified in Section 2.2 would eliminate the impacts to human safety created by the proposed action.
Migratory Birds	**NO**	**NO**	Not present.
Native American Religious Concerns	**NO**	**NO**	There are no known Native American Religious Concerns regarding this project.
Noxious Weeds	**YES**	**NO**	To prevent the risk for spread, any noxious weeds or non-native invasive weeds would be avoided when establishing and accessing trap sites and holding facilities.
Prime or Unique Farmlands	**NO**	**NO**	Not present.
Riparian-Wetland Zones	**YES**	**YES**	Discussed below.
T&E Species	**YES**	**YES**	Discussed below.
Water Quality	**YES**	**NO**	Locate trap sites and temporary holding facilities away from any riparian areas to avoid impacts to water quality.
Waste (Hazardous or Solid)	**NO**	**NO**	Not present.
Wild and Scenic Rivers	**NO**	**NO**	Not present.
Wilderness and Wilderness Study Area	**YES**	**NO**	Wilderness Study Areas of: Palomino Hills (3-114) and Saddle Butte (3-111) are located within the Vale District; Wildcat Canyon (2-72C) and Sheepshead Mountain (2-72D) are located within the Vale and shared with the Burns District are located within the HMA. To prevent any impacts to WSA values, trap sites and temporary holding facilities would be located in previously disturbed areas. Use of trap sites or holding facilities outside existing areas of disturbance would not be located in areas with existing WSA values.

In addition to the critical elements listed in Table 2, the following resources may be affected by the Action Alternatives and/or the No Action Alternative. The existing situation (affected environment) relative to these resources is described below.

3.2.1 Wild Horses

Coyote Lake/Alvord-Tule Springs HMA

The Coyote Lake/Alvord-Tule Springs HMA is comprised of portions of four allotments in both Vale and Burns District BLM. The topography of the HMA ranges from relatively flat to mountainous. Elevation varies from approximately 3,900 to 6,100 feet, with the southern end of the Sheepshead Mountains being the highest prominent landmark.

The area's designation as a herd management area was analyzed in the SEORMP/FEIS (2001). AML is established at a population range of 198 - 390 wild horses. Forage is allocated for 4680 animal unit months (AUMs). Inventory data show the majority of wild horses historically concentrating on Red Mountain, Tule Springs, and Mickey Basin throughout the spring, summer, and fall as water sources and forage become scarce. During most winters, horses tend to disperse across the lower elevations in the HMA as water becomes available in potholes and playas.

The current estimated population of 250 wild horses in the Coyote Lake/Alvord-Tule Springs HMA is based on a direct count aerial population survey completed in April 2010. Calculation of population growth has been difficult to determine by individual HMA as horses continually move into adjacent HMAs. The current population is approximately 50 horses over the AML lower limit.

In the early 1970's, wild horses within the Coyote Lake portion of the HMA were very varied in color while the Alvord-Tule Springs portion of the HMA was dominated by bay, black, brown, sorrel, palomino, and buckskin. Adult horses in the HMA weigh an average of 950 to 1150 pounds and stand between 14.3 and 15.3 hands, with some stallions being slightly larger. The horses exhibit saddle stock conformation with quarter horse and thoroughbred influence.

Baseline genetic diversity samples were taken in 2001. These samples indicate that genetic variability within the Coyote Lake/Alvord-Tule Springs HMA is moderate to good. The herd appears to be of mixed origins from light racing and riding breeds which includes the thoroughbred and quarter horse. In comparison with other Oregon herds, the Coyote Lake/Alvord-Tule Springs herd shows closest resemblance to the Jackies Butte and Paisley herds.

The last removal of excess wild horses from the Coyote Lake/Alvord-Tule Springs HMA was completed in November 2008 when 393 horses were gathered and 244 were removed. Seventy-four mares were fertility control treated and released with 70 stallions/geldings for a post-removal 50/50 % male/female sex ratio.

Sheepshead/Heath Creek HMA

The Sheepshead/Heath Creek HMA is comprised of portions of two allotments in both Vale and Burns District BLM. The topography of the HMA ranges from relatively flat to mountainous. Elevation varies from approximately 3,900 to 6,300 feet, with the northern end of the Sheepshead Mountains being the highest prominent landmark. Approximately 20% of this HMA burned in the Sheepshead fire in 2001. There have been several smaller fires within this area from the 1980's to present. The location of the Sheepshead burn is where most of the horses are currently congregated within the HMA.

The area's designation as a herd management area was analyzed in the AMURMP/FEIS (2004). AML is established at a population range of 161-302 wild horses. Forage is allocated for 3624 AUMs. Inventory data show the majority of wild horses historically concentrating on the north end of the Sheepshead Mountains, Bone Springs Canyon, and Wildcat Canyon yearlong.

The current estimated population of 350 wild horses in the Sheepshead/Heath Creek HMA is based on a direct count aerial population survey completed in April 2010. Calculation of population growth has been difficult to determine by individual HMA as horses continually move into adjacent HMAs, but the overall population growth in the Barren Valley Complex has been over 20% since the previous gather. The increase in numbers in Sheepshead/Heath Creek HMA indicates there has most likely been movement from adjacent HMAs. The current population in this HMA is approximately 190 horses over the AML lower limit.

In the early 1970's, wild horses within the Sheepshead portion of the HMA were very varied in color while the Heath Creek portion of the HMA was dominated by dun, bay, black, brown, sorrel, and an occasional paint. Adult horses in the HMA weigh an average of 950 to 1150 pounds and stand between 14.3 and 15.3 hands, with some stallions being slightly larger. The horses exhibit saddle stock conformation with quarter horse and thoroughbred influence.

Baseline genetic diversity samples were taken in 2002. These samples indicate that genetic variability within the Sheepshead/Heath Creek HMA is low. The herd appears to be of mixed origins from North American gaited breeds and occasional draft horse influence. In comparison with other Oregon herds, this HMA shows closest resemblance to the Paisley HMA.

The last removal of excess wild horses from the Sheepshead/Heath Creek HMA was completed in November 2008 when 233 horses were gathered and 145 were removed. Forty-six mares were fertility control treated and released with 43 stallions/geldings for a post-removal 40/60% male/female sex ratio.

Since the Barren Valley Complex was identified as individual HMAs in the 1970's, management and gathers have occurred independently and cooperatively. Therefore, the table below reflects the gather history for all of the HMAs in the Barren Valley Complex except for Sand Springs HMA.

Table 3: Coyote Lake/Alvord-Tule Springs and Sheepshead/Heath Creek Gather History

HMA	Year	Captured	Removed	Released	Died/Euthanized
Coyote Lake/Alvord-Tule Springs	2008	393	244	184	4
Sheepshead/Heath Creek	2008	212	145	84	1
Coyote Lake/Alvord-Tule Springs	2005	367	367		
Sheepshead/Heath Creek	2002	320	257	63	
Coyote Lake/Alvord-Tule Springs	2001	303	303		
Alvord-Tule Springs	1997	113	113		
Coyote Lake	1996	151	134		
Sheepshead	1994	190	190		
Sheepshead	1992	115	115		
Coyote Lake	1991	203	203		
Alvord/Barren Valley/Sheepshead/Tule Springs	1988	6	6		
Sheepshead	1988	214	214		
Coyote Lake	1987	5	5		
Coyote Lake	1986	547	545	0	2
Alvord/Barren Valley/Sheepshead/Tule Springs	1985	207	207	0	0
Sheepshead	1985	258	258	0	0
Sheepshead /Heath Creek	1984	389	388	0	1
Alvord/Barren Valley/Sheepshead/Tule Springs	1981	338	336	0	2
Barren Valley/Sheepshead/Alvord	1979	518	510	5	3
Sheepheads/Coyote Lake	1977	599	598	0	1
Barren Valley/Sheepshead/Alvord	1976	8	8	0	0
Barren Valley/Sheepshead/Alvord	1975	20	20	0	0
Barren Valley/Sheepshead/Alvord	1974	38	35	0	3

Sand Springs HMA

The Sand Springs HMA is comprised of portions of two allotments in the Vale District. The topography of the HMA ranges from relatively flat to river canyon country. Elevation varies from approximately 3,900 to 4,500 feet, with Saddle Butte being the highest prominent landmark just outside the north boundary of the HMA. Approximately 35% of this HMA burned in the Happy Valley fire in 2006. There have been several smaller fires within this area from the 1980's to present. The location of the Happy Valley fire is where the perennial upland water sources are located in the HMA, thereby being in the heart of summer range and habitat.

The area's designation as a herd management area was analyzed in the SEORMP/FEIS (2001). AML is established at a population range of 100-200 wild horses. Forage is allocated for 2400 AUMs. Inventory data show the majority of wild horses historically concentrating on the rims above the Owyhee River during the winter and around the few perennial springs during the spring, summer, and fall.

The current estimated population of 90 wild horses in the Sand Springs HMA is based on a direct count aerial population survey completed in April 2010. Calculation of population growth has been difficult to determine by individual HMA as horses continually move into adjacent HMAs, but the increase in numbers in this HMA indicates there has most likely been movement from adjacent HMAs. The current population in this HMA is approximately 10 horses under the AML lower limit.

In the early 1970's, wild horses within the Sand Springs HMA were very predominantly pinto and buckskin colors. Adult horses in the HMA weigh an average of 950 to 1050 pounds and stand between 14.2 and 15.2 hands, with some stallions being slightly larger. The horses exhibit saddle stock conformation with quarter horse and thoroughbred influence.

Baseline genetic diversity samples have not been analyzed for this herd.

The last removal of excess wild horses from the Sand Springs HMA was completed in September 2006 when 130 horses were gathered and 115 were removed in an emergency gather following a large wildfire.

Table 4: Sand Springs Gather History

HMA	Year	Captured	Removed	Released	Died/Euthanized
Sand Springs	2006	130	115	15	5
Sand Springs	2005	280	220	60	2
Sand Springs	2000	194	138	56	1
Sand Springs	1992	189	189		
Sand Springs	1988	208	208		
Sand Springs	1985	327	325	0	2
Sand Springs	1981	350	246	0	4
Sand Springs	1977	280	277	0	3

Most of the wild horses observed in the Barren Valley Complex in 2010 were a Body Condition Score of 4-6 using the Henneke Body Condition Chart.

3.2.2 Grazing Management

Coyote Lake/Alvord-Tule Springs HMA

The Coyote Lake portion of the HMA is located within the Coyote Lake Allotment and part of the Whitehorse Butte Allotment in the Vale Distrit. Coyote Lake has two livestock operators. One operator is authorized to utilize 344 active AUMs from November 16 to February 14 and the other operator is authorized to utilize 3014 active AUMs and 1945 exchange of use AUMs from October 1 to January 31. Livestock grazing reductions

have been made on a voluntary basis by past owners of the White Horse Ranch in the Red Mountain North pasture due to lack of water and impacts on Willow Creek.

The BLM allocated forage for livestock use in the SEORMP/ROD (2002,p. E-181). The allocation was carried forward from the Southern Malheur Rangeland Program Summary Update (December 1986). The allocation will be revisited during activity planning associated with evaluation and assessment within Barren Valley Geographic Management Area as described in the SEORMP.

The Alvord-Tule Springs portion of the HMA is located within the Alvord Allotment in the Burns District. There are four pastures within the allotment. Three of the pastures are authorized as spring/summer use and one pasture is authorized as winter use. There is one livestock operator authorized to graze a total of 7,355 AUMs of annually from December 1 to April 15 on the winter pasture and from April 16 to June 30 on the spring pastures.

Forage for livestock use was most recently allocated by the BLM in the 2005 AMURMP/ROD. The allocation will be revisited during activity planning associated with evaluation and assessment within the Alvord Allotment as described in the Andrews RMP.

Table 5 summarizes information about livestock grazing and its relationship to wild horse management within the Coyote Lake/Alvord-Tule Springs HMA.

Table 5: Livestock Use Information

Allotment	Total Allotment Acres	% of Allotment in HMA	Number of Permittees	Number of Authorized Livestock	Authorized Season of Use	Authorized Livestock AUMs in Allotment	Average Actual Livestock Use (AUMs) (Past 5 years)
Coyote Lake	162,858 PD 15,500 Pvt 80 State	100%	2	Cattle	10/01-02/14	3,358	414 minimum 1,737 average 4,257 maximum
Whitehorse Butte	124,821 PD 14,544 Pvt 166 State	19%	1	Cattle	03/16-08/31	9,287	5152 average 3130 minimum 8089 maximum
Alvord	223,895 PD 5,600 Pvt	38%	1	700 Cattle 1254 cattle	4/16 – 6/30 12/01-4/15	7,355	1660 minimum 6035 average 8859 maximum

Sheepshead/Heath Creek HMA

The Sheepshead portion of the HMA is located within the Sheephead Allotment in the Vale District. There is one operator authorized to graze cattle annually, and who is authorized to utilize 3,949 active Animal Unit Months (AUMs) of forage within the allotment each year between March 01 and February 28. Currently there is no Allotment Management Plan (AMP) for the Sheephead Allotment. There is also no established grazing system within the Sheephead Allotment; however the permittee rotates his cattle around four unfenced use areas which are Palomino Hills, East and West Ryegrass and Sheephead.

The BLM allocated forage for livestock use in the SEORMP/ROD (2002, p. E-178). The allocation was carried forward from the Southern Malheur Rangeland Program Summary Update (December 1986). The allocation will be revisited during activity planning associated with evaluation and assessment within Barren Valley Geographic Management Area as described in the SEORMP.

The Heath Creek portion of the HMA is located within the Pollock Allotment in the Burns District. There are seven pastures within the allotment. Six of the pastures are authorized as spring/summer use and one pasture is authorized as winter use. There is one livestock operator authorized to graze a total of 4107 AUMs annually between November 15 to March 31 on the winter pasture and from April 1 to September 15 on the spring/summer pastures.

Table 6 summarizes information about livestock grazing and its relationship to wild horse management within the Sheepshead/Heath Creek HMA.

Table 6: Livestock Use Information

Allotment	Total Allotment Acres	% of Allotment in HMA	Number of Permittees	Number of Authorized Livestock	Authorized Season of Use	Authorized Livestock AUMs in Allotment	Average Actual Livestock Use (AUMs) (Past 5 years)
Sheepshead	152,037 PD 1,102 Pvt 602 State	100%	1	597 Cattle	03/01-02/28	3,949	3,966 minimum 4,057 average 4,233 maximum
Pollock	76,758 PD 4,895 Pvt 5,561 State	32%	1	520 Cattle 530 cattle	4/01 – 9/15 11/15-3/31	4107	1431 minimum 4305 average 4783 maximum

Sand Springs HMA

The Sand Springs HMA is located primarily within the Saddle Butte Allotment in the Vale District. There are a total of seven livestock operators authorized to graze cattle annually. The operators are authorized to utilize 6,314 Animal Unit Months (AUMs) of forage within the allotment each year between November 1 and March 31. An AUM is the amount of forage needed to sustain one cow, five sheep, or five goats for a month. The allotment is comprised of one pasture which is grazed by livestock. The Saddle Butte Allotment Management Plan (AMP) acknowledged that no formal grazing system was developed given that the allotment was to be grazed during the winter. Within the Saddle Butte Allotment the approximate growth period for the key forage species, bottlebrush squirreltail *(Elymus elymoides)*, is March 25 through August 1. Winter grazing use in the Saddle Butte Allotment is based on that year's forage production.

The BLM allocated forage for livestock use in the SEORMP/ROD (2002, p. E-208). The allocation was carried forward from the Southern Malheur Rangeland Program Summary Update (December 1986). The allocation will be revisited during activity planning associated with evaluation and assessment within Saddle Butte Geographic Management Area as described in the SEORMP.

Table 7 summarizes information about livestock grazing in Saddle Butte Allotment and its relationship to wild horse management within the Sand Springs HMA.

Table 7: Livestock Use Information

Allotment	Total Allotment Acres	% of Allotment in HMA	Number of Permittees	Number of Authorized Livestock	Authorized Season of Use	Authorized Livestock AUMs in Allotment	Average Actual Livestock Use (AUMs) (Past 5 years)
Saddle Butte	175,841 PD 9,172 Pvt 623 State	100%	7	1,714 Cattle	11/1 – 3/31	6,314	1,999 minimum 4,081 average 5,971 maximum

3.2.3 Wildlife

The project area includes habitat for approximately 300 wildlife species. Many of these species use the area for part of the year and/or as connective habitat while traveling to more productive ground.

Big Game: Pronghorn Antelope are widely distributed across the project area and represent the primary big game species in this area. Mule deer use this area throughout the year, but are only found in large numbers during the winter. During October through May this area is particularly important as Mule deer winter range habitat. Elk also use this area in the winter, but are less frequent than pronghorn antelope and mule deer. California Bighorn sheep are common on the Sheepshead Mountains, Red Mountain, and around Tule Springs, but only use Coyote Lake in winter.

Raptors: A variety of Raptor species use the project area at various times during the year. Most Raptors species migrate through and are only in the area for a short time. These include: the bald and golden eagle, Swainson's, Ferruginous, red- tailed and sharp shinned hawk, prairie and peregrine falcon, northern harrier, American Kestrel, and Western burrowing owl.

Other important game species known to occur in the HMA include chukar and Hungarian partridge, mourning doves, and sage grouse.

The area lacks perennial water and habitat for native species of fish.

3.2.4 Threatened and Endangered/Special Status Species

No threatened and/or endangered animal species are known or suspected to occur within the Barren Valley Complex. Special status wildlife species likely to occur within the project area include kit fox, spotted bat, pygmy rabbit, peregrine falcon, and sage grouse.

Greater sage grouse: The greater sage grouse is currently a species that is considered "warranted for listing under the Endangered Species Act of 1973. A small number of sage grouse use this area, but wildfire has reduced the amount of sage brush and diminished the habitat. Approximately half of the Barren Valley HMA is considered category 2 sage grouse habitat. The northern quarter of the unit is category 1. The management goals for category 2 sage grouse habitat are: avoid development within these areas, or if impacts are unavoidable, maintain the current habitat quantity or quality and provide a net benefit to sage grouse habitat. Category 1 habitat is priority habitat for sage grouse. Actions within category 1 habitat should not diminish or reduce the quality of habitat for sage grouse.

Information on the remaining special status species is limited, but the likelihood of additional special status species using this area for a significant portion of their life history requirements is low.

Special Status Plant Species:

In February 2008 a new interagency special status species list for Oregon/Washington BLM and Region 6 of the USFS was developed (BLM 2008). The interagency list is an attempt to coordinate management for rare species across land ownership boundaries. This new and most current list used new criteria to determine if species are sensitive and eliminated the categories of Bureau Assessment and Bureau Tracking Species. As a result, some previously listed species have come off the list and some species' status has changed from sensitive to strategic. There are now three categories of special status species for Oregon/Washington BLM and Region 6 of the USFS: Federally listed species under the ESA, and Sensitive species or Strategic species as designated by the State Director or the Regional Forester.

A number of previously known special status plants in the Barren Valley complex are no longer special status. This change in status could be due to a number of factors such as the new criteria in determining if species are sensitive or expansion of a species range as a result of increased inventory and survey. For this reason only those plants presently designated as Bureau Sensitive or Bureau Strategic will be addressed within this section.

Within the Barren Valley Complex nine different special status species occur: Davis' peppergrass (*Lepidium davisii*), Desert chaenactis (*Chaenactis xantiana*), Transmontane sand-verbena (*Abronia turbinata*), Wheeler's skeleton-weed (*Chaetadelpah wheeleri*), Lyrate malacothrix (*Malacothrix sonchoides*), Salt heliotrope (*Heliotropium curassavicum*), Iodine bush (*Allenrolfea occidentalis*) and Desert needlegrass (*Achnatherum speciosum*). Aside from Davis' peppergrass, abundance of all other species occurrences is low with isolated infrequent pockets throughout the complex. Wild horses do not pose a known threat to the aforementioned species aside from Davis' peppergrass.

Davis' peppergrass (*Lepidium davisii*), is a Bureau Sensitive specie with known occurrences in five playas within the Coyote Lake/Alvord – Tule Springs HMA and seven playas within Sand Springs HMA. Recent and past monitoring reveals heavy horse use on the playas. The use generally occurs during times when the playas are wet and being used as a water source for the horses. This continues through most of the summer, causing extensive soil compaction and heavy disturbance. In Oregon wild horse impact is the primary threat to this species.

3.2.5 Vegetation

Shrub steppe vegetation communities in the area result from cold winters and hot dry summers. Historically, the project area supported a wide variety of sagebrush/perennial grassland cover types. Disturbance factors that have occurred within the complex include historic domestic livestock grazing use, wild horse grazing use, wildfire and invasive plants. Outside of seedings and cheatgrass, this vast area consists of a sagebrush (*Artemisia*) overstory with an understory dominated by perrenial bunchgrass. Dominant species of shrub and bunchgrass include: Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*), mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*), low sagebrush (*Artemisia arbuscula*), shadscale (*Atriplex confertiflora*), spiny hopsage (*Grayia spinosa*), Sandberg bluegrass (*Poa sandbergii*), Cusick's bluegrass (*Poa cusickii*), bluebunch wheatgrass (*Pseudorogneria spictata*), Thurber's needlegrass (*Achnatherum thurberiana*), Idaho fescue (*Festuca idahoensis*) and Indian ricegras (*Achnatherum hymenoides*). Riparian habitats are very infrequent throughout the complex. Riparian vegetation consists of but is not limited to the following: Beardless wildrye (*Leymus triticoides*), Coyote willow (*salix exigua*), yellow willow (*Salix lutea*), Nebraska sedge (*Carex nebraskensis*), *Carex* sp., spikerush (*Eleocharis* sp.), Baltic rush (*Juncus balticus*), tufted hairgrass (*Deschampsia caespitosa*), alpine timothy (*Phleum alpinum*), Leiberg's bluegrass (*Poa leibergii*), hardstem bulrush (*Schoenoplectus acutus*), and cattail (*Typha* sp.).

Portions of the complex are infested with a conglomerate of mostly annual weeds or weedy species. Perennial pepperweed (*Lepidium latifolium*), whitetop (*Lepidium spp.),* Russian knapweed (*Acroptilon repens*), halogeton (*Halogeton glomeratus*) and saltcedar (*Tamorix ramosissima*) are common in the Upper and Lower Willow Creek Waterholes areas (Little Coyote Playa). With the exception of saltcedar, these species as well as Scotch (*Onapordum acanthium*) and Canada thistle (*Circium arvense*), occur as small, isolated patches within other portions of the area.

3.2.6 Soils

The soils found in the Barren Valley Complex were surveyed and described in Oregon's Long Range Requirements for Water 1969, Appendix I-11, Owyhee Drainage Basin and Appendix I-12. They are mainly a

combination of Unit 75 and 77 soils on slopes varying from three to twelve percent and Unit 30 and 55 soils on slopes varying from zero to twelve percent. The area also contains smaller amount of Units 57, 76, and 99.

Unit 30 soils are deep, poorly drained soils formed in fine-textured alluvium on flat basins and old playa bottoms. The native vegetation consists mostly of western wheatgrass, silver sagebrush, big sagebrush, and rabbitbrush.

Unit 55 soils are shallow, loamy, well drained soils with cemented pans. These soils occur on very extensive to moderately steep old fans and high terrace remnants. Native vegetation consists mostly of big sagebrush, low sagebrush, rabbitbrush, budsage, Atriplex spp., needlegrass, squirreltail grass, and Sandberg bluegrass.

Unit 57 soils are deep, well drained soils derived from loamy materials on old fans. Sloes are dominantly nearly level to gently sloping. Native vegetation consists mostly of big sagebrush, low sagebrush, rabbitbrush, budsage, Atriplex spp., needlegrass, and squirreltail grass.

Unit 75 soils are loamy, shallow, very stony, well drained soils over basalt, rhyolite, or welded tuff. Unit 75 soils occur on gently undulating to rolling lava plateaus with some very steep faulted and dissected terrain. The soil profile consists of very stony silt loam, stony loam, and stony silt loam over bedrock at 15+ inches.

Unit 76 soils are shallow, clayey, very stony, well drained soils over basalt, rhyolite, or welded tuff. They occur on gently undulating to rolling lava plateaus and some very steep faulted and dissected terrain. The soil profile consists of very stony, silt loam, stony silty clay, to stony and channery, heavy, silty clay loams over fractured bedrock at 18+ inches.

Unit 77 soils are very shallow, very stony, rocky, well-drained soils over basalt, rhyolite, or welded tuff. These soils occur on gently undulating to rolling lava plateaus. Native vegetation consists mostly of big sagebrush, low sagebrush, and Sandberg bluegrass.

Unit 99 is a miscellaneous land unit consisting of recent lava flows. These flows are generally on low slopes, but do have extremely irregular, rough surfaces. There do tend to be small pockets of soil development on which there is some vegetation.

3.2.7 Riparian Areas and Water Resources

In the Coyote Lakes portion of the HMA, Willow Creek is the only perennial riparian area. Willow Creek is on the State of Oregon's 303(d) list which indicates that the stream is unable to meet water quality standards. Oregon Department of Environmental Quality (ODEQ) has initiated a Total Maximum Daily Load study on Willow Creek (Willow Creek Total Maximum Daily Load, ODEQ, March 1999). This study identifies wild horse grazing in the riparian area as one of the factors contributing to the stream's degraded condition.

In the Alvord-Tule Springs portion of the HMA, the only perennial water sources are springs in Mickey Basin, Mickey Hot Springs, and Tule Springs. Wells provide some extension of range during the winter. Season long grazing near perennial springs, (Tule Springs, Buckbrush Springs, Big Sand Gap Springs, Little Sand Gap Springs), and Calderwood Desert Well, becomes a resource concern as horse numbers continue to increase. Table Mountain pasture has historically perennial springs and water holes fed from Wildcat Creek which are currently dry. There are a few scattered playas and seeps throughout the rest of the HMA that provide limited riparian habitat.

Riparian areas in the Sheepshead/Heath Creek HMA are associated with a few perennial springs, Heath Creek, Wildcat Canyon, and bone Canyon. The entire riparian habitat in this HMA is associated with the higher elevations along the ridges associated with the Sheepshead Mountains. Most of the limited water sources in the lower elevations of this HMA are associated with reservoirs.

Riparian vegetation in the Sand Springs HMA is extremely limited, existing primarily at a few scattered springs and reservoirs. Most of the riparian areas are related to several perennial springs and seeps along the eastern edge of the HMA above the Owyhee River Rim. There are also several natural water gaps on the Owyhee River that wild horses rarely use.

While not extensive, riparian zones are an important resource for wildlife, wild horses, and livestock. Because of the demands on riparian areas, management considerations have focused on protecting these areas. Maintaining AML of wild horse herds is important to keeping utilization at acceptable levels and preserving riparian habitat.

4.0 Environmental Consequences

4.1 Introduction

This section of the EA documents the potential environmental impacts which would be expected with implementation of the Action Alternatives (Alternatives 1-4) and/or the No Action Alternative. These include the direct impacts (those that result from the management actions) and indirect impacts (those that exist once the management action has occurred).

4.2 Predicted Effects of Alternatives

The direct and indirect impacts to these resources which would be expected to result with implementation of the Action Alternatives or No Action Alternative are discussed in detail below.

4.2.1 Wild Horses

Results of Win Equus Population Modeling

The Alternatives were modeled using Version 3.2 of the Win Equus population model (Jenkins, 2000). The purpose of the modeling was to analyze and compare the effects of the Action Alternatives on population size, average population growth rate, and average removal number. Another objective of the modeling was to identify if any of the alternatives "crash" the population or cause extremely low population numbers or growth rates.

Minimum population levels and growth rates were found to be within reasonable levels and adverse impacts to the population are not likely. There was not a significant difference in the Action Alternatives for the number average number of horses removed over the next 11 years. See Appendix C for additional detail.

Impacts Common to Action Alternatives (1-4)

Over the past 35 years, various impacts to wild horses as a result of gather activities have been observed. Under the Proposed Action, impacts to wild horses would be both direct and indirect, occurring to both individual horses and the population as a whole.

The BLM has been conducting wild horse gathers since the mid-1970s. During this time, methods and procedures have been identified and refined to minimize stress and impacts to wild horses during gather implementation. The SOPs (Appendix A) would be implemented to ensure a safe and humane gather occurs

and would minimize potential stress and injury to wild horses. In addition to implementation of the SOPs, BLM would temporarily close roads in the HMA where gather operations were occurring to assist in ensuring the safety of the public, BLM contractors, BLM personnel, and wild horses.

In any given gather, gather-related mortality averages about one half of one percent (0.5%), which is very low when handling wild animals. Approximately another six-tenths of one percent (0.6%) of the captured animals could be humanely euthanized due to pre-existing conditions and in accordance with BLM policy (IM-2009-041). These data affirm that the use of helicopters and motorized vehicles has proven to be a safe, humane, effective, and practical means for the gather and removal of excess wild horses (and burros) from the public lands. The BLM also avoids gathering wild horses by helicopter during the 6 weeks prior to and following the peak foaling season (i.e., March 1 through June 30).

Individual, direct impacts to wild horses include the handling stress associated with the roundup, capture, sorting, handling, and transportation of the animals. The intensity of these impacts varies by individual, and is indicated by behaviors ranging from nervous agitation to physical distress. When being herded to trap site corrals by the helicopter, injuries sustained by wild horses may include bruises, scrapes, or cuts to feet, legs, face, or body from rocks, brush or tree limbs. Rarely, wild horses may encounter barbed wire fences and may receive wire cuts. These injuries are very rarely fatal and are treated on-site until a veterinarian can examine the animal and determine if additional treatment is indicated.

Other injuries may occur after a horse has been captured and is either within the trap site corral, the temporary holding corral, during transport between facilities, or during sorting and handling. Occasionally, horses may sustain a spinal injury or a fractured limb but based on prior gather statistics, serious injuries requiring humane euthanasia occur in less than 1 horse per every 100 captured.

To minimize the potential for injuries from fighting, the animals are transported from the trap site to the temporary (or short-term) holding facility where they are sorted as quickly and safely as possible, then moved into large holding pens where they are provided with hay and water.

Indirect individual impacts are those which occur to individual wild horses after the initial event. These may include miscarriages in mares, increased social displacement, and conflict in studs. These impacts, like direct individual impacts, are known to occur intermittently during wild horse gather operations. An example of an indirect individual impact would be the brief skirmishes between older studs which ends when one stud retreats. Injuries typically involve a bite or kick with bruises which do not break the skin. Like direct individual impacts, the frequency of these impacts varies with the population and the individual. Observations following capture indicate the rate of miscarriage varies, but can occur in about 1 to 5% of the captured mares, particularly if the mares are in very thin body condition or in poor health.

Gathering the wild horses during the Fall/Winter reduces risk of heat stress, although this can occur during any gather, especially in older or weaker animals. Adherence to the SOPs as well and techniques used by the gather contractor help minimize the risks of heat stress. Heat stress does not occur often, but if it does, death can result. Most temperature related issues during a gather can be mitigated by adjusting daily gather times to avoid the extreme hot or cold periods of the day.

A few foals may be orphaned during a gather. This can occur if the mare rejects the foal, the foal becomes separated from its mother and cannot be matched up following sorting, the mare dies or must be humanely euthanized during the gather, the foal is ill or weak and needs immediate care that requires removal from the mother, or the mother does not produce enough milk to support the foal. On occasion, foals are gathered that

were previously orphaned on the range (prior to the gather) because the mother rejected it or died. These foals are usually in poor, unthrifty condition. Every effort is made to provide appropriate care to orphan foals. Veterinarians may administer electrolyte solutions or orphan foals may be fed milk replacer as needed to support their nutritional needs. Orphan foals may be placed in a foster home in order to receive additional care. Despite these efforts, some orphan foals may die or be humanely euthanized as an act of mercy if the prognosis for survival is very poor.

Wild horses not captured may be temporarily disturbed and moved into another area during the gather operation. With the exception of changes to herd demographics from removals, direct population impacts have proven to be temporary in nature with most, if not all, impacts disappearing within hours to several days of release. No observable effects associated with these impacts would be expected within one month of release, except for a heightened awareness of human presence.

It is not expected that genetic health would be negatively impacted by the Proposed Action. The AML range in the Barren Valley Complex of 559-892 should provide for acceptable genetic diversity.

By maintaining wild horse population size within the AML within the Sheepshead/Heath Creek HMA, there would be a lower density of wild horses across all of the HMAs within the complex. This results in reducing competition for resources and allowing wild horses to utilize their preferred habitat. Maintaining population size within the established AMLs would be expected to improve forage quantity and quality and promote healthy, self-sustaining populations of wild horses in a thriving natural ecological balance and multiple use relationship on the public lands in the area. Deterioration of the range associated with wild horse overpopulation would be avoided. Managing wild horse populations in balance with the available habitat and other multiple uses would lessen the potential for individual animals or the herd to be affected by drought, and would avoid or minimize the need for emergency gathers, which would reduce stress to the animals and increase the success of these herds over the long-term.

Transport, Short Term Holding, and Adoption (or Sale) Preparation

About 275 excess horses would be removed. Animals would be transported from the capture/temporary holding corrals to the designated BLM short-term holding corral facility(s) according to SOPs (Appendix A). During transport, potential impacts to individual horses can include stress, as well as slipping, falling, kicking, biting, or being stepped on by another animal. Unless wild horses are in extremely poor condition, it is rare for an animal to be seriously injured or die during transport.

Upon arrival at the short term holding facility, most wild horses begin to eat and drink immediately and adjust rapidly to their new situation. Recently captured wild horses, generally mares, in very thin condition may have difficulty transitioning to feed. Some of these animals are in such poor condition that it is unlikely they would have survived if left on the range. Similarly, some mares may lose their pregnancies. Every effort is taken to help the mare make a quiet, low stress transition to captivity and domestic feed to minimize the risk of miscarriage or death.

After recently captured wild horses have transitioned to their new environment, they are prepared for adoption or sale. During the preparation process, potential impacts to wild horses are similar to those that can occur during handling and transportation. Serious injuries and deaths from injuries during the preparation process are rare, but can occur.

At short-term corral facilities, a minimum of 700 square feet is provided per animal. Mortality at short-term holding facilities averages approximately 5% per year (GAO-09-77, page 51), and includes animals euthanized

due to a pre-existing condition; animals in extremely poor condition; animals that are injured and would not recover; animals which are unable to transition to feed; and animals which are seriously injured or accidentally die during sorting, handling, or preparation.

From there, they would be made available for adoption or sale to qualified individuals or to long-term holding (grassland) pastures.

Adoption or Sale with Limitations, and Long Term Holding
Other indirect impacts include transportation to adoptions, sales, or long-term pastures (LTP). Adoptions are conducted in accordance with 43 CFR 5750. Sales of wild horses are conducted in accordance with Bureau policy. The BLM has maintained long-term pastures (LTP) in the Midwest for over 20 years. Potential impacts to wild horses from transport to adoption, sale or LTP are similar to those previously described.

LTPs are designed to provide excess wild horses with humane, life-long care in a natural setting off the public rangelands. Handling by humans is minimized to the extent possible although regular on-the-ground observation and weekly counts of the wild horses to ascertain their numbers, well-being, and safety are conducted.

Impacts of Alternative 1 (Proposed Action)
PZP application would be done according to SOPs (Appendix B). When injected, PZP (antigen) causes the mare's immune system to produce antibodies and these antibodies bind to the mare's eggs, and effectively block sperm binding and fertilization (Zoo Montana, 2000). PZP is relatively inexpensive, meets BLM requirements for safety to mares and environment, and can easily be administered in the field. In addition, among mares, PZP contraception appears to be completely reversible.

The highest success for fertility control has been obtained when applied during the timeframe of November through February. This gather would require PZP application outside the window for highest success. Below is the efficacy for the application of the two-year PZP vaccine:

		Year 1	Year 2	Year 3	Year 4
Summer Application	Normal	80%	65%	50%	
Winter Application	Normal	94%	82%	68%	

One-time application at the capture site would not affect normal development of the fetus, hormone health of the mare or behavioral responses to stallions, should the mare already be pregnant when vaccinated (Kirkpatrick, 1995). The vaccine has also proven to have no apparent effect on pregnancies in progress, the health of offspring, or the behavior of treated mares (Turner, 1997). Mares would foal normally in 2012 (Year 1).

Mares receiving the vaccine would experience slightly increased stress levels associated with handling while being vaccinated and freeze-marked. Serious injection site reactions associated with fertility control treatments are rare in treated mares. Any direct impacts associated with fertility control, such as swelling or local reactions at the injection site, would be minor in nature and of short duration. Most mares recover quickly once released back to the HMA, and none are expected to have long term consequences from the fertility control injections.

Under Alternative 1, some captured wild horses would be released back to the range to achieve a post-gather sex ratio of 60% studs or geldings and 40% mares. Under this alternative, band size would be expected to decrease, competition for mares would be expected to increase, recruitment age for reproduction among mares

would be expected to decline, and size and number of bachelor bands would be expected to increase. These effects would be slight, as the proposed sex ratio is not an extreme departure from normal sex ratio ranges. Modification of sex ratios for a post-gather population favoring studs or geldings would further reduce growth rates in combination with fertility control.

Impacts of Alternative 2 (Removal Only)
Implementation of Alternative 2 would result in capturing fewer wild horses than would be captured in Alternative 1. Alternative 2 would not involve fertility control; mares would not undergo the additional stress of receiving fertility control injections or freeze-marking and would foal at normal rates until the next gather is conducted. The post-gather sex ratio would be about 50:50 mares to studs. This would be expected to result in fewer and smaller bachelor bands, increased reproduction on a proportional basis within the herd, larger band sizes, and individual mares would likely begin actively producing at a slightly older age.

Impacts of Alternative 3 (Removal and Fertility Control)
Alternative 3 would involve fertility control, therefore, impacts related to fertility control would be the same as Alternative 1. The post-gather sex ratio would be about 50:50 mares to studs. This would be expected to result in fewer and smaller bachelor bands, increased reproduction on a proportional basis within the herd, larger band sizes, and individual mares would likely begin actively producing at a slightly older age.

Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)
Alternative 2 would not involve fertility control, therefore, impacts related to fertility control would not apply. The post-gather sex ratio would be about 60:40 studs or geldings to mares. These impacts would similar to those discussed in Alternative 1.

Impacts of Alternative 5 (No Action)
Under the No Action Alternative, there would be no active management to control the population size within the established AML at this time. In the absence of a gather, wild horse populations would continue to grow at an average rate of 25% per year. Without a gather and removal now, the population would grow to 449 in four years based on the average annual growth rate.

Use by wild horses would continue to exceed the amount of forage allocated for their use. Competition between wildlife, livestock and wild horses for limited forage and water resources would continue. Damage to rangeland resources would continue or increase. Over time, the potential risks to the health of individual horses would increase, and the need for emergency removals to prevent their death from starvation or thirst would also increase. Over the long-term, the health and sustainability of the wild horse population is dependent upon achieving a thriving natural ecological balance and sustaining healthy rangelands. Allowing wild horses to die of dehydration or starvation would be inhumane and would be contrary to the WFRHBA which requires that excess wild horses be immediately removed. Allowing rangeland damage to continue to result from wild horse overpopulation would also be contrary to the WFRHBA which requires the BLM to "*protect the range from the deterioration associated with overpopulation*", "*remove excess animals from the range so as to achieve appropriate management levels*", and "*to preserve and maintain a thriving natural ecological balance and multiple-use relationship in that area*."

4.2.2 Grazing Management
Direct impacts to livestock and management practices from activity associated with gathering, including disturbance resulting from moving horses with a helicopter, would be minimal within the HMAs. Removal of horses from outside the HMAs may result in minimal livestock disturbance while herding wild horses into the HMAs.

Impacts Common to Action Alternatives (1-4)

Removal of approximately 275 head of horses from inside and outside the complex would reduce competition between livestock and wild horses for the available forage and water resources. Indirect impacts would include an increase in the quality and quantity of the available forage in the short-term. Over the longer-term, improved vegetation resources would lead to a thriving natural ecological condition.

Impacts of Alternative 1 (Removal, Sex Ratio Adjustment, and Fertility Control) – Impacts of removal would benefit livestock management opportunities due to limitations for forage and water resources as identified above. Those benefits would be extended by limiting future growth of the horse herd through sex ratio adjustments and fertility control.

***Impacts of Alternative 2 (Removal Only)* –** Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

***Impacts of Alternative 3 (Removal and Fertility Control)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

***Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)

The current wild horse population is 10-20% above their forage allocation. Heavy to severe utilization is occurring in areas where wild horses concentrate. The indirect impacts of No Action (Defer Gather and Removal) would be continued damage to the range, continuing competition between livestock, wild horses and wildlife for the available forage and water, reduced quantity and quality of forage and water, and undue hardship on the livestock operators who would continue to be unable to fully use the forage resource they are authorized to use.

4.2.3 Wildlife

Impacts Common to Action Alternatives (1-4)

Wildlife currently using the project area would be exposed to the activities associated with horse gathers. The use of a helicopter would result in disturbance to wildlife, proportionate to the amount of time the helicopter is in use and the amount of ground that is covered. Effects would be of short duration (8 to 10 days) and localized. The effects to wildlife from trapping would be specific to the trap site.

Reducing horse numbers in this area would reduce the competition between horses and wildlife for the limited water and riparian habitat.

***Impacts of Alternative 2 (Removal Only)* –** Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

***Impacts of Alternative 3 (Removal and Fertility Control)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

***Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)
Wildlife populations in the HMA would be forced to compete more for limited water and forage, which would most likely alter use patterns. Habitat degradation would decrease wildlife populations and wildlife use in the HMA.

4.2.4 Threatened and Endangered/Special Status Species

Impacts Common to Action Alternatives (1-4)
Reducing the number of wild horses in the HMA will lead to increased herbaceous cover as well as maintained or improved vegetative conditions. This will benefit sage grouse and other special status species identified in the affected environment by limiting habitat fragmentation and providing increased forage and vegetative structure required by the various life processes of these species. Habitat quality and quantity would be increased reducing potential threats to the species viability within the Barren Valley Complex.

Reducing the number of wild horses in the HMA would avoid unnecessary adverse impacts from wild horses to special status plants and their potential habitats in the Barren Valley Complex. Trap sites and off road access points used for the gather would be surveyed for special status plants prior to the gather in order to avoid known occurrence and potential habitats.

***Impacts of Alternative 2 (Removal Only)* –** Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

***Impacts of Alternative 3 (Removal and Fertility Control)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

***Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)
Special status species would be affected by increased horse numbers through degradation of habitat conditions which could reduce productivity of these species. Riparian vegetation browsing and trampling springs, primarily due to wild horse use, would further degrade habitat conditions for wildlife.

4.2.5 Vegetation

Impacts Common to Action Alternatives (1-4)
In the immediate vicinity of the catch pens or corrals and the loading chute, short-term disturbance would occur. The soil would be compacted and vegetation would be trampled during panel installation by personnel and vehicles and severely trampled in the catch pen area by wild horses, domestic horses, and the wranglers. It is estimated and anticipated that 1 to 3 years would be required for native vegetation to become reestablished or regain vigor under average conditions with no reclamation. The total area of impact per trap would be approximately 2 acres, with less than ¼ acre severely disturbed. Less than one AUM of livestock forage would be temporarily lost for one grazing season at each trap site used.

There would be a positive impact to the upland and riparian vegetation by reducing the total numbers of wild horses grazing year long within the HMA. Lessened utilization would allow critical growth period rest for key cool season grasses. The composition of vegetation would change to a higher percentage of desirable plants, soil cover would increase and the potential for erosion would decrease.

Impacts of Alternative 1 (Removal, Sex Ratio Adjustment, and Fertility Control) – Impacts of removal would benefit vegetation resources as identified above. Those benefits would be extended by limiting future growth of the horse herd through sex ratio adjustments and fertility control.

***Impacts of Alternative 2 (Removal Only)* –** Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

***Impacts of Alternative 3 (Removal and Fertility Control)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

***Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)
Areas which are presently over utilized, such as areas adjacent to water sources, would continue to be used excessively. The area of over utilization would continue to increase in both size and degree. The composition of vegetation would change to a higher percentage of undesirable plants, soil cover would be reduced, and the potential for erosion would increase.

4.2.6 Soils

Impacts Common to Action Alternatives (1-4)
Soil loss and compaction would be expected to decrease in those areas near water sources where horses are forced to concentrate. Lower populations of horses would result in less hoof traffic, thereby decreasing negative impacts to soil micro biotic crusts.

Soil would be displaced and/or compacted on approximately two acres at each site in the construction of the trap, use of the access routes, and in the round-up and loading of the wild horses. The area of severe surface disturbance is normally less than 2,000 square feet. Minimal surface wind and water erosion is expected on these areas during the vegetative rehabilitation period (approximately 1 to 3 years).

***Impacts of Alternative 2 (Removal Only)* –** Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

***Impacts of Alternative 3 (Removal and Fertility Control)* -** Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

Impacts of Alternative 4 (Removal and Sex Ratio Adjustment) - Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)
Soil loss and compaction would be expected to increase in those areas near water sources where horses are forced to concentrate. Increased wild horse numbers on uplands and riparian areas would negatively impact soil surface features and would increase erosion in the HMA.

4.2.7 Riparian Areas and Water Resources

Impacts Common to Action Alternatives (1-4)
The proposed action would limit the intensity of use at water sources and surrounding uplands. Regulating the number of wild horses in the HMA would reduce use near water sources and riparian areas by minimizing degradation to these resources.

The trap sites would not be located adjacent to any surface water sources or riparian areas, therefore, there would be no anticipated direct impact due to the gather.

Impacts of Alternative 2 (Removal Only) – Impacts would not be as beneficial as in Alternative 1 and 3 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures or adjusting the sex ratio of the herd.

Impacts of Alternative 3 (Removal and Fertility Control) - Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not adjusting the sex ratio of the herd.

Impacts of Alternative 4 (Removal and Sex Ratio Adjustment) - Impacts would not be as beneficial as in Alternative 1 due to the wild horse population increasing at a faster rate as a result of not conducting fertility control measures.

Impacts of Alternative 5 (No Action)
Increasing numbers of wild horses in the HMA would result in greater use and degradation of riparian areas. This would result in an unacceptable decline in water quality through increased sedimentation and water temperatures. Riparian area vegetation would be degraded as additional horse use would decrease vegetation recruitment, reproduction, and survivability. In addition, riparian vegetation community types and distribution would be changed, root density lessened, and canopy cover reduced. This would lead to reduced stream channel and spring/seep dynamics and further deterioration of these systems.

4.3 Cumulative Effects for All Alternatives

The NEPA regulations define cumulative impacts as impacts on the environment that result from the incremental impact of the Proposed Action when added to other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions (40 CFR 1508.7). Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time. The cumulative impacts study area (CSA) for the purposes of evaluating cumulative impacts is the Barren Valley Complex.

According to the 1994 BLM *Guidelines for Assessing and Documenting Cumulative Impacts*, the cumulative analysis should be focused on those issues and resource values identified during scoping that are of major importance. Accordingly, the issues of major importance to be analyzed are maintaining rangeland health and proper management of wild horses.

Past and Present Actions

4.3.1 Wild Horses

Currently, management and AML of wild horses within the complex conforms to decisions in the SEORMP/ROD (2002) and the AMURMP/ROD (2005).

The actions which have influenced today's wild horse population are primarily wild horse gathers, which have resulted in the capture of some 7,352 wild horses, the removal of 6,919 excess horses, and release of 336 horses back into the HMA (see Table 3, Section 3.2.2).

4.3.2 Vegetation

Through land use planning decisions, the BLM has allocated the available forage to livestock, wildlife and domestic livestock. Additional benefits provided by healthy vegetation resources have resulted in land use planning decision to limit unacceptable impacts to vegetation. While the present livestock grazing system and efforts to manage the wild horse population within AML has reduced past historic impacts, monitoring indicates that the current overpopulation of wild horses is continuing to contribute to areas of heavy vegetation utilization, trailing and trampling damage and is preventing the BLM from managing for rangeland health and a thriving natural ecological balance and multiple use relationship on the public lands in the area.

4.4 Reasonably Foreseeable Future Actions

4.4.1 Wild Horses

Over the next 10-20 year period, reasonably foreseeable future actions include gathers about every 4 years to remove excess wild horses in order to manage population size within the established AML range. The excess animals removed would be transported to short-term corral facilities where they would be prepared for adoption, sale (with limitations), or LTPs. Any future wild horse management would be analyzed in appropriate environmental documents following site-specific planning with public involvement.

4.4.2 Vegetation

Continuing to graze livestock in a manner consistent with grazing permit terms and conditions would be expected to achieve or make significant progress towards achieving rangeland health standards and guidelines. Livestock grazing is expected to continue at similar stocking rates and utilization of the available vegetation (forage) would also be expected to continue at similar levels, with appropriate corrective actions implemented if current livestock management practices are found to contribute to not meeting rangeland health standards or guidelines. Similarly, appropriate actions would be implemented to adjust livestock grazing authorization terms and conditions, including livestock numbers and seasons of use, in the event that current livestock grazing practices are found to contribute to not meeting resource management objectives.

4.5 Summary of Past, Present, and Reasonably Foreseeable Future Actions

Impacts Common to Action Alternatives (1-4)

The cumulative effects associated with the capture and removal of excess wild horses includes gather-related mortality of less than 1% of the captured animals, about 5% per year associated with transportation, short term holding, adoption or sale with limitations and about 8% per year associated with long-term holding. This compares with natural mortality on the range ranging from about 5-8% per year for foals (animals under age 1), about 5% per year for horses ages 1-15, and 5-100% for animals age 16 and older (Stephen Jenkins, 1996, Garrott and Taylor, 1990). In situations where forage and/or water are limited, mortality rates increase, with the greatest impact to young foals, nursing mares and older horses.

The other cumulative effects which would be expected when incrementally adding either of the Action Alternatives to the CSA would include continued improvement of upland vegetation conditions, which would in turn benefit permitted livestock, native wildlife, and wild horse population as forage (habitat) quality and quantity is improved over the current level. Benefits from a reduced wild horse population would include fewer animals competing for limited forage and water resources. Cumulatively, there should be more stable wild horse populations, healthier rangelands, healthier wild horses, and fewer multiple use conflicts in the area over the short and long-term. Over the next 15-20 years, continuing to manage wild horses within the established AML range would achieve a thriving natural ecological balance and multiple use relationship on public lands in the area.

Impacts of Alternative 1 (Proposed Action)

Application of fertility control and adjustment in sex ratios to favor males should slow population growth and result in fewer gathers and less frequent disturbance to individual wild horses and the herd's social structure. However, return of wild horses back into the HMA could lead to decreased ability to effectively gather horses in the future as released horses learn to evade the helicopter.

Impacts of Alternative 2 (Removal Only)

Removal only of wild horses has been the predominant method of population control used in the past on this herd. This alternative will result in more frequent gathers and disturbance to the wild horses than Alternatives 1, 3 or 4. As wild horses are gathered and sorted through for selecting which animals to release back into the HMA, there could be a decrease in the ability to effectively gather horses in the future as released horses learn to evade the helicopter.

Impacts of Alternative 3 (Removal and Fertility Control)

Application of fertility control should slow population growth and result in fewer gathers and less frequent disturbance to individual wild horses and the herd's social structure. However, return of wild horses back into the HMA could lead to decreased ability to effectively gather horses in the future as released horses learn to evade the helicopter.

Impacts of Alternative 4 (Removal and Sex Ratio Adjustment)

Adjusting the sex ratio of the herd should slightly slow population growth and result in fewer gathers and less frequent disturbance to individual wild horses and the herd's social structure. However, return of wild horses back into the HMA could lead to decreased ability to effectively gather horses in the future as released horses learn to evade the helicopter.

Impacts of Alternative 5 (No Action)
Under the No Action Alternative, the wild horse population could exceed the low end of AML by approximately four or five times in four years. Movement outside the HMA would be expected as greater numbers of horses search for food and water for survival, thus impacting larger areas of public lands. Heavy to excessive utilization of the available forage would be expected and the water available for use could become increasingly limited. Eventually, ecological plant communities would be damaged to the extent that they are no longer sustainable and the wild horse population would be expected to crash.
Emergency removals could be expected under this alternative in order to prevent individual animals from suffering or death as a result of insufficient forage and water. These emergency removals could occur as early as FY 2012. During emergency conditions, competition for the available forage and water increases. This competition generally impacts the oldest and youngest horses as well as lactating mares first. These groups would experience substantial weight loss and diminished health, which could lead to their prolonged suffering and eventual death. If emergency actions are not taken, the overall population could be affected by severely skewed sex ratios towards stallions as they are generally the strongest and healthiest portion of the population. An altered age structure would also be expected.

Cumulative impacts would result in foregoing the opportunity to improve rangeland health and to properly manage wild horses in balance with the available forage and water and other multiple uses. Attainment of site-specific vegetation management objectives and Standards for Rangeland Health would not be achieved. AML would not be achieved and the opportunity to collect the scientific data necessary to re-evaluate AML levels, in relationship to rangeland health standards, would be foregone.

5.0 Monitoring and Mitigation Measures

The BLM Contracting Officer's Representative (COR) and Project Inspectors (PIs) assigned to the gather would be responsible for ensuring contract personnel abide by the contract specifications and the SOPs (Appendix A). Ongoing monitoring of forage condition and utilization, water availability, aerial population surveys, rangeland resources health, and animal health would continue.

Fertility control monitoring would be conducted in accordance with the SOPs (Appendix B). Monitoring the herd's social behavior would be incorporated into routine monitoring. The objective of this additional monitoring would be to determine if additional studs form bachelor bands or are more aggressive with breeding bands for the forage and water present.

If genetic monitoring indicates a loss of genetic diversity, then mares would be introduced into the Barren Valley Complex from an HMA with similar characteristics.

6.0 List of Preparers

The following list identifies the interdisciplinary team member's area of responsibility:

Shaney Rockefeller - Wild Horse and Burro Specialist, Vale District
Gary McFadden - Wild Horse and Burro Specialist, Burns District
Aimee Huff - Rangeland Management Specialist, Vale District
Marcella Egger - Rangeland Management Specialist, Vale District
Louis Clayburn - Rangeland Management Specialist, Burns District
Garth Ross - Wildlife Biologist, Fisheries, Vale District
Diane Pritchard – Archaeologist, Vale District
Lynne Silva, Weed Specialist, Vale District
Linus Meyer – Hydrologist, Soil Scientist, Riparian Management, Vale District
Gillian Wigglesworth – Botanist, ACEC/RNA Coordinator, Vale District
Caryn Meinicke – Botanist, Burns District
Kari Frederick – Recreation, VRM, Wilderness, Vale District
Eric Haakenson – Recreation, VRM, Wilderness, Burns District
Steve Christensen - Planning and Environmental Coordinator, Vale District
Rhonda Karges - Planning and Environmental Coordinator, Burns District
Carolyn Freeborn – Field Manager, Jordan Resource Area, Vale District
Joan Suther – Field Manager, Andrews Resource Area, Burns District

7.0 Consultation and Coordination

Public hearing(s) are held as a single state-wide hearing at the Burns District Office regarding the use of helicopters and motorized vehicles to capture wild horses (or burros). During the hearing(s), the public is given the opportunity to present new information and to voice any concerns or opinions regarding the use of these methods to capture wild horses (or burros). The Burns BLM Office held a hearing on May 4, 2010. One member of the pubic attended the meeting. BLM reviewed its Standard Operating Procedures in response to the views and issues expressed at the hearing and determined that no changes to the SOPs were warranted.

A notice of the action was sent to the groups and individuals on the Vale and Burns District Mailing Lists including wild horse and burro interest groups.

Livestock operators in the Barren Valley Complex have been consulted.

Coordination has been conducted with Oregon Department of Fish and Wildlife.

8.0 References

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9.0 Appendices

Appendix A – Standard Operating Procedures (Gather Operation)
Appendix B – Standard Operating Procedures (Fertility Control Application and Monitoring)
Appendix C – Win Equus Population Modeling Results
Map 1 – Barren Valley Complex

APPENDIX A

Standard Operating Procedures (SOPs) for Wild Horse (or Burro) Gathers

Gathers are conducted by utilizing contractors from the Wild Horse (or Burros) Gathers-Western States Contract or BLM personnel. The following procedures for gathering and handling wild horses apply whether a contractor or BLM personnel conduct a gather. For helicopter gathers conducted by BLM personnel, gather operations will be conducted in conformance with the *Wild Horse Aviation Management Handbook* (January 2009).

Prior to any gathering operation, the BLM will provide for a pre-capture evaluation of existing conditions in the gather area(s). The evaluation will include animal conditions, prevailing temperatures, drought conditions, soil conditions, road conditions, and a topographic map with wilderness boundaries, the location of fences, other physical barriers, and acceptable trap locations in relation to animal distribution. The evaluation will determine whether the proposed activities will necessitate the presence of a veterinarian during operations. If it is determined that a large number of animals may need to be euthanized or capture operations could be facilitated by a veterinarian, these services would be arranged before the capture would proceed. The contractor will be apprised of all conditions and will be given instructions regarding the capture and handling of animals to ensure their health and welfare is protected.

Trap sites and temporary holding sites will be located to reduce the likelihood of injury and stress to the animals, and to minimize potential damage to the natural resources of the area. These sites would be located on or near existing roads whenever possible.

The primary capture methods used in the performance of gather operations include:

1. Helicopter Drive Trapping. This capture method involves utilizing a helicopter to herd wild horses into a temporary trap.
2. Helicopter Assisted Roping. This capture method involves utilizing a helicopter to herd wild horses or burros to ropers.
3. Bait Trapping. This capture method involves utilizing bait (e.g., water or feed) to lure wild horses into a temporary trap.

The following procedures and stipulations will be followed to ensure the welfare, safety and humane treatment of wild horses in accordance with the provisions of 43 CFR 4700.

A. Capture Methods used in the Performance of Gather Contract Operations

1. The primary concern of the contractor is the safe and humane handling of all animals captured. All capture attempts shall incorporate the following:

All trap and holding facilities locations must be approved by the Contracting Officer's Representative (COR) and/or the Project Inspector (PI) prior to construction. The Contractor may also be required to change or move trap locations as determined by the COR/PI. All traps and holding facilities not located on public land must have prior written approval of the landowner.

2. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who will consider terrain, physical barriers, weather, condition of the animals and other

factors. Under normal circumstances this travel should not exceed 10 miles and may be much less dependent on existing conditions (i.e. ground conditions, animal health, extreme temperature (high and low)).

3. All traps, wings, and holding facilities shall be constructed, maintained and operated to handle the animals in a safe and humane manner and be in accordance with the following:

a. Traps and holding facilities shall be constructed of portable panels, the top of which shall not be less than 72 inches high for horses and 60 inches for burros, and the bottom rail of which shall not be more than 12 inches from ground level. All traps and holding facilities shall be oval or round in design.

b. All loading chute sides shall be a minimum of 6 feet high and shall be fully covered, plywood, metal without holes larger than 2"x4".

c. All runways shall be a minimum of 30 feet long and a minimum of 6 feet high for horses, and 5 feet high for burros, and shall be covered with plywood, burlap, plastic snow fence or like material a minimum of 1 foot to 5 feet above ground level for burros and 1 foot to 6 feet for horses. The location of the government furnished portable fly chute to restrain, age, or provide additional care for the animals shall be placed in the runway in a manner as instructed by or in concurrence with the COR/PI.

d. All crowding pens including the gates leading to the runways shall be covered with a material which prevents the animals from seeing out (plywood, burlap, plastic snow fence, etc.) and shall be covered a minimum of 1 foot to 5 feet above ground level for burros and 2 feet to 6 feet for horses

e. All pens and runways used for the movement and handling of animals shall be connected with hinged self-locking or sliding gates.

4. No modification of existing fences will be made without authorization from the COR/PI. The Contractor shall be responsible for restoration of any fence modification which he has made.

5. When dust conditions occur within or adjacent to the trap or holding facility, the Contractor shall be required to wet down the ground with water.

6. Alternate pens, within the holding facility shall be furnished by the Contractor to separate mares or jennies with small foals, sick and injured animals, estrays or other animals the COR determines need to be housed in a separate pen from the other animals. Animals shall be sorted as to age, number, size, temperament, sex, and condition when in the holding facility so as to minimize, to the extent possible, injury due to fighting and trampling. Under normal conditions, the government will require that animals be restrained for the purpose of determining an animal's age, sex, or other necessary procedures. In these instances, a portable restraining chute may be necessary and will be provided by the government. Alternate pens shall be furnished by the Contractor to hold animals if the specific gathering requires that animals be released back into the capture area(s). In areas requiring one or more satellite traps, and where a centralized holding facility is utilized, the contractor may be required

to provide additional holding pens to segregate animals transported from remote locations so they may be returned to their traditional ranges. Either segregation or temporary marking and later segregation will be at the discretion of the COR.

7. The Contractor shall provide animals held in the traps and/or holding facilities with a continuous supply of fresh clean water at a minimum rate of 10 gallons per animal per day. Animals held for 10 hours or more in the traps or holding facilities shall be provided good quality hay at the rate of not less than two pounds of hay per 100 pounds of estimated body weight per day. The contractor will supply certified weed free hay if required by State, County, and Federal regulation.

An animal that is held at a temporary holding facility through the night is defined as a horse/burro feed day. An animal that is held for only a portion of a day and is shipped or released does not constitute a feed day.

8. It is the responsibility of the Contractor to provide security to prevent loss, injury or death of captured animals until delivery to final destination.

9. The Contractor shall restrain sick or injured animals if treatment is necessary. The COR/PI will determine if animals must be euthanized and provide for the destruction of such animals. The Contractor may be required to humanely euthanize animals in the field and to dispose of the carcasses as directed by the COR/PI.

10. Animals shall be transported to their final destination from temporary holding facilities as quickly as possible after capture unless prior approval is granted by the COR for unusual circumstances. Animals to be released back into the HMA following gather operations may be held up to 21 days or as directed by the COR. Animals shall not be held in traps and/or temporary holding facilities on days when there is no work being conducted except as specified by the COR. The Contractor shall schedule shipments of animals to arrive at final destination between 7:00 a.m. and 4:00 p.m. No shipments shall be scheduled to arrive at final destination on Sunday and Federal holidays, unless prior approval has been obtained by the COR. Animals shall not be allowed to remain standing on trucks while not in transport for a combined period of greater than three (3) hours in any 24 hour period. Animals that are to be released back into the capture area may need to be transported back to the original trap site. This determination will be at the discretion of the COR/PI or Field Office horse specialist.

B. Capture Methods That May Be Used in the Performance of a Gather

1. Capture attempts may be accomplished by utilizing bait (feed, water, mineral licks) to lure animals into a temporary trap. If this capture method is selected, the following applies:

    a. Finger gates shall not be constructed of materials such as "T" posts, sharpened willows, etc., that may be injurious to animals.

    b. All trigger and/or trip gate devices must be approved by the COR/PI prior to capture of animals.

    c. Traps shall be checked a minimum of once every 10 hours.

2. Capture attempts may be accomplished by utilizing a helicopter to drive animals into a temporary trap. If the contractor selects this method the following applies:

    a. A minimum of two saddle-horses shall be immediately available at the trap site to accomplish roping if necessary. Roping shall be done as determined by the COR/PI. Under no circumstances shall animals be tied down for more than one half hour.

    b. The contractor shall assure that foals shall not be left behind, and orphaned.

3. Capture attempts may be accomplished by utilizing a helicopter to drive animals to ropers. If the contractor, with the approval of the COR/PI, selects this method the following applies:

    a. Under no circumstances shall animals be tied down for more than one hour.

    b. The contractor shall assure that foals shall not be left behind, or orphaned.

    c. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who will consider terrain, physical barriers, weather, condition of the animals and other factors.

C. Use of Motorized Equipment

1. All motorized equipment employed in the transportation of captured animals shall be in compliance with appropriate State and Federal laws and regulations applicable to the humane transportation of animals. The Contractor shall provide the COR/PI, if requested, with a current safety inspection (less than one year old) for all motorized equipment and tractor-trailers used to transport animals to final destination.

2. All motorized equipment, tractor-trailers, and stock trailers shall be in good repair, of adequate rated capacity, and operated so as to ensure that captured animals are transported without undue risk or injury.

3. Only tractor-trailers or stock trailers with a covered top shall be allowed for transporting animals from trap site(s) to temporary holding facilities, and from temporary holding facilities to final destination(s). Sides or stock racks of all trailers used for transporting animals shall be a minimum height of 6 feet 6 inches from the floor. Single deck tractor-trailers 40 feet or longer shall have at least two (2) partition gates providing at least three (3) compartments within the trailer to separate animals. Tractor-trailers less than 40 feet shall have at least one partition gate providing at least two (2) compartments within the trailer to separate the animals. Compartments in all tractor-trailers shall be of equal size plus or minus 10 percent. Each partition shall be a minimum of 6 feet high and shall have a minimum 5 foot wide swinging gate. The use of double deck tractor-trailers is unacceptable and shall not be allowed.

4. All tractor-trailers used to transport animals to final destination(s) shall be equipped with at least one (1) door at the rear end of the trailer which is capable of sliding either horizontally or vertically. The rear door(s) of tractor-trailers and stock trailers must be capable of opening the full width of the trailer. Panels facing the inside of all trailers must be free of sharp edges or holes that could cause injury to the animals. The material facing the inside of all trailers must be strong enough so that the

animals cannot push their hooves through the side. Final approval of tractor-trailers and stock trailers used to transport animals shall be held by the COR/PI.

5. Floors of tractor-trailers, stock trailers and loading chutes shall be covered and maintained with wood shavings to prevent the animals from slipping as much as possible during transport.

6. Animals to be loaded and transported in any trailer shall be as directed by the COR/PI and may include limitations on numbers according to age, size, sex, temperament and animal condition. The following minimum square feet per animal shall be allowed in all trailers:

11 square feet per adult horse (1.4 linear foot in an 8 foot wide trailer);
8 square feet per adult burro (1.0 linear foot in an 8 foot wide trailer);
6 square feet per horse foal (.75 linear foot in an 8 foot wide trailer);
4 square feet per burro foal (.50 linear feet in an 8 foot wide trailer).

7. The COR/PI shall consider the condition and size of the animals, weather conditions, distance to be transported, or other factors when planning for the movement of captured animals. The COR/PI shall provide for any brand and/or inspection services required for the captured animals.

8. If the COR/PI determines that dust conditions are such that the animals could be endangered during transportation, the Contractor will be instructed to adjust speed.

D. Safety and Communications

1. The Contractor shall have the means to communicate with the COR/PI and all contractor personnel engaged in the capture of wild horses utilizing a VHF/FM Transceiver or VHF/FM portable Two-Way radio. If communications are ineffective the government will take steps necessary to protect the welfare of the animals.

a. The proper operation, service and maintenance of all contractor furnished property is the responsibility of the Contractor. The BLM reserves the right to remove from service any contractor personnel or contractor furnished equipment which, in the opinion of the contracting officer or COR/PI violate contract rules, are unsafe or otherwise unsatisfactory. In this event, the Contractor will be notified in writing to furnish replacement personnel or equipment within 48 hours of notification. All such replacements must be approved in advance of operation by the Contracting Officer or his/her representative.

b. The Contractor shall obtain the necessary FCC licenses for the radio system

c. All accidents occurring during the performance of any task order shall be immediately reported to the COR/PI.

2. Should the contractor choose to utilize a helicopter the following will apply:

a. The Contractor must operate in compliance with Federal Aviation Regulations, Part 91. Pilots provided by the Contractor shall comply with the Contractor's Federal Aviation Certificates, applicable regulations of the State in which the gather is located.

b. Fueling operations shall not take place within 1,000 feet of animals.

E. Site Clearances

No personnel working at gather sites may excavate, remove, damage, or otherwise alter or deface or attempt to excavate, remove, damage or otherwise alter or deface any archaeological resource located on public lands or Indian lands.

Prior to setting up a trap or temporary holding facility, BLM will conduct all necessary surveys (archaeological, T&E, etc). All proposed site(s) must be inspected by a government archaeologist. Once archaeological survey has been conducted, the trap or temporary holding facility may be set up. Said surveys shall be arranged for by the COR, PI, or other BLM employees.

Gather sites and temporary holding facilities would not be constructed on wetlands or riparian zones.

F. Animal Characteristics and Behavior

Releases of wild horses would be near available water. If the area is new to them, a short-term adjustment period may be required while the wild horses become familiar with the new area.

G. Public Participation

Opportunities for public viewing (i.e. media, interested public) of gather operations will be made available to the extent possible; however, the primary considerations will be to protect the health, safety and welfare of the animals being gathered and the personnel involved. The public must adhere to guidance from the on-site BLM representative. It is BLM policy that the public will not be allowed to come into direct contact with wild horses or burros being held in BLM facilities. Only authorized BLM personnel or contractors may enter the corrals or directly handle the animals. The general public may not enter the corrals or directly handle the animals at anytime or for any reason during BLM operations.

H. Responsibility and Lines of Communication

Contracting Officer's Representative/Project Inspector
Shaney Rockefeller

Contracting Officer's Representative/Project Inspector
Gary McFadden

The Contracting Officer's Representatives (CORs) and the project inspectors (PIs) have the direct responsibility to ensure the Contractor's compliance with the contract stipulations. The Malheur Resource Area Assistant Field Manager and Field Manager will take an active role to ensure the appropriate lines of communication are established between the field, Field Office, State Office, National Program Office, and BLM Holding Facility offices. All employees involved in the gathering operations will keep the best interests of the animals at the forefront at all times.

All publicity, formal public contact and inquiries will be handled through the District Public Affairs Officer. This individual will be the primary contact and will coordinate with the COR/PI on any inquiries.

The COR will coordinate with the contractor and the BLM Corrals to ensure animals are being transported from the capture site in a safe and humane manner and are arriving in good condition.

The contract specifications require humane treatment and care of the animals during removal operations. These specifications are designed to minimize the risk of injury and death during and after capture of the animals. The specifications will be vigorously enforced.

Should the Contractor show negligence and/or not perform according to contract stipulations, he will be issued written instructions, stop work orders, or defaulted.

APPENDIX B

Standard Operating Procedures for Population-level Fertility Control Treatments

One-year liquid vaccine: The following implementation and monitoring requirements are part of the Proposed Action:

1. PZP vaccine would be administered through darting by trained BLM personnel or collaborating research partners only. For any darting operation, the designated personnel must have successfully completed a Nationally recognized wildlife darting course and who have documented and successful experience darting wildlife under field conditions.

2. Mares that have never been treated would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA) and loaded into darts at the time a decision has been made to dart a specific mare. Mares identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA).

3. The liquid dose of PZP vaccine is administered using 1.0 cc Pneu-Darts with 1.5" barbless needles fired from either Dan Inject® or Pneu-Dart® capture gun.

4. Only designated darters would mix the vaccine/adjuvant and prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of a capture gun.

5. Delivery of the vaccine would be by intramuscular injection into the left or right hip/gluteal muscles while the mare is standing still.

6. Safety for both humans and the horse is the foremost consideration in deciding to dart a mare. The Dan Inject® gun would not be used at ranges in excess of 30 m while the Pneu-Dart® capture gun would not be used over 50 m, and no attempt would be taken when other persons are within a 30-m radius of the target animal.

7. No attempts would be taken in high wind or when the horse is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal is when the dart would strike the skin of the horse at a perfect 90° angle.

8. If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another horse. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart the next day. Refrigerated darts would not be used in the field.

9. No more than two people should be present at the time of a darting. The second person is responsible for locating fired darts. The second person should also be responsible for identifying the horse and keeping onlookers at a safe distance.

10. To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting.

11. Attempts will be made to recover all darts. To the extent possible, all darts which are discharged and drop from the horse at the darting site would be recovered before another darting occurs. In exceptional situations, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine.

12. All mares targeted for treatment will be clearly identifiable through photographs to enable researchers and HMA managers to positively identify the animals during the research project and at the time of removal during subsequent gathers.

13. Personnel conducting darting operations should be equipped with a two-way radio or cell phone to provide a communications link with the Project Veterinarian for advice and/or assistance. In the event of a veterinary emergency, darting personnel would immediately contact the Project Veterinarian, providing all available information concerning the nature and location of the incident.

14. In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected horse until the dart falls out or the horse can no longer be found. The darter would be responsible for daily observation of the horse until the situation is resolved.

22-month time-release pelleted vaccine: The following implementation and monitoring requirements are part of the Proposed Action:

1. PZP vaccine would be administered only by trained BLM personnel or collaborating research partners.

2. The fertility control drug is administered with two separate injections: (1) a liquid dose of PZP is administered using an 18-gauge needle primarily by hand injection; (2) the pellets are preloaded into a 14-gauge needle. These are delivered using a modified syringe and jabstick to inject the pellets into the gluteal muscles of the mares being returned to the range. The pellets are designed to release PZP over time similar to a time-release cold capsule.

3. Delivery of the vaccine would be by intramuscular injection into the gluteal muscles while the mare is restrained in a working chute. The primer would consist of 0.5 cc of liquid PZP emulsified with 0.5 cc of Freunds Modified Adjuvant (FMA). The pellets would be loaded into the jabstick for the second injection. With each injection, the liquid or pellets would be injected into the left hind quarters of the mare, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).

4. In the future, the vaccine may be administered remotely using an approved long range darting protocol and delivery system if or when that technology is developed.

5. All treated mares will be freeze-marked on the hip or neck HMA managers to positively identify the animals during the research project and at the time of removal during subsequent gathers.

Monitoring and Tracking of Treatments:
1. At a minimum, estimation of population growth rates using helicopter or fixed-wing surveys will be conducted before any subsequent gather. During these surveys it is not necessary to identify which foals were born to which mares; only an estimate of population growth is needed (i.e. # of foals to # of adults).

2. Population growth rates of herds selected for intensive monitoring will be estimated every year post-treatment using helicopter or fixed-wing surveys. During these surveys it is not necessary to identify which foals were born to which mares, only an estimate of population growth is needed (i.e. # of foals to # of adults). If, during routine HMA field monitoring (on-the-ground), data describing mare to foal ratios can be collected, these data should also be shared with the NPO for possible analysis by the USGS.

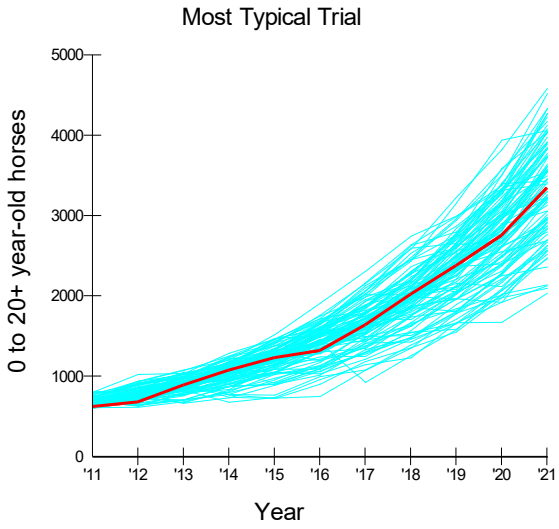
3. A PZP Application Data sheet will be used by field applicators to record all pertinent data relating to identification of the mare (including photographs if mares are not freeze-marked) and date of treatment. Each applicator will submit a PZP Application Report and accompanying narrative and data sheets will be forwarded to the NPO (Reno, Nevada). A copy of the form and data sheets and any photos taken will be maintained at the field office.

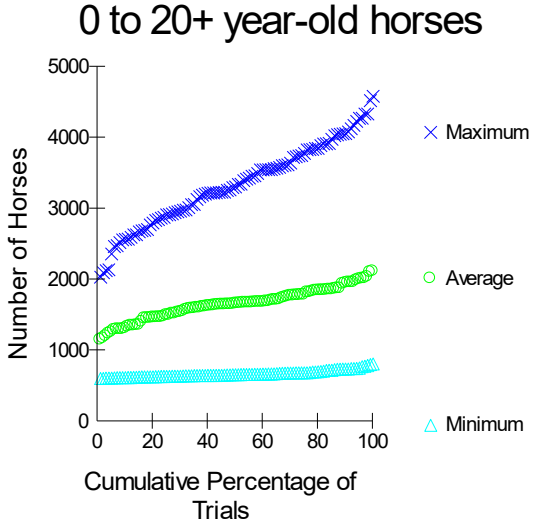
4. A tracking system will be maintained by NPO detailing the quantity of PZP issued, the quantity used, disposition of any unused PZP, the number of treated mares by HMA, field office, and State along with the freeze-mark(s) applied by HMA and date.

APPENDIX C

Population Modeling

Barren Valley Complex – No Action



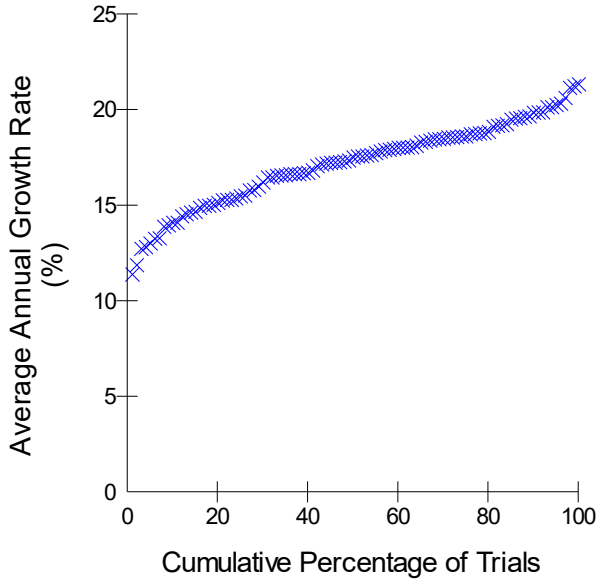


Population Sizes in 11 Years

	Minimum	Average	Maximum
Lowest Trial	606	1148	2037
10th Percentile	620	1321	2572
25th Percentile	637	1497	2918
Median Trial	660	1660	3328
75th Percentile	687	1799	3808
90th Percentile	741	1952	4075
Highest Trial	812	2113	4589

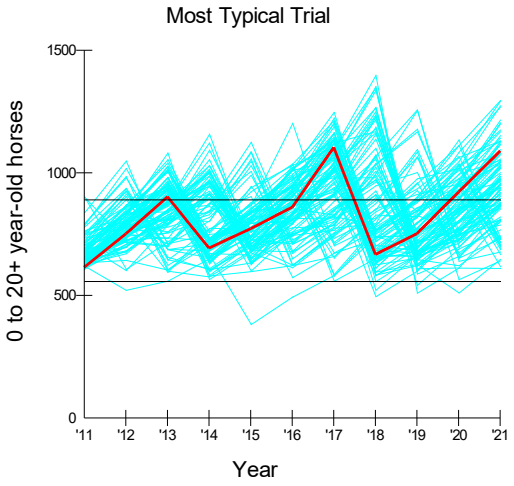
Barren Valley Comples – No Action (cont.)

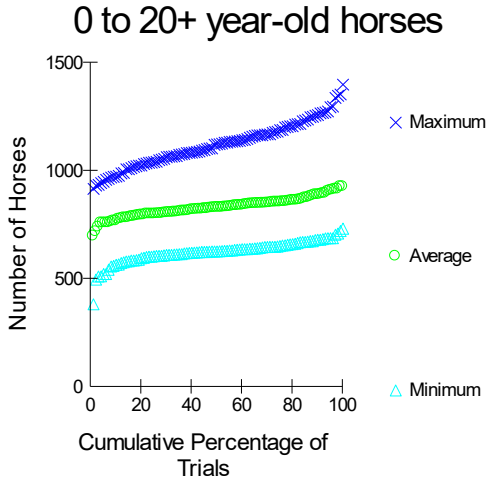
Explanation:
In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 606 and the highest was 4589. In half the trials, the minimum population size in 11 years was less than 660 and the maximum was less than 3328. The average population size across 11 years ranged from 1148 to 2113.



Average Growth Rate in 10 Years	
Lowest Trial	11.4
10th Percentile	14.1
25th Percentile	15.5
Median Trial	17.6
75th Percentile	18.7
90th Percentile	19.9
Highest Trial	21.4

Barren Valley Complex – Removal Only





Population Sizes in 11 Years*

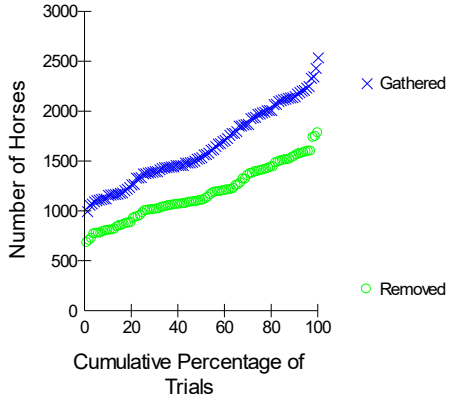
	Minimum	Average	Maximum
Lowest Trial	383	697	917
10th Percentile	566	771	976
25th Percentile	606	800	1042
Median Trial	628	830	1124
75th Percentile	654	856	1190
90th Percentile	682	890	1263
Highest Trial	733	926	1400

* 0 to 20+ year-old horses

Explanation:
In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 383 and the highest was 1400. In half the trials, the minimum population size in 11 years was less than 628 and the maximum was less than 1124. The average population size across 11 years ranged from 697 to 926.

Barren Valley Complex – Removal Only (cont.)

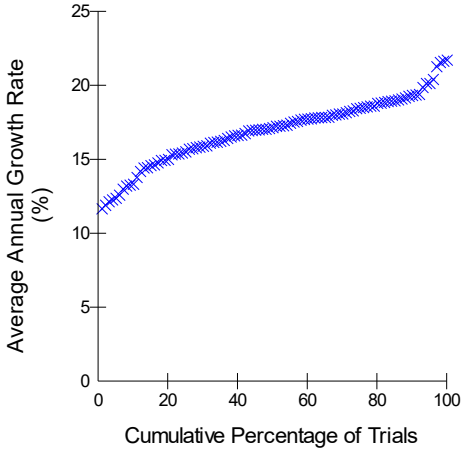
0 to 20+ year-old horses



Totals in 11 Years*

	Gathered	Removed
Lowest Trial	1000	682
10th Percentile	1164	805
25th Percentile	1378	992
Median Trial	1554	1106
75th Percentile	1984	1404
90th Percentile	2166	1553
Highest Trial	2541	1782

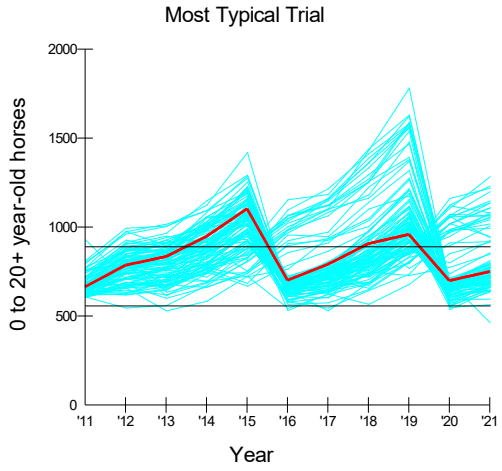
* 0 to 20+ year-old horses

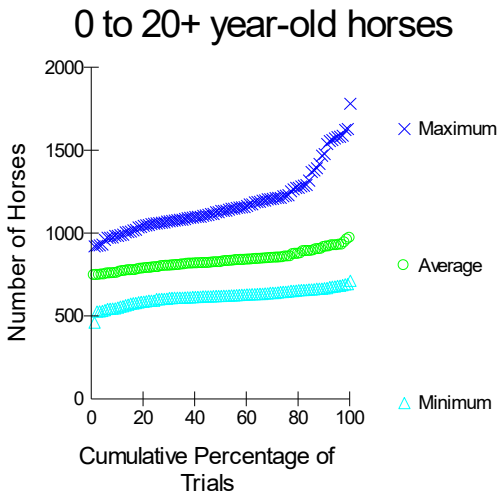


Average Growth Rate in 10 Years

Lowest Trial	11.7
10th Percentile	13.6
25th Percentile	15.6
Median Trial	17.2
75th Percentile	18.5
90th Percentile	19.4
Highest Trial	21.8

Barren Valley Complex – Removal and Fertility Control and Removal with Fertility Control and Sex Ratio Skew





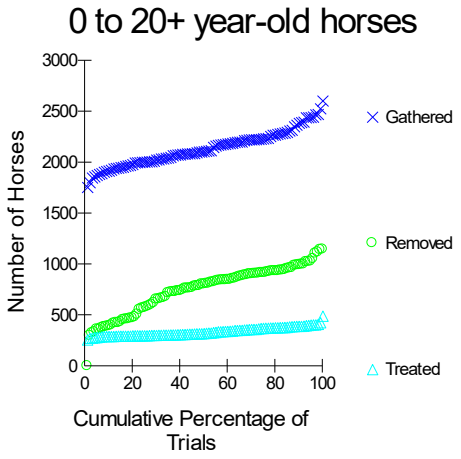
Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	464	746	924
10th Percentile	557	763	990
25th Percentile	605	793	1064
Median Trial	626	826	1138
75th Percentile	651	858	1232
90th Percentile	672	916	1511
Highest Trial	716	971	1784

* 0 to 20+ year-old horses

Explanation:
In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 464 and the highest was 1784. In half the trials, the minimum population size in 11 years was less than 626 and the maximum was less than 1138. The average population size across 11 years ranged from 746 to 971.

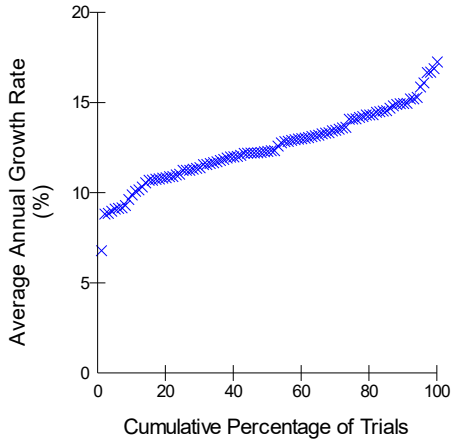
Barren Valley Complex – Removal and Fertility Control and Removal with Fertility Control and Sex Ratio Skew (cont.)



Totals in 11 Years*

	Gathered	Removed	Treated
Lowest Trial	1758	0	259
10th Percentile	1923	396	293
25th Percentile	2007	576	300
Median Trial	2110	806	322
75th Percentile	2236	917	373
90th Percentile	2391	996	392
Highest Trial	2607	1145	492

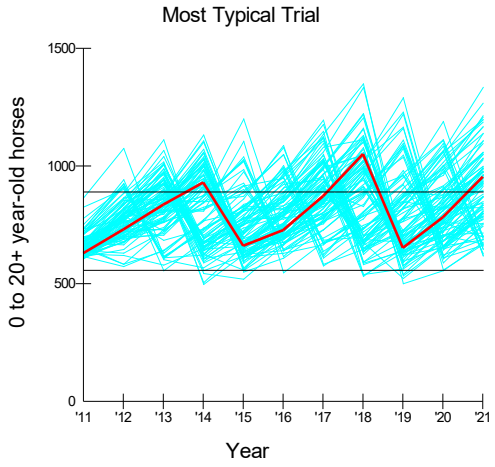
* 0 to 20+ year-old horses

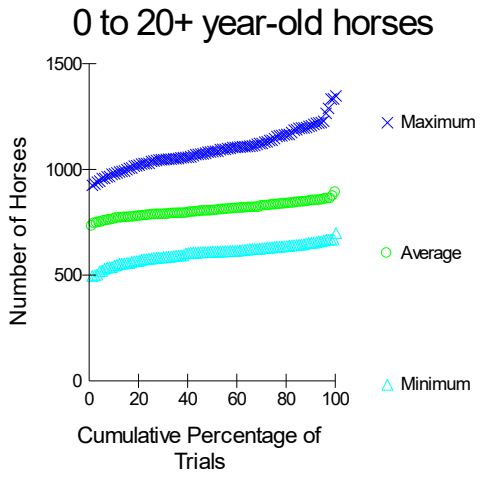


Average Growth Rate in 10 Years

Lowest Trial	6.8
10th Percentile	10.0
25th Percentile	11.3
Median Trial	12.3
75th Percentile	14.1
90th Percentile	15.0
Highest Trial	17.3

Barren Valley Complex – Removal and Sex Ratio Skew





Population Sizes in 11 Years*

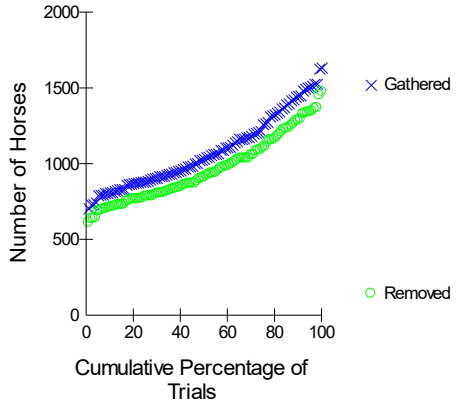
	Minimum	Average	Maximum
Lowest Trial	499	732	928
10th Percentile	550	767	984
25th Percentile	582	786	1042
Median Trial	614	805	1088
75th Percentile	636	833	1154
90th Percentile	656	852	1213
Highest Trial	702	892	1351

* 0 to 20+ year-old horses

Explanation:
In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 499 and the highest was 1351. In half the trials, the minimum population size in 11 years was less than 614 and the maximum was less than 1088. The average population size across 11 years ranged from 732 to 892.

Barren Valley Complex – Removal and Sex Ratio Skew (cont.)

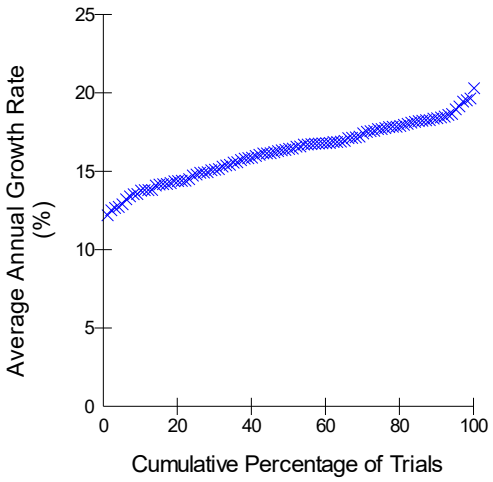
0 to 20+ year-old horses



Totals in 11 Years*

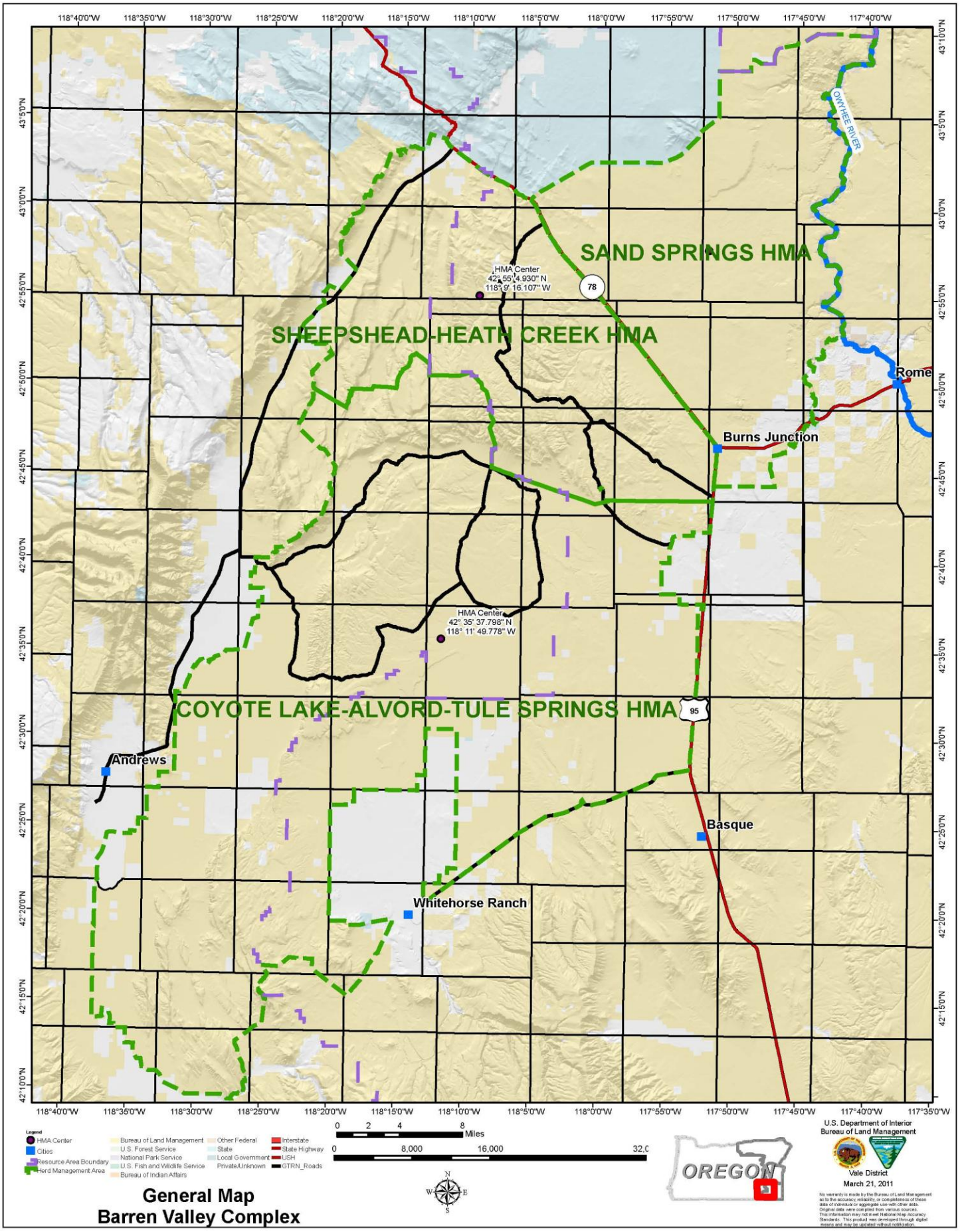
	Gathered	Removed
Lowest Trial	705	612
10th Percentile	812	716
25th Percentile	886	786
Median Trial	1038	916
75th Percentile	1264	1110
90th Percentile	1450	1292
Highest Trial	1635	1477

* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	12.2
10th Percentile	13.8
25th Percentile	14.9
Median Trial	16.5
75th Percentile	17.8
90th Percentile	18.5
Highest Trial	20.3



General Map
Barren Valley Complex

United States Department of the Interior
Bureau of Land Management

Final Environmental Assessment
Challis Wild Horse Gather Plan

Environmental Assessment DOI-BLM-ID-1030-2012-0006-EA

U.S. Department of the Interior
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1.0 Purpose and Need for Action

1.1 Introduction

The Bureau of Land Management (BLM) is proposing to gather excess wild horses in the Challis Herd Management Area (CHMA) in order to achieve the Appropriate Management Level (AML). The gather would occur for a 2 week period in the fall between September 15 and November 15. Approximately 274 wild horses would be gathered, and remove approximately 137 excess wild horses from within and outside the CHMA. Up to 137 of the captured wild horses would be released; of these, about 55 would be mares treated with fertility control and about 82 would be studs or geldings to maintain the sex ratio and slow population growth.

This Environmental Assessment (EA) is a site-specific analysis of the potential impacts that could result with the implementation of the Proposed Action or alternatives to the Proposed Action. Preparation of an EA assists the BLM authorized officer to determine whether to prepare an Environmental Impact Statement (EIS) if significant impacts could result, or a Finding of No Significant Impact (FONSI) if no significant impacts are expected.

This document is tiered to the Challis Resource Management Plan/Final EIS (RMP/EIS, 1999a).

1.2 Background

The CHMA is found in Custer County Idaho and consists of 168,720 total acres (154,150 BLM, 9,454 acres of State of Idaho lands, and 1,116 acres private lands near the East Fork of the Salmon River). The herd management area is bordered on the north by the Salmon River, on the west by the East Fork of the Salmon River, on the south by the ridgeline between Herd Creek and Road Creek and on the east by U.S. Highway 93 and the watershed boundary between the Salmon River drainage and the Lost River drainage.

The Appropriate Management Level (AML) for wild horses within the CHMA is 185. The AML was established in the October 1999 Challis Resource Management Plan EIS following an in-depth analysis of habitat suitability and resource monitoring and population inventory data, with public involvement. The AML for the CHMA is 185. The Challis RMP (CRMP) did recognize that between gathers the population would range up to 253. Two hundred and fifty-three (253) is the maximum number of wild horses that can graze in a thriving natural ecological balance and multiple use relationship on the public lands in the area.

The current estimated population of wild horses is 322 including wild horses residing in the CHMA and those outside the CHMA. This number is based on an aerial population inventory conducted as a direct count with multiple experienced observers immediately following the July 2009 gather, and prior to the release of gathered horses. The 57 animals the inventory identified on the range when coupled with the 167 animals released back into the CHMA following the gather, and the 3 following years foal increase result in a population of 322. Wild horse numbers have increased an average of 17% per year since the HMA was last gathered. The current population is about 174 animals over the AML.

The last wild horse gather of the CHMA occurred in July 2009, when 366 horses were captured, 141 horses were removed, 167 horses were released back into the CHMA. The 167 horses that

were released joined 57 horses that were not gathered, resulting in a 2009 post-gather population of 224 horses.

There are six allotments that are either partially or completely encompassed within the CHMA; including: Bradbury Flat, Bradshaw Basin, Mountain Springs, Road Creek, Split Hoof and Warm Springs. There are also two Areas of Critical Environmental Concern (ACEC) designated in the Challis RMP (USDI-BLM, 1999a), Malm Gulch/Germer Basin and Sand Hollow totaling 19,428 acres of "Frail Lands" where tuftaceous volcanic ash soils exist. These ACECs are part of the CHMA where wild horses have access, but are excluded to livestock grazing. Other ACECs in the CHMA include: Lone Bird, Antelope Flat, and East Fork Salmon River Bench. These ACECs contain a variety of unique features including pristine vegetation, rare plants, unusual plant assemblages, fragile soils, and cultural resource significance. The gather area involves two Wilderness Study Areas (WSAs): Corral-Horse Basin and Jerry Peak.

This environmental assessment (EA) evaluates the environmental effects of various alternatives the BLM has considered to maintain the CHMA wild horse AML.

This EA ensures compliance with the National Environmental Policy Act (NEPA) by providing site-specific analysis of potential direct, indirect, and cumulative effects to the human environment associated with completion of a gather and removal of excess wild horses in the CHMA. Should a determination be made that implementation of the Proposed Action or alternative actions would not result in significant environmental impacts, a FONSI would be prepared to document that determination, and a Decision Record providing the rationale for approving the chosen alternative would be issued.

Since the passage of the Wild Free-Roaming Horses and Burros Act (WFRHBA) of 1971, management knowledge regarding wild horse population levels has increased**.** By law, BLM is required to remove excess animals once a determination has been made that excess animals are present and removal is necessary. In the past two decades, program goals have shifted beyond establishing a *thriving natural ecological balance* (i.e. establishing AML for individual herds) and conducting gathers to achieve and maintain wild horse populations within the established AML so as to manage for a healthy wild horse populations and healthy rangelands. Management actions resulting from shifting the program emphasis include increasing fertility control and adjusting sex ratios to reduce population growth rates, increased gather intervals, improving the accuracy of population inventories and collecting genetic baseline data to support genetic health assessments. Decreasing removal numbers while reducing population growth rates and ensuring the welfare of wild horses on the range is pertinent to these program goals and consistent with findings and recommendations from the American Association of Equine Practitioners (AAEP), who recently issued a BLM Task Force Report in August 2011 following their evaluation of handling procedures and animal welfare at wild horse gathers, and short and long term sorting/holding facilities. The Executive Summary of this report stated: ―*Clearly the mission of the BLM Program – Healthy Ranges, Healthy Horses – is not a simple one. A central issue for all discussions involving the care and management of the wild horse population is controlling the reproductive rate of the wild horses on the range. The AAEP encourages the BLM to prioritize research and application of effective fertility control methods in order to reduce the foaling rate in wild herds"*

Public sentiment received for BLM gathers over the past few years have emphasized the desire for BLM to increase the use of fertility control in order to reduce the number of wild horses that have to be removed from the range or maintained in Long Term Pastures. This proposed gather is consistent with National BLM direction to increase the use of fertility control to maintain wild horses within AML with fewer necessary removals.

The following is a message from the BLM Director Bob Abbey: *"The BLM finds itself in the predicament of needing to gather overpopulated herds from the Western range each year while its holding costs keep rising – with no end in sight. Recognizing this unsustainable situation, the Government Accountability Office, in a report issued in October 2008, found the Bureau to be at a "critical crossroads" because of spiraling off-the-range holding costs and its limited management options concerning unadopted horses. In response, Secretary of the Interior Ken Salazar and I announced on October 7, 2009, a new and sustainable way forward for managing our nation's wild horse horses and burros. We recommended* ***applying new strategies aimed at balancing wild horse and burro population growth rates with public adoption demand to control holding costs*** **[emphasis in original]**. *This effort would involve slowing population growth rates of wild horses on Western public rangelands through the aggressive use of fertility control, the active management of sex ratios on the range, and perhaps even the introduction of non-reproducing herds in some of the BLM"s existing Herd Management Areas in 10 Western states"*. Refer to the entire message at
http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/national /about/director.html

The following is a quote from the Humane Society for the United States (HSUS): *"The HSUS strongly supports an increase in the use of fertility control – specifically the Porcine Zona Pellucida (PZP) immunocontraception vaccine – and sex ratio adjustments to slow population growth. This work should immediately be expanded to as many herds as possible as an alternative to gathers and long term holding. With an efficacy rate of over 90%, a comprehensive contraception program could dramatically reduce the financial burden on the agency and allow the BLM to once again focus its resources and efforts on range management programs" (HSUS 2010).*

To further implement this strategy of increasing population controls as a management tool, the 2012 BLM wild horse gather schedule increased emphasis to apply fertility control to reduce growth rates and thus the number of horses that must ultimately be removed from the range and either found adoptive homes or kept in long-term grazing pastures. This strategy‘s expected result is the removal of only limited numbers of excess wild horses for which there is the greatest adoption demand.

1.3 Purpose of and Need for Action

The Bureau of Land Management (BLM) Challis Field Office (CFO) is proposing to conduct a wild horse gather to remove excess wild horses from the Challis Herd Management Area (CHMA). The BLM is proposing to gather approximately 274 wild horses and remove approximately 137 excess wild horses from within and outside the CHMA in the fall. The purpose of the action is to address an excess population of wild horses, relative to the AML by

removing wild horses from the CHMA and all wild horses that have moved outside designated CHMA boundaries and to increase the interval between gathers.

The action is needed to be consistent with the established AML (USDI-BLM, 1999a) by removing excess wild horses from CHMA, and all horses outside the CHMA. This action would slow population growth rates, balance wild horse populations with herd health and other resources (Table 1). The action is needed to help prevent undue or unnecessary degradation of the public lands, protect rangeland resources from deterioration associated with excess wild horses within the CHMA, and would "restore a thriving natural ecological balance and multiple use relationship on the public lands consistent with the provisions of (P.L. 92-195 Section 1332 (b) (2) (as amended) of the *Wild Free-Roaming Horses and Burros Act WFRHBA of* 1971." Further, the action conducted in a humane, safe, efficient, and environmentally sound manner is needed to ensure current and future populations of healthy wild horses.

The Interior Board of Land Appeals (IBLA) has also held that "*Proper range management dictates removal of horses before the herd size causes damage to the rangeland. Thus, the optimum number of horses is somewhere below the number that would cause resource damage*" Animal Protection Institute, 118 IBLA 63, 75 (1991). The AML for CHMA needs to be achieved and maintained to prevent the decline of wild horse habitat and wildlife habitat and ensure long-term health and well-being of the wild horses. Maintaining wild horse populations consistent with the established AML would promote RMP and Allotment specific objectives, healthy riparian habitat, and maintaining or making progress towards attainment of Standards for Rangeland Health, and protection of ACECs and wilderness values in WSAs.

1.4 Land Use Plan Conformance

The Proposed Action is in conformance with the Challis Resource Management Plan (USDI-BLM, 1999a) under Wild Horses and Burros, Goal 1, Decision #1 "Manage the wild horse herd for an AML of 185 animals in accordance with the 1983 U.S. District Court Consent Judgment and the current activity plan for the wild horse CHMA"; Decision #3 "Monitor wild horse use of the Malm Gulch and Sand Hollow areas, and remove wild horses as necessary to protect fragile watersheds"; and Decision #7 "Adjust wild horse management to ensure progress toward the riparian and aquatic habitat conditions described in Attachment 1."

1.5 Appropriate Management Level (AML)

The AML was established through the Challis RMP (USDI-BLM, 1999a) process signed in 1999. The purpose of the RMP is to "*identify resource condition, objectives, land use allocations, and management actions and direction necessary to guide resource management on a long term, sustainable basis during the next 15-20 years*" (USDI-BLM, 1999a). The Challis RMP set the AML of 185 wild horses for the CHMA utilizing monitoring data, the Challis Experimental Stewardship Program sub-committee information, the 1976 Challis Herd Management Plan (CHMAP) and the 1989 CHMAP Update, as well as the Consent Judgment by United States District Judge Charles R. Richey. The Challis RMP also established objectives and goals of the CHMA stating "*The Herd would vary from 185 to 253 animals between roundups"*. The CRMP also allows the CFO to, "A*djust horse numbers to a lower level if causing unacceptable levels of resource degradation*".

The most recent helicopter inventory flight of the CHMA was conducted in 2009 immediately following the 2009 gather and before horses were released back to the CHMA, which resulted in a direct count of 57 wild horses. Once horses were released the wild horse population was 224. The 2012 estimated population based on 17% average annual increase (long-term average from inventory flights) is 322 wild horses (including foals) in the CHMA. The PZP fertility control was factored in for the 2011 year at 10% (fertility control for the treatment window, plus 90 mare's equaled 10% reproduction). The population estimate exceeds the established AML in the CHMA by 137 wild horses. Prior to the proposed gather, an inventory will be conducted to obtain a recent population inventory.

1.6 Relationship to Statutes, Regulations, Policy, Plans or other Environmental Analysis

The Proposed Action is in conformance with the WFRHBA of 1971 (Public Law 92-195, as amended), Section 302 (a) and (b) of the Federal Land Policy and Management Act (FLPMA) of 1976, the Public Rangelands Improvement Act of 1978 (Pub. L. 95-514, Sec. 4), the Code of Federal Regulations (CFR) at 43 CFR §4700, and policies. Applicable excerpts are as follows: *Where the Secretary determines . . . that overpopulation exists . . .* ***he shall immediately remove excess animals from the range*** *so as to achieve appropriate management levels. Such action shall be taken . . . until all excess animals have been removed so as to restore a thriving natural ecological balance to the range, and protect the range from the deterioration associated with overpopulation.*

The law also provides that determinations will be made ―*whether appropriate management levels should be achieved by the removal or destruction of excess animals,* ***or other options (such as sterilization, or natural controls on population levels)"*** [emphasis added].

FLPMA amended the WFRHBA with ―*In administering this Act, the Secretary may use or contract for the use of helicopters or, for the purpose of transporting captured animals, motor vehicles. Such use shall be undertaken only after a public hearing and under the direct supervision of the Secretary or of a duly authorized official or employee of the Department".*

PRIA directs the continued *"policy of protecting wild free-roaming horses and burros from capture, branding, harassment, or death, while at the same time facilitating the removal and disposal of excess wild free-roaming horses and burros which pose a threat to themselves and their habitat and to other rangeland values.*

BLM's Washington Office policy IM 2010-140, states in Section E: ―*During gather or herd management area planning, the authorized officer will consider a range of alternatives to reduce (slow) population growth rates and extend gather cycles for all wild horse herds with annual growth rates greater than or equal to 5%. These alternatives may include (but are not limited to): fertility control, adjustments in the sex ratio in favor of males, a combination of fertility control and sex ratio adjustment, and management of selected HMA for non-reproducing wild horses".* Similar direction is also located at Section 4.5.3 of the Wild Horses and Burros Management Handbook H 4700-1.

Statutes and Regulations
This action is governed by the **Wild Free Roaming Horse and Burro Act of 1971 (WFRHBA)** (Public Law (PL) 92-195 as amended) and Title 43 **Code of Federal Regulations (CFR)** part 4700. Gathering and disposal of the wild horses would be in accordance with PL 92-195 as amended by PL 94-579 **Federal Land Policy and Management Act (FLPMA)** and PL 95-514 **Public Rangelands Improvement Act (PRIA)**. Included are: **Section 302(b)** of FLPMA states that all public lands are to be managed so as to prevent unnecessary or undue degradation of the lands.

This action is in accordance with the following Federal Regulations:

43 CFR 4710.1, **Land Use Planning**
Management activities affecting wild horses and burros including the establishment of herd management areas shall be in accordance with approved land use plans pursuant to part 1600 of this title.

43 CFR 4710.2 Inventory and monitoring
The authorized officer shall maintain a record of the herd areas that existed in 1971, and a current inventory of the numbers of animals and their areas of use. When herd management areas are established, the authorized officer shall also inventory and monitor herd and habitat characteristics

43 CFR 4710.3-1 Herd Management Areas
Herd management areas shall be established for the maintenance of wild horse and burro herds. In delineating each herd management area, the authorized officer shall consider the appropriate management level for the herd, the habitat requirements of the animals, the relationships with other uses of the public and adjacent private lands, and the constraints contained in 4710.4. The authorized officer shall prepare a herd management area plan, which may cover one or more herd management area plans.

43 CFR 4710.4 Constraints on management.
Management of wild horses and burros shall be undertaken with the objective of limiting the animals' distribution to herd areas. Management shall be at the minimum feasible level necessary to attain the objectives identified in approved land use plans and herd management area plans.

43 CFR 4720.1 Removal of excess animals from public lands
Upon examination of current information and a determination by the authorized officer that an excess of wild horses or burros exists, the authorized officer shall remove the excess animals immediately.

43 CFR 4720.2-2 Removal of excess animals from private lands
If the authorized officer determines that proper management requires the removal of wild horses and burros from areas that include private lands, the authorized officer shall obtain the written consent of the private owner before entering such lands. **Flying aircraft over lands does not constitute entry.**

CFR 4740.1(a) (b) Use of motor vehicles or aircraft

(a) Motor vehicles and air craft may be used by the authorized officer in all phases of the administration of the Act, except that no motor vehicles or aircraft, other than helicopters, shall be used for the purpose of herding or chasing wild horses or burros for capture or destruction. All such use shall be conducted in a humane manner.
(b) Before using helicopters or motor vehicles… the authorized officer shall conduct a public hearing in the area where such use is to be made.

43 CFR 4770.1 Prohibited acts
The following acts are prohibited:

(a) Maliciously or negligently injuring or harassing a wild horse or burro;
(b) Removing or attempting to remove a wild horse or burro from the public lands without authorization from the authorized officer;
(c) Destroying a wild horse or burro without authorization from the authorized officer except as an act of mercy;
(d) Selling or attempting to sell, directly or indirectly, a wild horse or burro or its remains;
(e) Commercially exploiting a wild horse or burro;
(f) Treating a wild horse or burro inhumanely;
(g) Violating a term or condition of the Private Maintenance and Care Agreement;
(h) Branding a wild horse or burro
(i) Removing or altering a freeze mark on a wild horse or burro;
(j) Violating an order, term, or condition established by the authorized officer under this part.

43 CFR 4180.2(b), which states: "Standards and guidelines must provide for conformance with the fundamentals of rangeland health 4180.1."

The National Historic Preservation Act of 1966, as amended (NHPA; with regulations under 36 CFR 800) established the federal government's policy and programs on historic preservation. Section 106 of NHPA requires agencies to consider the effects of their actions on historic properties (defined as cultural resources determined to be eligible for listing on the National Register of Historic Places) prior to project implementation. The NHPA specifically requires federal agencies to identify and manage historic properties on federally owned and managed lands. Consultation under NHPA for the permit renewals has been conducted in accordance with BLM's National Programmatic Agreement and the implementing Protocol Agreement between Idaho BLM and the Idaho State Historic Preservation Office.

The *Fort Bridger Treaty of 1868* (15 Stat. 673) specifically reserves the rights of the Shoshone-Bannock Tribes to hunt, fish, gather, and exercise other traditional uses and practices on unoccupied federal lands, including public lands managed by the BLM-Challis Field Office. The federal government has a federal trust responsibility to manage public lands to provide for the continued exercise of tribal treaty rights, consistent with management policies, on all unoccupied lands within their jurisdiction. Part of the Federal trust responsibility entails conducting government-to-government consultation with Indian groups when a proposed project has the potential to impact the exercise of treaty-reserved rights.

The Clean Water Act (CWA) (33 USC §1251 et seq.) requires that states and tribes restore and maintain the chemical, physical, and biological integrity of the nation's waters. States and tribes must adopt water quality standards necessary to protect fish, shellfish, and wildlife while providing for recreation in and on the waters whenever possible.

Section 303(d) of the CWA establishes requirements for states and tribes to identify and publish a prioritized list of water bodies that are impaired (not fully supporting their designated beneficial uses, every two years). The most recent publication for Idaho was in 2010 by the Idaho Department of Environmental Quality (IDEQ). For waters identified on this list, the State must define total maximum daily loads (TMDLs) for each pollutant causing the water body not to fully support its designated beneficial uses. The TMDL is the amount of pollutant that could be added to the water body per day and the given water body would still fully support all of its designated beneficial uses. This is then used to establish allowable pollutant loads set at levels to achieve water quality standards required for the designated beneficial uses.

Section 7 of the Endangered Species Act (ESA) of 1973, as amended, outlines the procedures for Federal interagency cooperation to conserve federally listed species and designated critical habitat. Section 7(a) (2) states that each federal agency shall, in consultation with the Secretary, insure that any action it authorizes, funds, carries out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of its habitats.

Pursuant to Section 305(b)(2) of the Magnuson-Stevens Act, federal agencies must consult with the National Marine Fisheries Service (NMFS) regarding any of their actions authorized, funded, or undertaken, or proposed to be authorized, funded, or undertaken that may adversely affect Essential Fish Habitat (EFH). The Magnuson-Stevens Act, Section 3, defines EFH as “those waters and substrate necessary for fish for spawning, breeding, feeding, or growth to maturity.”

In 1995, the BLM adopted the Interim Strategy for Managing Anadromous Fish-Producing Watersheds in Eastern Oregon and Washington, Idaho, and Portions of California, commonly referred to as PACFISH (USDI-BLM 1995b). Also in 1995, the BLM implemented the Bull Trout Habitat Conservation Strategy known as INFISH (USDI-BLM 1995c). INFISH is virtually identical to PACFISH except that it applies to land management activities that influence bull trout habitats rather than anadromous fisheries habitats. INFISH standards apply to watersheds that are not already covered by PACFISH.

Sensitive plant inventories have been conducted on all proposed temporary capture sites and no populations have been discovered. If additional capture sites are needed plant clearances will occur prior to any action.

BLM Manual 6330, Management of Wilderness Study Areas, provides guidance on Wild Horse and Burro Management: Wild horse and burro herds are managed in WSAs only within geographic areas identified as having been used as a herd as its habitat in 1971 as directed by the Wild Free-Roaming Horse and Burro Act. Wild horses and burros are managed to remain in balance with the productive capacity of the habitat; this includes managing horses so as not to impair the wilderness characteristics. Wild horse and burro populations must be managed at appropriate management levels so as not to exceed the productive capacity of the habitat (as determined by available science and monitoring activities, to ensure a thriving natural ecological

balance, and to prevent impairment of the wilderness characteristics, watershed function, and ecological processes.

Helicopters and fixed wing aircraft may be used for the installation of new temporary facilities, for aerial surveys, for law enforcement activities, and for the gathering of wild horses and burros.

1.7 Conformance with Rangeland Health Standards and Guidelines

As stated in 43 CFR 4180.2(b) - ―Standards and guidelines must provide for conformance with the fundamentals of 43 CFR 4180.1. The Standards and Guidelines for Grazing Management for public lands have been reviewed by the Departmental Review Team who found that they comply with the requirements of the regulations. Gathering excess horses conforms to the Idaho standards and guides which are in conformance with appropriate land use plans.

1.8 Decision to be Made

The authorized officer's decision would determine whether to implement the proposed gather in order to bring the wild horse population to AML by maintaining or adjusting sex ratios and/or implementing fertility control measures (PZP-22 or current formula) in order to maintain population size within the established AML and avoid the deterioration of the range that can result from wild horse overpopulation. The authorized officer's decision would not set or adjust AML nor would it adjust livestock use, as these were set through previous decisions including the Challis RMP.

1.9 Scoping and Identification of Issues

As part of the preparation of this EA, a scoping letter dated February 3, 2012 was mailed to 41 individuals, agencies, and organizations on the interested public list for the Challis Herd Management Area, Wilderness List, and the associated Allotment Lists. In addition to the scoping letter an open house was held at the Challis Field Office on February 24, 2012 to answer questions and receive comments. Six comments were received at the Open House. Additionally, over 4,000 comments were received from interested publics during the scoping process. A statewide Helicopter Hearing for the use of motorized vehicles in the management of wild horses was held in Kimberly, Idaho on March 7, 2012. Six individuals attended and four commented on the use of helicopters. All of the individuals that commented supported the use of motorized vehicles in particular helicopters in the management of wild horses. Time was available following the meeting to comment on the proposed gathers in Challis and Boise.

The following issues were identified as a result of consultation/coordination and public scoping relative to the BLM's management of wild horses in the planning area:

- Population size and growth rate;
- Expected impacts to individual wild horses from handling stress;
- Expected impacts to herd social structure;
- Expected effectiveness of proposed fertility control application;
- Potential effects to genetic diversity;
- Potential impacts to animal health and condition;
- Impacts to individual wild horses and the Challis Herd.

2.0 Proposed Action and Alternatives

2.1 Introduction

This section of the EA describes the Proposed Action and alternatives, including any that were considered but eliminated from detailed analysis.

The authorized officer would determine whether to implement the proposed gather in order to bring the wild horse population back to AML by maintaining or adjusting sex ratios and/or implementing fertility control measures (PZP-22 or current formula) in order to maintain population size within the established AML and avoid the deterioration of the range that can result from wild horse overpopulation.

This section of the EA describes the Proposed Action and alternatives, including any that were considered but eliminated from detailed analysis. Five alternatives are considered in detail:

2.1.1 Alternative 1: Proposed Action

- Capture 274 wild horses or 85% of the population;
- Remove approximately 137 excess wild horses; including approximately 10 horses outside the CHMA;
- Treat all mares released back into the CHMA with PZP-22 fertility control vaccine at the sorting/holding facility
- Maintain the 60% male sex ratio as specified in the CHMAP revision of 1989.
- Wild horses captured from outside of the CHMA boundaries would be removed regardless of age and would not be released back into the CHMA.
- Field dart mares on private land adjacent to the CHMA if cooperating land owners and volunteers are willing to participate
- Photos of each mare treated with PZP-22 at the sorting/holding facility will be taken prior to release to assist in photo recognition to facilitate field darting trials in the future in the CHMA
- Photos would also be taken of individual mares prior to being field darted for photo recognition

2.1.2 Alternative 2: Removal Only

Capture and remove approximately 137 excess wild horses; including approximately 10 horses outside the CHMA with no fertility control and a 50/50 sex ratio adjustment.

2.1.3 Alternative 3: Removal with Fertility Control and Release of Geldings

- Selective removal of excess wild horses;
- Implementation of fertility control vaccine (PZP-22 or current formula);
- Maintain sex ratios to favor males (60:40);
- Achieving a post-gather population of 185 wild horses in the CHMA;
- A portion of the male population would be managed as a non-breeding population. The target sex ratio for the remaining breeding population would be 10:50:40 with a 10% gelding component;

2.1.4 Alternative 4: Removal with Fertility Control; without Sex Ratio Adjustment

- Capture and removal of approximately 137 excess wild horses, including approximately 10 wild horses from outside the CHMA;
- Administer PZP-22 fertility control vaccine to released mares and adjust the sex ratio to 50:50 from 60:40.

2.1.5 Alternative 5: No Action (Deferred Gather)

No Action — Defer gather and removal for a ten year period based on the Jenkins modeling program. This will display the possible ramifications of not gathering for a ten year period.

2.2 Management Actions Common to Alternatives 1-4

- The gather would begin about Mid-October and take 10 to 14 days to complete. Several factors such as animal condition, herd health, weather conditions, or other considerations could result in adjustments in the schedule.

- Gather operations would be conducted in accordance with the Standard Operating Procedures (SOPs) (Appendix B) described in the National Wild Horse and Burro Gather Contract (Appendix A). The primary gather (capture) methods would be the helicopter drive method with occasional helicopter assisted roping (from horseback).

- Existing capture sites and temporary sorting/holding facilities are located outside of riparian areas in previously used sites or other disturbed areas. Additional capture sites determined to be necessary for humane and effective gather operations will be located outside of stream riparian habitat conservation area (RHCA) buffers, consistent with the Challis RMP (USDI-BLM 1999a, Attachment 4, p. 83 – 84).

- Additional capture sites or sorting/holding facilities determined to be necessary for humane and effective gather operations would be inventoried for cultural resources and sensitive plants, and if present the capture sites and/or facilities would be established elsewhere.

- An Animal and Plant Health Inspection Service (APHIS) Doctor of Veterinary Medicine (DVM) or other licensed DVM will be on-site during the gather to examine animals and make recommendations to BLM for care and treatment of wild horses, and in accordance the 1999 Challis RMP Record of Decision (ROD) (pg. 90.)

- Decisions to humanely euthanize animals in field situations will be made in conformance with BLM policy (Washington Office Instruction Memorandum 2009-041). Current policy reference: http://www.blm.gov/wo/st/en/info/regulations/Instruction_Memos_and_Bulletins/national_instruction/2009/IM_2009-041.html

- Data including sex and age distribution, condition class information (using the Henneke rating system), color, size and other information may also be recorded, along with the disposition of that animal (removed or released).

- In accordance with IM 2009-062 hair samples should be collected every 10-15 years. DNA samples were last collected in 2002 placing the CHMA in the proper time frame for data collection. Hair samples would be collected on about 30 animals to assess the genetic diversity of the herd. Samples would also be collected during future gathers as needed to determine whether BLM's management is maintaining acceptable genetic diversity (avoiding inbreeding depression).

- Excess animals would be transported to the Challis BLM preparation facility where they will be prepared (freeze-marked, vaccinated and de-wormed) for adoption, sale (with limitations) or long-term holding.

- Temporary closure of roads within the CHMA during gather operations may be instituted as necessary to allow for safe and effective operations to proceed.

2.3 Description of Alternatives

2.3.1 Alternative 1. Proposed Action

The Proposed Action would gather about 274 or 85% of the population of wild horses beginning about Mid-October, in order to remove approximately 137 excess wild horses, apply PZP-22 fertility control vaccine to 55 released mares, and maintain a 60% male sex ratio. Wild horses captured from outside of the CHMA boundaries would be removed regardless of age and would not be released back into the CHMA. Animals would be removed using the National Selective Removal Policy IM 2010-164 (Appendix F). Selective removal criteria for the CHMA include: 1) First Priority: Age Class – Four Years and Younger; 2) Second Priority: Age Class – Eleven to Nineteen Years 3) Third Priority: Age Class Five to Ten Years 4) Fourth Priority: Age Class Twenty Years and Older should not be removed from the CHMA unless specific exceptions prevent them from being turned back to the range.

Mares would be treated with a two-year PZP-22 or most current formulation or similar vaccine and released back to the range. Fertility control treatment would be conducted in accordance with the approved standard operating and post-treatment monitoring procedures (SOPs, Appendix B). Mares would be selected to maintain a diverse age structure, herd characteristics and conformation (body type).

Stallions and geldings would be selected for release with the objective of maintaining the 60% male sex ratio. Stallions would be selected to maintain a diverse age structure, herd characteristics and body type (conformation).

The primary gather technique would be the helicopter-drive trapping method. The use of roping from horseback could also be used when necessary. Multiple capture sites may be used to gather wild horses both from within and outside the CHMA. The existing potential capture sites are depicted on Map 8. Any new capture sites or areas designated for sorting/holding facilities would be analyzed prior to use.

Capture sites would be limited to roads or outside active riparian areas or cultural resource sites. Capture sites within WSAs would be restricted to existing roads. No additional capture sites would be set up within known populations of Sensitive Species; any new additional capture sites not depicted on Map 8 would be constructed outside of the RHCA to preclude impacts to riparian areas or aquatic resources. Sites may be monitored for invasive or non-native species for the next several years to verify that these species are not present and do not become established. The capture sites and sorting/holding facilities may be seeded with native seed mixes if deemed necessary by the authorizing officer. These capture sites are used for short time frame of one to three days. However; prior experience has shown sorting/holding facilities may receive sufficient disturbance to require seeding due to longer duration of horses being present and larger numbers of horses. Sorting/holding site selections would follow SOPs (Appendix B).

Following the capture of wild horses, animals would be sorted by age and sex, and selected for release back to the CHMA or for transport to the Challis Wild Horse Preparation Facility. Table 2 displays the anticipated gather and removal figures.

Most foals would be removed and transported to the Challis wild horse preparation facilities as they would be 5-7 months of age and weaned from their dams. If foals too young to wean are encountered they would be released with their dam. If the dam is unable to be released they would be kept together and the foal would not be weaned until of an appropriate age. Due to the timing of the gather in mid-October the occurrence of foals too small to wean would be rare.

Herd health and characteristics data would be collected as part of continued monitoring of the wild horse herds. Other data, including sex and age distribution, condition class information (using the Henneke rating system), color, size and other information may also be recorded for all gathered wild horses. Genetic data would be collected from approximately 30 individuals to monitor the genetic health of the wild horses within the CHMA and compare samples taken in 2002.

Gathered wild horses would be transported to Challis BLM preparation facility or other BLM facilities where they would be prepared for adoption and/or sale to qualified individuals who could provide them with a good home or for transfer to long-term grassland pastures.

Limited bait (food/mineral) trapping may be utilized to achieve the management targets or to relieve specific resource or human safety concerns within and adjacent to the CHMA.

Wild horses would be selected and released back to the CHMA, so as to represent the historic characteristics of the CHMA. This would include selecting animals of moderate or larger stature, average or better confirmation, 20% grey coloration as well as the historic range of colors found within the CHMA. Animals that exhibit exceptional characteristics may be chosen for release outside of the selective removal priorities on a case by case basis. Wild horses to be released would be selected for health, stamina, strength and mothering abilities when these factors can be determined. Weak, unhealthy, and unthrifty animals would not be selected for release back into the CHMA. A helicopter inventory flight may be conducted following the gather to collect information about numbers and distribution of remaining wild horses within the CHMA.

An APHIS DVM or other licensed DVM would be on-site during the gather, as needed, to examine animals and make recommendations to the BLM for care and treatment of wild horses. All excess wild horses removed from within and outside the CHMA would be made available for adoption or sale to qualified individuals.

Any old, sick or lame horses unable to maintain an acceptable body condition (greater than or equal to a Henneke body condition score of 3 or with serious physical defects such as club feet, severe limb deformities, parrot mouth, or sway back would be humanely euthanized as an act of mercy. Decisions to humanely euthanize animals in field situations would be made in conformance with BLM policy (Washington Office Instruction Memorandum 2009-041).Refer to:
http://www.blm.gov/wo/st/en/info/regulations/Instruction_Memos_and_Bulletins/national_instruction/2009/IM_2009-041.html

2.3.2 Alternative 2: Removal Only

Alternative 2 would gather about 274 and remove approximately 137 excess wild horses from within and outside the CHMA beginning about Mid-October. Fertility control **would not be** applied; and the herd's sex ratio would be returned to 50:50.

2.3.3 Alternative 3: Removal with Fertility Control and Release of Geldings

Alternative 3 would gather about 274 and remove approximately 137 excess wild horses from within and outside the CHMA beginning about Mid-October. Fertility control would be applied, as stated in the proposed alternative; the herd's *breeding* sex ratio would be 50:40 stallions to mares. Geldings would make up an additional 10% of the herd acting as a non-reproductive component of the herd. The population would then be comprised of 40% mares and 60% mixed stallions and geldings.

2.3.4 Alternative 4: Removal with Fertility Control; without Sex Ratio Adjustment

Alternative 4 would gather about 274 and remove approximately 137 excess wild horses from within and outside the CHMA beginning about Mid-October. Fertility control would be applied but the sex ratio would return to 50:50.

2.3.5 Alternative 5: No Action (Deferred Gather)

No Action — Defer gather and removal for a ten year period based on the Jenkins modeling program. This would display the possible ramifications of not gathering for a ten year period.

The Proposed Action, Alternative 2, Alternative 3 and Alternative 4 were developed to respond to the identified resource issues and the Purpose and Need to differing degrees. The No Action Alternative would not achieve the identified Purpose and Need. However, it is analyzed in this EA to provide a basis for comparison with the other action alternatives, and to assess the effects of not conducting a gather at this time. The No Action Alternative is in violation of the WFRHBA which requires the BLM to immediately remove excess wild horses, additionally it is not in conformance with regulatory provisions for management of wild horses and burros as set forth at 43 CFR § 4700.

2.4 Alternatives Considered but Eliminated from Detailed Analysis

2.4.1 Water or Bait Trapping as the exclusive gather method

An alternative considered but dismissed from detailed analysis was use of water, bait (food/mineral) trapping as the primary gather method, instead of a helicopter gather. Though the use of water or bait trapping to remove excess wild horses and burros can be fairly effective on a small scale and under specific conditions and circumstances, this alternative was dismissed from detailed study for the following reasons: 1) the area within the CHMA is too rugged to access effectively during the most favorable times to gather; 2) the presence of widely spread water sources in the form of perennial springs and streams make it nearly impossible to restrict wild horse access to only selected water capture sites. The difficult terrain involved and the extended time necessary for implementation of this alternative would result in a substantial increase in gather costs and would make it difficult to limit the gather to the preferred winter months to take advantage of the recommended fertility control application period. Given the impracticalities of implementing this alternative for such a difficult geographic area, this alternative was eliminated from detailed study as the sole gather method. This method may be used in a limited approach on a case by case basis.

2.4.2 Remove or Reduce Livestock within the CHMA

This alternative would involve no removal of wild horses and instead address the excess wild horse numbers through the removal or reduction of livestock within the CHMA. This alternative was not brought forward for analysis because it is inconsistent with the Challis RMP and ROD and is inconsistent with multiple use management.

The proposal to reduce livestock would not meet the purpose and need for action identified in Section 1.3 "*to remove excess wild horses in the HMA. This action is needed in order to achieve a population size within the established AML, protect rangeland resources from further deterioration associated with the current wild horse overpopulation, and restore a thriving natural ecological balance and multiple use relationship in the area consistent with the provisions of Section 3(b) (2) of the Wild Free-Roaming Horses and Burros Act of 1971 (1971 WFRHBA)". R*educing horse populations to within AML is necessary in order to evaluate if wild horse management at AML would allow BLM to meet rangeland health standards.

The total authorized livestock AUMs with the upper range of AML in AUMs and the total wild horse population in AUMs for 2011 can be found in Table 9.

This alternative is also inconsistent with the WFRHBA, which directs the Secretary to immediately remove excess wild horses. Livestock grazing can only be reduced or eliminated if BLM follows regulations at 43 CFR § 4100 and must be consistent with multiple use allocations set forth in the land-use plan. Such changes to livestock grazing cannot be made through a wild horse gather decision, and are only possible if BLM first revises the land-use plans to allocate livestock forage to wild horses and to eliminate or reduce livestock grazing.

Furthermore, re-allocation of livestock AUMs to increase the wild horse/burro AMLs would not achieve a thriving natural ecological balance. Unlike livestock which can be confined to specific pastures, limited periods of use, and specific seasons-of-use so as to minimize impacts to

vegetation during the critical growing season or to riparian zones during the summer months, wild horses are present year-round and their impacts to rangeland resources cannot be controlled through establishment of a grazing system, such as for livestock. Thus, impacts from wild horses and burros can only be addressed by limiting their numbers to a level that does not adversely impact rangeland resources and other multiple uses. For the reasons stated above, this alternative was dropped from detailed analysis. Changes in forage allocations between livestock and wild horses/burros would have to be re-evaluated and implemented through the appropriate public decision-making processes to determine whether a thriving natural ecological balance can be achieved at a higher AML and in order to modify the current multiple use relationship established in the land-use plan.

2.4.3 Control the excess wild horses with only the use of fertility control treatment

An alternative to gather a significant portion of the existing population (95%) and implement fertility control treatments only, without removal of excess horses, was modeled using a three-year gather/treatment interval over a 20 year period. The wild horse population would continue to have an average population growth rate of 0.8% to 6.0 %, adding to the current wild horse overpopulation, albeit at a slower rate of growth. Over the next 20 years, on average the population would grow to between 322 and 1725 wild horses with the resulting population being between 130 and 1540 horses over AML. In order to accomplish the reduced rates of growth between 1435 and 1970 horses would need to be gathered and treated. This alternative would not bring the horse population to AML and would allow the wild horse population to continue to grow even further in excess of AML, resource concerns would escalate, and implementation of this alternative would result in significantly increased gather and fertility control costs without achieving a thriving natural ecological balance. This method has been used with small numbers of horses that are conditioned to human presence and easily approached. This alternative would not meet the purpose and need. It would not comply with the WFRHBA because it would not remove excess wild horses from the range and would not restore a thriving ecological balance to the ecosystem. The complete model trial data are described below in Table 1.

Table 1. WinEquus gather and treat data without removal

	Population Size in 11 Years*		
	Gathered	Removed	Treated
Lowest Trial	1052	0	306
10th Percentile	1328	0	415
25th Percentile	1530	0	465
Median Trial	1760	0	540
75th Percentile	1940	0	604
90th Percentile	2116	0	660
Highest Trial	2561	0	833

* 0 to 20+ year-old horses Parameters used in the modeling include the following assumptions:

Each time a horse is gathered it is counted, even though the same horse may be gathered multiple times during the 11 year period.

Each time a horse is treated with PZP-22 it is counted, even though the same horse may be treated multiple times over the 11 year period.

2.4.4 Gather Portion of Existing Population, Incremental reduction of Wild Horses, Evaluation of Habitat Response

An alternative to gather a significant portion of the existing population (85%) remove only a portion of the excess wild horse population (67 horses) and implement fertility control was recommended through the public review process. Implementation of this alternative would reduce the existing population by 16-18%, but the remaining wild horse population would be anticipated to increase at an average fertility rate of 17% (based upon average number of foals counted during population inventories) annually. This rate of increase would fully offset the 67 horses that would be removed as of the 2013 foal crop and no significant progress would be made in reducing resource impacts from the current overpopulation of wild horses within the CHMA. This alternative would not result in attainment of AML for the CHMA as required by under the WFRHBA.

Additionally, AML represents "*that optimum number of wild horses/burros which results in a thriving natural ecological balance and avoids a deterioration of the range*." (Animal Protection Institute, 109 IBLA 119 1989). The Interior Board of Land Appeals has also held that "Proper range management dictates removal of horses *before the herd size causes damage to the range land*. Thus, the optimum number of horses is somewhere below the number that would cause resource damage" Animal Protection Institute, 118 IBLA 63, 75 (1991) (italics added). The AML is set to prevent range deterioration and allow for allocation of forage for other use in compliance with our multiple use mission. For these reasons, this alternative was eliminated from further consideration.

2.4.5 Alternative Capture Techniques

An alternative using capture methods other than helicopters to gather excess wild horses was suggested through the public review process. The following optional methods were recommended through scoping; chemical immobilization, net gunning, and wrangler/horseback drive capture as potential methods for gathering horses. Net gunning techniques normally used to capture big game animals also rely on helicopters. Chemical immobilization is a very specialized technique and strictly regulated. Currently the BLM does not have sufficient expertise to implement either of these methods and it would be impractical to use given the size of the project area and the number of horses that would need to be removed, access limitations and difficulties in approachability of the horses. Each of these techniques may also increase mortality for wild horses as well as wranglers and domestic horses.

Use of wrangler on horseback drive-trapping to remove excess wild horses can be fairly effective on a small scale. However, given topography, terrain and difficulties in approaching the horses this technique would be ineffective and impractical. Horseback drive-trapping is also very labor intensive and can be very harmful to the domestic horses and the wranglers used to herd the wild horses. Domestic horses can easily be injured while covering rough terrain and the wrangler could be injured if he/she falls off. For these reasons, this alternative was eliminated from further consideration.

2.4.6 Density Dependent Mortality

While some members of the public have advocated "letting nature take its course", allowing horses to die of starvation would be inhumane treatment and would be contrary to the WFRHBA,

which mandates removal of excess wild horses. The damage to rangeland resources that results from excess numbers of wild horses is also contrary to the WFRHBA, which mandates the Bureau to "*protect the range from the deterioration associated with overpopulation*", "*remove excess animals from the range so as to achieve appropriate management levels*", and *"to preserve and maintain a thriving natural ecological balance and multiple-use relationship in that area"*. Once the vegetative resources are at these critically low levels due to excessive utilization by an over population of wild horses, the weaker animals, generally the older animals, and the mares and foals, are the first to be impacted. It is likely that a majority of these animals would die from starvation. The resultant population would be heavily skewed towards the stronger stallions which would lead to significant social disruption in the CHMA. By managing the public lands in this way, the vegetative resources would be impacted first and to the point that they have no potential for recovery. Competition between livestock, wildlife and wild horses for forage and water resources would continue, and worsen as wild horse numbers continue to increase above AML and would conflict with BLM's multiple use mission.

2.4.7 Designation of the CHMA to be Managed Principally for Wild Horses.

Designate the CHMA as "Wild Horse Range". This action under 43 CFR 4710.3-2 would require amendment of the 1999 Challis RMP, which is outside the scope of this EA. Only the BLM Director or Assistant Director (as per BLM Manual 1203: Delegation of Authority), may establish a Wild Horse and Burro Range after a full assessment of the impact on other resources through the land-use planning process. Wild Horse and Burro Range is not an "exclusive" designation. Designation would not necessarily exclude livestock use; therefore levels of livestock grazing permitted could remain the same. The CHMA has not been designated as a wild horse range under 43 CFR 4710.3-2.2. Currently there are four designated Wild Horse and Burro Ranges in the Western United States that are managed principally for wild horses and burros consistent with 43 CFR 4170.3-2.2. These are the Pryor Mountain Wild Horse Range in Montana; the Little Book Cliffs Wild Horse Range in Colorado; the Nevada Wild Horse Range and the Marietta Wild Burro Range in Nevada.

Table 2. Summary and Comparison of Impacts to Wild Horses by Alternative

	Alt 1: Proposed Action	**Alt 2: Removal Only**	**Alt 3: 10% Geldings Removal and Fertility Control only**	**Alt 4: Removal and Sex Ratio only**	**Alt 5: No Action**
Gather Number	274	274	274	274	0
Removal Number	137	137	137	137	0
Fertility Control - # Mares Treated	55	0	55	0	0
Post-Gather Sex Ratio	60/40*	50/50	60/40 or 10/50/40 Gelding/Stallion/ Mare	60/40	Unknown
Post-Gather Population	185	185	185	185	322

*The 1989 Challis Herd Management Plan Update changed the sex ratio to 60/40 males to females

3.0 Affected Environment and Environmental Effects

To comply with the National Environmental Policy Act (NEPA), the Bureau of Land Management is required to address specific elements of the environment that are subject to requirements specified in statute or regulation or by executive order. The following table outlines the elements that must be addressed in all environmental analyses, as well as other resources deemed appropriate for evaluation by the BLM, and denotes if the Proposed Action, sequential alternatives, or the No Action Alternative affects those elements.

Potential or expected impacts to the affected resources are discussed following the table. Direct impacts are those that result from the actual implementation of the chosen alternative. Indirect impacts are those impacts that occur once an alternative has been implemented.

Table 3. Resources Considered in Impact Analysis

Resource	**Present Yes/No**	**Affected Yes/No**	**Rationale**
Air Quality	**Yes**	**No**	The implementation of all alternatives including the no action alternative would not result in the production of vehicle or equipment emission or particulate matter above incidental levels. Short term dust would be present at capture sites.
Areas of Critical Environmental	**Yes**	**No**	There are five Areas of Critical Environmental Concern (ACEC) within the CHMA. They are:

Table 3. Resources Considered in Impact Analysis			
Resource	**Present Yes/No**	**Affected Yes/No**	**Rationale**
Concern (ACEC's)			Malm Gulch/Germer Basin, Lone Bird, Antelope Flat, East Fork Salmon River Bench and Sand Hollow. These ACECs contain a variety of unique features including pristine vegetation, rare plants, unusual plant assemblages, fragile soils and cultural resource significance. No capture sites would be built in an ACEC.
Cultural Resources	**Yes**	**No**	Intensive survey for cultural resources in and near gather-related congregation areas (e.g., proposed capture sites, sorting/holding facilities) has occurred in 1986, 1994, and 2004. These specific areas are used recurrently to support gathers. If additional gather-related congregation areas are determined necessary for the 2012 wild horse gather, inventory and review for cultural resources impacts would occur prior to project implementation. Impacts to properties eligible for listing on the National Register of Historic Places would be avoided, in accordance with the 2012 revision of the BLM's National Programmatic Agreement and the implementing Protocol Agreement between Idaho BLM and the Idaho State Historic Preservation Office. Adherence of the Standard Operating Procedures (SOPs) (Appendix B), would mitigate many potential impacts to historic properties.
Environmental Justice	**No**	**No**	There are no minority or low income populations residing near the proposed project area.
Existing and Potential Land Uses	**No**	**No**	The proposed action would not affect the study areas current and likely future use as a grazing allotment.
Farmlands (prime and unique)	**No**	**No**	There are no prime or unique farmlands in the vicinity of the project area.
Floodplains	**No**	**No**	Based on the FEMA Flood Insurance Study, this allotment occurs in Zone D, an area where no floodplains have been delineated and no flood hazards have been identified (FEMA 1988).
Forest Resources	**Yes**	**No**	There are forest resources in the project area located at high elevations with limited vehicle access. Therefore, Forest Resources would not be affected by the proposed action.
Grazing/Livestock Management	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Invasive, Non-Native Species	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Lands with Wilderness	**No**	**No**	The Challis CHMA overlaps with 6 LWC Inventory units. Three of the units were initially

Table 3. Resources Considered in Impact Analysis			
Resource	**Present Yes/No**	**Affected Yes/No**	**Rationale**
Characteristics			inventoried in the late 1970s (0407-01, Dry Gulch; 0406-07, Lone Pine Peak; and 0406-09, Bradbury Flat), and three of the units were first inventoried for the purposes of this project (0406-16, Gossi Spring; 0406-17, Broken Wagon; and 0406-15, Warm Springs). These units are located on the flats and foothills west of Highway 93 between the town of Challis and Trail Creek Road. The units are primarily composed of low-lying vegetation (grasses and sagebrush) with some of the steeper, higher elevation slopes hosting mahogany and evergreen stands. With the exception of a portion of Unit 0406-07, all were found to be clearly and obviously lacking wilderness characteristics when initially inventoried in 1979. During re-inventory in 2012 it was found that this continues to be the case. The portion of Unit 0406-07 not found to be clearly and obviously lacking wilderness characteristics during initial inventory (identified as unit 46-7) was not evaluated further in the 1980 Idaho Intensive Wilderness Inventory Proposed Decision. However, during re-inventory in 2012 it was determined that this area does not have outstanding opportunities for either solitude or primitive and unconfined recreation. Therefore, there are no Lands with Wilderness Characteristics present or affected within the project area.
Migratory Birds	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Mineral Resources	**Yes**	**No**	There are mineral resources (e.g., material that could be used as fill) in essentially all of the Challis Field Office area, including the project area. However, there would be no meaningful affects to such resources from the Proposed Action or alternatives.
Native American Religious Concerns	**Yes**	**No**	There are no known ceremonial sites or resources associated with ceremonial practices in the proposed project area.
Paleontological Resources	**No**	**No**	There are no known paleontological resources located in the proposed project area.
Range Resources	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Recreational Use	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Socio-economics	**Yes**	**Yes**	The proposed action is consistent with the prevalent economic and social values characteristic of this area. Many of the permittees within the CHMA are running far below permitted use in part due to the high

Table 3. Resources Considered in Impact Analysis			
Resource	**Present Yes/No**	**Affected Yes/No**	**Rationale**
			numbers of horses. With lower horse numbers they may be able run more livestock and add economic growth to Custer County. It is not known if there is revenue generated from Wild Horse viewing. If there is it may be temporarily reduced by fewer horses available. Impacts are disclosed under Environmental Effects.
Soils	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Threatened, Endangered, and Sensitive Fish; Fisheries	**Yes**	**Yes**	There is an absence of threatened / endangered fish or habitat throughout most of the CHMA. Where fisheries resources occur in the Road Creek watershed, and limited gather activities would also occur, measures are included in the action alternatives to prevent effects fish, stream channel habitat, or adjacent riparian areas. The distribution of fish and designated critical habitat along with description of activities and elements intended to prevent effects are disclosed under Affected Environment and Environmental Effects.
Threatened, Endangered, and Sensitive Animals	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Threatened, Endangered, and Sensitive Plants	**Yes**	**No**	There are 5 sensitive plant species found within the proposed project area. They are: Lemhi Milkvetch (*Astragalus aquilonius*), Challis Crazyweed (*Oxytropis besseyi* var. *salmonensis*), Wavy-Leaf Thelypody (*Thelypodium repandum*), Park Milkvetch (*Astragalus leptaleus*), and Challis Milkvetch (*Astragalus amblytropis*). No capture sites would be located within known plant populations. Capture sites not previously surveyed would be surveyed prior to gather site construction.
Tribal Treaty Rights and Interests	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Vegetation	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Visual Resources	**Yes**	**No**	All facilities would be temporary and be removed at the conclusion of the action.
Wastes, Hazardous and Solid	**Yes**	**No**	Hazardous material (DVM drugs (including PZP22) and liquid Nitrogen) in use during the gather operation would be kept, used and disposed of under the supervision of trained BLM staff, contract DVM or APHIS DVM.
Water Quality (Surface and Ground)	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Wetland and Riparian Zones	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.

Table 3. Resources Considered in Impact Analysis			
Resource	**Present Yes/No**	**Affected Yes/No**	**Rationale**
Wild and Scenic Rivers	**No**	**No**	There are no known Wild and Scenic Rivers in the area.
Wildlife	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Wild Horses	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.
Wilderness	**Yes**	**Yes**	Impacts are disclosed under Environmental Effects.

3.1 General Description of the Affected Environment

The CHMA encompasses 154,150 acres of BLM, 9,454 State of Idaho and 1,116 private lands near the East Fork of the Salmon River. The herd area is bordered on the north by the Salmon River, on the west by the East Fork of the Salmon River, on the south by the ridgeline between Herd Creek and Road Creek and on the east by U.S. Highway 93 and the watershed boundary between the Salmon River drainage and the Lost River drainage.

The climate of the region is semi-arid high desert typical of the Rocky Mountains in central Idaho. This climate is characterized by cold winters and hot dry summers which are affected by the Pacific Ocean maritime masses. Elevations, topography and aspect result in high variability in microclimates throughout the CHMA. The nearest national weather service station is located in Stanley, Idaho (1916-2010). This site recorded a mean monthly rainfall of 1.62 inches during January and 0.59 inches during July. Mean monthly max temperatures were 26.5°F in January and 78.5°F in July. The total annual precipitation varies with elevation as a result of orographic lifting resulting in 20-30 in (based upon Snotel Data from Mill Creek Summit site 627 at 8800 feet amsl) of annual precipitation near mountain tops and as little as approximately 7 inches of annual precipitation occurring on the valley floor. Most of the precipitation in the valley occurs as snow at higher elevations during fall and winter months.

The upland vegetation of the East Fork Watershed is dominated by coniferous forests with deciduous wooded areas interspersed along the watercourses. Highly productive mixed conifer stands at low to middle elevations consisting mainly of Douglas-fir, and lodgepole pine. At higher elevations, the moderately productive conifer species are Engleman spruce, subalpine fir, and lodgepole pine. Understory vegetation in the forested areas consists of various shrubs, forbs and grasses. Drier areas support grassland vegetation on sites where trees are scattered or absent. These lower elevation lands consist of a sagebrush/grass complex. Varieties of sagebrush (Artemesia spp.) dominate the watershed below 16 inches of annual precipitation. Mixed salt shrub types (Atriplex spp) are also present below 10 inches of annual precipitation. Herbaceous understory generally includes bluebunch wheatgrass, blue grasses, Idaho fescue, needle grasses, squirreltail, and a variety of perennial and annual forbs.

3.2 Wild Horses

3.2.1 Affected Environment

Congress passed the Wild Free Roaming Horses and Burros Act (WFRHBA) in 1971. The Act recognized the value of wild horses and burros on public lands, and directed public lands management agencies, including BLM, to establish herd management areas where wild horses

and burros could be part of a thriving ecological balance. Within herd management areas, BLM establishes "Appropriate Management Levels" (or AMLs), which is a wild horse and burro population range that can be accommodated within the herd management area without adversely affecting other resources.

Since the passage of the WFRHBA, management knowledge regarding wild horse population levels has increased. By law, BLM is required to remove excess animals once a determination has been made that excess animals are present and removal is necessary. In the past 2 decades, program goals have shifted beyond establishing appropriate management levels for individual herds and conducting gathers to achieve and maintain wild horse populations within the established AML. Management actions resulting from shifting the program emphasis include increasing fertility control and adjusting sex ratios to reduce population growth rates, increased gather intervals, improving the accuracy of population inventories and collecting genetic baseline data to support genetic health assessments. Decreasing removal numbers while reducing population growth rates and ensuring the welfare of wild horses on the range is pertinent to these program goals.

The CHMA has been gathered 14 times since October 1979. Approximately 2000 horses have been gathered since that time. One hundred eighty five wild horses would remain in the CHMA after the gather; of these, about 55 would be mares treated with PZP-22 or latest formula and about 111 would be stallions or geldings; maintaining the sex ratio to 60:40 in favor of males to slow population growth rates and assist in maintaining wild horse population levels consistent with the established AML. The proposed gather would occur in October/November and would be conducted in accordance with the Gather Plan and Standard Operating Procedures (SOPs) located in Appendices A and B. Map 8 displays the proposed gather area, capture sites, wild horse winter range, livestock grazing allotments and pastures.

Wild horses have a long life-span (20-30 years), adapt well to a variety of habitats, and have few natural predators. They also reproduce at a prolific rate; 15-20% (17% in the CHMA) annual reproduction rates are not unusual. Consequently, it is very difficult to maintain the appropriate management level through natural means. Over the years, the BLM has attempted a variety of management techniques to control wild horse and burro populations. The most common method is removal of excess horses, with the intent that the horses would be adopted and trained for domestic use. However, interest in adoptions has declined in recent years, primarily because of the cost of care. Therefore, horses and burros that are removed from the range are often transported to holding facilities, where they are cared for until they are adopted or die. Currently, there are approximately 48,000 horses in short-term corrals and long-term holding facilities.

Long-term holding facilities are expensive to operate and, in the view of wild horse and burro advocates, are contrary to the purpose of the WFRHBA. In response to sky-rocketing costs and public sentiment, the BLM has focused on other methods of population control, including the injection of contraceptives in mares, and adjusting male-female ratios. The most common equine contraceptive used, PZP is effective for two-to-three years, and must be re-applied to maintain effectiveness. Similarly, male-female ratios change over time. Thus, while

contraceptive use and manipulation of the male-female ratio tends to reduce the reproductive rate, it does not preclude the need to periodically remove excess horses from the HMA.

The wild horses in the herd management area are descendants of domestic horses that were released into the wild in the 1800s and early 1900s. For many years, local residents captured the wild horses and bred them with a variety of stock. Presently, the Challis wild horses are a mixture of draft (primarily Percheron) and American Saddle types. This lineage explains the significant number of gray horses in the herd, although there are a variety of colors, including buckskin, sorrel, appaloosa, pinto and bay. Adult horses in the Challis wild horse herd weigh an average of 1,000 pounds and stand between 14.2 and 15.2 hands, with some individuals weighing over 1,200 pounds. The herd is generally healthy, with good genetic diversity. Herd size has varied over the years, primarily as a result of horses being gathered from the range.

Oral history provided by local ranchers was submitted in a court document dated April 15, 1980 (Thomas, 1980) and related that Morgan mares escaped from a ranch and were never recaptured while other Morgans were released, with no attempt to recapture. James Bennett's grandfather released good stallions on the range gathering the offspring each year. Some good saddle horses were also lost and never recovered. Tom Chivers stated that his grandfather ran range mares that were then bred to Shire and Belgian stallions (imported from England), as well as light saddle horses and coach horses. Lawrence Bradbury's grandfather had two or three French Coach Stallions that were turned loose on the range to breed horses for stagecoach horses. Pat Lynch raised both saddle and draft horses, letting the mares run on the range gathering the young draft horses as three year olds to halter brake and then gather again as 5 year olds to sell as teams. Joe Anderson raised saddle stock and catered to the Calvary remount program which added Thoroughbred genetics. One outstanding Thoroughbred or Standardbred stallion named Bally Hall was turned out on the range between the years 1910-1915. Kenneth (Kenny) Bradshaw's father raised Percheron and Belgian horses for his teams (he made his living as a freight driver), pasturing many of his horses on the range. Ken also remembered some good American Saddlers and Hambletonians that were later added to the range herds (Thomas 1980). In summary the CHMA is made up of Percherons, Belgians and Shires and a mix of light breeds. This lineage explains the significant number of gray horses in the CHMA.

Past gather results are listed in Table 4. Previous to and including 2002 all gathers in the CHMA were a "gate cut" action whereby the removal of animals stopped when the target number was reached. In 2004 and 2009 BLM's selective removal criteria was implemented. In 2004, 26 mares were treated with the fertility control PZP. Forty-three mares were treated and released as part of the 2009 gather and PZP treatment. A total of 1,775 horses have been removed since 1979 as part of 14 previous gathers.

Table 4. Challis Historical Gather Schedule

Date	Captured	Removed	Released	Died/Euthanized
Oct-79	148	148	0	0
Oct-80	307	306	1	0
Oct-83	311	311	0	0
Sep-86	88	81	7	0
Sep-88	99	99	0	0
Sep-90	33	33	0	0
Sep-92	82	82	0	0
Sep-94	136	136	0	0
Sep-96	90	86	4	0
Aug-98	111	104	7	0
Aug-00	105	97	8	0
Aug-02	106	95	11	0
Aug-04	140	86	51	1
July-09	366	141	167	2
Total	2119			

Forage is allocated for 185 horses or 2,220 Animal Unit Months (AUMs) in the CHMA. Monitoring data indicate that when the total horse population begins to reach the upper limit of 253 animals (3,036 AUMs), resource conditions begin to decline, especially in riparian areas. Winter Range is the limiting factor for the CHMA so AUMs are based on available forage in winter.

The first BLM population survey flight in 1971 counted 150 horses in the CHMA which helped to establish the original AML of 150 wild horses. In 1979 final grazing EIS and the CHMAP were completed. These decisions called for the "*horse population to average 162 animals with a sex ratio of 60% males to females to allow for no greater than 15% annual population increase*." On March 2, 1983 a Consent Judgment allowed the AML to fluctuate between 185-340 animals but allowed that "*levels may be adjusted, based on either monitoring evaluations, studies, any applicable land use plans or amendments, or upon the advice and recommendation of the Challis Experimental Stewardship Program (ESP) Steering committee. The 340 was based on maximum amount of winter forage available.*" (USDI-BLM 1989). On July 1, 1987 the ESP steering group concerned about heavy winter snows and spring range not being ready to be grazed by large numbers of animals determined to manage the herd numbers at a level of 185 horses. The 1989 CHMAP Revision upheld this number as does the 1999 Challis RMP. The 1999 RMP also recognized that while the AML is 185 that the wild horse population would increase to 253 between gathers.

Wild horses are a long-lived species with documented survival rates ranging between 92% and 95% for all age classes and do not have the ability to self-regulate their population size.

Table 5. Herd Management Areas' Age/Sex and Survival Rates used in WinEquus Modeling.

Wild Horse Range	Age/Sex Classes	Survival Rate
Pryor Mountain Wild Horse Range, Montana	Foal	>95%
	15 years and younger, except for foals, both sexes	93%
Granite Range HMA, Nevada	Foal	>95%
	15 years and younger, except for male foals	92%
Garfield Flat HMA, Nevada	Foal	> 95%
	24 years and younger, except both foals, both sexes	92%

Ecological carrying capacity of a population refers to the level at which density-dependent, population regulatory mechanisms would take effect within the herd. At that level, the herd would show obvious signs of ill fitness. These include poor individual animal condition, low birth rates, and high mortality rates in all age classes due to disease and/or increased vulnerability to predation. Predation and disease have not substantially regulated wild horse population levels within the proposed gather area. Wild horses in general are very resilient and adaptable animals that are able to survive in poor quality habitat. Wild horses typically do not begin to show signs of body condition decline until the habitat components are severely deficient. Once the decline begins, their health deteriorates rapidly.

Baseline genetic diversity and viability samples were gathered in 2002. A total of 46 blood samples were taken during the 2002 gather to create baseline data to establish the current level of genetic diversity for the CHMA. A summary of the data indicates the following:

- The total number of genetic variants was about what is average for domestic horse breeds and well above normal for wild horse populations.
- Genetic variation is well above mean values seen for wild herds and domestic breeds.
- Genetic similarity. The highest mean *(S*) was with the New World Spanish breeds, followed closely by North American Gaited breeds. However, a dendrogram of genetic similarity of the Challis herd to domestic breeds placed the Challis herd within the cluster of the Heavy Draft and True Pony breeds. This likely indicates a mixed breed origin for the herd which also accounts for the high variability and high proportion of rare alleles.
- Variation in the Challis herd is fairly high, most likely due to a mixed breed origin of the herd and a relatively high recent population size.
- The Challis herd is a single interbreeding group.
- No action to modify the genetic makeup of the herd was recommended at the time of the analysis (2003). Of most importance is that Dr. Cothran stated of the CHMA under the recommendation's heading that "*The **AML is high enough that no action should be required** for many generations unless there is a catastrophic reduction in population size.*" (Genetic Analysis of the Challis feral horse herd p.4 emphasis added)

Population Modeling Summary

The Wild Horse Population Model (*WinEqqus version 1.40*) developed by Dr. Steve Jenkins was used to estimate the population growth and size of herds five years after the gather. Dean Bolstad, Washington Office Wild Horse and Burro Specialist, stated "*We recognize that some of the data in the model may not be exact for any given herd management area, but the model has been useful to approximate the effects of management actions and to compare alternatives*". (Bolstad personal communication 2009).

Population modeling was completed for the proposed action and alternatives (Table 6) to analyze how the alternatives would affect wild horse populations. The primary objective of modeling was to identify if any of the alternatives crash the population or cause extremely low population numbers or growth rates. Modeling included: Alternative 1 removal of excess wild horses with implementation of fertility control and maintaining the sex ratio; Alternative 2 removal only; and Alternative 5 No Action (no removal) where any management on the CHMA would be delayed for a 10 year period. The adaptations in the population described by Alternatives 3 and 4 are not an available option in the model. It may be assumed that Alternative 3 numbers would range slightly lower than Alternative 1 and that Alternative 4 numbers would range higher than Alternative 1. The results of population modeling show that minimum population levels and growth rates would be within reasonable levels and adverse impacts to the population would not be likely under Alternatives 1 and 2. Graphic and tabular results are displayed in detail in Appendix D. The population model is run for a period of 11 years including the current year. In other words it is the current year and a 10 year period.

Table 6. Alternatives Modeled in WinEquus

Alternative	Number Gathered (11 years)*	Number Removed (11years)*	Number treated	Median Growth Rate Next 10 Years (%)	Projected Gather (Year)	Average population (11 Years)
Alternative 1 Proposed Action	658	344		12.2%	2022	192
Alternative 2 Removal Only	735		171	15.8%	2022	210
Alternative 5 No Action	N/A	N/A	N/A	15.5%	2022	512

* The WinEquus model runs analysis for 11 years including the current year so that it projects the information for the following 10 years.

3.2.2 Environmental Effects—Wild Horses

Impacts to wild horses would occur on either the individual or the population as a whole. Direct impacts include stress or injuries associated with gathering, sorting, and handling of animals. Indirect impacts include changes in herd dynamics or population numbers.

Proposed Action -Removal, Fertility Control, Maintain Sex Ratio, and Remote Darting

Individual animals would experience moderate levels of physical and psychological stress for short periods of time during gather operations. Heart rates would be elevated, especially during

the final move into a trap site. However, animals would be moving at a walk/trot during most of the gather and would not be moving more than 8-10 miles with the majority traveling 5 - 6 miles. While wild horses in the CHMA are habituated to low levels of human activity (recreation and livestock management) higher levels of disturbance related to gather operations could cause anxiety in individuals. Because all phases of the process would be carried out according to BLM policy, individual stress would be minimized. Animals would be expected to recover from stress within 24 hours of entering the capture site.

Helicopter pilots allow wild horses to travel at their own pace for most of the distance to the gather location. The pilots are very experienced and do not excessively pressure the horses until the horses enter the wings of the capture site, when it is important to move the horses safely into the capture site and prevent them from turning back or trying to disband at the last minute. This is to avoid the need to re-gather or to rope the horses from horseback which could expose them to additional stress or injury. Foals separated during the gather process are safely grouped and transported to the sorting/holding facility to be reunited with their mother.

BLM staff would coordinate with the contractor on a daily basis to determine animal locations in proximity to gather sites, and to discuss terrain, animal health, gather distances and other gather logistics to ensure animal health and safety. Injuries would be examined and treated if needed by a veterinarian at the sorting/holding corrals.

Transport and sorting is completed as quickly and safely as possible so as to move the horses into the large holding pens where they can settle in with hay and water. When releasing animals back to the range, they would be returned to the same general area from which they were gathered.

The BLM has been gathering excess wild horses from public lands since 1975, and using helicopter since the late 1970s. Refer to Appendix B for information about methods that are utilized to reduce injury or stress to wild horses and burros during gathers. The national average mortality rate is 0.5%. In the CHMA 2009 gather, out of 366 horses gathered the mortality rate averaged 0.5% which is very low when handling wild animals. Another 0.6% of the animals captured were humanely euthanized due to pre-existing conditions and in accordance with BLM policy number WO-IM-2009-041. Another 2% died or were euthanized while in short term holding due to liver failure, injuries caused by other horses or collisions with corrals. This data affirms that the use of helicopters and motorized vehicles has proven to be a safe, humane, effective and practical means for the gather and removal of excess wild horses from the range. BLM staff is on-site at all times to observe the gather, monitor animal health, and coordinate the gather activities with the contractor. The SOPs outlined in Appendix B would be implemented to ensure that the gather is conducted in a safe and humane manner, and to minimize potential impacts to or injury of the wild horses. In their August 2012 BLM Task Force Report, the AAEP concluded that the care, handling and management practices utilized by the agency are appropriate for the horses and generally support the safety, health status and welfare of the animals.

Over the past 35 years, various impacts to wild horses from gathers have been observed. Individual, direct impacts to wild horses include handling stress associated with the capture,

sorting, animal handling, and transportation of the animals. The intensity of these impacts varies by individual, and is indicated by behaviors ranging from nervous agitation to physical distress. Observations made through completion of gathers show that the majority of wild horses captured acclimate quickly to the sorting/holding facility situation, becoming accustomed to water tanks and hay, as well as human presence. Both the BLM Wild Horse and Burro Specialists, and the Gather Contractor and crew are very attentive to the needs of all wild horses captured during gathers, ensuring their health and safety.

Accidental death or the need to humanely euthanize animals, as a direct result of gather activities is infrequent and averages less than one half to one percent (0.5 -1%) of the wild horses gather. Injuries sustained by wild horses during gathers may include nicks and scrapes to legs, face, or body from brush or tree limbs while being herded to the capture sites by the helicopter. Rarely, wild horses would encounter barbed wire fences and would receive wire cuts. These injuries are generally not fatal and are treated with medical spray at the sorting/holding corrals until a veterinarian can examine the animal. During some gathers, due to temperament and/or body condition, injures are more frequent, while on other gathers no wild horses are injured or die.

Most injuries are sustained once the animal has been captured and is either within the capture sites, sorting/holding facility, or occurs during sorting. These injuries result from kicks and bites or from collisions with corral panels or gates. Transport and sorting is completed as quickly and safely as possible to reduce the occurrence of fighting and then the wild horses are moved into large holding pens to settle in with hay and water. Injuries received during transport and sorting most often consist of superficial wounds of the rump, face, or legs. Occasionally, horses may sustain a spinal injury or a fractured limb which requires humane euthanasia but these injuries are rare. Similar injuries could be sustained if wild horses were captured through water or bait trapping, as the animals still need to be sorted, aged, transported, and otherwise handled following their capture.

Indirect individual impacts are those impacts which occur to individual horses after the initial stress event, and may include spontaneous abortions in mares, and increased social displacement and conflict in stallions. These impacts, like direct individual impacts, are known to occur intermittently during wild horse gather operations. An example of an indirect individual impact would be the brief skirmish which occurs amongst older stallions following sorting and release into the stud pen. Traumatic injuries usually do not result from these conflicts.

Spontaneous abortion events among mares following capture is very rare. Observations following capture indicate the rate of miscarriage varies, but can occur in about 1 to 5% of the captured mares, particularly if the mares are in very thin body condition or in poor health. This is not likely to be a significant factor based on the timing of this gather and the healthy state of horses in the CHMA.

Stallions selected for release would be released to increase the post-gather sex ratio to approximately 60% stallions in the remaining herds. Stallions would be selected to maintain a diverse age structure, herd characteristics and body type (conformation). It is expected that releasing additional stallions to reach the targeted sex ratio of 60% males would result in smaller band sizes, larger bachelor groups, and some increased competition for mares. With more

stallions involved in breeding it should result in increased genetic exchange and improvement of genetic health within the herd.

Through the capture and sorting process, wild horses are examined for health, injury and other defects. BLM Washington Office Instruction Memorandum IM-2009-041 is used as a guide to determine if animals should be euthanized (refer to SOPs Appendix B). Animals that are euthanized for non-gather related reasons include those with old injuries (broken hip, leg) that have caused the animal to suffer from pain or prevents them from being able to travel or maintain adequate body condition; old animals that have lived a successful life on the range, but now have few teeth remaining, are in poor body condition, or are weak from old age; and wild horses that have congenital, hereditary, or serious physical defects such as club foot or sway back.

It is expected that a few foals may be orphaned during a gather. This can occur if the mare rejects the foal, the foal becomes separated from its mare and cannot be matched up following sorting, the mare dies or must be humanely euthanized during the gather, the foal is ill or weak and needs immediate care that requires removal from the mare, or the mare does not produce enough milk to support the foal. On occasion, foals are gathered that were previously orphaned on the range (prior to the gather) because the mother rejected it or died. These foals are usually in poor condition. Every effort is made to provide appropriate care to all horses, including orphaned foals. BLM staff or DVMs may administer electrolyte solutions or orphan foals may be fed milk replacer as needed to support their nutritional needs. Orphan foals may be placed in a foster home in order to receive additional care. Despite these efforts, some orphan foals may die or be humanely euthanized as an act of mercy if the prognosis for survival is very poor. Due to the timing of the proposed gather, it is unlikely that orphan foals would be encountered as the majority of the current year‘s foals would be weaned already from their mothers.

Wild horses are usually very fit and in good health when not stressed by lack of food and water and are able to endure the physical requirements of a gather. However, the environmental conditions and the overall health and well-being of the wild horses is continually monitored through both summer and winter gathers to adjust gather operations as necessary to protect the wild horses from gather-related health issues. For these reasons, flexibility in gather operations is an inherent part of all gathers.

Wild horses in the CHMA often use snow instead of water when available during fall, winter, and spring months. Wild horses in the CHMA have easy access to water so horses are well hydrated when moving to capture sites. In any case, wild horses are typically in top physical fitness and are able to endure the physical demands of a gather (whether in winter or summer) better than most domestic horses that are not on a top physical training routine due to the requirements of surviving in the wild. Most temperature related issues during a gather can be mitigated by adjusting daily gather times to avoid the extreme hot or cold periods of the day.

Snow accumulation may occur in October, which can increase fatigue and stress. The helicopter pilot, regardless of season, allows horses to travel slowly at their own pace. The Contractor may stomp trails in the snow leading to the capture sites to make it easier for horses to travel to the gather site.

BLM does not gather wild horses with a helicopter unless it is an emergency during the six weeks before or after the *peak* foaling period (April and mid-May) which correlates to the 4 month period between March 1 and June 30 when a majority of foals are born. It is not uncommon for a very small number of foals to be encountered during any month of the year; however most are born during March thru June. If newborn foals or foals too young to wean are gathered, they are matched up with their mares after being gathered. During the proposed gather, most foals would be 5-7 months old and of bigger body size, and can be weaned, if they have not been weaned naturally already. Fall and winter time-frames are less stressful to foals than summer gathers due to them being older and more self-sufficient. Young foals in summer months may be more prone to dehydration and complications from heat stress. Additionally, handling, sorting and transport can be a stress to young animals however, BLM staff on site takes every precaution to assure that horses are handled and maintained to reduce these concerns.

Removal of excess wild horses would improve herd health. Decreased competition for forage and water resources would reduce stress and promote healthier animals. Implementation of Alternative 1 would allow for healthy range conditions and animals over the long term. Reduced population growth rates would be expected to: extend the time until AML is exceeded; increase the intervals between gathers; reduce disturbance to individual animals and herd social structure over the foreseeable future. Modeling suggests that average population growth rates under the median trial for the Proposed Action would be 6.1%. Gathers would still need to occur every 3 years in order to re-apply fertility control, however, according to the modeling, Alternatives 1 and 3 would remove 289-560 fewer wild horses over 11 years.

According to the modeling, if follow-up gathers could be implemented on a regular basis, population control measures may be adequate to maintain the population within existing AMLs.

Population Growth Controls (Fertility Control treatments and sex ratio adjustments)

Under the Proposed Action and Alternatives 3 and 4, the objective for the gather would include the application of fertility control to approximately 55 mares which would be released. All mares selected for release would be treated with a single dose of two-year PZP-22 or similar vaccine/fertility control. Immuno-contraceptive (fertility control) treatments would be conducted in accordance with the approved standard operating procedures (SOPs, Appendix B). Mares selected for release would be selected to maintain a diverse age structure, herd characteristics and conformation (body type).

When injected, PZP (antigen) causes the mare‘s immune system to produce antibodies; these antibodies bind to the mare‘s eggs and effectively block sperm binding and fertilization (Zoo Montana, 2000). PZP is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and can easily be administered in the field. In addition, among mares, PZP contraception appears to be completely reversible. The vaccine has also proven to have no apparent effect on pregnancies in progress, the health of offspring, or the behavior of treated mares (Turner et. al, 1997). Available data from 20 years of application to wild horses contradicts the claim that PZP application in wild mares causes mares to foal out of season or late in the year (Kirkpatrick and Turner 2003). The PZP vaccine is currently being used on over 75 horse management areas for the BLM and its use is appropriate for all free-ranging wild horse

herds. The long-term goal is to reduce or eliminate the need for gathers and removals (Kirkpatrick et al. 2010).

The highest success obtained for fertility control has been achieved when applied during the timeframe of November through February. Efficacy for application of the two-year PZP vaccine based on winter application is as follows:

Table 7. Fertility Control Effectiveness				
	Year 1	**Year 2**	**Year 3**	**Year 4**
Winter Period	Normal	94%	82%	68%
Summer Period	Normal	80%	65%	50%

The treatment would be controlled, handled, and administered by a trained BLM employee (Fertility SOPs, Appendix B). Mares receiving the vaccine would experience slightly increased stress levels associated with handling while being vaccinated and freeze-marked. Serious injection site reactions associated with fertility control treatments are rare in treated mares. Any direct impacts associated with fertility control, such as swelling or local reactions at the injection site, would be minor in nature and of short duration. Most mares recover quickly once released back to the CHMA, and none are expected to have long term impact from the fertility control injections.

As the sole approach, contraception would not allow the BLM to achieve the original population objectives; however, in conjunction with other techniques (e.g., removals of excess animals and adoption) and through incorporation of other population control techniques (e.g., sex ratio adjustments), it now provides a valuable tool in a larger, adaptive management approach to wild horse and burro management.

Contraception may be a cost effective and humane treatment to employ in horses to prevent increases in populations, or with other techniques, to reduce horse populations (Bartholow 2007). In general, contraception would not remove horses from an CHMA‘s population which would result in some continuing environmental effects by those individuals. Horses are long-lived reaching 20 or more years of age in the wild and those horses returned to the CHMA may continue exerting throughout their life span negative effects on the environment as described above, as opposed to the removal of a horse. Contraception, if effective, reduces future reproduction. Limiting future population increases of horses would limit increases in environmental damage from higher densities of horses. It may also reduce the effect of horse gather activities on the environment (if it limits the numbers of horse gathers required). If application of contraception to horses requires capturing and handling horses, the risks and costs associated with capture and handling of horses may be roughly equivalent (not counting the cost of adoption). Application of contraception to older animals and returning them to the CHMA may reduce risks associated with horses that are difficult to adopt or handle in captivity.

Ransom et al. (2010) found no differences in how PZP-treated and control mares allocated their time between feeding, resting, travel, maintenance, and social behaviors in three populations of wild horses, which is consistent with Powell (1999) findings in another population. Likewise, body condition of PZP-treated and control mares did not differ between treatment groups in

Ransom et al's (2010) study. Turner and Kirkpatrick (2002) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation.

In two studies involving a total of four wild horse populations, both Nunez et al. (2009) and Ransom et al. (2010), found that PZP-treated mares were involved in reproductive interactions with stallions more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Wilhelm 1995, Heilmann et al. 1998, Curtis et al. 2002). Ransom et al. (2010) found that control mares were herded by stallions more frequently than PZP-treated mares, and Nunez et al. (2009) found that PZP-treated mares exhibited higher infidelity to their band stallion during the non-breeding season than control mares. Madosky et al. (in press) found this infidelity was also evident during the breeding season in the same population that Nunez et al. (2009) studied, resulting in PZP-treated mares changing bands more frequently than control mares. Long-term implications of these changes in social behavior are currently unknown. Kirkpatrick et al. (2010) conclude by stating that the larger question is, even if subtle alterations in behavior may occur, this is still far better than the alternative and that the other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that would only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not. (Kirkpatrick and Turner 2002, 2008; Turner and Kirkpatrick 2002, 2003; Willis et al. 1994.) Population wide indirect impacts are more difficult to quantify and would occur over time. A large percentage of inoculated mares would experience reductions in fertility. Recruitment of foals into the population would be reduced over a three-year period. The potential multi-year reprieve from foaling would greatly increase overall health and fitness of the mares, as well as the health of the foals born after fertility returns.

Following resumption of fertility, the proportion of mares that conceive and foal could be increased (rebound effect) due to the increased fitness. Additionally, fertility control treatment could cause breeding and foaling seasons to become out of sync with foals born earlier or later in the year, or throughout the year but is generally associated with the timing of the treatment and not the vaccine itself. Research is continuing to document and quantify these effects.

The indirect effect of fertility control and adjustment of sex ratios to favor stallions would extend the time before another gather is required when compared to a gather without implementation of either population growth control method. Wild horses would experience reduced stress and disruption to population dynamics as a result of less frequent gathers. However, it is being recommended that the BLM return to these areas every 2-3 years to re-apply fertility control in order to maintain its effectiveness in controlling population growth rates. By reducing population growth rates, the number of wild horses that would have to be removed from the CHMA during future gathers would also be reduced or possibly eliminated.

Fertility control application would allow the average population size to be maintained at a level consistent with the AML. Long term genetic and physical health and future reproductive success of mares within the herd would be sustained. Reduced population growth rates and smaller population sizes would also allow for improvements to range condition, which would have long-

term benefits to wild horse habitat quality and contribute to the achievement and maintenance of a thriving natural ecological balance.

Population control methods including the adjustment of sex ratios to favor stallions would be expected to have relatively minor impacts to overall population dynamics. Under the Proposed Action and Alternatives 3 and 4, impacts of additional stallions in the population could include: decreased band size, increased competition for mares, and increased size and number of bachelor bands. These effects would be slight, as the proposed sex ratio is not an extreme departure from normal sex ratio ranges. Conversely, a selection criterion, which leaves more mares than stallions, would be expected to result in fewer and smaller bachelor bands, increased reproduction on a proportional basis with the herd, and larger band sizes. With more stallions involved in breeding it should result in increased genetic exchange and improvement of genetic health within the herd.

Modification of sex ratios for a post-gather population favoring stallions is expected to reduce growth rates and subsequent population size, as a smaller proportion of the population would consist of mares that are capable of giving birth to foals. As a result, gather frequency could be reduced as well as the numbers of horses gathered and removed in future gathers.

Wild horses that are gathered would be subject to one or more of several scenarios listed below:

Wild Horses Remaining or Released into the CHMA following Gather

The post-gather goal would be for 185 wild horses to remain within the CHMA. Approximately 137 excess wild horses would be removed during the gather. Wild horses that are not captured may be temporarily disturbed and move into other areas during gather operations. With the exception of changes to herd demographics, direct population wide impacts have proven, over the last 33 years, to be temporary in nature and with most if not all impacts to individual wild horses disappearing within hours to several days of release. No observable effects associated with these impacts would be expected within one month of release except a heightened awareness of human presence.

Herd population dynamics, age structure or sex ratio, growth rates and population size over time are the primary attributes directly related to the proposed gather.

The National Selective Removal Criteria (Appendix B) would be followed to the extent possible, however it is expected that the majority of released and non-gathered animals would consist of all age groups greater than 5 years of age.

The effects of successive removals on populations causing shifts in herd demographics favoring younger horses (5 to 15 years) would also have direct effects on the population. These impacts are not thought of as adverse to a population. They include development of a population which is expected to be more biologically fit, more reproductively viable, and more capable of enduring stresses associated with traumatic natural and artificial events.

The genetic effective population size (Ne) is a measure of the total number of mares and stallions which contribute genetically to the next generation. A population with an age structure involving high numbers of young animals (<5 years of age) would have a lower value of Ne than a similar sized population with a larger component of older breeding-age animals (>5 years of age). Through implementation of the BLM selective removal policy, the wild horses aged 5-10 years of age would be the first priority for release back to the range. Most or all wild horses under five years of age would be removed, thus resulting in a potential increase to the Ne in the CHMA.

Temporary Sorting/Holding Facilities During Gathers

Wild horses gathered would be transported from the capture sites to a temporary sorting/holding facility within the CHMA, primarily in goose-neck trailers, however straight deck semi-trailers may be used. At the temporary sorting/holding facility wild horses would be aged and sorted into different pens based on sex, age and other variables. The horses would be fed certified weed free quality hay and fresh water while in the sorting/holding facility. Mares and their un-weaned foals (if encountered) would be kept in pens together and marked similarly for identification. Horses identified for retention in the CHMA and for fertility control treatment would be maintained in these temporary corrals until the fertility control treatment could be implemented and then be released back into the CHMA.

At the temporary sorting/holding facility recommendations to the BLM regarding care, treatment, and if necessary, euthanasia of the recently captured wild horses would be provided by a licensed DVM. Any animals affected by a chronic or incurable disease, injury, lameness or serious physical defect (such as severe tooth loss or wear, club foot, and other severe congenital abnormalities) would be humanely euthanized using methods acceptable to the American Veterinary Medical Association (AVMA) in accordance to WO-IM- 2009-041.

Transport, Short Term Holding, and Adoption Preparation

Wild horses removed from the range would be transported to the receiving short-term holding facility in a goose-neck stock trailer or straight-deck semi-tractor trailer. Trucks and trailers used to haul wild horses would be inspected prior to use to ensure they can be safely transported. Wild horses would be segregated by age and sex when possible and loaded into separate compartments. Mares and their un-weaned foals may be shipped together. Transportation of recently captured wild horses is limited to a maximum of 12 hours. During transport, potential impacts to individual horses can include stress, slipping, falling, kicking, biting, or being stepped on by another animal. Unless wild horses are in extremely poor condition, it is rare for an animal to die during transport.

Upon arrival, recently captured wild horses would be off-loaded by compartment and placed in holding pens where they would be provided good quality hay and water. Most wild horses begin to eat and drink immediately and adjust rapidly to their new situation. At the short-term holding facility a veterinarian would provide recommendations to the BLM regarding care, treatment, and if necessary, euthanasia of recently captured horses. Any animals affected by a chronic or incurable disease, injury, lameness or serious physical defect (such as severe tooth loss or wear, club foot, and other severe congenital abnormalities) would be humanely euthanized using methods acceptable to the AVMA. Wild horses in very thin condition or animals with injuries

would be sorted and placed in hospital pens, fed separately and/or treated for their injuries. Recently captured horses, generally mares, in very thin condition may have difficulty transitioning to feed. A small percentage of animals can die during this transition; however, some of these animals are in such poor condition that it is unlikely they would have survived if left on the range.

After the recently captured horses have transitioned to their new environment, they are prepared for adoption, sale, or transport to a long-term grassland pastures. Preparation involves freeze-marking the animals with a unique identification number, vaccination against common diseases, castration, and de-worming. During the preparation process, potential impacts to wild horses are similar to those that can occur during transport. Injury or mortality during the preparation process is rare, but can occur.

At short-term corral facilities, a minimum of 700 square feet is provided per animal. Mortality at short-term holding facilities averages approximately 5% (GAO-09-77, Page 51). This includes animals euthanized due to a pre-existing condition, animals in extremely poor condition, animals that are injured and would not recover, animals which are unable to transition to feed, and animals which die accidentally during sorting, handling, or preparation. Approximately 12,000 excess wild horses are being maintained within BLM's short-term holding facilities.

Adoption

Adoption applicants are required to have at least a 400 square foot corral with panels that are at least six feet tall. Applicants are required to provide adequate shelter, feed, and water. The BLM retains title to the horse for one year and the horse and facilities are inspected. After one year, the applicant may take title to the horse at which point the horse becomes the property of the applicant. Adoptions are conducted in accordance with 43 CFR § 5750.

Sale with Limitation

Buyers must fill out an application and be pre-approved before they may buy a wild horse. A sale-eligible wild horse is any animal that is more than 10 years old or has been offered unsuccessfully for adoption at least three times. The application also specifies that all buyers are not to sell to slaughter buyers or anyone who would sell the animals to a commercial processing plant. Sales of wild horses are conducted in accordance with the 1971 WFRHBA and congressional limitations.

Long-Term Grassland Pastures

Since fiscal year 2008, the BLM has removed over 31,680 excess wild horses or burros from the Western States. Most animals not immediately adopted or sold have been transported to long-term grassland pastures (LTP) in the Midwest.

Potential impacts to wild horses from transport to adoption, sale or LTP are similar to those previously described. One difference is that when shipping wild horses for adoption, sale or LTP, animals may be transported for up to a maximum of 24 hours. Immediately prior to transportation, and after every 24 hours of transportation, animals are offloaded and provided a minimum of 8 hours on-the-ground rest. During the rest period, each animal is provided access to unlimited amounts of clean water and two pounds of good quality hay per 100 pounds of body weight with adequate bunk space to allow all animals to eat at one time. The rest period may be

waived in situations where the anticipated travel time exceeds the 24-hour limit but the stress of offloading and reloading is likely to be greater than the stress involved in the additional period of uninterrupted travel.

Long-term grassland pastures are designed to provide excess wild horses with humane, and in some cases life-long care in a natural setting off the public rangelands. There, wild horses are maintained in grassland pastures large enough to allow free-roaming behavior and with the forage, water, and shelter necessary to sustain them in good condition. About 28,600 wild horses that are in excess of the current adoption or sale demand (because of age or other factors such as economic recession) are currently located on private land pastures in Oklahoma, Kansas, and South Dakota. Establishment of LTPs was subject to a separate NEPA and decision-making process. Located in mid or tall grass prairie regions of the United States, these LTPs are highly productive grasslands compared to more arid western rangelands. These pastures comprise about 256,000 acres (an average of about 10-11 acres per animal). Of the animals currently located in LTP, less than one percent is age 0-4 years, 49 percent are age 5-10 years, and about 51 percent are age 11+ years.

Mares and sterilized stallions (geldings) are segregated into separate pastures except at one facility where geldings and mares coexist. Although the animals are placed in LTP, they remain available for adoption or sale to qualified individuals; and foals born to pregnant mares in LTP are gathered and weaned when they reach about 8-12 months of age and are also made available for adoption. The LTP contracts specify the care that wild horses must receive to ensure they remain healthy and well-cared for. Handling by humans is minimized to the extent possible although regular on-the-ground observation by the LTP contractor and periodic counts of the wild horses to ascertain their well-being and safety are conducted by BLM personnel and/or veterinarians. A small percentage of the animals may be humanely euthanized if they are in very poor condition due to age or other factors. Horses residing on LTP facilities live longer, on the average, than wild horses residing on public rangelands, and the natural mortality of wild horses in LTP averages approximately 8% per year, but can be higher or lower depending on the average age of the horses pastured there (GAO-09-77, Page 52).

Euthanasia or Sale without Limitation

While euthanasia and sale without limitation has been limited by current Congressional appropriations, it is allowed under the WFRHBA. Neither option is available for horses under the Department of the Interior's fiscal year 2012 budgetary appropriations and is not expected to be available under the 2013 budgetary appropriations. Although the appropriations restrictions could be lifted in future appropriations bills, it would be contrary to Departmental policy to euthanize or sell without limitations to healthy excess wild horses.

Alternative 2: Removal Only

Gather methods are the same as described in Alternative 1.

Implementation of Alternative 2 would result in capturing fewer wild horses than would be captured in Alternative 1. Alternative 2 would not involve fertility control; mares would not undergo the additional stress of receiving fertility control injections or freeze-marking and would foal at normal rates until the next gather is conducted. The post-gather sex ratio would be about 60:40 stallions to mares with a small percentage of geldings from the 2009 gather. All other aspects of the wild horse gather would be the same as those described for Alternative 1.

Alternative 3: Removal with Fertility Control and Geldings Released as a Non Reproducing Component

Alternative 3 would involve fertility control; therefore, impacts related to fertility control would be the same as Alternative 1. The post-gather sex ratio would be 50:50 mares to stallions. This would be expected to result in fewer and smaller bachelor bands, increased reproduction on a proportional basis within the herd, larger band sizes, and individual mares would likely begin actively producing at a slightly older age.

Bands with a single stallion and mares with offspring would be expected to decline in both size and number while bachelor bands would increase in size and number. An increase in genetic diversity (heterozygosis [Ho]) may also occur within the CHMA with an increase in stallions. A greater number of smaller herds would allow more stallions a chance to express their genetics. These effects would be slight, as the proposed sex ratio is not an extreme departure from normal sex ratio ranges. Modification of sex ratios for a post-gather population favoring stallions or geldings would further reduce growth rates in combination with fertility control reducing the frequency of gathers. This is beneficial in reducing the fertility rate by adding a sterile component that is not reproducing. Field observations from geldings returned to the CHMA in 2009 have shown some geldings would express their stallion behavior; retaining mares and defending territories. Mares in bands held by geldings would not reproduce. All other aspects of the wild horse gather would be the same as those described for Alternative 1.

Alternative 4: Removal with Fertility Control without Sex Ratio Adjustment

Alternative 4 would involve fertility control; but would not include sex ratio alteration returning the sex ratio to 50:50 from the 60:40 stallions and geldings to mares after the 2009 gather. All other aspects of the wild horse gather would be the same as those described for Alternative 1.

Alternative 5: No Action (Deferred Gather)

Under the No Action Alternative, there would be no active management to control the population size within the established AML at this time. In the absence of a gather, wild horse populations would continue to grow at an average rate of 17% per year based on population inventories conducted within the CHMA. Without a gather and removal now, the population would grow to 1,630 in ten years based on the average annual growth rate.

Use by wild horses would continue to exceed the amount of forage allocated for their use. Competition between wildlife, livestock and wild horses would continue, particularly on winter range where quantity and quality of forage is the limiting factor. Damage to rangeland resources would continue or increase. Over time, the potential risks to the health of individual horses would increase, and the need for emergency removals to prevent their death from starvation would also increase.

Due to density dependent factors horses would disperse into less populated areas and outside the CHMA. Movement of wild horses into populated areas and on Highways 75 and 93 would create a public safety hazard. Auto/horse collisions would increase due to this dispersal. Over the long-term, the health and sustainability of the wild horse population is dependent upon achieving a thriving natural ecological balance and sustaining healthy rangelands. Allowing wild horses to die of starvation would be inhumane and would be contrary to the WFRHBA which requires that excess wild horses be immediately removed. Allowing rangeland damage to continue to result from wild horse overpopulation would also be contrary to the WFRHBA which requires the BLM to *protect the range from the deterioration associated with overpopulation, remove excess animals from the range so as to achieve appropriate management levels*, and *to preserve and maintain a thriving natural ecological balance and multiple-use relationship in that area*.

3.3 Vegetation Resources

3.3.1 Affected Environment

Forty-three upland vegetation communities have been identified within the Challis Field Office Area. All but four of these are found within the CHMA. The most dominate of these are sagebrush communities with varying understories of Idaho fescue (*Festuca idahoensis*), Bluebunch wheatgrass (*Pseudoroegneria spicata*), needle-and-threadgrass (*Hesperostipa comata*) or Sandbergs' bluegrass (*Poa secunda*) and Cusick's bluegrass (*Poa cusickii*) species. Five species of sagebrush are prevalent in CHMA those being Basin Big Sagebrush (*Artemisia tridentata tridentata*), Mountain Big Sagebrush (*Artemisia tridentata vaseyana*), Wyoming Big Sagebrush (*Artemisia tridentata Wyomingensis)*, Three Tip sagebrush (*Artemisia tripartita*) and Black Sagebrush (*Artemisia nova*). Also common are shadscale (*Atriplex confertifolia*) and chicken sage (*Sphaeromeria argentea*) communities with Swallen's needlegrass (*Achnatherum swallenii*), squirreltail (*Elymus elymoides*), and bluegrass understories on drier sites (<9 inch precipitation zones) and Douglas Fir (*Pseudotsuga menziesii*) and curled leaf mahogany (*Cercocarpus ledifolious*) communities on the higher elevation, wetter sites (>16" precipitation).

The upland communities are generally in a healthy state with adequate vegetative cover, good plant spacing, and relatively little invasion of noxious or invasive plants. Existing species composition (based on weight) places most of the sites in late seral stage. It is estimated that less than 30% of the CHMA is at mid to early seral stage.

There are 13 wild horse long term trend sites within the CHMA that were located in areas frequented by wild horses, but used infrequently by livestock due primarily to topography and/or distance to water. Seven of the Upland Trend study sites have been monitored in the last ten years and have applicable data. There are also 21 trend sites in Mountain Springs Allotment

within the CHMA, 4 on Bradbury Flat, 2 on Bradshaw Basin, 5 on Road Creek, 2 on Split Hoof, 5 on Warm Springs, and 1 in the Sand Hollow ACEC.

Table 8 below describes the ecological sites by study site and states trend and actual vs. expected vegetative cover. Only the studies that have been monitored in the last 10 years have been included in this analysis

Table 8. Trend and Actual Vegetative Canopy Cover and Expected Vegetation Canopy Cover based on NRCS Site Guides for Rangeland Health Assessment Sites from 2002 for the Challis CHMA

Site	Most Recent Year Monitored	Range Site, Vegetation Type and Landscape Position	Trend (Upward, Downward, or Static)	Actual Vegetative Cover (%)	Expected Vegetative Cover (%)
WH-01	2011	Zeelnot-Meegernot-Adek Association 5-40% Slope Loamy 13-16"ppt. Mountain big sagebrush/ Idaho Fescue 012XY012ID	Downward	20%	15-20%
WH-02	2006	Venom-Cronks Complex 20-50% Slope South Slope Gravelly 11-13"ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY005ID	Upward	83%	*50-75%
WH-03	2006	Nielsen-Gaciba Association 20-50% Slope North slope loamy 12-16" ppt. Threetip sagebrush/Idaho fescue 012XY010ID	Static	93%	*65-75
WH-07	2010	Donkehill-Zeebar Complex 8-50% Slope Shallow Loam 11-13" ppt. Mountain big sagebrush/ Idaho Fescue/Bluebunch wheatgrass 012XY002ID	Downward	14%	15-20%
WH-08	2006	Hutchley-Nurkey Complex Limestone 10-40% South Slope Gravelly 11-13"ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY005ID	Upward	59%	*50-75%
WH-11	2011	Nitchley-Skibo-Rock Outcrop Complex 20-50% Limey Gravelly 8-13" ppt. Black sagebrush/Bluebunch wheatgrass	Downward	9%	15-20%

Table 8. Trend and Actual Vegetative Canopy Cover and Expected Vegetation Canopy Cover based on NRCS Site Guides for Rangeland Health Assessment Sites from 2002 for the Challis CHMA

Site	Most Recent Year Monitored	Range Site, Vegetation Type and Landscape Position	Trend (Upward, Downward, or Static)	Actual Vegetative Cover (%)	Expected Vegetative Cover (%)
		012XY001ID			
WH-13	2010	Nitchley-Skibo-Rock Outcrop Complex 20-50% Limey Gravelly 8-13" ppt. Black sagebrush/Bluebunch wheatgrass 012XY001ID	Static	23%	15-20%
SF-01	2009	Resoot-Friedman Complex 5-35% slope Loamy 13-16"ppt. Mountain big sagebrush/ Idaho Fescue 012XY012ID	Upward	80%	*40-60%
SF-02	2006	Zeelnot-Gravelly Loam Low Precipitation 15-40% Slope Gravelly 13-16"ppt. Threetipped sagebrush/Idaho Fescue 012XY033ID	Downward	85%	*60-75%
SF-05	2006	Xeric Torrifluvents 1-3% Slopes Alluvial Bottom 8-13"ppt. Basin big sagebrush/Western wheatgrass 012XY011ID	Downward	3%	35-40%
SF-06	2005	Parkay-Friedman Association 20-50% Slope Loamy 16-22"ppt. Mountain big sagebrush/Idaho fescue 012XY021ID	Downward	10%	35-50%
SF-08	2005	Reck-Threedot Complex 5-35% Slope Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue 012XY034ID	Downward	87%	*60-75%
SF-10	2011	Dacont-Resoot-Nielsen Association 6-40% Slope South Slope Gravelly 11-13" ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY005ID	Downward	13%	15-25%
SF-11	2006	Simeroi Gravelly Loam 6-15% Gravelly Loam 8-12" ppt.	Static	52%	*35%

Table 8. Trend and Actual Vegetative Canopy Cover and Expected Vegetation Canopy Cover based on NRCS Site Guides for Rangeland Health Assessment Sites from 2002 for the Challis CHMA

Site	Most Recent Year Monitored	Range Site, Vegetation Type and Landscape Position	Trend (Upward, Downward, or Static)	Actual Vegetative Cover (%)	Expected Vegetative Cover (%)
		Wyoming big sagebrush/ Bluebunch wheatgrass 012XY004ID			
SF-12	2008	Zeelnot-Gravelly Loam Low Precipitation 15-40% Slope Gravelly 13-16"ppt. Threetipped sagebrush/Idaho Fescue 012XY033ID	Downward	71%	*60-75%
SF-17	2009	Dawtonia Cold-Dawtonia Complex 2-5% Windswept 8-11"ppt. Silver chicken sage/Fringed sagebrush/Sandberg's bluegrass/Swallen's ricegrass 012XY006ID	Downward	7%	5-10%
SF-18	2007	Friedman-Reck-Goldhill Complex 5-35% Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue 012XY021ID	Downward	11%	35-50%
SF-19	2005	Zeebar-Nielsen-Povey Complex 20-70% Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue 012XY021ID	Downward	2%	35-50%
WS-08	2003	Friedman-Reck-Goldhill Complex 5-35% Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue 012XY021ID	Downward	9%	35-50%
RCB-01	2010	Resoot-Friedman Complex 5-35% slope Loamy 13-16"ppt. Mountain big sagebrush/ Idaho Fescue 012XY012ID	Upward	87%	*40-60%
RCB-02	2010	Zeelnot-Meegernot-Adek Association 5-40% Slope Loamy 13-16"ppt. Mountain big sagebrush/ Idaho Fescue 012XY012ID	Static	92%	*40-60%
WS-05	2009	Dawtoni-Frailton Complex 20-50% Slope South Slope	Downward	10%	15-25%

Table 8. Trend and Actual Vegetative Canopy Cover and Expected Vegetation Canopy Cover based on NRCS Site Guides for Rangeland Health Assessment Sites from 2002 for the Challis CHMA

Site	Most Recent Year Monitored	Range Site, Vegetation Type and Landscape Position	Trend (Upward, Downward, or Static)	Actual Vegetative Cover (%)	Expected Vegetative Cover (%)
		Gravelly 11-13" ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY005ID			
WS-06	2009	Parkay-Zeebar Complex 5-20% Slope Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue/Bluebunch wheatgrass 012XY021ID	Downward	23%	35-50%
WS-07	2009	Friedman-Reck-Goldhill Complex 5-35%Slope Loamy 16-22" ppt. Mountain big sagebrush/Idaho fescue 012XY021ID	Downward	14%	35-50%
WS-14	2009	Parkay-Nurkey Complex 20-50% Slopes Loamy 16-22" ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY021ID	Downward	3%	35-50%
RC-07	2010	Resoot-Friedman Complex 5-35% slope Loamy 13-16"ppt. Mountain big sagebrush/Threetip sagebrush/Idaho Fescue 012XY012ID	Static	72%	*40-60%
SH-05	2006	Dacont-Resoot-Nielsen Association 6-40% slope South Slope Gravelly 11-13" ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY005ID	Downward	68	50-75
BF-01	2006	Snowslide Gravelly Loam Dry 1-10 Percent Slopes Alluvial fans, terraces and drainages 8-12"ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY009ID	Upward	82	35
BF-02	2006	Nitchly-Skibo Rock Outcrop 20-50 Percent Slopes	Upward	87	35

Table 8. Trend and Actual Vegetative Canopy Cover and Expected Vegetation Canopy Cover based on NRCS Site Guides for Rangeland Health Assessment Sites from 2002 for the Challis CHMA

Site	Most Recent Year Monitored	Range Site, Vegetation Type and Landscape Position	Trend (Upward, Downward, or Static)	Actual Vegetative Cover (%)	Expected Vegetative Cover (%)
		Alluvial/colluvial fans terraces and mountain slopes 8-13" ppt.Black sagebrush/Bluebunch wheatgrass 012XY001ID			
BF-03	2006	Resoot-Friedman Complex 5-35% slope Loamy 13-16"ppt. Mountain big sagebrush/ Threetip sagebrush/Idaho Fescue 012XY012ID	Upward	95	65-75
BF-08	2006	Gany Gravelly Loam 30-60 Percent Slopes Alluvial/colluvial fans, terraces, and gentle foothills 13-16" ppt. Low sagebrush,/Bluebunch wheatgrass 043AY001ID	Upward	79	15-20
BB-01	2006	Parkay-Nurkey Complex 20-50% Slopes Loamy 16-22" ppt. Wyoming big sagebrush/Bluebunch wheatgrass 012XY021ID	Static	85	50-75
BB-02	2006	Dacont-Zeebar Association 20-50% Slopes South Slope Gravelly 11-13" ppt. Mountain big sagebrush/Bluebunch wheatgrass	Static	85	35-50

*Total Ground Cover by Litter, Rock and Vegetation

3.3.2 Environmental Effects-Vegetation

Potential horse capture sites have been identified (Map 8), most of which have been used in the past; additional sites may be used, but would be selected to avoid sensitive resources (Appendix B).

Alternatives 1-4

Effects common to all alternatives except the "No Action".

Short-term disturbance would occur in the immediate vicinity of the capture sites and the loading chute. The soil would be compacted and vegetation would be trampled during panel installation by personnel and vehicles and severely trampled in the catch pen area by wild horses, domestic horses, and the wranglers. Crushing of standing vegetation would occur during gather activities from running horses. Previously used capture sites are visually unapparent and difficult to distinguish from adjacent areas. Native vegetation is typically restored in a single growing season with no sign of noxious or weedy species. Although roads and trails would be used where possible, certain impacts would occur. It is unlikely these impacts would result in significant numbers of plants affected or size of area affected. Large stature shrub communities in the wetter areas would experience minimal impacts, while the low stature shrub communities (chicken sage, fringed sage) occupying the drier, more fragile ranges may experience some mortality resulting in reduced ground cover and increased bare ground. Although these impacts may appear to be considerable on a site-specific basis, they are generally slight when viewed throughout the project area. The area may be seeded after the disturbance with a certified weed free native seed that is well suited for the site.

Many of the vegetation types occupying the Herd Management Area are dominated by bluebunch wheatgrass. This particular species is sensitive to grazing during certain times of its life cycle due to its growth form. When the growing points (apical meristems) reach grazing height (4-6 inches) the plant ceases all growth if it is grazed. This response may last several years depending upon the severity of grazing, climate year, and health of the plant affected. Nested frequency plots located within bluebunch wheatgrass communities throughout the CHMA have a trend as follows: 4 static sites, five upward trending sites, and seven downward trending sites. It has not been conclusively determined that the downward trending sites are a result of uncontrolled wild horse grazing or merely natural plant dynamics (i.e. responses to drought). By controlling wild horse numbers at a level anticipated to be in balance with ecological capabilities of the site, these bluebunch wheatgrass communities would remain healthy and vigorous. Vegetation would be impacted positively due to a decrease in herbivory. It would be expected that livestock numbers would not change despite the increased of vegetation. Many of the AUMs that are currently being unused may continue to be unused.

A very small percentage of vegetation would be affected by actual gather activities. Each capture site would disturb <1 acre of surface vegetation. Concentration areas of horses may extend out to < 5 acres. The sorting/holding facility may also disturb an additional five acres. There are typically 3-5 capture sites used each gather. Following this logic up to 10 acres or less than $1/10^{th}$ of 1% of the CHMA vegetation would be affected by alternatives 1-4.

Alternative 5: No Action, Deferred Removal (10 years)
This alternative would also allow for unrestricted increases in the number of horses in the CHMA. Ecological trend of upland sites across the CHMA would be expected to decline. Under Alternative 5, residual herbaceous cover and litter cover from native plants would decrease across the CHMA. During late fall, winter and early spring woody vegetation would be browsed. Utilization would be higher, plant vigor would be lower, plant mortality would be higher, and plant community shifts toward less palatable species would occur faster. Invasive

species would be expected to increase more rapidly, and special status plants habitat would be degraded.

3.4 Livestock Management

3.4.1 Affected Environment

The purpose of this section is to assess the potential direct and indirect effects to livestock management within the CHMA as a result of the Proposed Action and the Range of Alternatives. The information presented here is to supply the reader with a general background and degree of livestock use that occurs within the grazing allotments that occur within the CHMA.

Livestock grazing occurs on 88% of the CHMA; there are six cow/calf grazing allotments within the CHMA currently under deferred or deferred/rest rotation grazing systems with use periods of spring, summer and fall. Two additional areas (Malm Gulch and Sand Hollow) are closed to livestock grazing (Challis RMP, 1999a) due to fragile volcanic ash soils. Warm Springs, Mountain Springs, Road Creek, Split Hoof, Bradbury Flat and Bradshaw Basin allotments are within the CHMA. The pastures are relatively large and allow for a broad range of movement while livestock are present. Temporary electric fences are used to restrict livestock movement and to keep livestock out of the RHCA. Following the conclusion of the grazing season these temporary fences are disabled and gates are opened. The other primary resources associated with the rangeland environment include recreation activities, watershed protection, and wildlife habitat.

Actual use helps determine the current level of use and number of animals. Managers can then better balance the on the ground use with the numbers that are permitted onto public land. Actual Use is defined as "*means where, how many, what kind or classes of livestock graze on an allotment or on a portion or pasture of an allotment*" (43 CFR 4100.0-5). In 2011 49% of the allotted AUMs were permitted on BLM. Conversely, 51% of the available AUMs were not permitted for various reasons some of which are resource protection, numbers of wild horses, and range improvement maintenance. Table 9 below describes by allotment the percent of AUMs used and what percentage of the allotment falls within the CHMA.

Table 9. CHMA LIVESTOCK AUMS FOR THE 2011 BILLING CYCLE (March 1, 2011 to February 28, 2012)

Allotment	AUMs Available	Actual Use 2011	% of Use	% of Allotment in CHMA	Allotment Acres Within CHMA	% of CHMA
Bradbury Flat	839	273	33	100	15706	9
Bradshaw Basin	908	277	31	100	8184	5
Mt. Springs	8884	6688	77	59	55695	32
Frail Lands (unallotted)	0	0	0	100	19428	12

Table 9. CHMA LIVESTOCK AUMS FOR THE 2011 BILLING CYCLE (March 1, 2011 to February 28, 2012)						
Allotment	**AUMs Available**	**Actual Use 2011**	**% of Use**	**% of Allotment in CHMA**	**Allotment Acres Within CHMA**	**% of CHMA**
Road Creek	534	37	7	98	9933	6
Split Hoof	268	102	38	100	8520	5
Warm Springs	4632	587	13	79	50774	31
Total	**16186**	**7964**	**49%**	**--**	**168648**	**100**

The CHMA has relatively few fences; most are "open ended" or drift fences that allow movement around the ends. There are some pasture fences that could limit travel. This is mitigated by gates being open except when livestock are present for 2-6 weeks during the year.

Table 10 below depicts the authorized livestock use (number of animals and dates of use) in the CHMA on all of the allotments except Mountain Springs which is depicted in Table 11.

Table 10. AUTHORIZED LIVESTOCK USE

Allotment	Pasture	Rotation Type	Livestock		Grazing Period		AUMS
			Number	Kind	Begin	End	
Bradbury Flat	Lime Rock	Deferred/Rest	166	Cattle	5/16	7/30	415
	Lime Rock North	Deferred/Rest	166	Cattle	5/16	7/30	415
	Bishop Spring	Deferred/Rest	166	Cattle	5/16	7/30	415
	RML	Deferred/Rest	166	Cattle	5/16	7/30	415
	Jensen Basin	Deferred/Rest	166	Cattle	5/16	7/30	415
	Lone Pine	Deferred/Rest	166	Cattle	5/16	7/30	415
Bradshaw Basin	Upper Bradshaw Basin	Deferred	279	Cattle	5/16	7/30	425
	Lower Bradshaw Basin	Deferred	279	Cattle	5/16	7/30	425
Split Hoof	Morraffio	Deferred	183	Cattle	5/16	6/15	187
	Pappas	Deferred					
Road Creek	Chicken Creek	Deferred	130	Cattle	5/16	8/31	207
	Dry Hollow	Deferred					
Warm Springs	Spar Canyon	Deferred/Rest	490	Cattle	5/9	8/24	1705
	Antelope Flat	Deferred	225	Cattle	5/15	6/15	213
	Antelope Flat	Deferred	225	Cattle	10/16	11/15	206
	Warm Springs	Deferred/Rest	512	Cattle	5/1	7/10	1171
	Warm Springs	Deferred/Rest	525	Cattle	11/1	1/7	1105

Table 11. Mountain Springs Authorized Livestock Use and Rotation

	Year 1		Year 2		Year 3		Year 4	
	2012	2012	2013	2013	2014	2014	2015	2015
	Date	#/ AUMS	Date	#/ AUMS	Date	#/ AUMS	Date	#/ AUMS
Gooseberry **	10/1-11/12	650/ 919	5/21-6/19	945/ 932	10/1-11/12	650/ 919	5/18-6/15	945/ 901
Antelope Flat	10/2-11/15	350/ 518	5/20-6/19	525/ 535	10/2-11/15	350/ 518	5/19-6/16 9/12 T	525/ 501 700 /23
Sheep Creek**	8/25-9/15	1400/ 1013	6/20-7/27	945/ 1181	8/13-9/11	1400/ 1381	REST	
Two Buttes**	5/18-6/15	840/ 801	9/9-9/13 10/2-11/12	1400/ 230 600/ 849	5/18-6/15	840 801	10/2-11/14	600/ 868
Sage Creek**	5/18-6/15	630/ 601	10/1-11/15	400/ 592	5/20-6/15	630/ 601	10/1-11/15	400/ 592
Dry Canyon	7/28-8/24	735/ 677	8/8-9/8 8/8-8/24	600/ 612 30/ 17	7/17-8/12	735/653	6/16 T 8/15-9/12	945/ 31 735/ 701
Spring Basin*	7/28-8/24	735/ 677	8/11-9/8 8/11-8/24	800/ 782 40/ 19	REST 7/14-7/16 T	735/ 73	6/17 T 7/17-8/14	735/ 24 735/ 677
Broken Wagon	Rest	--------	6/20-7/15 7/28-8/7	525/ 449 630/ 228	7/17-8/12	735/ 653	8/14-9/11	700/ 667
N. Fork Sage Road Creek*	6/16-7/13	735/ 677	REST		6/16-7/13	735/677	7/16-8/12	735/ 653
Horse Basin	7/14-7/27	1470/ 677	7/16-8/10 7/28-8/10	525/ 449 315/ 145	REST 7/14-7/16 T	735/ 73	6/17-7/15 6/17-7/15 8/1-8/4 T	525/ 501 210/ 200 735/ 24
Mosquito/Bear Creek	6/16-7/13	735/ 677	REST		6/16-7/13	735 /677	6/18-7/16	735/ 701
Total		1470/ 7237		1470/ 7020		1470/ 7026		1470/ 7064

**Outside CHMA, *Portion in CHMA, T- Trailing Action

There are also numerous range improvement projects located within the CHMA which have been built in cooperation with livestock operators in order to improve distribution of livestock, as well as conditions of the range. These projects include spring developments, stock ponds, vegetation treatments, water tanks, and guzzlers. Much of the maintenance of these projects, especially water developments is performed by livestock operators. Maintenance includes such actions as repair or replacement of pipelines and head boxes, and repair of water tanks. The construction and maintenance of these improvements benefit livestock, wild horses, and wildlife.

3.4.2 Environmental Effects—Livestock Management

Alternatives 1-4
Livestock may be present on the Warm Springs Allotment during gather. Because gates would be opened between allotments to facilitate movement of wild horses to capture sites, livestock could move between allotments during the gather. To prevent accidental trespass of livestock, permittees may be asked to move livestock to areas away from allotment boundaries or concentrations of wild horses. Livestock may experience some level of stress or may be displaced when the helicopter is in the area. This would put an additional burden on the livestock operator to ensure his cattle are out of the area. Impacts from humans and horses at capture site locations to livestock would be slight, localized per capture site, and for a limited period (<4 days).

Maintaining wild horse numbers within AMLs would result in slight reductions in forage utilization levels, around water developments, over a four year period. Overlap between wild horse and livestock use areas would also be limited resulting in appropriate utilization levels (40-50%) for each of the above allotments.

Alternative 5. No Action (Deferred Gather)
Increased horse numbers would result in increased competition for water and forage. This would result in vegetation utilization rates that would exceed the capacity of the area, degrading the forage resource and deteriorating the habitat. As the productivity and composition of desirable forage species decreases, an increase in the invasion of undesirable species would occur. This would result in greater competition for desirable forage between livestock and wild horses. It may also result in livestock traveling to more sensitive areas looking for more desirable forage. This decline would continue to the point that there would be both insufficient plant cover for range site protection and insufficient forage for all rangeland users, which in turn would reduce stocking rates and possible closure of the allotments. Livestock stocking rates may have to be reduced as wild horse population increases.

3.5 Invasive Species

3.5.1 Affected Environment
Invasive species and their encroachment on BLM administered lands represent a serious threat to the continued productivity, diversified use and aesthetic value of the CFO's public lands. BLM currently has an active noxious weed management program which emphasizes cooperation with Custer County, private landowners and BLM permitted land users. The CFO weed management program is based in part on the 2007 *Vegetation Treatments Using Herbicides on Bureau of*

Land Management Lands in 17 Western States Programmatic Environmental Impact Statement. (USDI-BLM, 2007), and the Challis-Salmon Field Office Integrated Weed Control Program, DOI-BLM-ID-330-2008-EA-30.

Under this integrated program, most known weed sites are inspected and treated on an annual basis. Use of herbicides has been evaluated by BLM in the *2007 Vegetation Treatments Using Herbicides on BLM Lands in 17 Western States PEIS* (USDI-BLM, 2007). The Challis Field Office tiered to this document in preparing the 2009 Challis -Salmon Integrated Weed Control EA.

Invasive species have been documented at 86 sites within or immediately adjacent to the CHMA. The species which have been documented and their number of occurrences are: Spotted knapweed *(Centaurea stoebe)* - 56; Leafy spurge (*Euphorbia esula*) – 13; Whitetop/Hoary cress (*Cardaria draba*) – 12; Diffuse knapweed (*Centaurea diffusa*) – 3; Canada thistle (*Cirsium arvense*) - 3; Black henbane (*Hyoscyamus niger*) -3; Russian knapweed (*Acroptilon repens*) -1; and Rush skeletonweed (*Chondrilla juncea*) -1. The vast majority of these sites are less than half an acre in size. Half of the inventoried sites occur along Highways 93 and 75, which are boundaries of the CHMA. In 2011, a total of 10.5 acres of chemical treatment was applied to weed sites throughout the CHMA.

Cheatgrass inventories have not yet been completed in the CHMA, so the extent of cheatgrass infestation is difficult to quantify.

3.5.2 Environmental Effects—Invasive Species

Alternatives 1-4
Wild horse gather activities would disturb soils in localized areas, primarily associated with capture sites and sorting/holding facility. Follow-up inspections by the BLM of these sites and treatment of any noxious weeds would prevent noxious weeds from invading and dominating adjacent native plant communities. There have been 14 gathers conducted in the CHMA since 1979. All of these have involved the use of trucks, trailers, domestic horses, and capture sites. An increase of invasive species has not been documented at any of the known capture sites; in most cases these sites are difficult, if not impossible to identify due to the recovery of native vegetation.

The BLM anticipates that the removal of wild horses over time would decrease overall impacts of wild horse use and proliferation of invasive, non-native species. As wild horses and livestock are managed within allocated forage levels, grazing pressure is reduced on native vegetation. Those native vegetation communities are then more resilient and better able to compete with non-native species.

Alternative 5 – No Action (Deferred Gather)
No impacts from the gather would occur. However, wild horse populations would remain over appropriate management levels and the impacts to native vegetation from wild horse grazing or trampling would increase. Impacts to the present plant communities could lead to an expansion of noxious weeds and invasive non-native species.

3.6 Wetlands/Riparian Zones

3.6.1 Affected Environment

Riparian-wetland habitats (i.e., riparian ecosystems) are generally defined as a body of water with its adjacent soil and vegetation (Hall and Bryant 1995). Healthy riparian ecosystems possess the following indicators: 1) adequate riparian-wetland vegetative cover present to protect banks and dissipate energy during high flows; 2) streambank vegetation, often called the "greenline" is comprised of those plants or plant communities that have root masses capable to stabilize streambanks; and 3) age class and structural diversity of riparian/wetland vegetation. Riparian-wetland vegetation should also reduce erosion by stabilizing streambanks, providing shade, filtering sediment, aiding floodplain development, dissipating energy, delaying flood water, and increasing groundwater recharge.

The riparian land cover includes natural vegetation dominated or characterized by shrub and/or herbaceous species requiring environmental conditions of moderate moisture and temperature. In semi-arid environments, riparian-wetland areas make up a relatively small but productive and resilient portion of the landscape, exhibiting vegetative or physical attributes reflective of the influence of water. They are important for the ecosystem functions they serve, such as floodplain and ground-water storage, water transport, improved water quality, and fish/wildlife habitat. The majority of the drainage network located within the CHMA consists of dry channels receiving water only during periods of high snow melt and/or intense precipitation. Relatively few stream miles (5% within the CHMA) convey water perennially; the stream miles by stream type are given in Table 12.

Proper Functioning Condition (PFC) is a qualitative method for assessing the condition of riparian-wetland areas. The term PFC is used to describe both the assessment process, and a defined, on the-ground condition of a riparian-wetland area. There are PFC methods for both streams (lotic) and springs (lentic) (USDI-BLM, 1998, 2003). PFC was designed as a baseline for the purpose of inventorying riparian areas and wetlands managed by BLM. While PFC is meant to establish a baseline, it is not a quantitative monitoring approach and is not meant for monitoring trend. Multiple Indicator Monitoring (MIM) has been developed for the purpose of monitoring stream channels and stream side vegetation on those streams and small wadeable rivers designated for monitoring. This is done for the purpose of determining the condition and trend of streambanks, channels and streamside vegetation. MIM measures the effectiveness of management strategies over 5 to 10 year time scales establishing trend. The metrics include but are not limited to: greenline-to-greenline width, streambank alterations, greenline vegetation composition, stubble height, woody species age class, and utilization on woody species. These metrics are then implemented in determining the ecological status of the greenline vegetation, the site wetland rating, and the streambank stability (USDI-BLM 2011a). Greenline analysis was conducted prior to MIM for the purpose of monitoring the streambank vegetation and determined the seral status and site wetland rating for a given DMA [Designated Monitoring Areas] (Winward, 2000). While both methods are similar the key difference is that the Winward Greenline Method was based upon steps of community type whereas the MIM method uses a plot (50 cm x 20 cm) placed at 80 or more locations within the DMA. The MIM method is considered more precise and/or repeatable than the Winward Greenline method. Photo monitoring has also been commonly employed by the CFO at streams and springs for implementation monitoring and to qualitatively assess conditions and trend.

Stream flow regimes in CHMA are indicative of a snow melt dominated system with highest flows occurring in July (870 CFS [Cubic Feet per Second] mean monthly discharge on East Fork Salmon River at USGS Gauge) and lowest flows occurring in January and February (79 CFS mean monthly discharge on East Fork Salmon River at USGS Gauge). The USGS Gauge is located near the mouth of the East Fork of the Salmon River and recorded surface flow from September 1928 – September 1939 & May 1973 – September 1981. The maximum flow 4,020 CFS was recorded on June 17, 1974 and the minimum flow 26 CFS was recorded on November 21, 1977. High magnitude flow events are typically associated with intense precipitation and/or high snow melt. This area is subject to high intensity short duration thunderstorms which result in episodic intense stream flow. Base flow occurs in the winter as a result of the majority of the precipitation being stored as snow.

Table 12. Miles of Stream by Stream Type

Stream Name	Stream Miles	Stream Type
Unnamed Streams	550.5	Intermittent to Ephemeral
Bear Creek	1.6	Intermittent to Ephemeral
Broken Wagon Creek	6.4	Intermittent to Ephemeral
Corral Basin Creek	0.9	Intermittent to Ephemeral
Horse Basin Creek	3.6	Intermittent to Ephemeral
Lone Pine Creek	8.5	Intermittent to Ephemeral
Mosquito Creek	1.6	Intermittent to Ephemeral
Road Creek	2.1	Intermittent to Ephemeral
Warm Spring Creek	2.7	Intermittent to Ephemeral
Unnamed Stream	17.2	Perennial
Bear Creek	4.1	Perennial
Broken Wagon Creek	1.3	Perennial
Corral Basin Creek	6.1	Perennial
East Fork Salmon River	0.2	Perennial
Horse Basin Creek	6.1	Perennial
Lone Pine Creek	0.3	Perennial
Mosquito Creek	6.1	Perennial
Road Creek	13.9	Perennial

Data compiled and distances estimated with a GIS using the USGS National Hydrology Dataset

Table 13. Proper Functioning Condition Inventory Summary for the Horse Management Area

Stream	Proper Functioning Condition (PFC)	Functional at Risk-Upward Trend (FAR-up)	Functional at Risk-Static (FAR-Trend Not Apparent)	Functional at Risk-Downward Trend (FAR-down)	Functional at Risk-Trend Not Assessed	Non Functional (NF)	Total Stream Miles
Bear Creek	1.8	0.4			2.9		5.1
Broken Wagon Creek					2.5*	1*	3.5
Corral Basin Creek					2.4	1.6	4
East Fork River	0.2						0.2
Horse Basin Creek	1.2	1			2.8	1.4	5.6
Lone Pine Creek		0.8					0.8
Mosquito Creek	3.9				1.2		5.1
Pine Creek				1.4	1.6		3
Road Creek					10	1.1	11.1
Warm Springs Creek					0.5		0.5
Percent of total	18%	6%	0%	4%	61%	13%	100%

All stream distances are given in miles and were estimated using GIS
*Data obtained from PFC Database (USDI-BLM, 2011b)

The CHMA is divided between the East Fork Salmon River and the Warms Spring Creek watersheds. The underlying geologic units are predominantly limestone and dolostone formations (Fisher and Johnson, 1995) which favor infiltration over stream runoff, resulting in the presence of springs with consistent perennial flow where portions of this infiltrated water re-emerges on these allotments. Riparian habitats involve not only riverine ecosystems, but also include vegetation associated with seeps, springs brooks, lakes, and ponds. The greenline plant community structure within riparian areas may vary depending on frequency of flooding, amount of scouring, and past disturbance. The riparian areas in the CHMA commonly support woody plant species including black cottonwood, quaking aspen, douglas-fir, mountain maple, wood's rose, willow species, red-osier dogwood, chokecherry, gooseberry, currant; as well as mesic forbs, grasses and desirable hydric species like nebraska sedge, beaked sedge and baltic rush.

The Spar Canyon sub-watershed is the first major drainage on the east side of the East Fork Salmon River traveling upstream from its mouth. Spar Canyon has no perennial tributary streams. Spar Canyon itself is 10 miles of deeply incised (10-20 feet) ephemeral stream channel that only conveys water during spring runoff and during occasional summer thunderstorms, and does not support riparian vegetation corridors. Spar Canyon has a number of ephemeral tributaries and springs including Bear Wallow, Tub Spring, Gossi Spring, White Colt Spring, Grey Stud Spring, and Sorrel Spring (IDEQ, 2003).

The entirety of the Road Creek Watershed with the exception of a small portion of privately owned lands proximal to the East Fork Salmon River is located in the southwestern portion of the CHMA. Road Creek is a tributary of the East Fork of the Salmon River and is perennial downstream of a spring located in the NW¼SW¼ & SW¼NW¼ sec. 36, T. 10 N., R 20 E., B. M. Road Creek has three perennial tributaries; Mosquito Creek, Horse Basin Creek and Bear Creek as well as multiple unnamed ephemeral dry washes; Pine Creek is an intermittent to ephemeral spring dominated tributary to Corral Creek which is a tributary to Horse Basin Creek. Road Creek and its perennial tributaries were assessed using the PFC protocol and the functionality determinations are given in Table 13 (Map 1).

Riparian monitoring occurs on Road Creek at five key areas. The key area MIM and Greenline Analysis monitoring results are given in Table 14. While the PFC data from 1994 indicated Road Creek was functioning at risk to nonfunctioning throughout the entire reach, the MIM and Greenline Analysis data indicate that riparian areas within Road Creek are at or near potential community and therefore likely functioning appropriately at this time. Based on monitoring horse use has been apparent in the Horse Basin and Corral Basin Creek drainages as well as along Road Creek. Wild Horse use in Horse and Corral Basin drainages is widely distributed and often season long; whereas use along Road Creek is seasonally focused in winter and apparent at localized areas. Horse use has not been apparent along Bear and Mosquito Creeks above their confluences into Road Creek. In those portions of the CHMA where surface water predominately occurs as upland springs (outside of the Road Creek Watershed) wild horse use of riparian areas/wetlands is focused at upland springs.

There were two lentic PFC's conducted on two springs located at the head of Chicken Creek on September 28, 1999. The spring located furthest up the drainage was approximately 0.1 acres and was rated as functioning at risk with trend not apparent. The second spring was downstream, 1.5 acres in size and was rated in proper functioning condition. Wild horses do not typically use the Chicken Creek drainage and therefore they were not a factor contributing to functionality at these springs. The majority of the springs within the CHMA were surveyed (1993-1994) for the Snake River Basin Adjudication of water rights. This survey found there are 12 ponds, 252 seeps and 26 spring brooks (Map 2). This survey found that use by wild horses, cows or big game was common throughout the CHMA.

Table 14. Riparian MIM and Greenline Analysis Monitoring Summary (1999-2012)							
Stream	Year	Key Area	Monitoring Method	Greenline Ecological Status Rating	Site Wetland Rating	Streambank Stability (%)	% Fines
Road Creek	1999	1	Greenline Analysis	Late Seral	Good		
Road Creek	2010	1	MIM	Potential Natural Community	Good	94%	49%
Road Creek	2005	3	Greenline Analysis	Potential Natural Community	Good		
Road Creek	2006	4	Greenline Analysis	Potential Natural Community	Good		
Road Creek	2010	4	MIM	Potential Natural Community	Very Good	97%	56%
Road Creek	2012	5	MIM	Potential Natural Community	Good	99%	39%
Horse Basin Creek	2007	2	MIM	Late Seral	Good	56%	

Lone Pine Creek is located in the northeastern portion of the CHMA located on Bradbury Flat. The upper reaches of Lone Pine Creek are known to be perennial, but surface water is discontinuous and the channel becomes dry typically more than 1 mile before its confluence with Warm Springs Creek with xeric species growing within the conveyance. Riparian monitoring occurs at a single photo point on Lone Pine Creek. Approximately 0.8 mile of the creek is located within an exclosure and this reach was assessed using the PFC protocol in October 1998, and was determined to be functioning at risk with an upward trend. The exclosure was constructed in 1992 for the purpose of excluding the majority of the perennial reach from grazing. Lone Pine creek within the exclosure is characterized by a narrow incised channel and receives sediment from its ephemeral tributaries during high intensity precipitation events resulting in siltation and sedimentation along this reach. The stream is also limited in its floodplain extent to the width of the stream bottom within the incised reach. Other water sources in the vicinity of Bradbury Flat are associated with ephemeral water courses; and springs which create spring brooks which flow for short distances before sinking. Population inventories and field observations indicate wild horses routinely use this area year round.

Broken Wagon Creek is located in the southeastern portion of the CHMA. Water distribution proximal to Broken Wagon Creek consists of the creek drainage including tributaries as well as upland seeps and springs, most of which do not convey surface water to the creek. There are multiple unnamed upland springs forming the headwaters of Broken Wagon Creek. The creek

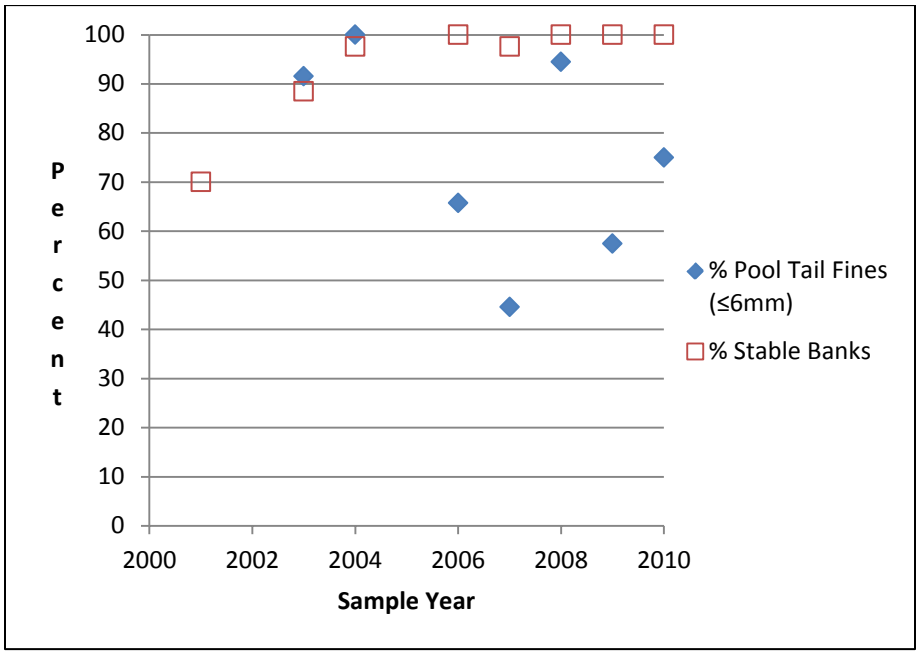
consists of approximately 1.3 miles of perennial reaches with the remainder classified as intermittent to ephemeral (see Table 12). Therefore it is likely that the reaches of the streams classified as non-functional during the 1994 PFC assessments were dry and may not have the potential to support a thriving riparian vegetation community. Population inventories and field observations indicate wild horses routinely use this area year round.

The 0.2 miles of the East Fork of the Salmon River is located along the southwestern margin of the CHMA at the mouth of Dry Hollow. This reach is in Proper Functioning Condition and is bounded to the west by steep topography which is prohibitive to horse access. Horse access is further limited by an east-west drift fence located at the northern margin of the mouth of Dry Hollow. Wild horses have not been observed in Dry Hollow during the last 7 population inventories (2000-2012).

The Pacific Anadromous Fish Strategy (PACFISH) and Inland Fish Strategy (INFISH) Biological Opinion Effectiveness Monitoring Program (PIBO – EM) for aquatic and riparian resources began development in 1998 following the ESA listing of steelhead and bull trout. A federal interagency effort to develop a large-scale monitoring program to determine whether PACFISH/INFISH management practices are maintaining, degrading, or improving biological and physical attributes, processes, and functions of riparian and aquatic habitats throughout the Upper Columbia River Basin. Interagency monitoring efforts at randomly selected streams in the upper Columbia River Basin began in 2001, typically on 5-year cycles. More information regarding the PIBO-EM program is available online (www) at http://www.fs.fed.us/biology/fishecology/emp/index.html. Relative to the CHMA, Road Creek has two monitoring locations associated with PIBO-EM. A DMA site, RC-KA-01, is used by the BLM for implementation monitoring (e.g. short term indicators of grazing use), and roving PIBO-EM crews conduct effectiveness monitoring (e.g. long term indicator of habitat condition or recovery). Approximately 0.1 miles upstream of RC-KA-01 there is an "Integrator" monitoring site. At this site PIBO-EM crews collect effectiveness monitoring data. This occurs typically at shorter intervals than 5 years (see Map 1).

At the Integrator reach on Road Creek effectiveness monitoring was conducted by PIBO monitoring crews 8 times from 2001 to 2010 (Figure 1). Between 2005 and 2010 pool tail fines appear cyclic; likely resultant of periodic transport limited deposition in this carex dominated reach, and periods of sufficient energy to transports fines. The high levels of bank stability are related to abundant root-binding greenline vegetation, resistant to hoof alteration. This data shows that this reach has attained/ maintained achievement of the riparian monitoring objective (>= 90% bank stability) pursuant to the Challis RMP (USDI-BLM, 1999) and that major decreases in fine sediment may not be possible due to geomorphology, beaver influence and perturbations resultant from yearlong wild horse and wildlife presence, seasonal grazing and the presence of the road along the creek.

Figure 1. Effectiveness monitoring data collected by PIBO crews 2001 to 2010 at Integrator Reach on Road Creek upstream of BLM DMA RC-KA-1.



3.6.2 Environmental Effects-- Wetlands/Riparian Zones

Alternative 1

Direct effects of horse gathering on riparian areas would vary based upon the distribution of the horses across the CHMA. Gather personnel would select capture sites based upon wild horse's distribution at the time of the gather. If the horses are distributed within the vicinities of riparian areas/wetlands a potential exists that horses may need to be gathered proximal to riparian areas. The gathering of horses near riparian areas has the potential to result in localized effects as a result of riparian areas/wetlands being traversed by the horses. For example during the 2009 gather 4 horses crossed Road Creek once each during the gather. These effects could consist of bank alterations from horses stepping on the stream banks. Moreover, the effects of horses crossing riparian areas/wetlands during a gather are not anticipated to be discernible from the number of crossings horses would make if a gather were not occurring because the horses cross the streams in the CHMA regularly.

There are three existing potential wild horse capture sites adjacent to Road Creek within the RHCA (Riparian Habitat Conservation Area), identified in the Challis RMP (P. 83-84, Attachment 4). These are categorized in the Challis RMP as: 1) a 300-foot buffer along perennial, fish bearing streams; 2) a 150-foot buffer along perennial non-fish bearing streams; 3) a 150-foot buffer around ponds, wetlands, lakes, reservoirs and wetlands greater than 1-acre in size; 4) and a 100-foot buffer around wetlands less than 1-acre and landslides / landslide prone areas (USDI-BLM, 1999a; USDI-USDA 1995b,c) the potential capture sites are proposed for use during the 2012 gather, and have been used in previous gathers. One site is located just downstream of the confluence of Horse Basin Creek and another located on the road approximately 1/3 mile downstream of the Bear Creek confluence. The most upstream proposed

capture site is near the North Fork Sage Road, between RC-KA-03 and RC-KA-04 (see Map 1). However, it is likely that only one site in the Road Creek watershed would be used during proposed wild horse gather operations. For example, during the 2004 and 2009 gathers only one capture site along Road Creek was used. The capture sites are located on the upstream side of the road among xeric flora. The effects of holding wild horses within these capture sites for a short duration (most would be only for hours, some could be held overnight) would be trampling of vegetation as well as loosening of surface soil, which could result in increased runoff from this site during subsequent precipitation events. Any excess sediment would be primarily buffered by the existing road. Furthermore, because the gather is likely to occur in the fall any subsequent precipitation would likely occur as snow not resulting in immediate runoff. The area would likely re-vegetate during the following spring growing season. Therefore, the gather is not likely to result in increased percent fines in perennial streams. In addition the immediate downstream reaches are influenced by beaver activity that has created multiple ponds acting as natural sediment traps. Any new capture sites would be outside of the RHCA to eliminate effects to streambanks and riparian vegetation.

Under this alternative wild horse population levels would undergo a short term (3-5 years) population reduction; this would likely result in an in kind short term reduction in the effects of horses to riparian areas and wetlands allowing for streams within the CHMA to continue to maintain or progress towards potential natural community. The AML was established by the RMP and where greenline monitoring and MIM have occurred these sites have continued to rate as late seral or potential natural community (see Table 14). The high bank stability indicates that the maintenance of AML in the CHMA has resulted in maintenance or recovery of riparian areas. Where wild horses effect upland springs (e.g. trampling, foraging, defecation) a short term reduction in the intensity and frequency of wild horse use would occur. There is a non-uniform spatial distribution of wild horse use on the upland springs within the CHMA. Those springs that receive relatively focused wild horse use are anticipated to continue to receive relatively focused wild horse use.

Alternative 2

The environmental effects to riparian and wetlands as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 3, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 3

The environmental effects to riparian and wetlands as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 4
The environmental effects to riparian and wetlands as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 3 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 5
Under this alternative no direct effects related to horse gather activities would. Rather than a short term reduction of horse population (as described under alternatives 1-4), the horse population would continue increasing. An increase of use by wild horses at riparian areas and upland springs is anticipated. For example monitoring data indicates that when the Appropriate Management Level of 185 is exceeded, wild horse effects (trampling, vegetation utilization, defection etc.) can intensify at riparian areas and upland springs. Livestock use criteria are used to manage the intensity of livestock grazing. Stubble height and bank alteration criteria have been set for the perennial streams in the CHMA by the Challis RMP. Use of riparian areas by wild horses has resulted in exceedance of these livestock grazing standards. For example, in 2003 there were 203 horses counted during the census conducted that year and livestock grazed from June 16 to July 11. When livestock were done grazing at Horse Basin Creek Key Area 2 the stubble height was 4 inches, on October 23 the stubble height was at 2 inches (the standard is 3 inches pursuant to the Challis RMP) and the bank shears were 26% (the Standard is less than 10% pursuant to the Challis RMP [USDI-BLM, 1999a]). In 2006 Horse Basin was rested from cattle grazing and 231 horses were counted during the census that year, stubble height was measured at Horse Basin Creek Key Area 2 at 2 inches. In both of these cases the exceedance of the grazing use criteria was attributable to horse use (see Appendix K).

Therefore as a result of this alternative it is likely that the springs and stream riparian areas in the CHMA would receive increased intensity and frequency of wild horse use. This has a potential to result in altered vegetation communities typically resulting in a lower seral stage. Lower seral stages have less recruitment of woody species reducing shade and stability of the site because binding roots associated with hydric herbaceous and mature trees/shrubs are not as prevalent. This results in an overall reduction in the spatial extent of the riparian area whether it is a stream or a spring site (USDI- BLM, 1998, 2003). This would result in riparian areas within the CHMA being more vulnerable to future disturbance such as drought, high flow events etc. and becoming nonfunctional.

3.7 Water Quality

3.7.1 Affected Environment

Approximately 346 stream miles were assessed within the CHMA by the IDEQ (Idaho Department of Environmental Quality) pursuant to section 305(b) of the CWA (Clean Water Act 33 U.S.C. §1251 et seq. 1972). Streams which are assessed pursuant to this section but do not meet the IDWQS (Idaho Water Quality Standards) are 303(d) listed, meaning that water body is classified as water quality impaired. Standard 7 of the Idaho Rangeland Health Standards states that surface and ground waters on public land shall comply with the IDWQS. The 305(b) water bodies assessed included perennial, intermittent and ephemeral stream reaches (see Table 12). Of these, approximately 95 stream miles were listed as impaired because they did not meet one or more of the IDWQS.

The majority of the stream miles within the CHMA are ephemeral to intermittent (see Table 12) under Riparian Areas and Wetlands) and only flow during periods of intense precipitation and/or high snow melt. Of particular importance to the streams located on the CHMA is the relationship between IDWQS and intermittent waters. An intermittent stream is defined (in Idaho Administrative Code 58.01.02.003.51) as a stream that has a period of zero flow for at least one week in most years. Where flow records are available a stream with a seven day average flow of less than 0.1 CFS is considered intermittent. Section 070.07 of IDWQS (Idaho Administrative Code 58.01.02.070.07) addresses the application of the IDWQS to these types of streams. IDWQS apply to intermittent streams during periods of optimum flow sufficient to support the beneficial uses for which the intermittent stream has been designated. In many cases, intermittent streams have not been designated and are protected for the default uses of cold water biota and secondary contact recreation (recreation that does not result in prolonged and intimate contact with the water when small amounts of water are likely to be ingested). Optimum flow is described as at least 1 CFS for aquatic life (cold water aquatic life) and at least 5 CFS for recreation uses. When flows drop below these threshold values, the IDWQS no longer apply to the given water body (IDEQ, 2001). The statuses of streams which are located upon the CHMA are given in Table 15.

Table 15. 305(b) Streams located in CHMA		
STREAM	Status	length (miles)
Unnamed Streams		207.50
Unnamed Streams	303D LISTED	76.99
Bear Creek		5.35
Broken Wagon Creek	303D LISTED	7.76
Corral Basin Creek		7.78
East Fork Salmon River		0.20
Horse Basin Creek		6.87
Lone Pine Creek		8.50
Mosquito Creek		1.61
Mosquito Creek	303D LISTED	6.05
Road Creek		12.23
Road Creek	303D LISTED	2.90
Sheep Creek	303D LISTED	0.32
Warm Spring Creek	303D LISTED	1.00

Due to the large scope of assessing all of the water bodies in Idaho the IDEQ has divided these bodies into lumped assessment units. Many of these assessment units have multiple monitoring sites for conducting assessments, if any of the assessment sites do not meet the criteria for being fully functioning the entire assessment unit is typically considered impaired and consequently 303(d) listed. The majority of unnamed stream miles which were 303(d) listed were lumped into the Warm Springs Creek source to Hole in the Rock Creek unit; the reason this assessment unit is 303(d) listed is for not supporting cold water aquatic life with low flow alterations and sedimentation /siltation listed as the pollutants. This assessment unit includes areas east of Highway 93 outside of the CHMA. The majority of monitoring sites used by IDEQ were assessed when no water was present in the channel, and the sites were selected in reaches which are classified as intermittent to ephemeral see Appendix H. Warm Springs Creek below Hole in the Rock Creek is not 303(d) listed. Population inventories and field observation indicate that wild horses are present in this portion of the CHMA but they do not affect the perennial and or ephemeral nature of the streams at the locations where assessments were conducted.

There is a short reach of Broken Wagon Creek (1.3 miles) which is perennial in the CHMA. Broken Wagon Creek is listed from source to mouth for not supporting cold water aquatic life with low flow alteration and sedimentation/siltation listed as pollutants. The site monitored by

IDEQ is not within the perennial reach and was monitored at a time when the stream was dry (see Appendix H for IDEQ monitoring data). The portion of Sheep Creek between Highway 93 and the confluence with Warm Springs Creek as well as the entirety of the ephemeral to intermittent unnamed streams above the confluence of Broken Wagon Creek and Warm Springs Creek were lumped into the Broken Wagon Creek source to Mouth Assessment Unit. Population inventories and field observation indicate that wild horses are present in this portion of the CHMA but they do not affect the perennial and or ephemeral nature of the streams at the locations where assessments were conducted.

The majority of perennial miles within the CHMA occur in the Road Creek watershed. Mosquito Creek is a tributary to Road Creek and is 303(d) listed from source to mouth for not supporting cold water aquatic life and salmonid spawning with combined biota/habitat bio-assessments (aquatic habitat conditions) listed as the pollutant. This determination was based upon a single 1998 sample taken 30 meters above the confluence into Road Creek and is not representative of typical habitat conditions within Mosquito Creek; during this sample IDEQ did not document salmonids, however westslope cutthroat have been documented by BLM electrofishing efforts in this reach. Furthermore IDEQ attempted to monitor a location in upper Mosquito Creek in 2004 and found the site to be inaccessible due to dense woody vegetation. Road Creek is 303(d) listed from confluence of Bear Creek downstream to the confluence of Horse Basin Creek for not supporting salmonid spawning with biota/habitat bioassessments (aquatic habitat conditions). Population inventories and field observation indicate that wild horses are present in this portion of the CHMA and the relative influence of wild horses on water quality determinations is unknown and the effects to aquatic habitat conditions as a result of the presence of wild horses is disclosed under fisheries.

3.7.2 Environmental Effects

Alternative 1

There is one capture site located along the 303(d) listed reach of Road Creek between the confluences of Mosquito Creek and Bear Creek. Any new capture sites would occur outside of the RHCA. Sedimentation and Siltation is the pollutant which is most likely to result from use of the capture sites, however a substantial contribution to streams within the CHMA is not anticipated as a result of the of capture sites. A low potential exists for wild horses to step in streams while being gathered (see riparian area and wetlands for further discussion). This could result in bank shearing and/or stepping in fine substrate sediments creating short duration localized turbidity. Turbidity is the measure of the cloudiness or haziness of a water body and is used as a method to determine sediment loading. The IDWQS for meeting cold water aquatic life beneficial use is: *Turbidity, below any applicable mixing zone set by the Department, shall not exceed background turbidity by more than fifty (50) Nephelometric Turbidity Units (NTUs) instantaneously or more than twenty-five (25) NTU for more than ten (10) consecutive days* (IDAPA 58.01.02). IDWQS would not be violated as a result of gathering horses because of the short duration of the gather (typically less than 3 days at any capture site) and the low potential for horses to cross streams as a result of this action. Effects to salmonid spawning and TES Fish (cold water aquatic life) are disclosed under Fisheries.

As a result of this gather, a short term decrease in horse population would occur. As discussed under section 3.6 there are effects to riparian areas which in turn affect water quality. These effects are a reduction in; 1) foraging which allows for canopy (shade) maintenance/recovery which influences water temperature, 2) defecation which leads to fecal coliform presence in surface waters and 3) bank destabilization which can result in sedimentation/siltation. The intensity and frequency of use of wild horses at streams and springs would receive a short term in kind reduction. However those water bodies associated with riparian areas which receive relatively focused wild horse use would continue to do so.

Alternative 2
The environmental effects to water quality as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 3, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 3
The environmental effects to water quality as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 4
The environmental effects to water quality as a result of a gather result from population size and the extent of gather operations (e.g. number of trucks on roads, number of captures sites etc.). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 3 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to riparian and wetland resources would be the same under this alternative as alternative 1.

Alternative 5
Under Alternative 5, it is likely that the springs and stream riparian areas in the CHMA would receive increased intensity and frequency of wild horse use. This has a potential to result in altered vegetation communities typically resulting in a lower seral stage. Lower seral stages have less recruitment of woody species reducing shade and stability of the site because binding roots associated with hydric herbaceous and mature trees/shrubs are not as prevalent. This results in an overall reduction in the spatial extent of the riparian area whether it is a stream or a spring site (USDI- BLM, 1998, 2003). A decrease in canopy/shade would result in an increase in water temperatures. A decrease in bank stability would result in increased sedimentation. The increase in numbers of wild horses would increase the fecal loading of springs and streams. Therefore the

cessation of gathering wild horses for period of up to 10 years would affect water quality such that more water bodies would likely not meet the IDWQS and thereby increase the miles of 303(d) listed streams on the CHMA.

3.8 Threatened, Endangered, and Sensitive Fish; Fisheries

3.8.1 Affected Environment

Species and Critical Habitat

This section focuses on the distribution of threatened / endangered fish species, BLM sensitive and Idaho State fish species of concern, and water bodies designated as critical habitat that border or lie within the CHMA. The United States Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) have, under ESA, identified the following threatened and endangered fish species and habitat that occur in the area administered by the CFO:

- **Snake River sockeye salmon** (*Oncorhynchus nerka*) (Endangered) (Federal Register 56FR58619). Critical habitat has been designated for Snake River sockeye salmon (Federal Register 58FR68543). However, the designation is limited to the Salmon River.

- **Snake River spring/summer Chinook salmon** (*Oncorhynchus tshawytscha*) (Threatened) (Federal Register 57FR14653). Critical habitat has been designated for Snake River spring/summer Chinook salmon and includes "river reaches presently or historically accessible…to Snake River spring/summer Chinook salmon" (Federal Register 58FR68543).

- **Snake River steelhead** (*Oncorhynchus mykiss*) (Threatened) (Federal Register 62FR43937). Critical habitat has been designated for Snake River Basin steelhead (Federal Register 70FR52630) and includes numerous streams in the Upper Salmon River watershed and the BLM Challis Resource Area.

- **Columbia River bull trout** (*Salvelinus confluentus*) (Threatened) (Federal Register 63FR31647). Critical habitat was designated for bull trout on September 26, 2005, and initially did not include the Salmon River. On October 18, 2010, a revised final designation of critical habitat was published which included mainstem rivers and numerous tributaries the Upper Salmon River watershed and BLM Challis Resource Area (Federal Register 75FR63898).

Although not ESA listed as threatened or endangered, westslope cutthroat trout (*Oncorhynchus clarki lewisi)* is an Idaho State species of concern and a BLM sensitive species. Westslope cutthroat trout occur in portions of the CHMA (USDI-BLM 1999a), and that benefit peripherally from stipulations and conservation measures intended to prevent effects to listed salmonids and designated critical habitat. Therefore, westslope cutthroat trout are discussed concurrently. Although designated critical habitat abounds in in the Salmon River, East Fork Salmon River and many tributaries, relatively few historically accessible streams and little designated critical

habitat occurs in the CHMA. Designated critical habitat for ESA listed salmonids (Map 3) and documented presence within and adjacent to the CHMA is summarized below in Table 16.

Table 16. Documented T/E and Sensitive Fish Species Presence and ESA Designated Critical Habitat Distribution Within the CHMA.

Stream Name	Stream Miles[1]	Stream Type[1]	T/E and Sensitive Fish Presence[2]	ESA Designated Critical Habitat
Unnamed Stream	550.5	Intermittent to Ephemeral	None	None
Bear Creek	1.6	Intermittent to Ephemeral	None[3]	CHS
Broken Wagon Creek	6.4	Intermittent to Ephemeral	None	None
Corral Basin Creek	0.9	Intermittent to Ephemeral	None	CHS
Horse Basin Creek	3.6	Intermittent to Ephemeral	UNK	CHS
Lone Pine Creek	8.5	Intermittent to Ephemeral	None	None
Mosquito Creek	1.6	Intermittent to Ephemeral	UNK	CHS
Road Creek	2.1	Intermittent to Ephemeral	WSCT[2]	CHS
Warm Spring Creek	2.7	Intermittent to Ephemeral	None	None
Unnamed Stream	17.2	Perennial	None	None
Bear Creek	4.1	Perennial	WSCT[2]	CHS
Broken Wagon Creek	1.3	Perennial	None	None
Corral Basin Creek	6.1	Perennial	None[2]	CHS
East Fork Salmon River	0.2	Perennial	BLT, CHS, STH, RBT, WSCT	BLT, CHS, STH
Horse Basin Creek	6.1	Perennial	WSCT, RBT[2]	CHS
Lone Pine Creek	0.3	Perennial	None	None
Mosquito Creek	6.1	Perennial	WSCT[2]	
Road Creek	13.9	Perennial	RBT[2], WSCT	

[1]Data compiled and distances estimated with a GIS using the USGS National Hydrology Dataset. [2]Based on known documented observations collected by BLM-CFO between 1994 and 2012, IDEQ, or IDFG (IDEQ, 2010); [3]The intermittent / ephemeral reach of Bear Creek occurs in a headwaters mesic meadow with insufficient surface water / stream channel habitat to support salmonids.

BLT = bull trout, CHS = Chinook salmon, STH = anadromous steelhead, RBT = resident / fluvial rainbow trout, WSCT = westslope cutthroat trout, None = no T/E or sensitive species present, UNK = fish presence unknown but potentially accessible by fish in downstream occupied habitat

Land ownership within the CHMA encompasses approximately 154,176 acres of BLM, 9,472 acres State of Idaho, 1,088 acres of private lands, with approximately 55 miles of perennial stream within the CHMA. The entire CHMA occurs in the Upper Salmon River Basin – 4th Field Hydrologic Unit Code (HUC) or USGS Cataloging Unit 17060201 (ISU 2012). The Upper Salmon River Basin (1.5 million acres) includes the mainstem Salmon River and numerous fish bearing tributaries (including the East Fork Salmon River) from the headwaters near Stanley, ID extending downstream to the mouth of the Pahsimeroi River near Ellis, Idaho. The CHMA is also included in the Upper Salmon River Major Population Group, used by NMFS to track the status and recovery of ESA listed anadromous (e.g. ocean going) salmonids (NMFS 2011; Ford

2010). However, relatively little fish habitat occurs in the CHMA because of distinctly volcanic geology and a preponderance of naturally intermittent or ephemerally wetted stream channels that historically and currently preclude occupancy by fish in most drainages which have only infrequent surface water conveyance to mainstem streams and rivers. Native fish and/or introduced salmonids are only known to occur in ≈5.1% of the total stream / conveyance miles within the CHMA, almost entirely located within Road Creek drainage (Table 16; Map 3).

The northwestern margins of the CHMA are bordered by State Highway 75 and the mainstem Salmon River and the southwest margins are bordered by the East Fork Salmon River and the East Fork Road. The southern boundary of the CHMA extends to the ridgeline drainage divide between Herd Creek and Road Creek in the Upper Salmon River watershed and the divide between Road Creek and Sage Creek in the Big Lost River Basin (4th Field HUC or USGS Cataloging Unit 17040218). The eastern margins of the CHMA are bordered by State Highway 93 and Warm Springs Creek, predominately occurring on private lands. The northern apex of the Challis CHMA is bounded by private lands developed for agriculture and home sites and State Highway 93 (Map 3).

The Salmon River, East Fork Salmon River, and Herd Creek all bound portions of the CHMA and are occupied designated critical habitat for ESA listed fish species including, Chinook salmon, steelhead, and bull trout. Spawning and rearing by anadromous Snake River Chinook salmon and Snake River steelhead has been assessed by Idaho Department of Fish and Game via aerial Chinook Salmon spawning redd counts, hatchery weir operations, and stream sampling and spawning surveys conducted by several state, federal, and tribal fisheries personnel. Resident Columbia Basin bull trout, westslope cutthroat, and rainbow trout *(Oncorhynchus mykiss)* are widely distributed throughout the Upper Salmon River Basin and occur in reaches of the Salmon River, East Fork Salmon River, and Herd Creek drainage which border the CHMA (NMFS 2011; USFWS 2010; USFWS 2002; USDI-BLM 1999a, 1999b, 1999c, 1999d, 1999e). The presence of Sockeye Salmon relative to the CHMA is limited to upstream migration by adults and downstream migration by juveniles in the adjacent reaches of the mainstem Salmon River en-route to Redfish Lake and the Sawtooth Fish Hatchery near Stanley ID, and the Pacific Ocean, respectively (IDFG 2011).

Within the boundaries of the CHMA a limited reach (≈0.2 miles long) of the East Fork Salmon River exists (Table 16) but is bounded by steep bedrock topography and results in infrequent wild horse presence proximal to this reach. Relative to the CHMA the remainder of designated critical habitat and stream reaches occupied by native fish and/or introduced salmonids is limited to the Road Creek drainage (USDI-BLM 1999a, 1999b, 1999c, 1999d, 1999e). Road Creek and its tributaries, while not designated by USFWS as critical habitat for bull trout or by NMFS for Snake River Steelhead, were included in the broader designation of critical habitat for Chinook salmon and essential fish habitat under the Magnuson-Stevenson Act. Other drainages within the CHMA such as Malm Gulch, Spar Canyon, and the Warm Springs Creek watershed with tributaries Lone Pine Creek and Broken Wagon Creek have limited perennial reaches and were unlikely to be historically accessible by Chinook salmon or other native fish (Table 16). This is because surface flows infiltrate into alluvium and often do not convey surface water adequate to maintain riparian corridors or stream channels capable of supporting fish. As ephemeral drainages approach their receiving waterways, their surface water conveyances are often absent

or become diffuse which further limits ephemeral surface water connectivity to only during extreme episodic precipitation events. In the case of Warm Springs Creek, historical disconnection from the Salmon River and geothermal influences has prevented the establishment of a native resident fish population in the Warm Springs Creek watershed (NMFS 2011; IDEQ 2003). Thus, with the exception of Road Creek, drainages in the CHMA were unlikely to be historically accessible by fish in the mainstem and East Fork Salmon Rivers, are not occupied by fish, and thus are not considered critical habitat.

Road Creek and its tributaries drain towards the East Fork of the Salmon River and constitute vast majority (≈79.2%) of perennial reaches on named streams in the CHMA (Table 16). Perennial Road Creek tributaries known to be occupied by native salmonids include: Mosquito Creek, Bear Creek, and Horse Basin Creek (Table 16). Snake River spring/summer Chinook salmon and steelhead are unlikely to currently occupy potentially historic spawning and rearing habitats in Road Creek due to a combination of the county road culvert, stream diversions, and flow-losing intermittent reaches in the lower ≈3 miles of Road Creek (USDI-BLM 2004; USDI-BLM 1999b). Beaver activity influences most BLM administered reaches of Road Creek from approximately Mosquito Creek to Chicken Creek and among private lands in lower Road Creek which can influence fish distribution and create a dynamic influence on habitat (Meehan 1991; Swanston 1991). For example, beaver ponds provide excellent rearing habitat juvenile salmonids, and increased aquatic productivity and low-velocity overwintering habitats are benefits. However, beaver ponds can displace spawning gravels via sediment deposition inherent to low-gradient stream impoundments and elevated stream temperatures detrimental to salmonids can also result.

The ability of salmonids to freely move within a watershed is essential to reproduction, foraging, and seeking thermal refugia during temperature limiting times of the year (July – August). Westslope cutthroat trout are the most widely distributed salmonid within Road Creek and its perennial tributaries. This distribution is supported by perennial surface water connectivity among its major tributaries, which allows fish to seek thermal refugia or spawning habitats. In Road Creek, naturally occurring intermittent reaches can necessitate such fish movement, and human barriers can further fragment habitats and impede fish migration (Meehan 1991). A culvert fish passage inventory was conducted for the Challis Field Office in 2004. Three Road Creek culverts were assessed as barriers to fish movement based on excessive slope and perched outlets, which one located near the East Fork Salmon River confluence on the East Fork Road (downstream of the CHMA boundary), and two in upper Road Creek on BLM lands (USDI-BLM, 2004). Road culverts which impeded fish passage have been reduced in Road Creek since 2006; 2 culverts in upper Road Creek between RC-KA-03 and RC-KA-04 (Map 3) were replaced, and in 2008 westslope cutthroat trout were observed at RC-KA-04 during macroinvertebrate sampling near the upstream terminus of perennial reaches of Road Creek in spring-dominated habitat. The culvert over Horse Basin Creek, just above its confluence into Road Creek, was assessed as a possible impediment to fish passage and also replaced in 2006. However, intermittent surface waters and anthropogenic barriers (e.g. culvert at East Fork Road below CHMA boundary) in the lower reaches of Road Creek which fragment fish distribution would likely persist into the foreseeable future.

The Road Creek watershed within the CHMA, including mainstem Road Creek on BLM lands, and Mosquito, Bear, and Horse Basin creeks have documented to be occupied by resident westslope cutthroat trout, rainbow trout, possible hybrids and mottled sculpin, but steelhead, Chinook salmon and bull trout were documented as being absent, and attributed to anthropogenic impediments to fish passage (IDEQ, 2010; USDI-BLM 1999b, 1999d). This is based on past BLM electrofishing surveys in the 1990's and more recent visual observations. Limited sampling was conducted by IDEQ, and documented unknown salmonids in upper Road Creek, westslope cutthroat trout in Horse Basin and Bear Creeks, and several rainbow trout in lower Horse Basin Creek in 1997 and a possible 50-mm rainbow trout / unknown salmonid in Road Creek near RC-KA-01 on 8/1/2006.

Current fish presence, based on BLM electrofishing surveys conducted 6/7/2012 to 6/20/2012 throughout the Road Creek watershed from mouth to headwater terminus, included the same suite of species within the CHMA. It also appears fish from the East Fork Salmon River are unlikely to access and occupy habitat in Road Creek within the CHMA. A combination of westslope cutthroat trout, rainbow trout, salmonids visibly indicative of cutthroat x rainbow hybrids (Behnke 1992), and mottled sculpin were present at all sites sampled in the CHMA, except the most downstream parcel of BLM lands. Given intermittent reaches in upper (below DMA RC-KA-04) and the lower reaches of Road Creek, numerous beaver impoundments, volcanic geology and with substrate composition dominated by relatively fine particles, the presence of spawning gravel habitat is likely limited in distribution. This may suggest limited reproductive isolation, further complicated by anthropogenic fragmentation, and the presence of resident rainbow trout. While fish stocking was historically common place in the Upper Salmon River Basin, the contribution of this potential anthropogenic influence on fish presence or hybridization, also known to occur naturally, is unknown (Kozfkay 2012). Furthermore, spawning habitat for steelhead and access are likely precluded by substrate composition dominated by fine sediments inhibitive to steelhead spawning and passage impediments, respectively.

The June 2012 BLM electrofishing effort also included the lower reaches of Road Creek downstream of the East Fork County Road, and outside of the CHMA boundary. Here the confluence into the East Fork Salmon River occurs and there are perennial flows, apparently influenced by flow-gaining / recharge upstream on private lands. There was also a distinctly different fish assemblage, consisting of rearing anadromous salmonids and fish from the East Fork Salmon River, than potentially isolated resident populations upstream on BLM lands within the CHMA. The distribution of fish from the East Fork Salmon River into Road Creek may not extend beyond the lower ≈0.25 to 1.0 miles on private lands near the confluence with where access/egress to the river can occur. Two reaches (18 and 75m in length) below the East Fork Road on private lands near the mouth were electrofished by the BLM on 6/20/2012; relative to all reaches sampled in the Road Creek watershed, here the largest relative abundance of individuals occurred with a total of 29 rainbow trout and / or rearing steelhead (≈60 to 250mm TL) and 82 rearing Chinook salmon (≈30 - 90mm TL), a single bull trout (≈180mm TL), and mottled sculpin. However, upstream of this important spring influenced flow-gaining reach; there is a distinctly flow-losing reach, likely exacerbated by diversions and historic channel incision, where dry channel in the summer/fall or flows low enough to limit migration and occupancy occur. For example, of the 13 electrofishing reaches (stream length ranged 18 –

200m of sampled habitat) the most downstream portion of BLM lands on Road Creek, concurrent with the aforementioned intermittent reach, was the only reach sampled (stream length 200m) to not contain any fish.

Water Temperature

Removal of canopy cover over small streams can result in detrimental increases in water temperature (Powell et al. 2000). In a comparison of ungrazed exclosures to areas subject to season-long grazing in the Blue Mountain region of Oregon, Claire and Storch (1983) noted that the grazed section was devoid of shrub cover and daily water temperatures were 12°F higher than water temperatures inside exclosures. Elevated stream temperatures can be influenced by impacts such as increased stream widths, decreases in stream depth, reductions in overhanging bank cover and vegetation, or reductions in stream flow. Intermittent reaches which lose flow to infiltration can experience naturally elevated water temperatures, and beaver impoundments also can result in elevated stream temperature (Swanston 1991). The seasonal occurrence of livestock grazing and the persistent use by wild horses has resulted in localized accessible areas with destabilized streambanks and reduced vegetative cover, but predominately riparian vegetation is functioning appropriately and providing stream shading throughout most of Road Creek and its perennial tributaries. Further descriptions of riparian vegetation and bank stability are discussed under Wetlands / Riparian Zones (Table 14).

Relative to the CHMA, flow diversion and intermittent reaches likely influence stream flow and water temperature in the lower reaches of Road Creek. Further upstream where BLM administered lands contain most of the Road Creek drainage, recreational use and congregation of livestock and wild horses has resulted in localized areas of decreased bank stability and/or riparian vegetation density (Table 14). However, stream reaches have predominately dense intact riparian corridors resistant to streambank impacts from wild horses or livestock. Natural influences on water temperature in Road Creek occur as beaver impoundments which decrease velocity, accumulate fine sediments and increase water temperature, potentially to detrimental levels during temperature limiting times of the year (July-August). In combination with climactic variability, other natural influences on water temperature are likely spring dominated flow-gaining reaches where temperature maxima can be mediated by groundwater contribution; other apparent flow-losing reaches occur as well, which can exacerbate temperature maxima (Kondolf 1989).

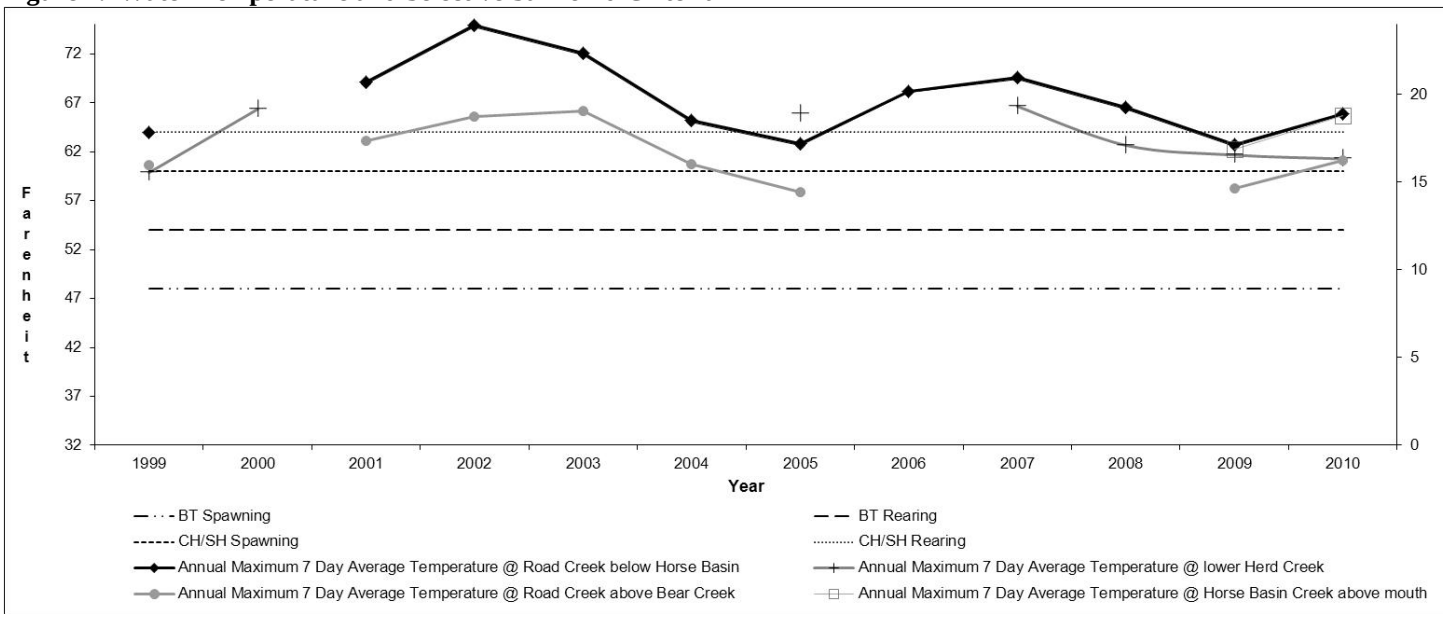
Table 17. PACFISH and INFISH Instream Temperature [1] Thresholds for ESA Listed Salmonids

Species	Spawning Season	Spawning Temperature (°C/ °F)	Rearing Temperature (°C/ °F)	Migration/Holding Temperature (°C/ °F)
Chinook Salmon	Summer	≤ 15.6/60	≤ 17.8/64	≤ 17.8/64
Steelhead Trout	Spring	≤ 15.6/60	≤ 17.8/64	≤ 17.8/64
Bull Trout	Fall	≤ 8.8/48	≤12.0/54	≤ 15.0/59

[1] *Measured as the 7 day maximum average instream temperature.*

Table 17 identifies PACFISH and INFISH instream temperature thresholds (USDI-BLM, 1995b, 1995c; USDI-BLM, 1999 b) important to listed salmonid behaviors and figure 2 displays annual temperature maxima displayed as the 7-day maximum average temperature in Road Creek and Horse Basin Creek. Water temperature is also presented neighboring Herd Creek, which contains spawning and rearing Chinook salmon and steelhead, and also contains reproductive populations of resident salmonids. Temperature thresholds for steelhead are presented below, and because they are similar to temperature thresholds for westslope cutthroat trout they are adequate for comparison. Based on annual temperature maxima presented in Figure 2, and a review of the seasonal progression of daily-water temperatures at Road Creek below Horse Basin, water temperatures likely exceed Chinook salmon and steelhead rearing thresholds for a portion of July and August, during most years; during 2009 the temperature maxima did not exceed this threshold (Figure 2). However, thermal refugia in the form of ground water gaining reaches and upper portions of Bear and Mosquito creeks likely exist, and seasonal water temperature maxima in Road Creek are unlikely to preclude occupancy by listed salmonids. For example, similar temperature maxima occur in neighboring Herd Creek which supports spawning and rearing by ESA listed salmonids (Chinook salmon, steelhead, and bull trout). Relative to westslope cutthroat trout, figure 2 suggests temperatures in lower Road Creek within the CHMA boundary exceed optimal growth conditions (>17°C) more often than Road Creek above its perennial tributaries, and has experienced temperature maxima (three seasons where data was available) greater that 20°C which can reduce survival of juvenile westslope cutthroat trout (Bear et al. 2007). However, downstream of the CHMA boundary flow-gaining reaches on private lands mediate what would otherwise be relatively high temperatures at the mouth, or even dry-channel.

Figure 2. Water Temperature and Selective Salmonid Criteria



Temperature data presented as the highest 7 day average maximum temperature recorded annually 1999 to 2010. BT Spawning = bull trout spawning temperature threshold, BT Rearing = bull trout juvenile rearing temperature threshold, CH/SH Spawning = Chinook salmon and steelhead trout spawning temperature threshold, CH/SH Rearing = Chinook salmon and steelhead trout juvenile rearing temperature threshold.

3.8.2 Environmental Effects

Alternative 1

The potential for proposed horse gather operations to have effects on fish or habitat is inherently low based upon the location of streams relative to the distribution of gather activities, and the limited extent of occupied fish habitat in the CHMA. In addition, elements of the proposed gather alternatives described below are expected to prevent any effects to ESA listed fish or habitat within and adjacent to the CHMA. The majority existing proposed capture sites used in previous wild horse gathers and sorting/holding facilities (Map 3) would occur ≥3-miles from habitat occupied by listed fish in Herd Creek, the mouth of Road Creek, the East Fork Salmon River or the Salmon River. These areas lie outside the potential gather area and horses occur there infrequently. During gather operations any wild horses proximal to these areas would be directed toward the CHMA interior, away from these substantial rivers or occupied habitat often bounded by State Highways, County roads, fenced private lands, and / or steep topography. Areas outside of the CHMA where horses are expected to be gathered would likely occur in the Warm Springs drainage, without surface water connectivity to the Salmon River, and where no stream habitat occupied by species of concern or native fish exist.

A portion of the wild horse gather activities under alternative 1 would occur in the Road Creek watershed on public lands, where unoccupied designated critical habitat for Chinook salmon occurs, and streams are known to be occupied by westslope cutthroat trout and rainbow trout (Table 17; Map 3). Occupied habitat in Road Creek downstream of the CHMA would not be influenced by gather operations, which would not occur nearby, because the pattern of horse presence is not known to result in their presence on private lands near the mouth. The majority of existing capture sites depicted on Map 3 occur away from perennial fish bearing streams, with substantial buffers to prevent effects to riparian areas, water, or fish habitat. Furthermore, horse gather operations include an SOP identified under Appendix B which establishes that *"the capture sites would be constructed so that no riparian vegetation is contained within them. No vehicles would be operated on riparian vegetation or on saturated soils associated with riparian/wetland areas."* Thus, all existing CHMA capture sites and potential additional sites would occur outside of active riparian zones (e.g. based on lateral extent of riparian vegetation). Most existing sites occur outside of riparian habitat conservation areas (RHCA), identified in the Challis RMP (P. 83-84, Attachment 4). These are categorized in the Challis RMP as: 1) a 300-foot buffer along perennial, fish bearing streams; 2) a 150-foot buffer along perennial non-fish bearing streams; 3) a 150-foot buffer around ponds, wetlands, lakes, reservoirs and wetlands greater than 1-acre is size; 4) and a 100-foot buffer around wetlands less than 1-acre and landslides / landslide prone areas (USDI-BLM, 1999b; USDI-BLM, 1995b). Gather personnel may need to establish additional capture sites, beyond those depicted on Map 3, based upon the locations of the horses at the time of the gather.

Under Alternative 1, a single sorting/holding facility would be established within or adjacent to the CHMA. There are three proposed locations where a single sorting/holding facility could be established to facilitate gather operations, fertility control, trailer-loading, and selective removal. One location is along the Spar Canyon Road ≈ 1.5 miles from the East Fork Salmon River, in an existing gravel pit resilient to impact which should preclude effects to fish or habitat associated

with the East Fork Salmon River. Temporary sorting/holding facilities would not extend beyond the road towards the Spar Canyon drainage, which does not support aquatic resources or riparian vegetation. The other locations, far from a fish bearing streams, occur in uplands on the Warm Springs side of the CHMA (Map 3). However, based on the distribution of wild horses during the proposed gather, implementation may require establishing a sorting/holding facility elsewhere in the CHMA. This would not result in effects to fisheries resources because any "new" locations would be established outside of the RHCA, and unlikely to even be proximal to a water body, and would not occur within the Road Creek watershed. Similarly, the Challis Horse Corrals would serve as preparation facility and is located in upland habitat ≈2.5 miles from the nearest fish bearing habitat in the mainstem Salmon River, and no surface water conveyance to the Salmon River occurs proximal to the corrals and thus no effects to fish or habitat in the mainstem Salmon River would occur.

There are three existing wild horse capture sites adjacent to Road Creek, which based on the location of the road and topography do occur within the RHCA (Map 3). One site is located just downstream of the confluence of Horse Basin Creek and another located on the road approximately 1/3 mile downstream of the Bear Creek confluence into Road Creek. Another capture site is located upstream of these, approximately one mile from the Bear Creek confluence. The most upstream capture site is outside of the RHCA on the Road Creek Road, near the North Fork Sage Road, between riparian DMAs RC-KA-03 and RC-KA-04. However, it is important to note that only one site in the Road Creek watershed is likely to be used during proposed horse gather operations. This is consistent with past gathers, including those in 2004 and 2009, when only one capture site in the entire Road Creek was used.

Holding horses within these capture sites would cause temporary localized impacts to upland vegetation from trampling as well as loosening of surface soil. No effects to substrate composition in Road Creek are expected because any potential sediment transport would be buffered by the existing road prism and both upland and riparian vegetation. Further, the proposed capture sites along Road Creek are used infrequently and post-gather impacts are not apparent and sites are not readily discernible. For example, persistent disturbance and increases of invasive weeds have not occurred. During the following spring / summer season minor impacts to upland vegetation and soils should be repairable upon regrowth of vegetation.

Chinook salmon or bull trout spawning and incubation does temporally align with fall-season gather activities, but only occurs in habitat outside or bordering the CHMA in Herd Creek, East Fork and mainstem Salmon River. No effects would occur because gather activities would not occur near them (e.g. capture sites and facilities are all greater than 1.5 miles from occupied habitat), and predominately fragmented ephemeral drainages throughout the CHMA and beaver impoundments and intermittent reaches in Road Creek preclude downstream indirect effects. Within Road Creek and its tributaries, October gather operations would not overlap with spring/summer spawning and egg incubation for westslope cutthroat trout or rainbow trout (USBWP 2005), where sedentary redds can be susceptible to trampling or other disturbance.

Under Alternative 1, it is anticipated that helicopter-assisted gathering of horses would primarily occur along upland trails and existing roads used by and familiar to wild horses, which is consistent with past wild horse gather operations. Based on the distribution of capture sites

relative to fish bearing streams in the Road Creek watershed and observations during past gathers, there is a low likelihood of a wild horse herd being "gathered" across a fish bearing stream. For example, during the 2009 wild horse gather, no horses were actively gathered across fish bearing streams, but 4 individuals were observed to deviate from the band and forded Road Creek after an unexpected vehicle ascended the road they were being gathered along. Because stream crossings by wild horses, resultant of gather operations, are expected to occur infrequently or not-at-all and capture site destination would be located within the CHMA interior where no listed fish species are known to occur in Road Creek or its tributaries, the horse gather operations in the proposed gather area would not affect Chinook salmon, steelhead, bull trout, or sockeye salmon or habitat. In the event some horses forded a fish bearing stream occupied by westslope cutthroat trout or rainbow trout, no effects to these individuals are expected. For example, if wild horses forded Road Creek or its tributaries, predominately dense woody vegetation along the streambanks and undercut banks would provide suitable escape cover from the threat individual or small groups of wild horses would present.

Under Alternative 1, it is anticipated that after gather operations the population would undergo a short term (≈3-5 years) reduction. This may result in an in kind short term reduction in horse use in uplands, at uplands springs / seeps and perennial stream reaches where horses have been observed to occur. Horse gather operations are the active management approach described in the Challis RMP and employed to maintain the AML. Effectiveness monitoring at DMAs and PIBO-EM sites in the Road Creek watershed can be used to assess effectiveness of management relative to PACFISH, INFISH, and Challis RMP objectives. For example, high bank stability (≥80%) and late-seral greenline vegetation currently predominate along fish bearing streams in the Road Creek watershed, and is well documented at the PIBO-EM site (Figure 1 and Table 14). Therefore the ongoing approach to management and the 2012 horse gather would not prevent attainment of PACFISH riparian objectives and would allow for continued maintenance or recovery of riparian vegetation, bank stability, and fish habitat associated with perennial fish bearings streams, Road Creek, Bear Creek and Mosquito Creek.

However there are localized riparian area reaches in the Road Creek watershed, at both upland springs and fish bearing stream reaches, where wild-horse use is known to be focused. Often these locations have apparent bank alteration, regardless of the wild horse herd being at or above AML, and in the absence of cattle use. For example, along lower Horse Basin Creek (within the cattle exclosure) wild horses use is relatively frequent, and decreased bank stability from utilization by horses would persist. Streambank alterations attributable to wild horses present along Road Creek occur relatively infrequently. During March 2012 visual observations along Road Creek at monitoring site RC-KA-02 where horse presence has been observed in past winter / spring seasons , indicated no signs of wild horse streambank impacts were apparent, with streambank ice shelves that would preclude impacts much of the winter and early spring. In addition, the relative difference in horse use along Horse Basin Creek and Road Creek is related to the general distribution of wild horses being strongly related to portion of the CHMA north of Road Creek. Because impacted reaches are not distributed widely in the watershed and maintenance of the AML is proposed, the pattern and intensity of wild horse use relative to fish bearing streams is unlikely to increase and thus proposed horse gather operation would not have an effect on fish habitat within the CHMA.

Alternative 2
The potential for environmental effects to threatened, endangered and sensitive fish and fisheries from horse gather operations under this alternative are related to population size and the extent of gather operations (e.g. captures sites within the RCHA associated with fish bearing streams). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 3, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to fish bearing streams and rivers would be the same under this alternative as alternative 1.

Alternative 3
The potential for environmental effects to threatened, endangered and sensitive fish and fisheries from horse gather operations under this alternative are related to population size and the extent of gather operations (e.g. captures sites within the RCHA associated with fish bearing streams). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 4 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to fish bearing streams and rivers would be the same under this alternative as alternative 1.

Alternative 4
The potential for environmental effects to threatened, endangered and sensitive fish and fisheries from horse gather operations under this alternative are related to population size and the extent of gather operations (e.g. captures sites within the RCHA associated with fish bearing streams). The post gather wild horse population as a result of this alternative would not differ from alternatives 1, 2, and 3 and any subsequent changes in population growth rate would not have a differentiable effect to riparian and wetlands resources over the 3 – 5 year time period prior to the next anticipated gather. The spatial and temporal extent of the gather operations relative to fish bearing streams and rivers would be the same under this alternative as alternative 1.

Alternative 5
Under this alternative, no horse gather activities would occur, and rather than a short term reduction of the horse population and maintenance of the AML (as described under alternatives 1-4), the horse population would continue to increase. Therefore increase of use by wild horses at riparian areas and upland springs is anticipated. This may cause intensified horse use at localized areas along fish bearing streams in the Road Creek watershed. For example, monitoring data indicates that when the AML is exceeded, wild horse effects (trampling, vegetation utilization, defection etc.) can intensify at riparian areas and upland springs. Grazing use criteria are used to manage the intensity of livestock grazing. Stubble height and total bank alteration criteria have been set for the perennial streams in the CHMA by the Challis RMP. Use of riparian areas by wild horses has resulted in use of livestock exceeding grazing standards. In 2003 there were 203 horses counted during the census and livestock grazed from 6/16-7/11; when livestock were done grazing at Horse Basin Creek Key Area 2 the stubble height was 4", on October 23 the stubble height was at 2" (the standard is 3" pursuant to the Challis RMP) and the bank shears were 26% (the Standard is less than 10% pursuant to the Challis RMP). In 2006, Horse Basin was rested from cattle grazing and 231 horses were counted during the census that

year, and a location known to receive focused wild horse use, stubble height was measured at Horse Basin Creek Key Area 2 at two inches. In both of these cases the grazing use criteria was exceeded and it was attributable to only horse use (see Appendix I). Based on patterns of wild horse use, under this alternative bank stability and greenline riparian vegetation important to instream fish habitat would be degraded along Horse Basin Creek where horses are known to concentrate relative to all other fish bearing habitats in the CHMA. This alternative may also result in changes in the spatial pattern of horse use and additional effects to unoccupied designated critical habitat for Chinook salmon and resident salmonids along Road Creek on BLM lands where wild horse use is relatively infrequent.

3.9 Wilderness Study Areas

3.9.1 Affected Environment

Approximately 47% of the CHMA is located within lands managed as WSAs (see Map 6). All 48,500 acres of the Corral-Horse Basin WSA fall within the CHMA. The Jerry Peak WSA makes up 46,150 acres; 23,269 acres of which are in the CHMA. WSAs are managed according to the Challis RMP and the Bureau's Manual 6330 – Management of Wilderness Study Areas (7/13/2012), and BLM pays careful and particular attention to proposals that could limit Congress' ability to designate areas as wilderness. The Manual directs that in a WSA the preservation of wilderness values within the WSA is paramount and should be the primary consideration when evaluating any action that may conflict with or be adverse to those wilderness values. Any proposed use or facility within a WSA must be evaluated to determine whether the action would impair wilderness values. The Manual states, "Wild horse and burro populations must be managed at appropriate management levels so as not to exceed the productive capacity of the habitat (as determined by available science and monitoring activities), to ensure a thriving natural ecological balance, and to prevent impairment of wilderness characteristics, watershed functions, and ecological processes. The BLM should limit population growth or remove excess animals as necessary to prevent the impairment of the WSA." (1.6.D.10). The BLM, therefore, considers proposals in the inventory area on a case-by-case basis to determine the potential impacts on wilderness characteristics.

As discussed above under the heading of *Relationship to Statutes, Regulations, Policy, Plans or Other Environmental Analysis*, the Manual provides guidance specific to wild horse and burro management which directs the BLM to manage populations at the appropriate management level to ensure a thriving natural ecological balance.

Wild horse census data from 1980 (the time of WSA designation) shows that 621 horses were counted on June 12th, 306 were gathered and removed from the CHMA in October, and 319 horses remained in December. The 1980 Intensive Wilderness Inventory does not include a narrative description of the wilderness characteristics for either the Corral-Horse Basin or Jerry Peak WSAs. The following is a synopsis of the characteristics as described in the 1991 Idaho Wilderness Study Report:

Corral-Horse Basin WSA:

Size and Physical Characteristics

The WSA contains 48,500 acres of BLM managed land roughly bounded on the north by Spar Canyon, on the west by the East Fork of the Salmon Road, on the south by Road Creek, and on the east by Dry Gulch Road. The WSA is composed of rolling hills surrounding Corral and Horse Basins. Anderson Peak, at 9,342 feet, dominates the WSA. The vegetation is predominantly sagebrush and grass with strips of timber on streams, sideslopes, and bottoms. There is one large stand of Douglas-fir on the east slope of Anderson Peak.

Naturalness

The Corral-Horse Basin WSA presents the visitor with a general appearance of naturalness. The majority of human imprints are grazing activities, fences, and waterhole developments. These imprints are not considered to be major impacts to naturalness and blend into the landscape when viewed from more than .5 mile. Impacts to the natural appearance of the WSA consist of two cherry-stem roads which breach the boundaries for 6 miles and two vehicle ways traversing the WSA on the east.

Solitude

Rolling hills, wide basins, lack of vegetative screening, and outside sights and sounds from the cherry stems, boundary roads, and private ranches all detract from the solitude of the WSA, despite the WSA's size.

Primitive and Unconfined Recreation

The relatively large size of the unit, lack of man-made or natural barriers, and lack of developments result in outstanding opportunities for primitive and unconfined recreation. Opportunities are available for hiking, backpacking, fishing, hunting, horseback riding, cross-country skiing, snowshoeing, photography, bird-watching, and sightseeing. The lack of barriers and rolling terrain also encourage off-road vehicle use, especially in the fall.

Supplemental values

The excellent opportunities for wild horse viewing were identified as special features for this WSA (USDI-BLM 1991).

Jerry Peak WSA:

Size and Physical Characteristics

The WSA contains 46,150 acres of BLM managed land roughly bounded on the south by the Challis National Forest, on the west by the East Fork of the Salmon Road, on the north by Road Creek, and on the east by Pecks Canyon Road. The WSA is composed of rolling hills with sagebrush and grass on the northern half of the unit while the southern portion has steeper terrain culminating with Jerry Peak (10,010 ft. elevation). Large areas of timber are located in the upper reaches of Bear, Mosquito, Sage, and Lake Creeks. Herd Lake, located in the southwest portion of the WSA is popular for fishing and sightseeing.

Naturalness

The Jerry Peak WSA presents the visitor with a general appearance of naturalness. The overall influence of human imprints on the naturalness of the area, as perceived by the

visitor, is minimal due to the wide dispersal and low impact developments. Imprints exist from fences and spring developments associated with range management, access roads, and jeep trails. Range improvements tend to be small and blend in with the natural landscape. The four dead-end roads (Herd Lake, Mosquito Creek, Sage Creek, and Bradshaw Creek), while not technically part of the WSA, influence the perception of naturalness.

Solitude
Topographic relief and vegetative screening, especially in the Bear Creek and Lake Creek drainages, provide for outstanding opportunities for solitude.

Primitive and Unconfined Recreation
Outstanding opportunities for primitive and unconfined recreation exist in this WSA due to the large size of the WSA, lack of barriers and developments in or near the eastern portion of the unit. Opportunities are available for hiking, backpacking, fishing, hunting, horseback riding, cross-country skiing, snowshoeing, photography, bird-watching, and sightseeing.

Supplemental values
Excellent opportunities for viewing wild horses and the landslide (which created Herd Lake) were identified as special features for this WSA (USDI-BLM 1991).

During the Challis BLM travel management planning effort from 2002-2009, historic aerial photo analysis and an intensive inventory review were performed to determine the presence of motorized vehicle routes in the WSAs. Any vehicle routes not present within the WSAs at the time of inventory were removed from consideration for designation, not included on the final travel maps, and remain closed to motorized vehicle use. All routes were analyzed; however, some of these routes were left open to motorized vehicle use and some routes were closed. Cross country travel by motorized or mechanized means is prohibited in all WSAs managed by the Challis Field Office, including the Corral-Horse Basin and Jerry Peak WSAs.

3.9.2 Environmental Effects

Proposed Action-Alternative 1
Wild horses were identified during the Corral-Horse Basin and Jerry Peak WSA inventory processes as a supplemental wilderness value within both of the WSAs. Appropriate management techniques identified in the Wilderness IMP have been built into each alternative. Each of the action alternatives would capture enough horses to reduce the herd size to the Appropriate Management Level (185 head). This alternative would also utilize identical capture site locations and gather techniques (helicopters for aerial herding, temporary capture sites, sorting/holding facilities and use of motorized vehicles only on routes which existed at the time of inventory and designated for vehicle use).

It is likely that during the gather there would be some amount of surface disturbance due to the concentration of horses and humans within a temporary capture area. This surface disturbance would primarily occur outside of WSA boundaries due to project designs incorporated for the protection of WSA values. Where surface disturbance does occur within the WSA boundaries it

would not be to a degree which requires reclamation. By maintaining the wild horse herd at the AML, degradation caused by overgrazing would be reduced — thus maintaining the overall health and appearance of the WSAs.

Nothing within the proposed action alternative would affect the size and physical characteristics of either the Corral-Horse Basin or Jerry Peak WSAs. Wilderness values such as outstanding opportunities for primitive recreation, solitude and naturalness would be temporarily impacted by the presence of human activity including helicopters, trucks, riders and temporary capture sites during the gather. The duration of the gather, and the impacts identified above, is expected to last about two weeks. The supplemental values noted in the original inventories include excellent wild horse viewing in Corral-Horse Basin WSA and the geological value of the Herd Lake landslide in Jerry Peak WSA. Wild horse viewing opportunities would be temporarily reduced due to fewer visible animals.

This project would be substantially unnoticeable once the temporary capture facilities are removed. These actions would not affect the area's wilderness characteristics or values in a manner which would constrain Congress' ability to determine suitability for preservation as wilderness. All of the action alternatives are consistent with the BLM Manual 6330 – Management of Wilderness Study Areas.

Alternative 2
Under this alternative effects would be similar to the proposed action alternative. It is anticipated that the effects of the gather (i.e. reduced wild horse numbers) would have a shorter lasting duration.

Alternative 3
Under this alternative effects would be similar to the proposed action alternative. It is anticipated that the effects of the gather (i.e. reduced wild horse numbers) would last for a longer duration of time. Adding geldings to the population may reduce or limit reproduction.

Alternative 4
Under this alternative effects would be similar to the proposed action alternative. It is anticipated that the effects of the gather (i.e. reduced wild horse numbers) would have a shorter duration of time.

Alternative 5
Under the No-action alternative, there would be no direct impacts to the wilderness characteristics of either the Jerry Peak or Corral-Horse Basin WSAs. However, indirect impacts would result from the unmanaged population growth of the wild horse herd. Effects to the soil, water, vegetation, wildlife and aesthetic qualities of the WSA would mount as the wild horse population continued to expand. This could ultimately impact the naturalness of the units and the outstanding opportunities for primitive and unconfined recreation. The No-action alternative would not be consistent with the Manual which states, "… the Bureau must still endeavor to make every effort not to allow populations within WSAs to degrade wilderness values, or vegetative cover as it existed on the date of the passage of FLPMA. Wild horse and burro

populations must be managed at appropriate management levels as determined by monitoring activities to ensure a thriving natural ecological balance."
(USDI-BLM, 1991)

3.10 Recreation

3.10.1 Affected Environment

The CHMA falls primarily within the Challis Extensive Recreation Management Area (ERMA), while a sliver along the East Fork and Main Salmon Rivers falls within the Upper Salmon River Special Recreation Management Area (SRMA). The Upper Salmon River SRMA attracts and concentrates substantial numbers of recreationists who come to the area due to the outstanding opportunities for river recreation, ease of access, international name recognition, and proximity of the area to other prominent recreation centers. The SRMA is managed to accommodate this concentration of use by providing developed recreation opportunities such as campgrounds, day use areas, trails, interpretive signage, and boating facilities. The majority of recreational use in the Main Salmon River corridor, and recreational developments, occur between highway 75 and the river. Along the East Fork Salmon River, most use occurs along the East Fork Road and at the Little Boulder Creek Recreation Site.

In contrast to an SRMA, recreation management within an ERMA is custodial in nature and is not the driving management factor in the area. Recreation in the ERMA is generally dispersed in nature and includes hunting, fishing, hiking, wildlife viewing, scenic driving, and off-highway vehicle (OHV) use. Dispersed recreation activities allow visitors to experience an area in an unstructured manner, remove themselves from every day pressures, and to challenge themselves in an outdoor setting. Benefits resulting from these experiential opportunities are many and include a closer relationship to the natural world, improved physical and mental health, and interpersonal connections with other people who enjoy similar experiences.

Elements of the physical, administrative, and social setting can have an impact on both the opportunities and benefits for recreationists to the area. Approximately 47% of the Challis CHMA is managed by the BLM as WSAs. In order to protect the wilderness characteristics of these areas, and to provide a non-motorized hunting experience, the BLM implemented annual seasonal motorized closures from October 1-December 31 in the Corral-Horse Basin and Jerry Peak WSAs. Herd Creek, Road Creek, North Fork of Sage Creek, and Spar Canyon Roads are the only routes available to motorized use during the seasonal closure period. Due to these administrative actions affecting the ERMA, recreationists visiting the WSAs during this period of time have an expectation of a primitive recreational opportunity unaffected by the presence of motorized vehicle use.

The gather is scheduled to occur in October which coincides with the commencement of big game rifle hunting seasons in this area (Idaho Department of Fish and Game Hunt Unit 36-A). In 2011, the following hunts took place in Unit 36-A during the month of October:

Table 18. October 2011 Hunts within Hunt Unit 36-A and Number of Hunters

Date	Hunt #/Description of Hunt	# of Hunters
9/25-10/24	#4037/Pronghorn, Controlled Tag	10
10/10-10/24	NA/Regular Deer, General Tag	Unlimited
10/10-10/31	NA/White-tailed Deer, General Tag	Unlimited
10/26-10/31	#1019/Regular Deer, Controlled Tag	230
10/1-10/31	#2010/Antlered Elk, Controlled Tag	58
10/1-10/31	#2161/Outfitter Allocated, Antlered Elk, Controlled Tag	3

Unit 36-A is 494,000 acres, of which the CHMA affects 154,150 acres of public lands. The portion of the CHMA, and Unit 36-A, which is managed under the seasonal motorized closure is 72,500 acres.

3.10.2 Environmental Effects

Proposed Action-Alternative 1

Under the Proposed Action alternative 1, impacts to recreation would be limited. Due to the timing of the proposed gather (October), the user group most likely to be affected are big game hunters. The largest impact is likely to be the presence of the helicopter as it circles the area, directing bands of wild horses toward the capture sites. A second, and less disrupting impact, would be the possible restriction to vehicle traffic along Road Creek Road in the event that one of three capture sites on Road Creek drainage are utilized.

The greatest impact to recreational use of the ERMA would be expected to be a result of the use of a helicopter to gather wild horses during hunting season. The helicopter is expected to be in the air 5-8 hours a day for up to 1-2 weeks during the middle to late October. The sights and sounds of a low flying helicopter in the air for a majority of the daylight hours would impact the opportunity for solitude for any visitor to the area, regardless of the purpose of the visit (hunting, hiking, camping, etc.). The impact on hunting opportunity, and more specifically hunting success, would be entirely situation specific. It is possible that a hunt could be spoiled if the helicopter is situated in such a manner as to drive big game away from a hunter's position. However, it is equally possible that the flight pattern of the helicopter could direct the game toward a hunter, thus improving the odds of success.

A second likely impact to recreational visitors to the area would be the potential of the Arrowhead capture site to block through traffic on Road Creek. Road Creek Road is one of two routes which connect from east to west between Highway 93 and the East Fork area. It is also one of just a handful of routes not affected by the seasonal motorized closures in place to protect wilderness values and to provide a non-motorized hunting experience in the WSAs. However, even if capture sites along Road Creek are utilized for the proposed gather, the impacts to recreational use should be minimal for the following reasons; 1) Road Creek Road is a through route, so access would be available from either end at all times, 2) the capture site would only be expected to be in place 3-7 days, and 3) when not actively in use (containing, or soon to contain gathered horses), the capture would be opened up to allow vehicle access along Road Creek Road.

Alternative 2
Under this alternative effects to recreation would be nearly identical to the Proposed Action. The duration of higher wild horse reproduction increases the frequency of the gather cycle.

Alternative 3
Under this alternative effects to recreation would be nearly identical to the Proposed Action. The duration of lower wild horse reproduction decreases the frequency of the gather cycle.

Alternative 4
Under this alternative effects to recreation would be nearly identical to the Proposed Action. The duration of higher wild horse reproduction increases the frequency of the gather cycle.

Alternative 5
Under the No-action alternative, there would be no direct impacts to recreational use of the area. However, indirect impacts would result from the unmanaged population growth of the wild horse herd. Effects to the soil, water, vegetation, wildlife and aesthetic qualities of the CHMA would mount as the wild horse population continued to expand. This could ultimately impact the desirability of the area for dispersed recreation activities such as hunting and camping. The ability of visitors to view wild horses would increase if the gather were to be deferred, though the condition of the landscape and of the horses themselves might equally impact the viewing experience.

3.11 Soils

3.11.1 Affected Environment
The Challis Field Office area has 190 mapped soil units (USDA-NRCS 2002); 72 of these soil units occur within the CHMA. The soils within the CHMA are shallow to very deep, gravelly to stony loams to clay loams derived from extrusive igneous rocks; some of these soils contain calcic horizons due to limestone deposits. Soils occurring at the higher elevations have a thick surface horizon (mollic); however, most are dry for at least half the growing season (aridic) (USDA-NRCS 2002).

The erosion hazard across most of the CHMA is slight to moderate (USDA-NRCS 2002); this indicates soils in the CHMA area are able to capture store and safely release nearly all precipitation, except during occasional high intensity thunderstorms or intense short-duration (1-2 days) spring snowmelt. In such cases, surface flow and sediment transportation into streams can be pronounced, particularly on very steep slopes (>60 %). An exception to this is the "badlands" soils mostly associated with Malm Gulch and Sand Hollow Allotments (domestic livestock grazing privileges have been relinquished on both allotments) which make up approximately 8% (≈ 13,764 Acres) of the HMA. These soils are Dawtonia-Frailton Complex (20-50% Slopes), Farvant-Badland-Gradco Complex (25-60% Slopes), Frailton-Dawtonia Complex (15-50% Slopes), Frailton-Gradco Complex 35-60% Slopes), and Gradco-Farvant Complex (30-60% Slopes); these soils are considered sensitive due to their highly erosive nature. All the soil types within the CHMA have rapid infiltration rates although some may have a clay or calcic horizon within 20 inches of the surface that may perch surface water (USDA-NRCS 2002).

Biological crusts are soil particles bound together by organic materials and are formed by living organisms and their by-products (USDA-NRCS 1997). In cool desert environments biological crusts generally increase water infiltration by acting as holding structures for water (USDI-BLM/USGS 2001). Biological soil crusts do occur within the CHMA, however the extent to which they are present is fairly limited and highly dispersed.

Ground cover is affected by the timing, duration, frequency, and intensity of grazing use. Domestic livestock grazing is controlled by the various grazing systems (numbers and kind of livestock, season of use, and number of AUMs) applied to the allotments within in the CHMA. Wild horse grazing is manipulated by controlling the population (the intensity of use) as the timing, duration and frequency of wild horse use is unmanaged.

Other factors impacting ground cover are outside of management control, such as climatic conditions resulting in annual variations in forage production. Climatic conditions (i.e. timing of precipitation and temperature) are primary factors determining the rate of photosynthesis and thus plant growth.

3.11.2 Environmental Effects

3.11.2.1 Alternative 1-4

Areas of heavy use occur around existing water sources such as springs, troughs, water gaps, salt licks, and areas providing cover or shade. The soils within and closely surrounding these areas receive heightened use as compared to surrounding areas and may, in some instances (e.g. areas of low soil moisture) show signs of soil compaction, erosion, and reduced productivity. These areas of decreased vegetation and litter cover are generally more susceptible to soil erosion and increased runoff. Conversely, congregation areas with high soil moisture that undergo several freeze-thaw cycles each year are less likely to remain compacted. This freeze-thaw action promotes soil stability by decreasing compaction and increasing surface area and sites for seed germination and vegetative establishment.

Impacts to the soil resource from large ungulate grazing activities include compaction (Wheeler 2002) and disruption or destruction of physical or micro-biotic soil crusts (Memmott 1998), which can result in soil erosion. Surface disturbance that disrupts or destroys physical or micro-biotic soil crusts can also lead to erosion. Erosion of the soil surface following disturbances would remove the litter layer and potentially portions of the A horizon (the uppermost, organic soil layer). Of the entire soil profile, the litter layer and the A horizon contain the greatest amount of organic matter which is the source of plant-available nutrients (Neff 2005). Alterations to the nutrient cycle via erosion can reduce plant community productivity and create conditions that are conducive to the introduction of nonnative species (Kourtev 2002). Impacts, which are generally caused by hoofing action, would be greatest in high use areas such as water troughs, fence lines, and crossings. However, the majority of the soil surface would be stabilized by root mass, organic matter (e.g., herbaceous/woody litter, manure from domestic livestock, wild horses and wildlife), decomposition products, and/or biological crust.

Impacts directly related to the gather operation are likely to be limited to the capture sites and the sorting facilities. Most of the capture sites and sorting facilities are located in areas that were used in previous gathers and are already disturbed sites. Any additional or new capture sites necessary to facilitate a more efficient and safer gather would be cleared prior to placement, and would only be set up on existing roads so new disturbances would be minimal. The existing capture sites are located in areas where little or no biological soil crust occur, however the potential exists for disturbance to soil crusts to occur during the course of moving the wild horses to the capture sites. This could result in an increase in the erosive potential on a site by site basis as the soil holding and precipitation infiltration benefits that biological crusts provide could be reduced (Brotherson and Rushforth. 1983).

Immediately following the gather the horse population would have a short term reduction and this would likely result in a short term reduction in the intensity of horse use at areas of concentrated use, however as this is only a short term decrease in the population no substantial improvement in soil condition (compaction, infiltration ability, etc.) is expected to occur.

3.11.2.2Alternative 5

Under this alternative, the number of wild horses would increase leading to a potential increase and intensify of horse use across the CHMA. Of concern would be areas where concentrated use already occurs, where use would be intensified, and the possibility for initiating new areas of concentrated use. Population estimates have the herd size growing to 1,630 horses over the next 10 years, which equates to 19,560 AUM's. Both the projected wild horse numbers and projected AUMs are substantially over the designated herd size of 185 horses or 2,220 AUM's. At areas of concentrated use this increase could lead to reduction of cover which could increase soil erosion on a site by site basis. Land areas covered by plant biomass, living or dead, are protected and experience reduced soil erosion because raindrop and wind energy is dissipated by the biomass layer (Pimentel and Kounang 1998). Impacts to biological soil crusts would likely increase if the deferral is for an extended period of time and could lead to an increase in the erosion potential of the soil on a site by site basis.

3.12 Threatened/Endangered/Sensitive animals/birds/wildlife

3.12.1 Affected Environment

Threatened, Endangered, Candidate and Special Status Species

There are four terrestrial wildlife species that are identified by USFWS for consideration under Endangered Species Act (ESA) that have been documented or have the potential to occur in the CFO (Table 19).

Table 19. Threatened, Endangered, and Candidate Present on Lands Administered by the CFO.			
Common Name	**Scientific Name**	**Status**	**General Habitat**
Canada lynx	*Lynx canadensis*	Threatened	Conifer
Wolverine	*Gulo gulo luscus*	Candidate	Alpine, boreal, and arctic habitats
Yellow-billed cuckoo	*Coccyzus americanus*	Candidate	Riparian
Greater sage grouse	*Centrocercus urophasianus*	Candidate	Sagebrush steppe

The CHMA is not within a designated Canada Lynx (*Lynx canadensis*) Analysis Unit (LAU). A portion of lynx linkage exists on the southeast portion of the CHMA.

Wolverines (*Gulo gulo luscus*) seek higher altitude, colder habitats with persistent spring snow during denning and for physiological reasons the rest of the year. No designated habitat for wolverines exists within the CHMA.

Suitable yellow-billed cuckoo breeding habitat is defined as needing "large blocks of riparian..." with "dense understory foliage" (USFWS 2001). Likewise, Reynolds and Hinckley (2005) define breeding habitat requirements as "a minimum of approximately five acres of *prime* riparian habitat…of old growth cottonwoods, with a dense understory of willow or dogwood" (*emphasis added*). Reynolds and Hinckley (2005) conducted both a review of historic records and site sampling in Idaho, and concluded: 1) "yellow-billed cuckoos have never been particularly abundant in Idaho," and 2) the nesting strongholds are limited and few with "the statewide nesting population at fewer than 20 pairs, and in all likelihood closer to 10 pairs in an average year." Based on data from the USFWS (2001) and Reynolds and Hinckley (2005), potential, suitable breeding habitats do not exist in the CHMA for the yellow-billed cuckoo.

The Canada lynx, wolverine, and yellow-billed cuckoo would not be discussed further in this document due to a lack of suitable habitat and/or the lack of any known presence within the lands encompassed by the CHMA.

Greater Sage-Grouse

In response to declines in Greater sage-grouse populations, the *National Sage-grouse Habitat Conservation Strategy* (National Assessment) was developed to assess "the ecological status and potential factors that influenced greater sage-grouse and sagebrush habitats across their entire distribution" (Connelly 2004). The same year BLM developed the *National Sage-grouse Habitat Conservation Strategy* (National Assessment) "to guide future actions for conserving sage-grouse and associated sagebrush habitats and to enhance BLM's ongoing conservation efforts" with the purpose "to set goals and objectives, assemble guidance and resource materials, and provide a comprehensive management direction for the BLM's contributions to the on-going multi-state sage-grouse conservation effort in cooperation with the WAFWA" (USDI-BLM 2004b). In Idaho, state-wide guidance is provided by the *Conservation Plan for the*

Greater Sage-grouse in Idaho (Idaho Sage-grouse Advisory Committee 2006). The primary goal of the plan is to:

> "Maintain, improve, and where possible increase, sage-grouse population and habitats in Idaho, while considering the predictability and long-term sustainability of a variety of other land uses."

In order to meet this goal, the plan outlines a series of conservation measures designed to minimize impacts associated with a wide array of potential threats to sage-grouse and their habitats.

The Idaho Sage-grouse Management Plan was completed in 1997. This plan called for the "creation of Local Working Groups (LWG) that would develop sage-grouse management plans for each of Idaho's sage-grouse planning areas" (Idaho Sage-grouse Advisory Committee 2006). The Challis Sage-grouse Local Working Group (Challis LWG) was formed in 2002. The Challis LWG is made up of interested parties; both private citizens and public agencies. The Challis LWG completed the Challis Sage-grouse Conservation Plan (Challis SCP) in 2007. The goal of Challis SCP is to plan and oversee the implementation of conservation measures within the Challis Sage-grouse Planning Area "that will result in stable, healthy sage-grouse population within sustainable habitats" and that "management should occur in a socially, economically, and ecologically focused manner" (CSGLWG 2007). The Challis Sage Grouse LWG designated priority areas within the Challis SCP and has mapped seasonal sage-grouse habitat throughout the LWG area. Seasonal habitat is delineated by sage-grouse observations and habitat type encompassing those locations. Seasonal maps are updated as new data points are collected.

On March 5, 2010, the USFWS (FWS) found the greater sage-grouse "warranted, but precluded by higher priority listing actions" and placed it on the Candidate species list. Until sage-grouse can be addressed by USFWS, it is managed by the state and addressed by BLM as a special status species (USDI-BLM 2008b). In response to the petition to list, the FWS identified habitat conversion and fragmentation from wildfire, invasive plants, energy and infrastructure development, urbanization, and agricultural conversion to be the primary threats to greater sage-grouse throughout its range. Through the development of planning strategies to address FWS concerns for "improved regulatory mechanisms", BLM has identified wildfire, habitat loss due to invasive plant species, and habitat fragmentation as the major threats in the western portion of the existing sage-grouse range, of which Idaho is part.

On December 27, 2011, the BLM released Instruction Memorandum No. 2012-043, Greater Sage-grouse Interim Management Policies and Procedures (Interim Management), to provide interim conservation policies and procedures to be applied to ongoing and proposed authorizations and activities that affect the Greater Sage-grouse and its habitat until long term conservation measures can be incorporated into Land Use Plans. These policies and procedures apply to Preliminary Priority Habitats (PPH) and Preliminary General Habitats (PGH). PPH is defined as "areas that have been identified as having the highest conservation value to maintain sustainable Greater Sage-Grouse populations" and PGH is defined as areas "of occupied seasonal or year-round habitat outside of priority habitat." The majority of the CHMA is identified PPH (~124,119 acres) by Interim Management. The remainder of the CHMA is considered PGH

(~43,468 acres) except for two small portions (~261 acres for both) on the southern end of the CHMA that are not identified as either PPH or PGH (see Map 4). For PPH, policies and procedures are "to maintain, enhance, or restore conditions for Greater Sage-grouse and its habitat" (USDI-BLM 2011b). Specifics to wild horse and burro management from the policies and procedures include "Manage wild horse and burro population levels within established Appropriate Management Levels (AML)."

Sage-grouse habitat, which consists of large expanses of sagebrush-dominated shrub steppe, was delineated cooperatively by the BLM, IDFG, and other partners during the development of the *Conservation Plan for the Greater Sage-grouse in Idaho* (Idaho Sage-grouse Advisory Committee 2006). The majority of public lands administered by the Challis Field Office, including the CHMA, are designated as "key" sage grouse habitat. Key sage-grouse habitat is defined as "areas of generally intact sagebrush that provides sage-grouse habitat during some portion of the year" (Idaho Sage-grouse Advisory Committee 2006).

It is estimated that greater sage-grouse range wide distribution in potential habitat pre-settlement was 1,200,483 km^2 and current distribution is 668,412 km^2 (Connelly 2004). The CHMA and the Big Lost Valley in general represent a portion of the Big Lost sage-grouse sub-population which takes in 550,791 acres of the Warm Spring and Big Lost watersheds. Lek count data presented by Garton et al. (2011:348), suggests that this subpopulation has declined from an estimated high of 22,000 males in 1969 to approximately 5,500 males in 2007. The subpopulation appears to have stabilized at around 5,000 males since 1992. In comparison the National Assessment states:

> "Annual rates of change suggest a long term decline for greater sage-grouse in western North America and support the trend information obtained from lek attendance (males/lek) data. Sage-grouse populations declined at an overall rate of 2.0% per year from 1965 to 2003. From 1965-85, the population declined at an average of 3.5%. From 1986 to 2003, the population declined at a lower rate of 0.4% and fluctuated around a level that was 5% lower than the 2003 population (Connelly 2004)."

Beever noted in Studies of Avian Biology, Greater Sage-grouse: ecology and conservation of a landscape species and its habitats (2011) that "Past research elaborated that free-roaming horses can exert notable influences in sagebrush (*Artemisia spp.)* communities, on structure and composition of vegetation and soils, as well as indirect influences on numerous animal groups whose abundance collectively may indicate the ecological integrity of such communities."

Pygmy Rabbit

Pygmy rabbits (*Brachylagus idahoensis*) are known to be distributed historically (Green 1980) and presently (BLM files) throughout southern and eastern Idaho where preferred habitat conditions exist. Pygmy rabbits are sagebrush obligates and are found in landscapes with tall, dense stands of sagebrush and deep soils for construction of burrow systems (Green 1980). On September 20, 2010, the USFWS 12-month finding on the petition to list found pygmy rabbits

"not warranted at this time" for the populations found outside the Columbia River distinct populations segment.

Migratory Birds

Migratory birds have the potential to exist throughout the CHMA on all habitat types. The majority of wild horse use takes place in shrub steppe habitat and riparian corridors. Fragmentation and removal of habitat has been shown to have impacts on the presence of sagebrush obligate passerine species (Knick and Rotenberry 1995). Potential impacts to migratory birds are; direct disturbance, and removal of potential nesting habitat.

Big Game Species

Gray Wolf

Wolf populations have grown from the initial release of 66 wolves within the Intermountain West in 1995 and 1996 to an estimated 705 by the end of 2010 in Idaho alone (Holyan et al. 2011). Gray wolf management and population control efforts have been conducted in response to livestock depredation problems under the Memorandum of Agreement between The Secretary of the Interior and The State of Idaho (dated January 5, 2006), and in accordance with Idaho Wolf Population Management Plan (IDFG 2008). Idaho Department of Fish & Game conducted a hunt for wolves following the initial wolf delisting by USFWS in which 173 were harvested. Recorded wolf mortalities, including control efforts, have ranged from 15 wolves being removed in 2003 to 273 in 2009. On May 5, 2011, the USFWS, as directed by legislative language in the fiscal year 2011 appropriations bill, reinstated the 2009 rule removing gray wolves in the Northern Rocky Mountains Distinct Population Segment, which included the state of Idaho, from the endangered species list. Recreational hunting began in 2011 in the hunting unit on and around the CHMA with a quota limit that can be taken by recreationists. The quota for each hunting unit would be adjusted as needed to meet the management goals in IDFG's Idaho Wolf Population Management Plan. The Castle Peak Pack territory overlaps with the CHMA. Individuals not associated with a pack can be present throughout the CHMA. Potential impacts to gray wolf would be through direct disturbance and indirectly through removal of a potential food source.

Other Big Game

The Challis Field Office RMP designates habitat for antelope, bighorn sheep, elk and mule deer within the CHMA. These species would be referred to collectively as Big Game in this section. Of most concern are areas considered for winter use, especially those that have been designated as "crucial winter range." These crucial winter range areas are those that are the most readily accessible to big game and utilized most often in a severe winter. Potential impacts to big game would be displacement and vegetation removal.

Table 20 Current designated big game winter ranges within the CHMA:

Table 20. Acres of Winter or Crucial winter Range by big game species:		
Species	Winter Range	Crucial Winter Range
Mule deer (*Odocoileus hemionus*)	67,563	15,713
Elk (*Cervus elaphus*)	54,254	3,360
Pronghorn Antelope (*Antilocapra americana*)	24,314	11,101
Bighorn Sheep (*Ovis canadensis*)	61	51
*Acreages Assessed in GIS		

3.12.2 Wildlife Environmental Effects

Alternatives 1-4
Threatened, Endangered, Candidate and Special Status Species

Greater Sage-Grouse

Potential impacts to sage-grouse would be disturbance of individuals by gathering activities and vegetation removal at capture sites. Disturbances by gathering activities would be short term and would be confined to small portions of the CHMA leaving the rest of the CHMA available for use without such disturbances. Capture sites would be temporary, limiting the time that horses would be present within them. Vegetation removal at capture sites is rarely entire and the percent of vegetation removal is dependent on time and intensity of activity at each site. Vegetation damage is likely, but is limited to a very small area (<0.01%) within the CHMA. Vegetation removal creates potential sites for invasive weeds to establish. SOPs (Appendix B) for horse gathers are to monitor these sites for weeds and treat if they are found. Several of the capture sites have been used for past gathers and remain weed free and not visually apparent. The gather would be performed at a time when there would be no lekking or nesting and young of the year chicks would already be capable of flight.

Pygmy Rabbit

Pygmy rabbits have the potential to be present throughout the CHMA where their preferred habitat exists. Potential impacts to pygmy rabbits would be vegetation removal at occupied burrows. SOP's for capture site placement is that it would be placed as to cause "as little damage to the natural resources of the area, as possible" and would have to be approved by an Authorized Officer. The Authorized Officer would be trained in recognition of pygmy rabbit sign so as to avoid capture placement on burrows. Alternatives 1-4, would be in compliance with BLM Manual 6840 and would not lead to the listing of the species.

Migratory Birds

Horse gather activities would take place after nesting of migratory birds, eliminating impacts associated with these activities. Gathering activities would still have the possibility of disturbing individuals. Adult and healthy young of the year would be capable of flight. Gather activities would be short term and limited to a portion of the CHMA. The remainder of the CHMA and surrounding landscape would be available for use by individuals that might be disturbed. Any

disturbances to vegetation would be limited to capture and staging sites. SOP's would be used to limit vegetation disturbance by gather activities to previously disturbed sites or locate new sites in areas to cause as little resource damage as possible. Individual birds that might be using activity sites would have the surrounding habitat to relocate to. Alternatives 1-4, would be in compliance with BLM Manual 6840 and would not lead to the listing of the species.

Big Game Species

Gray Wolf

Gray Wolves are a highly mobile species capable of using habitat at a landscape scale. Wolves that might be present in the CHMA could be disturbed by gather activities. This would be short term and habitat impacted by gather activities would be available to wolves following those activities. The horse gather would reduce herd size and remove potential prey for wolves. Wolves have not been documented preying on horses within the CHMA. Additionally, cow elk herd numbers are meeting population objectives for hunt units within and around the CHMA. Alternatives 1-4, would be in compliance with BLM Manual 6840 and would not lead to the re-listing of the species.

Other Big Game

Impacts from gather activities would take place outside of the crucial winter period for big game eliminating the potential for disturbances during this sensitive time. Disturbances to big game present within the CHMA during the gather would be short term and limited to isolated areas. The remainder of the CHMA and the surrounding habitat would be available for use by big game that could be disturbed by gather activities. Vegetation disturbances would be limited to capture and staging sites. There are 8 possible capture sites located in designated mule deer winter habitat and 7 possible capture sites in elk winter (see Map 5). There are no possible capture sites located in antelope or bighorn sheep winter range and no possible traps sits located in crucial winter range for any of the big game species. To the extent possible these sites would be placed in previously disturbed sites and would represent a small portion of the CHMA. Potential vegetation damage from this alternative would be of a scale as not to be a factor in big game herd health.

Alternative 5
Threatened, Endangered, Candidate and Special Status Species

Greater Sage-Grouse

The AML would continue to be exceeded, if horses were not gathered under Alternative 5. "Long-term conservation objectives should consider the appropriate management levels of horses and burros that can be maintained, because free-roaming equids can influence the structure and function of sagebrush ecosystems" (Conelly, 2011). The horse herd has exceeded the AML and is consuming more AUM's than is set aside for them within the Challis RMP, and is leading to indirect effects of a reduction of nesting and brooding success for sage-grouse. Riparian areas are important to sage-grouse brood rearing. Ungulate utilization beyond that

allocated within the RMP would lead to increased impacts to riparian areas and increased potential for invasive weed infestations in areas of focused utilization such as riparian areas.

Pygmy Rabbit

Increased forage consumption by a larger horse herd could lead to a decrease in competition of grasses and forbs with sagebrush. An increase in sagebrush would favor pygmy rabbits in areas where burrow construction is possible. However, incidences of this would be scattered and small in scope of the CHMA. If horse herd numbers were to grow to the point of damaging mature sagebrush, individual pygmy rabbits could be impacted. Wild horse herd numbers in a magnitude to have an impact on sagebrush and indirectly on the pygmy rabbit population throughout the majority of the CHMA is not likely within the 10-year scope of Alternative 5. This alternative would be in compliance with BLM Manual 6840 and would not lead to the listing of pygmy rabbits.

Migratory Birds

An increased horse herd has a higher potential for impacts to nesting migratory birds. Whether or not the horse herd could grow to a size to have a measurable impact on nest success within the CHMA during the ten year period of this alternative is not known. If wild horse herd size were to increase to the point of having measurable impacts to migratory birds, they would be sporadic, both spatially and temporally, within the CHMA. The impacts would not exclude migratory bird use within the CHMA. The CHMA only occupies a portion (21%) of the CFO. CFO lands outside of the CHMA would be available for use by migratory birds without the wild horse herd being present. For these reasons, Alternative 5, would be in compliance with BLM Manual 6840 and would not lead to the listing of migratory birds.

Big Game Species

Gray Wolf

Greater forage consumption by horses in the CHMA would lead to greater competition with native ungulates, the wolves' primary prey. This could lead to a decreased native prey species. Horses could replace native ungulates as the preferred prey species of wolves who utilize the CHMA as a territory. This alternative would be in compliance with BLM Manual 6840 and would not lead to the re-listing of wolves.

Other Big Game

Utilization of forage by horses beyond what is authorized in the Challis RMP would be in direct competition with other authorized ungulate forage utilization. Big game forage utilization would likely shift to areas outside of the CHMA. This would lead to increased completion in big game areas outside of the CHMA. It could also lead to degradation of winter and crucial winter big game within and along the border of the CHMA.

3.13 Tribal Treaty Rights

3.13.1 Affected Environment

The 1868 Fort Bridger Treaty between the United States and the Shoshone and Bannock Tribes reserves the Tribes' right to hunt, fish, gather, and exercise other traditional uses and practices on unoccupied federal lands. The federal government has a unique trust relationship with federally-recognized American Indian Tribes including the Shoshone-Bannock Tribes. BLM has a responsibility and obligation to consider and consult on potential effects to natural resources related to the Tribes' treaty rights or cultural use. Resources or issues of interest to the Tribes that could have a bearing on their traditional use and/or treaty rights include: tribal historic and archaeological sites, sacred sites and traditional cultural properties, traditional use sites, water, fisheries, traditional use plant and animal species, vegetation (including noxious and invasive, non-native species), air and water quality, wildlife, access to lands and continued availability of traditional resources, land status, and the visual quality of the environment.

The CHMA includes unoccupied federal lands, Idaho State lands, and private lands located within the Shoshone-Bannock Tribes' aboriginal territory. Therefore, tribal treaty rights, as defined, are applicable to the project area. The current condition and nature of specific affected resources associated with tribal rights and interests are described in the following sections: Vegetation, Wildlife, and Fisheries.

3.13.2 Tribal Treaty Rights Environmental Effects

3.13.2.1 Alternatives 1-5

There would be no changes in land status or access associated with the proposed actions and the project area would retain its unoccupied Federal land status. During the actual wild horse gather, short term access limitations might be instituted to protect the health and safety of the horses, BLM employees and contractors. Information about where daily activities are expected to take place would be made available via a Wild Horse Gather Information Phone Line. Limitations would be expected to take place in specific areas for no more than 4-8 working days over no more than a 14 day period.

Discussion of potential impacts to specific resources associated with Tribal rights and interests can be found in the Vegetation, Wildlife, and Fisheries sections of this document.

3.14 Human Health and Safety

3.14.1 Affected Environment

Members of the public can inadvertently wander into areas that put them in the path of wild horses that are being herded or handled during the gather operations, creating the potential for injury to wild horses and to the BLM employees and contractors conducting the gather and/or handling the horses as well as to the public themselves. Because these horses are wild animals, there is always the potential for injury when humans get too close or inadvertently get in the way of gather activities.

The helicopter work is done at various heights above the ground, from as little as 10-15 feet (when herding the animals the last short distance to the capture site) to several hundred feet (when doing a reconnaissance of the area). While helicopters are highly maneuverable and the pilots are very skilled in their operation, unknown and unexpected obstacles in their path can impact their ability to react in time to avoid members of the public in their path. These same unknown and unexpected obstacles can impact wild horses being herded by the helicopter in that they may not be able to react and can be potentially harmed or caused to flee which can lead to injury and additional stress. When the helicopter is working close to the ground, the rotor wash of the helicopter is a safety concern by potentially causing loose vegetation, dirt, and other objects to fly through the air which can strike or land on anyone in close proximity as well as cause decreased vision. Though rare, helicopter crashes and hard landings can and have occurred (approximately 10) over the last 30+ years while conducting wild horse and burro gathers which necessitates the need to follow gather operations and visitor protocols at every wild horse and burro gather to assure safety of all people and animals involved. Flying debris caused by a helicopter incident poses a safety concern to BLM and contractor staff, visitors, and the wild horses.

During the herding process, wild horses would try to flee if they perceive that something or someone suddenly blocks or crosses their path. Fleeing horses can go through wire fences, traverse unstable terrain, and go through areas that they normally don‘t travel in order to get away, all of which can lead them to injure people by striking or trampling them if they are in the animal‘s path.

Disturbances in and around the capture sites and sorting/holding have the potential to injure the government and contractor staff who are trying to sort, move and care for wild horses by causing them to be kicked, struck, and possibly trampled by the animals trying to flee. Such disturbances also have the potential for similar harm to the public themselves.

The BLM is committed to allowing access by interested members of the public to the fullest possible degree without compromising safety or the success of operations. To minimize risks to the public from helicopter operations, the gather Contractor is required to conduct all helicopter operations in a safe manner, and to comply with FAA regulations (FAR) 91.119 (Appendix G) and BLM IM No. 2010-164 (Appendix D). Public observations sites would also be established in locations that reduce safety risks to the public (e.g., from helicopter-related debris or from the rare helicopter crash landing, or from the potential path of gathered horses), to wild horses (e.g., by ensuring observers would not be in the line of vision of horses being moved to the capture site) and to contractors and BLM employees who must remain focused on the gather operations and the health and well-being of the wild horses. The Visitor Protocol and Ground Rules for public observation found in Appendix E provide the public with the opportunity to safely observe the gather operations. Every attempt would be made to identify observation site(s) at the gather location that offers good viewing opportunities, although there may be circumstances (flat terrain, limited vegetative cover, private lands, etc.) that require viewing locations to be at greater distances from the capture site to ensure safe gather operations.

At recent gathers, public observers have ranged in number from only a handful of individuals to a maximum of between 15-25 members of the public. At these numbers, BLM has determined

that the current level of public visitation to gather operations falls below the threshold of an open air assembly under the FAR regulations. 14 CFR § 91.119.

At current populations, wild horses are moving across Highways 93 and 75. This presents a safety hazard to both wild horses and motorists. A number of collisions have been reported on Highway 75.

3.14.2 Environmental Effects

3.14.2.1 Alternatives 1-4

All helicopter operations must be in compliance with FAR 91.119. Public safety as well as that of the BLM and contractor staff is always a concern during the gather operations and is addressed through the implementation of Visitor and Ground Rules that have been used in recent gathers to ensure that the public remains at a safe distance and does not impede gather operations. Appropriate BLM staffing (public affair specialists and law enforcement officers) would be present to assure compliance with visitation protocols at the site. These measures minimize the risks to the health and safety of the public, BLM staff and contractors, and to the wild horses themselves during the gather operations.

Gathering wild horses that occur near the highway corridors would reduce the risk of motorist/wild horse collisions.

3.14.2.2 Alternative 5

There would be no gather related safety concerns for BLM employees, contractors or the general public as no gather activities would occur.

3.15 SocioEconomics

3.15.1 Affected Environment

The CHMA is located in Central Idaho within Custer County, which is among the most rural communities in the United States; it comprises approximately 3,160,320 acres with a total population of 4,368 with 0.9 persons per square mile. The population of Custer County grew by 0.6 percent between 2000 and 2010. The median age is 47 years old with 53 percent males and 47 percent females. The dominant areas of employment in Custer County are management and professionals at 35 percent; sales and office occupations at 20 percent; construction, extraction, and maintenance at 17 percent; service 13 percent; production/transportation 10 percent; and farming, fishing and forestry 5 percent. The unemployment rate in Custer County is currently 10.2 percent. The median household income is $41,910.00. Nearly 14 percent of the population lives below the poverty line. 93 percent of the population has a high school education (United States Department of Commerce 2010).

3.15.2 Environmental Effects

3.15.2.1 Alternatives 1-4

Gather operations are so transient in nature, only being within Custer County for less than a month, BLM does not anticipate direct socio-economic impacts would be measurable. However,

indirect impacts may result from impacts to other economic features. Timing of gather operations during hunting seasons may adversely affect or displace hunters which bring direct revenue stream into the local economy (i.e. spending on lodging, fuel, food, supplies, licenses, and other indirect expenditures). These same opportunities for local revenue may be replaced by the contractors and additional BLM staff that would be residing in Challis during the gather.

The CHMA contains a number of County and BLM roads. Travelers in the area may experience a short increase in area traffic due to gather operations, and small delays in traffic to allow for fueling of helicopters, and other gather operations.

3.15.2.2 Alternative 5

While the effects described under Alternatives 1-4 above would not occur under the No Action Alternative other economic impacts may develop. If wild horses were not gathered for a period of ten years or more, this action or lack of action could necessitate livestock being reduced or removed from allotments within the CHMA. Without federal land grazing, it would be difficult for any of the 10 livestock operators to remain viable operations. Custer County livestock operators depend on public land grazing because 97% of the county is Federal or state lands. Therefore, private land for leasing is difficult to find. If they were able to stay in the livestock industry it would be at a greatly reduced level.

As rangeland health deteriorates wildlife species including upland and big game numbers would suffer, thus limiting tags, hunting opportunity and harvest. These reductions would affect the number of hunters that would purchase goods within the county. This also would directly affect the outfitters and guides within the area.

4.0 Cumulative Impact Analysis For All Alternatives

NEPA regulations define cumulative impacts as impacts on the environment that result from the incremental impact of the Proposed Action when added to other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions (40 CFR 1508.7). Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time. The time frame for analysis is from the passage of the Wild Free-Roaming Horses and Burros Act of 1971 to 2022 ten years past the proposed gather which is a reasonable time frame to consider potential future actions within this analysis.

The Cumulative Impacts Assessment Area (CIAA) for this project includes the CHMA; Bradbury Flat, Bradshaw Basin, Mountain Springs, Road Creek, Split Hoof, Willow Creek, and Warm Springs livestock grazing allotments in their entirety; and portions of the Sage Creek and Herd Creek allotments; Malm Gulch/Germer Basin, Lone Bird, Antelope Flat, East Fork Salmon River Bench and Sand Hollow ACEC's; and all of Jerry Peak West and Corral/Horse Basin WSA's; and sage grouse habitat connected to the CHMA (see Map 7). Landscape characteristics within the CIAA are consistent with those described in the Affected Environment for the CHMA.

The CIAA intersects four watersheds, East Fork Salmon River, mainstem Salmon River, Big Lost River, and Warm Springs Creek. Warm Spring Creek is fully allocated for irrigation and is influenced by geothermal springs and does not contain a native fish assemblage and was likely

historically inaccessible to listed salmonids in the Salmon River, and is not designated critical habitat. The tributaries to Warm Spring within the CIAA are disconnected from Warm Spring Creek and only convey water during intense precipitation and or high snow melt events. Warm Springs Creek is anthropogenically altered by a combination of existing roads, extensive land conversion for agriculture, and diversion arrays and channel networks utilized primarily in the Round Valley of Challis, ID. Consequently these streams have little potential to convey water or sediment to the Salmon River, or result in contributions discernible from background conditions.

A portion of the Big Lost Watershed is located in the southeastern part of the CIAA and consists primarily of the Sage Creek drainage. Sage Creek does not flow perennial along its entire reach, it typically sinks near the canyon mouth where hydric species terminate approximately 4.5 miles upstream of the ephemeral confluence into Chilly Slough. The Sage Creek drainage tributaries consist of North Fork Sage Creek, Bradshaw Creek, Corral Creek, and the South Fork of Sage Creek. The Sage Creek subwatershed does not contain a fish population (native or introduced) and was likely historically inaccessible by native fish assemblages in the Big Lost River. The Big Lost River watershed does not contain ESA listed salmon or trout, but does contain the Big Lost River whitefish, a candidate species for listing under ESA. Their distribution is limited to mainstem reaches and perennial tributaries in the upper watershed outside of the CIAA.

Road Creek drainage is described under the Affected Environment for Wetlands/Riparian Zones, Water Quality, and Threatened, Endangered, and Sensitive Fish; Fisheries because it is entirely contained within the CHMA.

4.1 Past and Present Actions

According to the 1994 BLM Guidelines for Assessing and Documenting Cumulative Impacts, the cumulative analysis should be focused on those issues and resource values identified during scoping that are of major importance. Accordingly, the issues of major importance identified are Livestock Grazing and Mining/Infrastructure Development.

Past, present, and reasonably foreseeable actions that have impacted the watershed to varying degrees include livestock grazing and mining/infrastructure development. Although these actions probably do not account for all of the impacts that have or are likely to occur in the watershed, GIS analysis, agency records, and professional judgment suggest that they have contributed to the vast majority of cumulative impacts that have occurred in the CIAA.

Table 21. Past, Present, and Reasonably Foreseeable Future Actions within the Pahsimeroi River Watershed.		
Type of Activity	**Past and Present**	**Reasonably foreseeable**
Livestock Grazing		
Number of Permits/Leases	BLM-15 active permits USFS-2 active permits State-8 leases	BLM- renewing/modifying grazing permits as they expire; USFS – no projected changes to grazing permits. State – renewing/modifying leases as they expire.
Animal unit Months	BLM-15,794 State- 943	Adjustments in AUMs may be proposed based on allotment- specific conditions, though no adjustments are proposed at this time.
Range Improvements	Fences-157 miles Pipelines-43 miles Developed Springs-67 Troughs-170 Stock ponds-35	Additional fencing, pipelines, and troughs may be constructed based on allotment-specific needs. At this time, 3 spring exclosures are planned in Horse Basin Pasture on the Mountain Springs Allotment.
Mining/ Infrastructural Developments		
Powerlines	64 miles	0 miles
Power Poles	806 poles	0 poles
Motorized Routes	BLM—506 miles USFS—19 miles	0 miles
Non-motorized Routes (Closed Motorized Routes)	11 mile	0 miles
Surface Mining	145 acres	0 acres
Sand and Gravel	124 acres	0 acres
Irrigation Diversions	7	0

*Data derived from Challis Road Layer dated 2011.

**Forest Service roads include open and seasonally closed natural and gravel surfaced roads and primitive trails. Data derived from Forest Service Roads layer dated 2008.

Livestock Grazing

Livestock grazing in the CIAA began in the 1870's when miners came into the area looking for gold in the nearby mountains, specifically Yankee Fork and Bayhorse. Livestock operators soon followed. Open range ranchers began moving into the valley in 1872 with cattle, horses, and sheep. It soon became apparent that winter forage was a limiting factor to ranching in the area. At that time, the Federal Government was encouraging settlement through the Homestead Act which forced open range ranchers to build fences and produce hay. As a result of the location of the CIAA relative to active mining and homesteading private lands in the CIAA were not developed until the early 1900's with the first patent of land being granted in 1912 and the majority of the private lands being patented by 1930.

In 1934, the Taylor Grazing Act was passed and the first grazing permits were issued. Livestock grazing was consistently reduced from 1948 to 1975 (USDI-BLM 1977). AUMs were further reduced through implementation of the 1977 Challis Grazing EIS.

There are currently 15 livestock grazing operators on 10 allotments (see Map 8) that are permitted to graze 15,794 AUMs on BLM-administered lands within the CIAA. In support of these operations, 43 miles of pipeline, 157 miles of fence, 170 troughs, and 35 stock ponds have been constructed.

Each of the allotments found in the CIAA have similar histories and have been continuously grazed since the 1870's. An example of this history is found in the Warm Springs Allotment. Warm Springs Allotment has been grazed since the inception of the US Grazing Service in 1936. The first permit allowed 9,800 sheep, 175 cattle and 20 horses. In 1957, a range adjudication reduced livestock grazing privileges by 20%. In 1960 a partial conversion was made from sheep to cattle at a ratio of 5 sheep to 1 cow. In 1963, following a range survey, grazing privileges were established at 1265 cattle for 7453 AUMs (Harbour 1975).

A number of vegetation manipulation projects have occurred within the CIAA in the past. These have occurred as either seedings, brush removals, prescribed fire and broadleaf spray directed at removing or reducing sagebrush canopy. These treatments occurred as follows: Warm Springs Allotment at 10,695 acres, Mountain Springs Allotment at 8,062 acres, Split Hoof Allotment at 980 acres and Bradbury Flat Allotment at 300 acres for a total treated acres of 20,037 acres.

Past livestock grazing has influenced the composition of vegetation due to dietary preference and selectivity of forage by livestock. Within the CIAA, 91% of the lands are available for livestock grazing managed by State and Federal agencies. Additional grazing occurs on private lands within the CIAA, but is not quantifiable. Livestock grazing impacts plants by removing the leaf area available for photosynthesis, removing of growing points, and reducing the ability of the plant to maintain a favorable shoot to root balance. This ultimately impacts the vegetation composition and the ability to meet long-term resource objectives for plant communities. Further, livestock grazing can mechanically impact vegetation through trampling. This is especially true in areas of livestock congregation (e.g. salt licks, water sources, and trails between foraging and watering areas) that potentially would be devoid of vegetation. Impacts to vegetation would also occur as a result of construction of range improvements. These impacts

include: removal of vegetation and/or crushing or trampling vegetation, as well as altering of streambanks and wetland areas. In response to patch grazing, spring and riparian corridor exclosures have been installed. However exclosures have only been shown to reduce cattle grazing along streams and upland springs not wild horse use.

Mining and Infrastructure Development

Infrastructural development in the CIAA has increased over time, although the majority remains undeveloped (see Table 22). Most of the development has occurred along the Herd, Road and Warm Spring Creek's bottom where private lands have been modified to accommodate farming and ranching activities; irrigation diversions occur along each of these creeks for the purpose of irrigating the private lands. The prominent land use within these private lands is irrigated pasture. The majority of open water which occurs in the CIAA is located on private lands in ponds used primarily for irrigation storage.

Table 22. Land Use/ Land Cover within the CIAA

Land Use/ Land Cover Type	Acres	Percent of CIAA
Open Water	30	0.01
Developed Open Space	524	0.21
Developed Low Intensity	992	0.40
Developed Medium Intensity	518	0.21
Barren Land (bedrock/sand/clay)	2218	0.89
Evergreen Forest	14293	5.73
Shrub/Scrub	173653	69.57
Grassland/Herbaceous	56071	22.46
Pasture/Hay	1150	0.46
Cultivated Crops	132	0.05
Woody Plants (hydric)	20	0.01
Herbaceous Plants (hydric)	13	0.01
*All data derived from USGS(2006) using a GIS		

A road network has been developed in the CIAA which accommodates a wide variety of vehicular traffic. To date, approximately 446 miles of primitive road (4WD) and ATV trails, less than 1 mile of naturally surfaced road, 30 miles of gravel-surfaced roads, and 30 miles of paved roads including US 93 crisscross the CIAA. As a result of a recent Travel Management Plan decision, 11 miles of BLM-administered roads/trails have been closed to motorized vehicles. The majority of these roads were developed to accommodate ranching and recreation.

Infrastructural development has resulted in the removal of acreage from existing riparian habitat. Within the CIAA, approximately 31% of perennial streams miles contain a road within their riparian corridor, resulting in the removal of as much as approximately 28 acres or ≈1% of the RHCA present in the CIAA. Road crossings in the form of culverts can result in contributions of sediment, but more importantly can influence juvenile and adult fish passage, often due to

undersized diameter and/ or slope which results in excessive velocity and perched outlets which create vertical impediments to fish passage. Road crossings of fish bearing streams are limited to tributary habitats; bridges associated with the Salmon and East Fork Salmon rivers do not limit fish passage. Due to the distribution of fish bearing stream and designated critical habitat, culverts which impede fish passage are limited to the Road Creek watershed. However, culvert replacements by the BLM have improved connectivity among habitats in upper Road Creek and its tributaries. Private lands and the East Fork Road near the mouth have culverts likely to impede fish passage under some conditions, and these are likely to foreseeably to persist.

There are irrigation diversions that likely influence stream flow seasonally in the lower reaches of Road Creek, which can limit fish habitat relative to volume and temperature. There are approximately 1.4 miles of Road Creek in which irrigation occurs within the RHCA for a total of 30 acres or ½ of 1% of the total RHCA available in the CIAA. This irrigation has a further effect on the downstream 0.4 miles of Road Creek in the CIAA by reducing the water available for hydric species. Due to the past construction of roads and their ongoing existence and maintenance there has been a cumulative removal of approximately 28 acres or ≈0.5% of RHCA. In lower Road Creek the RHCA along the ≈2 miles of Road Creek (≈3% of the RHCA relative to CIAA) have been affected by stream diversion, riparian conversion to pasture, and other private land development.

Power lines within the CIAA are located along the US 93 corridor through Warm Springs Valley, a power line bisects the CIAA near Spar Canyon connecting US 93 with the East Fork Salmon River Valley. A power line also runs up the lower portion of Road Creek delivering power to the private residences. In total there are 64 miles of power lines in the CIAA.

The largest mine with the CIAA is the Three Rivers Stone Quarry which has a total disturbed area of 124 acres. The Three River Stone Quarry has a National Pollution Discharge Elimination System permit from the U.S. Environmental Protection Agency authorizing the discharge of sediment. It was determined and concurred upon (January 2009) by USFWS and NMFS that the Three River Stone Quarry would result in only insignificant effects to habitat and T&E fish associated with the East Fork and mainstem Salmon rivers resultant from disturbance (e.g. blasting) or sediment conveyance from the quarry (USDI-BLM, 2008; USDI-BLM, 2009).

There are 21 additional acres associated with surface mining in the CIAA. These mine sites include a building stone prospect, zeolite prospect, precious gemstone prospect, and a gold prospect (there are no known occurrences of gold in the CIAA). There are 124 acres of common variety sand/gravel/rock sites within the CIAA. The majority of these sites are no longer active; there are 6 active sites for a total of 20 acres or 16% of the 124 acres of disturbance within the CIAA. There no active oil and or gas leases within the CIAA.

The great majority of the infrastructure and mines in the CIAA has been in place for decades. Relatively recent changes include the expansion of the Three Rivers Stone Quarry near the confluence of the East Fork into the main stem Salmon River. These alterations of the lands within the CIAA are generally widespread geographically and have not resulted in a noticeable progression toward a more urban orientation.

Reasonably Foreseeable Actions

Reasonably foreseeable future actions include continuation of past and present actions as described above. The level and character of livestock grazing and infrastructure are anticipated to remain consistent into the foreseeable future. Within the next 10 years the 15 livestock grazing permits are projected for renewal. Each of the livestock permittees has applied for their permits to be renewed. The permits would be renewed with similar Terms and Conditions or may be modified to ensure compliance with Fundamentals of Rangeland Health.

Future range improvement projects in the CIAA include 3 spring exclosures in the Mountain Springs Allotment in the Horse Basin Pasture (USDI –BLM 1999f). These spring exclosures would address a number of resource management issues including heavy use by wild horses and livestock. There are no mining and/or infrastructure developments in the reasonably foreseeable future in the CIAA.

4.1.1 Cumulative Impacts Associated with Past, Present and Reasonably Foreseeable Future Actions

Each of the past, present, and reasonably foreseeable future actions contribute a specific incremental environmental effect that can be described or accounted for with the same indicators as used in the alternative analysis presented earlier in the document. The proper indicator depends upon the analysis method used and the resource affected by the action. Indicators might be measured by the acres of soil disturbed, acres of big game habitat affected, miles of stream affected, percentage change in ground cover, number of springs developed, or whichever indicator of effect is best used to describe and account for the accumulated effect to the resource of concern. The accumulated effect of past, present, and reasonably foreseeable future actions on a given resource provides a baseline from which to evaluate the contribution of the alternatives to the collective impact on that resource. The purpose of this section of the document is to provide that baseline. The effects of the various alternatives on the baseline are presented in the subsequent section.

4.1.1.1 Wild Horses

The indicator for wild horses is horse health and safety. Under Alternative 1-4 wild horses would be maintained within AML, this in addition to the livestock grazing AUMs would continue to be within the carrying capacity and provide a thriving natural ecological balance. Alternative water sources (i.e. troughs, water holes) provide water for wild horses as well as for domestic livestock. Mining, sand and gravel operations, and the related actions have displaced wild horses from 269 acres within the CIAA. The 525 miles of road within the CIAA allow for motorized vehicle travel which may affect wild horse distribution and behavior. No additional roads would be created for the horse gather. Power lines /poles do not affect wild horse health and safety.

Under Alternative 5 wild horse numbers would exceed the AML. This in addition to the permitted livestock grazing AUMs would be above the carrying capacity for the public lands. Deterioration of uplands and riparian areas through an overpopulation of wild horses would not

improve habitat for future generations of wild horses or wildlife; cumulatively resulting in reduced AML or elimination of wild horses within this HMA.

No other past, present or reasonably foreseeable actions would offset the damage to the range caused by an ever increasing population of wild horses.

4.1.1.2 Vegetation Type, Communities, and Rangeland Resources

The indicator for vegetation is acres of concentrated disturbance. Areas associated with this are 170 troughs, 35 water holes, 157 miles of fence and 43 miles of pipelines within the CIAA. Assuming a disturbance diameter of 0.7 acres per water source (e.g., trough and stock ponds), an 8-foot disturbance width for fences, and an 18-inch disturbance width for pipelines, a total of approximately 304 acres or about 0.1% of surface vegetation within the CIAA has been altered from their natural state by livestock grazing and associated activities.

The duration and intensity of the effects of these disturbances are not equivalent. The 144 acres of disturbance associated with water sources tends to be relatively intense and long-term, while the 152 acres associated with trailing along fences is more likely to be locally intense, but discontinuous. This disturbance includes removal of vegetation along the fence, trampling of vegetation from livestock trailing along the fence, and from T-posts being pounded into the ground. The 8 acres of disturbance associated with pipeline installation occurred as single events with disturbances being relatively short-term (i.e., 2 to 3 years).

Mining, sand and gravel operation, and associated activities have resulted in 269 acres of concentrated disturbance of vegetative communities. There are 525 miles of road in the CIAA. Assuming an average road width of 20 feet there are approximately 1,300 acres of vegetation disturbance. The disturbance related to power lines/poles is related to the roads as described above and the individual poles. Disturbance of the individual poles would be negligible. Cumulatively, this has resulted in a total vegetative disturbance of 1,873 acres.

Under Alternatives 1-4 approximately 10 acres of new concentrated disturbance would occur to vegetative communities. Cumulatively this would result in a 0.5% increase in the number of disturbed acres within the CIAA.

Under Alternative 5 there would be no new concentrated disturbance associated with gather activities. However, no other past, present or reasonably foreseeable actions would offset the effects caused by an increasing population of wild horses. Complete removal of permitted livestock would not compensate for unregulated population growth within the CHMA. The population would eventually reach a level in which forage is inadequate to meet demand.

4.1.1.3 Livestock Management

The indicator for livestock grazing is number of permitted AUMs. Mining/infrastructure development has not affected permitted AUMs. Alternatives 1-4 would not affect permitted AUMs because the AML would be maintained providing a thriving natural ecological balance.

Under Alternative 5 livestock management would need to be modified such that permitted AUMs may be reduced or cancelled, because forage availability for livestock would be reduced as a result of increased wild horse populations.

4.1.1.4 Invasive/Non-native Species

The indicator for invasive/non-native species is acres of disturbance because activities that remove native vegetation and expose bare soil are likely to create niches where there is a potential for weed invasion. Generally, invasive species have been introduced to the CIAA as "hitchhikers" on vehicles or animals, as evidenced by the pattern of infestations. The acres of disturbance associated with these type of activities are disclosed under section 4.1.1.2 Vegetation Type, Communities, and Rangeland Resources.

There are 86 noxious weed locations mapped across ownerships in the CIAA for an approximate total of 75 acres (USDI-BLM 2009c). This total comprises approximately .03% of the total acreage within the CIAA. Cheatgrass infestations have not yet been mapped on BLM lands within the CIAA. General field observations indicate that infestations are occurring to some degree along roadways and on drier southwest facing aspects.

Alternatives 1-4 would result in a direct effect of approximately 10 or less acres of disturbance for noxious weeds to invade. Cumulatively this would result in a 0.5% increase in the number of disturbed acres for invasive species to establish.

Under Alternative 5 there would be no new concentrated disturbance associated with gather activities. Due to increased horse populations it is anticipated that invasive/non-native species occurrences would increase due to reduced vegetation and increased ground disturbance. This would result in an increased effort to manage invasive/non-native species.

4.1.1.5 Wetlands/Riparian Zones

The indicator for wetlands/riparian areas is acres of riparian affected. Livestock grazing has locally affected streams and upland springs. In response to these localized effects, grazing exclosures have been constructed. There are three proposed upland spring exclosures at sites where wild horse and livestock use have affected the springs. Mining/Infrastructural development has resulted in the removal of acreage from existing riparian habitat. Within the CIAA, approximately 31% of perennial streams miles contain a road within their riparian corridor, resulting in the removal of as much as approximately 28 acres or approximately ½ of 1% of the RHCA present in the CIAA. Installation of power poles within riparian corridors has removed approximately 0.004 acres or less than 1/10th of 1% of riparian areas present in the CIAA (Table 23).

Irrigation diversions dewater Road Creek and reduce water available for maintenance of riparian areas. The importance of the presence of water to sustained healthy riparian corridors cannot be understated. There are approximately 1.4 miles of Road Creek in which irrigation occurs within the RHCA for a total of 30 acres or ½ of 1% of the total RHCA available in the CIAA. This irrigation has a further effect the downstream 0.4 miles of Road Creek in the CIAA by reducing

the water available for hydric species. The irrigation of 30 acres typically requires a diversion rate of 0.6 cubic feet per second, any diversion in excess of this amount would be returned to Road Creek as ground water flow.

Table 23. Cumulative Effects on Riparian Areas and Vegetation

Resource	Total within CIAA	Total within the CHMA
Acres of RHCA(300-ft buffer of Perennial Streams)	5,914	3,487
305(b) Streams miles	306	251
303(d) Streams miles	214	95
Perennial Stream Miles	91.8	54.5
Road Miles Within RHCA (300-ft buffer of Perennial Streams)	28.8	14.4
Miles of Perennial Stream Affected by Mining	0	0
Miles of Power lines within the RHCA (300-ft buffer of Perennial Streams)	0.8	0.7
Number of Power Poles within the RHCA (300-ft buffer of Perennial Streams)	18	12
Number of Developed Springs (based upon USFS and BLM Range Improvement GIS Data)	27	19

1. Data were compiled from various sources using and at various scales (e.g. watershed scale, allotment scale) GIS. The values given are best estimates derived using this GIS data.
2. These data were used for the majority of the percentage calculations given

Cumulatively, past and present actions have resulted in the removal of approximately 28 acres or ≈0.5% of RHCA due to the construction of roads; approximately 145 acres (the RHCA along the 2 miles of Road Creek) or ≈3% of the RHCA have been impaired or affected as a result of stream dewatering and private land development; all of the acres of RHCA have been potentially affected by livestock and/or wild horses. All sites for which monitoring data is available are in late seral status or at potential natural community and have shown a static to improved trend from 1999-2012 (Table 14).

Under Alternatives 1- 4 no new acres of disturbance would occur within RHCAs therefore no cumulative effects are anticipated as a result of gather activities. Under Alternative 5, it is anticipated that use of riparian vegetation and alteration of wetland areas by wild horses would increase.

4.1.1.6 Water Quality

The indicator for water quality is macroinvertebrate assemblages. Aquatic macroinvertebrates can be used to assess water quality and the influence of organic pollutants. The assemblage of macroinvertebrate taxa are relatively sedentary and can be quantitatively sampled; some of the metrics used to describe the macroinvertebrate assemblage and contributions of different taxa include the number of pollutant intolerant species and number of clinger taxa (which can decrease as a result of sedimentation/siltation). Quantitative aquatic macroinvertebrate sampling conducted by the BLM between 1994 and 2008 indicates a static to upward trend in water quality for perennial streams across the CIAA (See Appendix H). These data, combined with relatively high levels of bank stability and predominately late-seral riparian vegetation (see Table 14), suggest that ongoing grazing actions managed with an array of passive and active methods and periodic horse gather operations to maintain AML have allowed for maintenance and/or recovery of elements of stream habitat important to water quality for fish bearing streams. Furthermore, macroinvertebrate data collected by IDEQ for streams across the CIAA indicate that macroinvertebrate communities are appropriate for the bioregions in which they were collected (See Appendix H). Cumulatively, at the CIAA scale, water quality has been affected by increased sedimentation and siltation due to grazing, horse use and road developments. Mines, sand and gravel operations, and power lines/poles within the CIAA do not occur proximal to RHCAs. Livestock and wild horse use have also resulted in fecal loadings to waters within the CIAA.

Under Alternatives 1-4, it is anticipated that macroinvertebrate assemblages would continue to show a static to upward trend in water quality. Under Alternative 5, macroinvertebrate assemblages (e.g. relative abundance of clinger and pollutant taxa) could reflect increased wild horse use in riparian areas.

4.1.1.7 Threatened / Endangered and Sensitive Fish; Fisheries

The indicator for Threatened/Endangered and Sensitive Fish; Fisheries is miles of ESA designated critical habitat for fish, which includes all fish bearing streams (see Table 24) affected. Under Alternatives 1- 4 no new acres of disturbance would occur within RHCAs therefore no cumulative effects are anticipated for the miles of ESA designated critical habitat as

a result of gather activities. Under Alternative 5, it is anticipated that the pattern of wild horse use of riparian vegetation and alteration of streambanks would expand.

Table 24. Threatened/Endangered and Sensitive Fish; Fisheries Resources and Cumulative Effects		
Resource	Total within CIAA	Total within the CHMA
Acres on State and Federal Lands Subject to Grazing	227,270	149,343
Perennial Stream Miles	91.8	54.5
Approximate Fish Bearing Stream Miles	43.5	32.5
Miles of Designated Critical Habitat for Chinook Salmon	44.8	37.3
Miles of Designated Critical Habitat for Steelhead	3.3	1.6
Miles of Designated Critical Habitat for Bull Trout	1.8	1.5
Road Miles Within RHCA (300-ft buffer of Perennial Streams)	28.8	14.4
Miles of Perennial Streams where grazing is authorized	89.2	52.3
Acres of Grazing Exclosures	138	138
Miles of Perennial Streams within Exclosures	1.5	1.5

4.1.1.8 Wilderness

For the purposes of analyzing cumulative impacts, the CIAA for wilderness values includes the entirety of the Jerry Peak and Corral-Horse Basin WSAs (92,650 acres, combined). Due to the nature of WSAs some of the actions which have cumulatively affected the rest of the CIAA have not, and would not, occur within the boundaries of these special designations (agricultural development, road construction, and land use conversions). Both WSAs were found during Intensive Wilderness Inventory in 1980 to have certain wilderness values (size, naturalness, outstanding opportunities for either solitude or primitive and unconfined recreation, and supplemental values).

There have been no past and present actions which have impacted the size of the either WSA. There has been some amount of primitive road development (motorized routes created by the passage of vehicles) since the time of inventory, however, these routes were inventoried and closed during the 2008 travel planning effort. There have been no road construction or maintenance efforts that have affected the boundaries, and subsequent size, of either WSA.

The Jerry Peak WSA contains 1.4 miles of buried pipeline, 17 miles of primitive routes, 27.3 miles of fence, and 7 troughs. Corral-Horse Basin WSA contains 2.4 miles of buried pipeline, 34 miles of primitive routes, 25.8 miles of fence, and 9 troughs. All of these developments affect the naturalness of the WSAs to some degree, though the pipelines effectively disappear after one or two growing seasons. The 51 miles of primitive routes are all single vehicle width and have no impacts associated with construction, resulting in an average disturbance width of 8' and 49 acres of disturbance across both WSAs. The 53 miles of fence result in 51 acres of disturbance (assuming a worst-case scenario of 8' of initial vegetative disturbance), and the 16 troughs impact 10.5 acres. Therefore, the cumulative physical impacts to naturalness associated with the existing developments are approximately 110 acres, or .1% of the combined acreage of the WSAs. There would be no new facilities associated with the proposed action or alternatives. There are eight potential capture sites which might be utilized 'within the boundaries' of the WSAs. Of these eight sites, one is located on private land, one on State land, and four are on Road Creek Road. Approximately one acre of vegetation could be trampled during gather operations at each of the 6 of the capture sites on or directly adjacent to the WSAs, resulting in an additional six acres of temporary physical disturbance (.006% of the WSA acreage).

Outstanding opportunities for solitude could be reduced during helicopter operations, though they would not cumulatively affect the long term availability of solitude (or primitive recreation) within the impact area. Finally, managing the wild horse herd for the appropriate management level would maintain the supplemental values identified during inventory at a healthy and sustainable level.

4.1.1.9 Recreational Use, Existing and Potential

In the CIAA, recreational use has been affected, and would continue to be affected, by certain elements of grazing management and mining/infrastructure development. The common denominators, when considering the cumulative impact of these broad activities to recreational

use, are the elements/indicators which have the potential to affect the naturalness (or scenic) quality of the landscape or the ability of visitors to freely traverse the landscape.

Past and present grazing related actions which have cumulatively combined to affect the natural across quality of the CIAA include the construction of 157 miles of fencing, 43 miles of pipeline, 170 water troughs, and 35 stockponds. The impact of pipelines is nearly non-existent because once the initial, short term disturbance recovers; their evidence on the landscape becomes virtually unnoticeable to the casual observer. The presence of 170 troughs within the CIAA equates to one trough for every 1,437 acres of publically accessible land within the CIAA. With an effective impact of approximately 0.7 acres/trough, the impact to the naturalness of the area becomes very limited (approximately 119 acres). Waterholes have a similar impact area of 0.7 acres/waterhole, resulting in approximately 25 acres of disturbance across the CIAA. The presence of fencing affects both the naturalness of the area, as well as the ability for a recreationist to move freely throughout the ERMA. With 244,349 acres of publicly accessible land within the CIAA (381 square miles), there currently exists approximately one mile of fence for every 2.5 square miles. At an average disturbance width for fence construction of 8 feet, this would directly affect approximately 152 acres. While additional fencing, troughs, and pipelines may be constructed in the future based on specific needs, the only additional grazing developments in the foreseeable future are the 3 potential spring exclosures in Mountain Springs Allotment. By nature, small exclosure fences result in minimal impacts to freedom of movement for recreationists and impact the naturalness of an area at a localized scale.

Motorized routes (trails, roads, and primitive roads) also affect both the naturalness of the area and the ability to traverse the landscape. Dependent on the activity, and more so on the perception of the user, motorized routes can add or detract from the recreational experience. However, regardless of the experience, 525 miles of designated motorized routes within the CIAA directly affects the naturalness of the area by the disturbance of approximately 1,536 acres of vegetation (assuming an average width of disturbance of 25 feet), or 0.6% of the CIAA. The BLM has recently completed a travel management plan which affects the CIAA and there are no known plans for designating new motorized routes in the foreseeable future. There are 11 miles of existing routes on the BLM within the CIAA which have been closed to motorized use and would be expected to return to a natural condition as use diminishes over time.

The remaining key elements within the CIAA that affect the naturalness of the area are land use conversions (irrigated private lands and mineral extraction developments) and infrastructure related to rural development (such as powerlines and homes). These actions tend to be concentrated in the valley bottom, within or close to, private lands and county roads. In total, these activities have reduced the quality of naturalness on approximately 2,282 acres or less than 1% (this is the acreage for Developed Open Space, Developed Low Intensity, Developed Mod Intensity, and the 248 acres of surface mining identified in the CIAA description) of the assessment area by removing natural vegetation, disturbing soils, and facilities construction. There are no known plans for additional land use conversions or infrastructure development in the foreseeable future.

The impacts associated with the proposed action and alternatives considered in this analysis would not result in any measurable cumulative impacts to recreational experiences or

opportunities. There would be no addition or subtractions to the road and fencing systems which impact recreational mobility, nor would there be any impacts to the naturalness of the area beyond short term impacts to vegetation at discrete capture sites (less than 20 combined acres across the CIAA, or .008%).

4.1.1.10 Soils

Soils within the CIAA have been impacted by disturbances. The majority of grazing impacts (livestock, and wild horse) occur around existing water sources such as springs, troughs, stock ponds, areas providing cover or shade and along fence lines where livestock and horses tend to trail. The soils within and closely surrounding these areas receive heightened use and may exhibit signs of soil compaction, erosion, and reduced productivity. These areas of decreased vegetation and litter cover are generally more susceptible to soil erosion, increased runoff, and infestation by invasive, non-native plant species. Pipelines, not typically areas of intensive use, also disturb vegetation and soils as a consequence of their installation for the short term, but recover within 2 to 3 years. The indicator for soils is acres of concentrated surface disturbance. The acres of concentrated disturbance are described under 4.1.1.2 Vegetation Type, Communities, and Rangeland Resources.

Under Alternatives 1-4 approximately 10 acres of new concentrated disturbance would occur to soils. Cumulatively this would result in a 0.5% increase in the number of disturbed acres within the CIAA.

Under Alternative 5, there would be no new concentrated disturbance associated with gather activities. However, no other past, present or reasonably foreseeable actions would offset the effects caused by an increasing population of wild horses. Complete removal of permitted livestock would not compensate for unregulated population growth within the CHMA. The population would eventually reach a level in which forage is inadequate to meet demand. The decrease in vegetation cover would result in increased erosion.

4.1.1.11 Threatened/Endangered/Sensitive Animals/Migratory Birds/Wildlife

Cumulative effects on wildlife are those impacts that alter or remove a measurable portion of natural processes that individual species have evolved to need during some portion of their life. BLM recognizes that individuals or portions of populations could move in and out of the representative CIAA. The CIAA used here is delineated to encompass the majority of the population of each specific species that use the habitat influenced by the actions proposed in this document in conjunction with the cumulative impacts occurring to the habitat within the CIAA boundary. The CIAA also fits within the scope of BLM CFO's purview and capabilities of accurate analysis for the time, intensity, and scope of cumulative impacts. The CIAA is also delineated as to accurately represent cumulative impacts to each species and not be overly large as to dilute potential impacts. Analysis under this section of cumulative effects would focus on habitat health within the CIAA as described in this document and how the health of the CIAA impacts different species that uses it.

Greater Sage-Grouse

The indicators for impacts to Sage-grouse are the removal of habitat and direct disturbance or removal of individuals by the above described activities. Cumulative effects analysis in this section would focus on sagebrush steppe health of the CIAA. However, sage-grouse population trend is indicative of the stochasticity (unpredictable spatiotemporal fluctuations in the environment) of the sagebrush ecosystem and population trend would be discussed in an effort to determine sagebrush steppe health. Interim Management (IM-2012-043) designated 27% (68,292 acres) of the CIAA as Preliminary General Habitat (PGH) and 69% (173,865 acres) as Preliminary Priority Habitat (PPH). PPH covers the majority of the CIAA with PGH only being present around the periphery of unsuitable sage-grouse habitat. Sage-grouse movements are not well understood, but site fidelity has been documented by sage-grouse. Males have been documented showing site fidelity to lekking sites and females showing fidelity for nesting areas (Connelly 2004). The gather is scheduled to take place outside of the lekking and nesting period. The scheduled gather period takes place during the sage-grouse summer period (7/1 – 11/15). Mapped CSGLWG summer seasonal habitat occurs on the southern half of the CIAA and is generally associated with higher elevations of the CIAA. According to Garton (2011) the Big Lost sage-grouse subpopulation extents into the south east half of the CIAA.

Summer seasonal habitat for the CHMA within the alternatives equates to 64% (37,822 acres) of that mapped by CSGLWG (59,180 acres) within the CIAA.

Greater sage-grouse within the CIAA have been impacted in varying degrees by all of the past, present, and reasonable foreseeable future actions. Impacts from ungulate grazing occur in the form of altering the sagebrush and herbaceous cover needed for sage-grouse habitat, and nest trampling. Grazing of wild ungulates occurred throughout the region at various levels prior to European settlements. Livestock grazing prior to the passage of the Taylor Grazing Act and pre-European settlement native ungulate grazing intensities are not known. After passage of the Taylor Grazing Act in 1934 livestock have been present throughout the CIAA in different forms, seasons, and rates. Impacts associated with livestock developments include fences, troughs, waterholes, and pipelines. The total acreage impacted by these developments is 304 acres or approximately 0.1% of the CIAA. These developments have different levels of impacts on sage-grouse. Impacts associated with waterholes and pipelines are generally limited to construction. Troughs pose a potential for entrapment if bird ramps are not maintained and fences have the potential for impacts to low flying individuals. There are 170 trough locations within the CIAA. Using the 0.7 acres of disturbance of sage-grouse habitat per water trough equates to a loss of approximately 119 acres or 0.04% of the CIAA. There are 157 miles of fence on State and Federal lands within the CIAA. The density of fencing within the CIAA is 0.4 miles of fence per square mile. This density of fencing is below the 1.6 miles of fence per square mile that poses a higher risk for sage-grouse (USDI-BLM 2011).

Infrastructure development can have different forms and different direct and indirect impacts on sage-grouse that occur within the CIAA. There is 64 miles of powerlines within the CIAA. Power poles that have the potential to be used by raptors as a perch but do not have perch deterrents installed and exist within the sage grouse habitat would continue to provide raptors an opportunity to be used as such. There are 506 miles of roads within the CIAA. Existing routes

have the potential to aid the spread of invasive weeds. Possible impacts from roads within sage-grouse habitat include the spread of invasive weeds, habitat fragmentation, and direct disturbance or mortality depending on the amount occurring and the frequency used. There is 145 acres of surface mining occurring in the CIAA. Open pit mining activities remove habitat from use by sage-grouse for typically long periods of time.

Pygmy Rabbit

The indicators for impacts to pygmy rabbits are the removal of habitat and direct disturbance or removal of individuals by the above described activities. Power poles can provide perches for raptors. There are 64 miles of powerlines within the CIAA. Power poles that are present around pygmy rabbit activity and do not have perch deterrents installed would continue to provide a perching opportunity for raptors. Roads can promote invasive weeds and fragment habitat. Mining activities can remove habitat for pygmy rabbits during active mining.

Migratory Birds

The indicators for impacts to migratory birds are the removal of habitat and direct disturbance or removal of individuals by the above described activities. Migratory birds have the potential to exist throughout the CIAA on all habitat types. The majority of ungulate use takes place in shrub steppe habitat. Ungulate grazing has the potential to be present throughout the CIAA during the entire breeding/nesting season. Habitat conversions are not detrimental on a species level if other habitat is available. Open pit mining activities of all scales typically remove vegetation during normal operations. Vegetation removal and disturbances during breeding/nesting season have the potential to cause breeding/nesting failure. The extent to which this has happened in the CIAA is not known. Infrastructure development also has the ability to impact birds positively and negatively. Vehicle collisions increase as roads develop and speeds increase. Powerlines can provide perches for birds and act as a surrogate to natural structures. Some powerline designs can also cause electrocutions when birds come in contact with them.

Gray Wolf

The indicators for gray wolves are direct disturbance or removal and the altering of prey abundance and composition. Factors influencing how many wolves might be removed because of depredation are numerous. Wolves that have been removed because of past depredation have not inhibited wolf population growth in Idaho since their reintroduction in 1995. Wolves are highly mobile and use habitat on a landscape scale. The remaining elements as described in the past and present actions are at a scale that has not shown impacts to wolves as a species. Wolves have continued to expand their range and population since their reintroduction.

Other Big Game

The indicators for big game are direct competition for resources, removal of vegetation, and direct disturbance. Horse and livestock grazing takes place throughout the majority of the CIAA. Infrastructure development can remove big game habitat, cause mortalities, and influence behavior. There are 506 miles of designated routes within the CIAA. There is 145 acres of

surface mining in the CIAA. Infrastructure development can remove or fragment available big game habitat. Vegetation treatments are generally favorable to big game. There have been 20,037 acres of vegetation treatments in the past in the CIAA. Vegetation treatments act as a controlled surrogate to wildfire and reset successional changes to provide a mosaic of vegetation availability that can benefit big game.

Cumulative Impacts Common to All Species

Under Alternatives 1-4 approximately 10 acres of new concentrated disturbances would occur to vegetative communities. Cumulatively this would result in a 0.5% increase in the number of disturbed acres. The proposed actions within this alternative are isolated and limited to this one action. The proposed gather actions (herding, trapping, and transportation) would have a negative impact to individual wildlife species present during activities. The actions would be limited both spatially and temporally. A reduction in the horse herd would lesson forage removal/consumption and the potential for disturbances by horses. These actions, when added to other past, present, and reasonably foreseeable actions, would not lead to the listing of any of the species described in this document and present within the CIAA.

Under Alternative 5 horse herd number would increase and lead to greater consumption of forage and a greater potential for disturbances to wildlife. The proposed actions in this alternative would lead to the horse herd numbers increasing. There are no gather activities associated with this alternative. Horse herd number would continue to increase. The AML would continue to be exceeded and forage not allocated to horses would be removed. This would lead to an increase in completion for forage and potential for disturbance of wildlife, as well as, a decrease in cover for certain wildlife. However, these actions, when added to other past, present, and reasonably foreseeable actions, would not lead to the listing of any of the species described in this document and present within the CIAA within the proposed time period in this alternative of ten years.

4.1.1.12 Tribal Treaty Rights

Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time. Within the Challis Horse Management Area (CHMA), there are 154,150 acres of BLM lands, 9,454 acres of State of Idaho lands, and 1,116 acres of privately owned lands. Access to treaty reserved resources within the Shoshone-Bannock Tribes' aboriginal territory may no longer be possible on privately owned lands within the CHMA because of land status and/or access restrictions.

The federal government has a federal trust responsibility to manage public lands to provide for the continued exercise of tribal treaty rights, consistent with management policies, on all unoccupied lands within their jurisdiction. The proposed action and alternatives analyze potential management actions designed to address appropriate and effective wild horse management on lands within the CHMA. Provisions are also made to protect the safety and health of the horses, BLM employees and contractors during actual gather activities, and may include short-term area closures within the CHMA.

Any short-term temporary areal closures deemed necessary would not alter federal land status, but would necessarily limit access to and utilization of treaty-reserved resources on unoccupied

federal lands during actual wild horse gather activities. Impacts to access of specific areas within the CHMA would last between 4-8 days over an approximate two-week period in October.

Detailed discussion of potential cumulative impacts to resources of special interest to the Shoshone-Bannock Tribes such as water quality, fisheries, vegetation, ad wildlife are analyzed and discussed in detail within those specific sections (e.g., Fisheries, Vegetation) located elsewhere within this document.

4.1.1.13 Human Health and Safety

Members of the public can inadvertently wander into areas that put them in the path of wild horses that are being herded or handled during the gather operations, creating the potential for injury to wild horses and to the BLM employees and contractors conducting the gather and/or handling the horses as well as to the public themselves. Because these horses are wild animals, there is always the potential for injury when humans get too close or inadvertently get in the way of gather activities.

The helicopter work is done at various heights above the ground, from as little as 10-15 feet (when herding the animals the last short distance to the capture site) to several hundred feet (when doing a reconnaissance of the area). While helicopters are highly maneuverable and the pilots are very skilled in their operation, unknown and unexpected obstacles in their path can impact their ability to react in time to avoid members of the public in their path. These same unknown and unexpected obstacles can impact wild horses being herded by the helicopter in that they may not be able to react and can be potentially harmed or caused to flee which can lead to injury and additional stress. When the helicopter is working close to the ground, the rotor wash of the helicopter is a safety concern by potentially causing loose vegetation, dirt, and other objects to fly through the air which can strike or land on anyone in close proximity as well as cause decreased vision. Though rare, helicopter crashes and hard landings can and have occurred (approximately 10) over the last 30+ years while conducting wild horse and burro gathers which necessitates the need to follow gather operations and visitor protocols at every wild horse and burro gather to assure safety of all people and animals involved. Flying debris caused by a helicopter incident poses a safety concern to BLM and contractor staff, visitors, and the wild horses.

4.1.1.14 Socioeconomics

The CIAA is located in Custer County, a rural area with an estimated population of 4,368 (U.S. Department of Commerce 2010). Over the last ten years, the county's population has remained more or less static, increasing by 0.6%. The primary population centers within the county are Challis (pop. 1,081), Mackay (pop.517), and Stanley (pop. 63). Population density within the county is low at an estimated 0.88 persons per square mile, in part because 97% of the county is in federal or state ownership.

Median household income as of 2010 was $41,773.00, slightly below the state median of $44, 640.00.

Traditionally, the largest employment sectors in the county have been mining, government and agriculture, though employment patterns have shifted markedly through time. According to the U.S. Department of Commerce (2011), employment in the mining sector shrunk from approximately 38% of total employment in 1998 to 10% in 2003. Since 2003, the mining sector has seen more or less steady growth, reaching approximately 37% of total employment by 2009.

Employment in government fluctuated to a lesser degree. In 1998, the percentage of total jobs in federal, county, or local government was approximately 16%; it grew markedly to 24% by 1993 and then shrank to its 2009 level of 17.4%.

In contrast to employment in the mining and government sectors, employment in the services sector has seen steady growth within the county and as of 2009, represented 65.2 % of total employment (U.S. Department Commerce 2011). Within the services sector, relatively low paying jobs in accommodation, food service and retail trade now rival higher paying jobs in mining as the dominant source of income in the county.

The trend in agricultural employment, on the other hand, has been one of slow, but steady decline. Since 1970, farm employment shrank by approximately 21% and, as of 2009, comprised 10.5 % of total employment in the county (U.S Department of Commerce 2011, 2001).

One reason for the slow and steady decline in the agricultural sector is suggested by farm operator earnings. As indicated in Table 25, net average cash income from farms has been well below the median income for the county as a whole (when adjusted for inflation). A substantial number of farms have operated with a net loss. It is clear that one means to keep the farm operating under these circumstances is to supplement their income by working off the farm. As indicated in Table 25, more than half of the operators in the county worked off the farm for at least some portion of the year during the 1992-2007 period, many for more than 200 days a year.

Table 25. Farm operators net average cash income and days worked off the farm, Custer County, 1992-2007.

	Net average cash income from farms (dollars adjusted for inflation, 2011)	Operators with net losses (percentage of total)	Operators working off farm for any number of days (percentage of total)	Operators working off farm more than 200 days in a year
1992	13,819	169 (63.3)	144 (53.9)	84 (31.5)
1997	13,292	158 (59.0)	158 (59.0)	95 (35.4)
2002	(4,407)	212 (74.4)	164 (57.5)	111(38.9)
2007	27,676	74 (28.4)	182 (69.7)	76 (29.1)

United States Department of Agriculture (1992b, 1997a, 1997b, 2002, 2007c)

Several researchers have noted that profit is not the primary motivating factor in the choice to remain on the farm. In their study of ranching in Colorado, Bartlett et al. (1989) concluded that rate of return was of little or no importance in the rancher's decision to stay in the cattle business with most respondents citing the ranching way of life as their principal motivating factor. Similarly, Rowe et al. (2001) indicate that tradition, place attachment, and family were the primary motivating factors to stay in agriculture. Similar motivations have also been discussed by Liffman et al. (2000) and Bartlett et al. (2002). When ranchers do choose to sell, typical motivations cited are a lack of heirs, impending retirement, and detrimental public policy with lack of profitability being one of the least cited reasons. These factors may explain the rather slow pace of decline in the agricultural sector within the county.

The steep level of growth in the retail trade and accommodation/food service sectors and the gradual decline of the more traditional agricultural sector are accompanied by potential social consequences. In their study of Owyhee County, Idaho, Harp and Rimby (2004) found that communities characterized by ranching displayed higher levels of cohesion, integration, and attachment than those that did not, and argue that declines in ranching would result in similar reductions in these societal characteristics. Winkler et al. (2007) describe a similar decline in extractive-oriented economic structures in communities throughout the Intermountain West and a new orientation towards natural and cultural amenities. The result has been an in-migration of "outsiders", the presence of seasonal populations, increasing tourism and higher housing values; a phenomenon characteristic of the "New West."

While declines in societal cohesion and integration and the transformations characteristic of the "New West" phenomenon are not readily apparent in the communities within Custer County, these types of changes may become more apparent in the future given current trends in economic indicators.

5.0 Monitoring and Mitigation Measures

The BLM Contracting Officer's Representative (COR) and Project Inspectors (PIs) assigned to the gather would be responsible for ensuring contract personnel abide by the contract specifications and the SOPs (Appendix B). Ongoing monitoring of forage condition and utilization, water availability, aerial population surveys, rangeland resources health, and animal health would continue.

Fertility control monitoring would be conducted in accordance with the SOPs (Appendix B). Monitoring the herd's social behavior would be incorporated into routine monitoring. The objective of this additional monitoring would be to determine if additional stallions form bachelor bands or are more aggressive with breeding bands for the forage and water present.

If genetic monitoring indicates a loss of genetic diversity, then mares would be introduced into the CHMA from another HMA with similar characteristics.

6.0 List of Preparers

The following list identifies the interdisciplinary team member's area of responsibility:
Kevin Lloyd, Wild Horse and Burro Specialist/Rangeland Management Specialist
Peggy Redick, Supervisory Resource Management Specialist

Kyle Jackson, Rangeland Management Specialist
Bart Zwetzig, Wildlife Biologist
Ryan Beatty, Fisheries Biologist
Carol Hearne, Archeologist/Supervisory Resource Management Specialist
Nate Arave, Hydrologist
Leigh Redick, Invasive Species
Jeff Christenson, Outdoor Recreation Planner

7.0 Government to Government Consultation

Government to government consultation with the Shoshone-Bannock Tribes was initiated. The Tribal Business Committee was notified concerning the gather through the scoping letter BLM staff and Tribal technical staff meetings occurred on 4/26/2012. To date, no concerns about short- or long-term impacts to tribal treaty rights or access have been expressed by the Tribes.

7.1 Public Consultation and Coordination

A scoping letter dated February 3, 2012 was mailed to 41 individuals, agencies, and organizations on the interested public list for the Challis Herd Management Area, Wilderness List, and the associated Allotment Lists. In addition to the scoping letter an open house was held at the Challis Field Office on February 24, 2012 to answer questions and receive comments. Six comments were received at the Open House. Additionally, over 4,000 comments were received from interested publics during the scoping process. All scoping comments were carefully considered and addressed in the Environmental Assessment (EA).

A statewide Helicopter Hearing for the use of motorized vehicles in the management of wild horses was held in Kimberly, Idaho on March 7, 2012. Six individuals attended and four commented on the use of helicopters. All of the individuals that commented supported the use of motorized vehicles in particular helicopters in the management of wild horses. Time was available following the meeting to comment on the proposed gathers in Challis.

News releases throughout the state invited interested publics to attend the meetings.

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9.0 Appendices

Appendix A: Gather Plan

The purpose of the gather plan is to outline the methods and procedures for conducting a gather to remove excess wild horses from public lands administered by the CFO. Implementation of the Proposed Action would require the capture of 274 and removal of approximately 137 wild horses to achieve a post-gather population of 185 wild horses. An adoption will be held at the Challis Wild Horse Facility following vaccinations, freezmarking, deworming, gelding and blood being drawn for Coggin's test.

A. Gather Area

The Proposed Gather Area includes the CHMA and areas outside of the CHMA boundary east of Highway 93. The CHMA is 168,468 total acres including federal, state and private lands. Refer to Map 8, which displays the CHMA, grazing allotments and the gather area.

B. Administration of the Contract /Gather Operations

The National Wild Horse and Burro Gather Contract would be used to conduct the wild horse gather tentatively scheduled for October 2012. BLM personnel would be responsible for overseeing the contract for the capture, care, aging, and temporary holding of wild horses from the capture area. BLM WH&B Specialists would be present during all aspects of the gather activities.

Standard Operating Procedures (SOPs) described within this document (Appendix B) would be utilized for the capture and handling of wild horses and burros, and have been developed over time to ensure minimal impacts associated with gathering, handling, and transporting wild horses and burros and collecting herd data.

It is estimated that 3-5 capture sites and 1 set of central sorting/holding facility would be necessary to complete the gather. Ideally, capture sites would be established away from riparian vegetation and stream channels in areas of previous soil or vegetation disturbance (such as gravel pits and roads), to avoid impacts to aquatic habitat, unaltered vegetation, cultural resources and soils.

Branded and/or claimed horses would be transported to a temporary sorting/holding facility. Ownership would be determined under the estray laws of the State of Idaho by an Idaho Brand Inspector. Collection of gather fees and any appropriate trespass charges would be collected per BLM policy and regulation.

An APHIS DVM or other licensed DVM would be on-site for the duration of the gather to provide recommendations to WH&B Specialists for care and treatment of sick or injured wild horses as per the 1983 Consent Decree. Consultation with the DVM may take place prior to the euthanasia of wild horses in accordance with Washington Office Instruction Memorandum (IM 2009-041). Refer to Appendix B, Part E for more information about the euthanasia policy.

Precautions would be taken to ensure that young or weak horse foals are safely gathered and cared for appropriately. If a band has a small foal that can't keep the mare and foal are left and not gathered. If the pilot is unable to determine which mare the foal belongs to than the entire band may be left ungathered. In some instances the may be gathered by wranglers that are guided to the foals location by the pilot. Every effort is made to reunite mares and foals as soon as they arrive at the sorting facility. If a foal were determined to be an orphan, qualified adopters would be contacted immediately to provide proper care for the foal. Milk replacer formula and electrolytes would be available to care for orphan foals if necessary.

C. General Overview of Wild Horse and Horse Gather Methods

The contractor supplies and transports all equipment needed to conduct a gather to a central location where a sorting/holding facility is constructed, which consists of six or more pens constructed of sturdy panels 6 feet high, with a central alleyway and working/squeeze chute in the center. Corral panels are covered with snow fencing to keep animals calm and to create a visual barrier reducing disturbances and to reduce collisions, and water tanks are located within the pens. The central alley and pen arrangement allows the BLM staff and the contractor to sort recently captured animals, separating animals to ship to the adoption facilities, and mares and foals from stallions to prevent fighting and injury. The pen arrangement allows the contractor to off-load wild horses from stock trailers into the pens, and facilitates the loading of the horses to be transported to facilities onto large straight deck trucks.

At various locations throughout the CHMA, smaller sets of capture sites are constructed in order to gather horses in the proximal vicinity of where wild horse herds are located. The capture sites or capture sites consist of a series of pens made out of sturdy steel panels six feet tall, and wings made out of jute (a fibrous material) and "T" posts that funnel wild horses into the corrals as they are captured. Once captured, the horses are loaded into stock trailers and transported to the central sorting/holding facility. Horses typically remain less than 24 hours at the capture site before being transported to the sorting/holding facility. Horses may also spend up to an hour one stock trailers while other groups of horses are brought to the capture sites.

The contractor utilizes a helicopter and pilot to conduct gathers. Use of a helicopter is humane, safe and effective. Methods for use of helicopter are well established after over 30 years of BLM WH&B experience. Contract pilots are certified for the moving of animals under governmental contract. Experience and field observations have shown that wild horses settle down once gathered and are more concerned with intra-species dominance than the helicopter. The pilot locates groups of wild horses within the CHMA and guides them towards the capture corrals. In most cases, horses are allowed to travel at their own pace, and are not pushed. Distances average 4-7 miles over mixed terrain which may consist of rolling foothills or steeper terrain, drainages, ridges and valley bottoms. The horses often follow their own trails. The pilot, BLM staff (including the Contract Officer Representative and the Project Inspector) and APHIS DVMs monitor the condition of the horses to ensure their safety, checking for signs of exhaustion, injuries etc. The contractor and pilots are very skilled at designing and building capture corrals, and safely herding the horses to them. Generally, wild horses are very fit, and recover quickly from being captured. Distances that the horses travel are modified to account for summer temperatures, snow depth, animals in weakened condition, young foals, or older/lame animals.

Under ideal conditions, some horses could be herded 10 miles at the discretion of the COR/WH&B Specialist.

Once near the capture site, the contractor holds a Prada horse (a tame trained horse) at the mouth of the wings. As the pilot pushes the wild horses closer, the Prada horse is released, which then runs into the capture corrals, leading all of the wild horses with him. Crewmembers rush in to secure gates once the horses are within the corrals. During summer gathers, the crew often separates foals from adults at the capture site so that they may be transported to the Sorting/holding facility separately and avoids being injured by adult horses. Foals may be loaded into a separate stock trailer where they can have shade, water, and electrolyte if necessary. Once unloaded at the sorting/holding facility, foals may be rejoined with the mothers if not old enough to wean, and monitored to ensure that all of the foals "join-up" or reunited with their dams. Often paint marks are applied to the foals and mothers to assist the contractor and BLM staff in identifying pairs.

Occasionally (and more frequently for difficult to gather areas) helicopter-assisted roping is implemented, in which the pilot moves a small group of horses to the gather area, and the crewmembers rope the animals from horseback. This method often prevents overstressing the horses from repeated attempts to move them into the capture corrals. The roped horses are then led to the corrals, to awaiting stock trailers, or immobilized on the ground (less than 30 minutes) until they can be loaded into stock trailers.

Once horses are loaded and transported to the sorting/holding facilities, they are sorted by the contractor‘s staff and BLM employees. The contractor looks at the horse‘s teeth to estimate age while held in the chute, and the BLM staff documents age, color, body condition and lactation status of the horse. Aging wild horses is a process of estimation due to the type of wear that can occur to the teeth of a wild horse on the range. Injuries are noted and treated if needed. Once sorted, the horses are given hay and unlimited water, if no health concerns exist. During this time, the BLM may consult with a DVM to treat sick or injured animals, or make recommendations for euthanasia.

When sufficient numbers of horses to be removed are available at the sorting/holding facility, they are loaded into the straight deck trailers that hold 35-45 wild horses depending upon their size semi-tractor trailer will transport horses to the Challis Preparation Facility on Upper Hot Springs Road in Challis, Idaho. This requires separating horses remaining in the CHMA from those to be removed. The trailers have three compartments so that mares, stallions and foals can be transported separately. Transportation time is estimated to be an hour from the sorting/holding facility to the Challis Preparation Facility. If additional room is required or for any other reason horses are not able to be shipped to the Challis Facility, they will be shipped the Boise Idaho Facility or the Rock Springs Wyoming Facility, both approximately 5-7 hours away.

During sorting, BLM staff identifies wild horses to be released back to the CHMA based on the National Selective removal guidance, fertility control and historic characteristics. Mares may be held until the end of the gather so that fertility control can be given to them to slow future population growth rates. When it is time for the release, the mares and stallions are each loaded into separate stock trailers and transported back inside the CHMA. The rear of the trailer is

opened up, and the horses are allowed to step off and travel back into the CHMA. Sometimes the horses are released directly from the sorting/holding facility located within the CHMA.

Before the wild horses are released back to CHMA, hair samples would be collected for genetic testing from 40 random individuals that are gathered from varying parts of the CHMA. Data collected during the gather in conjunction with previous genetic analysis reports will be used to monitor the genetic health of the CHMA.

D. Data Collection

The WH&B Specialist would be responsible for collecting population data on individual HMAs. The extent to which data is collected may vary among the field offices to meet specific needs pertaining to each HMA.

1) Hair Samples/Genetics Analysis

Hair samples would be collected for genetic testing from 40 random individuals that are gathered from varying parts of the CHMA and analyzed for genetic trend data (last collected in 2002) of wild horses (genetic diversity (heterozygosity), historical origins and unique markers). These data determine adequacy of herd size, genetic diversity, and historic characteristics. WHB Specialists would collect a minimum sample size of 25 hair samples from both mares and stallions in a ratio similar to the sex ratio released. Age would not be a defining factor in determining which animals to sample. Samples would be sent to Texas A&M University for analysis.

2) Herd Health and Viability Data Collection

WHB Specialists would document information related to age, sex, color, overall health, pregnancy, or nursing status from each animal captured. An estimate of the number of horses evading capture would also be recorded. Information on reproduction would be collected to the extent possible, through documentation of the wild horses captured during the gather, and the age of those released following the gather.

3) Characteristics

WHB Specialists would record color and size of the animals, and any characteristics as to type would be noted, if determined. Any incidence of negative genetic traits (parrot mouth, club foot etc.) or other abnormalities would be noted as well.

4) Condition Class

A body condition class score would be recorded based on the Henneke System. This would be recorded for the population in general and/or for specific animals if necessary.

E. Euthanasia

The Authorized Office (or designee) will make decisions regarding euthanasia, in accordance with BLM policy as expressed in Washington Office Instructional Memorandum No. 2009-041. A DVM may be called to make a diagnosis and final determination. Current BLM SOP is to have a DVM from APHIS on site throughout the gather to observe animal health and condition and provide input to BLM staff regarding the potential need to euthanize wild horses on gathers. Euthanasia shall be done by the most humane method available. Authority for humane

euthanasia of wild horses or burros is provided by the Wild Free-Roaming Horses and Burros Act of1971, Section 3(b)(2)(A), 43 CFR 4730.1, BLM Manual 4730 - Euthanasia of Wild horses and Burros and Disposal of Remains. The following are excerpted from IM 2009-41:

A Bureau of Land Management (BLM) authorized officer may authorize the euthanasia of a wild horse or Burro in field situations (includes free-roaming horses and burros encountered during gather operations) as well as short- and long-term wild horse and Burro holding facilities with any of the following conditions:
(1) Displays a hopeless prognosis for life;
(2) suffers from a chronic or incurable disease, injury or serious physical defect; (includes severe tooth loss or wear, severe club feet, and other severe acquired or congenital abnormalities)
(3) would require continuous treatment for the relief of pain and suffering in a domestic setting;
(4) is incapable of maintaining a Henneke body condition score greater than two, in its present environment;
(5) has an acute or chronic injury, physical defect or lameness that would not allow the animal to live and interact with other horses or burros , keep up with its peers or exhibit behaviors which may be considered essential for an acceptable quality of life constantly or for the foreseeable future;
(6) suffers an acute or chronic infectious disease where State or Federal animal health officials order the humane destruction of the animal as a disease control measure.

There are three circumstances where the authority for euthanasia would be applied in a field situation:
(A) If an animal suffers from a condition as described in 1-6 above that causes acute pain or suffering and immediate euthanasia would be an act of mercy, the authorized officer has the authority and the obligation to promptly euthanize the animal. If the animal is euthanized during a gather operation, the authorized officer will describe the animal''s condition and report the action using the gather report in the comment section that summarizes gather operations (See attachment 1). If the euthanasia is performed during routine monitoring, the Field Manager will be notified of the incident as soon as practical after returning from the field.

(B) Older wild horses and burros encountered during gather operations should be released if, in the opinion of the authorized officer, the criteria described in 1-6 above for euthanasia do not apply, but the animals would not tolerate the stress of transportation, adoption preparation, or holding and may survive if returned to the range. This may include older animals with significant tooth wear or tooth loss that have a Henneke body condition score greater than two. However, if the authorized officer has inspected the animal''s teeth and feels the animal''s quality of life will suffer and include health problems due to dental abnormalities, significant tooth wear or tooth loss; the animal should be euthanized as an act of mercy.

(C) If an animal suffers from any of the conditions listed in 1-6 above, but is not in acute pain, the authorized officer has the authority to euthanize the animal in a humane manner.

I. Special Stipulations

1) Private landowners or the proper administering agency(s) would be contacted and authorization obtained prior to setting up capture corrals on any lands which are not administered by BLM. Wherever possible, capture sites would be constructed in such a manner as to not block vehicular access on existing roads.

2) Capture sites would be constructed so that no riparian vegetation is contained within them. No vehicles would be operated on riparian vegetation or on saturated soils associated with riparian/wetland areas.

3) The helicopter would avoid eagles and other raptors, and would not be flown repeatedly over any identified active raptor nests. No unnecessary flying would occur over big game on their winter ranges or active fawning/calving grounds during the period of use.

4) Standard operating procedures in the site establishment and construction of capture sites will avoid adverse impacts from capture site construction or operation to wildlife species, including threatened, endangered, or sensitive species.

5) Capture sites and sorting/holding facility

No personnel working at gather sites may excavate, remove, damage, or otherwise alter or deface or attempt to excavate, remove, damage or otherwise alter or deface any archaeological resource located on public lands or Tribal lands.

Prior to setting up a trap or temporary holding facility, BLM will conduct all necessary surveys (archaeological, T&E, etc). All proposed site(s) must be inspected by a government archaeologist. Once archaeological survey has been conducted, the trap or temporary holding facility may be set up. Said surveys shall be arranged for by the COR, PI, or other BLM employees.

Gather sites and temporary holding facilities would be constructed outside the RHCA.

Locations would be placed only in areas that have been surveyed and reviewed for potential impacts to cultural resources. Capture site capture sites capture sites. capture sites and sorting/holding facilities

Wilderness Study Areas: When gathering wild horses from within Wilderness Study Areas (WSAs), applicable policy will be strictly adhered to. Only approved roads will be traveled on. A Wilderness Specialist or designee would be present to ensure that only inventoried ways or cherry stemmed roads are traveled on by vehicles within the WSA.

Capture sites and sorting/holding facilities will be constructed outside of weed infested areas to limit the spread of weedy species.

Appendix B: Standard Operating Procedures for Wild Horse and Horse Gathers

Gathers are conducted by utilizing contractors from the Wild Horse Gathers-Western States Contract, or BLM personnel. The following procedures for gathering and handling wild horses would apply whether a contractor or BLM personnel conduct a gather. For helicopter gathers conducted by BLM personnel, gather operations will be conducted in conformance with the *Wild Horse Aviation Management Handbook* H-4740-1 (January 2009).

Prior to any gathering operation, the BLM will provide for a pre-capture evaluation of existing conditions in the gather area(s). The evaluation will include animal conditions, prevailing temperatures, snow depth, drought conditions, soil conditions, road conditions, and a topographic map with wilderness boundaries, the location of fences, other physical barriers, and acceptable capture locations in relation to animal distribution. The evaluation will determine whether the proposed activities will necessitate the presence of a licensed DVM during operations. If it is determined that a large number of animals may need to be euthanized or capture operations could be facilitated by a DVM, these services would be arranged before the capture would proceed. The contractor will be apprised of all conditions and will be given instructions regarding the capture and handling of animals to ensure their health and welfare is protected. Capture sites and temporary sorting/holding facilities will be located to reduce the likelihood of injury and stress to the animals, and to minimize potential damage to the natural resources of the area. Wherever possible, capture sites would be located on or near existing roads to limit ground disturbance.

All capture sites and sorting/holding facility locations must be approved by the Authorized Officer prior to construction. All capture sites and sorting/holding facilities not located on public land must have prior written approval of the landowner.

Capture sites would be located to cause as little injury and stress to the animals, and as little damage to the natural resources of the area, as possible. Sites would be located on or near existing roads. Additional capture sites may be required, as determined by the Authorized Officer, to relieve stress to the animals caused by specific conditions at the time of the gather (i.e. dust, rocky terrain, temperatures, etc.).

The primary capture methods used in the performance of gather operations include:
1. Helicopter Assisted Gathering. This capture method involves utilizing a helicopter to direct wild horses into a temporary corral.
2. Helicopter Assisted Roping. This capture method involves utilizing a helicopter to herd wild horses or burros to ropers on horseback.
3. Bait Trapping. This capture method involves utilizing bait (food/mineral) to lure wild horses into a temporary corral.

A. Capture Methods used in the Performance of Gather Contract Operations

1. The primary concern of the contractor is the safe and humane handling of all animals captured. All capture attempts shall incorporate the following:

All capture sites and sorting/holding facilities locations must be approved by the Contracting Officer's Representative (COR) and/or the Project Inspector (PI) prior to construction. The Contractor may also be required to change or move trap locations as determined by the COR/PI. All traps and sorting/holding facilities not located on public land must have prior written approval of the landowner.

2. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who would consider terrain, physical barriers, weather, condition of the animals and other factors. Under normal circumstances this travel should not exceed 10 miles and may be much less dependent on existing conditions (i.e. ground conditions, animal health, extreme temperature (high and low)).

3. All traps, wings, and sorting/holding facilities shall be constructed, maintained and operated to handle the animals in a safe and humane manner and be in accordance with the following:

    a. Captures sites and sorting/holding facilities shall be constructed of portable panels, the top of which shall not be less than 72 inches high for horses and 60 inches for burros, and the bottom rail of which shall not be more than 12 inches from ground level. All traps and sorting/holding facilities shall be oval or round in design.

    b. All loading chute sides shall be a minimum of 6 feet high and shall be fully covered, plywood, metal without holes larger than 2”x4”.

    c. All runways shall be a minimum of 30 feet long and a minimum of 6 feet high for horses, and 5 feet high for burros, and shall be covered with plywood, burlap, plastic snow fence or like material a minimum of 1 foot to 5 feet above ground level for burros and 1 foot to 6 feet for horses. The location of the government furnished portable fly chute to restrain, age, or provide additional care for the animals shall be placed in the runway in a manner as instructed by or in concurrence with the COR/PI.

    d. All crowding pens including the gates leading to the runways shall be covered with a material which prevents the animals from seeing out (plywood, burlap, plastic snow fence, etc.) and shall be covered a minimum of 1 foot to 5 feet above ground level for burros and 2 feet to 6 feet for horses

    e. All pens and runways used for the movement and handling of animals shall be connected with hinged self-locking or sliding gates.

4. No modification of existing fences would be made without authorization from the COR/PI. The Contractor shall be responsible for restoration of any fence modification which he has made.

5. When dust conditions occur within or adjacent to the trap or sorting/holding facility, the

Contractor shall be required to wet down the ground with water.

6. Alternate pens, within the sorting/holding facility shall be furnished by the Contractor to separate mares or jennies with small foals, sick and injured animals, estrays or other animals the COR determines need to be housed in a separate pen from the other animals. Animals shall be sorted as to age, number, size, temperament, sex, and condition when in the sorting/holding facility so as to minimize, to the extent possible, injury due to fighting and trampling. Under normal conditions, the government would require that animals be restrained for the purpose of determining an animal's age, sex, or other necessary procedures. In these instances, a portable restraining chute may be necessary and would be provided by the government. Alternate pens shall be furnished by the Contractor to hold animals if the specific gathering requires that animals be released back into the capture area(s). In areas requiring one or more satellite traps, and where a centralized sorting/holding facility is utilized, the contractor may be required to provide additional sorting/holding facility to segregate animals transported from remote locations so they may be returned to their traditional ranges. Either segregation or temporary marking and later segregation would be at the discretion of the COR.

7. The Contractor shall provide animals held in the traps and/or sorting/holding facilities with a continuous supply of fresh clean water at a minimum rate of 10 gallons per animal per day. Animals held for 10 hours or more in the traps or sorting/holding facilities shall be provided good quality hay at the rate of not less than two pounds of hay per 100 pounds of estimated body weight per day. The contractor would supply certified weed free hay if required by State, County, and Federal regulation.

8. It is the responsibility of the Contractor to provide security to prevent loss, injury or death of captured animals until delivery to final destination.

9. The Contractor shall restrain sick or injured animals if treatment is necessary. The COR/PI would determine if animals must be euthanized and provide for the destruction of such animals. The Contractor may be required to humanely euthanize animals in the field and to dispose of the carcasses as directed by the COR/PI.

10. Animals shall be transported to their final destination from temporary sorting/holding facilities as quickly as possible after capture unless prior approval is granted by the COR for unusual circumstances. Animals to be released back into the CHMA following gather operations may be held up to 21 days or as directed by the COR. Animals shall not be held in traps and/or temporary sorting/holding facilities on days when there is no work being conducted except as specified by the COR. The Contractor shall schedule shipments of animals to arrive at final destination between 7:00 a.m. and 4:00 p.m. No shipments shall be scheduled to arrive at final destination on Sunday and Federal holidays, unless prior approval has been obtained by the COR. Animals shall not be allowed to remain standing on trucks while not in transport for a combined period of greater than three (3) hours in any 24 hour period. Animals that are to be released back into the capture area may need to be transported back to the original trap site. This determination would be at the discretion of the COR/PI or Field Office horse specialist.

B. Capture Methods That May Be Used in the Performance of a Gather

1. Capture attempts may be accomplished by utilizing bait (food/mineral) to lure animals into a temporary trap. If this capture method is selected, the following applies:

    a. Finger gates shall not be constructed of materials such as "T" posts, sharpened willows, etc., that may be injurious to animals.

    b. All trigger and/or trip gate devices must be approved by the COR/PI prior to capture of animals.

    c. Traps shall be checked a minimum of once every 10 hours.

2. Capture attempts may be accomplished by utilizing a helicopter to drive animals into a temporary trap. If the contractor selects this method the following applies:

    a. A minimum of two saddle-horses shall be immediately available at the trap site to accomplish roping if necessary. Roping shall be done as determined by the COR/PI. Under no circumstances shall animals be tied down for more than one half hour.

    b. The contractor shall assure that foals shall not be left behind, and orphaned.

3. Capture attempts may be accomplished by utilizing a helicopter to drive animals to ropers. If the contractor, with the approval of the COR/PI, selects this method the following applies:

    a. Under no circumstances shall animals be tied down for more than one hour.

    b. The contractor shall assure that foals shall not be left behind, or orphaned.

    c. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who would consider terrain, physical barriers, weather, condition of the animals and other factors.

C. Use of Motorized Equipment

1. All motorized equipment employed in the transportation of captured animals shall be in compliance with appropriate State and Federal laws and regulations applicable to the humane transportation of animals. The Contractor shall provide the COR/PI with a current safety inspection (less than one year old) for all motorized equipment and tractor-trailers used to transport animals to final destination.

2. All motorized equipment, tractor-trailers, and stock trailers shall be in good repair, of adequate rated capacity, and operated so as to ensure that captured animals are transported without undue risk or injury. Equipment will need to be washed to remove invasive species seed.

3. Only tractor-trailers or stock trailers with a covered top shall be allowed for transporting animals from capture site site(s) to temporary sorting/holding facilities, and from temporary sorting/holding facilities to final destination(s). Sides or stock racks of all trailers used for transporting animals shall be a minimum height of 6 feet 6 inches from the floor. Single deck tractor-trailers 40 feet or longer shall have two (2) partition gates providing three (3) compartments within the trailer to separate animals. Tractor-trailers less than 40 feet shall have at least one partition gate providing two (2) compartments within the trailer to separate the animals. Compartments in all tractor-trailers shall be of equal size plus or minus 10 percent. Each partition shall be a minimum of 6 feet high and shall have a minimum 5 foot wide swinging gate. The use of double deck tractor-trailers is unacceptable and shall not be allowed.

4. All tractor-trailers used to transport animals to final destination(s) shall be equipped with at least one (1) door at the rear end of the trailer, which is capable of sliding either horizontally or vertically. The rear door(s) of tractor-trailers and stock trailers must be capable of opening the full width of the trailer. Panels facing the inside of all trailers must be free of sharp edges, protrusions or holes that could cause injury to the animals. The material facing the inside of all trailers must be strong enough so that the animals cannot push their hooves through the side. Final approval of tractor-trailers and stock trailers used to transport animals shall be held by the COR/PI.

5. Floors of tractor-trailers, stock trailers and loading chutes shall be covered and maintained with wood shavings to prevent the animals from slipping.

6. Animals to be loaded and transported in any trailer shall be as directed by the COR/PI and may include limitations on numbers according to age, size, sex, temperament and animal condition. The following minimum square feet per animal shall be allowed in all trailers:

11 square feet per adult horse (1.4 linear foot in an 8 foot wide trailer);
8 square feet per adult burro (1.0 linear foot in an 8 foot wide trailer);
6 square feet per horse foal (.75 linear foot in an 8 foot wide trailer);
4 square feet per burro foal (.50 linear feet in an 8 foot wide trailer).

7. The COR/PI shall consider the condition and size of the animals, weather conditions, distance to be transported, or other factors when planning for the movement of captured animals. The COR/PI shall provide for any brand and/or inspection services required for the captured animals.

8. If the COR/PI determines that dust conditions are such that the animals could be endangered during transportation, the Contractor will be instructed to adjust speed of transporting vehicles.

D. Treatment of Injured or Sick; Disposition of Terminal Animals

The contractor would restrain sick or injured animals if treatment is necessary. A veterinarian may be called to make a diagnosis and final determination. Destruction would be done by the most humane method available. Authority for humane destruction of wild horses is provided by the Wild Free-Roaming Horse and Burro Act of 1971, Section 3(b)(2)(A), 43 CFR 4730.1, BLM

Manual 4730 - Destruction of Wild Horses and Burros and Disposal of Remains, and is in accordance with BLM policy as expressed in Instructional Memorandum No. 98-141.

The Authorized Officer would determine if injured animals must be destroyed and provide for destruction of such animals. The contractor may be required to dispose of the carcasses as directed by the Authorized Officer.

The carcasses of the animals that die or must be destroyed as a result of any infectious, contagious, or parasitic disease would be disposed of by burial to a depth of at least 3 feet.

The carcasses of the animals that must be destroyed as a result of age, injury, lameness, or noncontagious disease or illness would be disposed of by removing them from the capture site or sorting/holding corral and placing them in an inconspicuous location to minimize visual impacts. Carcasses would not be placed in drainages regardless of drainage size or downstream destination.

E. Safety and Communications

1. The Contractor shall have the means to communicate with the COR/PI and all contractor personnel engaged in the capture of wild horses utilizing a VHF/FM Transceiver or VHF/FM portable Two-Way radio. If communications are ineffective the government will take steps necessary to protect the welfare of the animals.

    a. The proper operation, service and maintenance of all contractor-furnished property is the responsibility of the Contractor. The BLM reserves the right to remove from service any contractor personnel or contractor-furnished equipment which, in the opinion of the contracting officer or COR/PI violate contract rules, are unsafe or otherwise unsatisfactory. In this event, the Contractor will be notified in writing to furnish replacement personnel or equipment within 48 hours of notification. All such replacements must be approved in advance of operation by the Contracting Officer or his/her representative.

    b. The Contractor shall obtain the necessary FCC licenses for the radio system

    c. All accidents occurring during the performance of any task order shall be immediately reported to the COR/PI.

2. Should the contractor choose to utilize a helicopter the following will apply:

    a. The Contractor must operate in compliance with Federal Aviation Regulations, Part 91. Pilots provided by the Contractor shall comply with the Contractor's Federal Aviation Certificates, applicable regulations of the State in which the gather is located.

    b. Fueling operations shall not take place within 1,000 feet of animals.

F. Site Clearances

Personnel working at gather sites will be advised of the illegality of collecting artifacts. Prior to setting up a capture site or temporary sorting/holding facility on a site not previously cleared or disturbed, BLM will conduct all necessary clearances (archaeological, T&E, etc). All proposed site(s) must be inspected by a government archaeologist. Once archaeological clearance has been obtained, the capture site or temporary sorting/holding facility may be set up. Said clearance shall be arranged for by the COR, PI, or other BLM employees.

No personnel working at gather sites may excavate, remove, damage, or otherwise alter or deface or attempt to excavate, remove, damage or otherwise alter or deface any archaeological resource located on public lands.

New capture sites and temporary holding/sorting facilities would be constructed outside RHCA or weed infested areas.

G. Public Participation

Opportunities for public viewing (i.e. media, interested public) of gather operations will be made available to the extent possible; however, the primary considerations will be to protect the health, safety, and welfare of the animals being gathered and the personnel involved. The public must adhere to guidance from the on-site BLM representatives. It is BLM policy that the public will not be allowed to come into direct contact with wild horses or burros being held in BLM facilities. Only authorized BLM personnel or contractors may enter the corrals or directly handle the animals. The general public may not enter the corrals, climb on or lean through corrals, or directly handle the animals at any time or for any reason during BLM operations (refer to Appendix F and G).

H. Responsibility and Lines of Communication

Incident Command

Chris Robbins—Idaho Wild Horse and Burro State Lead

Contracting Officer's Representative

Kevin Lloyd—Challis Field Office Wild Horse and Burro Specialist

Alternate Contracting Officer's Representative

Scott Fleur—Lander (WY) Field Office Wild Horse and Burro Specialist

Project Inspector

Kent Benson– Burley Field Office BLM Range Technician

The Contracting Officer‘s Representatives (CORs) and the project inspectors (PIs) have the direct responsibility to ensure the Contractor‘s compliance with the contract stipulations. Kevin Lloyd, Challis Field Office WH&B Specialist would serve as the primary COR. Todd Kuck, CFO Field Manager, other CFO employees, Idaho Falls District employees and members of the Idaho Wild Horse Team will take an active role to ensure the appropriate lines of communication are established between the field, Field Office, State Office, National Program Office, and BLM

Sorting/holding Facility offices. All employees involved in the gather operations will keep the best interests of the animals at the forefront at all times.

All publicity, formal public contact and inquiries will be handled through the Idaho State Office and the Idaho Wild Horse Team Public Affairs Officer. These individuals will be the primary contact and will coordinate with the COR on any inquiries.

The COR would coordinate with the contractor and the BLM Corrals to ensure animals are being transported from the sorting/holding facility in a safe and humane manner and are arriving in good condition.

The contract specifications require humane treatment and care of the animals during removal operations. These specifications are designed to minimize the risk of injury and death during and after capture of the animals. The specifications would be vigorously enforced.

Should the Contractor show negligence and/or not perform according to contract stipulations, he would be issued written instructions, stop work orders, or defaulted by the COR.

I. Additional requirements for personnel conducting gather operations also include:

1. Electric prods (hotshots) would not be used routinely on horses. They can be used when animal or human safety is in jeopardy or as a last resort. Handlers do not constantly carry prods. Prods are picked up only when necessary and then put away. Electric prods are never applied to sensitive areas such as the eyes.
2. Electric prod use will not be disguised, but used openly and transparently.
3. Handling aids, including electric prods and flags will not be used abusively.
4. Flagging will be used strategically, as excessive flagging desensitizes the animal and becomes useless if used too much.
5. Gates and doors will not be deliberately slammed or shut on horses or burros passing through.
6. Excessive yelling and unnecessary noises will not be utilized in the loading and unloading process.
7. There will be no hitting, kicking, or striking a horse.
8. Loading or unloading of transport vehicles is performed during daylight hours, or supplemental light will be provided in the area to facilitate visibility.
9. Holes, gaps, or openings will be eliminated in the loading/unloading area to avoid injury.
10. Transport vehicles will be properly aligned with the loading/unloading ramps or docks. No gaps will exist between the unloading/loading docks or ramps and the bottom or floor of the trailer's exit. No gaps will exist between the trailer and the side walls of the unloading area, whereby a horse's limbs or head can become stuck or injured.

Appendix C Standard Operating Procedures for Population-level Fertility Control Treatments

One-year liquid vaccine: The following implementation and monitoring requirements are part of the Proposed Action:

1. PZP vaccine would be administered through darting by trained BLM personnel or collaborating research partners only. For any darting operation, the designated personnel must have successfully completed a nationally recognized wildlife darting course and who have documented and successful experience darting wildlife under field conditions.
2. Mares that have never been treated would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA) and loaded into darts at the time a decision has been made to dart a specific mare. Mares identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA).
3. The liquid dose of PZP vaccine is administered using 1.0 cc Pneu-Darts with 1.5" barbless needles fired from either Dan Inject® or Pneu-Dart® capture gun.
4. Only designated darters would mix the vaccine/adjuvant and prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of a capture gun.
5. Delivery of the vaccine would be by intramuscular injection into the left or right hip/gluteal muscles while the mare is standing still.
6. Safety for both humans and the horse is the foremost consideration in deciding to dart a mare. The Dan Inject® gun would not be used at ranges in excess of 30 m while the Pneu-Dart® capture gun would not be used over 50 m, and no attempt would be taken when other persons are within a 30-m radius of the target animal.
7. No attempts would be taken in high wind or when the horse is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal is when the dart would strike the skin of the horse at a perfect 90° angle.
8. If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another horse. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart the next day. Refrigerated darts would not be used in the field.
9. No more than two people should be present at the time of a darting. The second person is responsible for locating fired darts. The second person should also be responsible for identifying the horse and keeping onlookers at a safe distance.
10. To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting.
11. Attempts would be made to recover all darts. To the extent possible, all darts which are discharged and drop from the horse at the darting site would be recovered before another darting occurs. In exceptional situations, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine.

22-month time-release pelleted vaccine:
The following implementation and monitoring requirements are part of the Proposed Action:

1. PZP vaccine would be administered only by trained BLM personnel or collaborating research partners.
2. Mares that have never been treated would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund‘s Modified Adjuvant (FMA). Mares identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund‘s Incomplete Adjuvant (FIA).
3. The fertility control drug is administered with two separate injections: (1) a liquid dose of PZP is administered using an 18-gauge needle primarily by hand injection; (2) the pellets are preloaded into a 14-gauge needle. These are delivered using a modified syringe and jabstick to inject the pellets into the gluteal muscles of the mares being returned to the range. The pellets are designed to release PZP over time similar to a time-release cold capsule.
4. Delivery of the vaccine would be by intramuscular injection into the gluteal muscles while the mare is restrained in a working chute. The primer would consist of 0.5 cc of liquid PZP emulsified with 0.5 cc of Freunds Modified Adjuvant (FMA). The pellets would be loaded into the jabstick for the second injection. With each injection, the liquid or pellets would be injected into the left hind quarters of the mare, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).
5. In the future, the vaccine may be administered remotely using an approved long range darting protocol and delivery system if or when that technology is developed.
6. All treated mares will be freeze-marked on the hip or neck CHMA to positively identify the treated animals during the research project and during subsequent gathers. Within the Challis CHMA, treated mares are freeze branded with an AA on their left (right?) hip.

Monitoring and Tracking of Treatments:
1. At a minimum, estimation of population growth rates using helicopter or fixed-wing surveys will be conducted before any subsequent gather. During these surveys it is not necessary to identify which foals were born to which mares; only an estimate of population growth is needed (i.e. # of foals to # of adults).
2. Population growth rates of herds (CHMA is not selected for intensive monitoring) selected for intensive monitoring will be estimated every year post-treatment using helicopter or fixed-wing surveys. During these surveys it is not necessary to identify which foals were born to which mares, only an estimate of population growth is needed (i.e. # of foals to # of adults). If, during routine CHMA field monitoring (on-the-ground), data describing mare to foal ratios can be collected, these data should also be shared with the NPO for possible analysis by the USGS.
3. A PZP Application Data sheet will be used by field applicators to record all pertinent data relating to identification of the mare (including photographs if mares are not freeze-marked) and date of treatment. Each applicator will submit a PZP Application Report and accompanying narrative and data sheets will be forwarded to the NPO (Reno, Nevada). A copy of the form and data sheets and any photos taken will be maintained at the field office.
4. A tracking system will be maintained by NPO detailing the quantity of PZP issued, the quantity used, disposition of any unused PZP, the number of treated mares by HMA, field office, and State along with the freeze-mark(s) applied by HMA and date.

Appendix D: WinEquus Population Modeling Summary

Summary of Genetic Viability and Population Modeling in Wild Horse Herds

Genetic Viability
The concept of Minimum Viable Populations (MVP), or the minimum number of breeding individuals required for a population to survive over a period of time has been a central issue to conservation biology for years. The BLM is now taking MVP into consideration in the management of its wild horse herds in response to the challenge of balancing wild horse impacts on their ecosystem with a herd size large enough to constitute a viable population size. A wild horse population managed at numbers low enough to prevent ecological damage may pose problems to the long-term health and genetic diversity of a herd.

Genetic diversity, within wild horse and burro populations, refers to the entire complement of genetic material representative of all individuals (or a sample of individuals) from within the population. Some populations may possess genetic uniformity to a certain "type" or breed of horse, but management interests are specific to maintaining a maximum diversity of genetic material which appears representative of each herd. Promotion of diversity will minimize the effects of genetic drift, or the random loss of genetic material due to mating processes, and maximize genetic health of the herds.

The genetic effective population size (Ne) is a measure of the total number of mares and stallions which contribute genetically, through successful breeding, to the next generation. Although no standard goal for Ne currently exists for wild horse and burro herds, a goal of Ne=50, which comes from domestic breeding guidelines, can be conservatively applied**.** Populations, where Ne is calculated to be less than 50, may experience higher rates of loss of genetic diversity than would be considered acceptable under recommended management goals.

Viable management Alternatives for conserving genetic diversity within managed wild horse herds may take several forms. Some options to be considered might include: altering population age structure (through removals) to promote higher numbers of reproductively-successful animals; altering breeding sex ratios (through removals) to encourage more breeding males; increasing generation intervals (and reducing the rate of loss of genetic material) by removing (or sterilizing) younger versus older mares; and/or introducing breeding animals periodically from other genetically similar herds to help in conservation efforts. In this last scenario, only one or two breeding animals per generation (~10 years) would need to be introduced in order to maintain the genetic resources in small populations of less than 100 animals.

Population Modeling
The **Wild Horse Population Model** (*WinEquus version 1.40*) developed by Dr. Steve Jenkins was used to estimate the population growth and size of herds five years after the gather. There are three data sets from three Herd Management Areas (Garfield, Granites, and Pryor Mountain) built into and available for use in the Jenkins Model. An infinite number of data sets from other sources can also be entered into the model for local herds. Most population projections are based on the Garfield data. These data are the best available for many areas and are based on substantial field work and research. The model's projections using the Garfield data are very

close to what actually occurs in the herds. BLM has not conducted an independent analysis to compare the model's projections with observed growth rates. The Jenkins Model is a tool that BLM uses compare the relative effects of one proposed management action to other alternative management actions including removals and fertility control. Dean Bolstad stated "*We recognize that some of the data in the model may not be exact for any given herd management area, but the model has been useful to approximate the effects of management actions and to compare alternatives*". (Bolstad personal communication 2009)

The wild horse population data used in the statistical analysis modeling was extrapolated from the 2009 gather/removal and population inventory, including numbers, age and sex structure of the remaining horses in the CHMA as well as the ones released back into CHMA.

Sex ratio at Birth: 43% Females 57% Males

The following percent effectiveness of fertility control was utilized in the population modeling for Alternative I:

Year 1: 94%, Year 2: 82%, Year 3: 68%

The environmental and demographic model option was selected as a means to project population growth while weighing both environmental and demographic variables during 'good' and 'bad' years. Results of the Jenkins population model are not considered a 'prediction' of what will happen to the herds in the future. Results of the model are being used as an aid to evaluate the management practices that are identified in this document and to project population growth.
The modeling analysis made the following assumptions for all Alternatives:
1. The current age selection policy would continue through the lifetime of the modeling analysis.
2. Gathers would be completed every four years with the herds lowered to AML.
3. The herd would rise to at least the threshold limit prior to a gather.
4. Foals are included in the herd appropriate management level.
5. 85% of the herd can be located during gather operations; 15% are not found.
6. All simulations used the survival and foaling rates supplied with the WinEquus population model for the Garfield Flat HMA.
7. Simulations were run for ten years with 100 trials each.
Assumptions: Population Modeling Criteria/Management Options by Alternative, Proposed Alternative, Fertility Control
Gather interval: regular interval of four years
Gathers for fertility treatment only occur if population exceeds threshold.
Gathers continue after removals to treat additional females to be released.
Percent effectiveness of fertility control: Year 1: 94%, Year 2: 84%, Year 3: 62% (November window).
Percentages of released mares treated: 5-20+: 100%.
100% of 0-4 year olds removed.

The modeling trials are based on Alternatives1, 2 and 5. Alternatives 3 and 4 are too specific for WinEquus to model. It may be expected that were trials conducted the numbers for Alternative 3 numbers would range lower than Alternative 1 and Alternative 4 numbers would range higher than Alternative 1.

Alternative 1-Proposed Action
Remove Wild Horses using selective removal criteria, administer Fertility Control on mares returning to the CHMA and maintaining the 60:40 sex ratio of males to females

Alternative 2-Removal only following the selective removal criteria without the fertility control or skewing the sex ratio

Alternative 3-Removal with fertility control with geldings being released as a non-reproducing component of the wild horse herd

Alternative 4- Removal with fertility control but no sex ratio adjustment

Alternative 5- No Action
Modeling under this alternative shows no management of wild horses where they are allowed to reproduce uncontrolled until natural parameters begin to take effect.

Objectives of Population Modeling
Review of the data output for each of the simulations completed with the population model provided many useful comparisons of the possible outcomes for each Alternative. The creator of the modeling program, Stephen Jenkins stresses that it is important to think about the range of possible outcomes, not just focus on one average or typical trial. Some of the questions that needed to be answered through the modeling include the following:
• Do any of the Alternatives "crash" the population?
• What effect does fertility control have on population growth rate?
• What effects do the different Alternatives have on the average population size?

Population size in ten years
Out of the 100 trials in each simulation run, the model tabulated minimum, average and maximum population sizes. The model was run for a period of ten years from 2009 to 2018, and gives output through 2019 (which is actually eleven years). These numbers are useful to make relative comparisons of the different Alternatives, and potential outcomes under different management options. The data displayed within the Table 26 is broken down into different levels. The 10th percentile, 90th percentile and the average are all shown on the Table 26. This output, together with the summary graph of population sizes, is probably the most important representation of the results of the program in terms of assessing the effects of the management plan because it shows not only expected average results but also extreme results that might be possible. The data is for all horses from 0 to 20 years of age.

None of the results obtained for any of the Alternatives indicate that a crash of the population would occur if the Alternative were implemented.

Table 26. Population Size Graph

Modeling Statistics CHMA	Alternative 1 Proposed Action	Alternative 2 Removal Without Additional Population Controls	No Action Alternative 5
Population in Year One	185	185	322
Median Growth Rate	14.5	17.4	16.9
Average Population	204	234	292
10th Percentile	178	212	229
90th Percentile	230	257	362

Alternative 1—Proposed Action
The Proposed Action Alternative includes the gather and removal of horses as well as implementing fertility control and skewing the sex ratios 60:40 in favor of males.

Figure 3. Management Efforts of Gathering Approximately Every Three Years

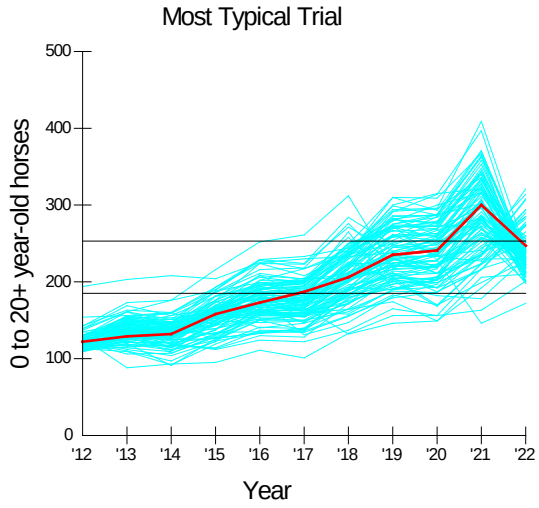


Figure 3 above shows management efforts including gatherings approximately every three years.

Figure 4. Maximum, Average, and Minimum Growth Rates of the Proposed Action

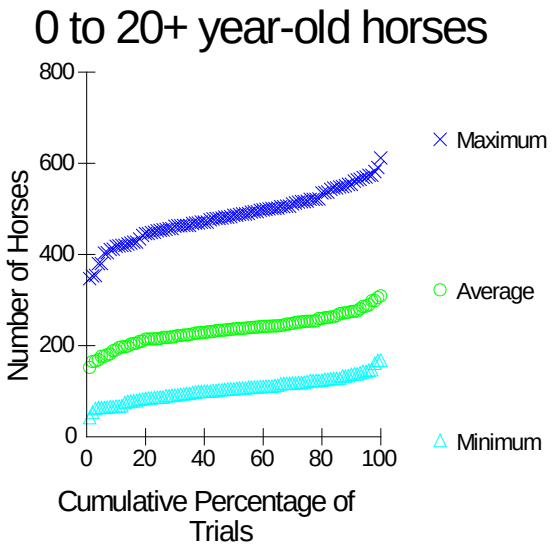


Figure 4 above shows the maximum, average, and minimum growth rate with the proposed action management tools.

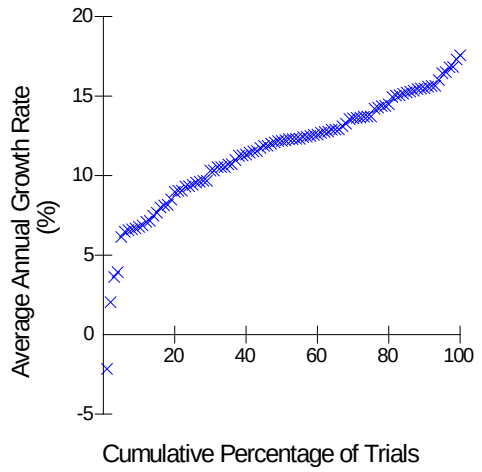
Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	88	137	201
10th Percentile	110	166	256
25th Percentile	111	178	274
Median Tria	117	192	296
75th Percentile	124	206	322
90th Percentile	130	218	350
Highest Trial	194	227	409

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 88 and the highest was 409. In half the trials, the minimum population size in 11 years was less than 117 and the maximum was less than 296. The average population size across 11 years ranged from 137 to 227.

Figure 5. Cumulative Percentage of Trials with Average Annual Growth Rate of Proposed Action



Average Growth Rate in 10 Years	
Lowest Trial	2.2
10th Percentile	6.8
25th Percentile	9.5
Median Trial	12.2
75th Percentile	14.0
90th Percentile	15.5
Highest Trial	17.6

Alternative 2—Removal Only

Figure 6. Alternative 2 Trial

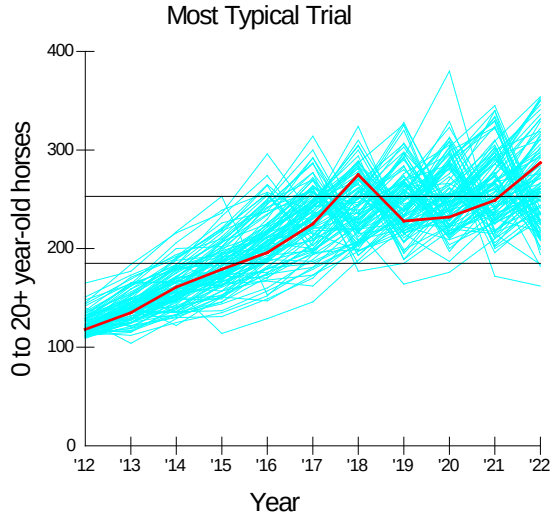
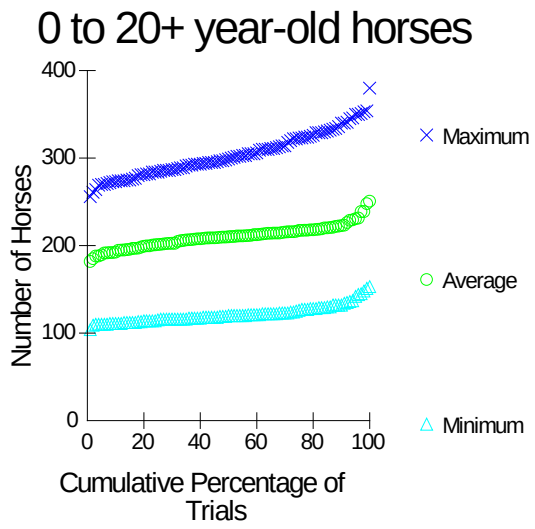


Figure 7. Cumulative Percentage of Trials with Average Annual Growth Rate of Alternative 2



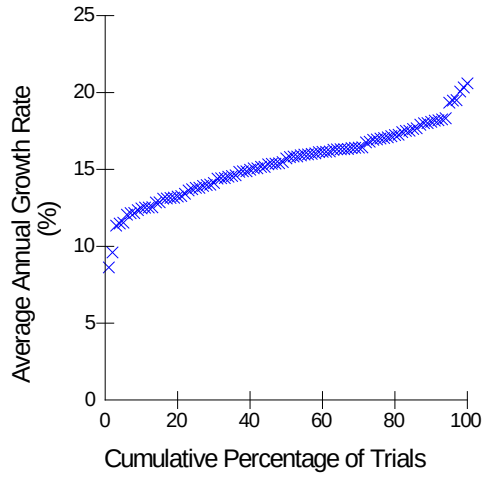
Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	104	182	256
10th Percentile	111	194	274
25th Percentile	116	201	284
Median Trial	120	210	300
75th Percentile	126	217	323
90th Percentile	133	223	340
Highest Trial	153	251	380

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 104 and the highest was 380. In half the trials, the minimum population size in 11 years was less than 120 and the maximum was less than 300. The average population size across 11 years ranged from 182 to 251.

Figure 8. Cumulative Percentage of Trials with Average Annual Growth Rate



Average Growth Rate in 10 Years	
Lowest Trial	8.6
10th Percentile	12.5
25th Percentile	13.8
Median Trial	15.8
75th Percentile	17.0
90th Percentile	18.1
Highest Trial	20.6

Alternative 5--No Action

No Management is the No Action Alternative where no management of the CHMA would occur to alter the population.

Sex ratio at birth: 57% males
Scaling factors for annual variation: survival probabilities = 1.00, foaling rates = 1.00
Correlation between annual variation in survival probabilities and foaling rates = 0.00
Management by removals and fertility control
Starting year is 2012
Gathering occurs at minimum interval of 3 years
Initial gather year is 0
Gathers for fertility treatment occur regardless of population size.
Gathers do not continue after removals to treat additional females.
Threshold population size for gathers is 0.
Target population size following removals is 0.
Foals are included in AML.
Percent of population that can be gathered = 80%.
Percent effectiveness of fertility control: year 1 is 90%, year 2 is 0%, year 3 is 0%, year 4 is 0%, year 5 is 0%.

Figure 9. Cumulative Percentage of Trials with Average Annual Growth Rate of Alternative 5.

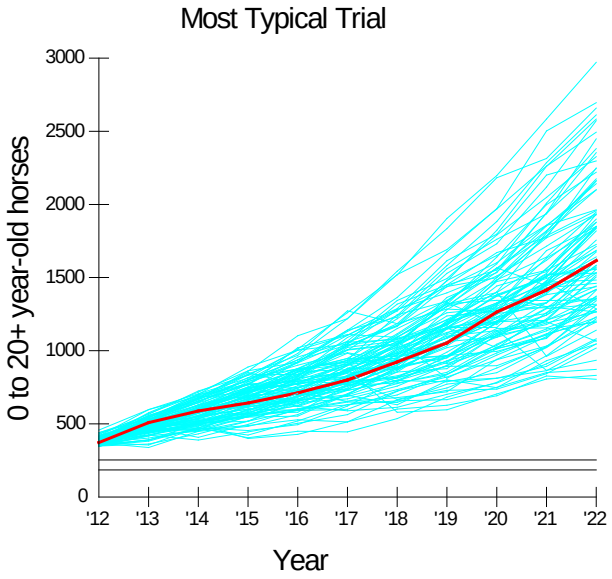
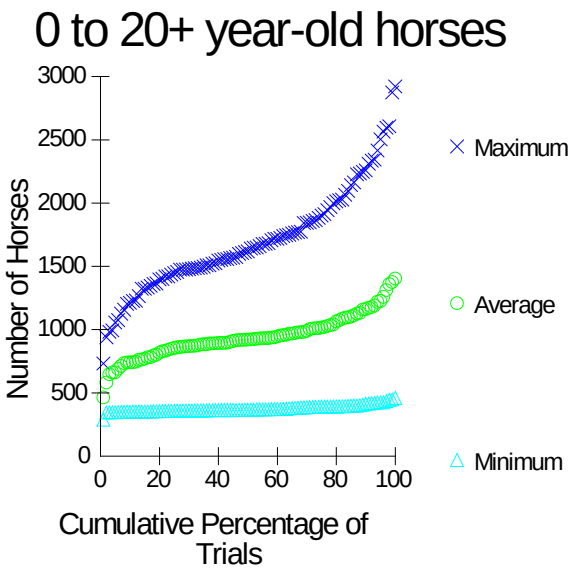


Figure 10. No Management-Typical Trial



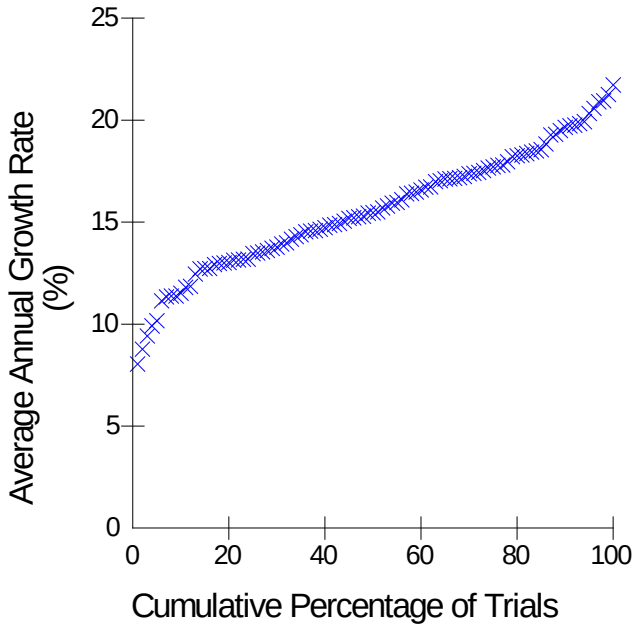
Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	288	463	732
10th Percentile	350	742	1220
25th Percentile	358	856	1451
Median Trial	366	921	1630
75th Percentile	391	1018	1908
90th Percentile	414	1167	2285
Highest Trial	460	1404	2922

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number of 0 to 20+ year-old horses ever obtained was 288 and the highest was 2922. In half the trials, the minimum population size in 11 years was less than 366 and the maximum was less than 1630. The average population size across 11 years ranged from 463 to 1404.

Figure 11. No Management-Population Graph



Average Growth Rate in 10 Years	
Lowest Trial	8.0
10th Percentile	11.7
25th Percentile	13.5
Median Trial	15.5
75th Percentile	17.7
90th Percentile	19.7
Highest Trial	21.7

Average Growth Rates in 10 years

Growth rates were obtained from running the model for 100 trials for four years under management options for each Alternative. As expected, the Alternative implementing fertility control reflects the lowest overall growth rate. The type of gather (gatecut vs. selective removal) seems to have minimal impacts to the growth rates as there are little differences between Alternatives I and 2. The range of growth rates are reasonable and do not indicate that any of the Alternatives would result in growth rates that are so low as to put the population at risk.

Growth Rate Graph

The growth rate graph shows the distribution of average growth rate across all trials in graphical format. Each point on the graph represents one of the 100 trials run for each simulation. The direct effects of removals are not counted in computing annual growth rates, although a selective

removal may change the average foaling rate or survival rate of individuals in the population and may indirectly affect the growth rate. Fertility control was reflected in a reduction of population growth rate compared to the observed rate from previous censuses.
The following graphs illustrate the results obtained from the model for growth rates for each Alternative.

Proposed Alternative I
Population Modeling Summary
To summarize the results obtained by simulating the range of Alternatives for the CHMA wild horse gather, the original questions can be addressed.
• Do any of the Alternatives "crash" the population?

None of the Alternatives indicate that a crash is likely to occur to the population under any of the Alternatives. Minimum population levels and growth rates are all within reasonable levels, and adverse impacts to the population are not likely.
• What effect does fertility control have on population growth rate?

As expected, the Alternative implementing fertility control reflects the lowest overall growth rate of 14.5%.
• What effect do the different Alternatives have on the average population size?

The growth rate does not vary between the different alternatives that do not include fertility control. Growth is similar between Alternative I and Alternative II. Populations of wild horses have the capacity to increase at rates as high as 30% per year. Research has shown that unmanaged populations of wild horses and might eventually stabilize (due to density-dependent regulatory mechanisms) at very high numbers, near what is known as their food-limited ecological carrying capacity. At these levels, however, the herds would show obvious signs of ill-fitness including poor individual animal condition, low birth rates, and high mortality rates in all age classes due to disease and/or increased vulnerability to predation. In addition, supporting range conditions would be noticeably deteriorated, with much of the available habitat showing symptoms of irreparable over-grazing. The CHMA is currently managed to allow the herd to be healthy with strong foal production and survival rates. The model analysis indicates that with fertility control and removals, production rates would decline and the herds could be managed at a four year gather cycle.

Appendix E: Protocol and Ground Rules Daily Visitation Protocol and Ground Rules for the Challis Wild Horse Gather

BLM recognizes and respects the right of interested members of the public and the press to observe the Challis wild horse gather. At the same time, BLM must ensure the health and safety of the public, BLM's employees and contractors, and America's wild horses. Accordingly, BLM developed these rules to maximize the opportunity for reasonable public access to the gather while ensuring that BLM's health and safety responsibilities are fulfilled. Failure to maintain safe distances from operations at the gather and temporary sorting/holding sites could result in members of the public inadvertently getting in the path of the wild horses or gather personnel, thereby placing themselves and others at risk, or causing stress and potential injury to the wild horses.

General Daily Protocol

A Wild Horse Gather Information Phone Line will be set up prior to the gather so the public can call for daily updates on gather information and statistics. Visitors are strongly encouraged to check the phone line the evening before they plan to attend the gather to confirm the gather and their tour of it is indeed taking place the next day as scheduled (weather, mechanical issues or other things may affect this) and to confirm the meeting location.

Visitors must direct their questions/comments to either their designated BLM representative or the BLM spokesperson on site, and not engage other BLM/contractor staff and disrupt their gather duties/responsibilities - professional and respectful behavior is expected of all. BLM may make the BLM staff available during down times for a Q&A session on guided pubic-observation days. However, the contractor and its staff will not be available to answer questions or interact with visitors.

Observers are prohibited from riding in government and contractor vehicles and equipment.

Observers must provide their own 4-wheel drive high clearance vehicle, appropriate shoes, winter clothing, food and water.

Gather operations may be suspended if bad weather conditions create unsafe flying conditions.

BLM will establish one or more observation areas, in the immediate area of the gather and sorting/holding sites, to which individuals will be directed. These areas will be placed so as to maximize the opportunity for public observation while providing for a safe and effective horse gather. The utilization of such observation areas is necessary due to the use and presence of heavy equipment
and aircraft in the gather operation and the critical need to allow BLM personnel and contractors to fully focus on attending to the needs of the wild horses while maintaining a safe environment for all involved. In addition, observation areas will be located so as to protect the wild horses from being spooked, startled or impacted in a manner that results in increased stress.

BLM will delineate observation areas with yellow caution tape (or a similar type of tape or ribbon).

Visitors will be assigned to a specific BLM representative on guided-observation days and must stay with that person at all times.

Visitors are **NOT** permitted to walk around the gather site or temporary sorting/holding facility unaccompanied by their BLM representative.

Observers are prohibited from climbing/trespassing onto or in the trucks, equipment or corrals, which is the private property of the contractor.

When BLM is using a helicopter or other heavy equipment in close proximity to a designated observation area, members of the public may be asked to stay by their vehicle for some time before being directed to an observation area once the use of the helicopter or the heavy machinery is complete.

When given the signal that the helicopter is close to the gather site bringing horses in, visitors must sit down in areas specified by BLM representatives and must not move or talk as the horses are guided into the corral.

Individuals attempting to move outside a designated observation area will be requested to move back to the designated area or to leave the site. Failure to do so may result in citation or arrest. It is important to stay within the designated observation area to safely observe the wild horse gather.

Observers will be polite, professional and respectful to BLM managers and staff and the contractor/employees. Visitors who do not cooperate and follow the rules will be escorted off the gather site by BLM law enforcement personnel, and will be prohibited from participating in any subsequent observation days.

BLM reserves the right to alter these rules based on changes in circumstances that may pose a risk to health, public safety or the safety of wild horses (such as weather, lightening, wildfire, etc.).

Public Observation
Guided public observation will provide for interested members of the public to see the wild horse gather activities at a given site. On this day, BLM attempts to allow the public to get an overall sense of the gather process and has available staff who can answer questions that the public may have. The public may rendezvous at a designated place and may be escorted by BLM representatives to and from the gather site. If not escorted, maps and meeting locations/times will be provided.

The number of guided observation days, and which days they are, will be determined prior to the gather and will be announced through a press release and on the website. Interested observers should RSVP ahead through the BLM-Challis Field Office. A meeting place will be set for each observation day and the RSVP list notified. BLM representatives will escort observers on guided observation days to and from the gather site and temporary sorting/holding facility.

Appendix F: BLM Instruction Memorandum Number 2010-164

UNITED STATES DEPARTMENT OF THE INTERIOR BUREAU OF LAND MANAGEMENT WASHINGTON, D.C. 20240 http://www.blm.gov
July 22, 2010
In Reply Refer To:
4710 (260) P

EMS TRNASMISSION 07/23/2010
Instruction Memorandum No. 2010-164
Expires: 09/30/2011

To: All Field Officials (except Alaska)
From: Assistant Director, Renewable Resources and Planning
Subject: Public Observation of Wild Horse and Burro Gathers

Program Area: Wild Horse and Burro Program

Purpose: The purpose of this Instruction Memorandum (IM) is to establish policy for public observation of wild horse and burro (WH&B) gathers.

Policy/Action: The Bureau of Land Management's (BLM's) policy is to accommodate public requests to observe a gather primarily through advance appointment, on days and at times scheduled by the authorized officer. Planning for one public observation day per week is suggested.

Specific viewing opportunities will be based on the availability of staff with the necessary expertise to safely and effectively host visitors, as well as other gather-specific considerations (e.g., weather, terrain, road access, landownership). The public should be advised that observation days are tentative and may change due to unforeseen circumstances (e.g., weather, wildfire, trap relocation, equipment repair, etc.). To ensure safety, the number of people allowed per observation day will be determined by the District Manager (DM) and/or Field Office Manager (FM) in consultation with the Contracting Officer's Representative/WH&B Specialist (COR) for the gather.

The DM/FM has the primary responsibility for effectively planning and managing public observation of the gather operation. Advance planning will:
· Ensure that the public have opportunities to safely observe wild horse gathers;
· Minimize the potential for disruption of the gather's execution;
· Maximize the safety of the animals, visitors, and the BLM and contractor personnel;
· Provide for successful management of visitors; and
· Ensure preparedness in the event of unanticipated situations.
The authorized officer will consider the following when planning for public observation of WH&B gather operations. Also see Attachment 1 (Best Practices When Planning for Public Observation at Gathers).

A. Safety Requirements
During WH&B gathers, the safety of the animals, the BLM and contractor personnel, and the public is of paramount importance. Because of the inherent risk involved in working with WH&B, the public will not be allowed inside corrals or pens or be in direct contact with the animals. Viewing opportunities during the gather operation must always be maintained at a safe distance (e.g., when animals are being herded into or worked at the trap or temporary sorting/holding facility, including sorting, loading) to assure the safety of the animals, the BLM and contractor personnel, and the public.

Unless an emergency situation exists, the BLM's policy prohibits the transportation of members of the public in Government or Contractor-owned or leased vehicles or equipment. Therefore, observers are responsible for providing their own transportation to and from the gather site and assume all liability for such transportation.

The helicopter/aircraft is the private property of the gather contractor. Due to liability and safety concerns, Bureau policy prohibits observers from riding in or mounting cameras onto the aircraft. Should observers create unsafe flying and gathering conditions, for example, by hiring an aircraft to film or view a gather, the COR, in consultation with the gather contractor, will immediately cease gather operations.

The COR has the authority to stop the gather operation when the public engage in behavior that has the potential to result in harm or injury to the animals, employees, or other members of the public.

B. Planning for Public Observation at WH&B Gathers
During advance planning for public observation at WH&B gathers, the authorized officer should consult with the State External Affairs Chief or appropriate Public Affairs office. An internal communications plan will be developed for every gather (Attachment 2). It may also be helpful to prepare answers to frequently asked questions (Attachment 3).

C. Law Enforcement Plan
A separate Law Enforcement Plan should be developed if the need for law enforcement support is anticipated. The Law Enforcement Plan must be approved in advance by the Special Agent-In-Charge (SAC) or the State Staff Ranger of the State in which the gather is occurring.

D. Temporary Closure to Public Access
Under the authority of section 303(a) of the Federal Land Management and Policy Act (43 U.S.C. 1733(a)), 43 CFR 8360.0-7, and 43 CFR 8364.1, the authorized officer may temporarily close public lands within all or a portion of the proposed gather area to public access when necessary to protect the health and safety of the animals, the public, contractors and employees. Completion of a site-specific environmental analysis of the environmental impacts associated with the proposed closure and publication of a Federal Register Notice is required.

E. Gather Contract Pre-Work Conference
· Talk to the contractor about how many members of the public are expected and when. Discuss, and reach mutual agreement, about where best to position the public at the individual trap-sites to

allow the gather to be observed, while accomplishing the gather objectives and assuring the humane treatment of the animals and the safety of the BLM and contractor personnel, and public.
· No deviation from the selected viewing location(s) should be made, unless the gather operation is being adversely impacted. The COR will consult with the gather contractor prior to making any changes in the selected viewing locations.
· The BLM's policy prohibits it from ferrying observers in the helicopter or any other mode of conveyance unless an emergency situation exists. Review this policy with the contractor during the pre-work conference.

F. Radio Communication
· Assure there is effective radio communication between law enforcement personnel, gather COR or project inspectors (PIs), and other BLM staff.
· Identify the radio frequencies to be used.
· Communication with the gather contractor is through the BLM COR or PI, and from the gather contractor to the helicopter pilot. Direct communication between BLM personnel (other than the COR) and the helicopter pilot is not permitted, unless agreed upon by the BLM authorized officer and the contractor in advance, or the pilot is requesting information from the COR.

G. Pre- and Post-Action Gather Briefings
· Pre-briefings conducted by knowledgeable and experienced BLM staff can be helpful to the public.
· The pre-gather briefing is an opportunity to explain what individuals will see, why the BLM is conducting the gather, how the animals will be handled, etc.
· Post-action briefings may also be helpful in interpreting and explaining what individuals saw, what happened, why certain actions were taken, etc.

H. Summary of Individual Roles and Responsibilities
1. District and/or Field Office Managers
DMs and/or FMs are responsible for keeping the State Director and State WH&B Lead fully informed about the gather operation. Included is working with State/local public affairs staff to prepare early alerts if needed. An additional responsibility is determining if a law enforcement presence is needed.
2. Public Affairs Staff
The local district/field office public affairs staff is responsible for working with the COR, DM/FM, other appropriate staff, the State WH&B Program Lead, and the State Office of Communications to implement the communications strategy regarding the gather.
3. Law Enforcement
Develop and execute the law enforcement plan in consultation with District/Field Office Managers, the COR/PI, and the State's Special Agent-In-Charge or State Staff Ranger.
4. Contracting Officer's Representative (COR)/Project Inspectors (PIs)
The COR and the PI's primary responsibility is to administer the contract and manage the gather. A key element of this responsibility is to assure the safe and humane handling of WH&B. The COR is also responsible for working closely with the DM/FM and Public Affairs Staff to develop the communication plan, and for maintaining a line of communication with State, District, and Field Office managers, staff and specialists on the progress of, and any issues related to, the gather operation.

Timeframe: This instruction memorandum is effective immediately.

Budget Impact: Higher labor costs will be incurred while accommodating increased interest from the public to attend gather events. The budget impacts of unanticipated situations which can occur during WH&B gathers include substantial unplanned overtime and per diem expense. Through advance planning, necessary support staff can be identified (e.g., law enforcement, public affairs, or other BLM staff) and the cost-effectiveness of various options for providing staff support can be evaluated. In situations where public interest in a gather operation is greater than anticipated, the affected state should coordinate with the national program office and headquarters for assistance with personnel and funding.

Background: Heightened interest from the public to observe WH&B gathers has occurred. Advance planning for public observation of gather operations can minimize the potential for unanticipated situations to occur during WH&B gathers and assure the safety of the animals, the BLM and contractor personnel, and the public.

Manual/Handbook Sections Affected: No change or affect to the BLM manuals or handbooks is required.

Coordination: This IM was coordinated among WO-200 and WO-260 staff, State WH&B Program Leads, field WH&B Specialists, public affairs, and law enforcement staff in the field.

Contact: Questions concerning this policy should be directed to Susie Stokke in the Washington Office at (202) 912-7262 or Lili Thomas in the National Program Office at (775) 861-6457.

Signed by:
Bud C. Cribley
Acting, Assistant Director Division of IRM
Governance, WO-560
Renewable Resources and Planning

Authenticated by:
Robert M. Williams

Appendix G: Federal Aviation Administration

General Operating and Flight Rules Sec. 91.119

Part 91 GENERAL OPERATING AND FLIGHT RULES
Subpart B--Flight Rules General
Sec. 91.119
Minimum safe altitudes: General.

Except when necessary for takeoff or landing, no person may operate an aircraft below the following altitudes:
(a) Anywhere. An altitude allowing, if a power unit fails, an emergency landing without undue hazard to persons or property on the surface.

(b) Over congested areas. Over any congested area of a city, town, or settlement, or over any open air assembly of persons, an altitude of 1,000 feet above the highest obstacle within a horizontal radius of 2,000 feet of the aircraft.

(c) Over other than congested areas. An altitude of 500 feet above the surface, except over open water or sparsely populated areas. In those cases, the aircraft may not be operated closer than 500 feet to any person, vessel, vehicle, or structure.

(d) Helicopters, powered parachutes, and weight-shift-control aircraft. If the operation is conducted without hazard to persons or property on the surface—

(1) A helicopter may be operated at less than the minimums prescribed in paragraph (b) or (c) of this section, provided each person operating the helicopter complies with any routes or altitudes specifically prescribed for helicopters by the FAA; and

(2) A powered parachute or weight-shift-control aircraft may be operated at less than the minimums prescribed in paragraph (c) of this section.]
Amdt. 91-311, Eff. 4/2/10

Appendix H. Water Quality and Macroinvertebrate Data

The inventory of streams pursuant to section 305(b) of the CWA (Clean Water Act 33 USC §1251 et seq. 1972) is accomplished using quantitative criteria set forth in the IDWQS (Idaho Water Quality Standards, Idaho Code 58.01.02) and the Idaho Small Stream Ecological Assessment Framework (IDEQ, 2002). The assessments typically involve collection of habitat data, macroinvertebrate samples and an electro-fish sample. These data are then in turn used to derive stream habitat (SHI), macroinvertebrate (SMI) and fish (SFI) indexes, these indexes are then used to calculate a 1 – 3 score based upon bioregion with 3 being the highest score, the specific criteria is given in Table 27 below. The scores for each index are then averaged. A stream with an average score greater than or equal to two is considered fully functioning. Due to the large scope of assessing all of the water bodies of the state the IDEQ (Idaho Department of Water Quality) have divided these bodies into lumped assessment units. Many of these assessment units have multiple monitoring, if any of the assessment sites do not meet the criteria for being fully functioning the entire assessment unit is considered impaired and consequently 303(d) listed.

Table 27. Index Scoring Criteria Based upon Bioregion

	Bioregion Classification			
Condition Category	**Northern Mountains**	**Central and Southern Mountains**	**Basins**	**Condition Rating**
SMI Bioregion Scoring Criteria				
Above the 25th percentile of reference condition	≥65	≥59	≥51	3
10th to 25th percentile of reference condition	57-64	51-58	43-50	2
Minimum to 10th percentile of reference condition	39-56	33-50	33-42	1
Below minimum of reference condition	<39	<33	<33	Minimum Threshold
SFI Bioregion Scoring Criteria				
	Rangeland	Forest		
Above the median of reference condition	≥82	≥81		3
25th percentile to median of reference condition	62-81	67-80		2

Table 27. Index Scoring Criteria Based upon Bioregion				
	Bioregion Classification			
	Northern Mountains	**Central and Southern Mountains**	**Basins**	**Condition Rating**
5th to 25th percentile of reference condition	39-61	34-66		1
Below 5th percentile of reference condition	<39	<34		Minimum Threshold
SHI Bioregion Scoring Criteria				
	Northern Rockies	Northern Basin and Range	Snake River Basin/ High Desert	
Above 25th percentile of reference condition	≥66	≥63	≥58	3
10th to 25th percentile of reference condition	58-65	50-62	55-57	2
below 10th percentile of reference condition	<58	<50	<55	1

Table 28. Stream Index Data for CHMA from IDEQ										
Stream	Assessment Unit	Date	Flow (CFS)	SMI Bioregion	SMI/ Score	SHI Bioregion	SHI / Score	SFI Bioregion	SFI/ Score	Average Score
Lone Pine Cr. upper	Warm Springs Cr., Hole in the Rock Cr. - Mouth	6/3/2008	0							
Lone Pine Cr. Lower	Warm Springs Cr., Hole in the Rock Cr. - Mouth	6/3/2008	0							
Warm Springs Cr.	Warm Springs Cr., Hole in the Rock Cr. - Mouth	6/29/1995	3.5		Macro-invertebrates collected but no MBI score reported					
Warm Springs Cr.	Warm Springs Cr., Source to Hole in the Rock Cr.	7/13/2005	0							
Broken Wagon Cr. Source to Mouth	Broken wagon Cr. Source to Mouth	6/3/2008	0							
Corral Basin Cr.	Corral Basin Cr. Source to Mouth	7/3/2007	0*							
Horse Basin Cr. upper	Horse Basin Cr. Source to Mouth	6/3/2008	0*							
Horse Basin Cr. lower	Horse Basin Cr. Source to Mouth	7/26/2006	0*							

Table 28. Stream Index Data for CHMA from IDEQ										
Stream	Assessment Unit	Date	Flow (CFS)	SMI Bioregion	SMI/ Score	SHI Bioregion	SHI / Score	SFI Bioregion	SFI/ Score	Average Score
Horse Basin near Corral Basin Cr. Confluence	Corral Basin Cr. Source to Mouth	9/12/2001	0							
Horse Basin Near Road Cr.	Corral Basin Cr. Source to Mouth	7/7/1997	2.5		Macro-invertebrates collected but no MBI score reported				Fish Sampled no SFI reported	
Bear Cr. near conflu. with Road Cr.	Road Cr. Source to Conflu. with Horse Basin Cr.	8/4/1998	3.38		Macro-invertebrates collected but no MBI score reported				Fish Sampled no SFI reported	
Mosquito Cr. near conflu. with Road Cr.	Mosquito Cr. Source to Mouth	8/4/1998	1.13		Macro-invertebrates collected but no MBI score reported				Fish Sampled no SFI reported	
Mosquito Cr. near conflu. with Road Cr.	Mosquito Cr. Source to Mouth	8/17/2004	0*							
Road Cr. 1/2 mile dwnstrm spring source	Road Cr. Source to Conflu. with Horse Basin	6/27/1995	0.5		Macro-invertebrates collected but no MBI				Fish Sampled no SFI reported	

Table 28. Stream Index Data for CHMA from IDEQ										
Stream	Assessment Unit	Date	Flow (CFS)	SMI Bioregion	SMI/ Score	SHI Bioregion	SHI / Score	SFI Bioregion	SFI/ Score	Average Score
	Cr.				score reported					
Road Cr. dwnstrm of Bear Cr. Conflu.	Road Cr. Source to Conflu. with Horse Basin Cr.	8/11/1997	1.1		Macro-invertebrates collected but no MBI score reported				Fish Sampled no SFI reported	
Road Cr. Dwnstrm of Mosquito Cr. Conflu.	Road Cr. Source to Conflu. with Horse Basin Cr.	7/7/1997	5.1		Macro-invertebrates collected but no MBI score reported				Fish Sampled no SFI reported	
Road Cr. upstrm of Chicken Cr. Conflu.	Road Cr. Source to Conflu. with Horse Basin Cr.	8/1/2006	2.29	Central and Southern Mountains	70.33/ 3	Northern Mountains	51.0/ 1	Forested	61.42 /1	1.67
Road Cr. Upstrm of Pvt. Land Boundary	Road Cr. Conflu. with Horse Basin Cr. to Mouth	9/11/2001	1.68	Central and Southern Mountains	60.70/ 3	Northern Mountains	63.0/ 2			2.5
Road Cr. Upstrm of Pvt. Land Boundary	Road Cr. Conflu. with Horse Basin Cr. to Mouth	8/13/2003	0							

Table 28. Stream Index Data for CHMA from IDEQ										
Stream	Assessment Unit	Date	Flow (CFS)	SMI Bioregion	SMI/ Score	SHI Bioregion	SHI / Score	SFI Bioregion	SFI/ Score	Average Score
Unnamed Trib. to Road Cr. near Pvt. Boundary	Road Cr. Conflu. with Horse Basin Cr. to Mouth	6/3/2008	0							
Road Cr. Upstrm of Conflu. East Fork Salmon River	Road Cr. Conflu. with Horse Basin Cr. to Mouth	7/29/2010	1.33							
Spar Canyon Upper	Spar Canyon Source to Mouth	6/3/2008	0							
Spar Canyon Lower	Spar Canyon Source to Mouth	6/3/2008	0							
Bradshaw Gulch Creek near East Fork	Bradshaw Gulch Creek Source to Mouth	6/3/2008	0							
Unnamed Tributary to Salmon River	Salmon River East Fork to Garden Creek	7/2/2007	0							
* **Site Listed as inaccessible**										

Table 29. Sage Creek IDEQ Beneficial Use Reconnaissance Program Data: 1st Order N. Fork and Main Sage Creek above Corral

Burp Site location	SMI Score	SHI Score	Flow	Date Sampled
1 mi. above Forks	3	3	8.16	6/29/1998
Below Corral Cr.	3	3	2.56	6/29/1998

Burp Site location	SMI Score	SHI Score	Flow	Date Sampled
North Fk. 200m above Forks	2	3	2.75	6/29/1998

Table 30. Water Quality Data for CHMA Streams										
Site Name	Time	Date	Easting	Northing	TempC	Specific Conductance µs/cm	SAL PPT	PH	FLOW (CFS)	Comment
Road Creek	14:20	20100830	721256	4896256	11.1	198.9	0.13	8.14		Above private property boundary
Road Creek KA1	14:00	6/15/2011	725207.0	4894276.0	10	140.1	0.07		11.35	Flow Tracker used to Measure Flow
Horse Basin Creek KA1		11/1/2011	725677	4896722	0.8	349.3	0.17	7.39		
Horse Basin Creek KA2	10:45	11/1/2011	732079	4896002	0.2	281.1	0.13	7.96		

Table 31 summarizes multiple metrics from fixed-area quantitative aquatic benthic macroinvertebrate sampling conducted by the BLM Challis Field Office within the CHMA. Sample processing, organism identification, method development, and calculation of metrics conducted by the National Aquatic Monitoring Center, Utah State University, Logan, UT (NAMC, 2009). * = Site is located outside of the CHMA, but within the cumulative analysis area. The stream and location where sample reach occurred are presented with associated metrics: *Richness = an estimate of community structure and stream health based on the number of distinct taxa; # of Families = Similar to richness it is the number of taxanomic groups (families) present in the sample; # of EPT Taxa = the number of distinct taxa within the insect Orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddis flies) commonly considered sensitive to pollution; # of Intolerant Taxa & # of Tolerant Taxa= The Hilsenhoff Biotic Index [(HBI) (Hillsenhoff 1987)] summarizes the overall pollution tolerances of the taxa collected, and has been used to detect nutrient enrichment, high sediment loads, low dissolved oxygen, and thermal impacts. Taxon HBI values can also be used to determine the number of pollution intolerant and tolerant taxa occurring at a site, where taxa with HBI values < 2 were considered intolerant clean water taxa and taxa with HBI values > 8 were considered pollution tolerant taxa; # of Clinger Taxa = The number of taxa characterized as those which generally cling to the tops of rocks and can be reduced by sedimentation or abundant algal growths.*

Table 31. Macroinvertebrate Sampling Within the CHMA Summary							
Stream (Location)	**Date**	**Richness**	**# of Families**	**# of EPT taxa**	**# of intolerant taxa**	**# of tolerant taxa**	**# of clinger taxa**
Bear Creek (BRC-KA-01)	8/16/1994	19	16	11	6	0	9
	8/16/1995	18	15	9	5	0	6
	8/18/1996	18	16	10	4	1	8
	8/18/1997	23	20	15	9	1	9
	8/12/2008	27	20	17	9	1	11
Horse Basin Creek (HBC-KA-01)	8/23/1993	18	16	7	4	0	6
	8/2/1994	15	12	5	3	0	8
	8/9/1995	16	14	6	4	0	7
	8/8/1996	17	15	8	5	0	6
	8/5/1997	17	16	6	4	0	9
	8/13/2008	23	18	7	5	1	6
Horse Basin Creek (HBC-KA-02)	9/23/1995	20	17	12	6	0	12
	8/13/1996	19	16	12	6	0	11
	8/12/1997	16	15	9	5	1	8
	8/6/2008	28	19	13	6	1	13
Lower Horse Basin Creek (LHB-KA-01)	8/8/1996	19	17	6	4	0	6
	8/5/1997	22	19	7	5	0	7
	8/6/2008	30	22	12	7	0	16
Mosquito Creek (MQC-KA-01)	7/27/1994	17	15	9	4	1	4
	7/27/1995	20	17	12	6	0	6
	7/31/1996	17	13	7	3	0	6
	8/7/1997	25	23	15	9	0	9
	8/15/2008	30	25	15	9	1	11
North Fork Sage Creek (NFS-KA-01)	8/5/2008	36	23	21	14	0	18
Road Creek (RC-KA-01)	8/2/1995	18	13	10	8	0	9
	8/8/1996	16	12	6	4	1	10
	8/7/1997	17	13	8	4	0	11
	8/11/2008	21	15	10	8	1	11
Road Creek (RC-KA-02)	8/2/1995	17	15	8	6	0	6
	8/8/1996	18	14	10	6	0	11

Table 31. Macroinvertebrate Sampling Within the CHMA Summary							
Stream (Location)	**Date**	**Richness**	**# of Families**	**# of EPT taxa**	**# of intolerant taxa**	**# of tolerant taxa**	**# of clinger taxa**
	8/5/1997	17	13	8	6	0	11
	8/11/2008	27	19	13	10	1	14
Road Creek above Bear Creek confluence (RCABEAR)	8/7/2008	29	22	16	12	0	18
Road Creek (RC-KA-03)	8/3/1995	14	12	6	3	0	6
	8/8/1996	13	12	7	3	0	5
	8/5/1997	17	15	7	4	0	6
	8/5/2008	28	19	11	5	1	9
Road Creek (RC-KA-04)	8/19/1994	23	18	6	3	0	4
	8/21/1995	16	14	3	0	0	3
	8/5/2008	17	12	7	4	1	4

Appendix I Annual Indicator Monitoring

2001

Horse Basin grazing AUM Total - 475 from 7/16 – 8/7

- 7/23/2001 – 232 Horses Counted
- March 30, 2001 – Use along Horse Basin Creek was observed to have exceeded use standards. This was a pre livestock assessment
  July 12, 2001 HBC-KA1, 7.1" stubble height & HBC-KA2, 10.1" stubble height
  July 27, 2001 HBC-KA2, 7" stubble height
  Aug 15, 2001 HBC-KA1, 3.5" stubble height, 5% shearing- livestock use not apparent
  HBC-KA2, 5" stubble height, less than 5% shearing
  Oct 12, 2001 HBC-KA2, 2" stubble height
  Oct 16, 2001 HBC-KA1, 3" stubble height, less than 5% shears

2002

- Horse Basin Rested – 0 cattle AUMs
- 7/25/2002 – 251 horses Counted
- July 16, 2002 HBC-KA2, 8.5" Stubble Height

2003

- Horse Basin grazing AUM Total - 519 from 6/16 – 7/11
- 8/28/2003 – 203 horses counted
- June 17, 2003 LHB-KA1, 6" stubble height
- June 17, 2003 HBC-KA1, 12" stubble height
- June 17, 2003 HBC-KA2, 8" stubble height
- July 1, 2003 LHB-KA1, 4" stubble height
- July 1, 2003 HBC-KA2, 4" stubble height
- Oct. 23, 2003 LHB-KA1, 4" stubble height, 15% bank shears – horse use was apparent
- Oct. 23, 2003 HBC-KA2, 2" stubble height, 26% bank shears – exceedance of 3" stubble height and 5% bank shears attributed to wild horse use

2004

- Horse Basin grazing AUM Total - 609 from 6/21 – 7/12
- 6/28/2004 – 241 horses counted
- July 28, 2004 HBC-KA2, 4" stubble height
- Oct. 12, 2004 LHB-KA1, 3.5" stubble height, 19% shears – very little livestock sign; exceedance of 5% attributed to wild horse use
- Oct. 12, 2004 HBC-KA1, 12+" stubble height, 0% shears

2005

- Horse Basin grazing AUM Total - 383 from 7/16 – 8/11
- 9/29/2005 – 208 horses counted
- Aug. 31, 2005 HBC-KA2, 6" stubble height, slight use on willows
- Oct. 18, 2005 HBC-KA2, 3" stubble height, light to moderate use on willows

2006

- Horse Basin Rested – however cattle trailing occurred through this pasture
- 9/21/2006 – 231 horses counted
- June 21, 2006 HBC-KA2(Upstream)- 6" stubble height, alteration 3.6%, shearing <1%
- June 21, 2006 HBC-KA2(Downstream)- 5" stubble height, alteration 9.6%, shearing 5%
- Oct. 11, 2006 HBC-KA2 - 2" stubble height, 13.6% alteration, shearing <5%

2007

- Horse Basin grazing AUM Total - 618, from 6/16-7/13 use was focused in upper horse basin
- 9/2007 – 360 horses estimated
- July 17, 2007 HBC-KA2 – 4.1" stubble height, alteration 40%, slight use on willows
- Nov 20, 2007 HBC-KA2 – 2" stubble height, alteration not measured, slight use on willows

2008

- Horse Basin grazing AUM Total – 691, from 7/13-7/29
- 9/18/2008 – 413 horses counted

2009

- Horse Basin grazing AUM Total – 378, from 7/13 - 8/1
- 9/2009 224 - horses estimated
- 7/16/2009 HBC-KA2 – 5" stubble height, Alterations were 24% on right bank and 22.5% on left bank (measures taken prior to grazing indicating horse use)
- 9/11/2009 HBC-KA2 – 3.5" stubble height

2010

- Horse Basin Rested, 73 grazing AUMS attributed to trailing on 7/14-7/16
- 9/2010 263 - horses estimated
- 8/10/2010 HBC-KA1 – 12" plus stubble height, alterations not measured
- 11/03/2010 HBC-KA2 – 4.1" left bank & 3.1" right bank stubble height, 39% alterations

2011

- Horse Basin Grazing AUM Total – 606, 7/12 – 8/8
- 9/2011 275 - horses estimated
- 6/29/2011 HBC-KA1
- 6/29/2011 HBC-KA2
- 8/25/2011 HBC-KA1
- 8/25/2011 HBC-KA2
- 11/1/2011 HBC-KA1 – 5.3" stubble height, 12% alterations
- 11/1/2011 HBC-KA2 – 4.7" stubble height, 25% alterations
- 11/4/2011 LHB-KA1 – 3.9" stubble height, 31% alterations

U.S. Department of the Interior
Bureau of Land Management
Final Environmental Assessment
DOI-BLM-UT-W020-2015-0017-EA

Population Control Research Wild Horse Gather for the Conger and Frisco Herd Management Areas

May 2016

U.S. Department of the Interior
Bureau of Land Management
West Desert District
Fillmore Field Office
Cedar City Field Office

BLM

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1.0 Purpose of and Need for the Proposed Action

1.1 Introduction

This Environmental Assessment (EA) has been prepared to disclose and analyze the environmental consequences of the Population Control Research Wild Horse Gather for the Conger and Frisco Herd Management Areas (HMAs) as proposed by the Bureau of Land Management's Fillmore and Cedar City field offices and in cooperation with United States Geological Survey (USGS) Fort Collins Science Center and Colorado State University (CSU). The EA is a site-specific analysis of potential impacts that could result in the implementation of a proposed action or alternatives to the proposed action. The EA assists the BLM in project planning and ensuring compliance with the National Environmental Policy Act (NEPA), and in making a determination as to whether any "significant" impacts could result from the analyzed actions. "Significance" is defined by NEPA and is found in regulation 40 CFR 1508.27. An EA provides evidence for determining whether to prepare an Environmental Impact Statement (EIS) or a statement of "Finding of No Significant Impact" (FONSI). If the decision maker determines that this project has "significant" impacts following the analysis in the EA, then an EIS would be prepared for the project. If not, a Decision Record may be signed for the EA approving the selected alternative, whether the proposed action or another alternative. A Decision Record (DR), including a FONSI statement, documents the reasons why implementation of the selected alternative would not result in "significant" environmental impacts (effects) beyond those already addressed in the Warm Springs Resource Area Proposed Resource Management Plan and Final Environmental Impact Statement (RMP/EIS), 1986 and Pinyon Management Framework Plan/Final EIS, 1983.

1.2 Background

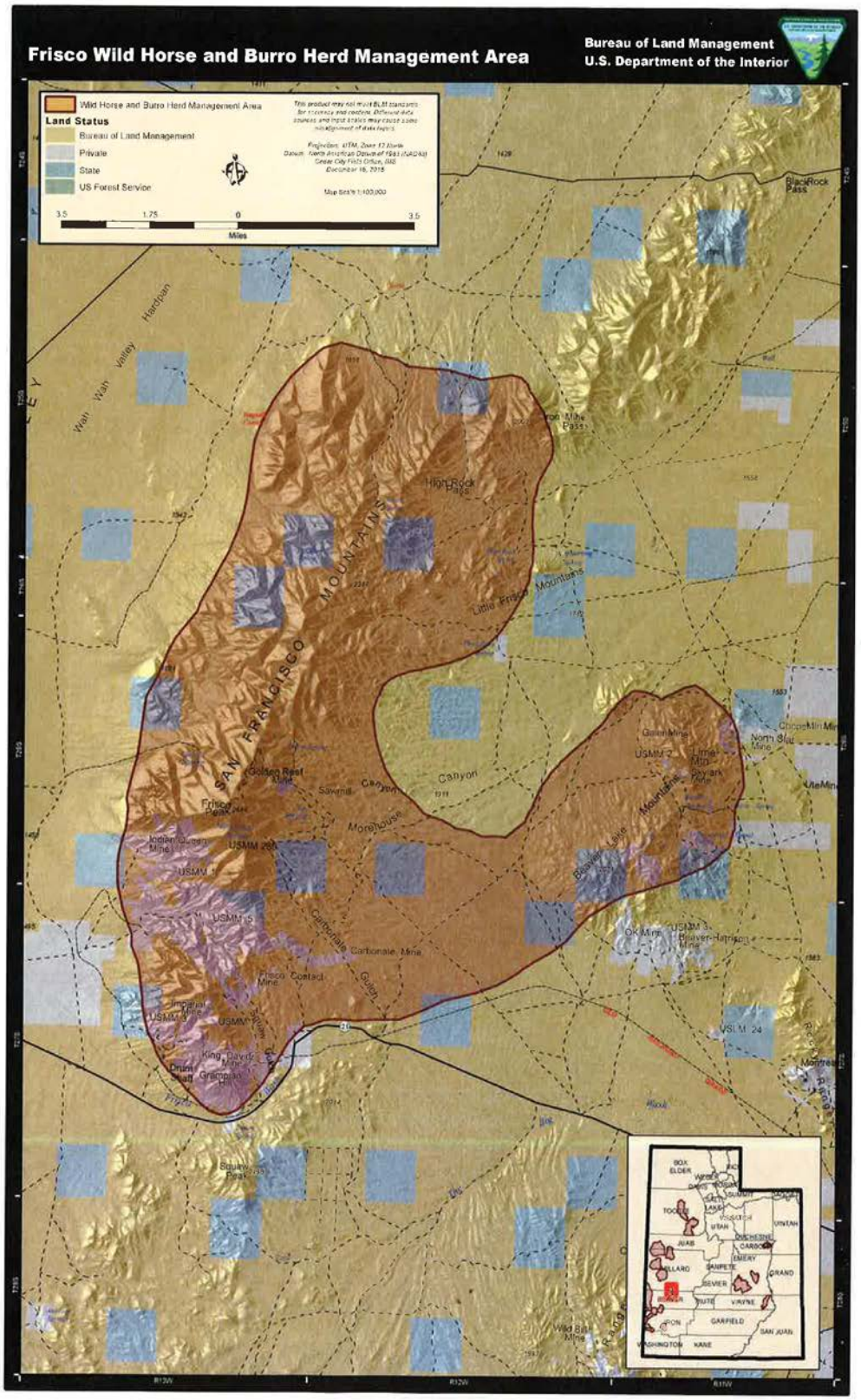
Since the passage of the Wild Free-Roaming Horses and Burros Act (WFRHBA) of 1971, BLM has refined its understanding of how to manage wild horse population levels. By law, BLM is required to control any overpopulation, by removing excess animals, once a determination has been made that excess animals are present and removal is necessary. Program goals have always been to establish and maintain a "thriving natural ecological balance," which requires identifying the Appropriate Management Level (AML) for individual herds. In the past two decades, goals have also explicitly included conducting gathers and applying contraceptive treatments to achieve and maintain wild horse populations within the established AML, so as to manage for healthy wild horse populations and healthy rangelands. Management actions resulting from shifting the program emphasis include the use of fertility control, and adjusting sex ratios. Both of these management actions can reduce total population growth rates in the short term, and increase gather intervals. Other efforts include improving the accuracy of population inventories and collecting genetic baseline data to support genetic health assessments. Decreasing the numbers of excess wild horses removed while also reducing population growth rates and ensuring the welfare of wild horses on the range are all consistent with findings and recommendations from the National Academy of Sciences (NAS), American Horse Protection Association (AHPA), the American Association of Equine Practitioners (AAEP), Humane Society of the United States (HSUS), Government Accountability Office (GAO), Office of Inspector General (OIG) and current BLM policy. BLM's management of wild horses must also

be consistent with Standards and Guidelines for Rangeland Health and for Healthy Wild Horse Populations developed by the Northeastern Great Basin Resource Advisory Council (RAC).

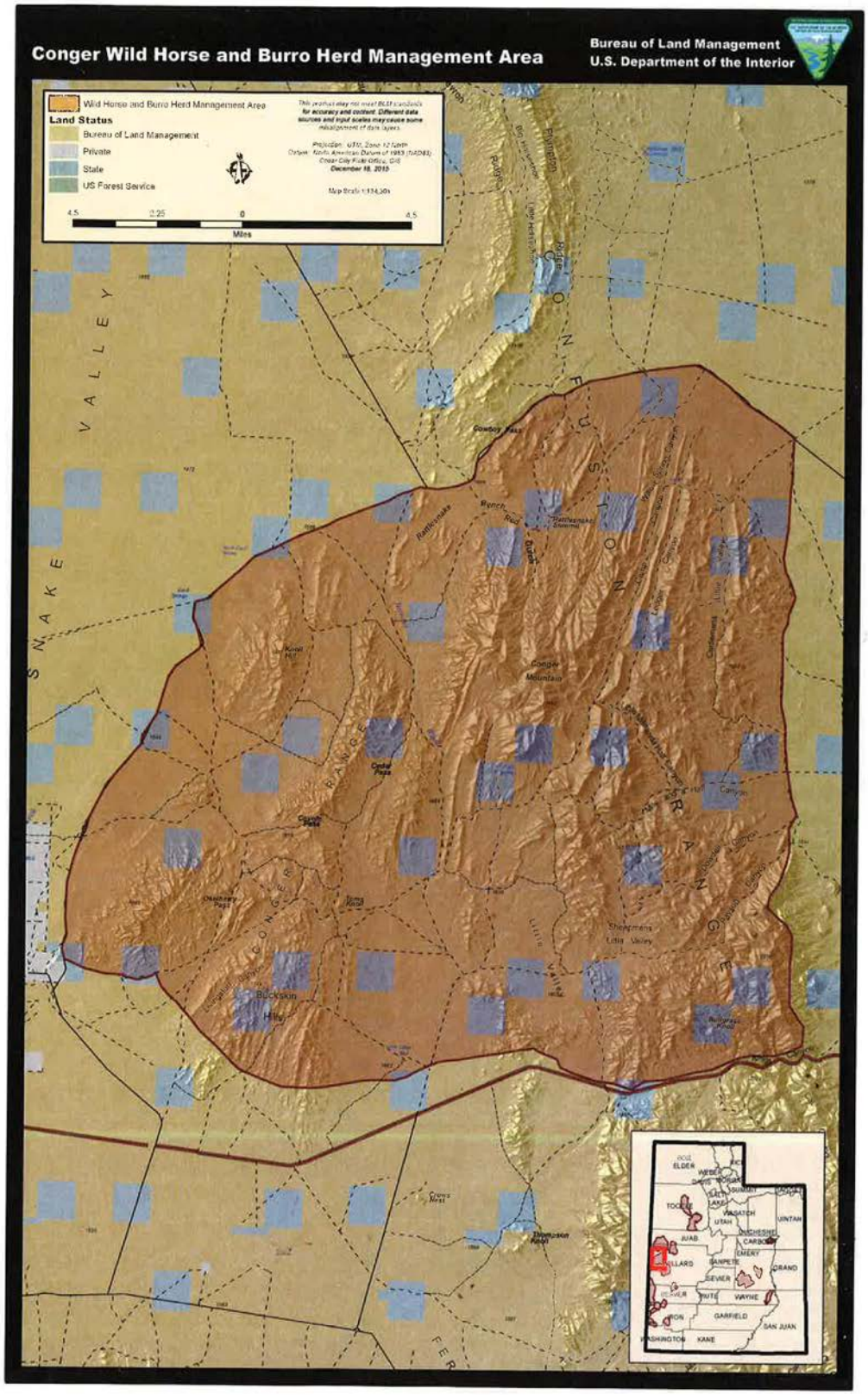
The current estimated population of wild horses in the Frisco HMA (Map A) is 288 (see Appendix I). This number is based on the Utah BLM's population statistics (2/28/2016) compiled from the most recent aerial survey of the HMA that took place in February 2016. The current population is about 4 times over the AML upper limit. It was last gathered in December of 2012.

The current estimated population of wild horses in the Conger HMA (Map B) is 310 (see Appendix I). This number is based on the Utah BLM's population statistics (2/28/2016) compiled from the most recent aerial survey of the HMA that took place in February 2016. The current population is about 4 times over the AML upper limit. It was last gathered in September 2010. Since the passage of the WFRHBA, management knowledge regarding horse population levels has increased. For example, wild horses are capable of increasing numbers 15-20% annually (NAS 2013), resulting in the doubling of wild horse populations about every 3 years.

MAP 1


Frisco Wild Horse and Burro Herd Management Area
Bureau of Land Management
U.S. Department of the Interior
Wild Horse and Burro Herd Management Area
Land Status
Bureau of Land Management
Private
State
US Forest Service
Miles
SAN FRANCISCO MOUNTAINS
High Rock Pass
Little Frisco Mountains
Canyon
Morehouse
Carbonate Mine
Frisco Contact Mine
Beaver Lake Mountains
Wah Wah Valley Hardpan
Black Rock Pass
Squaw Peak
Wild Bill Mine

MAP 2


Conger Wild Horse and Burro Herd Management Area
Bureau of Land Management
U.S. Department of the Interior
Wild Horse and Burro Herd Management Area
Land Status
Bureau of Land Management
Private
State
US Forest Service
Miles
Conger Mountain
Buckskin Hills
Sheepmans Little Valley
Thompson Knoll
Crows Nest
Cedar Pass
Cowboy Pass
Rattlesnake
Ranch Rd
Knoll Hill
Oakhurst Pass
Toms Knoll
Little Valley
Canyon
Ridge
Plympton
Big Hollenbeck
Little Hollenbeck
Ridge
SNAKE VALLEY
CONFUSION RANGE
CONGER RANGE
ELDER
WEBER
DAVIS
MORGAN
SUMMIT
TOOELE
SALT LAKE
WASATCH
UTAH
UINTAH
DUCHESNE
JUAB
CARBON
EMERY
MILLARD
SANPETE
GRAND
SEVIER
BEAVER
PIUTE
WAYNE
IRON
GARFIELD
SAN JUAN
WASHINGTON
KANE

1.3 Need for the Proposed Action

This action is needed to achieve population sizes that would provide an appropriate sample size of study animals for both the treated and control populations for statistical analysis; remove wild horses from areas not designated for wild horse use; slow population growth rates; remove excess wild horses from within the HMA; protect rangeland resources from deterioration associated with an overpopulation of wild horses; achieve population sizes that are consistent with the established AMLs; and restore and maintain a thriving natural ecological balance and multiple use relationship on the public lands consistent with the provisions of Section 3(b) (2) of the WFRHBA. Furthermore, the action is needed to ensure current and future populations of healthy wild horses.[1]

1.4 Purpose of the Proposed Action

The purpose of the Proposed Action is to remove excess wild horses within the Conger Mountain and Frisco HMAs to bring the populations of both HMAs towards AML, while achieving a population of approximately 100 animals in first year on each HMA for the purpose of gathering data for USGS and CSU studies. The population size has been identified appropriate for a comparative statistical analysis for the proposed studies by USGS and CSU that would measure core demographic parameters. Any wild horses located outside the HMAs (in areas not designated for their use) would also be removed. In addition, the USGS research project would provide empirical data for population and ecosystem modeling, to improve future management of wild horses, and to contribute to a better understanding of the behaviors and ecology of wild horses. The behavioral studies at Conger HMA (treatment population) would inform BLM about the behavioral effects of gelding a portion of a wild horse herd. That type of information is not currently available. Studies at both Conger HMA and Frisco HMA would improve BLM's understanding of wild horse demography. The NAS committee recommended the use of statistical models for the understanding of herd demography, but little empirical data exists on which to build such models. The data collected from the studies would contribute to population dynamics models that may point to environmental factors that have the greatest influence on demographic parameters. The data would also be used to improve, update, and revise the WinEquus population model currently being used by BLM wild horse specialists. Following the completion of the research study, additional gathers in both HMAs would be necessary to remove excess wild horses to achieve a thriving ecological balance with multiple resources by achieving and maintain AMLs within the HMAs. Some gelded animals would remain on the Conger HMA as a non-reproductive segment of the population for further observation and to achieve management objectives.

---

1 The Interior Board of Land Appeals (IBLA) defined the goal for managing wild horse (or burro) populations in a thriving natural ecological balance as follows: "As the court stated in Dahl vs. Clark, supra at 594, the 'benchmark test' for determining the suitable number of wild horses on the public range is 'thriving natural ecological balance.' In the words of the conference committee which adopted this standard: 'The goal of WH&B management should be to maintain a thriving ecological balance (TNEB) between WH&B populations, wildlife, livestock and vegetation, and to protect the range from the deterioration associated with overpopulation of wild horses and burros.'"

1.5 Land Use Plan Conformance

The action alternatives are subject to the:

- ❒ Warm Springs Resource Area Resource Management Plan/Record of Decision Rangeland Program Summary (RMP/ROD), 1987.
- ❒ Pinyon Management Framework Plan/Final EIS, 1983.
- ❒ Frisco Herd Management Area Plan (HMAP), 2012.

The Pinyon MFP (RM 1.8, WH1.1…) states, "…remove horses as required to maintain horse numbers at or below 1982 inventory levels…". The MFP also states that the number of herd units and the population of each herd would depend on the results of monitoring studies, range condition, viewing opportunities, cooperative management, and range developments.

The Warm Springs Resource Area RMP states, "Horse numbers in the Conger Mountain…will be maintained near the following allocation levels. Horse numbers will be kept between 80 and 40 head in the Conger HMA…". The RMP also states that this will require periodic removals and that vegetation monitoring will be used to determine if management objectives are being met.

The Frisco HMAP states, "The current Appropriate Management Level (AML) for the area is 60 horses with not less than 30." This plans monitoring objectives includes monitoring; use of vegetation and waters by wild horses; individual and herd behavior; individual and herd health; movements of identified wild horses to determine use patterns and seasonal migrations and range of travel.

1.6 Relationship to Laws, Regulations, and Other Plans

The action alternatives are in conformance with the WFRHBA (as amended), applicable regulations at 43 CFR § 4700 and BLM policies. Included are:

❒ 43 CFR 4710.3-1 Herd Management Areas.
Herd management areas shall be established for the maintenance of wild horse and burro herds. In delineating each herd management area, the authorized officer shall consider the appropriate management level for the herd, the habitat requirements of the animals, the relationships with other uses of the public and adjacent private lands, and the constraints contained in 4710.4. The authorized officer shall prepare a herd management area plan, which may cover one or more herd management areas.

❒ 43 CFR 4710.4 Constraints on Management.
Management of wild horses and burros shall be undertaken with limiting the animals' distribution to herd areas. Management shall be at the minimum feasible level necessary to attain the objectives identified in approved land use plans and herd management area plans.

❒ 43 CFR 4720.1 Removal of excess animals from public lands.
Upon examination of current information and a determination by the authorized officer that an

excess of wild horses or burros exists, the authorized officer shall remove the excess animals immediately.

❒ 43 CFR 4740.1 Use of motor vehicles or aircraft.

(a) Motor vehicles and aircraft may be used by the authorized officer in all phases of the administration of the Act, except that no motor vehicle or aircraft, other than helicopters, shall be used for the purpose of herding or chasing wild horses or burros for capture or destruction. All such use shall be conducted in a humane manner.

(b) Before using helicopters or motor vehicles in the management of wild horses or burros, the authorized officer shall conduct a public hearing in the area where such use is to be made.

❒ All supplemental authorizations contained in Appendix 1 of the National NEPA Handbook 1790-1.

1.7 Scoping and Identification of Issues

Consultation and coordination with BLM, the Utah State Historic Preservation Office (SHPO), the Utah Division of Wildlife Resources (UDWR), US Fish & Wildlife Service (USFWS), Native American Indian tribes and routine business contacts with livestock operators and others, has underscored the need for the BLM to maintain wild horse and burro populations within the AML.

The Proposed Action was posted on the Electronic Notification Bulletin Board (ENBB) September 24, 2015 for public notification and then posted on the BLM's ePlanning site November 13, 2015. Responses were received from Utah's Public Lands Policy Coordinating Office and American Wild Horse Preservation Campaign.

The following issues were identified as a result of consultation/coordination and internal scoping relative to the BLM's management of wild horses in the planning area:

1. Impacts to individual wild horses and the herd. Measurement indicators for this issue include:
    - Expected impacts to individual wild horses from handling stress
    - Expected impacts to herd social structure
    - Expected effectiveness of proposed fertility control application
    - Potential effects to genetic diversity
    - Potential impacts to animal health and condition

2. A need to implement different or additional population control methods in order to maintain population size within AML over the long-term. Measurement indicators for the issue include:
    - Projected population size and annual growth rate (WinEquus population modeling)
    - Projected gather frequency
    - Projected number of excess animals to be removed and placed in the adoption, sale, and short and long-term holding pipelines over the next 10 years

3. Impacts to Rangeland Heath Standards/livestock grazing. Measurement indicators for this issue include:

- Expected forage utilization and potential competition for forage and water over time
- Potential impacts to Rangeland Health Standards/vegetation and resources

Impacts could also be expected to wildlife and safety. Other resources which were considered are described in Appendix E.

2.0 Proposed Action and Alternatives

2.1 Introduction

This section of the EA describes the Proposed Action and alternatives, including any that were considered but eliminated from detailed analysis. Three alternatives are considered in detail:

- **Alternative 1** Frisco HMA: **Proposed Action** – Capture wild horses in order to remove excess animals above the target research control population of 100 wild horses and establish a 50% male to female sex ratio and all age classes. A 5 year research study would be implemented. Target population would be above high AML. Following the completion of the research study, 2 to 3 additional gathers would be necessary to remove excess wild horses to achieve and maintain AML over the 10 year life of this document.
- **Alternative 1** Conger Mountain HMA: **Proposed Action** – Capture wild horses in order to remove excess animals above the target research treatment population of 100 and establish a 50% male to female sex ratio and all age classes. A 5 year research study would be implemented. A portion of the male population (up to 75%) would be treated (gelded) during a subsequent gather in Year 2 to evaluate the effects of maintaining a population of gelded males on the behavior and spatial ecology of the overall population, and to determine their health and short-term survival. Target population would be above high AML. Following the completion of the research study, 2 to 3 additional gathers would be necessary to remove excess wild horses to achieve and maintain AML over the 10 year life of this document.
- **Alternative 2** Conger and Frisco HMAs: **Removal Only** – Capture and remove excess animals to achieve and maintain AML. No research study would be implemented.
- **Alternative 3** Conger and Frisco HMAs: **No Action** — Defer gather, removal and research on these HMAs.

The Proposed Action and Alternative 2 were developed to respond to the identified resource issues and the Purpose and Need to differing degrees. The No Action Alternative would not achieve the identified Purpose and Need. However, it is analyzed in this EA to provide a basis for comparison with the other action alternatives, and to assess the effects of not conducting a gather at this time. The No Action Alternative is in violation of the WFRHBA which requires the BLM to immediately remove excess wild horses.

2.2 Description of Alternatives Considered in Detail

2.2.1 Alternative 1 -Proposed Action

Frisco HMA

The Proposed Action would reduce the population of wild horses on the Frisco HMA to 100 head by gather approximately 250 and removing approximately 175 head of excess wild horses from within and outside the HMA beginning on or about July 1, 2016. All adults would be freeze marked with a unique 4-digit hip mark to facilitate individual identification and monitoring. Population aerial inventories would be conducted prior to and after the gather and removal. Animals would be removed using a selective removal strategy that would allow for

success within the research study parameters without compromising the established herd dynamics. Approximately 75 of the captured wild horses would be released with an objective of establishing a 50%/50% male/female sex ratio and meeting the desired research population objective of 100 animals. Radio/GPS tags would be braided into their tails or manes on 20 adult males and radio/GPS collars or tags would be placed on 40 adult females and up to 15 yearling females (Year 1 of research study). Once tags are braided into the tails or manes they would be held in place with a non-toxic, low temperature curing epoxy resin. Mares collared for the multiple year study would be 3 years old or older. All mares being collared would be in Henneke body condition score 4 or greater. Animals that are "thin" (Henneke score of <3), deformed, or who have any apparent neck problems wouldn't be fitted with a collar. As tags are small (<200g) and are not worn around the neck they are considered of low burden to the animal, and therefore could potentially be worn by animals in lower boy condition. All collars would be fitted according to procedures outlined in Appendix H. All radio collars would have a manual release mechanism that can be remotely activated in case of emergency and a timed release mechanism which would be programmed to release at the end of the study period. No collars would remain on wild horses indefinitely. If the collar drop-off mechanism fails at the end of the study those individual horses would be captured and the collars manually removed. Each collared horse would be observed once a month while collared. Every effort would be made to release horses to the same general area from which they were gathered. Dependent foals would be released with their mare. Radio marked females would be monitored to check for foals. Foals and radio marked animals would be monitored through summer 2017 and data would additionally be recorded related to survival rates, movement rates, and spatial ecology. Global positioning system (GPS) and very high frequency (VHF) radio collars and tags can be used to provide high spatial and temporal resolution information for detecting free-roaming horse movement, locations and for other research purposes including but not limited to effectiveness of population inventories and demographics.

Year 2 (Oct 2017-Sept 2018)
Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. In the fall of 2017, collect fecal samples of radio marked mares to determine pregnancy; collect non-invasive fecal samples from foals to determine paternity in order to inform individual male reproductive success. In winter 2017/2018, conduct an aerial inventory to assess population size. In spring and summer 2018, monitor radio marked females to check for foals, and monitor foals and radio marked adults through the summer for survival, movement rates, and spatial ecology.

Year 3 (Oct 2018-Sept 2019)
Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. In the fall of 2018, collect fecal samples of radio marked mares to determine pregnancy; collect fecal samples from foals to determine paternity for male reproductive success analyses. In winter 2018/2019, conduct an aerial inventory to assess population size. In spring and summer 2019, monitor radio marked females to check for foals and monitor foals and radio marked adults throughout summer for survival, movement rates, and spatial ecology.

Year 4 (Oct 2019-Sept 2020)
Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. In the fall of 2019, collect fecal samples of radio marked mares to determine pregnancy; collect fecal samples from foals to determine paternity for male reproductive success analyses. In winter 2019/2020, conduct an aerial inventory to assess population size. In spring and summer 2020, monitor radio marked females to check for foals and monitor foals and radio marked adults through the summer for survival, movement rates, and spatial ecology.

Year 5 (Oct 2020-Sept 2021)
Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. In the fall of 2020, collect fecal samples of radio marked mares to determine pregnancy; collect fecal samples from foals to determine paternity. In winter 2020/2021, conduct an aerial inventory to assess population size. In spring and summer 2021, monitor radio marked females to check for foals and monitor foals and radio marked adults through the summer for survival and movements, and to check that radio collars drop off as programmed. The data would begin to be analyzed, population models would be developed, and results written up and submitted to peer-reviewed journals.

Year 6-10 (Oct 2021 – Sept 2026)
Upon the conclusion of the research study the BLM would conduct gathers to remove excess wild horses to achieve a thriving ecological balance with multiple resources by achieving and maintain AML. Gathers and management of the Frisco HMA would be in accordance with the Frisco HMAP where the sex ratio would be returned to a 60/40 male to female ratio pending study results. Study results would be used in future management objectives and incorporated in the Frisco HMAP. Several gathers may be necessary to achieve and maintain AML based on National holding space availability and National gather priorities.

Conger Mountain HMA
The Proposed Action would gather up to 300 and remove approximately 225 excess wild horses from within and outside the Conger Mountain Herd Management Area (HMA) beginning on or about July 1, 2016. All adults would be freeze marked with a unique 4-digit hip mark to facilitate individual identification and monitoring. Population aerial inventories would be conducted prior to and after the gather and removal. Animals would be removed using a selective removal strategy that would allow for success in achieving the necessary population parameters for the research study, without compromising the established herd dynamics. Approximately 75 of the captured wild horses would be released with an objective of establishing a 50%/50% male/female sex ratio and meeting the desired research population objective of 100 animals. At the beginning of the study, Year 1, 30 mature (≥3 years old) males would be marked with radio tags braided into their tails or manes held in place with a non-toxic, low temperature curing epoxy resin, and 30 mares would be radio collared. Mares collared for the multiple year study would be 3 years old or older. All mares being collared would be in Henneke body condition score 4 or greater. Animals that are "thin" (Henneke score of <3), deformed, or who have any apparent neck problems wouldn't be fitted with a collar. As tags are small (<200g) and are not worn around the neck they are considered of low burden to the animal, and therefore could potentially be worn by animals in lower boy condition. All collars would be fitted according to

procedures outlined in Appendix H. All radio collars would have a manual release mechanism that can be remotely activated in case of emergency and a timed release mechanism which would be programmed to release at the end of the study period. No collars would remain on wild horses indefinitely. If the collar drop-off mechanism fails at the end of the study those individual horses would be captured and the collars manually removed. Each collared horse would be observed once a month while collared. Every effort would be made to release horses to the same general area from which they were gathered. Dependent foals would be released with their mare. Radio collared mares would be observed 1-2x/month over winter to check radio collars. In the spring and summer 2017, radio marked females would be monitored to check for foals and data would be recorded on all radio marked individuals related to survival rates, movement rates and spatial ecology. We would collect fecal samples of radio marked mares to determine pregnancy and collect fecal samples from foals to determine paternity for male reproductive success analyses. Pre-treatment behavioral data would be collected on 16 tagged males (harem stallions and bachelors) and 4 collared females, plus their social associates, between March and September 2017.

Year 2 (Oct 2017-Sept 2018)
In fall 2017 conduct a gather of all males and their groups. Based on consultation with BLM specialists, 50% to 75% of all adult males in the population would be gelded. This would be approximately 37 males, which would be randomly selected for treatment (gelding), other males would not be treated and would serve as control animals. All captured horses (males and females, plus juveniles and foals) would be brought to the Delta Wild Horse Facility. The gelding of the selected males would be conducted there by a veterinarian in a controlled environment, and those treated animals would be provided sufficient time to heal and be monitored for complications. Both treated and untreated males would be released back to the HMA along with females in the population at approximately the same time. Of the radio-tagged males, 8 treatment and 8 control males (plus 4 collared mares) would continue as focal study animals for intensive behavioral data collection. This would consist of over 1000 hours of behavioral observations collected between March and September each year of the research study. The remaining radio-marked individuals would be monitored less frequently, in order to record data related to survival rates, movement rates, fertility and fecundity and spatial ecology. We would collect fecal samples of radio marked mares to determine pregnancy and collect fecal samples from foals to determine paternity for male reproductive success analyses.

Year 3 (Oct 2018-Sept 2019)
In winter 2018/2019, conduct an aerial inventory to assess population size. Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. Record post-treatment data on behavioral variables during March to September. Researchers would collect fecal samples of radio marked mares to determine pregnancy and collect fecal samples from foals to determine paternity for male reproductive success analyses, and record survival, presence of foals, and other population parameters.

Year 4 (Oct 2019-Sep 2020)
In winter 2019/2020, conduct an aerial inventory to assess population size. Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month

throughout the year to check individuals wearing collars. Record post-treatment data on behavioral variables during March to September. Researchers would collect fecal samples of radio marked mares to determine pregnancy and collect fecal samples from foals to determine paternity for male reproductive success analyses, and record survival, presence of foals, and other population parameters.

Year 5 (Oct 2020-Sep 2021)
In winter 2020/2021, conduct an aerial inventory to assess population size. Continue gathering data with the use of radio collars and tags, locating individuals approximately 1-2x/month throughout the year to check individuals wearing collars. Record final year of post-treatment data on behavioral variables during March to September. Researchers would collect fecal samples of radio marked mares to determine pregnancy and collect fecal samples from foals to determine paternity for male reproductive success analyses, and record survival, presence of foals, and other population parameters, and to check that radio collars drop off as programmed. The data would begin to be analyzed, and results written up and submitted to peer-reviewed journals.

Year 6-10 (Oct 2020 – Sept 2025)
Upon the conclusion of the research study the BLM would conduct gathers to remove excess wild horses to achieve a thriving ecological balance with multiple resources by achieving and maintain AML. Gathers and management of the Conger HMA would be in accordance to Warm Springs Resource Area RMP and recent management practices where the sex ratio would be adjusted to favor males (60/40 male to female ratio) pending research results. Several gathers may be necessary to achieve and maintain AML based on National holding space availability and National gather priorities. During the gathers, any gelding from the research study that is captured would be evaluated for return based on the health of the individual and management objectives.

Description of Gelding Procedure
Stallions between 5 and 20 years of age and with a Henneke body condition score of 3 or higher (Henneke 1983) would be randomly selected for gelding. No animals which appear to be distressed, injured, or in poor health or condition would be selected for gelding. Stallions would not be gelded within 72 hours of capture. The surgery would be performed at a BLM-managed holding center by a veterinarian using general anesthesia and appropriate surgical techniques (see Colorado State University Institutional Animal Care and Use Committee protocol Appendix A and Gelding SOPs in Appendix C). The final determination of which specific animals would be gelded would be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer.

The animal would be sedated with Xylazine at 1.1mg/kg administered intravenously followed 2-3 minutes later with Ketamine to induce anesthesia. The Ketamine is given at a dose of 2.2mg/kg intravenously. They are placed in lateral recumbency and the surgical site is prepped using a Chlorhexidine scrub. The surgeon would wear sterile gloves. The scrotum is incised over the testicles and the testicles are removed using a Henderson castrating tool. The incision is left open to drain. Each stud would be given a Tetanus shot, also an intramuscular injection of Procaine Penicllin G at a rate of 22,000 units/kg and an intravenous injection of Flunixin Meglumine at 2.2mg/kg.

Any males that have an inguinal or scrotal hernias would be removed from the population, sent to a regular BLM facility and be treated surgically as indicated if possible or euthanized if they have a poor prognosis for recovery according to BLM policy (IM 2009-041, IM 2009-063). Horses with only one descended testicle may be removed from the population and managed at a regular BLM facility according to BLM policy or anesthetized with the intent to locate the undescended testicle for castration. If an undescended testicle cannot be located, the animal may be recovered and removed from the population if no surgical exploration has started. Once surgical exploration has started those that cannot be completely castrated would be euthanized prior to recovering them from anesthesia according to BLM policy. All animals would be rechecked by a veterinarian the day following surgery. Those that have excessive swelling, are reluctant to move or show signs of any other complications would be held in captivity and treated accordingly as they normally would in a BLM facility. Once released to the wild no further veterinary interventions are possible.

Selected stallions would be shipped to the facility, gelded, and returned to the range within 30 days. Gelded animals would be monitored periodically for complications for approximately 7-10 days following release. This monitoring may be completed either through aerial recon if available or field observations from major roads and trails. The goal of this monitoring is to detect complications if they are occurring and determine if the horses are freely moving about the HMA. All adults would have been freeze-marked at the first gather with a 4 digit freezemark number high on their hip to facilitate post-treatment and routine field monitoring. Post-gather monitoring would be used to document whether or not geldings form bachelor bands or intermix with the breeding population as expected. Other periodic observations of the long term outcomes of gelding would be recorded during routine resource monitoring work. Such observations would include but not be limited to band size, social interactions with other geldings and harem bands, distribution within their habitat, forage utilization and activities around key water sources. More intensive observations of gelded and non-gelded wild horses would be conducted by a CSU/USGS research team. Periodic population inventories and future gather statistics would assist BLM to determine if managing a portion of the herd as non-breeding animals is an effective approach to slowing the annual population growth rate by replacing breeding mares with sterilized animals, and thereby extending the gather cycle when used in conjunction with other population control techniques.

It should be noted that adequate reduction of female horse fertility rates is expected to result only if a large proportion of male horses in the population are sterile, because of their social behavior (Garrott and Siniff 1993). By itself, it is unlikely that sterilization (gelding) would allow the BLM to achieve its horse and burro population management objectives since a single stallion is capable of impregnating multiple mares, and stallions other than the dominant harem stallion may also breed with some mares. Therefore, to be fully effective, use of sterilization to control population growth requires that either the entire male population be gathered and treated (which is not practical) or that some percentage of the female wild horses/burros in the population be gathered and treated. If the treatment is not of a permanent nature (e.g., application of the PZP-22 vaccine to mares) the animals would need to be gathered and treated on a cyclical basis.

2.2.2 Alternative 2 - Conger and Frisco HMAs: Gather and Removal Only

Alternative 2 would gather and remove about 150 - 200 excess wild horses from within and outside the Frisco HMA and gather and remove about 200 -250 excess wild horses within and outside the Conger HMA beginning on or about July 1, 2016 to achieve and maintain the AMLs for both HMAs. Based on administrative factors (population estimates, budget, holding space, National gather priorities, etc.) multiple gathers may be conducted within the 10 year life of this document in order to achieve and maintain wild horse populations within the AML and achieve a thriving ecological balance in keeping with the BLM's multiple use mission. Post-gather sex ratios for both HMAs would be expected to remain at 60%/40% males to females.

2.2.3 Alternative 3 - No Action

Under the No Action Alternative, no gather would occur and no additional management actions would be undertaken to control the size of the wild horse population at this time. Populations would be expected to continue growing exponentially from their current sizes, which are far above established AMLs.

2.3 Summary Comparison of Alternatives

<u>Management Actions Common to Alternatives 1-2</u>

- ❑ The research gathers would begin in July 2016 and take about 7 days to complete in each HMA. Several factors such as animal condition, herd health, weather conditions, or other considerations could result in adjustments in the schedule.
- ❑ Gather operations involve areas beyond the HMA boundaries as displayed in Maps 1 and 2.
- ❑ Gather operations would be conducted in accordance with the Comprehensive Animal Welfare Program (CAWP) for Wild Horses and Burro Gathers (Appendix B), which includes provisions of the Comprehensive Animal Welfare Program (BLM Instructional Memorandum 2015-151). The primary gather (capture) methods would be the helicopter drive method with occasional helicopter assisted roping (from horseback). Water trapping could be used in addition, to capture individually selected animals for the research study as well as to address management needs in regards to public safety, emergency situations and private land issues.
- ❑ Trap sites and temporary holding facilities would be located in previously used sites or other disturbed areas whenever possible. Undisturbed areas identified as potential trap sites or holding facilities would be inventoried for cultural resources. If cultural resources are encountered, these locations would not be used unless they could be modified to avoid impacts to cultural resources.
- ❑ Gather operations in Wilderness Study Areas (WSAs) would be conducted by herding animals by helicopter to the temporary gather sites located outside WSA boundaries. No landing of aircraft would occur in WSAs except for emergency purposes and no motorized vehicles would be used in WSAs in association with the gather operation unless such use is consistent with the minimum requirements for management of WSAs and is preapproved by the authorized officer.
- ❑ Decisions to humanely euthanize animals in field situations would be made in conformance with BLM policy (Washington Office Instruction Memorandum 2009-041). Current policy

reference:
http://www.blm.gov/wo/st/en/info/regulations/Instruction_Memos_and_Bulletins/national_instruction/2009/IM_2009-041.html

- ❑ Data including sex and age distribution, condition class information (using the Henneke rating system), color, size and other information may also be recorded, along with the disposition of the animal (removed or released).
- ❑ Hair samples would be collected on all animals returned to the range from each HMA to assess the genetic diversity and pedigree of the herds. Samples would also be collected during future gathers as needed to determine whether BLM's management is maintaining acceptable genetic diversity (avoiding inbreeding depression).
- ❑ If at any time in the future the genetic diversity there is determined to be relatively low, then a large number of other HMAs could be used as sources for fertile wild horses that could be transported into Conger Mountain and Frisco HMAs.
- ❑ Excess animals would be transported to the BLM Delta Wild Horse Facility where they would be prepared (freeze-marked, vaccinated and de-wormed) for adoption, sale (with limitations) or long-term holding.
- ❑ A BLM contract Veterinarian, Animal and Plant Health Inspection Service (APHIS) Veterinarian or other licensed Veterinarian would be on site as the gather is started and then as needed for the duration of the gather to examine animals and make recommendations to the BLM for the care and treatment of wild horses, and ensure humane treatment. Additionally, animals transported to the BLM Delta Wild Horse Facility are inspected by facility staff and the BLM contract Veterinarian, to observe health and ensure the animals have been cared for humanely.
- ❑ Noxious weed monitoring at gather sites and temporary holding corrals would be conducted in the spring and summer of 2017by BLM. Treatment would be provided, if necessary, following guidance from the Noxious Weed Control EA# J-010-099-015EA. Mitigation measures would be followed to eliminate the spread of noxious/invasive weeds as outlined in Noxious Weed Clearance Fillmore Field Office dated December 15, 2009.
- ❑ Monitoring of rangeland forage condition and utilization, water availability, aerial population surveys and animal health would continue.
- ❑ A comprehensive post-gather aerial population inventory would occur within 12 months following the completion of the gather operation. The inventory would be planned to include the Conger and Frisco HMAs and adjacent areas outside HMA boundaries.

Helicopter

If the local conditions require a helicopter drive-trap operation, the BLM would use a contractor or in-house gather team to perform the gather activities in cooperation with BLM and other appropriate staff. The contractor would be required to conduct all helicopter operations in a safe manner and in compliance with Federal Aviation Administration (FAA) regulations 14 CFR § 91.119 and BLM IM No. 2010-164. Helicopter landings would not be allowed in wilderness except in the case of an emergency.

Helicopter drive trapping involves use of a helicopter to herd wild horses into a temporary trap. The CAWP outlined in Appendix B would be implemented to ensure that the gather is conducted in a safe and humane manner, and to minimize potential impacts or injury to the wild horses. Traps would be set in an area with high probability of access by horses using the topography, if possible, to assist with capturing excess wild horses residing within the area. Traps consist of a

large catch pen with several connected holding corrals, jute-covered wings and a loading chute. The jute-covered wings are made of material, not wire, to avoid injury to the horses. The wings form an alley way used to guide the horses into the trap. Trap locations are changed during the gather to reduce the distance that the animals must travel. A helicopter is used to locate and herd wild horses to the trap location. The pilot uses a pressure and release system while guiding them to the trap site, allowing them to travel at their own pace. As the herd approaches the trap the pilot applies pressure and a prada horse is released guiding the wild horses into the trap. Once horses are gathered they are removed from the trap and transported to a temporary holding facility where they are sorted.

If helicopter drive-trapping operations are needed to capture the targeted animals, BLM would assure that an Animal and Plant Health Inspection Service (APHIS) veterinarian or contracted licensed veterinarian is on-site during the gather to examine animals and make recommendations to BLM for care and treatment of wild horses. BLM staff would be present on the gather at all times to observe animal condition, ensure humane treatment of wild horses, and ensure contract requirements are met.

Bait/Water Trapping
Bait and/or water trapping would not necessarily be used, but may be used if circumstances require it. Bait and/or water trapping generally require a longer window of time for success than helicopter drive trapping. Although the trap would be set in a high probability area for capturing excess wild horses residing within the area, and at the most effective time periods, time is required for the horses to acclimate to the trap and/or decide to access the water/bait.

Trapping involves setting up portable panels around an existing water source or in an active wild horse area, or around a pre-set water or bait source. The portable panels would be set up to allow wild horses to go freely in and out of the corral until they have adjusted to it. When the wild horses fully adapt to the corral, it is fitted with a gate system. The acclimation of the horses creates a low stress trapping method. During this acclimation period the horses would experience some stress due to the panels being setup and perceived access restriction to the water/bait source.

When actively trapping wild horses, the trap would be staffed or checked on a daily basis by either BLM personnel or authorized contractor staff. Horses would be either removed immediately or fed and watered for up to several days prior to transport to a holding facility. Existing roads would be used to access the trap sites.

Gathering excess horses using bait/water trapping could occur at any time of the year and traps would remain in place until the target number of animals are removed. Generally, bait/water trapping is most effective when a specific resource is limited, such as water during the summer months. For example, in some areas, a group of wild horses may congregate at a given watering site during the summer because few perennial water resources are available nearby. Under those circumstances, water trapping could be a useful means of reducing the number of horses at a given location, which can also relieve the resource pressure caused by too many horses. As the proposed bait and/or water trapping in this area is a low stress approach to gathering wild horses, such trapping can continue into the foaling season without harming the mares or foals.

Gather Related Temporary Holding Facilities (Corrals)
Wild horses that are gathered would be transported from the gather sites to a temporary holding corral in goose-neck trailers. At the temporary holding corral, wild horses would be sorted into different pens based on sex. The horses would be aged and provided good quality hay and water. Mares and their un-weaned foals would be kept in pens together. At the temporary holding facility, a veterinarian, when present, would provide recommendations to the BLM regarding care and treatment of the recently captured wild horses. Any animals affected by a chronic or incurable disease, injury, lameness or serious physical defect (such as severe tooth loss or wear, club foot, and other severe congenital abnormalities) would be humanely euthanized using methods acceptable to the American Veterinary Medical Association (AVMA).

Transport, Off-range Corrals, and Adoption Preparation
All gathered wild horses would be removed and transported to BLM holding facilities where they would be inspected by facility staff and if needed a contract veterinarian to observe health and ensure the animals are being humanely cared for.

Those wild horses that are removed from the range and are identified to not return to the range would be transported to the receiving off-range corrals (ORC, formerly short-term holding facility) in a goose-neck stock trailer or straight-deck semi-tractor trailers. Trucks and trailers used to haul the wild horses would be inspected prior to use to ensure wild horses can be safely transported. Wild horses would be segregated by age and sex when possible and loaded into separate compartments. Mares and their un-weaned foals may be shipped together. Transportation of recently captured wild horses is limited to a maximum of 12 hours.

Upon arrival, recently captured wild horses are off-loaded by compartment and placed in holding pens where they are provided good quality hay and water. Most wild horses begin to eat and drink immediately and adjust rapidly to their new situation. At the off-range corral, a veterinarian provides recommendations to the BLM regarding care, treatment, and if necessary, euthanasia of the recently captured wild horses. Wild horses in very thin condition or animals with injuries are sorted and placed in hospital pens, fed separately and/or treated for their injuries.

After recently captured wild horses have transitioned to their new environment, they are prepared for adoption, sale, or transport to long-term grassland pastures. Preparation involves freeze-marking the animals with a unique identification number, vaccination against common diseases, castration, and de-worming. At ORC facilities, a minimum of 700 square feet of space is provided per animal.

Adoption
Adoption applicants are required to have at least a 400 square foot corral with panels that are at least six feet tall. Applicants are required to provide adequate shelter, feed, and water. The BLM retains title to the horse for one year and inspects the horse and facilities during this period. After one year, the applicant may take title to the horse, at which point the horse becomes the property of the applicant. Adoptions are conducted in accordance with 43 CFR Subpart 4750.

Sale with Limitations
Buyers must fill out an application and be pre-approved before they may buy a wild horse. A sale-eligible wild horse is any animal that is more than 10 years old or has been offered unsuccessfully for adoption at least three times. The application also specifies that buyers cannot sell the horse to slaughter buyers or anyone who would sell the animals to a commercial processing plant. Sales of wild horses are conducted in accordance with the 1971 WFRHBA and congressional limitations.

Off-Range Pastures
When shipping wild horses for adoption, sale, or Off-Range Pastures (ORPs) the animals may be transported for up to a maximum of 24 hours. Immediately prior to transportation, and after every 24 hours of transportation, animals are offloaded and provided a minimum of 8 hours on-the-ground rest. During the rest period, each animal is provided access to unlimited amounts of clean water and two pounds of good quality hay per 100 pounds of body weight with adequate space to allow all animals to eat at one time.

Mares and sterilized stallions (geldings) are segregated into separate pastures, except at one facility where geldings and mares coexist. Although the animals are placed in ORP, they remain available for adoption or sale to qualified individuals; and foals born to pregnant mares in ORP are gathered and weaned when they reach about 8-12 months of age and are also made available for adoption. The ORP contracts specify the care that wild horses must receive to ensure they remain healthy and well-cared for. Handling by humans is minimized to the extent possible although regular on-the-ground observation by the ORP contractor and periodic counts of the wild horses to ascertain their well-being and safety are conducted by BLM personnel and/or veterinarians.

Euthanasia or Sale without Limitations
While the destruction of healthy excess animals and sale without limitations is allowed under the WFRHBA, neither option is currently available for disposition of excess horses, due to Congressional restrictions in the Department of the Interior's appropriations bills. This appropriations language has been in effect for much of the past twenty years and BLM, accordingly, does not destroy healthy excess animals or allow their sale without limitations.

Any old, sick or lame horses unable to maintain an acceptable body condition (greater than or equal to a Henneke BCS of 3) or with serious physical defects would be humanely euthanized either before gather activities begin or during the gather operations. Decisions to humanely euthanize animals in field situations would be made in conformance with BLM policy (Washington Office Instruction Memorandum (WO IM) 2015-070 or most current edition). Conditions requiring humane euthanasia occur infrequently and are described in more detail in Washington Office Instruction Memorandum 2009-041.

Public Viewing Opportunities
Opportunities for public observation of the gather activities on public lands would be provided, when and where feasible, and would be consistent with WO IM No. 2013-058 and the Visitation Protocol and Ground Rules for Helicopter WH&B Gathers within Utah. This protocol is intended to establish observation locations that reduce safety risks to the public during helicopter gathers

(e.g., from helicopter-related debris or from the rare helicopter crash landing, or from the potential path of gathered wild horses), to the wild horses (e.g., by ensuring observers would not be in the line of vision of wild horses being moved to the gather site), and to contractors and BLM employees who must remain focused on the gather operations and the health and well-being of the wild horses. Observation locations would be located at gather or holding sites and would be subject to the same cultural resource requirements as those sites.

Summary Comparison of the Alternatives

Item	Alternative 1: Proposed Action	Alternative 2	Alternative 3: No Action
• Impacts to Wild Horse Gather Number • Removal Number • Research Treatment (gelding) - # Stallions • Radio Collars and Tags on Marked Animals • Post-Gather Population Size	• Wild horses (gather and removal) would experience handling stress associated with gather operations which would vary by individual and intensity and range from nervous agitation to physical distress. • Stallions gathered in year 2 would be held in the Delta Wild Horse Facility for a short time (expected to be ~7 days or less) • Stallions that would be gelded would experience moderate stress and pain levels from increased handling levels while being sedated and immediately after performance of the gelding procedure. • Mares being fitted with radio collars would have a short acclimation period but would quickly return to normal behaviors based on observationsof captive wild horses at a BLM facility. Males would have a	• Impacts to wild horses gathered and removed would be the same as Alternative 1: Proposed Action • No impacts to wild horses from research study and treatment. • Post gather population would have better access to available space, forage, water, cover and genetic diversity within HMAs for long-term existence.	• No impacts to wild horses from gather operations, or research study. • Population levels would continue to rise above levels that the HMAs could sustain long term. Horses would expand outside established HMAs looking for forage, water, space and cover increasing impacts to those areas where there is no allocation for wild horse use.

	Global Positioning System/VHF radio transmitter tag braided into their tails and would experience no discomfort, nor require an acclimation period. • Post gather population would have access to available space, forage, water, cover and genetic diversity within HMAs for long-term existence.		
Impacts to Rangeland Health Standards/Livestock Grazing/ and Vegetation Resources	• Utilization levels on forage species would be improved compared to current conditions, would be within appropriate utilization levels after the study and AML is achieved and maintained.	• Same as Alternative 1: Proposed Action	• Increased levels of utilization on vegetation resulting in the loss of vegetation/habit at and not in conformance with Rangeland Health Standards

2.4 Alternatives Considered but Dismissed from Detailed Analysis

Use of Bait and/or Water Trapping

It would not be timely, cost-effective or practical to use bait and/or water trapping as the primary gather method to remove the excess horses located within the Conger and Frisco HMAs in order to initiate the research study and achieve near high AML without risking increased degradation to the rangelands. As a result, this alternative was dismissed from detailed analysis.

Remove or Reduce Livestock within the HMA

This alternative would involve no removal of wild horses and instead address the excess wild horse numbers through the removal or reduction of livestock within the HMA. This alternative was not brought forward for detailed analysis because it is outside of the scope of the analysis, is inconsistent with the Pinyon MFP, Warm Springs Resource Area RMP and the WFRHBA, which directs the Secretary to immediately remove excess wild horses, and is inconsistent with BLM's mission of multiple use, sustained yield management, under the Federal Land Policy and Management Act (FLPMA). Livestock grazing can only be reduced following the process outlined in the regulations found at 43 CFR Part 4100. Several reductions and changes have been made to livestock grazing within allotments associated to the Conger and Frisco HMAs

through this process. The elimination of livestock grazing in an area would require an amendment to the Pinyon MFP and Warm Springs Resource Area RMP. Such changes to livestock grazing cannot be made through a wild horse gather decision.

Livestock permit renewals were completed from 2007 – 2014 on the allotments within and adjacent to the Conger and Frisco HMA. Each of these renewals had Environmental Assessments and Decision Records completed. These decisions established stocking rates for livestock. The decisions also established seasons of use, areas of use, kind and class of livestock and management actions to improve livestock distribution. These management actions included the establishment of grazing systems, allowable use levels, salting and herding practices. Some livestock reductions were made in these decisions on allotments within the Frisco HMA. Livestock grazing continues to be evaluated for allotments and use areas within the Conger and Frisco HMAs. Monitoring and evaluation of livestock grazing is in accordance with the Pinyon MFP's Rangeland Program Summary Section IV, 17, which states:

"Rangeland studies and monitoring programs will be continued and/or initiated to determine if rangeland management objectives are being achieved and if proposed grazing use levels must be adjusted. This monitoring program will continue on all allotments. Particular attention will be given those areas where there is high resource conflict or there is the possibility of rapid improvement or deterioration of the rangeland resources. The concentration of rangeland monitoring will be on those allotments in the "I" category.

The monitoring program will evaluate changes in range condition and trend which includes determination of plant vigor, plant character, plant density, plant phenology, ground cover and degree of forage utilization on key species. Four primary studies will be used in this evaluation: (1) actual grazing use, (2) forage utilization, (3) range trend, and (4) climate analysis. In addition, data on wildlife habitat, riparian vegetation, and watershed condition will be collected and used as needed. When results of studies are evaluated and it is determined that the objectives are not being achieved on a specific allotment, modifications could include changes in grazing systems, livestock numbers, season of use, additional rangeland developments, or any combination of these alternatives."

Livestock preference as reflected in existing permits for the allotments that overlap Frisco HMA has remained essentially the same from 1983 to present. For the past ten years actual livestock use with the HMAs has been substantially reduced or even eliminated during the years of drought and during years when the wild horse estimated population was above AML. All of the livestock permits have been renewed through the NEPA process. Adjustments to livestock grazing permits have included seasons-of-use, kind-of-livestock, AUM's, and numbers of livestock, in order to improve or maintain the vegetative condition on the allotments. As livestock grazing permits are evaluated, additional adjustments to the total number of AUM's of specified livestock grazing on each allotment, seasons-of-use, and kind-of-livestock may be made. Detailed information about the authorized livestock use within the HMA is provided in Term Grazing Permit Renewal EAs EA-UT- 040-06-36, DOI-BLM-UT-C010-2011-0034-EA, EA-UT- 040-06-35, and DOI-BLM-UT-W020-2014-0010-EA for those allotments.

The BLM is currently authorized to remove livestock from the HMA. "…if necessary to provide

habitat for wild horses or burros, to implement hard management actions, or to protect wild horses or burros from disease, harassment or injury" under CFR 4710.5. This authority in usually applied in cases of emergency and not for general management of wild horses or burros in a manner that would be inconsistent with the land use plan and the separate decisions establishing the appropriate levels of livestock grazing and wild horse use, respectively. Available data also indicates that wild horse use – including where livestock use has been excluded – has resulted in excessive vegetative utilization and impacts to rangelands that are recovering from wildfire.

Remove and Gather to the Upper AML
A post-gather population size at the upper level of the AML range would result in the AML being exceeded the next foaling season. This would be unacceptable for several reasons.

The AML represents "…that 'optimum number' of wild horses which results in a thriving natural ecological balance and avoids a deterioration of the range" (Animal Protection Institute, 109 IBLA 119;1989). The Interior Board of Land Appeals (IBLA) has also held that, "Proper range management dictates removal of horses before the herd size causes damage to the rangeland. Thus, the optimum number of horses is somewhere below the number that would cause resource damage" (Animal Protection Institute, 118 IBLA 63, 75; 1991).

The upper level of the AML established within a HMA represents the maximum population at which a thriving natural ecological balance would be maintained. The lower level represents the number of animals to remain in a HMA following a wild horse gather, in order to allow for a periodic gather cycle, and to prevent the population from exceeding the established AML between gathers.

Additionally, gathering to the upper range of AML would result in the need to follow up with another gather within one year (with resulting stress on the wild horse population), and could result in overutilization of vegetation resources and damage to the rangeland if the BLM were unable to gather the excess horses in the HMAs on an annual basis. This alternative would not reduce the wild horse population growth rate of 20 - 25 percent in the Frisco and Conger HMAs and the BLM would not be able to conduct periodic gathers and still maintain a thriving natural ecological balance. For these reasons, this alternative did not receive further consideration in this document.

Wild Horse Management Implementing Fertility Control without Removals to Achieve AML
This alternative would not allow for population regulation by removing wild horses to achieve AML on the Conger and Frisco HMAs. Wild horse management under this alternative would involve inoculating mares with PZP or other population growth suppression vaccines. Gather, data collection, and handling techniques would be followed in accordance with the proposed action. Mares inoculated during the summer of 2016 and other years the vaccine was administered would foal normally in the spring following treatment. Reproduction would be limited the following year or years after treatment.

In addition to not meeting the selection criteria for implementing population growth suppression

research, this alternative was eliminated from further consideration due to the inability to achieve population objectives and research needs. The current populations within the Conger and Frisco HMAs exceeds the AML as established in the Pinyon MFP and Warm Springs Resource Area RMP. Implementing population growth suppression without removing wild horses would not address the immediate issue of achieving AML for decades. Population modeling shows that using this alternative with the current immunocontraceptives available would not control the population of wild horses and would not be in conformance with the WFRHBA, Pinyon MFP, and Warm Springs Resource Area RMP. The WFRHBA mandates the BLM to prevent the range from deterioration associated with overpopulation and preserve and maintain a thriving natural ecological balance in consideration with multiple use relationships.

Fertility Control Treatment Only Including Using Bait/Water Trapping to Dart Mares with PZP Remotely (No Removal)

Population modeling was completed to analyze the potential impacts associated with conducting gathers about every 2-3 years over the next 20 year period to treat captured mares with population growth suppression. Under this alternative, no excess wild horses would be removed. Under such an alternative, the use of bait or water trapping would still not remove excess wild horses. While the average population growth would be reduced to about 16% per year, AML would not be achieved and the damage to the range associated with wild horse overpopulation would continue. This alternative would not meet the Purpose and Need for the Action, would be contrary to the WFRHBA, and was dismissed from further study.

The use of remote darting to administer PZP within an HMA where the horses are not accustomed to human activity has been shown to be very difficult. In the Cedar Mountain HMA during a two-year study where administration of PZP by remote darting was to occur, not a single horse was successfully darted. This method has been effective in some HMAs where the wild horses are more approachable, but horses in the Conger and Frisco HMAs are not approachable, so this method of administering PZP was dismissed from further study.

Wild Horse Numbers Controlled by Natural Means

This alternative was eliminated from further consideration because it is contrary to the WFRHBA which requires the BLM to prevent the range from deterioration associated with an overpopulation of wild horses. It is also inconsistent with the Pinyon MFP and Warm Springs Resource Area RMP, which directs the Fillmore and Cedar City Field BLM Offices to conduct gathers as necessary to achieve and maintain the AML. The alternative of using natural controls to achieve a desirable AML has not been shown to be feasible in the past. Wild horses in the Conger and Frisco HMAs are not substantially regulated by predators. In addition, wild horses are a long-lived species with documented foal survival rates exceeding 95% and they are not a self-regulating species. This alternative would result in a steady increase in numbers which would continually exceed the carrying capacity of the range until severe and unusual conditions that occur periodically-- such as blizzards or extreme drought-- caused catastrophic mortality of wild horses.

Use Alternative Capture Techniques Instead of Helicopters to Capture Excess Wild Horses

An alternative using capture methods other than helicopters and bait/water trapping was suggested by the public. As no specific alternative methods were suggested, the BLM identified

chemical immobilization, net gunning, and wrangler/horseback drive trapping as potential methods for gathering horses. Net gunning techniques normally used to capture big game also rely on helicopters. Chemical immobilization is a very specialized technique, is strictly regulated, and also typically relies on helicopters for close access to the animal. Currently, the BLM does not have sufficient expertise to implement either of these methods and they would be impractical to use given the size of the Conger and Frisco HMAs, access limitations and approachability of the horses.

Use of wrangler on horseback drive-trapping to remove excess wild horses can be fairly effective on a small scale, but due to the number of excess horses to be removed, the large geographic size of the Conger and Frisco HMAs, access limitations and approachability of the horses this technique would be ineffective and impractical. Horseback drive-trapping is also very labor intensive and can be very harmful to the domestic horses and the wranglers used to herd the wild horses. For these reasons, this alternative was eliminated from further consideration.

Gather and Release Excess Wild Horses Every Two Years and Apply PZP to Horses for Release

Another alternative considered was to gather a substantial portion of the existing population (90%) and implement population growth suppression treatment only, without removal of excess horses was modeled using a two-year gather/treatment interval over a 10 year period. PZP pellets that were intended to work for 22 months have been shown in recent pen trials to actually be effective for only one year. Based on WinEquus population modeling (See Appendix D), this alternative would not result in attainment of AML for the HMAs. The wild horse population would continue to have an average population growth rate of 5% to 15% on Conger and 4% to 12% on Frisco, adding to the current wild horse overpopulation, albeit at a slower rate of growth than the No Action Alternative. The modeling reflected an average population size in 11 years of 343 to 1497 wild horses on Conger and 381 to 710 wild horses on Frisco under a two year treatment interval. None of the trials in this alternative would decrease the existing overpopulation of wild horses, resource concerns and rangeland deterioration would continue, and implementation would result in substantially increased gather and population growth suppression costs relative to the alternatives that remove excess wild horses to the AML range. In addition to not achieving AML, the time needed to complete a gather would also increase over time, because the more frequently an area is gathered, the more difficult wild horses are to trap. They become very evasive and learn to evade the helicopter by taking cover in treed areas and canyons. Wild horses would also move out of the area when they hear a helicopter, thereby further reducing the overall gather efficiency. The horses would also become so wary of traps used in water or bait traps that they would avoid any waters where traps are or were set up. Frequent gathers would increase the stress to wild horses, as individuals and as entire herds. It would become increasingly more difficult over time to repeat gathers every two years to successfully treat a large portion of the population. For these reasons, this alternative was dropped from detailed study.

3.0 Affected Environment

This section of the EA briefly discusses the relevant components of the human environment which would be either affected or potentially affected by the Action Alternatives or No Action (refer to Table 2). Direct impacts are those that result from the management actions while indirect impacts are those that exist once the management action has occurred.

3.1 General Description of the Affected Environment

Frisco HMA

The Frisco HMA is approximately 60,367 acres and is located approximately 15 miles northwest of Milford, Utah (Map 1). Access is provided to the HMA by several dirt roads that originate from Utah State Highway 21. However, the condition of the roads can very on a daily basis due to weather conditions. Temperatures range from 105°F in the summer, to sub-zero in the winter. The wild horses primarily use the lower benches in the winter and the higher elevations in the summer. The HMA is heavily forested with pinyon/juniper trees. The soils within the area are primarily loams. There are considerable amounts of surface rock and scattered rocky outcrops within canyons resulting in wild horses having difficulty traveling long distances and having to take circuitous routes between forage and water.

The HMA ranges from 5,600 feet in the valleys to 9,500 feet in elevation at the top of Frisco Peak. The HMA supports vegetation types of big sagebrush and pinyon/juniper. The pinyon/juniper vegetation type dominates the HMA and can be dense with minimal understory forage. Open areas outside of the pinion/juniper canopy are dominated by sagebrush/grasslands. Indian ricegrass (*Oryzopsis hymenoides*), needle-and-thread grass (*Stipa comata*), and small amounts of curlygrass (*Hilaria jamesii*) are the primary forage species. Forage has been suffering from drought conditions of below normal precipitation in 2007, 2008, 2009, 2012, 2104 and 2015. Spring moisture in 2015 was only 50% of normal, which reduced both water flows at springs and forage production. Forage conditions have not made sufficient improvement since 2012. Minimal vegetative growth of plants and heavy grazing have already reduced much of the available vegetation. Vegetation near water has been impacted heavily.

The HMA has twelve springs. Five of the springs are developed with the rest undeveloped. Most of these water sources were dry in 2015. Only three springs (Dipper, Moorehouse, and High Rock Springs) have sufficient water to sustain wild horses and wildlife through the summer. Dipper and High Rock Springs are in the northeast portion of the HMA. Moorehouse spring is in the central eastside of the HMA. This limits the wild horse movement to the east side of the HMA. These springs also rely on pipelines and troughs to provide the water sources for the wild horses. Currently the pipelines and troughs are in working order, but if they fail these sources could go dry quickly.

There are estimated to be 288 wild horses within the HMA at present with 29 of these being yearlings (last year's foals). As forage within close proximity of water sources is depleted, the wild horses will need to range greater distances for forage. The distance the animals must travel can result in rapid physical deterioration of the animals. In addition, an overlapping dependence of wildlife for the same habitat as wild horses, necessitates action to reduce competition for

limited resources and to preserve physical condition of all animals. Rather than maximizing the number of a single species on the range, it is within BLM's multiple use mission to ensure, as much as possible, that forage and water are adequate to sustain healthy populations of native wildlife as well as horses.

Conger Mountain HMA

The Conger Mountain HMA is approximately 151,506 acres of public and private land, and is located approximately 20 miles northeast of Garrison, Utah (Map 2). Access is provided to the HMA by several dirt roads that originate from Utah State Highways 6 and 50. However, the condition of the roads can very on a daily basis due to weather conditions. Temperatures range from 100°F in the summer, to sub-zero in the winter. The wild horses primarily use the lower benches and valley bottoms in the winter and the higher elevations in the summer. The HMA has some forested areas comprised of pinyon/juniper trees primarily along ridge tops and across Conger Mountain. The soils within the area are primarily loams. Terrain features may require horses to travel several miles between water and forage along well defined trails.

The HMA ranges from 5,200 feet in the valleys to 8,070 feet in elevation at the top of Conger Mountain. The HMA supports vegetation types of black sagebrush and pinyon/juniper. Open areas outside of the pinyon/juniper sites are dominated by blacksage/grasslands. There are a few juniper trees that occur on the valley floors and low hills among the blacksage/grasslands. Key species include indian ricegrass (*Oryzopsis hymenoides*), bottlebrush squirreltail (*Sitanion hystrix*), galletta (*Hilaria jamesii*), needle-and-thread grass (*Stipa comata*), sand dropseed (*Sporobolus cryptandrus*) and winterfat (*Ceratoides lanata*). Forage has been suffering from drought conditions of below normal precipitation in 2008, 2009, 2010, 2012, 2104 and 2015. Winter and spring moisture in 2015 was only 50% of normal which reduced both water flows at springs and forage production. Forage conditions have not made sufficient improvement since 2012. Minimal vegetative growth of plants and heavy grazing have already reduced much of the available vegetation. Vegetation near water has been impacted heavily.

The HMA has four major springs that have been developed providing water storage across the HMA. These springs (Conger, Skunk, Willow, and Knoll Springs) provide permanent water to sustain wild horses and wildlife through the summer. Skunk and Willow Springs are in the north portion of the HMA. Conger Spring is in the central and Knoll Spring is on the west side of the HMA. Skunk and Conger Springs have been developed with pipelines and a series of storage tanks and troughs to provide the water sources for the wild horses, wildlife and livestock during authorized grazing seasons. The northern portion of the HMA, including the WSA, has been designated as crucial habitat for wild horses. Skunk, Willow and the upper Conger Springs are located within this designation and all available surface water is available for wild horses and wildlife. Livestock grazing in the crucial habitat is limited to sheep only. All authorized cattle grazing takes place within the open portion of the HMA where water is piped to storage tanks and troughs.

There are estimated to be 310 wild horses within the HMA at present with 50 of these being yearlings (last year's foals). As forage within close proximity of water sources is depleted, the wild horses will need to range greater distances for forage. The distance the animals must travel can result in rapid physical deterioration of the animals. In addition, an overlapping dependence

of wildlife for the same habitat as wild horses, necessitates action to reduce competition for limited resources and to preserve physical condition of all animals. Rather than maximizing the number of a single species on the range, it is within BLM's multiple use mission to ensure, as much as possible, that forage and water are adequate to sustain healthy populations of native wildlife as well as horses.

3.2 Description of Affected Resources

Elements of the human environment identified as present and potentially affected by at one of the alternatives include livestock grazing, rangeland health standards (including soils, vegetation and riparian), wild life and wild horses. The existing situation (affected environment) relative to these resources is described below. Other resources considered which would not be affected by the alternatives are described in Appendix E.

Livestock Grazing

The Skunk Springs, Ledger Canyon, Conger Spring, Buckskin, Painter Spring, Browns Wash, Crystal Peak, Red Rock, Beaver Lake, Frisco, and Wah Wah Lawson allotments are within the two HMAs. There are a total of 18 livestock operators who are currently authorized to graze livestock in these allotments annually. The operators are authorized to use 40,021 Animal Unit Months (AUMs) of forage each year. An AUM is the amount of forage needed to sustain one cow, five sheep, or five goats for a month. The allotments consist of various pastures grazed in rest-rotation and deferred rotation grazing systems. The season of use may vary by 1-2 weeks annually based upon forage availability, drought conditions, and other management criteria.

The BLM allocated forage for livestock use and AML for the Conger HMA was established as a population range 40 – 80 through the Warm Springs Resource Area RMP ROD, 1987. The BLM allocated forage for livestock use and as suitable for wild horses in the Pinyon Management Framework Plan (PMFP) (1983). The Frisco Herd Management Area Plan (2012) provides direction for management specific to the Frisco HMA and adjusted the AML to 30-60. Adjustments in permitted use have been made through Allotment Management Plans and permit renewals as conditions have changed such as drought and class of livestock changes. The following table summarizes the livestock use information for the allotments in the HMA.

Livestock Use

Allotment	Total Allotment Acres	% of Allotment in HMA	Permittee	Livestock	Authorized Season of Use	% Public Land	Authorized Livestock AUMs (Preference Entire Allotment)
Conger HMA							
Skunk Springs	37,061	15%	1 2	1372 Sheep 22 Cattle	11/09 – 04/15 05/10 – 10/15	100% 100%	1426 115
Ledger Canyon	17,811	12%	1	1957 Sheep	11/16 – 04/15	90%	1749
Conger Spring	70,425	34%	1	526 Sheep 316 Cattle	11/01 – 5/10 11/01 – 5/10	80% 85%	581* 1826
Buckskin	21,898	14%	1	2062 Sheep	11/16 – 04/30	100%	2264
Browns Wash	26,112	17%	1	2017 Sheep	11/01 – 04/30	86%	2003
Painter Spring	33,486	8%	1	1947 Sheep	11/01 – 04/15	100%	2125
Crystal Peak	69,099	28%	1 2	403 Sheep 3700 Sheep	11/01 – 04/30 10/14 – 04/30	100% 91%	430 4361
Frisco HMA							
Red Rock	20,769	8%	1	2465 Sheep	03/01 – 04/30	81%	801
Beaver Lake	71,901	31%	1	496 Cattle 100 Cattle	11/01 – 05/31 06/0/ - 06/30	100% 100%	3457 99
Frisco	65,227	23%	1	2640 Sheep 1800 Sheep 50 Cattle	10/16 – 03/31 04/01 – 05/31 10/16 – 05/31	92% 92% 100%	2683 664 376
Wah Wah Lawson	141,180	11%	1	335 Cattle 1280 Cattle 1100 Cattle	10/01 – 10/15 10/16 – 02/28 03/01 – 06/15	87% 87% 87%	144 4999 3367

*AUMs are the same. Operator does not use sheep AUMs (581 sheep AUMs in nonuse).

Rangeland Health Standards

The Standards for Rangeland Health address the potential for soil erosion (Standard 1. Upland soils exhibit permeability and infiltration rates that sustain or improve site productivity, considering the soil type, climate, and landform), the health of riparian and wetland areas (Standard 2. Riparian and wetland areas are in properly functioning condition. Stream channel morphology and functions are appropriate to soil type, climate and landform), the maintenance of desired species (Standard 3. Desired species, including native, threatened, endangered and special-status species, are maintained at a level appropriate for the site and species involved) and water quality (Standard 4. BLM will apply and comply with water quality standards established by the state of Utah (R3172) and the federal clean water and safe drinking water acts.). Standards 1-3 are addressed below and in other sections, while standard 4 is not addressed as this standard was being met on both HMAs and the quantity of surface water in these HMAs is very limited.

Soils

Soils within the proposed gather area are highly variable in terms of parent material, erosion potential, productivity and other aspects. Detailed soil descriptions and maps may be found in the Soil Survey of Beaver County, Utah and West Millard-Juab Area, Utah (Natural Resource Conservation Service (NRCS), 1996).

BLM is required to keep an inventory of how well grazing allotments are meeting Utah BLM's Rangeland Health (RLH) Standards, which includes rating soil conditions in terms of current conditions and causal factors for those conditions. The results of RLH assessments are the basis of soils analysis for this proposal. RLH Standard 1 requires productive upland soils as evidenced by sufficient cover and litter to protect soil surfaces from erosion, the absence of erosion indicators and appropriate kind and amounts of vegetation to allow properly functioning ecological conditions. The Key Forage method has been used as recently as spring 2015 to monitor how much vegetation has been removed (primarily by large ungulates), and may be used to reflect whether or not adequate protective vegetation cover and litter has been left on-site to protect soils surfaces from erosion.

Vegetation

Rangeland Health Studies have been completed on all of the livestock grazing allotments that are or have a portion of the allotment within the Conger and Frisco HMA. These studies can be found within the allotment files at the BLM Fillmore and Cedar City Field Offices. The methodology of each study was completed using technical reference 1734-6. Vegetation production and vigor has been reduced by drought (Standard and Guideline Studies). Drought is defined as prolonged dry weather generally when precipitation is less than 75% of average annual amount (Society for Range Management 1974). Precipitation is the most important single factor determining the type and productivity of vegetation in an area. Forage production increases rapidly as precipitation increases up to about 20 inches per year (Holechek, 1989). Slight reduction from normal precipitation can cause severe reductions in plant yield in areas with less than 12 inches of precipitation (Klages 1942). The valleys within the Conger and Frisco HMA average less than 8 inches per year. During the period from 2007-2009 the precipitation was below 75% for that area. Need to update precipitation information.

The current drought cycle has had a tremendous influence on rangeland vegetation. As described above, year-long grazing by wild horses has put additional stress on key forage species already affected by drought. Some key forage species have been lost. Recovery could take 5 to 15 years, depending on how severely the drought affected a particular area. Two or more years of drought have far greater impact on vegetation than one year of drought followed by normal or above-normal precipitation.

The Conger and Frisco HMA supports multiple vegetation types including: Pinyon-Juniper (PJ), sagebrush, salt brush and grasslands. The PJ woodland type dominates the Frisco HMA and is very dense with minimal understory forage. Open areas outside the PJ canopy are dominated by big sagebrush with Indian ricegrass, wheatgrass, bluegrass, and squirreltail grass as the primary forage species. Only 12% of the HMA produces forage that can be used by ungulates, with only 3% of the HMA considered to be good forage production area. The Conger HMA has more open areas dominated by salt brush and grasslands producing more forage.

Monitoring data collected within the Frisco HMA indicated the Utah BLM Standards and Guidelines for Healthy Rangelands were not being fully met and that causal factors for non-attainment of Standard 2 and 3 include dewatering of riparian resource, excessive use by wild horses and elk, the prevalence of invasive species including cheatgrass and halogeton, pinyon and juniper tree encroachment, historic livestock grazing and climatic conditions (drought).

Utah BLM Standards and Guidelines for Healthy Rangelands are being met, but the causal factors (i.e. climatic conditions and wild horse and livestock grazing) are contributing to the plant communities not being able to maintain "*viable levels commensurate with the species and habitats potential*" within the Conger HMA. During the past several years, climatic events have occurred at critical times which have allowed the habitat to respond positively but continued drought conditions are evident within the plant communities.

Utilization studies that have been completed during the past 20 years, along with CCFO/FFO staff observations, suggest that as wild horse populations increase they contribute to the decrease of forage species. This is especially true in grassland, sagebrush/grassland, and seeded areas. The High Rock seeding has been overtaken by PJ woodland and sagebrush with little grass understory left. The Frisco fire continues to be primarily grasses, but no utilization studies have been completed on it due to its remoteness.

Utilization studies completed on the Beaver Lake Allotment at the end of June, 2012, showed that in a pasture used only by cattle the utilization on Indian Ricegrass was Slight (13%), while the two adjacent pastures that received use by cattle and wild horses was Moderate to Heavy use (41%-65%).

Seven trend studies were set up within and adjacent to the Frisco HMA by the BLM. These studies describe the soils as being in a stable trend with browse trending slightly down and herbaceous species trending from slightly down to slightly up depending on location within the HMA. These Frequency trend studies suggest the trend is in general stable or static condition. Additional information on the vegetation studies have been summarized in Term Grazing Permit Renewal EAs for the allotments within the HMA.

Year-long grazing by wild horses has been one contributing factor to the downward trend of the grasses and the change from cool season grasses to warm season grasses. Horses, because they are territorial, are grazing the same areas repeatedly throughout the spring during critical growing periods for grasses. High populations of wild horses can reduce the available forage for not only the year the grasses are grazed, but also for years to come. Horses will graze the most desirable forage plants first before grazing on other species. Wild horses are capable of cropping forage much more closely than wild or domestic ruminants, causing a loss of the most desirable forage species and reducing plant diversity.

From 1998 to 2003 and 2008 to present the excess number of wild horses (numbers over AML) within the HMA reduced the amount of available forage for all grazing animals.

Riparian/Wetland

Several small wetland/riparian areas are present within the Frisco HMA and consist of streams, seeps, and springs that all occur on BLM lands. There are approximately 0.5 miles of lotic (stream) habitat and a total of approximately 2.6 acres of lentic (spring/seep) areas in the Frisco HMA that have been inventoried. An unknown amount of riparian/wetland area that occurs within the Frisco HMA still needs to be inventoried. Common riparian/wetland species are willows, cottonwoods, sedges, rushes, Woods rose, and Kentucky bluegrass. The riparian/wetland areas that have been inventoried since 1995 have approximately 0.9 acres rated in proper functioning condition, 0.23 acres rated as functioning at risk with no apparent trend, 0.86 acres functioning at risk with downward trend, and 0.5 linear miles and 0.67 acres rated as nonfunctional. Riparian habitats represent less than 1 percent of the total acreage of public lands in the Frisco HMA. Reptiles, amphibians, mammals, and bird species routinely use riparian areas for food, water, cover or migration routes. Many neotropical migratory birds are riparian obligates.

Lentic Resources for Frisco HMA

		Riparian Functional Rating – Acres of Riparian					
Site Name	Year Assessed	PFC	FAR-UP	FAR-NA	FAR-DN	NF	Total
Bardsley Spring	2006	0.01					0.01
Cattail Spring	2007	0.4					0.4
Diaper Spring	2007					0.03	0.03
Horse Spring	2010				0.01		0.01
West Three Kiln	1995			0.23			0.23
Lower Morehouse Spring	2010	0.26					0.26
Smith Spring	2007	0.17					0.17
West Spring	2007	0.06					0.06
Tub Spring	2007				0.01		0.01
Sawmill Seep 1	2007					0.17	0.17
Sawmill Seep 2	2007					0.06	0.06
Sawmill Seep 3	2007					0.38	0.38
Coyote Spring	2007				0.8		0.8
Douglas Spring	1995					0.03	0.03
Armstrong Spring (0.4 miles outside HMA boundary)	2007				0.04		0.04
Frisco HMA Lentic Total Acres		0.9		0.23	0.86	0.67	1.83
Percent of Total Acres						100%	100%

PFC=Proper Functioning Condition FAR-UP= Functional at risk with upward trend
FAR-NA= Functional at risk, trend not apparent FAR-DN= Functional at risk with downward trend
NF= Non-functional

Lotic Resources for Frisco HMA

Site Name	Year Assessed	Riparian Functional Rating – Miles of Stream PFC	FAR-UP	FAR-NA	FAR-DN	NF	Total
Sawmill Canyon	2007					0.5	0.5
Frisco HMA LoticTotal Miles						0.5	0.5
Percent of Total Miles						0.5	100%

PFC=Proper Functioning Condition FAR-UP= Functional at risk with upward trend
FAR-NA= Functional at risk, trend not apparent FAR-DN= Functional at risk with downward trend
NF= Non-functional

Causal Factors

The rationale for the less than PFC rating was water development, dewatering, road encroachment, upstream channel conditions, juniper encroachment, rabbitbrush encroachment, recreation, and riparian exclosure maintenance. Livestock, wild horses, and wildlife were also noted as causal factors for portions of the streams not rating at PFC. Wild horses, wildlife, and livestock graze riparian areas due to the presence of water, shade, and succulent vegetation. Riparian areas are vulnerable to the effects of overgrazing due to heavy concentration of wild horses, wildlife, and livestock within these areas. Livestock, wildlife, and wild horse grazing impacts water in many ways. Grazing impacts can alter the chemical, physical and biological integrity of the water. Grazing impacts also have the ability to modify the hydrologic response of watersheds by reducing infiltration, reducing vegetative cover, stream channel/floodplain degradation, accelerated erosion processes, surface roughness, and increase compaction. All of these impacts are known to occur, but the impacts cannot be quantified in a predictive manner. Many of the causal factors are within the control of management.

Riparian-wetland areas support a wide variety of avian fauna, mule deer, elk, pronghorn, greater sage grouse, Townsend's big-eared bat and many other small mammals, reptiles, and amphibians. Riparian-wetland resources provide food, shelter, breeding ground, and migration corridors for a variety of wildlife species. Mule deer and elk are attracted to riparian areas due to cooler summer temperatures, valuable forage, water availability and, in treed sites, the ability of the communities to provide hiding cover as well as thermal cover in the winter. Lowland riparian areas provide a valuable source of water and succulent forage for pronghorn. Mule deer utilize riparian-wetland areas during fawn rearing because riparian vegetation along springs, streams, meadows, and aspen stands are a source of succulent grasses and forbs; which provide important nutrition during gestation and lactation.

Below are photos of Armstrong Spring in 1995 (wild horse population within AML) and in 2012 (368% over upper AML). The only use that occurs on Armstrong Spring is wild horses, a few elk and occasional non-permitted livestock.

Armstrong Spring 1995
Non-Functioning with upward trend.

Armstrong Spring 2012
Non-Functioning with downward trend

Wild Horses

The Frisco HMA was formally designated as a Herd Management Area (HMA) through the Pinyon Management Framework Plan (PMFP), 1983, which allows for, "the removal of horses as required to maintain horse numbers at or below 1982 inventory levels, but not less than 1971 levels." The Frisco Herd Management Area Plan (2012) provides direction for management specific to the Frisco HMA. The Frisco HMAP can be found at the following link: http://www.blm.gov/style/medialib/blm/ut/natural_resources/wild_horses_and_burros/frisco.Par.53144.File.dat/FriscoFinalEA.pdf.

The Conger Mountain HMA was formally designated as a Herd Management Area (HMA) through the Warm Springs Resource Area RMP/ROD, 1987. AML was established through site vegetation inventory monitoring and data collection as a population range 40 - 80 in the Warm Springs Resource Area RMP/EIS, 1986. The following table summarizes the AML, estimated population, and estimated removal numbers for the HMAs under the Proposed Action.

Summary of Wild Horse Population Information

HMA	Acres	AML Range	Estimated Pop.	Proposed Target Gather	Proposed Target Remove	Target Treat, Yr 2 (# Studs)	Adjust Sex Ratio (% Studs)	Estimated Post Gather Pop. Size
Frisco	60,367	30 - 60	345*	300	245	0	50	100
Conger Mtn.	170,990	40 - 80	372*	330	272	25-35	50	100

* Population estimates as of March 2016 include the 20% population increase of 2016 foals based on population inventories completed in February 2016. Based on the National Academy of Science (NAS) report released in 2013 the estimated population could be 20%-30% lower than the actual population.

Utilization levels by wild horses on the rangelands within the HMAs have shown increases as the

population has increased. Potential for loss of key forage species has increased as the amount of sustainable forage is depleted through higher levels of use. The past two years have exhibited unfavorable climatic conditions that have had below normal to precipitation, but cooler temperatures and timely rain events have allowed key vegetative species to survive. Drought events over the past ten years have shown the effects of limited resources for wild horses through body condition and range condition. Areas outside the HMAs are experiencing increased un-allotted use on forage species and resources by wild horses which have expanded outside the HMAs. Excess wild horses above AML need to be removed in order to protect the resources outside the HMAs and those resources within the HMAs to allow for proper rangeland health and herd sustainability.

Wild horses within the Conger and Frisco HMAs are currently in moderately thin to moderate body class conditions or a body condition score (BCS) class 3 – 5 on the Henneke BCS chart. Increased utilization levels have been observed by wild horses within key areas, which adversely impacts range health and inhibits recovery of the native vegetative communities in these key areas. Monitoring also indicates that wild horses have moved and are residing outside the HMA boundaries.

The genetic variability of the Conger Mountain HMA is high. According to the "Genetic Analysis of the Conger, Ut HMA" conducted by Dr. E. Gus Cothran published June 30, 2009, "the values related to the allelic diversity in particular suggest a herd with highly mixed ancestry. This view is consistent with the similarity values seen and the heterozygosity measures. The herd ancestry most likely is from North American breeds." The results indicated that genetic diversity in the Conger Mountain HMA is not unique. Moreover, if at any time in the future the genetic diversity there is determined to be relatively low, then a large number of other HMAs could be used as sources for fertile wild horses that could be transported into Conger Mountain HMA.

Blood samples for genetic testing were taken in 2006 to create a baseline for the wild horses that occur within the Frisco HMA. Hair samples for genetic testing were collected and analyzed in 2012 by Dr. Gus Cothran and Texas A&M University. The report released in 2013 states that the genetic variability of the Frisco HMA in general is on the high side but there is a high percentage of variation that is at risk. The herd appears to be in genetic equilibrium. There is no evidence of gene flow into the herd or of recent low population size. Genetic similarity results suggest a herd with mixed ancestry. No action is needed at this point to maintain genetic variability, but the herd should be monitored closely. Hair samples would be collected on both the Conger and Frisco HMAs for genetic analysis.

Wildlife

No federally listed threatened, endangered or candidate species have been identified within the Conger and Frisco HMAs and they will not be discussed further in this document.

BLM Sensitive Wildlife Species

The following list summarized the Special Status Wildlife Species (excluding species listed

under ESA) recognized by management under BLM's 6840 Manual and Instruction Memorandum No. UT2007-078. These species are known to occur or have a high probability of occurrences within the Conger or Frisco HMAs.

Ferruginous Hawk *(Buteo regalis):* The Ferruginous hawk may occur within the Conger or Frisco HMAs. Primary breeding habitat is pinyon-juniper and secondary breeding habitat is shrub-steppe. Edges of pinyon-juniper woodland, utility structures (transmission poles), cliffs and isolated trees serve to provide nesting as well as perching structures for ferruginous hawk.

Townsend Big-Eared Bat *(Corynorhinus townsendii)*: Townsend big-eared bat primary breeding habitat consists of pinyon-juniper woodlands and mountain shrub communities. Small moths and a variety of soft-bodied insects are typical food habits.

Big Game

Mule Deer *(Odocoileus hemionus*: Mule deer habitats in the Conger and Frisco HMAs have been identified as crucial winter range. During spring, summer and early fall, deer fed primarily on a variety of forbs and grasses, with light use on big sagebrush, black sagebrush and antelope bitterbrush. In fall and winter, deer shift their diets to shrubs including big sagebrush, black sagebrush, antelope bitterbrush, Gambel oak and curlleaf mountain mahogany.

Rocky Mountain Elk *(Cervus Canadensis)*: The Frisco HMA has been identified as yearlong elk habitat. Elk primarily forage on grasses, but also utilize shrubs, trees and forbs.

Pronghorn *(Antilocapra Americana)*: Pronghorn typically utilize a variety of vegetation with shrubs being highest in composition followed by forbs and grasses. The Conger and Frisco HMAs have been identified as yearlong pronghorn habitat.

Upland Game

Chukar *(Alectoris chukar)*: Chukar prefers to inhabit open, rocky mountain slopes and forage on seeds from a variety of shrubs, grass and forbs within the Conger and Frisco HMAs.

Migratory Birds and Raptors

The Migratory Bird Treaty Act (16 U.S.C. §703-712, July 3, 1918, as last amended in 1989) prohibits taking, killing, or possessing migratory birds including nests and eggs. In 2001, Executive Order 13186 was issued to outline responsibilities of federal agencies to protect migratory birds under the Migratory Bird Treaty Act (66 FR 3853-3856). Instruction memorandum 2008-050 provides interim guidance to enhance coordination and communication towards meeting BLM's obligations to the Migratory Bird Treaty Act and Executive Order 13186.

BLM entered into a Memorandum of Understanding (BLM- MOU WO-230-2010-04) with USFWS to promote the conservation of migratory birds; specifically, *to strengthen migratory*

bird conservation by identifying and implementing strategies that promote conservation and avoid or minimize adverse impacts on migratory birds through enhanced collaboration between the Parties, in coordination with state, tribal, and local governments.

Golden Eagle (Aquila chrysaetos)
Golden eagles may occur on the Conger and Frisco HMAs year round. The SWreGAP Animal Habitat Model has shown know or probable winter habitat. A majority of the HMAs would be used for foraging.

Health and Safety

In recent gathers, members of the public have increasingly traveled to the public lands to observe BLM's helicopter gather operations. Because these horses are wild animals, there is always the potential for injury when individuals get too close or inadvertently get in the way of gather activities. The helicopter work is done at various heights above the ground, from as little as 10-15 feet (when herding the animals the last short distance to the gather corral) to several hundred feet (when doing a recon of the area). When the helicopter is working close to the ground, the rotor wash of the helicopter is a safety concern by potentially causing loose vegetation, dirt, and other objects to fly through the air which can strike or land on anyone in close proximity as well as cause decreased vision. During the herding process, wild horses or burros will try to flee if they perceive that something or someone suddenly blocks or crosses their path. The public would not be allowed to view the actual trapping activities during bait or water trapping, as described in Section 2.3. Safe viewing areas would be identified for the public to view loading, and sorting. Public observation of the helicopter gather activities on public lands will be allowed and would be consistent with BLM IM No. 2010-164 and in compliance with visitation protocols for scheduled and non-schedule visitation.

4.0 Environmental Consequences

4.1 Introduction

This section of the EA documents the potential environmental impacts which would be expected with implementation of the Action Alternatives (Alternatives 1-2), and/or the No Action Alternative. These include the direct impacts (those that result from the management actions) and indirect impacts (those that exist once the management action has occurred).

4.2 Potential Impacts

The direct and indirect impacts to these resources which would be expected to result with implementation of the Action Alternatives or No Action Alternative are discussed in detail below.

Livestock Grazing

Alternatives 1 and 2

Livestock located near gather activities may be temporarily disturbed or displaced by helicopter use and increased vehicle traffic during the gather operation. This displacement would be temporary and the livestock would move back into the area once gather operations moved. Past experience has shown that gather operations have little impacts on grazing cattle and sheep.

Indirect impacts to livestock grazing would be an increase in forage availability and quality, reduced competition for water and forage, and improved vegetative resources that would lead toward a thriving ecological condition over the course of 6 to 10 years.

Annual authorized livestock use may be adjusted due to a number of factors, including rangeland health or drought. Managing wild horses at the AML through gather and removals with or without Population growth suppression would help with long-term sustainability of authorized livestock use within the HMAs at the permitted levels. Managing wild horses within AML would reduce the likelihood of adjustments to current active livestock permits attributable to overuse of resources by wild horses. This action would have no direct impact on current livestock permits in terms of active AUMs, season of use and/or terms and conditions. The fences would provide better control of livestock from getting on Highway 21 and causing livestock/vehicle collisions. Adjustments to livestock permits (if any) would be made during the livestock allotment permit renewal process.

Alternative 3 (No Action)

Utilization by authorized livestock has been directly impacted due to the current overpopulation of wild horses, both within and outside the HMA boundaries. Livestock operators have been asked to take voluntary reductions due to the impacts of the wild horse population on range vegetation/forage conditions. The current wild horse population is 3-5 times above their forage allocation. Moderate to heavy utilization is occurring. The indirect impacts of No Action (Defer Gather and Removal) would be continued damage to the range, continuing competition between livestock, wild horses and wildlife for the available forage and water, reduced quantity and quality of forage and water, and undue hardship on the livestock operators who would continue to be unable to fully use the forage they are authorized to use.

Rangeland Health Standards

Alternatives 1 and 2
Rangeland Health Standards are directly impacted by the levels of use experienced upon upland soils, riparian and wetland areas, desired plant species including native, threatened, endangered and special status species. The Standards for Rangeland Health indicate that, under alternatives 1 or 2, the potential for soil erosion would be reduced (Standard 1. Upland soils exhibit permeability and infiltration rates that sustain or improve site productivity, considering the soil type, climate, and landform) and riparian areas would receive less grazing pressure which in turn would reduce the impacts to these riparian areas (Standard 2. Riparian and wetland areas are in properly functioning condition. Stream channel morphology and functions are appropriate to soil type, climate and landform) and would contribute to the maintenance of desired species (Standard 3. Desired species, including native, threatened, endangered and special-status species, are maintained at a level appropriate for the site and species involved). Therefore, the potential for maintenance of rangeland health would be increased by removing the wild horses to keep their numbers on the HMA at levels that are closer to the appropriate management level. If no action is taken, rangeland health will deteriorate in areas where wild horses spend most of their time. Riparian vegetation would be affected and soil erosion would increase as desired vegetation is removed from the range. A reduction in the number of wild horses to the appropriate management levels within the HMA boundaries would allow increased maintenance of rangeland health. Over time as population levels are managed at AML, rangeland health would continue to improve allowing for the thriving ecological condition of all uses present.

Alternative 3 (No Action)
Deterioration of rangeland health would continue to increase as population levels increase with no action. Those areas where wild horses spend a majority of their time would suffer from the loss of riparian vegetation, increased soil erosion and compaction and the desired plant species are removed from the range. Indirect impacts from no action would occur in areas not suitable for wild horses. These areas outside the HMAs would experience increased levels of use and may not be resilient enough to recover. Wild horses exist within the HMAs because their basic needs of water, desirable vegetation, cover and space are met. Areas outside the HMAs lack some if not all of these needs and would suffer from increased use.

Soils

Alternative 1 (Proposed Action)
The Proposed Action would have a direct impact to soils directly in the trap area. These areas would be disturbed by the hoof action of wild horses when they are concentrated in the trap area to be loaded on the trailers. The disturbance would be ¼ to ½ acre in size at each trap and would normally be in areas already disturbed like a road, wash, or previous trap site. Most operations would occur when soils are dry or frozen reducing the impact to soils. Past trap site locations have recovered within a year with vegetation to stabilize the soils. No compaction of soils have occurred from past gather operations.

This analysis assumes that livestock use would continue at levels as established by grazing permit renewal decisions, big game numbers would continue as established by herd management

plans and state law and removal of wild horses would be as proposed to within the AML levels specified for the HMA at the end of the 10 year period.

The proposed action would have the indirect impact of aiding eleven grazing allotments (Skunk Springs, Ledger Canyon, Conger Spring, Buckskin, Browns Wash, Painter Spring, Crystal Peak, Red Rock, Beaver Lake, Frisco, Wah Wah Lawson) to move towards attainment or maintenance of Rangeland Health Standard 1. In general, the reduction of wild horses to proposed levels would reduce utilization levels, which would allow more residual vegetation and litter to remain on site and protect the soil resource. Increased litter would provide additional protection from wind and water erosion, promote infiltration, detain surface flows and retard soil moisture loss by evaporation, thus allowing for better vegetative productivity. Indicators, such as pedestals, bare ground, litter movement, flow patterns, etc. should lessen with implementation of the proposed action. Further, reduced numbers of horses should result in less compaction of wet sites, such as riparian areas and enhance soil and vegetation production there.

Alternative 2 (Gather and Removal Only)
Impacts to the soil resource would be essentially the same under Alternative 2 as under the Proposed Action Alternative. Protective vegetative cover and soil surfaces would respond as well as under the Proposed Action, or even better because Alternative 2 leads to the horse population reaching AML at an earlier date.

Alternative 3 (No Action)
Under the No Action Alternative, wild horse populations would continue to increase beyond the capacity of the habitat to provide water and forage. Heavy and severe use of desirable vegetation resources by wild horses would continue and increase. Horses are opportunistic feeders and as their populations increased, may eventually have to choose non-forage species, such as three-awn grass, rabbitbrush and junipers for their survival, which would result in even less litter and residual vegetation left on site than under the current situation. Current indicators of poor soil conditions would remain on allotments currently not meeting Rangeland Health Standards. Additional indicators, such as increased overland flows, rills and gullies could occur as additional soil was lost from the allotments. Wind erosion could become a factor, where it is not currently. Horses would have to expand their ranges because of the distances they would need to travel from water to obtain forage. Ultimately, the allotments currently meeting Rangeland Health Standard 1 would see soil condition deteriorated until they no longer meet Standard 1 (or other standards). It is also likely that wild horses would expand outside their current HMAs as long as they were not restricted by adequate fencing. Under the No Action Alternative, additional trailing, trampling and compaction would occur at riparian zones and other water sources. Decreased percolation and water holding capacity and increased surface runoff from these water sources would result.

Vegetation

Alternative 1 (Proposed Action)
Over a period of 10 years, competition for forage and water between wild horses, wild life and livestock would be directly reduced. A reduced number of wild horses over this period of time within the Conger and Frisco HMAs would improve and/or sustain rangeland health and lower

utilization levels.

Indirect impacts from gathering to the low-range of the AML include reduced trailing by wild horses (less vegetation trampling/disturbance) as they travel to/from water and forage. Actual forage utilization by wild horses would also be reduced from heavy (61+% of annual year's growth) at the present time to moderate or less (<40-60%) within a 1-3 mile radius of the available water. Over the long term, reduced forage utilization would promote vegetation re-growth and provide for natural recovery of overgrazed plants. A reduced demand for forage would help improve the vigor of vegetation, allow for seedling establishment, increase ground cover, and thereby maintain a thriving natural ecological balance. The recovery from the most recent drought and the extended drought in the past 15 years would be allowed to continue and should show improved vegetative trend of key forage species, if precipitation remains near or above long-term average levels. Long-term rangeland health would continue to be met within and/or improve within the allotments as key forage and riparian areas would receive less use, especially during time of drought when wild horse are hardest on vegetation near water.

Reducing the wild horse population to within the AML would contribute to maintaining sufficient vegetation and litter within the HMAs to protect soil from erosion, meet plant physiological requirements, facilitate plant reproduction, and reduce potential for spread of noxious weeds.

This alternative would result in periodic gathers to remove excess wild horses from the Conger and Frisco HMAs. The first gathers would reduce the population for the research studies, and then between the 5-10 year period gathers would try to achieve then maintain the population within AML. For helicopter round-ups, direct impacts to vegetation would include short-term (1 to 10 days) disturbance of native vegetation immediately in and around temporary trap sites, and holding and handling facilities. For bait trapping, the direct impacts to vegetation would be longer (5-365 days) but would still be considered short term. There would be direct impacts to the vegetation immediately in and around temporary trap sites, and holding, sorting and animal handling facilities. Impacts created by vehicle traffic and hoof action of penned horses can be locally severe in the immediate vicinity of the corrals or holding facilities. Keeping the sites approximately ½ acre in size would minimize the disturbance area. Since most trap sites and holding facilities are re-used during recurring wild horse gather operations, any impacts would remain site specific and isolated in nature. In addition, most trap sites or holding facilities are selected to enable easy access by transportation vehicles and logistical support equipment and would therefore, generally be near or on roads, pullouts, water haul sites or other previously disturbed flat spots. These common practices would minimize the cumulative effects of these impacts.

The research part of the proposed action would not impact rangeland resources and vegetation directly. However, the data gained through the research would be used to improve the current management of wild horses, and are expected to lead to reduced impacts to other resources including vegetation.

Alternative 2 (Removal Only)
Impacts of the gather and removal would be similar to those described in Proposed Action.

Removal of excess wild horses would be beneficial to vegetative resources. The improved management of wild horses within these HMAs and others that would come from the research proposal would not occur at this time.

Alternative 3 (No Action)
Under the No Action Alternative, wild horses would continue to increase in population size beyond the capacity of the habitat to provide water and forage. Heavy and severe use of vegetation resources by wild horses would continue and increase, resulting in further degradation of plant communities, increased soil erosion, and susceptibility to invasive species. Downward trends in key perennial species would be expected in conjunction with reductions in ecological condition and soil stability. The vegetative functional groups (i.e. grass, shrubs, trees etc.) would be changed as grasses are over-used during critical growing seasons. Vegetation would also experience reduced production resulting in reduced forage availability to wildlife, livestock, and wild horses. Eventually, rangeland health would be reduced below a threshold that would be difficult to recover from.

Riparian/Wetland

Alternative 1 (Proposed Action)
The only direct impact to riparian/wetland areas that could result from the Proposed Action would be from possible use of riparian areas for employment of water trapping. Impacts from water trapping would include construction of a temporary trap around a water source that is designed to hold the horses until they can be transported or treated. Also, trampling of riparian vegetation could occur while the horses are in the trap. Helicopter trap sites and temporary holding facilities would not be constructed on riparian resources.

The Proposed Action would indirectly impact riparian wetland zones and water quality due to the decreased utilization by wild horses in these sensitive areas allowing for the possibility of riparian wetland areas to improve through natural processes. With a plan to only gather and remove 50-200 wild horses from each HMA in each successive gather operation, the improvements would occur incrementally as the wild horses are gathered over the next ten years until the low end of AML is reached. Under this alternative, native plant health, soils and would slowly improve. Any opportunity to make progress toward achieving and maintaining riparian areas in properly functioning condition would not be possible until reaching the lower end of AML.

Implementing the Proposed Action would slightly decrease current competition for water sources and alleviate pressures exerted on riparian habitat due to wild horses congregating around these sensitive areas. The functionality of riparian resources would improve in condition towards a more properly functioning condition (PFC) with the removal of excess wild horses.

Alternative 2 (Gather and Removal Only)
Impacts to the riparian wetland zones would be essentially the same under Alternative 2 as under the Proposed Action Alternative. Direct impacts to the riparian/wetlands would only occur during water or bait trapping direct at a riparian area. Indirect impacts would improve riparian/wetlands as excess wild horses are removed.

Alternative 3 (No Action)
The No Action Alternative would not have any direct impacts to riparian/wetland resources. Indirect impacts would result from continued and increased utilization on riparian vegetation as wild horse populations continue to increase. Wild horse population size would continue to increase in excess of the established AML. Riparian areas currently rated at Proper Functioning Condition (PFC), could experience downward trends caused by utilization of riparian vegetation and browse, and trampling by populations of wild horses in excess of AML. Riparian areas rated below PFC (Functional at Risk and Non-Functional) would likely not improve, and downward trends would continue. Wild horses have been identified through Proper Functioning Condition Assessments as a contributing factor in riparian areas within the Frisco HMA not being in PFC. Standard 2 in the Standards for Rangeland Health which states *"Riparian and wetland areas are in properly functioning condition…"* is not currently being met for riparian areas within the HMA.

Wild Horses

Alternatives 1 and 2
Over the past 35 years, various impacts to wild horses as a result of gather activities have been observed. Under the Proposed Action, potential impacts to wild horses would be both direct and indirect, occurring to both individual horses and the population as a whole.

The BLM has been conducting wild horse gathers since the mid-1970s. During this time, methods and procedures have been identified and refined to minimize stress and impacts to wild horses during gather implementation. The CAWP in Appendix B would be implemented to ensure a safe and humane gather occurs and would minimize potential stress and injury to wild horses.

In any given gather, gather-related mortality averages only about one half of one percent (0.5%), which is very low when handling wild animals. Approximately, another six-tenths of one percent (0.6%) of the captured animals, on average, are humanely euthanized due to pre-existing conditions and in accordance with BLM policy (GAO-09-77). These data affirm that the use of helicopters and motorized vehicles has proven to be a safe, humane, effective, and practical means for the gather and removal of excess wild horses (and burros) from the public lands. The BLM also avoids gathering wild horses by helicopter during the 6 weeks prior to and following the expected peak of the foaling season (i.e., from March 1 through June 30).

Individual, direct impacts to wild horses include the handling stress associated with the roundup, capture, sorting, handling, and transportation of the animals. The intensity of these impacts varies by individual, and is indicated by behaviors ranging from nervous agitation to physical distress. When being herded to trap site corrals by the helicopter, injuries sustained by wild horses may include bruises, scrapes, or cuts to feet, legs, face, or body from rocks, brush or tree limbs. Rarely, wild horses will encounter barbed wire fences and will receive wire cuts. These injuries are very rarely fatal and are treated on-site until a veterinarian can examine the animal and determine if additional treatment is indicated.

Other injuries may occur after a horse has been captured and is either within the trap site corral,

the temporary holding corral, during transport between facilities, or during sorting and handling. Occasionally, horses may sustain a spinal injury or a fractured limb but based on prior gather statistics, serious injuries requiring humane euthanasia occur in less than 1 horse per every 100 captured. Similar injuries could be sustained if wild horses were captured through bait and/or water trapping, as the animals still need to be sorted, aged, transported, and otherwise handled following their capture. These injuries can result from kicks and bites, or from collisions with corral panels or gates.

To minimize the potential for injuries from fighting, the animals are transported from the trap site to the temporary (or short-term) holding facility where they are sorted as quickly and safely as possible, then moved into large holding pens where they are provided with hay and water. On many gathers, no wild horses are injured or die. On some gathers, due to the temperament of the horses, they are not as calm and injures are more frequent. Overall, direct gather-related mortality averages less than 1%.

Indirect individual impacts are those which occur to individual wild horses after the initial event. These may include miscarriages in mares, increased social displacement, and conflict in studs. These impacts, like direct individual impacts, are known to occur intermittently during wild horse gather operations. An example of an indirect individual impact would be the brief 1-2 minute skirmish between older studs which ends when one stud retreats. Injuries typically involve a bite or kick with bruises which do not break the skin. Like direct individual impacts, the frequency of these impacts varies with the population and the individual. Observations following capture indicate the rate of miscarriage varies, but can occur in about 1 to 5% of the captured mares, particularly if the mares are in very thin body condition or in poor health.

A few foals may be orphaned during a gather. This can occur if the mare rejects the foal, the foal becomes separated from its mother and cannot be matched up following sorting, the mare dies or must be humanely euthanized during the gather, the foal is ill or weak and needs immediate care that requires removal from the mother, or the mother does not produce enough milk to support the foal. On occasion, foals are gathered that were previously orphaned on the range (prior to the gather) because the mother rejected it or died. These foals are usually in poor condition. Every effort is made to provide appropriate care to orphan foals. Veterinarians may administer electrolyte solutions or orphan foals may be fed milk replacer as needed to support their nutritional needs. Orphan foals may be placed in a foster home in order to receive additional care. Despite these efforts, some orphan foals may die or be humanely euthanized as an act of mercy if the prognosis for survival is very poor.

Through the capture and sorting process, wild horses are examined for health, injury and other defects. Decisions to humanely euthanize animals in field situations would be made in conformance with BLM policy. BLM Euthanasia Policy IM-2009-041 is used as a guide to determine if animals meet the criteria and should be euthanized (refer to CAWP, Appendix B). Animals that are euthanized for non-gather related reasons include those with old injuries (broken or deformed limbs) that cause lameness or prevent the animal from being able to maintain an acceptable body condition (greater than or equal to BCS 3); old animals that have serious dental abnormalities or severely worn teeth and are not expected to maintain an acceptable body condition, and wild horses that have serious physical defects such as club feet,

severe limb deformities, or sway back. Some of these conditions have a causal genetic component such that the animals should not be returned to the range; this prevents suffering and avoids amplifying the incidence of the deleterious gene in the wild population.

Wild horses not captured may be temporarily disturbed and moved into another area during the gather operation. With the exception of changes to herd demographics from removals, direct population impacts have proven to be temporary in nature with most, if not all, impacts disappearing within hours to several days of release. No observable effects associated with these impacts would be expected within one month of release, except for a heightened awareness of human presence.

It is not expected that genetic health would be affected by the Proposed Action. Available indications are that these populations contain high levels of genetic diversity at this time. More information about the genetic diversity in these populations will become available as a result of Alternatives 1 or 2. The AML range of 40 – 60 on the Frisco HMA and 40 – 80 on the Conger Mtn. HMA should provide for acceptable genetic diversity. If at any time in the future the genetic diversity in either HMA is determined to be relatively low, then a large number of other HMAs could be used as sources for fertile wild horses that could be transported into the HMA of concern.

By maintaining wild horse population size near the AML, there would be a lower density of wild horses across the HMA, reducing competition for resources and allowing the wild horses that remain to use their preferred habitat. Maintaining population size near the established AML would be expected to improve forage quantity and quality and promote healthy, self-sustaining populations of wild horses in a thriving natural ecological balance and multiple use relationship on the public lands in the area. Deterioration of the range associated with wild horse overpopulation would be reduced. Managing wild horse populations in balance with the available habitat and other multiple uses would lessen the potential for individual animals or the herd to be affected by drought, and would avoid or minimize the need for emergency gathers. All this would reduce stress to the animals and increase the success of these herds over the long-term.

Alternative 1 (Proposed Action)

Over the next 10 years, implementation of the Proposed Action would allow for data to be gather and analyzed that might better assist land managers and specialists make better decisions about use of gelding as a management tool for population growth suppression. No previous study of this type has examined the effect of gelding on the behavior of free-roaming wild horses, nor how they interact with intact wild stallions. In addition, the data collected from the Frisco HMA herd would provide necessary empirical data to build population dynamics models that may predict environmental factors which influence demographic parameters like survival and fertility rates. Recording such data will allow BLM to further its use of science to improve the BLM Wild Horse and Burro Program, in keeping with the BLMs science strategy (Kitchell et al. 2015).

Alternative 1 (Proposed Action) would gather up to 275 horses, of which 200 would be removed

to achieve a wild horse population to the level prescribed for the research study on the Conger HMA and gather up to 250 horses and remove 175 to achieve a wild horse population size to the level prescribed for the research study as a control population on the Frisco HMA. A ratio of 50%/50% male to female would be released back to the Conger HMA after selection of those to marked and fitted with radio collars/tags. In the Year 1 gather, mares and studs would be selected for release to maintain a diverse age structure, herd characteristics, and conformation (body type). During Year 2 of the study, horses within the Conger Mtn. HMA would be gathered and brought to the Delta Wild Horse Facility. Approximately 37 stallions would be treated by gelding, after which all gathered horses would be released back to the Conger Mtn. HMA.

The impact of radio collars and tags is very minimal. From March 2015 through March 2016 researchers at the U.S. Geological Survey conducted a preliminary study on captive wild horses and burro jennies to determine proper fit and wear of radio collars (Schoenecker et al. 2014). The condition of wild horses wearing radio collars was compared to non-collared controls and documented with photographs. In addition, both collared individuals and controls were observed for 80 minutes each week for 14 weeks in order to quantify any impact of the collar on their behavior and health. At the end of the study period (March 2016) the collars were removed. Preliminary analyses indicate that mares had almost no impact in terms of rubbing or wear from radio collars, and behavior of collared and uncollared mares did not differ (Schoenecker et al. 2016 *in prep*). There was no impact of radio tags on behavior or wear, either.

Recent research on non-lethal methods for managing population growth of free-roaming wild horses has focused largely on suppressing female fertility through contraception (Ballou et al. 2008, Killian et al. 2008, Turner et al. 2008, Gray et al. 2010, Ransom et al. 2011). Very few studies have been conducted on techniques for reducing male fertility. Nelson (1980) and Garrott and Siniff (1992) modeled potential efficacy of male-oriented contraception as a population management tool, and both studies agreed that while slowing growth, sterilizing only dominant males (i.e., harem-holding stallions) would result in only marginal reduction in female fertility rates. Eagle et al. (1993) and Asa (1999) tested this hypothesis on herd management areas (HMAs) where dominant males were vasectomized. Their findings agreed with modeling results from previous studies, and they also concluded that sterilizing only dominant males would not provide the desired reduction in population growth rate, assuming that the numbers of fertile females is not changed. While bands with vasectomized harem stallions tended to have fewer foals, breeding by bachelors and subordinate stallions meant that population growth still occurred. Garrott and Siniff (1992) concluded from their modeling that male sterilization would effectively suppress population growth only if a large proportion of males (>85%) could be sterilized, regardless of social order. However, sterilization of >85% of males in a population may have genetic consequences, reducing heterozygosity and increasing inbreeding coefficients, as it would potentially allow a very small group of males to dominate the breeding (as seen in equid reintroductions: Saltz et al. (2000), King unpublished data). Although such genetic consequences could be mitigated, the question of how >85% gelded males in a population would interact with intact stallions and mares and with their habitat is unknown. Garrott and Siniff's (1992) model predicts that gelding 50-80% of mature males in the population would result in reduced, but not halted, population growth. However, it is predicted that within 2 years of this treatment an entire foal crop of fertile males would become sexually mature, so the 85%

treatment would have to be repeated until foaling was suppressed. Even then after just a few years there would be an accumulation of fertile males coming to maturity. No previous study has directly focused on the individual or population-level effects of gelding males in a free-roaming horse population. A central and compelling reason to complete the proposed study is to inform BLM with data and associated analyses about these questions.

A literature search was conducted by a research scientist at Colorado State University to find scientific publications on the effect of gelding on horses and mammals in general. This search using the Web of Science and BioOne research search engines involved terms about gelding and castration in relation to behavior, as well as general effects. While over 220 hits were obtained for the various search terms, very few of the resulting papers were relevant to the question of the effect of gelding on the behavior of male horses in the wild. Despite livestock being managed by castrating males for centuries, there has been remarkably little research on castrates (Hart and Jones 1975, Jewell 1997). It is therefore unknown exactly what effect gelding an adult stallion and releasing him back in to a wild horse population will have on his behavior and that of the wider population, and can only be hypothesized from the scarce existing literature. Scientific data collected on the animals involved in this project would help to document the effects of maintaining geldings as a portion of a wild population.

Feral horses typically form bands composed of an adult male with 1 to 3 adult females and their immature offspring (Feist and McCullough 1976, Berger 1986, Roelle et al. 2010). In many populations subordinate ‘satellite’ stallions have been observed associating with the band, although the function of these males continues to be debated (see Feh 1999, and Linklater and Cameron 2000). Juvenile offspring of both sexes leave the band at sexual maturity (normally around two or three years of age (Berger 1986), but adult females may remain with the same band over a span of years. Group stability and cohesion is maintained through positive social interactions and agonistic behaviors among all members, and herding and reproductive behaviors from the stallion (Ransom and Cade 2009). Group movements and consortship of a stallion with mares is advertised to other males through the group stallion marking dung piles as they are encountered, and over-marking mare eliminations as they occur (King and Gurnell 2006). Quantifying these key wild horse behaviors is an important tool in understanding how the presence of a large number of gelded males may influence social structure in the population and ultimately how animals congregate and distribute themselves on the range.

In horses, males play a variety of roles during their lives (Deniston 1979): after dispersal from their natal band they generally live as bachelors with other young males, before associating with mares and developing their own breeding group as a harem stallion or satellite stallion. In any population of horses not all males will achieve harem stallion status, so all males do not have an equal chance of breeding (Asa 1999). Stallion behavior is thought to be related to androgen levels, with breeding stallions having higher androgen concentrations than bachelors (Angle et al. 1979, Chaudhuri and Ginsberg 1990). A bachelor with low libido had lower levels of androgens, and two year old bachelors had higher testosterone levels than two year olds with undescended testicles who remained with their natal band (Angle et al. 1979).

Dogs and cats are commonly neutered, and it is also common for them to continue to exhibit reproductive behaviors several years after castration (Dunbar 1975). Dogs, ferrets, hamsters, and

marmosets continued to show sexually motivated behaviors after castration, regardless of whether they had previous experience or not, although in beagles and ferrets there was a reduction in motivation post-operatively (Hart 1968, Dunbar 1975, Dixson 1993, Costantini et al. 2007, Vinke et al. 2008). Ungulates continued to show reproductive behaviors after castration, with goats and llamas continuing to respond to females even a year later in the case of goats, although mating time and the ejaculatory response was reduced (Hart and Jones 1975, Nickolmann et al. 2008).

Although libido and the ability to ejaculate tends to be gradually lost after castration (Thompson et al. 1980) some geldings continue to intromit (Rios and Houpt 1995). Stallion-like behavior in domestic horse geldings is relatively common (Smith 1974), being shown in 20-33% of cases whether the horse was castrated pre- or post-puberty (Line et al. 1985, Rios and Houpt 1995, Schumacher 2006). While some of these cases may be due to cryptorchidism or incomplete surgery, it appears that horses are less dependent on hormones than other mechanisms for the maintenance of sexual behavior (Smith 1974). Domestic geldings exhibiting masculine behavior had no difference in testosterone concentrations than other geldings (Line et al. 1985, Schumacher 2006), and in some instances the behavior appeared context dependent (Borsberry 1980, Pearce 1980). Domestic geldings had a significant prolactin response to sexual stimulation, but lacked the cortisol response present in stallions (Colborn et al. 1991).

No study has quantified the effect of castration on aggression in horses, with only one report noting that aggression was a problem in domestic horse geldings who also exhibited sexual behaviors (Rios and Houpt 1995). Castration is thought to increase survival as males are released from the cost of reproduction (Jewell 1997). In Soay sheep castrates survived longer than rams in the same cohort (Jewell 1997), and Misaki horse geldings lived longer than intact males (Kaseda et al. 1997, Khalil and Murakami 1999).

Wild horses are rarely gelded and released back into the wild, resulting in few studies that have investigated their behavior in free-roaming populations. In a pasture study of domestic horses, Van Dierendonk et al. (1995) found that social rank among geldings was directly correlated to the age at which the horse was castrated, suggesting that social experiences prior to sterilization may influence behavior afterward. Of the two geldings present in a study of semi-feral horses in England, one was dominant over the mares whereas a younger gelding was subordinate to older mares; stallions were only present in this population during a short breeding season (Tyler 1972). A study of domestic geldings in Iceland held in a large pasture with mares and sub-adults of both sexes, but no mature stallions, found that geldings and sub-adults formed associations amongsteach other that included interactions such as allo-grooming and play, and were defined by close proximity (Sigurjónsdóttir et al. 2003). These geldings and sub-adults tended to remain in a separate group from mares with foals, similar to castrated Soay sheep rams (*Ovis aries*) behaving like bachelors and grouping together, or remaining in their mother's group (Jewell 1997). In Japan, Kaseda and Khalil (1996) reported that young males dispersing from their natal harem and geldings moved to a different area than stallions and mares during the non-breeding season. Although the situation in Japan may be the equivalent of a bachelor group in natural populations, in Iceland this division between mares and the rest of the horses in the herd contradicts the dynamics typically observed in a population containing mature stallions. Sigurjónsdóttir et al. (2003) also noted that in the absence of a stallion, allo-grooming between

adult females increased drastically. Other interesting findings included increased social interaction among yearlings, display of stallion-like behaviors such as mounting by the adult females, and decreased association between females and their yearling offspring (Sigurjónsdóttir et al. 2003). In the same population in Iceland Van Dierendonck et al. (2004) concluded that the presence of geldings did not appear to affect the social behavior of mares or negatively influence parturition, mare-foal bonding, or subsequent maternal activities. Additionally, the welfare of broodmares and their foals was not affected by the presence of geldings in the herd. These findings are important because treated males in our study will be returned to the range in the presence of pregnant mares and mares with foals of the year.

These few studies may not reflect behavior of free-roaming wild horses in the western US, where ranges are much larger, intact stallions are present year-round, and population size and density may be highly variable. Additionally no study exists on the behavior of wild stallions pre- and post-castration, and what effects this will have on their group membership, home range, and habitat use. Studies on sterilization of harem stallions to control population growth all acknowledge that success is dependent on a stable group structure, as strong bonds between a stallion and mares reduce the probability of a mare mating an extra-group stallion (Nelson 1980, Garrott and Siniff 1992, Eagle et al. 1993, Asa 1999). It is therefore vital to know whether any gelded stallions remain with mares and maintain a stable group membership.

This study will examine not only behavioral changes, but also the effect of gelding treatment on home range and habitat use. Bands of horses tend to have distinct home ranges, varying in size depending on the habitat and varying by season, but always including a water source, forage, and places where horses can shelter from inclement weather or insects (King and Gurnell 2005). By comparison, bachelor groups tend to be more transient, and can potentially use areas of good forage further from water sources, as they are not constrained by the needs of lactating mares in a group. It is unknown whether gelded stallions will behave like group stallions, bachelors, or form a group of their own concentrating in prime habitat or in the vicinity of water sources due to reduced desire for mare acquisition, maintenance, and reproductive behaviors. This study is necessary to quantify such behaviors and habitat use patterns.

Several public comments received suggested that castrating a stallion will preclude it from roaming freely and that gelding wild horses will essentially create populations of “domestic horses” on public land, which will have no value for scientists or the public. Those suggestions are not accurate. Gelding wild horses does not change their status as wild horses under the act. In terms of whether geldings will continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that geldings would continue to roam unhindered in the HMA where this action would take place. The scientific study that is part of this proposed action is structured in a way that would allow for a quantitative testing of the hypothesis that there are differences in movement behaviors between gelded and intact stallions. Although there may be differences, BLM would be surprised if the results indicate that gelded animals cease to roam freely. In contrast to the statement that there is no utility to the advancement of science and to the public, at least one value of initiating this study would be to test for differences in movement patterns between geldings and intact stallions, which would allow for more quantitative measures of free-roaming behavior.

The BLM does anticipate that gelded individuals may exhibit some behavioral differences, when compared to their own pre-treatment behaviors, or when compared to other intact stallions; such differences would be quantified under the proposed action. There is absolutely no evidence that would suggest that a gelded wild horse would have its movements hindered or would become docile or obedient simply as a result of castration. While it may be that a gelded horse could have a different set of behavioral priorities than an intact stallion, the expectation is that geldings will choose to act upon their behavioral priorities in an unhindered way, just as is the case for an intact stallion. In this sense, a gelded male would be just as much 'wild' as defined by the act as any intact stallion, even if his patterns of movement differ from those of an intact stallion.

Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a gelded animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior.

Under the proposed action, reproductive stallions would still be a component of the population's age and sex structure. The question of whether or not a given gelding would or would not attempt to maintain a harem is not germane to population-level management. The scientific study proposed will evaluate the demographic and behavioral effects of having some geldings within the larger population of wild horses. Gelding a subset of stallions in the proposed action would not prevent other stallions and mares from continuing with the typical range of social behaviors for sexually active adults.

Some comments received state that gelded stallions would have drastically lower testosterone levels and that males would lose much of their masculine behavior, which is largely responsible for stallion behavior and also plays a role in the "free roaming" nature of wild horses. The notion that a sudden decrease in testosterone would eliminate free-roaming behavior relies on an unsupported implication that free roaming behavior means something more than unhindered movement. The proposed study would be able to answer the question of whether gelded wild stallions change their behaviors with respect to other wild horses. BLM does not at all expect that geldings would become in any way docile. BLM fully expects that geldings would remain feisty and unruly with respect to humans. Similar statements state that testosterone is partially responsible for the stallion behavior that maintains these intricate family bands and that introducing large numbers of castrated males would impact family structures within the herds. It should be noted that BLM has no legal, regulatory, or policy mandate to maintain any given ratio of sexually reproductive stallions to mares within any population of wild horses. Despite that, in the Conger HMA there would continue to be a number of intact stallions that one would expect continue to compete for available sexually mature mares. As such, BLM would expect that family structures to continue to be exhibited under the proposed action. The BLM also is not required to manage populations of wild horses in order to ensure that any given individual maintains its social standing within any given harem or band.

Castration (the surgical removal of the testicles, also called gelding or neutering) is a well-established surgical procedure for the sterilization of domestic and wild horses. The procedure is relatively straight forward, rarely leads to serious complications and seldom requires postoperative veterinary care. Gelding adult male horses results in reduced production of

testosterone which directly influences reproductive behaviors. Although 20-30% of domestic horses, whether castrated pre- or post-puberty, continued to show stallion-like behavior (Line et al. 1985), it is assumed that free roaming wild horse geldings would exhibit reduced aggressive and reproductive behaviors. Gelding of domestic horses most commonly takes place before or shortly after sexual maturity, and age-at-gelding can affect the degree to which stallion-like behavior is expressed later in life. The behavior of wild horse geldings in the presence of intact male horses has not been studied or well documented. This study is necessary to quantify such behaviors.

Though gelding is a common surgical procedure, minor complications are not uncommon after surgery, and it is not always possible to predict when postoperative complications would occur. Fortunately the most common complications are almost always self-limiting, resolving with time and exercise. Individual impacts to the stallions during and following the gelding process should be minimal and would mostly involve localized swelling and bleeding. A small amount of bleeding is normal and generally subsides quickly, within 2-4 hours following the procedure. Some localized swelling of the prepuce and scrotal area is normal and may begin between one to 5 days after the procedure. Swelling should be minimized through the daily movements (exercise) of the horse during travel to and from foraging and watering areas. Most cases of minor swelling should be back to normal within 5-7 days, more serious cases of moderate to severe swelling are also self-limiting and resolve with exercise after one to 2 weeks. Serious complications (eviscerations, anesthetic reaction, injuries during handling, etc.) that result in euthanasia or mortality during and following surgery are rare and vary according to the population of horses being treated. Normally one would expect serious complications in less than 5% of horses operated under general anesthesia, but in some populations these rates can be as high as 12% (Shoemaker 2004). These complications are generally noted within 3 or 4 hours of surgery but may occur any time within the first 7 days following surgery (Hunt 1989). If they occur they would be treated with surgical intervention when possible or euthanasia when there is a poor prognosis for recovery.

Another concern raised by some public comments was that geldings are unable to contribute to the genetic diversity of the herd. This is true, but it does not lead to an expectation that the Conger HMA would experience inbreeding. Existing levels of genetic diversity were high when last measured, and expectations are that heterozygosity levels are even higher now that the population has continued to grow exponentially. In addition, many of the stallions that are gelded would have already had a chance to breed, or have already passed on genetic material to their offspring. BLM is not obligated to ensure that all stallions born within a population have the chance to sire a foal and pass on genetic material. The herd in which the proposed action is to take place is not at immediate risk of catastrophic loss of genetic diversity, nor does the genetic diversity in this band represent unique genetic information. This action does not prevent BLM from augmenting genetic diversity in the treated herd in the future, if future genetic monitoring indicates that would be necessary. Any statement that the herd would not be viable is conjecture, and is not supported by the fact that the treated population would still have mares and intact stallions at all times.

The Conger HMA is located such that a small number of horses can enter the population from neighboring areas. As such, there is the potential for some additional genetic information to

continually enter this population. The BLM allows for the possibility that if future genetic testing indicates that there is a critically low genetic diversity in the Conger population and other populations that interact with it genetically, then future management of the Conger population could include genetic augmentation, by bringing in additional stallions, mares, or both.

Another public concern voiced was the opinion that there are less intrusive, more humane, and more sustainable forms of fertility control available to the BLM. In terms of fertility control options that are effective on male horses, other available methods such as the injection of GonaCon-Equine immunocontraceptive vaccine apparently require multiple handling occasions to achieve long-term infertility. Insofar as the law indicates that management should be at the minimum level necessary to achieve management objectives (CFR 4710.4), and if gelding some fraction of a managed population can reduce population growth rates by replacing breeding mares, it then follows that gelding some individuals can lead to a reduced number of handling occasions, which is consistent with legal guidelines. Similarly, PZP immunocontraception that is currently available for use in mares requires handling or darting every year, which is hard to construe as consistent with a minimum level of management. Any such management activities that require multiple capture operations represent management that could be interpreted as being more intrusive, less humane, and less sustainable than an activity that requires only one period of handling.

Alternative 2

Implementation of Alternative 2 would result in impacts from gather and removal activities similar to those described in proposed action. Alternative 2 would not involve any form of population growth suppression; stallions would not be gelded and mares would not receive population growth suppression. No research study would be implemented.

Alternative 3 (No Action)

Under the No Action Alternative, there would be no active management to control the population size within the established AML at this time. In the absence of a gather, wild horse populations would continue to grow at an average rate of 20% per year within the Frisco and Conger HMAs. Without a gather and removal now, the population would grow to 504 on the Frisco HMA and 590 on the Conger Mtn. HMA in four years' time.

Use by wild horses would continue to exceed the amount of forage allocated for their use. Competition between wildlife, livestock and wild horses for limited forage and water resources would continue. Damage to rangeland resources would continue or increase. Over time, the potential risks to the health of individual horses would increase, and the need for emergency removals to prevent their death from starvation or thirst would also increase. Over the long-term, the health and sustainability of any wild horse population is dependent upon achieving a thriving natural ecological balance and sustaining healthy rangelands. Allowing wild horses to die of dehydration or starvation would be inhumane and would be contrary to the WFRHBA which requires that excess wild horses be immediately removed. Allowing rangeland damage to continue to result from wild horse overpopulation would also be contrary to the WFRHBA which requires the BLM to "*protect the range from the deterioration associated with overpopulation*", "*remove excess animals from the range so as to achieve appropriate management levels*", and "*to preserve and maintain a thriving natural ecological balance and multiple-use relationship in*

that area."

Wildlife

Alternatives 1 and 2
Wildlife and wildlife habitat would be indirectly affected by the improvements in resource health from the removal of excess horses and research implementing the proposed action would reduce utilization on key forage species, improving the quantity and quality of forage available to wildlife and decreasing competition for water sources.

Activities such as using helicopters and roping can have short-term effects on wildlife due to increased noise (i.e. helicopters, roping) and increased human presence in the project area.

Bait and water trapping impacts would vary by individual wildlife species. The intensity of these impacts would be indicated by behaviors ranging from nervous agitation to physical distress. Temporary disturbance and/or displacement would occur to wildlife during set up of traps or the inability to escape from traps; however, since traps are monitored, it is very unlikely wildlife would become trapped. Minimal impacts are expected since trap sites and temporary holding facilities would be located primarily in already disturbed sites. If traps are located in areas of intact wildlife habitat, a wildlife inventory clearance would be required.

The use of radio collars/tags, gelding and other parts of the research portion of the proposed action would not impact wildlife.

Special Status Wildlife Species

Ferruginous hawks could be impacted temporarily and short term through increased human disturbance and noise. It is expected that after gather activities have ceased, ferruginous hawks would return to the area. Destruction of riparian habitat could lead to potential temporary impacts on Townsend big-eared bats foraging opportunities. Reduction of wild horse populations to within AML would decrease the impacts to these habitats.

Big Game

Direct impacts would consist primarily of disturbance and short-term displacement of big game by the low-flying helicopter, construction of temporary trap/holding facilities and water trapping locations. A reduction of the wild horse population would decrease competition for available forage, cover, space and water between big game and wild horses once the AML has been achieved.

Reducing wild horse populations to within AML would provide protection of riparian areas which are important for big game that seek cooler summer temperatures, valuable forage, water availability, and hiding cover as well as thermal cover in the winter. Mule deer and elk use riparian-wetland areas during fawn rearing because riparian vegetation provides a source of succulent grasses and forbs, which are nutritionally important nutrition during gestation and lactation. Lowland riparian areas provide a valuable source of water and succulent forage for

pronghorn.

Upland Game

The reduction of wild horse population to within AML would reduce impacts to upland game species. There would be less competition between wild horses and upland game species for sagebrush and grassland areas. These areas are nesting habitat for upland game species.

Migratory Birds and Raptors

The proposed action may include wild horse gather operations trapping during the migratory bird nesting season, typically April 1 – July 30, however the gather would likely have a low potential for disturbance to individual nesting birds and no potential for impact to migratory bird populations. Riparian areas provide important habitat requirements for migratory birds. These areas are used as riparian corridors and for breeding and wintering habitat. The proposed action should reduce overuse by horses in riparian areas, by reducing the number of wild horse that visit those areas on a daily basis.

The proposed action is not expected to impact the golden eagle nesting season, the gather would likely have a low potential for disturbance to individual golden eagle nesting birds. If disturbance did occur, it would be briefly while a helicopter flew by.

Alternative 3 (No Action)

Special Status Wildlife Species

Under the No Action Alternative impacts would continue between BLM special status species and wild horses; such as destruction and degradation of foraging habitat.

Big Game

Under the No Action Alternative, competition between wild horse and big game would continue and likely increase as the horse population increases. Key perennial species vigor and production would be reduced, thus limiting available forage to big game.

Upland Game

Under the No Action Alternative, wild horses would compete with upland game species for habitat that is suitable for nesting and foraging.

Migratory Birds and Raptors

The No Action Alternative would have no direct impact to migratory birds and golden eagles since the gather would not occur. Indirect impacts would be decreased forage and cover, which would cause a loss of habitat for some species of migratory birds.

Health and Safety
Members of the public can inadvertently wander into areas that put them in the path of wild horses that are being herded or handled during the gather operations, creating the potential for injury to the wild horses or burros and to the BLM employees and contractors conducting the gather and/or handling the horses as well as to the public themselves. Because these horses are wild animals, there is always the potential for injury when individuals get too close or inadvertently get in the way of gather activities.

While helicopters are highly maneuverable and the pilots are very skilled in their operation, unknown and unexpected obstacles in their path can impact their ability to react in time to avoid members of the public in their path. These same unknown and unexpected obstacles can impact the wild horses or burros being herded by the helicopter in that they may not be able to react and can be potentially harmed or caused to flee which can lead to injury and additional stress. When the helicopter is working close to the ground, the rotor wash of the helicopter is a safety concern by potentially causing loose vegetation, dirt, and other objects to fly through the air which can strike or land on anyone in close proximity as well as cause decreased vision.

Fleeing horses can go through wire fences, traverse unstable terrain, and go through areas that they normally don't travel in order to get away, all of which can lead them to injure people by striking or trampling them if they are in the animal's path.

Disturbances in and around the gather and holding corral have the potential to injure the government and contractor staff who are trying to sort, move and care for the horses and burros by causing them to be kicked, struck, and possibly trampled by the animals trying to flee. Such disturbances also have the potential for similar harm to the public themselves

These potential impacts would be minimized by safety measures described in Section 2.3 and the safe viewing areas set up to watch loading and sorting.

4.3 Cumulative Effects for All Alternatives

The NEPA regulations define cumulative impacts as impacts on the environment that result from the incremental impact of the Proposed Action when added to other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions (40 CFR 1508.7). Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time. The cumulative impacts study area (CSA) for the purposes of evaluating cumulative impacts is the Conger and Frisco HMAs.

According to the 1994 BLM *Guidelines for Assessing and Documenting Cumulative Impacts*, the cumulative analysis should be focused on those issues and resource values identified during scoping that are of major importance. Accordingly, the issues of major importance to be analyzed are maintaining rangeland health and maintaining appropriate management level.

Past and Present Actions

Wild Horses

The Warm Springs Resource Area RMP/ROD Rangeland Program Summary (1987), designated the Conger Mountain HMA for the long-term management of wild horses. The HMA established in 1976 and identified in the "West Desert Wild Horse Capture Plan" (1977) are nearly identical in size and shape to the original herd areas identified in 1971. Management of wild horses within the HMA today is guided by the Warm Springs Resource Area RMP, 1987. AML was established as a population range of 40 – 80 on the Conger Mtn. HMA in 1987 through issuance of the Warm Springs Resource Area ROD.

The Pinyon Management Framework Plan (PMFP) (1983) and the Warm Springs Resource Area RMP/ROD Rangeland Program Summary (1987), identifies the Frisco HMA as suitable for wild horses, and allows for, "the removal of horses as required to maintain horse numbers at or below 1982 inventory levels, but not less than 1971 levels." (Pinyon MFP Wild Horse Amendment)(1983). The Frisco Herd Management Area Plan (2012) provides direction for management specific to the Frisco HMA and adjusted the AML to 30-60. The CCFO has records of six (6) wild horse gathers and removals that have occurred since 1971 within the Frisco HMA, resulting in the removal of approximately 463 wild horses from the area.

Congressional appropriations over the past ten years and most recently for the 2016 budget year prohibit the destruction of healthy animals that are removed or deemed to be excess. BLM policy is consistent with these appropriations provisions such that only sick, lame, or dangerous animals can be euthanized, and destruction is no longer used as a population control method. Nor does BLM sell excess wild horses for slaughter; rather BLM makes every effort to place excess wild horses with private citizens who can provide the animals with a good home.

Based on anecdotal observations from various BLM HMAs across the west, geldings released back to their home range: (1) tend to remain near where they were released (with adequate forage and water), (2) form small bachelor groups rather than join with a reproducing band, (3) maintain better body condition than the herd average because they are sexually inactive, (4) live longer in comparison to sexually active horses, and (5) were easy to recapture (many have been recaptured and released several times). (BLM Handbook H-4700-1) Those anecdotal observations were not based on a scientifically sound study design, and BLM will be able to update expectations about gelded wild stallions if the Preferred Alternative is selected.

Public interest in the welfare and management of wild horses continues to be very high. There are many different values pertaining to wild horse management from the public's perceptions. Some view wild horses as nuisance animals, while others strongly advocate management of wild horses as living symbols of the pioneer spirit.

Rangeland Health Standards

Through previous land use plan decisions, the BLM has allocated the available forage to wild horses, wildlife and domestic livestock. Other decisions, such as grazing permit renewals, have resulted in adjustments to livestock numbers and seasons of use and for implementation of grazing systems and the associated range improvements to promote rangeland health.

While the present livestock grazing system and efforts to manage the wild horse populations within AML has reduced past historic impacts, the current overpopulation of wild horses is continuing to contribute to areas of heavy vegetation utilization, trailing and trampling damage and is preventing the BLM from managing for rangeland health and a thriving natural ecological balance and multiple use relationship on the public lands in the area. Rangeland Health Assessments have been conducted within the Conger and Frisco HMAs for the associated livestock grazing allotments. Portions of the HMAs have been monitored over the past several years due to problems with drought, vegetation condition and the combined use of wild horses and domestic livestock. Adjustments have been made from these evaluations to the permitted use by livestock by way of season of use, livestock numbers, and grazing systems through the allotment evaluation and permit renewal processes.

The Proposed Action analyzed in this EA would result in the reduction in competition between wild horses and other users (i.e. native wildlife and domestic livestock) for the limited available forage and water resources. Direct improvements in soils and riparian condition would be expected in the short term and result in fewer multiple-use conflicts within and adjacent to the Conger and Frisco HMAs.

Over the long-term, improving the range would further benefit all users and the resources they depend on for forage and water.

Under the No Action (no removal) alternative, the current population of wild horses would not be reduced through the completion of a gather this year. Competition among wild horses, native wildlife and domestic livestock for limited resources would increase, and riparian conditions would continue to deteriorate. Over the long term, the health of wild horses and native wildlife would be expected to suffer as rangeland productivity further declines.

Wildlife

The greatest impacts to wildlife species in the area are the result of habitat degradation from drought, invasive weeds, livestock and wild horse grazing, OHV use and vegetation treatments on SITLA and private land. The proposed action would help to off-set these impacts by reducing the amount of forage utilized by wild horses.

4.4 Reasonably Foreseeable Future Actions

Future activities which could be expected to contribute to the cumulative impacts of implementing the Proposed Action or Alternatives within the next 10 years include continued mining exploration and development, oil and gas leasing, power line construction, solar, wind or other “green” energy production, livestock adjustments, treatment of invasive plants, noxious weeds, and pests, wild horse AML adjustments, wild horse population growth suppression, modification of wild horse sex ratios, non-reproducing wild horse herd components, herd augmentation, and wild horse removals.

Livestock grazing is expected to continue at similar stocking rates and utilization of the available

vegetation (forage) would also be expected to continue at similar levels. Rangeland Health Assessments would be planned to be completed in future years which could result in changes to livestock grazing systems such as changes to season of use, reduced or increased permitted use levels, or implementation of rotational grazing systems.

Any future actions that take place within the Conger and Frisco HMAs would be assessed through appropriate environmental documents in conformance with NEPA. For example, future mining plans of operation might require an EA or and EIS. Rangeland Health Assessments that adjust wild horse or livestock use might be accompanied by an EA, and future wild horse fertility treatment or gathers would be in conformance with this EA or new or amended EAs.

Livestock grazing is expected to continue at similar stocking rates and utilization of the available vegetation (forage) would also be expected to continue at similar levels. Continuing to graze livestock in a manner consistent with grazing permit terms and conditions would be expected to achieve or make significant progress towards achieving Rangeland Health Standards.

Past, present and future project with regards to properly planned vegetation and wildlife habitat improvement, invasive weed treatment, and range improvements are beneficial for wildlife. These projects generally ensure the quality of habitat and forage for wildlife species. Direct competition between wild horses, big game and other wildlife will continue to occur for perennial grasses, forbs, water and shelter. Wild horse populations have and would continue to influence the available forage for wildlife. As wild horse populations increase, the competition between wildlife and wild horses for limited resources would increase. As wild horses and wildlife are managed within the population goals and appropriate management levels (AML) this competition would be reduced. Abundance of small bird, mammal and reptile populations can be reduced because of habitat alteration. Wild horses can reduce the vegetation cover required to support adequate prey populations for raptor species.

Future actions that would affect vegetation in within the Conger and Frisco HMAs that are currently being developed and employed in surrounding areas within the Fillmore Field Office include the development of wind farms and pipelines, and the pursuance of the underground water resources within Snake Valley (Utah) by the Southern Nevada Water Authority for use within the Las Vegas, Nevada area. The loss of vegetation and water with the development of these activities would adversely affect the wild horse and native wildlife populations in the long-term through the loss of habitat.

While there is no anticipation for amendments to the WFRHBA, any amendments may change the management of wild horses on the public lands. The Act has been amended three times since 1971; therefore there is potential for amendment as a reasonably foreseeable future action.

4.5 Cumulative Impacts

Alternatives 1 and 2

The cumulative effects associated with the capture and removal of excess wild horses include gather-related mortality of less than 1% of the captured animals, about 5% per year associated with transportation, short term holding, adoption or sale with limitations and about 8% per year

associated with long-term holding. These rates are comparable to natural mortality on the range ranging from about 5-8% per year for foals (animals under age 1), about 5% per year for horses ages 1-15, and 5-100% for animals age 16 and older (Stephen Jenkins, 1996, Garrott and Taylor, 1990). In situations where forage and/or water are limited, mortality rates in the wild increase, with the greatest impact to young foals, nursing mares and older horses. Animals can experience lameness associated with trailing to/from water and forage, foals may be orphaned (left behind) if they cannot keep up with their mare, or animals may become too weak to travel. After suffering, often for an extended period, the animals may die. Before these conditions arise, the BLM generally removes the excess animals to prevent their suffering from dehydration or starvation.

While humane euthanasia and sale without limitation of healthy horses for which there is no adoption demand is authorized under the WFRHBA, Congress prohibited the use of appropriated funds between 1987 and 2004 and again in 2010 to present for this purpose.

The other cumulative effects which would be expected when incrementally adding either of the Action Alternatives to the cumulative study area would include continued improvement of upland and riparian vegetation conditions, which would in turn benefit permitted livestock, native wildlife, and wild horse population as forage (habitat) quality and quantity is improved over the current level. Benefits from a reduced wild horse population would include fewer animals competing for limited forage and water resources. Cumulatively, there should be more stable wild horse populations, healthier rangelands, healthier wild horses, and fewer multiple use conflicts in the area over the short and long-term. Over the next 15-20 years, continuing to manage wild horses within the established AML range would achieve a thriving natural ecological balance and multiple use relationship on public lands in the area.

Alternative 1 (Proposed Action)
In the future, application of population growth suppression techniques and adjustment in sex ratios to include some number of gelded males would be expected to slow total population growth rates, and to result in fewer gathers with less frequent disturbance to individual wild horses and the herd's social structure. However, return of wild horses back into an HMA could lead to decreased ability to effectively gather horses in the future as released horses learn to evade the helicopter.

Alternative 3 (No Action)
Under the No Action Alternative, the wild horse population within the Conger and Frisco HMAs combined could exceed 1000 in four years. Movement outside the HMAs would be expected as greater numbers of horses search for food and water for survival, thus impacting larger areas of public lands. Heavy to excessive utilization of the available forage would be expected and the water available for use could become increasingly limited. Eventually, ecological plant communities would be damaged to the extent that they would no longer be sustainable and the wild horse population would be expected to crash; this result could happen sooner than later under drought conditions.

Emergency removals could be expected in order to prevent individual animals from suffering or death as a result of insufficient forage and water. These emergency removals could occur as

early as 2016 with the current population levels, expected growth, and if there is persistent drought. During emergency conditions, competition for the available forage and water increases. This competition generally impacts the oldest and youngest horses as well as lactating mares first. These groups would experience substantial weight loss and diminished health, which could lead to their prolonged suffering and eventual death. If emergency actions are not taken, the overall population could be affected by severely skewed sex ratios towards stallions as they are generally the strongest and healthiest portion of the population. An altered age structure would also be expected.

Cumulative impacts of the no action alternative would result in foregoing the opportunity to improve rangeland health and to properly manage wild horses in balance with the available forage and water and other multiple uses. Attainment of site-specific vegetation management objectives and Standards for Rangeland Health would not be achieved. AML would not be achieved and BLM would forego the opportunity to collect the scientific data, which would include information about herd demography, effects of gelding, results from radio collar and GPS tags, fecal sampling, aerial survey enhancements, and population model development. All of those data would be necessary to re-evaluate AML levels in relationship to rangeland health standards, but would not be collected..

4.6 Monitoring and Mitigation Measures

The BLM Wild Horse Specialist, COR and PIs assigned to the gather would be responsible for ensuring all personnel abide by the CAWP (Appendix B).
Population research monitoring would be conducted by USGS/CSU in accordance with the protocol developed in the research proposal (Appendix A). Population inventories and vegetative monitoring (Rangeland Health, trend, utilization) would continue to be conducted as required by field office monitoring plans, BLM policy and the WFRHBA.

5.0 Consultation and Coordination

An annual single state-wide public hearing is held regarding the use of helicopters and motorized vehicles to capture wild horses (or burros) within the state of Utah. During the hearing, the public is given the opportunity to present new information and to voice any concerns or opinions regarding the use of these methods to capture wild horses (or burros). A hearing was held at the BLM Price Field Office, Price, Utah on December 8, 2015.

List of Preparers

The following list identifies the interdisciplinary team member's area of responsibility:

Name	Title	Area of Responsibility
Eric Reid	Assistant Field Manager	Project Lead/Wild Horses, Woodland/Forestry
Chad Hunter	Wild Horse and Burro Specialist	Wild Horses, ACECs, Environmental Justice, Farmlands (Prime or Unique), Floodplains, Invasive Species/Noxious Weeds, Rangeland Health Standards, Socio-Economics, Soils, Vegetation
James Priest	Wildlife Biologist	Wildlife, Migratory Birds, Threatened and Endangered and Special Status Species
Sheri Whitfield	Wildlife Biologist	Wildlife, Migratory Birds, Special Status Species
Stacey Whitman-Moore	Archeologist	Cultural Resources, Native American Religious Concerns
Jamie Palmer	Archeologist	Cultural Resources, Native American Religious Concerns
Teresa Frampton	Recreation Specialist	Wilderness, Visual Resources, Wild and Scenic Rivers, Lands/Access, ACECs, Recreation
David Jacobson	Recreation Specialist	Recreation, Wilderness, Visual Resources
Brian Taylor	Rangeland Management Specialist	Livestock Grazing, Standards for Rangeland Health, Vegetation
William Thompson	Rangeland Management Specialist	Farmlands (Prime or Unique), Riparian/Wetlands
Paul Caso	Rangeland Management Specialist	Soils, Floodplains, Air Quality, Water Quality, Water Rights
Adam Stephens	Rangeland Management Specialist	Air Quality, Greenhouse Gas Emissions, Hydrology, Water Quality, Riparian/Wetlands, Wild and Scenic Rivers
RB Probert	Weed Specialist	Invasive Species/Noxious Weeds
David Whitaker	Rangeland Management Specialist	Vegetation, Special Status Plant Species
Dan Fletcher	Assistant Field Manager	Special Status Plant Species
Gus Warr	Wild Horse and Burro Specialist	Wild Horses and Burros within the state of Utah

Alan Shepherd	Wild Horse and Burro Specialist	Wild Horses and Burros within the state of Nevada
Todd Leeds	Geologist	Geology/Mineral Resources, Wastes
Ed Ginouves	Geologist	Geology/Mineral Resources, Paleontology
Cindy Ledbetter	Environmental Coordinator	Greenhouse Gas Emissions, Environmental Justice, Paleontology, Socio-Economics
Gary Bishop	Assistant Fire Management Officer	Fire/Fuels Management
Melanie Mendenhall	Natural Resource Specialist	Fire/Fuels Management
Kyle Monroe	Engineering Technician	Property Boundary Evaluation
Michelle Campeau	Reality Specialist	Lands/Access
Glenn Pepper	Safety and Occupational Health Specialist	Wastes
Colby Peterson	Zone Forester	Woodlands/Forestry
Paul Griffin	Wild Horse and Burro Specialist	Population Statistical Modeling
Dr. Al Kane	APHIS Veterinarian	Animal Welfare
Dr. Kathryn Schoenecker	USGS	Research Proposal Lead
Dr. Sarah King	Colorado State University	Research Proposal Lead
Jared Bybee	Wild Horse and Burro Specialist	NEPA Compliance
Gina Ginouves	NEPA Specialist	NEPA Compliance

Public Involvement

Notification of the proposed action was listed on the ENBB and ePlanning (refer to section 1.7). A preliminary EA was made available for a 30-day public comment period. See Appendix G for a summary of the public comment period and comments received.

List of all Persons, Agencies and Organizations Consulted for Purposes of this EA

Name	Purpose & Authorities for Consultation or Coordination	Findings & Conclusions
Native American Tribes interested in projects within or interested in the state of Utah: Northwestern Band of Shoshoni Nation, Confederated tribes of the Goshute Reservation, Paiute Indian Tribe of Utah, Navajo	Consultation for undertaking, as required by the *Native American Graves Protection and Repatriation Act, the American Indian*	Identified tribes were notified October 1, 2015. The BLM has not received any letters expressing Native American concerns with the project.

Nation, Ute Indian Tribe of the Uintah and Ouray Reservation, Hopi Tribe, Southern Ute Tribe, Ute Mountain Ute Tribe, Pueblo of Zuni, Pueblo of Jemez, Shoshone Bannock Tribes, Eastern Shoshone Tribe	*Religious Freedom Act*, and various executive orders (e.g., Executive Order 13007)	
State of Utah, State and Institutional Trust Lands Administration, Renewable Resource Specialist	Consult with SITLA as the agency in control of state lands within the project area	
Millard County Commissioners	Consulted with Millard County	
Beaver County Commissioners	Consulted with Beaver County	

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Wood-Gush, D. G. M., and F. Galbraith. 1987. Social relationships in a herd of 11 geldings and two female ponies. Equine Veterinary Journal 19:129–132.

APPENDIX A

Colorado State University Institutional Animal Care and Use Committee protocol.

Colorado State University
Knowledge to Go Places

PROTOCOL
IACUC
Colorado State University

Protocol # [redacted]
Date Printed: February 05, 2016

Protocol Title:	Evaluating the behavior and ecology of geldings among a breeding population
Protocol Type:	IACUC
Date Submitted:	10/15/2015
Approval Period:	12/21/2015-12/20/2018
Important Note:	This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Personnel Information * * *

COLORADO STATE UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
ANIMAL USE APPLICATION

IACUC approval of this completed form is necessary prior to animals being obtained, housed or manipulated for research, testing or teaching purposes; performed at CSU or by CSU at other locations.

When you have completed all applicable sections of the protocol, you must also complete the certifications section and then click "Submit Form" link on the left-hand column.

All individuals listed on the protocol must have certified completion of the online CSU Animal Care and Use Training. Additionally, a "Training Record" should be uploaded in the Attachments section for the PI, Co-PI, and each person who will handle animals as a part of this study. Also, all individuals working with animals must be enrolled in the CSU Occupational Health and Safety Program (OHSP) via annual submission of a Risk Assessment Form to the OHSP.

Please contact an IACUC Coordinator if you have any questions.

Principal Investigator*

Name	Title	
[redacted]		
Email	**EID**	**Phone**
[redacted]	[redacted]	[redacted]
Department	**Mail Code**	
[redacted] Natural Resource Ecology Lab	[redacted]	
Will PI work with animals as part of this project?	Y	

Co-Principal Investigator

Name	Title	
[redacted]		
Email	**EID**	**Phone**
[redacted]		[redacted]
Department	**Mail Code**	
[redacted] Natural Resource Ecology Lab	[redacted]	
Will Co-PI work with animals as part of this project?	Y	

Department Head

Name of Department Head	Degree	Title
[redacted]		Professor
Email	**Phone**	**Fax**
[redacted]	[redacted]	
Department Name	**Campus Delivery Code**	
[redacted] Natural Resource Ecology Lab		

Will the Department Head work with animals as a part of this project? N

If this person will work with animals as a part of this protocol, upload a "Training Record" for this individual under the "Attachments" section of this protocol.

Other Personnel

Name	Title	
[redacted]	DVM	
Email	**EID**	**Phone**
[redacted]		[redacted]
Department	**Mail Code**	
Other		

Will this person be working with animals as a part of this project? Y

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* * * Species * * *

Species to be Used

Common Name	Horse			
Scientific Name	Horse			
Animal Sex	Male or Female			
Age Range	1	-	25	Year(s)
Weight Range	900	-	1500	lb(s)
Strain/Breed/Subline	Any strain			
Housing Location	Other			
	Wild animals on Conger Herd Management Area, Utah			
Room Number	N/A			
Maximum number of animals for three year project period	100			

USDA Pain Category (Choose all that will apply)

	Pain Category B	
X	Pain Category C	60
X	Pain Category D	40
	Pain Category E	

Pain Categories
Category B: Animals bred, conditioned or maintained for use in teaching, testing, or research, but not yet used for such purposes.
Category C: Animal use subjects them to no more than momentary or slight pain or distress and they do not receive pain-relieving drugs. Example : euthanasia prior to tissue collection; observation under normal conditions; positive rewards; routine injections (not Freund's adjuvant); tattooing; blood sampling.
Category D: Animal use subjects them to procedures where pain or distress is appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress which would otherwise be more than slight or momentary. Example: Needle biopsy non-survival or survival surgeries, terminal cardiac blood collection under terminal anesthesia; exposure of blood vessels for catheter implantation; induced infections or antibody production. PROCEDURES AT PAIN D REQUIRE VETERINARY CONSULTATION WITH THE UNIVERSITY VETERINARIAN OR DESIGNEE.
Category E: Animal use in which they must be subjected to unrelieved pain or distress for scientific reasons. Examples: toxicological or microbial testing or infectious disease research that requires continuation until severe clinical symptoms are evident or death occurs; application of noxious stimuli from which the animal cannot escape; prolonged restraint; use of paralyzing drugs for restraint of conscious animal; infliction of burns or trauma. PAIN E PROCEDURES REQUIRE CONSULTATION WITH THE UNIVERSITY VETERINARIAN OR DESIGNEE, AND MUST BE SCIENTIFICALLY JUSTIFIED IN THE PROTOCOL.

Source of Animals

Please indicate the source of the animals that will be used in the protocol. Be as specific as possible:
Outside Vendor (indicate whether purchased through LAR or by the investigator/department);
Transferred from another approved protocol (indicate protocol number);
Free-ranging Wildlife;
Faculty/Staff/Student-Owned ;
Client-Owned;
Other (please explain).

NOTE: If this is a study using Client Owned animals, you must provide a copy of the Informed Owner Consent Form along with approval from VMC Director in the Attachments section.

Free-ranging Wildlife managed by the Bureau of Land Management

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* * * Are You Using? * * *

Please indicate if you propose to use any of the following so the IACUC may better assess your protocol.

1. Will you be using live animals for teaching? N

   What are the goals of the course(s) and who is the intended audience(s)?

   Please describe the preparation the students will have prior to handling live animals (e.g. lecture, demonstrations, anatomical model use, videos)

2. Will you be using euthanized animals for teaching purposes? N

   What will be the source of the animals (LAR or Vendor) and what is the disposal plan?

   What are the goals of the course(s) and who is the intended audience(s)?

3. Will you be collaborating with another institution(s)? Y

Institution(s)

Institution Name	Other
Other (please specify)	US Geological Survey
PHS Assurance #	
USDA Registration #	
Collaboration institution personnel	[redacted]

Briefly explain how the collaboration or subcontract is structured

Funding comes to USGS from BLM. [redacted] is an ecologist at USGS who is Affiliate Faculty at CSU and co-PI.

Please summarize if animals will be purchased by, housed, or have procedures performed by CSU personnel at this other institution.

No.

Institution Name	Other
Other (please specify)	Bureau of Land Management
PHS Assurance #	
USDA Registration #	
Collaboration institution personnel	

Briefly explain how the collaboration or subcontract is structured

BLM manages the horses in the wild and maintains the Delta Wild Horse Facility where surgery will take place.

Please summarize if animals will be purchased by, housed, or have procedures performed by CSU personnel at this other institution.

4. Will you be using biohazardous agents?

   a) Recombinant DNA (rDNA), human fluids or human tissues — N

   b) Infectious Agents? — N
   If you indicated "Yes" to 4a. or b. above, please provide IBC protocol "PARF" number, or indicate "Submitted" or "Submission Pending," as appropriate.

   c) Will this protocol involve the generation of new transgenic or knockout lines using rDNA? — N

   d) If using an infectious agent or toxin, is it on the USDA or CDC Select Agent List (see Select Agents for the two lists of agents)? — N

5. Will studies be performed under Good Laboratory, Good Clinical, or Good Manufacturing Practices (GLP/GCP/GMP)? Such studies are regulated by the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA). Please contact the CSU Quality Assurance Manager for additional review and approval of GLP/GCP/GMP documentation. — N
   If yes, please provide the name of the individual who will be the Study Monitor, and briefly describe how the project involves GLP/GCP/GMP or preliminary product testing.

6. Will you be using controlled drugs?
   Will controlled drugs (including HCG and Ketamine) be used? — Y
   If yes, list whose CSU "drug cabinet" will be accessed. — [redacted], DVM in Delta, Utah

7. Will carcinogenic or chemical substances that are hazardous to humans or animals be used? N

Toxic Agent(s)

8. Will you be using radiological agents N

Isotope(s)

9. Will this be a field study (i.e. conducted on free-living wild animals in their natural habitat)? In addition to IACUC approval, the investigator is responsible for obtaining all necessary federal/state or other government permits for wildlife studies. Y

Field Study or Wildlife Study

Are state, federal or local permit(s) required?	Y
For which species or circumstances are permits required?	Wild horse
Do any of these species carry a zoonotic disease (e.g., rabies, hantavirus, bird flu)?	Y
Do you need additional information on protective measures for personnel?	Y
Are any species involved in this research under endangered or protected categories? (State, Federal or IUCN listed species)	Y

Indicate which species and explain why these species must be used for research

Wild horses are protected under the Wild Horse and Burro Act (PL 92-195). The purpose of this research is to examine the effect of castrating a proportion of males on the behavior and ecology of the population.

Other pertinent information regarding wildlife or fish studies that may help the IACUC review this protocol

We have a signed proposal from BLM which forms the permit to conduct this research. BLM will complete a NEPA document and any necessary Environmental Assessments for gelding stallions.

If this research is conducted in the field, note the person responsible for, and storage location of records detailing sedation and/or other materials administered to the animals in this study

Person Responsible	[redacted], BLM
Storage Location	Fillmore Field Office, Utah

If voucher specimens are collected, list the institution(s) where they will be deposited.

N/A

*** Funding Sources ***

Funding Checklist

Funding - Grants/Contracts

Funding Administered By	Other
	NREL
Y	Has the proposal related to this protocol been submitted?

Sponsored Programs PASS# (if available)

Sponsor Grant ID#	
CSU Fund #	
Funded By	Bureau of Land Management (BLM)
Principal Investigator	[redacted]
Grant/Contract Title	Evaluating the behavior and ecology of geldings among a breeding population
Y	For Federal projects, are contents of this protocol the same as described in Federal proposal application?
N	Is this protocol under an Umbrella protocol?

Funding - Other

Dept. Funding

Other Funding

This protocol is funded (in whole or in part) with funding from an agency in the U.S. Department of Defense (DoD)? This includes direct grant/contract funding or subcontract work that is flow-through of funding from DoD. N

If DoD funding is involved, the PI will be responsible for obtaining approval from the DoD Animal Care and Use Research Office (ACURO) for all new protocols and amendments to existing protocols prior to initiation of the work/change to the protocol.

Check here if this project is self-funded (No aspect of this work will have charges to a sponsored project, departmental account, other CSU-related account associated with it.)

NOTE: Applicable Federal Grant Application, including competing renewals must be attached. Applicable investigator's brochure and sponsor's protocol must be attached for all industry sponsored clinical trials. You will be prompted for these in the Attachments section.

Has this protocol received other internal reviews (check all that apply):

Reviewed for CRC Funding	Yes	No X
Reviewed by VTH/Clinical Sciences Clinical Research Review Board:	Yes	No X
I assure that the activities described with in this document submitted for IACUC review are consistent with those described in any related grant, contract, or subcontract that has been submitted or awarded.	YES X	NO

*** Rationale ***

1. PROJECT INFORMATION

a) Protocol title

Evaluating the behavior and ecology of geldings among a breeding population

b) Application type

Note: If you are editing a previously approved protocol for an Amendment or Continuing Review, please leave the answer to the questions under b. below as they were in the originally approved protocol.

This project is a: (check only one)

X New project

4th year renewal (please enter number of protocol that you are renewing below)

If this is a 4th year renewal, please indicate the number of the protocol it is renewing.

2. LAY SUMMARY

a) What is the overall goal or purpose of this animal use?
Provide a brief description which would convey to a lay audience the purpose for the proposed use of animals. Use language understandable to a layperson. Avoid overly technical terms and define acronyms. The readability should be similar to a newspaper article. For example, the goal of a study could be expressed as follows: "Disease XYZ is a serious threat to the health of.... This project will seek to test the efficacy of treatment ABC." Or, "This project seeks to understand the cellular mechanisms that influence X through in vitro analysis utilizing tissues harvested from the proposed species Y."
Note: A section from your grant application using highly technical terms is not acceptable.

We aim to look at the effect of gelding (castrating) 75% of males in a wild horse population, to examine the effects at both an individual and population level. Wild horses in the United States became a federally protected species after the passage of the Wild Free-Roaming Horses and Burros Act in 1971. Many wild horse populations across the western United States are increasing at high rates and occupy habitats of limited forage availability and diversity. The Bureau of Land Management (BLM) is responsible for managing wild horses on public lands, and currently only have two options for managing population growth: removal of horses to holding facilities for public adoption or long-term maintenance, and suppression of female fertility. Neither of these options are perfect, as holding facilities are approaching capacity, and female immunocontraception requires repeat applications. There is therefore a need for further effective, nonlethal approaches to reducing wild horse population growth rates on public lands. While previous research efforts have focused on controlling female fertility, male sterilization through gelding (testicular removal) could provide this alternative approach. Gelded males will not be able to breed, but there is no research on how their presence will affect the rest of the population. This project will seek to evaluate the effects of gelding males on their behavior and spatial ecology, and to determine how it affects their health and short-term survival. Additionally we will examine these same effects in females within the population. Information on the impacts of gelding on the grouping and movements of wild horses, and whether geldings potentially have a greater impact to the habitat by overconcentrating near water sources or prime habitat will allow managers to make informed decisions about the suitability of this management option.

b) What will the impact of the use of live animals in this project be for human OR animal health, the advancement of knowledge, or the good of society?
Regulations and ethical standards require that procedures involving the use of animals in research or teaching be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge or the good of society. Provide a brief description which would convey to a lay audience the impact the proposed research will have for one or more of the

above considerations. For example, "1 million people are estimated to contract disease XYZ each year. The proposed project will further the cause of developing effective treatments for the disease." Or "The cellular mechanisms X have previously been studied, but no studies have looked at aspect C of this mechanism. This study will advance the scientific understanding of X by exploring aspect C."
Note: Projects are not required to have application for human health to receive IACUC approval.

Wild horses are protected under Congress as they are considered cultural heritage, however their range is limited to where they were found when the Wild Free-Roaming Horses and Burros Act was passed in 1971. There are currently over 31,000 horses managed by the Bureau of Land Management (BLM) on public lands, with a potential for this number to increase by 20% every year. To control this growth the BLM removes horses from the range and places them in holding facilities where they are available for public adoption. Due to the low demand for these horses by the public there were 45,000 animals in holding facilities in 2012, maintenance of which consumes almost 60% of the BLM Wild Horse and Burro Program's budget. Little research has been conducted on the behavior and ecology of wild horses in the western United States over the past 20 years, despite a changing climate and different demands from the land. This study will impact animal health by providing a scientific understanding of the effects of gelding male horses in a free-roaming breeding population, and so inform its utility as a management tool for controlling horse population growth; it will advance knowledge of the behavior and ecology of wild horses, and be good for society in ensuring that cultural heritage remains.

3. JUSTIFICATION FOR USE OF ANIMALS

For parts a. and b. below, please answer "Yes" or "No" for each question.
There should be a Yes/No answer in all questions a)i. through a)vii. and b)i. through b)v.

a) Living animals are required for this project because:
(You should select either Y or N for each query.)

i)	Y		Complexity of the processes studied cannot be duplicated/modeled using in vitro models
ii)	Y		Not enough information known about processes being studied to design non-living models
iii)		N	Pre-clinical studies in living animals are necessary prior to human testing
iv)		N	This study requires tissue harvested from animals prior to in vitro testing
v)		N	Currently this is the best method to accomplish the required teaching
vi)	Y		Populations are being studied in natural or semi-natural environments
vii)	Y		Animal behavior is being studied

viii) Other (please specify):

b) This species has been selected because:
(You should select either Y or N for each query.)

i)	Y		Anatomy, physiology, behavior or agent susceptibility of species uniquely suited to the study
ii)		N	Lowest phylogenetic species providing adequate size, tissue, or anatomy for

proposed study

iii) N This species provides a particularly good model for the human or other animal disease or process

iv) N Previous studies which form the background for this project used this species

v) Y The objective of this study is to provide information about the target species

vi) Other (please specify):

4. JUSTIFICATION FOR NUMBER OF ANIMALS TO BE USED

The IACUC requires justification of proposed animal use numbers. A power calculation, confidence interval width, or an explanation why a power calculation is not feasible for this project should be provided. Complete one or more of the following (as appropriate) to justify the number of animals you will use (you may refer to Russ Lenth's U. Iowa stats website for statistical calculations). For experimental designs with multiple groups/treatments, it is suggested that a table of animal numbers per group be provided in the Attachments section. In addition make sure the animal numbers justified here agree with those mentioned in other sections of the application.

Answer N/A for any question (a-i) that is not applicable. There should be an answer or N/A in all boxes a-i.

a) This is an exploratory or pilot study. Describe how the proposed number of animals needed was determined. Note: A total of more than 12 animals indicates to the IACUC that the project may not be a pilot:

N/A

b) The group size was determined using a statistical package. Specify the statistical package used, effect size(s), estimate of variation used, and power level expected. (If multiple response variables are to be measured, the power calculation should be based on the most critical measures. When the objective is not to test but to estimate differences between mean or proportions, sample size may be justified based on confidence interval width criteria.):

N/A

c) This is a teaching protocol. Specify student-to-animal ratio, and explain how that was determined. There should be a clear correlation between the teaching objective and the number of animals per student:

N/A

d) This study involves tissue or cells harvested from animals for in vitro studies. Explain the number of animals requested for the amount of tissue needed to obtain a specified level of precision desired, or if an experiment involving the tissue samples will be conducted as part of this protocol, provide power calculations as described in b above. Cleary show the relationship between the number of animals requested and the number needed for the in vitro work:

N/A

e) This study involves breeding animals for later use in research, testing, or teaching. List the number of breeding males and females to be used/number of offspring produced each year, and describe how the animals are expected to be allocated to the subsequent experiment(s). If only a portion of the offspring will be usable in experiments, please indicate the number and reason for this:

N/A

f) This is a study of feral or wild animals where animals will be captured and released attempting to maximize sample size within logistical constraints. Describe and suggest a level of precision necessary to obtain useful information and the sample size required to obtain this precision:

The BLM has requested that we castrate 75% of males in the target population. Out of a population of 100 animals this will equate to about 40 males (we estimate around 37), as the others will be either female or juvenile males at the time of castration. Behavioral observations will be conducted on 16 focal male horses (8 of which will be castrated at the end of Year 1) and 4 focal female horses and their social associates (thus potentially including the majority of the 100 horses present at the HMA).

g) This is an observational, non-manipulative study in which animals will only be observed and animal numbers cannot be predicted. The animals will not be captured nor will their behavior be manipulated:

N/A

h) Sample size is government driven or agency mandated. Provide appropriate references documenting this requirement (e.g. product safety testing as mandated by FDA regulations):

See f.

i) Other. Please describe in detail:

N/A

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* * * Procedures * * *

Surgical Procedure

Procedure Type:	Surgical Procedure		
Procedure Title:	Gelding		
Species:	Horse (Wild animals on Conger Herd Management Area, Utah)	**Pain/Distress Category:**	D
Approximate number of animals to be used in this procedure:			40
All D and E studies require date of consultation with the University Veterinarian; or, the name of other vet who was consulted:			IACUC mtg 11/17/15

Use Location (Campus)	BLM Delta Adoption Facility	**Building Name:**	Off-Campus
		Room Number:	NA

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* * * Surgery Info * * *

Surgery Info

Specific room number where surgery is performed:	NA
Surgery Type:	S-Survival

ALSO NOTE: The Guide defines major surgery as one that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions, and the USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

Will this project include Multiple Major Survival Surgery (MMSS)? N

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Project Overview section of this form.

Number of animals per year:

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* * * Procedure Description * * *

Procedure Description

Procedure Description. Provide a brief description of how the procedure will be conducted. For blood/fluid collections include the route(s) of collection, volume, and frequency. For drug/compound dosing include route(s) of administration, volume, and frequency. For inoculations, include agent/vaccine information, route(s) of administration, volume, frequency, and dose. For procedures requiring administration of anesthesia, analgesia, provide the doses/route of administration; and for procedures requiring aseptic preparation, briefly describe animal, surgeon, and instrument preparation. Please DO NOT simply cut-and-paste from laboratory SOPs with superfluous or overly general information in them.

USGS or BLM will contract a clinical veterinarian to perform this procedure.

All animals will be sedated appropriately and then placed in lateral recumbency. A testicular block with a local anesthetic agent such as lidocaine or bupivacaine will also be done. The surgical site will be prepped using chlorhexidine scrub and the surgeon will wear sterile gloves to perform the castration procedure. Appropriate post-procedural medications will be given to the animals as well, e.g. tetanus shot, antibiotics, analgesics.

Please list any clinical effects or changes from normal health and behavior which may occur as a result of this procedure. This should include both short and longer-term effects of the procedure, as applicable.

The most common complications after gelding surgery are edema and excessive hemorrhage, with funiculitis, hydrocele, peritonitis, and penile damage reported as less common. Herniation and eventration has been reported, with incidences of these complications reduced if horses are checked for inguinal hernias prior to surgery. Behavior effects in a wild horse are not known.

Describe post procedure monitoring that will be performed. This should clearly indicate the frequency of monitoring, who will conduct it, and address the short- and longer-term complications that may result from the procedure.

Horses will be maintained in corrals for 24 to 72 hours after gelding, and then returned to the wild. In corrals they will be under veterinary supervision and visually checked at least twice a day. Once in the wild horses will be observed about once a week between April and August, and at least once a month through the rest of the year.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be given rescue analgesia, other clinical treatments, or euthanasia. Please include any scoring system that will be used to determine when humane intervention will be triggered in the Attachments section or provide the scoring criteria below, as applicable.

Any males that have an inguinal hernia or are cryptorchid will not receive surgery, but be euthanized as per BLM IM 2009-063 (attached). If within the first 24 hours after surgery animals show excessive swelling and/or are refusing food and not moving they will be checked by a veterinarian and given rescue analgesia (Flunixin Meglumine) as necessary. Once released to the wild no further veterinary interventions are possible.

*** Surgeon Details ***

Surgeon Details

Surgeon Name

[redacted]

Y

Does the Surgeon have prior specific experience with this surgery on this species?

Describe the previous experience and/or training plan to assure surgical proficiency.

[redacted] has been practicing veterinary medicine for 14 years and has been contracted by the BLM for veterinary care for the wild horses at the Delta Wild Horse Facility in Utah throughout this time. At the facility he provides general veterinary care (disease prevention and treatments) for horses brought in from the range and performs gelding surgeries on the males that are sent to other facilities for adoption.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Note: Documentation of training is not required if you are using VMC or LAR services

Anesthetists

Anesthetist Name	[redacted]
Describe previous experience and training in anesthesia. (Note: If you are using VMC or LAR services type N/A here.)	Veterinarian contracted by the BLM for care of wild horses at the Delta Wild Horse Facility. Has anesthetized many wild horses to perform this gelding surgery.

Parameters monitored during surgery:

Respiratory rate and mucous membrane color.

Anesthetic Agents

Agent Name	Ketamine, Horse, 0.12-2.2 mg/kg
Dosage (in mg/kg if possible)	2.2 mg/kg
Route	Intravenous injection (IV)

Agent Name	Lidocaine
Dosage (in mg/kg if possible)	~10-20 mls
Route	Other intra-testicular

Agent Name	Bupivicaine
Dosage (in mg/kg if possible)	~10-20 mls
Route	Other intra-testicular

Paralytic Agents

Other premedications not already listed above

Agent Name	Xylazine
Dosage (in mg/kg if possible)	1.1 mg/kg
Route	Intravenous injection (IV)

Duration and Frequency of Administration

Once for sedation

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* * * Perioperative Care * * *

Perioperative Care

Pre-emptive agents (analgesics given prior to procedure)

Intra-operative analgesics (local blocks;intracavity blocks).

Describe what parameters will be monitored during anesthesia/surgery to assure proper anesthesia.

Antibiotics or Anti-Microbials

Agent Name	**Penicillin G (procaine)**
Dosage (in mg/kg if possible)	22,000 units/kg
Route	**Intramuscular injection (IM)**

Duration and Frequency of Administration
Once

Post Operative Monitoring

Analgesic Agents

Agent Name	**Other** Flunixin Meglumine
Dosage (in mg/kg if possible)	2.2mg/kg
Route	**Intramuscular injection (IM)**

Duration and Frequency of Administration
Once

Recovery Location Building Name	Delta Wild Horse Facility, Delta, UT
Room Number	
Responsible Personnel	[redacted], [redacted] (BLM), [redacted] (BLM), [redacted], [redacted]
Parameters Monitored Note: Include any pain scale or scoring system as an attachment in attachments section.	We will monitor levels of swelling and whether the horse is eating post-surgery. Experience with horses will be used to assess pain, aided by the Horse Grimace Scale (attached) as necessary.
Monitoring Duration	24-72 hours
Monitoring Frequency	At least 2x day.

---

* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Agents

Agent Name	**Other**
	Tetanus
Dosage (in mg/kg if possible)	one shot
Route	**Intramuscular injection (IM)**
Frequency	

Behavior - observational

Procedure Type:	Behavior - observational		
Procedure Title:	Behavior and ecology of geldings in a breeding population		
Species:	Horse (Wild animals on Conger Herd Management Area, Utah)	**Pain/Distress Category:**	C

Approximate number of animals to be used in this procedure: 100

All D and E studies require date of consultation with the University Veterinarian; or, the name of other vet who was consulted:

Use Location (Campus)	Field	**Building Name:**	Off-Campus
		Room Number:	NA

---

* * * Procedure Description * * *

Procedure Description

Procedure Description. Provide a brief description of how the procedure will be conducted. For blood/fluid collections include the route(s) of collection, volume, and frequency. For drug/compound dosing include route(s) of administration, volume, and frequency. For inoculations, include agent/vaccine information, route(s) of administration, volume, frequency, and dose. For procedures requiring administration of anesthesia, analgesia, provide the doses/route of administration; and for procedures requiring aseptic preparation, briefly describe animal, surgeon, and instrument preparation. Please DO NOT simply cut-and-paste from laboratory SOPs with superfluous or overly general information in them.

Behavioral observations do not require any surgical procedure. Horses will be observed through binoculars or a spotting scope from on foot or a vehicle, at a distance sufficient that the observer does not influence behavior. We will have 20 focal animals (16 males, 4 females) on which we will record maintenance and social behaviors, as well as their nearest neighbor. Behavior of social associates during observation sessions of the 20 focal animals will also be recorded, meaning that a large proportion of the whole population (up to 100 animals) may be observed.

Please list any clinical effects or changes from normal health and behavior which may occur as a result of this procedure. This should include both short and longer-term effects of the procedure, as applicable.

NA

Describe post procedure monitoring that will be performed. This should clearly indicate the frequency of monitoring, who will conduct it, and address the short- and longer-term complications that may result from the procedure.

Each of the 20 focal horses and their social associates will be observed for 16-18 hours a month between April and August each year. The rest of the year they will be visually checked at least once a month.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be given rescue analgesia, other clinical treatments, or euthanasia. Please include any scoring system that will be used to determine when humane intervention will be triggered in the Attachments section or provide the scoring criteria below, as applicable.

Wild animals will not be given any veterinary care.

Herd Management

Procedure Type: Herd Management

Procedure Title: Gathering wild horses and handling them in captivity

Species: Horse (Wild animals on Conger Herd Management Area, Utah)

Pain/Distress Category: C

Approximate number of animals to be used in this procedure: 100

All D and E studies require date of consultation with the University Veterinarian; or, the name of other vet who was consulted:

Use Location (Campus) Field

Building Name: Off-Campus

Room Number: NA

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* * * Procedure Description * * *

Procedure Description

Procedure Description. Provide a brief description of how the procedure will be conducted. For blood/fluid collections include the route(s) of collection, volume, and frequency. For drug/compound dosing include route(s) of administration, volume, and frequency. For inoculations, include agent/vaccine information, route(s) of administration, volume, frequency, and dose. For procedures requiring administration of anesthesia, analgesia, provide the doses/route of administration; and for procedures requiring aseptic preparation, briefly describe animal, surgeon, and instrument preparation. Please DO NOT simply cut-and-paste from laboratory SOPs with superfluous or overly general information in them.

Gathers will be conducted by the BLM following BLM IM 2013-059 and BLM Comprehensive Animal Welfare Program (CAWP) for wild horse and burro gathers (attached). Experienced BLM personnel will gather horse groups into corrals using appropriate methods, as indicated in the attachments (helicopters and/or bait traps). Bait traps will be used whenever feasible. Bait traps are described in the CAWP and IM 2013-059 (attached). Bait traps use food, mineral supplements, water, or a mare in heat as an attractants in to a temporary trap. The trap will be observed at least every 12 hours and water and food will be provided if animals are held for longer than 12 hours. When water is used as bait other water sources may be closed off to lure the horses to the trap, but will not be closed for a period that would adversely affect the wellbeing of other animals using the area.

Once caught, stallions and mares will be moved in to different corrals, with mare-foal pairs kept together. All horses will then be loaded in to BLM approved stock trailers for transport to the BLM Delta

Wild Horse Facility.

Please list any clinical effects or changes from normal health and behavior which may occur as a result of this procedure. This should include both short and longer-term effects of the procedure, as applicable.

Experienced BLM personnel will perform gathers and herd animals through the chute. Neither gathers nor moving animals through chutes should cause any pain to the animal, but being in close proximity to humans is not natural for wild horses and will cause stress. We will work to reduce the stress on study animals by working as quickly and efficiently as possible, and using quiet voices and low tones.

Following the BLM Comprehensive Animal Welfare Program (CAWP) for wild horse and burro gathers helicopters will remain at a distance from horse groups and move them as slowly as possible to corrals.

Describe post procedure monitoring that will be performed. This should clearly indicate the frequency of monitoring, who will conduct it, and address the short- and longer-term complications that may result from the procedure.

All horses will be carefully monitored during the gather by the PIs, BLM personnel, and an on-site veterinarian if a helicopter gather. When in corrals they will be monitored at least once every day by BLM personnel.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be given rescue analgesia, other clinical treatments, or euthanasia. Please include any scoring system that will be used to determine when humane intervention will be triggered in the Attachments section or provide the scoring criteria below, as applicable.

Decision for euthanasia of horses will follow BLM IM 2009-041 (attached). As wild animals, horses other than those receiving surgery will not be given veterinary care.

---

* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Agents

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* * * Alternative Search * * *

Alternatives Search

Federal regulations require that the fewest number of live animals necessary are used for research, testing, or teaching, and that investigators document that they have given all due consideration to reducing or eliminating the use of potentially painful or distressful procedures (Pain Category D or E). The USDA considers automated literature searches the most effective and efficient method for demonstrating compliance with the above requirements.

For ALL projects, regardless of pain categorization, please conduct a literature search utilizing terms that would allow you to demonstrate that the proposed research or other animal use is not unnecessarily duplicative of previously documented work. Please enter the appropriate Search Data (click the "Add" button) and answer Question 1 below.

If the proposed project involves procedures at Pain Categories D and/or E, documentation of a literature search which demonstrates that the fewest number of the lowest order of animals will be used to obtain valid results, and alternatives to EACH potentially painful/distressful procedure proposed have been sought. Therefore please enter the appropriate Search Data and answer Questions 2 & 3 below. See USDA Policies #11 and 12).

For assistance with alternatives searches, please consult the CSU Libraries IACUC Alternatives Search Help page, see the Alternatives to Painful or Distressful Procedures document (prepared by the University Veterinarian), or contact an IACUC Coordinator.

Click the "Add" button below to enter information pertinent to your search(es). Please then address question 1 and, as appropriate to the procedures to be conducted, address, questions 2-3.

Search Data

Search Range From: 1965
To: 2015
Search Date: 08/25/2015
Search Terms

Please provide the Keywords and the Boolean terms such as AND, OR used to relate keywords (e.g. term#1 [AND] term#2) for searches for each of the three components of the Alternatives Search indicated above:

gelding behavior; gelding ecology; gelding aggression; gelding sexual; gelding health; castrat* male behavior; fertility control male horse; male contraception horse; "chemical castration" horse; "chemical castrat*"; equine castration

Databases Searched (you must search at least 2 databases):

	Agricola Data Base		Google Scholar
	ALTBIB - Bibliography on Alternatives to Animal Testing		HSVMA Alternatives in Education Database
	SCIRUS		Lab Animal
	AnimAlt-ZEBET		Lab. Animals Journal
	ATLA (FRAME--Alternatives to Laboratory Animal Journal)		Medline / PubMed
X	BioOne (access from CSU Libraries website)		NORINA
	BIOSIS (Note: CSU Libraries does not subscribe to this database)		TOXLINE
	CAB Abstracts (access from CSU Libraries website)	X	Web of Science (access from CSU Libraries Website)
			Other, please specify:

1. **N** **Did the search reveal that your project is duplicative of previously documented work?**
   a) Please provide the number of hits and an overview of the results.

Searches for "gelding behavior" (109 hits), "gelding ecology" (5 hits), "gelding aggression" (9 hits), "gelding sexual" (37 hits),"fertility control male horse" (236 hits), "castrat* male behavior" (title only: 170 hits). Most of the papers that resulted were veterinary in nature, or dealt with management of captive domestic horses. There was only one paper that involved the behavior of geldings in the wild, or with stallions and breeding mares, and it did not describe their behavior or interactions beyond that they dispersed to a certain area and lived longer than intact stallions (Kaseda et al. 1997). The other most relevant papers dealt with keeping mares and geldings in groups in a pasture (Van Dierendonck and Spruit 2012, Van Dierendonck et al. 2004, 2009, Sigurjonsdotiir et al. 2003). Some papers referred to the continuation of libidinous behavior after castration (Borsberry 1980, Pearce 1980, Smith 1974). Papers on other species also attested to sexual behavior being observed after castration, however most of these papers were not relevant as the animals were castrated prior to puberty.

   b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project anyway.

2. N **Did the search reveal any possible reductions or replacements that would allow the use of fewer animals or animals of a lower order?**

a) Please provide the number of hits and an overview of the results.

Searches for the terms "gelding behavior" (109 hits), "gelding ecology" (5 hits), "gelding aggression" (9 hits), "gelding sexual" (37 hits),"fertility control male horse" (236 hits), "castrat* male behavior" (title only: 170 hits), "male contraception horse" (34 hits), ""chemical castration" horse" (0 hits), "chemical castration horse" (9 hits), ""chemical sterilization" horse" (0 hits), ""chemical castrat*"" (146 hits). Although some of these results described the effects of castration in rodents (e.g., Achiraman et al. 2014, Constantini et al. 2007, Hume and Wynne-Edwards 2005, Turner et al. 1980) or primates (e.g. Dixson 1993) their results were not relevant to this study as the species had different social systems. Furthermore our sample size is determined by the Bureau of Land Management and as our aim is to examine the effect of gelding on a wild horse population we cannot use animals of a lower order.

b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project as proposed.

3. N **Did the search reveal any possible refinements that would allow the use of alternative procedures to those that will potentially cause pain and/or distress for the animals (Protocols utilizing procedures at pain category D and/or E)?**

a) Please provide the number of hits and an overview of the results.

We searched for the terms "fertility control male horse" (236 hits), "male contraception horse" (34 results), ""chemical castration" horse" (0 hits), "chemical castration horse" (9 results), ""chemical sterilization" horse" (0 results), ""chemical castrat*"" (146 results) in order to see if there were any options to surgical castration of males. Only one paper described a potential drug that could be used for chemical castration of stallions (Pozor et al. 2013), but the effects were reversible within about 71 days; we need permanent sterilization or a drug that would not need to be re-applied. Most of the chemical castration papers were about human sex offenders, with the remainder discussing chemical castration in other species. These papers were not applicable to our study as either the animals were chemically castrated pre-puberty, or else the paper did not state whether sterility was permanent or would need re-application. Furthermore we cannot use untested drugs on wild horses without checking their suitability first, which is out of the scope of this study. Three papers discussed vasectomies in horses: in zoo animals (Silber et al. 2013), in wild horses (Eagle et al. 1993), and a model of the effects in wild horses (Garrott and Siniff 1992). Prior to proposing this study we discussed the use of vasectomies with the Bureau of Land Management, but it was decided to focus this study on testicular removal rather than vasectomy.

b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project as proposed.

Teaching Protocols

1. If this is a teaching protocol, please specify why there are no alternatives to using live animals.

Protocols Involving Unrelieved Pain or Distress

1. For Pain Category E procedures, explain why drugs or other ameliorative treatments cannot be used to fully alleviate pain/distress. Please provide citations to the relevant literature.

Other Means of Determining Non-Duplication and Alternatives

The Animal Welfare Act allows other means of determining whether your project is duplicative AND whether it can be refined to decrease the animal number or order, AND to determine if alternatives to a potentially painful/distressful procedure can be used. For example, under some circumstances, colloquia, subject expert consultants, or other sources may provide relevant and up-to-date information regarding alternatives. When other sources are the primary means of considering alternatives, sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult should be provided. If you used an alternative search strategy, provide information on the strategy, methods, sources, and relevant findings.

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* * * Project Overview * * *

Project Overview

Provide a clear and concise sequential description of the procedures the animals will undergo. The description should include information on the experimental groups and the study endpoints. It should allow the reader to see the timing and relationship of all procedures that will be conducted with the animals. For lengthy or complex experiments with many groups and/or procedures, a table or flowchart showing the experimental manipulations by group should also be uploaded into the Attachments section. A response here is required.

January or February 2016 - gather horses and put radio collars on 30 male and 30 female horses (covered under FORT IACUC 2015-10 and the Inter-Institutional Agreement (IIA) with CSU, attached).

April to August 2016 - collect pre-treatment data on aggression, affiliative behavior, reproductive behavior, and band membership and fidelity of focal animals and their social group. Experimental groups will consist of: 4 adult females and their social groups, plus 16 adult male bachelors (n=8) and harem stallions (n=8) and their social groups. Focal individuals will include representatives of adult age groups and stallion social status present.

Fall 2016 – gather and treat animals (timing is dependent on BLM's gather schedule). All horses at Conger HMA will be gathered (as far as possible) and transported to the Delta BLM facility in BLM approved transport. Gathers and transport will follow BLM IM 2013-059 and follow their animal welfare standards (see attached). We will coordinate with the BLM and contract a veterinarian to perform the gelding surgery. Males for gelding will be selected randomly, blocked by age and social status, with a total of 75% of adult (≥3 years old) males receiving surgery (~37 horses). Animals with a Henneke body condition score of ≤3, and/or over the age of 18 (estimated by tooth wear) will not be gelded. Of the 16 focal stallions for behavioral observations, 8 will be gelded (4 bachelors and 4 harem stallions). Twenty four to 72 hours after gelding, depending on veterinary advice, the wild horses will be returned to the HMA and released. Exercise after gelding is recommended to reduce edema at the surgery site (Searle et al. 1999), and release of all horses at the same time will ensure that time in captivity is consistent between treatment and control animals. All collared horses will be visually checked at least once per month during the winter, with as many of the other geldings found as possible.

April to August 2017- collect post-treatment data on behavioral variables using the same focal individuals and whatever group they are in, whether or not they remain with the same group after gelding. Continue to visually observe radio marked individuals by locating them ≥1x/month to check collars and survival, presence of foals, and other population parameters.

April to August 2018- collect post-treatment data on behavioral variables using the same focal individuals and whatever group they are in. Continue to visually observe radio marked individuals by locating them ≥1x/month to check collars and survival, presence of foals, and other population parameters.

April to August 2019- collect post-treatment data on behavioral variables using the same focal individuals and whatever group they are in. Continue to visually observe radio marked individuals by locating them ≥1x/month to check collars and survival, presence of foals, and other population parameters. Check that collars drop off as programmed at the end of 2019, otherwise bait trap to

remove collars that fail. In 2020 initiate data analyses and conduct manuscript preparation.

Multiple Major Survival Surgery(MMSS) Description:

Describe why it is necessary to perform multiple major surgical procedures on the same animal.

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* * * Husbandry * * *

Animal Care/Husbandry

Emergency Contact Information

List all individuals/phone numbers that are to be notified by veterinary staff or others in the event of an emergency:

[redacted] (CSU) - [redacted] [USGS/CSU]
[redacted] (BLM) - [redacted]
[redacted] (BLM) - [redacted]

Will Lab Animal Resources provide the daily care **N**

If "No," specify who will provide the daily care:

Animals are wild, so will not receive daily care. When at the Delta Wild Horse Facility they will be under the daily care of the facility manager.

If "No," justify why LAR will not be providing animal care:

Animals will be located in Utah.

What veterinarian will provide medical care to animals? **Other**

If "Other" specify who:

[redacted] DVM

Contact information:

[redacted] Veterinarian Services, [redacted] or [redacted] (cell)

If "Other" justify why LAR will not be providing medical care:

Animals will be located in Utah

Location of medical records (indicate building/room or other applicable information):

Medical records will be maintained by BLM at the Fillmore Field Office in Fillmore, Utah.

Special Husbandry or Care

List any special or unusual requirements for care of the animals and who will provide this care (e.g. special diet, altered light cycle, variation from standard enrichment, etc.):

N/A

Non-standard Experimental Requirements (Procedures requiring Exemptions from the Guide).

Social Housing

If you are using a social species there are mandatory housing requirements. CSU considers social housing to include compatible housing with conspecifics, as well as housing in the same secondary containment with visual, auditory, olfactory or tactile contact with conspecifics. See the "Policy on Social Management of Animals" on the IACUC Policies and Guidelines Page.

Please indicate which of the following is true:

1. Animals will be provided with social housing (unless an animal has individual incompatibility or vet care concerns, or due to cohort attrition).

X 2. Animals will not be housed at CSU.

3. Animals will be housed singly because that is appropriate for this species (including hamsters, rabbits, male mice, tom cats, and livestock in stalls).

4. Animals will be housed singly because such housing is necessary for research, testing or teaching goals.

If you will be housing animals singly for research, testing or teaching purposes (#4 above), you must provide a written justification which indicates the experimental constraints that make the housing necessary:

Food or Fluid restriction (other than up to 12 hours prior to surgery/general anesthesia) X None

Food or Fluid restriction

Species	Food Restriction	Length of Restriction	Fluid Restriction	Length of Restriction	Reason for Restriction
Horse (Wild animals on Conger Herd Management Area, Utah)					

Description

Restraint of Conscious Animals (other than momentary restraint for routine procedrues, e.g. blood collections, injections, and such) X None

Restraint of Conscious Animals

Species	Type restraint (manual, commercial, manual and commercial)	Please describe Acclimation to restraint	Length of restraint
Horse (Wild animals on Conger Herd Management Area, Utah)			

Description

Non-standard housing requirements X None

Non-standard housing requirements

Species	Cage/Pen size	Cage Sanitation Interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from exercise (dogs only)
Horse (Wild animals on Conger Herd Management Area, Utah)					

Description

*** * * Disposition of Animals * * ***

Please provide the information requested below regarding what will happen to animals at study end. (Check all that apply)

	Animals will be adopted (Note, PI is required to follow the IACUC "Policy on Animal Adoptions" which is located on the page IACUC Policies and Guidelines Page.
	Sold at auction (hoof stock only)
X	Released into home territory (wildlife studies)
	Returned to client
	Transferred to other studies (please specify below)
	Animals will be euthanized (Please add method below)

If using CO_2 as the method of euthanasia for mice and rats, please be aware that the IACUC requires use of the "Directions for CO_2 Euthanasia of Rodents" (available on the IACUC Policies and Guidelines Page) unless the protocol provides scientific justification why that procedure cannot be used.

Euthanasia Method

Please briefly describe what will happen with the animals at the conclusion of the study in the text box below:

*** * * Attachments * * ***

PLEASE ATTACH ANY RELEVANT DOCUMENTS, INCLUDING:

Grant applications to any PHS agency, NSF, and USDA related to this activity
Training Records for all personnel on this protocol
Any scientific literature or articles relevant to the review of this project.

Please upload training records for the PI, Co-PI, and all individuals who will be working with animals as a part of this protocol. Click here to obtain the template for the Training Record.

Document Type	**Grant or Grant Proposal**
Attachment	Final Gelding Proposal_signed
Document Name	Final Gelding Proposal_signed

Document Type	**Protocol Supplement**
Attachment	IIA [redacted] field study

Document Name	IIA[redacted] field study
Document Type	**Literature**
Attachment	Bureau of Land Management 2015
Document Name	Bureau of Land Management 2015
Document Type	**Literature**
Attachment	IM 2013-059, Wild Horse and Burro Gathers Comprehensive Animal Welfare Policy
Document Name	IM 2013-059, Wild Horse and Burro Gathers Comprehensive Animal Welfare Policy
Document Type	**Training Record**
Attachment	[redacted]_TrainingRecord
Document Name	[redacted]_TrainingRecord
Document Type	**Literature**
Attachment	Dalla Costa 2014 PLoS ONE
Document Name	Dalla Costa 2014 PLoS ONE
Document Type	**Protocol Supplement**
Attachment	FORT IACUC 2015-10 ApprovalLetter_FINAL
Document Name	FORT IACUC 2015-10 ApprovalLetter_FINAL
Document Type	**Literature**
Attachment	BLM IM 2009 41_WHB Euthanasia
Document Name	BLM IM 2009 41_WHB Euthanasia
Document Type	**Literature**
Attachment	IM 2009-063, Gelding of Wild Horses and Burros and Gelding Vouchers
Document Name	IM 2009-063, Gelding of Wild Horses and Burros and Gelding Vouchers
Document Type	**Training Record**
Attachment	TrainingRecord_Rev_29FEB2012_[redacted]
Document Name	TrainingRecord_Rev_29FEB2012_[redacted]
Document Type	**Training Record**
Attachment	[redacted] training
Document Name	[redacted] training

*** Guidelines ***

Guidelines

The CSU IACUC Policies and Guidelines page can assist you and your staff in the protocol development and animal study process.

*** Certifications ***

I understand that changes in the approved protocol must be submitted in writing to the IACUC as a protocol amendment and approved by the IACUC prior to implementation. Such changes include, but are not limited to: species, animal numbers, animal-related procedures, animal restraint, food/water deprivation, euthanasia, PI, research staff, and the like. Minor changes can be reviewed by the IACUC via the designated member review process throughout the month; significant changes (e.g. a large increase in animal numbers, adding an invasive procedure) usually require a new protocol be submitted for review by the IACUC at its next regularly scheduled meeting.

Please contact an IACUC Coordinator if you have any questions about preparing new protocol applications, amendment requests, or continuing reviews.

Certification Test

By submitting this protocol to the CSU Institutional Animal Care and Use Committee (IACUC), the Principal Investigator certifies the following:

1) I assure that myself and all students, staff, and faculty on this project are familiar with the Animal Welfare Act (AWA) and AWA Regulations and the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Guide for the Care and Use of Agricultural Animals in Research and Teaching, as applicable, and all recognize their responsibility in strictly adhering to approved protocols.

2) I assure that all individuals listed on this project are qualified through education and/or training to conduct procedures involving animals under this proposal and have taken the online CSU Animal Care and Use Training, which includes information on the regulatory responsibilities of the institution, the IACUC, and investigators, as well as the concepts of research or testing methods that limit the use of animals or minimize distress, and the methods for reporting animal welfare concerns. Additionally, as applicable to their work with animals, all individuals on the protocol have received training in the biology, handling, and care of the species to be used; aseptic surgical methods and techniques; and the proper use of anesthetics, analgesics, and tranquilizers.

3) I assure that all procedures will be conducted in accordance with all applicable Colorado State University IACUC policies as well as Occupational and Biosafety requirements, including those pertaining to the use of personal protective equipment.

4) I assure that all individuals working on this proposed protocol are participating in the Occupational Health and Safety Program (OHSP).

5) I assure that ANY change in the care and use of animals involved in this protocol will be promptly forwarded to the IACUC for review. Such changes will not be implemented until approval is obtained from the IACUC. Animals will not be transferred between investigators without prior approval.

6) I assure that I have reviewed the pertinent scientific literature and the sources and/or databases and have found no valid alternative to any procedures described herein which may cause more than momentary or slight pain, distress, or generalized discomfort to animals, whether it is relieved or not.

7) I assure that every effort has been made to minimize the number of animals used and reduce the amount of pain, distress, and/or discomfort these animals must experience.

8) I assure that the activities described in this document submitted for IACUC review are consistent with those described in any related grant, contract, or subcontract that has been submitted or awarded.

9) I assure that the information contained in this application for animal use is accurate to the best of my knowledge.

10) I understand that this application and/or my animal use privileges may be revoked by the IACUC if I violate any of the aforementioned assurance statements.

X The Principal Investigator has read and agrees to abide by the above assurances

---

*** Event History ***

Event History

Date	Status	View Attachments	Letters
08/14/2015	NEW FORM CREATED		
10/15/2015	NEW FORM SUBMITTED	Y	
10/15/2015	NEW FORM PANEL ASSIGNED		
10/15/2015	NEW FORM REVIEWER(S) ASSIGNED		
10/28/2015	NEW FORM Comments Received (Cycle 1)		
11/12/2015	NEW FORM PANEL REASSIGNED		
11/12/2015	NEW FORM REVIEWER(S) ASSIGNED		
11/13/2015	NEW FORM Comments Received (Cycle 1)		
11/13/2015	NEW FORM REVIEWER(S) ASSIGNED		
11/15/2015	NEW FORM Comments Received (Cycle 1)		
11/16/2015	NEW FORM Comments Received (Cycle 1)		
11/17/2015	NEW FORM Comments Received (Cycle 1)		
11/17/2015	NEW FORM Comments Received (Cycle 1)		
11/24/2015	NEW FORM Comments Sent (Cycle 1)		
12/01/2015	NEW FORM Responses Received (Cycle 1)	Y	
12/01/2015	NEW FORM Responses Sent (Cycle 1)		

12/01/2015	NEW FORM Responses Sent (Cycle 1)		
12/01/2015	NEW FORM Responses Sent (Cycle 1)		
12/01/2015	NEW FORM REVIEWER(S) ASSIGNED		
12/02/2015	NEW FORM Comments Received (Cycle 2)		
12/02/2015	NEW FORM Recommended for Approval		
12/21/2015	NEW FORM APPROVED	Y	Y

APPENDIX B
Comprehensive Animal Welfare Policy/Program (CAWP) Standards

Developed by

The Bureau of Land Management
Wild Horse and Burro Program

in collaboration with

Carolyn L. Stull, PhD
Kathryn E. Holcomb, PhD
University of California, Davis
School of Veterinary Medicine

June 30, 2015

WELFARE ASSESSMENT STANDARDS for GATHERS
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STANDARDS

Standard Definitions
Major Standard: Impacts the health or welfare of WH&Bs. Relates to an alterable equipment or facility standard or procedure. Appropriate wording is "must," "unacceptable," "prohibited."
Minor Standard: unlikely to affect WH&Bs health or welfare or involves an uncontrollable situation. Appropriate wording is "should."
Lead COR = Lead Contracting Officer's Representative
COR = Contracting Officer's Representative
PI = Project Inspector
WH&Bs = Wild horses and burros

FACILITY DESIGN

A. Trap Site and Temporary Holding Facility
   1. The trap site and temporary holding facility must be constructed of stout materials and must be maintained in proper working condition, including gates that swing freely and latch or tie easily. (**major**)
   2. The trap site should be moved close to WH&B locations whenever possible to minimize the distance the animals need to travel.(minor)
   3. If jute is hung on the fence posts of an existing wire fence in the trap wing, the wire should be either be rolled up or let down for the entire length of the jute in such a way that minimizes the possibility of entanglement by WH&Bs unless otherwise approved by the Lead COR/COR/PI. (minor)
   4. Fence panels in pens and alleys must be not less than 6 feet high for horses, 5 feet high for burros, and the bottom rail must not be more than 12 inches from ground level. (**major**)
   5. The temporary holding facility must have a sufficient number of pens available to sort WH&Bs according to gender, age, number, temperament, or physical condition. (**major**)
      a. All pens must be assembled with capability for expansion. (**major**)
      b. Alternate pens must be made available for the following: (**major**)
         1) WH&Bs that are weak or debilitated
         2) Mares/jennies with dependent foals
      c. WH&Bs in pens at the temporary holding facility should be maintained at a proper stocking density such that when at rest all WH&Bs occupy no more than half the pen area. (minor)
   6. An appropriate chute designed for restraining WH&Bs must be available for necessary procedures at the temporary holding facility. This does not apply to bait trapping operations unless directed by the Lead COR/COR/PI. (**major**)
   7. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may cause escape or possible injury. (**major**)
   8. Padding must be installed on the overhead bars of all gates and chutes used in single file alleys. (**major**)
   9. Hinged, self-latching gates must be used in all pens and alleys except for entry gates into the trap, which may be secured with tie ropes. (**major**)
   10. Finger gates (one-way funnel gates) used in bait trapping must be constructed of

materials approved by the Lead COR/COR/PI. Finger gates must not be constructed of materials that have sharp ends that may cause injuries to WH&Bs, such as "T" posts, sharpened willows, etc. (**major**)

11. Water must be provided at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals, and environmental conditions, with each trough placed in a separate location of the pen (i.e. troughs at opposite ends of the pen). Water must be refilled at least every morning and evening. (**major**)
12. The design of pens at the trap site and temporary holding facility should be constructed with rounded corners. (minor)
13. All gates and panels in the animal holding and handling pens and alleys of the trap site must be covered with materials such as plywood, snow fence, tarps, burlap, etc. approximately 48" in height to provide a visual barrier for the animals. All materials must be secured in place.(**major**)

These guidelines apply:

a. For exterior fences, material covering panels and gates must extend from the top of the panel or gate toward the ground.(**major**)
b. For alleys and small internal handling pens, material covering panels and gates should extend from no more than 12 inches below the top of the panel or gate toward the ground to facilitate visibility of animals and the use of flags and paddles during sorting. (minor)
c. The initial capture pen may be left uncovered as necessary to encourage animals to enter the first pen of the trap. (minor)

14. Non-essential personnel and equipment must be located to minimize disturbance of WH&Bs. (**major**)
15. Trash, debris, and reflective or noisy objects should be eliminated from the trap site and temporary holding facility. (minor)

B. Loading and Unloading Areas

1. Facilities in areas for loading and unloading WH&Bs at the trap site or temporary holding facility must be maintained in a safe and proper working condition, including gates that swing freely and latch or tie easily. (**major**)
2. The side panels of the loading chute must be a minimum of 6 feet high and fully covered with materials such as plywood or metal without holes that may cause injury. (**major**)
3. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may cause escape or possible injury. (**major**)
4. All gates and doors must open and close easily and latch securely. (**major**)

5. Loading and unloading ramps must have a non-slip surface and be maintained in a safe and proper working condition to prevent slips and falls. Examples of non-slip flooring would include, but not be limited to, rubber mats, sand, shavings, and steel reinforcement rods built into ramp. There must be no holes in the flooring or items that can cause an animal to trip. (**major**)
6. Trailers must be properly aligned with loading and unloading chutes and panels such that no gaps exist between the chute/panel and floor or sides of the trailer creating a situation where a WH&B could injure itself. (**major**)
7. Stock trailers should be positioned for loading or unloading such that there is no more than 12" clearance between the ground and floor of the trailer for burros and 18" for horses. (minor)

I. CAPTURE TECHNIQUE

A. Capture Techniques

1. WH&Bs gathered on a routine basis for removal or return to range must be captured by the following approved procedures under direction of the Lead COR/COR/PI. (**major**)
   a. Helicopter
   b. Bait trapping
2. WH&Bs must not be captured by snares or net gunning. (**major**)
3. Chemical immobilization must only be used for capture under exceptional circumstances and under the direct supervision of an on-site veterinarian experienced with the technique. (**major**)

B. Helicopter Drive Trapping

1. The helicopter must be operated using pressure and release methods to herd the animals in a desired direction and should not repeatedly evoke erratic behavior in the WH&Bs causing injury or exhaustion. Animals must not be pursued to a point of exhaustion; the on-site veterinarian must examine WH&Bs for signs of exhaustion. (**major**)
2. The rate of movement and distance the animals travel must not exceed limitations set by the Lead COR/COR/PI who will consider terrain, physical barriers, access limitations, weather, condition of the animals, urgency of the operation (animals facing drought, starvation, fire, etc.) and other factors. (**major**)
   a. WH&Bs that are weak or debilitated must be identified by BLM staff or the contractors. Appropriate gather and handling methods should be used according to the direction of the Lead COR/COR/PI. (**major**)
   b. The appropriate herding distance and rate of movement must be determined on a case-by-case basis considering the weakest or smallest animal in the group (e.g., foals, pregnant mares, or horses that are weakened by body condition, age, or poor health) and the range and environmental conditions present. (**major**)
   c. Rate of movement and distance travelled must not result in exhaustion at the trap site, with the exception of animals requiring capture that have an existing severely compromised condition prior to gather. Where compromised animals cannot be left on the range or where doing so would only serve to prolong their suffering, euthanasia will be performed in accordance with BLM policy. (**major**)
3. WH&Bs must not be pursued repeatedly by the helicopter such that the rate of movement and distance travelled exceeds the limitation set by the Lead

COR/COR/PI. Abandoning the pursuit or alternative capture methods may be considered by the Lead COR/COR/PI in these cases. (**major**)

4. When WH&Bs are herded through a fence line en route to the trap, the Lead COR/COR/PI must be notified by the contractor. The Lead COR/COR/PI must determine the appropriate width of the opening that the fence is let down to allow for safe passage through the opening. The Lead COR/COR/PI must decide if existing fence lines require marking to increase visibility to WH&Bs. (**major**)
5. The helicopter must not come into physical contact with any WH&B. The physical contact of any WH&B by helicopter must be documented by Lead COR/COR/PI along with the circumstances. (**major**)
6. WH&Bs may escape or evade the gather site while being moved by the helicopter. If there are mare/dependent foal pairs in a group being brought to a trap and half of an identified pair is thought to have evaded capture, multiple attempts by helicopter may be used to bring the missing half of the pair to the trap or to facilitate capture by roping. In these instances, animal condition and fatigue must be evaluated by the Lead COR/COR/PI or on-site veterinarian on a case-by-case basis to determine the number of attempts that can be made to capture an animal.(**major**)
7. Horse captures must not be conducted when ambient temperature at the trap site is below 10ºF or above 95ºF without approval of the Lead COR/COR/PI. Burro captures must not be conducted when ambient temperature is below 10ºF or above 100ºF without approval of the Lead COR/COR/PI. The Lead COR/COR/PI will not approve captures when the ambient temperature exceeds 105 ºF. (**major**)

C. Roping

1. The roping of any WH&B must be approved prior to the procedure by the Lead COR/COR/PI. (**major**).
2. The roping of any WH&B must be documented by the Lead COR/COR/PI along with the circumstances. WH&Bs may be roped under circumstances which include but are not limited to the following: reunite a mare or jenny and her dependent foal; capture nuisance, injured or sick WH&Bs or those that require euthanasia; environmental reasons such as deep snow or traps that cannot be set up due to location or environmentally sensitive designation; and public and animal safety or legal mandates for removal. (**major**)
3. Ropers should dally the rope to their saddle horn such that animals can be brought to a stop as slowly as possible and must not tie the rope hard and fast to the saddle so as to intentionally jerk animals off their feet. (**major**)
4. WH&Bs that are roped and tied down in recumbency must be continuously observed and monitored by an attendant at a maximum of 100 feet from the animal. (**major**)
5. WH&Bs that are roped and tied down in recumbency must be untied within 30 minutes. (**major**)
6. If the animal is tied down within the wings of the trap, helicopter drive trapping within the wings will cease until the tied-down animal is removed. (**major**)
7. Sleds, slide boards, or slip sheets must be placed underneath the animal's body to move and/or load recumbent WH&Bs. (**major**)
8. Halters and ropes tied to a WH&B may be used to roll, turn, position or load a recumbent animal, but a WH&B must not be dragged across the ground by a halter or rope attached to its body while in a recumbent position. (**major**)

9. Animals captured by roping must be evaluated by the on-site/on-call veterinarian within four hours after capture, marked for identification at the trap site, and be re-evaluated periodically as deemed necessary by the on-site/on-call veterinarian. (**major**)

D. Bait Trapping
1. WH&Bs may be lured into a temporary trap using bait (feed, mineral supplement, water) or sexual attractants (mares/jennies in heat) with the following requirements:
   a. The period of time water sources other than in the trap site are inaccessible must not adversely affect the wellbeing of WH&Bs, wildlife or livestock, as determined by the Lead COR/COR/PI. (**major**)
   b. Unattended traps must not be left unobserved for more than 12 hours. (**major**)
   c. Mares/jennies and their dependent foals must not be separated unless for safe transport. (**major**)
   d. WH&Bs held for more than 12 hours must be provided with accessible clean water at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals and environmental conditions. (**major**)
   e. WH&Bs held for more than 12 hours must be provided good quality hay at a minimum rate of 20 pounds per 1000 pound adult animal per day, adjusted accordingly for larger or smaller horses, burros and foals. (**major**)
      1) Hay must not contain poisonous weeds, debris, or toxic substances. (**major**)
      2) Hay placement must allow all WH&Bs to eat simultaneously. (**major**)

II. WILD HORSE AND BURRO CARE

A. Veterinarian
1. On-site veterinary support must be provided for all helicopter gathers and on-site or on-call support must be provided for bait trapping. (**major**)
2. Veterinary support must be under the direction of the Lead COR/COR/PI. The on-site/on-call veterinarian will provide consultation on matters related to WH&B health, handling, welfare, and euthanasia at the request of the Lead COR/COR/PI. All decisions regarding medical treatment or euthanasia will be made by the on-site Lead COR/COR/PI. (**major**)

B. Care
1. Feeding and Watering
   a. Adult WH&Bs held in traps or temporary holding pens for longer than 12 hours must be fed every morning and evening with water available at all times other than when animals are being sorted or worked. (**major)**
   b. Water must be provided at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals, and environmental conditions, with each trough placed in a separate location of the pen (i.e. troughs at opposite ends of the pen). . (**major**)
   c. Good quality hay must be fed at a minimum rate of 20 pounds per 1000 pound adult animal per day, adjusted accordingly for larger or smaller horses, burros and foals. (**major**)
      i. Hay must not contain poisonous weeds or toxic substances. (**major**)
      ii. Hay placement must allow all WH&Bs to eat simultaneously. (**major**)
   d. When water or feed deprivation conditions exist on the range prior to the gather,

the Lead COR/COR/PI should adjust the watering and feeding arrangements in consultation with the onsite veterinarian as necessary to provide for the needs of the animals. (minor)

2. Dust abatement
   a. Dust abatement by spraying the ground with water must be employed when necessary at the trap site and temporary holding facility. (**major**)

Trap Site

   a. Dependent foals or weak/debilitated animals must be separated from other WH&Bs at the trap site to avoid injuries during transportation to the temporary holding facility. Separation of dependent foals from mares must not exceed four hours unless the Lead COR/COR/PI authorizes a longer time or a decision is made to wean the foals. (**major**)

3. Temporary Holding Facility
   a. All WH&Bs in confinement must be observed at least once daily to identify sick or injured WH&Bs and ensure adequate food and water. (**major**)
   b. Foals must be reunited with their mares/jennies at the temporary holding facility within four hours of capture unless the Lead COR/COR/PI authorizes a longer time or foals are old enough to be weaned during the gather. (**major**)
   c. Non-ambulatory WH&Bs must be located in a pen separate from the general population and must be examined by the BLM horse specialist and/or on-call or on-site veterinarian as soon as possible, no more than four hours after recumbency is observed. Unless otherwise directed by a veterinarian, hay and water must be accessible to an animal within six hours after recumbency.(**major**)
   d. Alternate pens must be made available for the following: (**major**)
      1) WH&Bs that are weak or debilitated
      2) Mares/jennies with dependent foals
   e. Aggressive WH&Bs causing serious injury to other animals should be identified and relocated into alternate pens when possible. (minor)
   f. WH&Bs in pens at the temporary holding facility should be maintained at a proper stocking density such that when at rest all WH&Bs occupy no more than half the pen area. (minor)

C. Biosecurity

1. Health records for all saddle and pilot horses used on WH&B gathers must be provided to the Lead COR/COR/PI prior to joining a gather, including: (**major**)
   a. Certificate of Veterinary Inspection (Health Certificate, within 30 days).
   b. Proof of:
      1) A negative test for equine infectious anemia (Coggins or EIA ELISA test) within 12 months.
      2) Vaccination for tetanus, eastern and western equine encephalomyelitis, West Nile virus, equine herpes virus, influenza, *Streptococcus equi*, and rabies within 12 months.
2. Saddle horses, pilot horses and mares used for bait trapping lures must not be removed from the gather operation (such as for an equestrian event) and allowed to return unless they have been observed to be free from signs of infectious disease for a period of at least three weeks and a new Certificate of Veterinary Examination is obtained after three weeks and prior to returning to the gather. (**major**)

3. WH&Bs, saddle horses, and pilot horses showing signs of infectious disease must be examined by the on-site/on-call veterinarian. (**major**)
    a. Any saddle or pilot horses showing signs of infectious disease (fever, nasal discharge, or illness) must be removed from service and isolated from other animals on the gather until such time as the horse is free from signs of infectious disease and approved by the on-site/on-call veterinarian to return to the gather. (**major**)
    b. Groups of WH&Bs showing signs of infectious disease should not be mixed with groups of healthy WH&Bs at the temporary holding facility, or during transport. (minor)
4. Horses not involved with gather operations should remain at least 300 yards from WH&Bs, saddle horses, and pilot horses being actively used on a gather. (minor)

III. HANDLING

A. Willful Acts of Abuse
1. Hitting, kicking, striking, or beating any WH&B in an abusive manner is prohibited. (**major**)
2. Dragging a recumbent WH&B without a sled, slide board or slip sheet is prohibited. Ropes used for moving the recumbent animal must be attached to the sled, slide board or slip sheet unless being loaded as specified in Section II. C. 8. (**major**)
3. There should be no deliberate driving of WH&Bs into other animals, closed gates, panels, or other equipment. (minor)
4. There should be no deliberate slamming of gates and doors on WH&Bs. (minor)
5. There should be no excessive noise (e.g., constant yelling) or sudden activity causing WH&Bs to become unnecessarily flighty, disturbed or agitated. (minor)

B. General Handling
1. All sorting, loading or unloading of WH&Bs during gathers must be performed during daylight hours except when unforeseen circumstances develop and the Lead COR/CO/PI approves the use of supplemental light. (**major**)
2. WH&Bs should be handled to enter runways or chutes in a forward direction. (minor)
3. WH&Bs should not remain in single-file alleyways, runways, or chutes longer than 30 minutes. (minor)
4. Equipment except for helicopters should be operated and located in a manner to minimize flighty behavior . (minor)

C. Handling Aids
1. Handling aids such as flags and shaker paddles must be the primary tools for driving and moving WH&Bs during handling and transport procedures. Contact of the flag or paddle end of primary handling aids with a WH&B is allowed. Ropes looped around the hindquarters may be used from horseback or on foot to assist in moving an animal forward or during loading. (**major**)
2. Electric prods must not be used routinely as a driving aid or handling tool. Electric prods may be used in limited circumstances only if the following guidelines are followed:
    a. Electric prods must only be a commercially available make and model that uses DC battery power and batteries should be fully charged at all times. (**major**)
    b. The electric prod device must never be disguised or concealed. (**major**)
    c. Electric prods must only be used after three attempts using other handling aids

(flag, shaker paddle, voice or body position) have been tried unsuccessfully to move the WH&Bs. (**major**)

d. Electric prods must only be picked up when intended to deliver a stimulus; these devices must not be constantly carried by the handlers. (**major**)
e. Space in front of an animal must be available to move the WH&B forward prior to application of the electric prod. (**major**)
f. Electric prods must never be applied to the face, genitals, anus, or underside of the tail of a WH&B. (**major**)
g. Electric prods must not be applied to any one WH&B more than three times during a procedure (e.g., sorting, loading) except in extreme cases with approval of the Lead COR/COR/PI. Each exception must be approved at the time by the Lead COR/COR/PI. (**majo**r)
h. Any electric prod use that may be necessary must be documented daily by the Lead COR/COR/PI including time of day, circumstances, handler, location (trap site or temporary holding facility), and any injuries (to WH&B or human). (**major**)

IV. TRANSPORTATION

A. General

1. All sorting, loading, or unloading of WH&Bs during gathers must be performed during daylight hours except when unforeseen circumstances develop and the Lead COR/CO/PI approves the use of supplemental light. (**major**)
2. WH&Bs identified for removal should be shipped from the temporary holding facility to a BLM facility within 48 hours. (minor)
   a. Shipping delays for animals that are being held for release to range or potential on-site adoption must be approved by the Lead COR/COR/PI. (**major**)
3. Shipping should occur in the following order of priority; 1) debilitated animals, 2) pairs, 3) weanlings, 4) dry mares and 5) studs. (minor)
4. Planned
5. transport time to the BLM preparation facility from the trap site or temporary holding facility must not exceed 10 hours. (**major**)
6. WH&Bs should not wait in stock trailers and/or semi-trailers at a standstill for more than a combined period of three hours during the entire journey. (minor)

B. Vehicles

1. Straight-deck trailers and stock trailers must be used for transporting WH&Bs. (**major**)
   a. Two-tiered or double deck trailers are prohibited. (**major**)
   b. Transport vehicles for WH&Bs must have a covered roof or overhead bars containing them such that WH&Bs cannot escape. (**major**)
2. WH&Bs must have adequate headroom during loading and unloading and must be able to maintain a normal posture with all four feet on the floor during transport without contacting the roof or overhead bars. (**major**)
3. The width and height of all gates and doors must allow WH&Bs to move through freely. (**major**)
4. All gates and doors must open and close easily and be able to be secured in a closed position. (**major**)
5. The rear door(s) of the trailers must be capable of opening the full width of the trailer.

(**major**)

6. Loading and unloading ramps must have a non-slip surface and be maintained in proper working condition to prevent slips and falls. (**major**)
7. Transport vehicles more than 18 feet and less than 40 feet in length must have a minimum of one partition gate providing two compartments; transport vehicles 40 feet or longer must have at least two partition gates to provide a minimum of three compartments. (**major**)
8. All partitions and panels inside of trailers must be free of sharp edges or holes that could cause injury to WH&Bs. (**major**)
9. The inner lining of all trailers must be strong enough to withstand failure by kicking that would lead to injuries. (**major**)
10. Partition gates in transport vehicles should be used to distribute the load into compartments during travel. (minor)
11. Surfaces and floors of trailers must be cleaned of dirt, manure and other organic matter prior to the beginning of a gather. (**major**)

C. Care of WH&Bs during Transport Procedures

1. WH&Bs that are loaded and transported from the temporary holding facility to the BLM preparation facility must be fit to endure travel. (**major**)
   a. WH&Bs that are non-ambulatory, blind in both eyes, or severely injured must not be loaded and shipped unless it is to receive immediate veterinary care or euthanasia. (**major**)
   b. WH&Bs that are weak or debilitated must not be transported without approval of the Lead COR/COR/PI in consultation with the on-site veterinarian. Appropriate actions for their care during transport must be taken according to direction of the Lead COR/COR/PI. (**major**)
2. WH&Bs should be sorted prior to transport to ensure compatibility and minimize aggressive behavior that may cause injury. (minor)
3. Trailers must be loaded using the minimum space allowance in all compartments as follows: (**major**)
   a. 12 square feet per adult horse.
   b. 6.0 square feet per dependent horse foal.
   c. 8.0 square feet per adult burro.
   d. 4.0 square feet per dependent burro foal.
4. The Lead COR/COR/PI in consultation with the receiving Facility Manager must document any WH&B that is recumbent or dead upon arrival at the destination. (**major**)
   a. Non-ambulatory or recumbent WH&Bs must be evaluated on the trailer and either euthanized or removed from the trailers using a sled, slide board or slip sheet. (**major**)
5. Saddle horses must not be transported in the same compartment with WH&Bs. (**major**)

V. EUTHANASIA or DEATH

A. Euthanasia Procedure during Gather Operations

1. An authorized, properly trained, and experienced person as well as a firearm appropriate for the circumstances must be available at all times during gather operations. When the travel time between the trap site and temporary holding facility

exceeds one hour or if radio or cellular communication is not reliable, provisions for euthanasia must be in place at both the trap site and temporary holding facility during the gather operation. (**major**)

2. Euthanasia must be performed according to American Veterinary Medical Association euthanasia guidelines (2013) using methods of gunshot or injection of an approved euthanasia agent. (**major**)
3. The decision to euthanize and method of euthanasia must be directed by the Authorized Officer or their Authorized Representative(s) that include but are not limited to the Lead COR/COR/PI who must be on site and may consult with the on-site/on-call veterinarian. (**major**)
4. Photos needed to document an animal's condition should be taken prior to the animal being euthanized. No photos of animals that have been euthanized should be taken. An exception is when a veterinarian or the Lead COR/COR/PI may want to document certain findings discovered during a postmortem examination or necropsy. (minor)
5. Any WH&B that dies or is euthanized must be documented by the Lead COR/COR/PI including time of day, circumstances, euthanasia method, location, a description of the age, gender, and color of the animal and the reason the animal was euthanized. (**major**)
6. The on-site/on-call veterinarian should review the history and conduct a postmortem physical examination of any WH&B that dies or is euthanized during the gather operation. A necropsy should be performed whenever feasible if the cause of death is unknown. (minor)

B. Carcass Disposal

1. The Lead COR/COR/PI must ensure that appropriate equipment is available for the timely disposal of carcasses when necessary on the range, at the trap site, and temporary holding facility. (**major**)
2. Disposal of carcasses must be in accordance with state and local laws. (**major**)
3. WH&Bs euthanized with a barbiturate euthanasia agent must be buried or otherwise disposed of properly. (**major**)
4. Carcasses left on the range should not be placed in washes or riparian areas where future runoff may carry debris into ponds or waterways. Trenches or holes for buried animals should be dug so the bottom of the hole is at least 6 feet above the water table and 4-6 feet of level earth covers the top of the carcass with additional dirt mounded on top where possible. (minor)

CAWP
Required Documentation and Responsibilities of Lead COR/COR/PI
Required Documentation

Section	Documentation
II.B.5	Helicopter contact with any WH&B.
II.C.2	Roping of any WH&B.
III.B.3.a and III.B.4.b	Reason for allowing longer than four hours to reunite foals with mares/jennies. Does not apply if foals are being weaned.
III.C.1	Health status of all saddle and pilot horses.
IV.C.2.h	All uses of electric prod.
V.C.4	Any WH&B that is recumbent or dead upon arrival at destination following transport.
VI.A.5	Any WH&B that dies or is euthanized during gather operation.

Responsibilities

Section	Responsibility
I.A.10	Approve materials used in construction of finger gates in bait trapping
II.A.1	Direct gather procedures using approved gather technique.
II.B. 2	Determine rate of movement and distance limitations for WH&B helicopter gather.
II.B.2.a	Direct appropriate gather/handling methods for weak or debilitated WH&B.
II.B.3	Determine whether to abandon pursuit or use other capture method in order to avoid repeated pursuit of WH&B.
II.B.4	Determine width and need for visibility marking when using opening in fence en route to trap.
II.B.6	Determine number of attempts that can be made to capture the missing half of a mare/foal pair that has become separated.
II.B.7	Determine whether to proceed with gather when ambient temperature is outside the range of 10°F to 95°F for horses or 10°F to 100°F for burros.
II.C.1	Approve roping of any WH&B.
II.D.1.a	Determine period of time that water outside a bait trap is inaccessible such that wellbeing of WH&Bs, wildlife, or livestock is not adversely affected.
III.A.2	Direct and consult with on-site/on-call veterinarian on any matters related to WH&B health, handling, welfare and euthanasia.
III.B.1.e	Adjust feed/water as necessary, in consultation with onsite/on call veterinarian, to provide for needs of animals when water or feed deprivation conditions exist on range.
III.B.4.c	Determine provision of water and hay to non-ambulatory animals.
IV.C.2.g	Approve use of electric prod more than three times, for exceptional cases only.
V.A.1	Approve sorting, loading, or unloading at night with use of supplemental light.
V.A.2.a	Approve shipping delays of greater than 48 hours from temporary holding facility to BLM facility.
V.C.1.b	Approve of transport and care during transport for weak or debilitated WH&B.
VI.A.3	Direct decision regarding euthanasia and method of euthanasia for any WH&B; may consult with on-site/on-call veterinarian.
VI.B.1	Ensure that appropriate equipment is available for carcass disposal.

APPENDIX C
Standard Operating Procedures for Field Castration (Gelding) of Wild Horse Stallions

June 2011

Gelding will be performed with general anesthesia and by a veterinarian. The combination of pharmaceutical compounds used for anesthesia, method of physical restraint, and the specific surgical technique used will be at the discretion of the attending veterinarian with the approval of the authorized officer (I.M. 2009-063).

Pre-surgery Animal Selection, Handling and Care

1. Stallions selected for gelding will be greater than 6 months of age and less than 20 years of age.
2. All stallions selected for gelding will have a Henneke body condition score of 3 or greater. No animals which appear distressed, injured or in failing health or condition will be selected for gelding.
3. Stallions will not be gelded within 36 hours of capture and no animals that were roped during capture will be gelded at the temporary holding corrals for rerelease.
4. Whenever possible, a separate holding corral system will be constructed on site to accommodate the stallions that will be gelded. These gelding pens will include a minimum of 3 pens to serve as a working pen, recovery pen(s), and holding pen(s). An alley and squeeze chute built to the same specifications as the alley and squeeze chutes used in temporary holding corrals (solid sides in alley, minimum 30 feet in length, squeeze chute with non-slip floor) will be connected to the gelding pens.
5. When possible, stallions selected for gelding will be separated from the general population in the temporary holding corral into the gelding pens, prior to castration.
6. When it is not possible or practical to build a separate set of pens for gelding, the gelding operation will only proceed when adequate space is available to allow segregation of gelded animals from the general population of stallions following surgery. At no time will recently anesthetized animals be returned to the general population in a holding corral before they are fully recovered from anesthesia.
7. All animals in holding pens will have free access to water at all times. Water troughs will be removed from working and recovery pens prior to use.
8. Prior to surgery, animals in holding pens may be held off feed for a period of time (typically 12-24 hours) at the recommendation and direction of the attending veterinarian.
9. The final determination of which specific animals will be gelded will be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer.
10. Whether the procedure will proceed on a given day will be based on the discretion of the attending veterinarian in consultation with the Authorized Officer taking into consideration the prevailing weather, temperature, ground conditions and pen set up. If these field situations can't be remedied, the procedure will be delayed until they can be, the stallions will be transferred to a prep facility, gelded, and later returned, or they will be released to back to the range as intact stallions.

Gelding Procedure

1. All gelding operations will be performed under a general anesthetic administered by a qualified and experienced veterinarian. Stallions will be restrained in a portable squeeze chute to

allow the veterinarian to administer the anesthesia.
2. The anesthetics used will be based on a xylazine/ketamine combination protocol. Drug dosages and combinations of additional drugs will be at the discretion of the attending veterinarian.
3. Animals may be held in the squeeze chute until the anesthetic takes effect or may be released into the working pen to allow the anesthesia to take effect. If recumbency and adequate anesthesia is not achieved following the initial dose of anesthetics, the animal will either be redosed or the surgery will not be performed on that animal at the discretion of the attending veterinarian.
4. Once recumbent, rope restraints or hobbles will be applied for the safety of the animal, the handlers and the veterinarian.
5. The specific surgical technique used will be at the discretion of the attending veterinarian.
6. Flunixin meglamine or an alternative analgesic medication will be administered prior to recovery from anesthesia at the professional discretion of the attending veterinarian.
7. Tetanus prophylaxis will be administered at the time of surgery.
8. Other medications may also be administered at the time of surgery at the professional discretion of the attending veterinarian.
9. All geldings will be allowed to recover from anesthesia within the working pen or the adjacent recovery pen. Once, fully recovered each gelding will be transferred to the gelding holding pen(s). Animals will remain segregated from intact stallions for at least 24 hours following surgery or until their release.
10. Any stallions determined or believed to be a cryptorchid will be allowed to recover from the anesthesia, marked for later recognition, and shipped to a BLM prep facility for appropriate surgery or euthanasia if it is determined that they cannot be fully castrated. At no time will a partial castration be performed. Because cryptorchidism is an inherited condition, cryptorchid stallions should never be released back into an HMA.
11. Gelded animals will be freeze marked on their left hip with an identifying mark to minimize the potential for future recapture and to facilitate post-treatment monitoring. Each State will establish its own marking system in compliance with their State Brand Board. For example, Nevada BLM will utilize the identifying freeze mark on the hip (to be determined) as well as a 2 inch “F” freeze mark on the left side of the neck per agreement with the NV Brand Board.

Post-operative handling, care and monitoring
1. All animals that have fully recovered from anesthesia will have free access to water and hay prior to subsequent release.
2. All geldings will be held at least overnight for observation. Animals will not be left unattended for at least 3 hours following the procedure.
3. The attending veterinarian will observe all animals 12-24 hours after the procedure or again prior to release. Geldings will be released no later than 48 hours following surgery near a water source in their home range when possible.
4. Any gelding observed have complications will be held at the gather site until his condition improves or be shipped to a holding facility until he is able to be returned to the range.
5. Gelded animals would be monitored periodically for complications for approximately 7-10 days post-surgery. This monitoring will be completed either through aerial recon if available or field observations from major roads and trails. It is not anticipated that all the geldings will be observed but the goal is to detect complications if they are occurring and determine if the horses are freely moving about the HMA.
6. Animals found on the range with serious gelding complications will either be recaptured for treatment, if possible or euthanized as an act of mercy if necessary.

7. Observations of the long term outcomes of gelding will be recorded during routine resource monitoring work. Such observations will include but may not limited to band size, social interactions with other geldings and harem bands, distribution within their habitat, forage utilization and activities around key water sources.

APPENDIX D
Win Equus Population Modeling Results

Population Model

Conger and Frisco 2015 Population Modeling

To complete the population modeling for the Conger and Frisco Herd Management Areas, version 1.40 of the WinEquus program, created April 2, 2002, was utilized.

Objectives of Population Modeling

Review of the data output for each of the simulations provided many use full comparisons of the possible outcomes for each alternative. Some of the questions that need to be answered through the modeling include:

• Do any of the Alternatives "crash" the population?

• What effect does fertility control have on population growth rate?

• What effects do the different alternatives have on the average population size?

• What effects do the different alternatives have on the genetic health of the herd?

Population Data, Criteria, and Parameters utilized for Population Modeling

All simulations used the survival probabilities, foaling rates, and sex ratio at birth that was supplied with the Winn Equus population for the Garfield HMA.

Sex ratio at Birth:

Frisco	Conger
41% Females	50% Females
59% Males	50% Males

A 94% effectiveness of PZP for 1 year in population growth suppression was used when fertility control was utilized in the modeling,

The following table displays the contraception parameters utilized in the population model for Alternative 2-4:

Contraception Criteria

Age	Percentages for Fertility Treatment
1	0%
2	100%
3	100%
4	100%
5	100%
6	100%

7	100%
8	100%
9	100%
10-14	100%
15-19	100%
20+	100%

Population Modeling Parameters Modeling Parameter	**Gather and Removals Only**		**Population Growth Suppression using PZP**		**Gather and Removal with Population Growth Suppression using PZP**		**No Gather and Removals or Population Growth Suppression using PZP**
Management by removal only	Yes		No		No		No
Threshold Population Size Following Gathers	Conger	Frisco	Conger	Frisco	Conger	Frisco	N/A
	80	60	80	60	80	60	
Target Population Size Following gather	40	30	40	30	40	30	N/A
Gather for Population Growth Suppression regardless of population size	No		Yes		Yes		N/A
Gather continue after removals to treat additional females	N/A		Yes		Yes		N/A

Population Modeling Criteria

The following summarizes the population modeling criteria that are common to the Proposed Action and all alternatives:

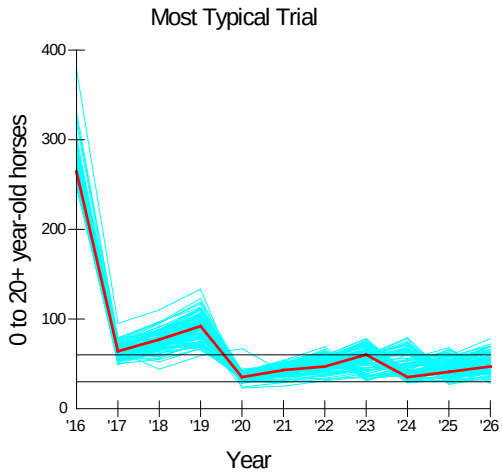
- Starting year: 2016
- Initial Gather Year: 2016
- Gather interval: regular interval of three years
- Gather for fertility treatment regardless of population size: No
- Continue to gather after reduction to treat females: Yes
- Sex ratio at birth: 59% males
- Percent of the population that can be gathered: 80%
- Minimum age for long term holding facility horses: Not Applicable
- Foals are included in the AML
- Simulations were run for 10 years with 100 trials each

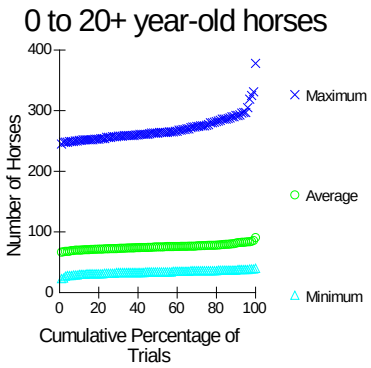
FRISCO

Results Removal Only

Alternative 1 and 2: Gather and Removal

Population Size



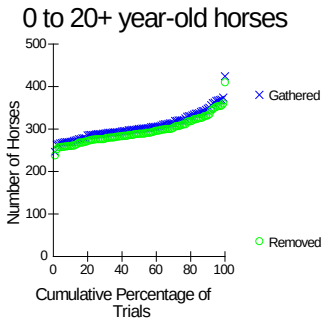


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	23	67	245
10th Percentile	30	70	251
25th Percentile	32	72	256
Median Trial	34	75	264
75th Percentile	36	78	276
90th Percentile	37	82	292
Highest Trial	40	91	378

* 0 to 20+ year-old horses

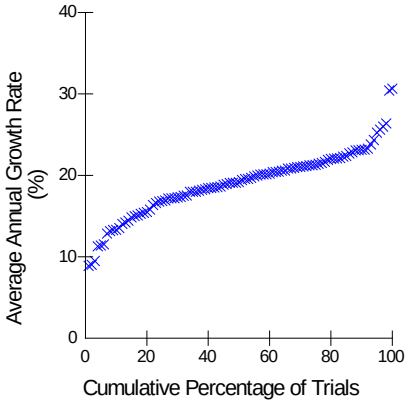
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 23 and the highest was 378. In half the trials, the minimum population size in 11 years was less than 34 and the maximum was less than 264. The average population size across 11 years ranged from 67 to 91.



Totals in 11 Years*

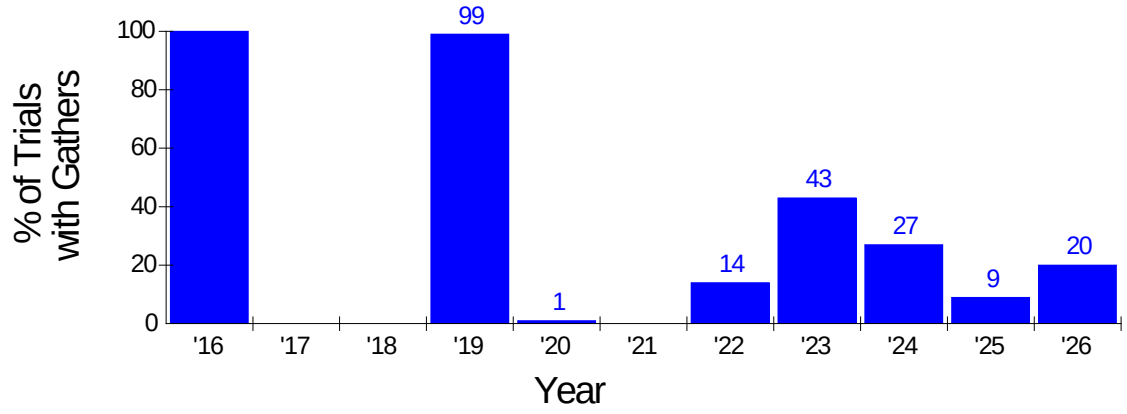
	Gathered	Removed
Lowest Trial	246	238
10th Percentile	270	260
25th Percentile	288	276
Median Trial	300	289
75th Percentile	320	310
90th Percentile	349	334
Highest Trial	424	410

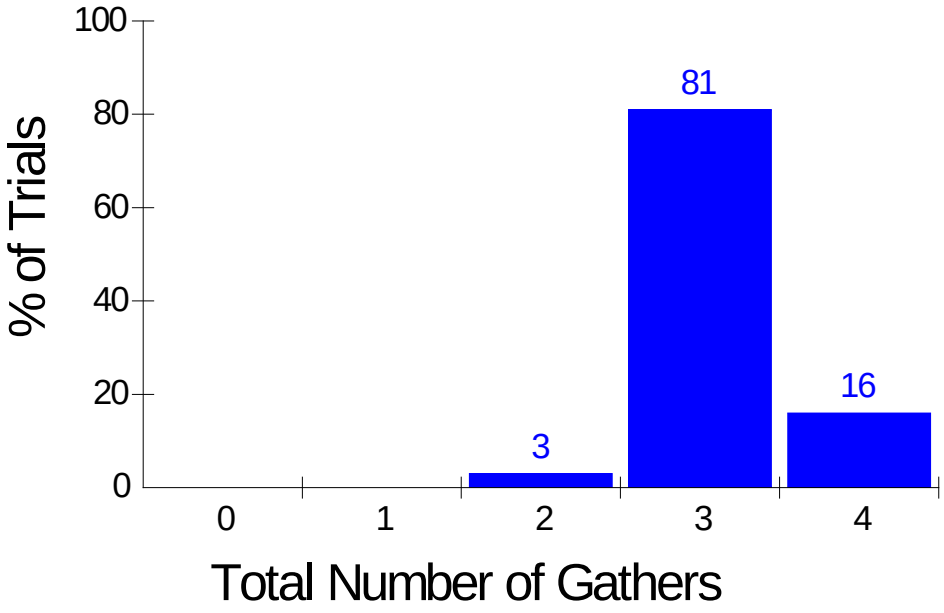
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	8.9
10th Percentile	13.4
25th Percentile	16.9
Median Trial	19.3
75th Percentile	21.4
90th Percentile	23.2
Highest Trial	30.6





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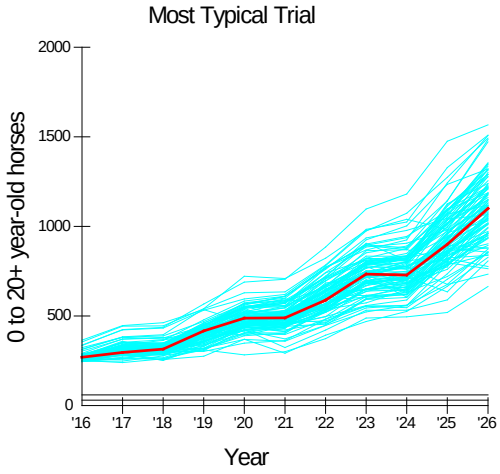
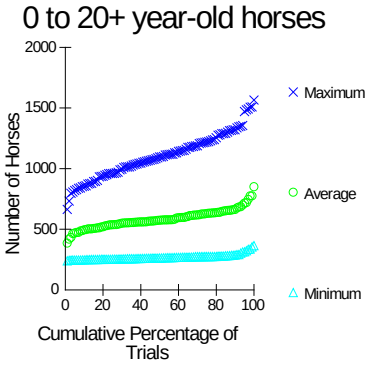
Results Fertility Only

Population Size



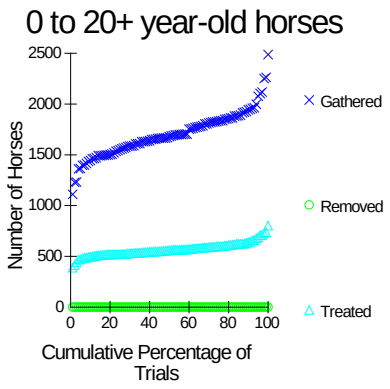


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	240	385	664
10th Percentile	248	497	857
25th Percentile	254	538	961
Median Trial	263	575	1097
75th Percentile	275	631	1222
90th Percentile	290	669	1328
Highest Trial	365	851	1566

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 240 and the highest was 1566. In half the trials, the minimum population size in 11 years was less than 263 and the maximum was less than 1097. The average population size across 11 years ranged from 385 to 851.

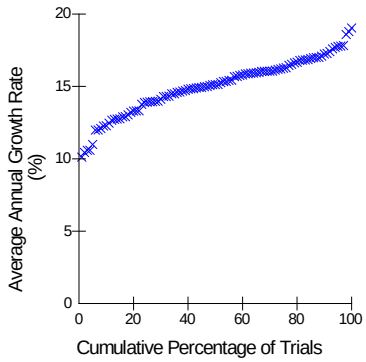


Totals in 11 Years*

	Gathered	Removed	Treated
Lowest Trial	1111	0	388
10th Percentile	1452	0	496
25th Percentile	1555	0	521
Median Trial	1682	0	560
75th Percentile	1834	0	599
90th Percentile	1946	0	640

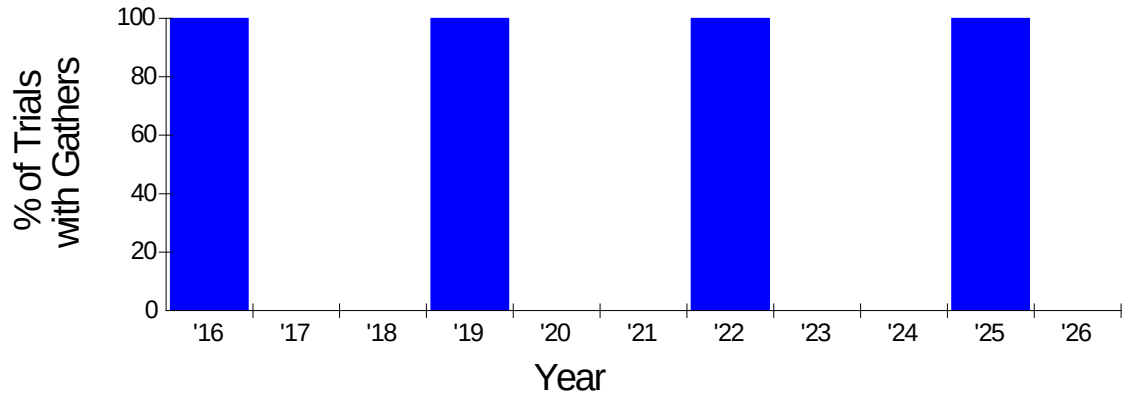
Highest Trial	2487	0	802

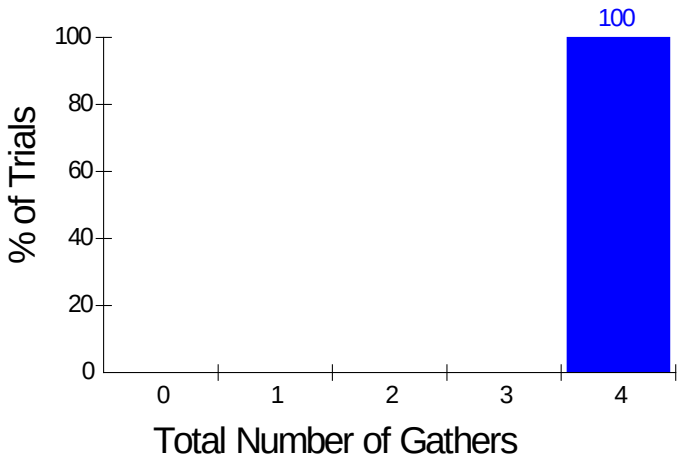
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	10.1
10th Percentile	12.4
25th Percentile	13.9
Median Trial	15.1
75th Percentile	16.3
90th Percentile	17.3
Highest Trial	19.0





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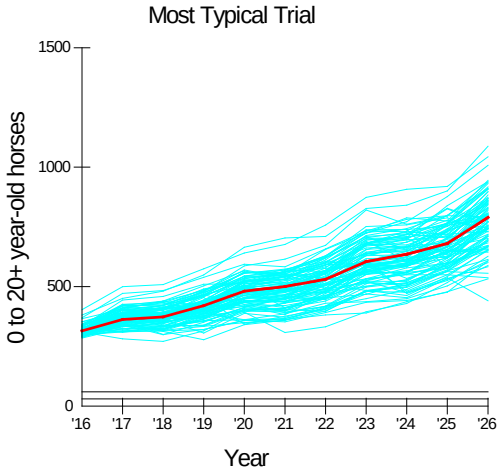
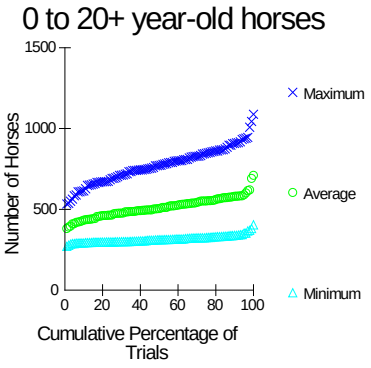
Results Fertility Only With 2-Year PZP

Note: This population modeling assumes that a 2-year PZP is available, but data from a recent trial

indicate that it is not. All available PZP vaccines are only available for 1 year at this time.

Population Size



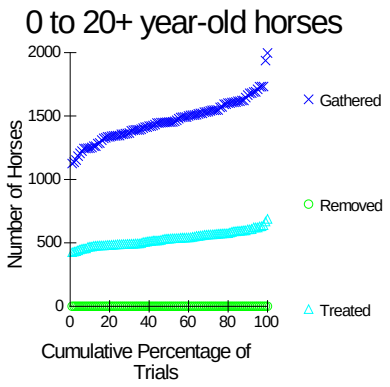


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	271	381	533
10th Percentile	293	434	620
25th Percentile	298	468	692
Median Trial	312	506	772
75th Percentile	326	552	848
90th Percentile	339	581	910
Highest Trial	404	710	1088

* 0 to 20+ year-old horses

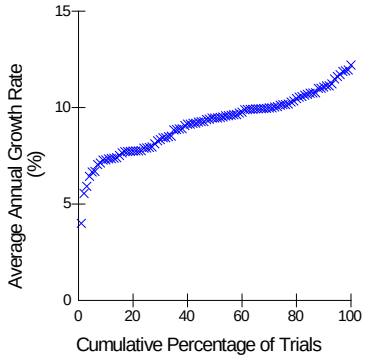
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 271 and the highest was 1088. In half the trials, the minimum population size in 11 years was less than 312 and the maximum was less than 772. The average population size across 11 years ranged from 381 to 710.



Totals in 11 Years*

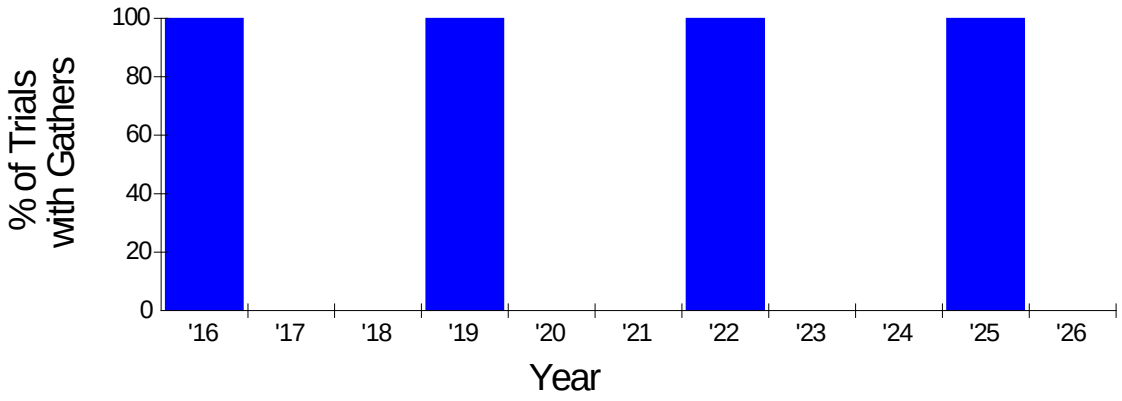
	Gathered	Removed	Treated
Lowest Trial	1126	0	427
10th Percentile	1250	0	471
25th Percentile	1351	0	490
Median Trial	1452	0	534
75th Percentile	1558	0	572
90th Percentile	1672	0	607
Highest Trial	1997	0	689

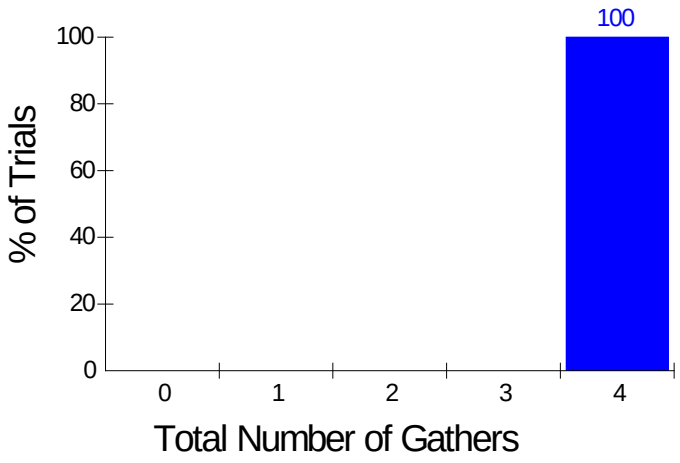
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	4.0
10th Percentile	7.3
25th Percentile	7.9
Median Trial	9.5
75th Percentile	10.2
90th Percentile	11.1
Highest Trial	12.2

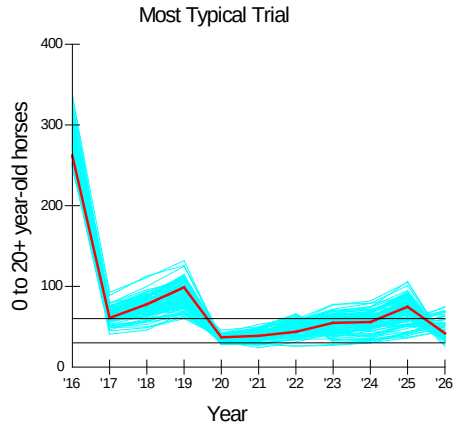




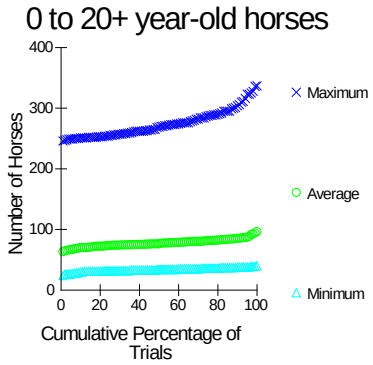
FRISCO

Results Gather and Removal with Fertility control.

Population Size



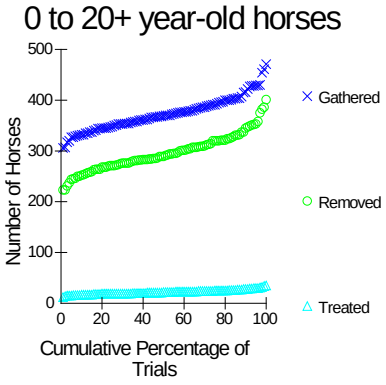
Population Sizes in 11 Years*



	Minimum	Average	Maximum
Lowest Trial	24	63	246
10th Percentile	30	70	251
25th Percentile	32	74	255
Median Trial	34	77	270
75th Percentile	36	82	288
90th Percentile	38	86	305
Highest Trial	40	96	337

* 0 to 20+ year-old horses

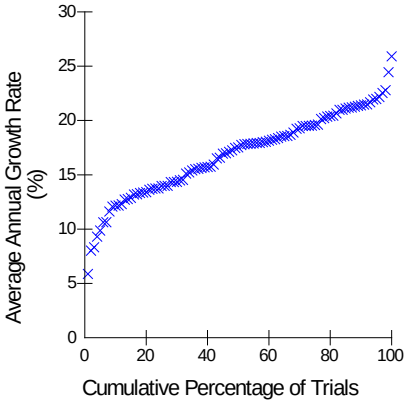
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 24 and the highest was 337. In half the trials, the minimum population size in 11 years was less than 34 and the maximum was less than 270. The average population size across 11 years ranged from 63 to 96.



Totals in 11 Years*

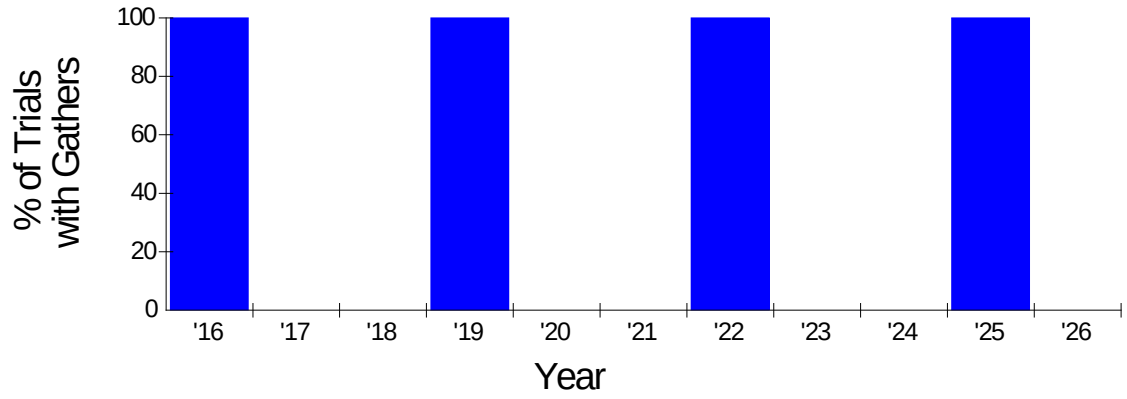
	Gathered	Removed	Treated
Lowest Trial	306	223	12
10th Percentile	332	252	17
25th Percentile	349	271	19
Median Trial	370	290	22
75th Percentile	392	320	25
90th Percentile	419	346	28
Highest Trial	471	401	35

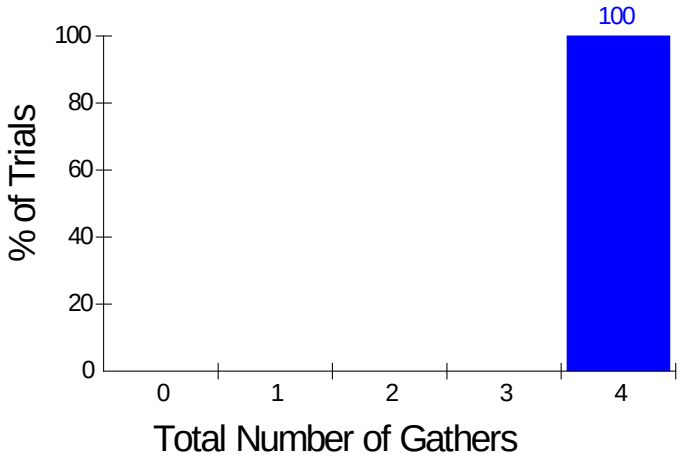
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	5.9
10th Percentile	12.2
25th Percentile	14.0
Median Trial	17.6
75th Percentile	19.6
90th Percentile	21.4
Highest Trial	25.9



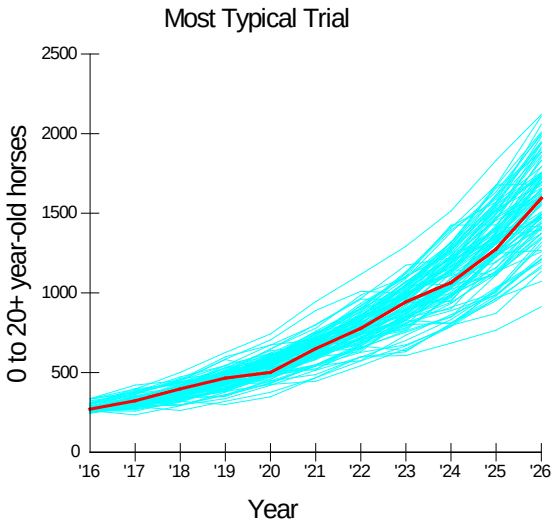


FRISCO

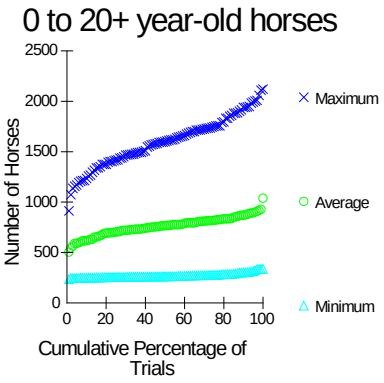
Results No Management

Results - No Action Alternative

Population Size



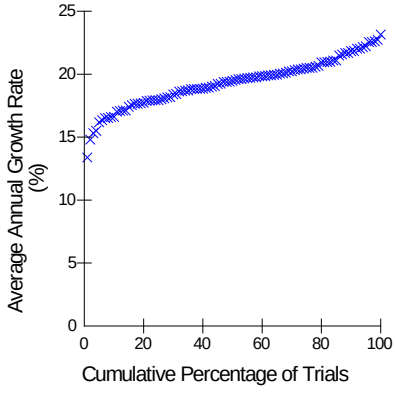
Population Sizes in 11 Years*



	Minimum	Average	Maximum
Lowest Trial	235	504	913
10th Percentile	250	620	1246
25th Percentile	256	704	1418
Median Trial	263	766	1603
75th Percentile	277	820	1752
90th Percentile	303	871	1939
Highest Trial	337	1038	2120

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 235 and the highest was 2120. In half the trials, the minimum population size in 11 years was less than 263 and the maximum was less than 1603. The average population size across 11 years ranged from 504 to 1038.



Average Growth Rate in 10 Years

Lowest Trial	13.4
10th Percentile	16.8
25th Percentile	18.0
Median Trial	19.5
75th Percentile	20.5

90th Percentile 21.8

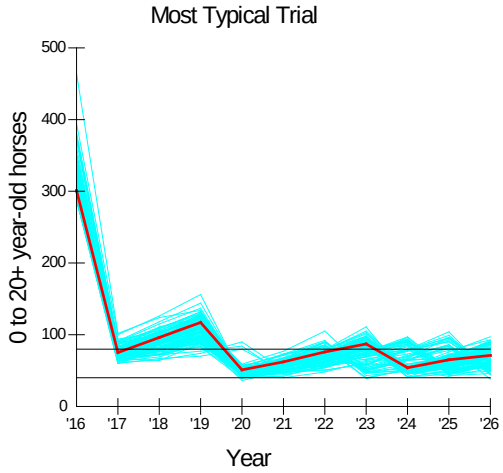
Highest Trial 23.1

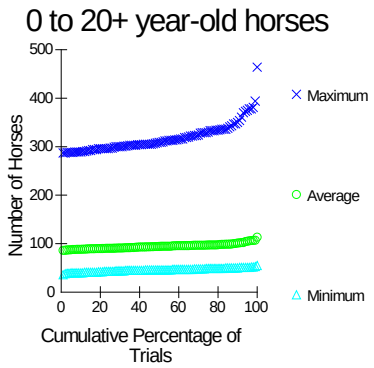
CONGER

Results Removal Only

Alternative 1 and 2: Gather and Removal

Population Size



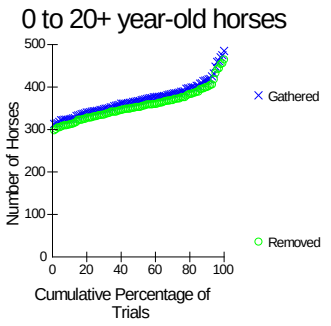


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	36	86	287
10th Percentile	40	88	290
25th Percentile	43	90	297
Median Trial	46	94	309
75th Percentile	49	97	332
90th Percentile	50	101	352
Highest Trial	55	113	464

* 0 to 20+ year-old horses

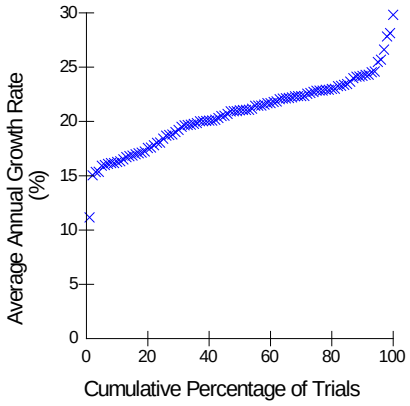
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 36 and the highest was 464. In half the trials, the minimum population size in 11 years was less than 46 and the maximum was less than 309. The average population size across 11 years ranged from 86 to 113.



Totals in 11 Years*

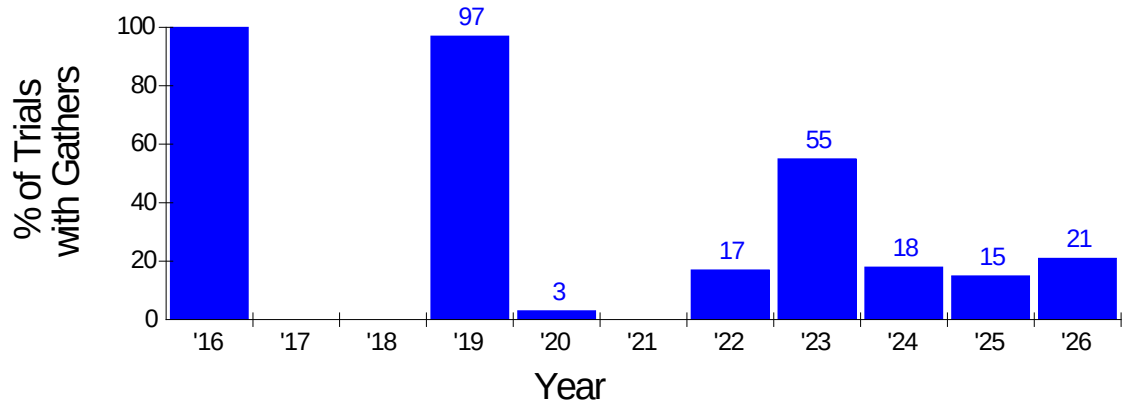
	Gathered	Removed
Lowest Trial	313	298
10th Percentile	324	312
25th Percentile	344	332
Median Trial	367	353
75th Percentile	389	376
90th Percentile	417	401
Highest Trial	485	465

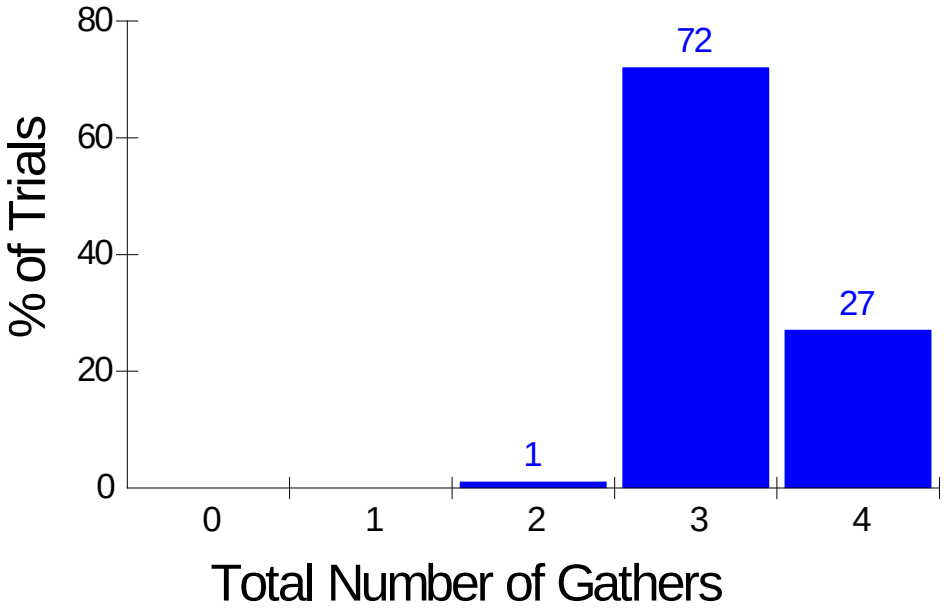
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	11.2
10th Percentile	16.3
25th Percentile	18.6
Median Trial	21.0
75th Percentile	22.8
90th Percentile	24.3
Highest Trial	29.8

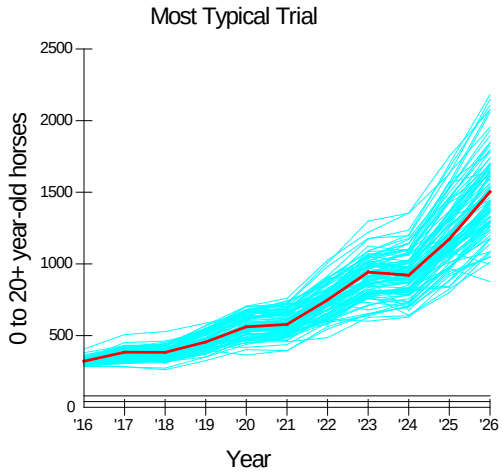


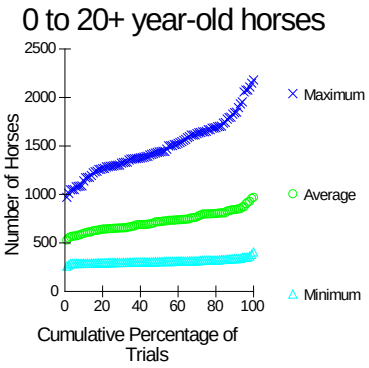


CONGER

Results Fertility Only

Population Size



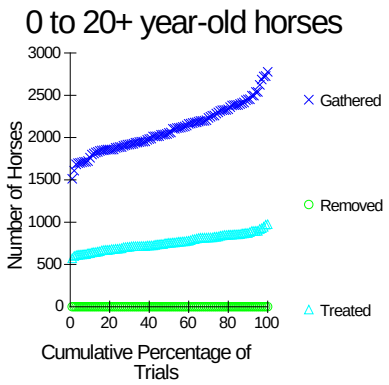


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	262	529	970
10th Percentile	290	602	1156
25th Percentile	297	649	1293
Median Trial	307	719	1440
75th Percentile	321	799	1659
90th Percentile	339	845	1846
Highest Trial	407	971	2181

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 262 and the highest was 2181. In half the trials, the minimum population size in 11 years was less than 307 and the maximum was less than 1440. The average population size across 11 years ranged from 529 to 971.

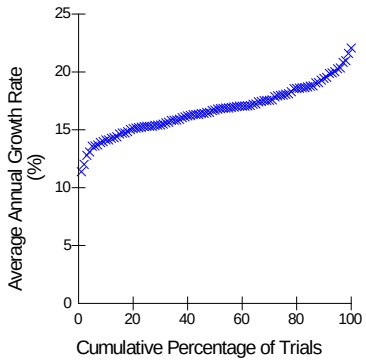


Totals in 11 Years*

	Gathered	Removed	Treated
Lowest Trial	1512	0	574
10th Percentile	1780	0	638
25th Percentile	1888	0	690
Median Trial	2058	0	756
75th Percentile	2306	0	835
90th Percentile	2452	0	878

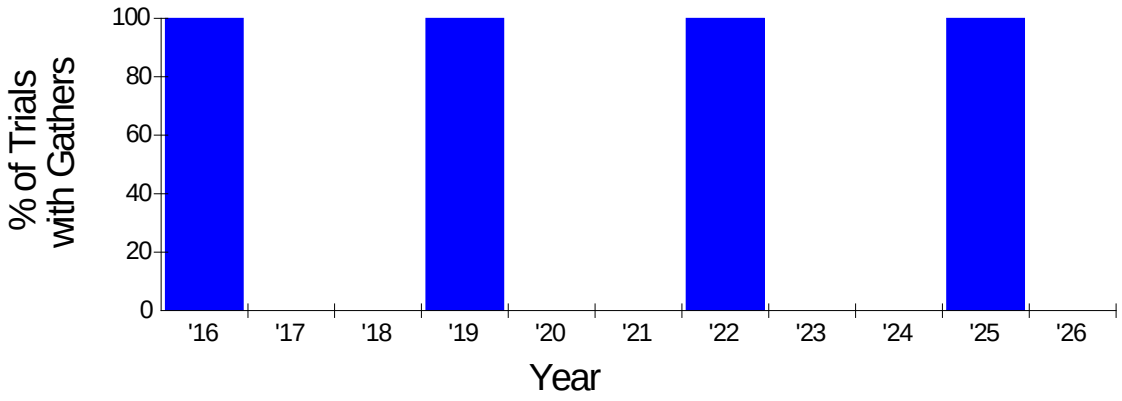
Highest Trial	2777	0	976

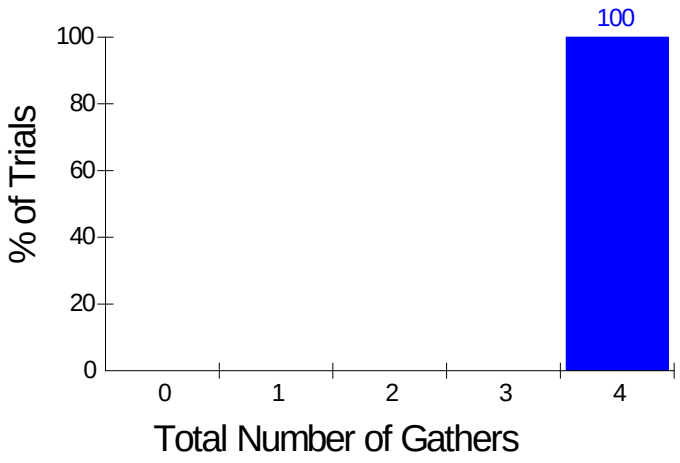
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	11.4
10th Percentile	14.1
25th Percentile	15.3
Median Trial	16.8
75th Percentile	18.0
90th Percentile	19.5
Highest Trial	22.1



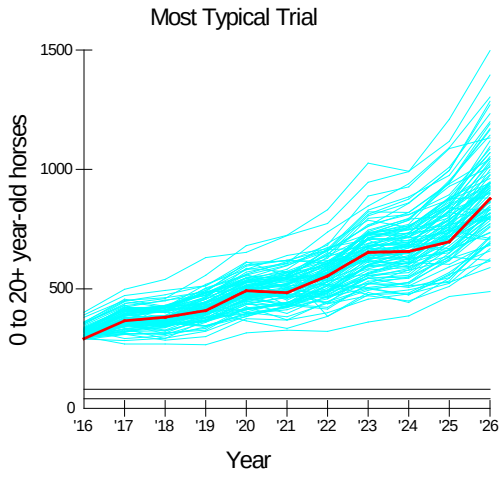


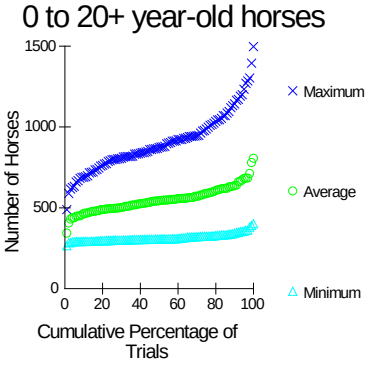
CONGER

Results Fertility Only With 2-Year PZP

Note: This population modeling assumes that a 2-year PZP is available, but data from a recent trial indicate that it is not. All available PZP vaccines are only available for 1 year at this time

Population Size



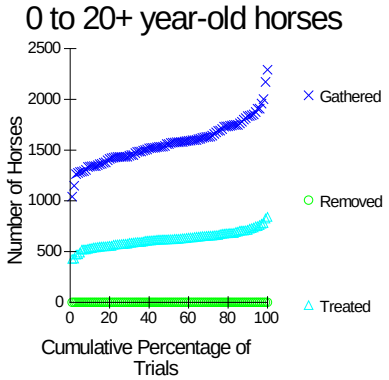


Population Sizes in 11 Years*

	Minimum	Average	Maximum
Lowest Trial	266	343	489
10th Percentile	292	465	690
25th Percentile	298	496	798
Median Trial	306	544	874
75th Percentile	324	589	994
90th Percentile	344	638	1154
Highest Trial	402	806	1497

* 0 to 20+ year-old horses

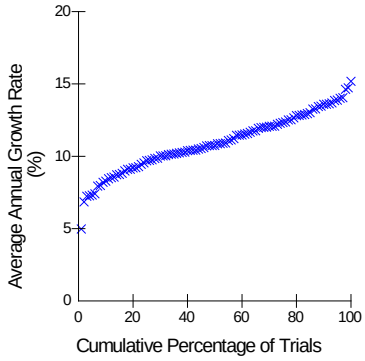
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 266 and the highest was 1497. In half the trials, the minimum population size in 11 years was less than 306 and the maximum was less than 874. The average population size across 11 years ranged from 343 to 806.



Totals in 11 Years*

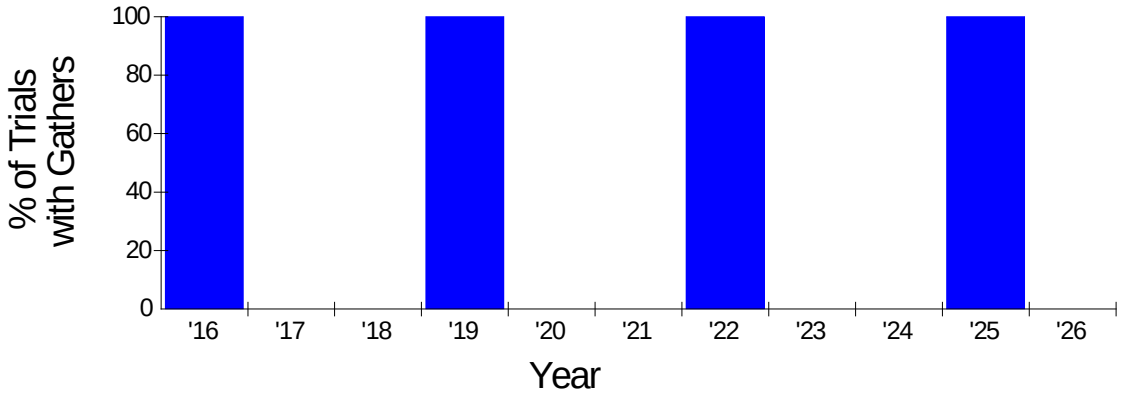
	Gathered	Removed	Treated
Lowest Trial	1041	0	428
10th Percentile	1340	0	534
25th Percentile	1433	0	574
Median Trial	1565	0	622
75th Percentile	1691	0	667
90th Percentile	1836	0	720
Highest Trial	2291	0	842

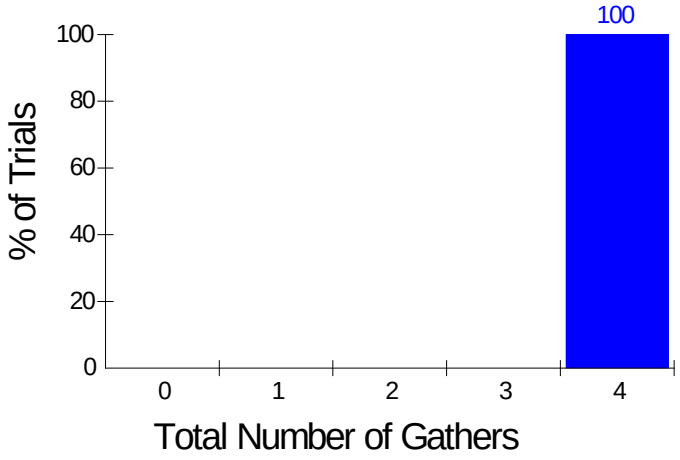
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	5.0
10th Percentile	8.4
25th Percentile	9.7
Median Trial	10.8
75th Percentile	12.4
90th Percentile	13.6
Highest Trial	15.2

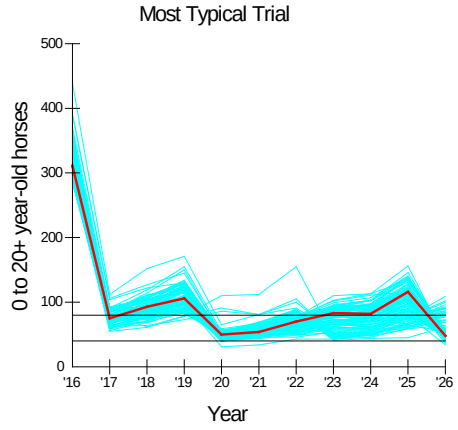




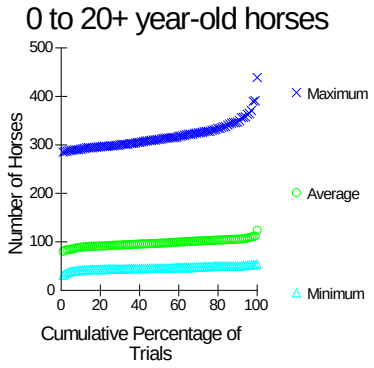
CONGER

Results Gather and Removal with Fertility control.

Population Size



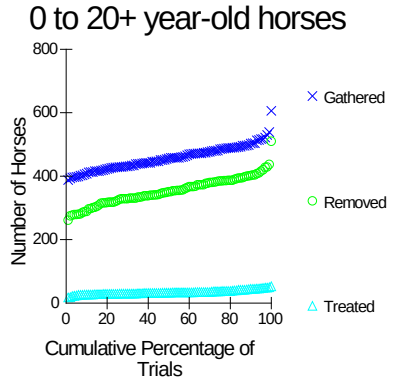
Population Sizes in 11 Years*



	Minimum	Average	Maximum
Lowest Trial	31	80	285
10th Percentile	42	89	292
25th Percentile	44	92	298
Median Trial	46	97	312
75th Percentile	49	103	328
90th Percentile	50	106	348
Highest Trial	54	124	439

* 0 to 20+ year-old horses

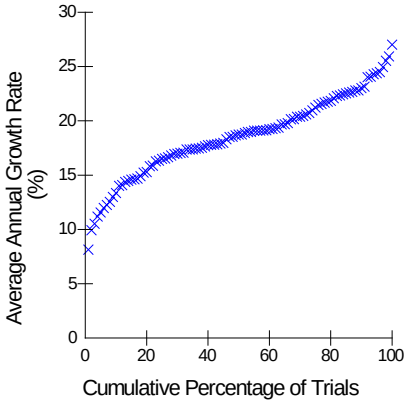
In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 31 and the highest was 439. In half the trials, the minimum population size in 11 years was less than 46 and the maximum was less than 312. The average population size across 11 years ranged from 80 to 124.



Totals in 11 Years*

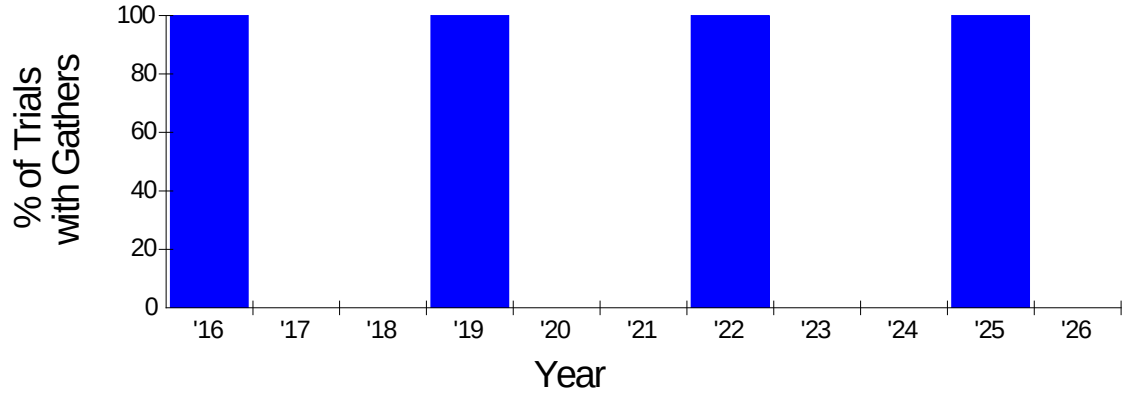
	Gathered	Removed	Treated
Lowest Trial	388	261	18
10th Percentile	410	293	27
25th Percentile	428	325	30
Median Trial	457	351	33
75th Percentile	486	385	38
90th Percentile	504	403	45
Highest Trial	606	510	53

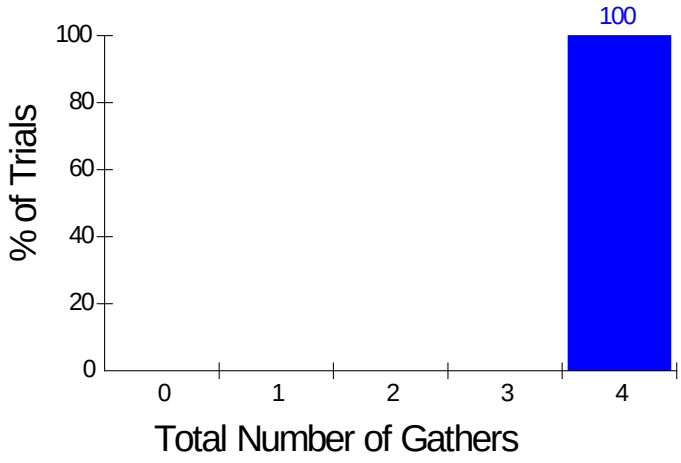
* 0 to 20+ year-old horses



Average Growth Rate in 10 Years

Lowest Trial	8.1
10th Percentile	13.7
25th Percentile	16.5
Median Trial	18.7
75th Percentile	21.3
90th Percentile	23.1
Highest Trial	27.0



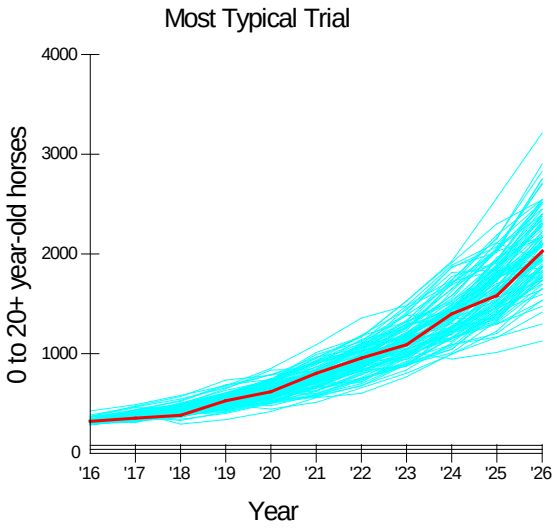


CONGER

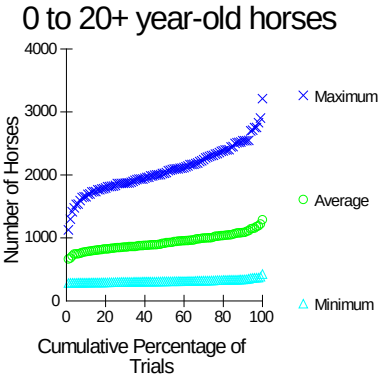
Results No Management

Results - No Action Alternative

Population Size



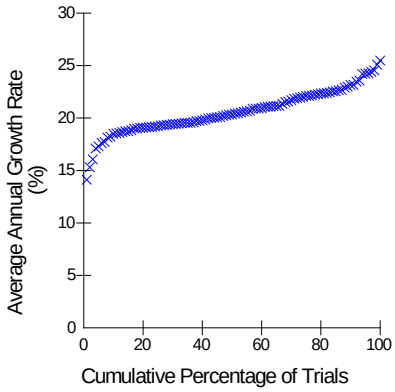
Population Sizes in 11 Years*



	Minimum	Average	Maximum
Lowest Trial	286	665	1128
10th Percentile	290	783	1673
25th Percentile	299	845	1848
Median Trial	311	920	2033
75th Percentile	327	1020	2321
90th Percentile	342	1087	2544
Highest Trial	427	1288	3212

* 0 to 20+ year-old horses

In 11 years and 100 trials, the lowest number 0 to 20+ year-old horses ever obtained was 286 and the highest was 3212. In half the trials, the minimum population size in 11 years was less than 311 and the maximum was less than 2033. The average population size across 11 years ranged from 665 to 1288.



Average Growth Rate in 10 Years

Lowest Trial	14.1
10th Percentile	18.5
25th Percentile	19.3
Median Trial	20.4
75th Percentile	22.1
90th Percentile	23.2
Highest Trial	25.5

APPENDIX E
Interdisciplinary Team Checklists

Project Title: Population Control Research Wild Horse Gather for the Conger and Frisco Herd Management Areas
NEPA Log Number: DOI-BLM-UT-W020-2015-0017-EA
File/Serial Number:
Project Leader: Eric Reid/Chad Hunter

DETERMINATION OF STAFF: (Choose one of the following abbreviated options for the left column)
NP = not present in the area impacted by the proposed or alternative actions
NI = present, but not affected to a degree that detailed analysis is required
PI = present with potential for relevant impact that need to be analyzed in detail in the EA

Determi-nation	Resource	Rationale for Determination	Signature	Date
RESOURCES AND ISSUES CONSIDERED (INCLUDES SUPPLEMENTAL AUTHORITIES APPENDIX 1 H-1790-1)				
NI	Air Quality	There are would be no impact to air quality as a result of the proposed action.	/s/ Paul Caso	10/9/15
NI	Areas of Critical Environmental Concern	There are two ACEC's (Wah Wah Mountains and Crystal Peak) that are located in the proposed project areas. They are both designated as closed to ORV use. The project, as proposed, would not impact these ACEC's.	/s/ Teresa Frampton	9/25/15
NP	Cultural Resources	No historic properties affected	/s/ Stacey Whitman Moore	9/28/15
NI	Greenhouse Gas Emissions	BLM does not have the ability to associate an action's contribution in a localized area to impact global climate change. Further, an IPCC assessment states that, "difficulties remain in attributing observed temperature changes at a smaller than continental scale"	/s/ Cindy Ledbetter	9/11/15
NI	Environmental Justice	Low income or minority populations would not be disproportionately impacted by the project.	/s/ Cindy Ledbetter	9/11/15
NI	Farmlands (Prime or Unique)	The only possible soil disturbance related to the proposed action would occur on shallow soils that are not suitable for farmlands.	/s/ Bill Thompson	11/19/15
NI	Floodplains	Floodplains would not be impacted by the proposed action.	/s/ Paul Caso	10/9/15
NP	Fire/Fuels Management	The project, as proposed sill not impact fire or fuels management.	/s/ Gary Bishop	9/25/15
NI	Geology / Mineral Resources/Energy Production	Geology / Mineral Resources are present but will not be impacted by Proposed Action.	/s/ Todd Leeds, P.G.	9/25/15
NI	Invasive Species/Noxious Weeds (EO 13112)	Noxious and/or invasive species would not be impacted by the proposed project.	/s/R.B. Probert	11/30/15
NI	Lands/Access	The project, as proposed, would not affect access to public lands.	/s/ Teresa Frampton	9/25/15
PI	Livestock Grazing	Removal of excess horses would benefit the livestock grazing program through reduced competition for vegetation and water resources.	/s/ Brian Taylor	10/5/15

Determi-nation	Resource	Rationale for Determination	Signature	Date
NI	Migratory Birds	Given the low magnitude and short duration of the proposed action, no impacts to migratory birds are anticipated. Migratory birds may benefit from the reduction of herd numbers.	/s/ James Priest	9/25/15
NP	National Historic Trails	No potential to affect NHT	/s/ Stacey Whitman Moore	9/28/15
NI	Native American Religious Concerns	Tribal consultation was initiated on October 1, 2015. Tribes did not express any concerns regarding the proposed project.	/s/ Stacey Whitman Moore	11/4/15
NP	Paleontology	There are no known paleontological resources within the project area.	/s/ Cindy Ledbetter	9/11/15
NI	Property Boundary Evaluation	The property markers will not be affected by the project.	/s/ Kyle Monroe	9/25/15
PI	Rangeland Health Standards	The potential for maintenance of Rangeland Health would be increased by removing wild horses to keep their numbers on the HMA within the appropriate management level. If no action is taken, rangeland health will deteriorate in areas where wild horses spend most of their time.	/s/ Brian Taylor	10/5/15
NI	Recreation	There are no developed recreational sites that would be affected by the proposed activity and casual recreation use would not be affected.	/s/ Teresa Frampton	9/25/15
NI	Sensitive Animal Species	Sensitive wildlife species that could be found within or near the proposed action include but limited to: golden eagle, bald eagle, burrowing owl, Ferruginous hawk, kit fox, Brewer's sparrow, loggerhead shrike, sage sparrow and various bat species. Given the low magnitude and duration of the proposed action, no substantial impacts to these species are anticipated to occur and therefore do no further analysis is required.	/s/James Priest	9/28/15
NI	Socio-Economics	No quantifiable increased or decreased economic impact to the local area would be caused by the proposed action.	/s/ Cindy Ledbetter	9/11/15
NI	Soils	Soils would not be impacted by the proposed action.	/s/ Paul Caso	10/9/15
NI	Threatened, Endangered, Candidate or Special Status Plant Species	There are no known federally-listed plants within the Conger HMA. There is one known BLM Sensitive Plant Species, sand-loving buckwheat (*Eriogonum nummulare* var. *ammophilum*) that is found on the northwest and southern fringes of the Conger HMA. The project proposal is to merely conduct a wild horse study to better understand and manage wild horse populations. This study is not anticipated to have negative impacts on this sensitive plant species.	/s/DWhitaker	10/20/15
NP	Threatened, Endangered, or Candidate Animal Species	There are no known federally listed fish or wildlife species known to occur within or near the proposed action.	/s/ James Priest	9/28/15
NP	Wastes (hazardous or solid)	There are no hazardous waste sites within the project area. The project will not generate hazardous waste.	/s/ Todd Leeds, P.G.	9/28/15
NI	Water Resources/Quality (drinking/surface/ground)	There are would be no impact to water resources/quality as a result of the proposed action.	/s/ Paul Caso	10/9/15

Determi-nation	Resource	Rationale for Determination	Signature	Date
NI	Water Rights	Water Rights would not be impacted by the proposed action.	/s/ Paul Caso	10/9/15
NI	Wetlands/Riparian Zones	Gathering wild horses would occur far from any riparian areas in the HMA. There would be no disturbance to riparian areas in the HMA.	/s/ Bill Thompson	11/19/15
NI	Wilderness/WSA	There are two WSA's (Conger Mountain and King Top) that are located within the proposed project area, but the project, as proposed, would not impact the WSA's.	/s/ Teresa Frampton	9/25/15
NI	Wildlife and Fish Excluding Designated/Special Status Species	General wildlife species, such as mule deer, antelope, mountain lion, coyote, rattle snakes, lizards and jack rabbits occur within the scope of the proposed action. Managing herd numbers will benefit wildlife overall by reducing competition and improving range condition.	/s/ James Priest	9/28/15
NI	Woodland / Forestry	There are would be no impact to woodland or forestry products with the implementation of the proposed action.	/s/Eric Reid	8/27/15
PI	Vegetation Excluding Designated/Special Status Species	There are no anticipated negative impacts to range vegetation from the proposed horse gather. Very little ground disturbance is proposed. The removal of the excess Wild Horses will have a positive impact on the vegetative resource by decreasing the amount of use occurring on forage plants.	/s/ Brian Taylor	12/15/15
NI	Visual Resources	The proposed project is located within a small portion of the VRM Class III Classification and the remaining portions are in the Class IV classification. The proposed project would not impact visual resources.	/s/ Teresa Frampton	9/25/15
PI	Wild Horses and Burros	Wild Horse population within the Conger HMA would be brought to be within the established appropriate management levels set for the HMA by the removal of the excess wild horses. The research proposal could provide valuable information for future management of wild horse populations throughout the BLM. Any negative effects from the research proposal could be remedied over time to return the population to a more normal balance of reproductive males though removals of the geldings and releasing stallions from similar HMAs with in the field office if necessary. Habitat would improve directly from the removal of the excess animals and reducing the amount of use on the range within the HMA.	/s/Eric Reid	8/27/15
NI	Lands with Wilderness Characteristics	The project, as proposed, would not affect Lands with Wilderness Character.	/s/ Teresa Frampton	10/22/15

FINAL REVIEW:

Reviewer Title	Signature	Date	Comments
Environmental Coordinator			
Authorized Officer			

INTERDISCIPLINARY TEAM NEPA CHECKLIST

Project Title: Conger and Frisco Wild Horse Gelding and Demography Research

NEPA Log Number: DOI-BLM-UT-W020-2015-0017-EA
File/Serial Number:

Project Leader: Eric Reid/Chad Hunter

DETERMINATION OF STAFF: ***(Choose one of the following abbreviated options for the left column)***

NP = not present in the area impacted by the proposed or alternative actions
NI = present, but not affected to a degree that detailed analysis is required
PI = present with potential for relevant impact that need to be analyzed in detail in the EA
NC = (DNAs only) actions and impacts not changed from those disclosed in the existing NEPA documents cited in Section D of the DNA form.
The rationale column should include NI and NP discussions.

RESOURCES AND ISSUES CONSIDERED:

Determi-nation	Resource	Rationale for Determination	Signature	Date
NI	Air Quality	Air quality in the area is good as is typical of relatively undeveloped areas of the western U.S. The area meets NAAQS. Nothing in the proposal would affect current conditions.	A. Stephens	12/7/15
NP	Areas of Critical Environmental Concern	None within Field Office boundaries.	C. Hunter	11/23/15
NI	Cultural Resources	This gather will have no effect to significant cultural resources. The corral locations will be located on an area of existing disturbance. The possibility of finding intact cultural resources in these areas is minimal to non-existent. If an existing disturbed area cannot be located for the corral area, a cultural resource inventory will take place prior to the gather. If cultural resources are located during this inventory, the corral area will be moved to another location, which does not contain cultural resources.	Jamie Palmer	11/6/2015
NI	Greenhouse Gas Emissions	Releases of greenhouse gasses (GHG's), such as carbon monoxide, would occur as a result of operation of internal combustion engines being operated during the gather. The removal would occur in a very remote portion of Iron, Beaver and Millard counties and occur using improved county roads and lesser roads. Release of GHG's would be consistent with current levels of releases in the area and very short term.	A. Stephens	12/7/15
NI	Environmental Justice	No minority or economically disadvantaged groups would be affected.	C. Hunter	11/23/15
NP	Farmlands (Prime or Unique)	There is no soil survey completed for much of this area. There are likely soils in and adjacent to the herd unit capable of being prime or unique farmlands, however only when irrigation water is supplied. Where there is no irrigation water supplied, there are no prime or unique farmlands present.	C. Hunter	11/23/15
PI	Fish and Wildlife	Cedar City FO: The area is mapped as UDWR crucial summer mule deer, crucial yearlong pronghorn, and substantial yearlong rocky mountain elk habitat. Fawning for pronghorn April 15- June 15 and mule deer May 1 – June 15.	S. Whitfield	11/12/15

Determi-nation	Resource	Rationale for Determination	Signature	Date
		The area is also considered substantial yearlong chuckar habitat. However, the gather operations would occur outside the fawning seasons and the research activities wouldn't be greater than general public use of the area. Reducing horse numbers within the HMA would be beneficial and ensure the required forage availability for big game species throughout the year and during crucial big game fawning periods.		
NI	Floodplains	Nothing in the proposal would affect the functioning of a floodplain, nor would any of the alternatives effect the function of a floodplain. Therefore the action is consistent with Executive Order 11988.	C. Hunter	11/23/15
NI	Fuels/Fire Management	No impact to fire or fuels management	M. Mendenhall	11/5/15
NI	Geology / Mineral Resources/Energy Production	The transient and superficial nature of the activities proposed would not substantially impact any mineral resources that may be present in the project area.	E. Ginouves	11/4/15
PI	Hydrologic Conditions	Hydrologic conditions are variable throughout the Frisco HMA, but in general are thought to be relatively good. The only recent soil data that exists is unpublished data for parts of Beaver and Millard Counties. Fragile soils occur within the HMA. These are considered fragile primarily due to steep slopes. Field examination of some of the allotments during rangeland health evaluations revealed small areas with a moderate or higher departure from normal in soil stability. Excess numbers of horses are removing protective vegetative cover and are contributing to localized active gullying and overland flows. Soil compaction is a localized problem, especially where horses are trailing to and from water sources. See EA text under "Rangeland Health Standards" for more details.	A. Stephens	12/7/15
NI	Invasive Species/Noxious Weeds	As long as there is a stipulation (as in the SOPs) of the use of weed free hay during any bait trapping, and for any feeding purposes of wild horses and/or domestic horses at the gather site or at holding areas on public land.	C. Hunter	11/23/15
NI	Lands/Access	Any pending or authorized lands and realty actions in the project area would not be substantially affected by the proposed action as long as measures are taken to assure all rights by grant, permit or lease holders are upheld. Prior to any surface disturbing activities in the vicinity of potential lands projects, the lands and realty staff should be notified to assist in locating existing or pending lands actions that may be impacted.	M. Campeau	11/16/15
PI	Livestock Grazing	Livestock and wild horses compete directly for vegetative, water, and cover resources. Higher populations of wild horses mean more competition with livestock. Wild horse populations that are within AML reduce competition. When wild horse populations are above AML the livestock numbers must be reduced to not over utilize the vegetative and water resources.	C. Hunter	11/23/15
PI	Migratory Birds	Multiple migratory songbirds and raptors occur within the Frisco HMA including ferruginous hawks, red-tailed hawks and golden eagles.	S. Whitfield	11/12/15
NI	Native American Religious Concerns	Based on previous government to government consultations with the Paiute Indian Tribe of Utah, the Hopi Tribe and data	Jamie Palmer	11/6/2015

Determi-nation	Resource	Rationale for Determination	Signature	Date
		from recent ethnographic studies, this action would not adversely affect the physical integrity or limit access to any known sacred sites.		
NI	Paleontology	The transient and superficial nature of the activities proposed would not substantially impact any fossil resources that may be present in the project area.	E. Ginouves	11/4/15
PI	Rangeland Health Standards	This is addressed as part of the Rangeland Health Standards section of the EA.	C. Hunter	11/23/15
NI	Recreation	Recreation in the project area is dispersed, and some displacement may occur during gather operations, however impacts will not be substantial. Coordination is necessary with the Utah Division of Wildlife Resources to notify public of operations, and to avoid conflicts during hunting season.	D. Jacobson	12/7/15
NI	Socio-Economics	The proposed action will not in its self change the socio-economics of the area.	C. Hunter	11/23/15
PI	Soils	Under the current situation of currently permitted livestock numbers, wildlife numbers being what they are and wild horses above AML, inadequate residual vegetation (forage) and litter remain on areas of grazing allotments within the analysis area (as evidenced by Rangeland Health Information). Lack of protective ground cover directly affects the soil's exposure to the erosive elements of wind and water. A reduction in horse numbers would allow additional vegetation to remain on these key areas, thus providing additional protection to the soil surface. For the purposes of the EA, hydrologic conditions and soils are combined.	C. Hunter	11/23/15
NI	Special Status Plant Species	There are 3 candidate plant species (*Eriogonum spathulatum var. kayeae, Leipidium ostleri, Trifolium friscanum)* occur within the project area. The gather is not anticipated to have impacts on the candidate plant species. There are no BLM sensitive plant species have been identified to occur within the project area.	D. Fletcher	12/08/2015
PI	Special Status Animal Species	Pygmy rabbits, ferruginous hawk and big-eared bat have the potential to occur within the Frisco HMA. Reducing horse numbers within the HMA would ensure adequate forage and cover for wildlife species using the area. No threatened, endangered or candidate wildlife species are within the Frisco HMA.	S. Whitfield	11/12/15
NP	Wastes (hazardous or solid)	There are no known hazardous or solid wastes created from this activity.	Glenn Pepper	12/7/2015
PI	Water Resources/Quality (drinking/surface/ground)	The proposed action would not be expected to impact drinking/ground water quality. However, surface water quality associated with riparian areas may improve with reduced grazing/trampling impacts from wild horses.	A. Stephens	12/7/15
PI	Wetlands/Riparian Zones	Project stipulations minimize impacts to wetland/riparian areas. A reduction in wild horse numbers would be expected to be beneficial to most riparian areas associated with the proposed action where wild horses have impacted riparian functionality.	A. Stephens	12/07/15
NI	Wild and Scenic Rivers	There are no Wild and Scenic Rivers present within CCFO	A. Stephens	12/07/15

Determi-nation	Resource	Rationale for Determination	Signature	Date
NI	Wilderness/WSA	Placement of gather sites in previously disturbed areas, and along existing roads would ensure no impacts to areas which may have wilderness characteristics.	D. Jacobson	12/7/15
NI	Woodland / Forestry	No impact to Woodland/Forestry management	C. Peterson	11/5/15
PI	Vegetation	The proposed management and removal of excess wild horses would benefit vegetative resources by reducing utilization, trampling, and leaving more residual cover.	C. Hunter	12/7/15
NI	Visual Resources	The proposed action includes only minor temporary disturbance. The actions will not measurable impact visual resources.	D. Jacobson	12/7/15
PI	Wild Horses and Burros	Project is within the Frisco HMA and there would be direct impacts to wild horses. See EA	C. Hunter	11/23/15
NI	Lands with Wilderness Characteristics	The project would not change the character of the land scape in areas that have been identified as having lands with wilderness characteristics such as units UT-C010-199. The areas would still have wilderness characteristics after the proposed gather.	D. Jacobson	12/7/15

FINAL REVIEW:

Reviewer Title	Signature	Date	Comments
Environmental Coordinator		2/24/16	
Authorized Officer		2/24/16	

APPENDIX F

Observation Protocol and Ground Rules

These rules were created to ensure the safety of both the humans and the animals at the gather site(s).
A scheduled public observation day provides a more structured mechanism for interested members of the public to see the wild horse gather activities at a given site. The BLM attempts to allow the public to get an overall sense of the gather process and has available staff who can answer questions that the public may have. The public rendezvous at a designated place and are escorted by BLM representatives to and from the gather site.

- The Bureau of Land Management (BLM) will schedule observation days to provide the media and public opportunities to view activities during the wild horse gather.
- To provide a safe environment for the animals, BLM staff, contractors and members of the public/media, requests will be accepted on a first come, first served basis and be limited to 10 people per observation day unless otherwise approved by authorized BLM official over the gather. The BLM recommends all appointments be made as far in advance as possible in order to help us schedule and confirm your request, and will make every reasonable effort to accommodate the public.
- Observation days and gather operations may be suspended if bad weather conditions create unsafe flying conditions.
- The BLM will notify observers as soon as possible if an observation day is canceled due to bad weather.
- Observers must provide their own 4-wheel drive high clearance vehicle, appropriate shoes, clothing and food.
- Observers are prohibited from riding in government and contractor vehicles and equipment.
- Visitors arriving at the rendezvous site without an appointment will not be allowed to participate in the observation day.
- BLM representatives will escort visitors to and from the gather and/or temporary holding facility.
- Visitors will be assigned to a BLM representative and must stay with that person at all times.
- Visitors are NOT permitted to walk around the gather site unaccompanied by a BLM representative.
- The BLM will clearly identify observation areas and visitors must stay within these designated areas.
- Observers are prohibited from climbing/trespassing onto or in the trucks, equipment or corrals, which is the private property of the contractor.
- Visitors must direct their questions/comments to either a designated BLM representative or the BLM spokesperson on site, and not engage other BLM/contractor staff and disrupt their gather duties/responsibilities.
- BLM may make the BLM/contractor staff available during down times for a Q&A session.
- When given the signal that the helicopter is close to the gather site bringing horses in, visitors must sit down in areas specified by BLM representatives and must not move or talk as the horses are guided into the corral.
- Observers will be polite, professional and respectful to BLM managers and staff and the

contractor/employees.

- Visitors who do not cooperate and follow the rules will be escorted off the gather site by BLM law enforcement personnel, and will be prohibited in participating in any subsequent observation days.

- Non- Scheduled Observation day Protocol and Ground Rules
- Non-scheduled observation days are days when the public is welcome to attend a gather on public land, or on specified private lands where permission was granted. The public is responsible for their own safety and health in their travels to and from the gather site.
- BLM staff may be limited on these days to answer questions.
- Visitors must direct their questions/comments to either a designated BLM representative or the BLM spokesperson on site, and not engage other BLM/contractor staff and disrupt their gather duties/responsibilities.
- The public will be expected to remain in designated observation areas.
- Visitors are NOT permitted to walk around the gather site unaccompanied by a BLM representative.
- The BLM will clearly identify observation areas and visitors must stay within these designated areas.
- Observers are prohibited from climbing/trespassing onto or in the trucks, equipment or corrals, which is the private property of the contractor.
- Observers must provide their own 4-wheel drive high clearance vehicle, appropriate shoes, clothing and food.
- When given the signal that the helicopter is close to the gather site bringing horses in, visitors must sit down in areas specified by BLM representatives and must not move or talk as the horses are guided into the corral.
- Gather operations may be suspended if bad weather conditions create unsafe flying conditions. Notification of suspension of gather operations will be made to the public that is present as soon as possible.
- Visitors must direct their questions/comments to either a designated BLM representative or the BLM spokesperson on site, and not engage other BLM/contractor staff and disrupt their gather duties/responsibilities.
- BLM may make the BLM/contractor staff available during down times for a Q&A session.

Observers will be polite, professional and respectful to BLM managers and staff and the contractor/employees.

Visitors who do not cooperate and follow the rules will be escorted off the gather site by BLM law enforcement personnel, and will be prohibited in participating in any subsequent observation days.

APPENDIX G

Public Comments and Responses

A Preliminary Environmental Assessment (EA) for the Population Control Research Wild Horse Gather for the Conger and Frisco Herd Management Areas DOI-BLM-UT-W020-2015-0017-EA was available to the public for a 30-day review/comment period beginning on April 18, 2016 and ending May 17, 2016. Written comments were received from 7 individuals by mail from Beaver County and the general public. E-mail comments and form letters were received from approximately 6,000 individuals. Approximately 5,980 of these letters were in a form letter format, of which over 5,000 were in support of Alternative 2. Comments received after May 17, 2016 were not accepted. Many of these comments contained overlapping issues/concerns which were consolidated into 72 comments and 14 distinct topics. Many of the comments could be clarified or answered by referring to sections within the EA. Others were outside the scope of the document. All comments were considered, but only those which included substantive comments were addressed below. Changes were made from the Preliminary EA to this Final EA based upon those comments and public involvement. Comments which are clearly addressed in the EA are not contained below. Comments which state personal opinion or support/opposition to the gather but are not substantive or are outside of the scope of this document are included in the case file at the Fillmore Field Office. Below is a detailed summary of the substantive comments received and how BLM used these comments in preparing the final environmental assessment. In addressing the comments the references are to the Preliminary EA unless otherwise specified.

No.	Commenter	Comment	BLM Response
GATHER METHODS			
1.	Several	Several comments were received which stated concerns with helicopter gathering of wild horses. These comments are represented by the following.	Wild horses are moved during gather operations by herding and are not stampeded. The WFRHBA mandates the gather and removal of excess wild horses and specifically authorizes the use of helicopters in Section 9 of the Act. "In administering this Act, the Secretary may use or contract for the use of helicopters or, for the purpose of transporting captured animals, motor vehicles. Such use shall be undertaken only after a public hearing and under the direct supervision of the Secretary or of a duly authorized official or employee of the Department." The Public Rangelands Improvement Act (PRIA) of 1978 (Pub. L. 95-514, Sec. 4, Oct. 25, 1978, 92
2.	Several	The many persuasive arguments against using helicopters to round up wild equines include, but are not limited to, the following: 1. They unavoidably traumatize, terrorize, injure, and kill wild horses. 2. Helicopter roundups also regularly force wild horses –including young foals and elderly or elderly animals– to run from helicopters at dangerous speeds over miles of rugged terrain, causing numerous serious veterinary injuries and fatalities, both during the roundup process itself and following it as a direct result of injuries sustained on these long stampedes. 3. Helicopter roundups also shatter closely knit family bands, forcibly separating foals from their mothers and stallions and mares, terrifying the already frightened animals and destroying the social fabric of wild horse herds.	

No.	Commenter	Comment	BLM Response
		4. They reduce population numbers to genetically unsustainable levels, because the BLM does not use accurate census data and because the agency unfairly skews forage allocations to vastly and unfairly favor privately owned livestock.	Stat. 1805.) also addresses this issue with the direction to "continue the policy of protecting wild free-roaming horses and burros from capture, branding, harassment, or death, while at the same time facilitating the removal and disposal of excess wild free-roaming horses and burros which pose a threat to themselves and their habitat and to other rangeland values." The BLM's helicopter-assisted gathers are conducted humanely, as affirmed by three recent independent reports (see below), and have proven to be more humane, effective, and efficient than other types of gather methods when large numbers of animals need to be removed over wide areas or rugged terrain. Helicopters start the horses moving in the right direction and then back off sometimes one-quarter to one-half mile from the animals to let them travel at their own pace; horses are moved at a more rapid pace when they need to be turned or as they reach the entrance to the capture site. Helicopter pilots are better able to keep mares and foals together than horseback riders; pilots can also more effectively move the animals around such barriers as deep ravines, fences, or roads. In Fiscal Year 2014, out of 1,863 wild horses and burros removed, a total of 18 animals, or approximately one percent (0.97 percent), died or were euthanized during gather

No.	Commenter	Comment	BLM Response
			operations; of those 18, nine animals, or about one-half of one percent (0.48 percent) of the removed animals, died or were euthanized because of acute injuries. Acute injury deaths include all animals that died or were euthanized because of acute injuries, such as spinal cord or head injuries, fractured limbs, or other severe injuries that occurred during gathers. Total deaths include all animals that died or were euthanized for any reason during gathers, including acute or sudden injuries or illnesses, as well as chronic or pre-existing conditions that required euthanasia (such as limb deformities, lameness, and poor body condition). Two reports issued in the fall of 2010 (one by four independent, credentialed equine professionals and one by the Interior Department's Office of Inspector General), plus another report released in 2011 by the American Association of Equine (Veterinary) Practitioners, found -- without any ideological or political bias -- that the BLM's gathers of wild horses are conducted in a humane manner. The Inspector General determined that the BLM's gathers are "justified" and reported that the agency "is doing its best to perform a very difficult job."
ALTERNATIVE METHODS			
3.	Individual	Would like to see grazing permittees exchange livestock AUMs for wild horse AUMs to better manage horse populations since they have expertise and equipment.	The exchange of livestock for wild horse AUMs is outside of the scope of this document.

No.	Commenter	Comment	BLM Response
4.	Several	Several comments were received which recommend alternative population control methods, such as predator management, natural management, and additional facilities.	These suggestions are outside of the scope of this document. Management of predators (wildlife) is outside the authority of the BLM.
5.	Several	Several comments were received which suggest using passive gather techniques such as bait trapping, on the range management bait trapping with volunteers, bait trapping and darting with Native PZP.	The proposed action includes the use of water and bait trapping dependent on herd health and the season (fall, winter, or summer) in which the gather is scheduled in order to maximize gather success and minimize impacts to wild horses. As stated in the EA, the use of bait and water trapping, though effective in specific areas and circumstances, would not be timely, cost-effective or practical as the primary gather method for this HMA due to the size of the area and the remoteness of many of the water sources. During the research part of the proposed action the use of water and bait trapping would not meet the gather objectives to implement the protocols. Water and bait trapping would be used as needed after the research studies are completed.
6.	Several	• Use Field Darting at the Appropriate time of the year (January through early spring), • Recruit volunteer citizen scientists to document the herds to ensure accurate population numbers • Identify band structure and mares to be targeted for fertility control. • Establish follow-up protocol to determine success or failure of the program. • Bait trap to document bands and to apply PZP done by Volunteer Scientists.	See Alternatives Considered but Eliminated from Further Analysis. The population of wild horses on the Conger and Frisco HMAs are over the AML. The water resources and forage within the HMAs cannot support the current number of wild horses. The use of PZP would slow the growth rate

No.	Commenter	Comment	BLM Response
			slightly, reducing the number of horses that need to be removed from the HMAs over time, but would not address the current over population. Remote darting has been shown to be ineffective on wild horse herds in Utah. A study by HSUS on the Cedar Mountain HMA in Utah has shown that after two years of trying to administer PZP through remote darting, not one horse has been darted. The wild horses in Utah (excluding the Onaqui HMA horses) are not use to the presence of people and are very wary. It is extremely difficult to get within 50 yards of the wild horses in the Conger and Frisco HMAs in order to dart them. However, this method would be included as population growth suppression and may be used in the future as one of many tools used in the management of the HMAs.
7.	Several	The EA failed to review, research and implement the Reserve Design strategy for the provision of adequate habitat suitable for long-term viable horse populations. Through appropriate restrictions on area occupied, we would see the "filling of the wild horse niche" and natural self-stabilization of the herd. This is what ecological "climax" species are capable of, given the chance but this requires patience and scientifically supportable research and credibility and common sense – all of which the BLM actions and proposals continually prove they are deficient in.	This suggested management scheme is outside of the scope of this document.
COST OF GATHER			
8.	Several	The EA does not consider the economic impacts of removing wild horses as well as long-term cost and adverse effects of adding	The Wild Free Roaming Horses and Burros Act (WFRHBA) does not require a cost-based

No.	Commenter	Comment	BLM Response
		wild horses to the already overburdened, unsustainable holding pen system.	decision-making process if excess horses are present. "Proper range management dictates removal of horses before the herd size causes damage to the range land (118 IBLA 75)." BLM has a responsibility per the WFRHBA to remove excess wild horses, ensuring the health of wild horses and the rangeland.
NUMBERS OF HORSES GATHERED			
9.	Several	Several comments were received which stated that AML levels should be changed.	This is a planning level decision and is outside of the scope of this document.
10.	Several	Several comments were received which questioned why the BLM proposes to gather horses down and manage at 100 horses in each HMA which is still well above high AML.	See section 1.4 in the Final EA.
11.	Several	Several comments were received which stated that the AML levels were too low to ensure genetic diversity and viability.	See, Section 3.2 Description of Affected Resources, Wild Horses (page 35) of Preliminary EA.
12.	Several	Several comments were received which stated that the population estimates and growth rate of wild horses is inaccurate. These comments are represented by the following.	See Appendix I for the estimated population on the Conger and Frisco HMAs. See section 1.4 in the Final EA.
13.	Cloud Foundation	Wild Horse populations are based solely on estimates that use a 20% annual population growth. Please provide the empirical data to substantiate this growth rate in the Conger and Frisco herds. We find no scientific basis for a 20% growth rate, which is used by the BLM across the board to estimate wild horse populations. Research monies would be better spent on accurately documenting wild horse populations on not only the Conger and Frisco HMAs but on all HMAs and HAs across the country	The Using Science to Improve the BLM Wild Horse and Burro Program A Way Forward published by the National Research Council of the Nation Academies is being used by BLM to develop new procedures and policies in the management of wild horses and burros. Some of the recommendation made by this report have been implemented (ex. population inventory methods) while others are being reviewed or developed within the laws, regulations,
14.	Friends of Animals	BLM's math doesn't add up. The agency states that "approximately 175" wild horses would be removed from the Frisco HMA to maintain an AML of 30-60 wild horses. However, according to BLM's population statistics, there	

No.	Commenter	Comment	BLM Response
		are now 293 wild horses in the Frisco HMA, and if BLM intends to reduce the Frisco population to even the high end of AML, a minimum of 233 wild horses would be removed from the Frisco HMA as a result of the proposed action. Similarly, BLM states that "approximately 225" wild horses would be permanently removed from the Conger Mountain HMA.4 Conger Mountain's population is approximately 342. If BLM intends to reduce the Conger HMA population to an AML of 40-80, the agency would have to remove a minimum of 262 wild horses from the Conger Mountain HMA. Thus, BLM's currently analysis is based on incorrect, confusing, or misleading information.	policies, budgets and other limits that were not considered by the report. However, there is no requirement for BLM to follow or implement any or all of the recommendation made in that report. The BLM utilizes well established scientific methods in the field of range monitoring, inventory and carrying capacity allocations, following approved methods outlined in official technical references and BLM handbooks and manuals. The CCFO and FFO have extensive vegetative trend, utilization, precipitation, actual use, riparian, and rangeland health studies which are contained in the Conger and Frisco HMAs and allotment monitoring files (4120 and 4710 files). Only the most current pertinent information has been summarized within this EA to show that excess wild horses occur within and outside, but adjacent to the HMAs. BLM use population growth rate not Birth Rate. The population inventory that was conducted in February of 2016 used simultaneous double-count method. Photos were taken of each band of horses that were observed. Photos, GPS coordinates and time of recorded observance were used to eliminate from the data any horses or bands that were double counted.
15.	Individual	Per the DOI/BLM herd stats the Frisco HMA wild horse population jumped to 67% in the last year and the Conger HMA wild horse population jumped to 83% last year! See chart below (in the comment letter). This is biologically impossible since mares give birth to only one foal per year at the MOST and stallions and foals (up to reproductive age of about 3 years) do not provide foals and therefore do not add to the annual population. As I stated, this annual population increase is physically unattainable in the wild.	

No.	Commenter	Comment	BLM Response
			According to the NAS 2013 report wild horses are capable of increasing numbers 15-20% annually, resulting in the doubling of wild horse populations about every 3 years.
WILD HORSE AND WILDLIFE IMPACTS			
16.	Return to Freedom Wild Horse Preservation	How many elk, deer and pronghorn graze this HMA? Targeting wild horses for removal to 'protect' the natural resources on the HMA while leaving all these other species on the range will result in continued damage on the HMA.	Management of wildlife is the responsibility of the Utah Division of Wildlife Resource (UDWR) outside the authority of the BLM.
WILD HORSE NUMBERS VS LIVESTOCK NUMBERS			
17.	Several	Several comments were received which stated that livestock numbers should be reduced rather than gather wild horses.	See, Section 3.2 Description of Affected Resources, Livestock Grazing (page 28) of EA. The BLM is not proposing to remove wild horses simply because the population is over AML. Through monitoring and review of other relevant factors, we have determined that excess wild horses are present and need to be removed not only to prevent degradation of the range, but to curtail existing impacts by wild horses and ensure wild horse health and welfare, as well as improvement and health of the habitat. The BLM utilizes well established scientific methods in the field of range monitoring, inventory and carrying capacity allocations, following approved methods outlined in official technical references and BLM handbooks and manuals.
18.	Cloud Foundation	The EA describes the impact of wild horses on every aspect of the environment but fails to consider the even greater impact of livestock grazing. Until EAs evaluate the greater impact of livestock grazing vs wild horse within HMAs and HAs - they have no validity and continue to turn a blind eye to the primary cause of rangeland degradation which is livestock grazing.	
19.	Several	BLM has failed to disclose to the public the actual allocation of forage made available to cattle, sheep, and wild horses in the Conger Mountain and Frisco HMAs.	
20.	AWHPC	At page 41 of the Draft EA, BLM makes the statement that "[o]ver a period of 10 years, competition for forage and water between wild horses, wildlife and livestock would be directly reduced." Given that this project is experimental in design and for the purposes of gathering information on the efficacy of the chosen herd management approach, this statement is appropriately framed as "could be" reduced.	

No.	Commenter	Comment	BLM Response
			Removal of livestock would not be in conformance with the existing Land Use Plan and is contrary to the BLM's multiple-use mission as outlined in the 1976 Federal Land Management and Policy Act (FLPMA) and PRIA, and would be inconsistent with the WFRHBA, which directs the Secretary to immediately remove excess wild horses. Additionally this would only be effective for the very short term as the horse population would continue to increase. Eventually the HMA and adjacent lands would no longer be capable of supporting the horse populations. Livestock adjustments have been made through other actions and documents. The purpose of the EA is not to adjust livestock use. There is no requirement of the WFRHBA or the regulations to reduce or eliminate livestock as a means to restore TNEB. Administration of Livestock grazing on public lands fall under 43 CFR Subpart D, Group 4100. Livestock grazing on public lands is also provided for in the Taylor Grazing act of 1934. The CCFO and FFO have extensive vegetative trend, utilization, precipitation, actual use, riparian, and rangeland health studies which are contained in the Conger and Frisco HMAs and allotment monitoring files (4120 and 4710 files). Only the most

No.	Commenter	Comment	BLM Response
			current pertinent information has been summarized within this EA to show that excess wild horses occur within and outside, but adjacent to, the HMAs. Rangeland Health Assessments were completed on grazing allotments within the gather area from 2007 through 2015 as indicated by the Monitoring Report for the Conger and Frisco HMA. This report showed that causal factors for not meeting standards included, but are not limited to, Pinyon Pine/ Juniper (PJ) encroachment, drought and grazing by livestock, wildlife and wild horses. These studies can be found within the allotment files and summaries of these studies are in the Monitoring Report for the Conger and Frisco HMA. The methodology of each study was completed using technical reference 1734-6. If it was determined that livestock were a causal factor toward the non-attainment of the Standards and Guidelines, changes to livestock grazing were made through the grazing permit renewal process. In the riparian section it states, "Damage to wetland and riparian areas often increases during drought years when wild horses may trample and dig in these areas in search of water. Because many of the springs within the Conger and Frisco HMA are non-functional due to drought conditions, the riparian vegetation is already stressed."

No.	Commenter	Comment	BLM Response
			While this referred to the riparian areas in the HMA in general, there are riparian areas that do not receive use by livestock and those show negative impact by wild horse and wildlife.
IMPACTS OF PERMANENT STERILIZATION ON WILD HORSES			
21.	Several	Several comments were received which stated that the EA did not adequately disclose the impacts to wild horses from permanent sterilization. These comments are represented by the following.	See Proposed Action and Impact Analysis sections in the Final EA. The practice of gelding horses is generally accepted throughout the veterinary community. The research proposal of the propose action is to study behaviors to gelded wild horses in a wild population. This research would then be published to improve BLM's understanding of wild horse demography and behavior to be used in future wild horse management throughout BLM. Other research studies are being conducted on various population growth suppression procedures. There are no genetic, scientific, cultural, or historical resources uniquely present among WH&B. The risks associated with the project are not unique and the risk of gelding alone is well known. The Proposed Action will not establish a precedent for future actions with significant effects or represent a decision in principle about a future consideration. Actions were
22.	AWHPC	There is a substantial question regarding several significance factors; □ Castration of 75% of the male stallions may cause loss or destruction of significant scientific, cultural, or historical resources; □ The use of gelding for wild horse population management is controversial among those in the scientific community. This issue is identified in the Draft EA, but inappropriately dismissed; □ The possible effects of this project are highly uncertain and involve unique and unknown risks; o The very nature of the project as a "research" project indicate that the outcome is unknown. □ The project may establish a precedent for future actions with significant effects; o The very nature of the project as a "research" project indicates the methods and outcomes are intended to be repeated. □ The impacts to the wild horses may result in a violation of federal law.	
23.	AWHPC	The BLM acknowledges the decision to engage in a project that goes against the NAS recommendations (DEA at 5). There is no alternative that included the option of vasectomy rather than castration and indeed, such an alternative was not even considered and later dismissed. This is a violation of the National Environmental Policy Act and renders the analysis in the Draft EA inadequate.	
24.	AWHPC	The BLM must rely upon the best available science when analyzing projects. For this	

No.	Commenter	Comment	BLM Response
		project, the BLM relied on the National Academy of Sciences (NAS) review of the Wild Horse and Burro Program titled "Using Science to Improve the BLM Wild Horse and Burro Program: A Way Forward," June 2013. Unfortunately, as we expressed in our scoping comments, the BLM has apparently used only the portions of the NAS document that support the pre-determined outcome for this project. The NAS findings that did not support the BLM proposal to castrate males from the Conger Mountain HMA were ignored.	considered by the Interdisciplinary Team within the context of past, present, and reasonably foreseeable future actions. Any future projects within the area or in the surrounding areas will be analyzed on their own merits and implemented or not, independent of the actions currently selected. A complete analysis of the direct, indirect, and cumulative effects of the selected alternative, and all other alternatives considered, is described in Chapter 4 of the EA. If significant negative impacts to the animals or rangelands are noted during the studies there would be no reason to repeat or continue the treatments being studied unless the methodology was significantly altered in response to those negative results. If the results turn out to be positive or show no negative impacts than similar work may be repeated elsewhere with some degree of monitoring to make sure the initial results carryover to other HMAs..
25.	AWHPC	The BLM dismisses our concerns about changes to stallion behavior post-gelding by stating that our concerns are unsupported (DEA at 50). However, we again refer the BLM to the NAS findings and recommendations and, indeed, point out that it is BLM's position that is unsupported, by their own admission. Statements in the Draft EA that "BLM fully expects the geldings would remain feisty and unruly with respect to humans" is unsupported by any scientific research.	
26.	Several	BLM is already aware of the impacts of castrating wild horses; the proposed "research study" is unnecessary. The purpose of the Conger Mountain gelding "study project" is to consider the behavioral effects of gelding on wild horse herds.5 BLM states that "[t]his type of information is not currently available."6 However, in 2013 the National Academy of Sciences report on BLM's wild horse program, National Research Council of the National Academies of Science, *Using Science to Improve the BLM Wild Horse and Burro Program, A Way Forward*, (2013)7, ("NAS Report") specifically noted the social and behavioral impacts of castration as a form of fertility control: A potential disadvantage of both surgical and chemical castration is loss of testosterone and consequent reduction in or complete loss of male-type behaviors necessary for maintenance of social organization, band integrity, and expression of a natural behavior repertoire.	
27.	Friends of Animals	So while it does not appear from BLM's horse fatality records that deaths from castration are	

No.	Commenter	Comment	BLM Response
		"rare," an adequate analysis of the actual impacts of surgical gelding requires that BLM provide detailed data regarding the number of horses castrated in BLM facilities per year, and the annual rate of complications, including deaths from evisceration and other causes now listed as "unexplained."	
28.	Several	The BLM must more fully analyze the impacts of the proposed gather, holding, and gelding of males on wild horses and their natural behaviors. The negative impacts of the gelding procedure must be analyzed for individual stallions and on the herd structure and dynamics. Reliance on studies from other areas around the world where herd structure, dynamics, and range are very different is not adequate.	
29.	Cloud Foundation	Gelding Alters Physical Health and Behavior The negative impact of gelding wild stallions has already been demonstrated in the South Steens HMA in Oregon where gelded males have been observed since 2009. Years later, in the South Steens HMA, gelding has had no impact on slowing population growth. Even one intact stallion can service receptive mares in season within the herd area. This is one reason the NAS did not recommend it. Male sterilization is ineffective, expensive, and dangerous.	
30.	Several	Sterilization of male horses causes the loss of male vigor in the stallions and this would have serious disruptive effects upon the individual bands and the surrounding herds to which each band belongs. This would result in less control over the young horses, which could actually result in more reproduction. It has been proven that the mature stable bands produce less offspring and come into balance with available resources. See ISPMB website and also www.thewildhorseconspiracy.org (various articles).	
USE OF POPULATION GROWTH SUPPRESSION			
31.	Several	Several comments were received which support or oppose the use of PZP. These comments are represented by the following.	The proposed action includes the use of population growth suppression using the currently

No.	Commenter	Comment	BLM Response
			approved vaccines and methods and allows for methods that are approved to be used in the future. The proposed action also allows for the use of water and bait traps along with other capture methods to use population growth suppression. These management tools would be used in the Conger and Frisco HMAs to reduce the annual population growth.
32.	Humane Society	We would like to note that some of the assertions regarding treatment with PZP contained within the EA are inaccurate. For instance, the EA notes that the horses in the Conger and Frisco HMAs are unapproachable, and as such a fertility control program using PZP is not feasible. To bolster this argument, the EA states that in the Cedar Mountain HMA, during a study where administration of PZP by remote darting, not a single horse was successfully darted. The EA then rules that because of this, a PZP program is not feasible within the HMA. While it is true that the HSUS study in Cedar Mountain HMA did find that darting horses was not easy, the fertility control program in that HMA was not unsuccessful due to this fact. Because PZP was highly effective in the HMA, more than 70% of the mares were treated in 2012, the 2013 population growth rate at Cedar Mountains was only 4.5%- less than a quarter of normal rates.	The primary use of PZP and any newly approved vaccines or methods would be to maintain the population within AML once achieved. These management tools could be used previous to achieving AML if gather success, holding capacity limitations, population growth rates, other national gather priorities or other circumstances prevent achieving AML during a gather. The population of wild horses on the Conger and Frisco HMAs are over the AML. The use of PZP would slow the growth rate slightly, reducing the number of horses that need to be removed from the HMAs over time, but would not address the current over population. Remote darting has been shown to be ineffective on wild horse herds in Utah. A study by HSUS on the Cedar Mountain HMA in Utah has shown that after two years of trying to administer PZP through remote darting, not one horse has been darted. The population growth

No.	Commenter	Comment	BLM Response
			rate on the Cedar Mountain HMA was reduced after gathering the population and administering PZP-22 on two separate occasions. The wild horses in Utah (excluding the Onaqui HMA horses) are not used to the presence of people and are very wary. It is extremely difficult to get within 50 yards of the wild horses in the Conger and Frisco HMAs in order to dart them. However, this method would be included as fertility control and may be used in the future.
DATA USED			
33.	Several	Several comments were received which questioned the data used in the analysis. These comments are represented by the following.	The BLM is not proposing to remove wild horses simply because the population is over AML. Through monitoring and review of other relevant factors, we have determined that excess wild horses are present and need to be removed not only to prevent degradation of the range, but to curtail existing impacts by wild horses and ensure wild horse health and welfare, as well as improvement and health of the habitat. The BLM utilizes well established scientific methods in the field of range monitoring, inventory and carrying capacity allocations, following approved methods outlined in official technical references and BLM handbooks and manuals. The CCFO and FFO have extensive vegetative trend, utilization, precipitation, actual use, riparian, and rangeland
34.	Individual	Horses are designated as “wild” under the FRWHBA. They do not compete with wildlife, They are wildlife. They are not designated livestock with cattle. Livestock are continually left out of the equation when assessing damage or reduction of forage and blame laid only on the horses.	
35.	Individual	The EA states that the "Key Forage" method was used to evaluate range-conditions. The full title of that approach is the "Key Forage Plant" (KFP) method. However, KFP is obsolete, having been replaced by the Landscape Appearance method as far back as 1996. Moreover, per Technical Reference 1734-7, Ecological Site Inventory, such qualitative assessments "may result in reduced accuracy, limiting use of the data." If for only this reason, I cannot rely on the EA's representations regarding conditions in the Conger and Frisco HMA.	

No.	Commenter	Comment	BLM Response
			health studies which are contained in the Conger and Frisco HMAs and allotment monitoring files (4120 and 4710 files). Only the most current pertinent information has been summarized within this EA to show that excess wild horses occur within and outside, but adjacent to the HMA. Rangeland Health Assessments were completed on the grazing allotments within the gather area from 2007 through 2015. Causal factors for not meeting standards included, but are not limited to, Pinyon Pine/Juniper (PJ) encroachment, drought and grazing by livestock, wildlife and wild horses. These studies can be found within the allotment files and summaries for the Conger and Frisco HMAs. The methodology of each study was completed using technical reference 1734-6. If it was determined that livestock were a causal factor toward the non-attainment of the Standards and Guidelines, changes to livestock grazing were made through the grazing permit renewal process. In the riparian section it states, "Damage to wetland and riparian areas often increases during drought years when wild horses may trample and dig in these areas in search of water. Because many of the springs within the Conger and Frisco HMAs are non-functional due to drought conditions, the riparian vegetation is already stressed". While this referred

No.	Commenter	Comment	BLM Response
			to the riparian areas in the Conger and Frisco HMAs in general, there are riparian areas that do not receive use by livestock and those show negative impact by wild horses and wildlife.
36.	Individual	A list of all range improvements done to manage and protect wild horses including, but not limited to, a list of all water sources, year-round availability of these water sources to wild horses and other wildlife	Not all data used to determine excess wild horses is contained within the EA. The purpose of the EA is to document the potential impacts associated with the Proposed Action and alternatives. Including this data in the EA would be cumbersome and would not change the impact analysis. Additional information is available at the BLM Fillmore and Cedar City Field Offices. The CCFO and FFO have extensive vegetative trend, utilization, precipitation, actual use, riparian, and rangeland health studies which are contained in the Conger and Frisco HMAs and allotment monitoring files (4120 and 4710 files). Only the most current pertinent information has been summarized within this EA to show that excess wild horses occur within and outside, but adjacent to the HMA. Added latest population inventories as an appendix to Final EA.
37.	Individual	Current and twenty-year historical documentation of herd population count/census numbers and a complete demographic breakdown of the Frisco and Conger wild horse populations (number of bands, stallion/mare ratio, number of foals, yearlings, two year olds and aged horses) including application, impact and results of past contraceptive use on the wild horses.	
38.	American Wild Horse Preservation	The EA failed to provide adequate alternative actions and fails to disclose or analyze the following prior to selecting the proposed action: • Rangeland assessment reports and results (and full assessments should be provided in the Appendix) for each the past five years for all areas in the HMA (including pastures, allotments, etc) – including all rangeland assessments used for the renewal of livestock permits, annual actual use of permitted livestock AUMs for each of the past five years (to better understand the cumulative impacts of livestock grazing on the HMA rangeland health), etc. • Methodology used to differentiate livestock usage impacts from wild horse impacts. Map of year-round and seasonal water sources within the HMA and a detailed description of all BLM actions (years, actions, locations) to protect and develop water sources. • Disclosure of the data utilized to determine the establishment of AML for the HMA and whether the conditions have changed	

No.	Commenter	Comment	BLM Response
		which would follow NAS recommendations of utilizing Adaptive Management to reassess AMLs in the EA. Include a complete list of all interested parties consulted when establishing AML and all scientific data used to set AML. • Mapping depicting the location of the wild horses in and outside the HMA, fence lines and water sources and all census data regarding the number of the wild horses.	
COMPLIANCE WITH POLICY			
39.	Individual	The EA fails to consider an alternative that includes working with wild horse advocacy organizations to re-evaluate and address the woefully inadequate Comprehensive Animal Welfare Program (CAWP) that pertains to the treatment of wild horses and burros during and after roundups as attached to this EA.	Considering alternatives to the Comprehensive Animal Welfare Program (CAWP) is outside the scope of this document. The CAWP policy is a national policy, not specific to the actions taken associated with the Conger and Frisco HMA. Fillmore and Cedar City Field Offices will follow the most current BLM policies pertaining to the CAWP. Minimal feasible level references the minimal level of management actions used to achieve Land Use Plan objectives, which the proposed action complies with.
40.	Several	Removing wild horses from the range, castrating them, and warehousing them in permanent holding facilities is not the "minimal feasible level" of wild horse management required under the Free-Roaming Wild Horses and Burros Act (WHBA), and the costs of doing so is an impact that must be disclosed under the National Environmental Policy Act (NEPA).	
COMPLIANCE WITH NEPA			
41.	AWHPC	This project presents a 10-year plan, using new research methods, over two HMAs, covering more than 200,000 acres of land. The length of the Draft EA alone suggests that an Environmental Impact Statement would have been more appropriate. Given the large amount of land covered, the large number of horses to be gathered and/or treated, and the current conditions on the ground, the BLM should conduct a more thorough analysis of the impacts of the proposed research project using an Environmental Impact Statement.	The analysis contained in the EA did not result in the expectation of any significant impacts to the human environment; consequently, an environment impact statement is not required. Please see the FONSI associated with the EA.

No.	Commenter	Comment	BLM Response
42.	Individuals (Chain)	The BLM should prepare an Environmental Impact Statement and cannot proceed on a Finding of No Significant Impact. The proposal to geld stallions as part of a research project to determine the efficacy of gelding as a population management tool has not been proven, is highly controversial, and will have significant impacts on the wild herd. All the impacts of this proposed action are not well understood, are uncertain, and are likely to be extremely detrimental to the well being of these wild horses. Further, the National Academy of Sciences already has clearly delineated the fundamental impacts of castration on wild stallions, stating that it will cause "loss of testosterone and consequent reduction in or complete loss of male-type behaviors necessary for maintenance of social organization, band integrity, and expression of a natural behavior repertoire.	
43.	AWHPC	The impacts to the wild horses may result in a violation of federal law.	The WFRHBA identifies sterilization as a means of population control. It also allows removals and research in the management of wild horses and burros.
44.	AWHPC	Tiering this Draft EA to the 1987 Warm Springs RMP and the 1983 Pinyon MFP is inadequate not only because those documents do not specifically address the type of research proposed and do not identify impacts specific to these two HMAs, but they are also both extremely outdated, 29 and 33 years old, respectively. Reliance on these documents and the extremely outdated analysis contained in both is inappropriate. Issues related to climate change, recent decades of drought, the American public's opinion on wild horses, and modern grazing practices are not included in those documents.	Any changes in the affected environment which have occurred since completion of these land use plans were considered in the EA. Projects do not need to be specifically addressed in a land use plan to be in conformance with the goals, objectives and decision of the land use plan.
GENETIC DIVERSITY			
45.	Several	Several comments were received concerning the loss of genetic diversity or viability. These comments are represented by the following.	See the Proposed Action and section 3.0 of the Final EA.
46.	Friends of Animals	BLM should consider the effect of this proposal on the genetic viability of the Frisco and the Conger Mountain herds. Under Alternatives 1	

No.	Commenter	Comment	BLM Response
		and 2, BLM proposes to subject both herds to repeated removals to reduce the population to dangerously low levels: the Frisco AML is 30-60, and the Conger Mountain AML is 40-80.23 In addition, up to 75% of the few Conger Mountain stallions released to the HMA will be subject to castration under alternative 1.	
47.	Cloud Foundation	Alternative 1 threatens genetic viability. (P 35 EA). According to the EA Hair Samples for genetic testing were collected and analyzed in 2012 by D. Gus Cothran. Dr. Cothran's analysis of the Frisco HMA states that "in general genetic viability is on the high side but there's a high percentage of variation at risk. Cothran's 2009 analysis of the Conger HMA states "if at any time in the future the genetic diversity is determined to be relatively low, a large number of the HMAs could be used as a source of fertile horses to be transferred to Conger HMA.	
RESEARCH			
48.	Several	Several comments were received which expressed concern with the use of radio collars. These comments are represented by the following.	Based on numerous studies that have used modern radio collars with remote releases and tags to study the ecology of wild ungulates and equids in particular, these devices have minimal effects on the animals wearing them. The impact of radio collars and tags is very minimal. From March 2015 through March 2016 researchers at the U.S. Geological Survey conducted a preliminary study on captive wild horses and burro jennies to determine proper fit and wear of radio collars (Schoenecker et al. 2014). The condition of wild horses wearing radio collars was compared to non-collared controls and documented with photographs. In addition, both collared individuals and controls were observed for 80 minutes each week for 14
49.	AWHPC	The EA/EIS must disclose and analyze all details of BLM data, information and research that resulted from implementing radio collar research on wild horses in Nevada in the 1980s and other BLM radio collar projects. The EA/EIS must disclose and analyze that BLM-sourced data – including the resulting harm that occurred to the collared horses, deaths, euthanasia, etc.	
50.	AWHPC	The EA/EIS must address how such deleterious effects of neck radio collaring of mares will be addressed or prevented given that mares also move their necks in manners that may allow the collar to become imbedded in the neck tissue, get caught on forage or fencing, be bitten by other horses, and cause discomfort or injury to the horse.	
51.	AWHPC	The fluctuating body condition of mares based on season, and the growth of younger mares as they mature must also be considered. The EA/EIS must consider and disclose the reason radio tail trackers are not used in mares as they	

No.	Commenter	Comment	BLM Response
		are proposed to be used on stallions. The EA/EIS must take a hard look at existing scientific data that outlines natural wild mare movements, behaviors, activities that may or likely will cause radio collars to move and tighten on the mares' necks.	weeks in order to quantify any impact of the collar on their behavior and health. At the end of the study period (March 2016) the collars were removed. Preliminary analyses indicate that mares had almost no impact in terms of rubbing or wear from radio collars, and behavior of collared and uncollared mares did not differ (Schoenecker et al. 2016 in prep). There was no impact of radio tags on behavior or wear, either. The reason collars are being used for mares instead of tail tags is because the retention of tags on the individual horse is shorter than collars, and the battery life for collecting data is shorter for tags than collars. The use of field observers and individuals to track and monitor wild horses on the ground, would not be time effectively, cost-effective or practical as the primary monitoring method for this HMA due to the size of the area, and the remoteness of many of the horses and large number of horses involved. However, some of this monitoring of unmarked horses would occur with the help of the collars and tags to help locate the animals. Also, locating wild horses by ground observers has flaws in terms of the scientific study design. With radio collars/tags, individually marked horses can be located using a randomized design, instead of "whenever the observers happen to find them." The latter method
52.	AWHPC	While the BLM may want to cite that "radio collar technology has been in regular use in other ungulate species for over 40 years" as a rational for the proposed experiment of putting neck radio collars on mares, the EA/EIS must provide scientific rational how this radio collar technology in "other ungulate species" applies to wild mares.	
53.	AWHPC	The EA/EIS must disclose and analyze any available data, reports, communications, etc. available within the various BLM offices related to wild horse and/or burro neck radio collar projects/pilots/programs/research which has occurred during the course of the agency's history of managing wild horses and burros. The implications of behavioral change to such individuals and deleterious effects of the neck radio collars must be disclosed and analyzed.	
54.	AWHPC	The EA/EIS must include as an appendix the results of radio collar research recently conducted by USGS at the BLM's Pauls Valley holding facility in Oklahoma.	
55.	AWHPC	The EA/EIS must disclose and analyze all details of BLM data, information and research that resulted from implementing radio collar research on wild horses in Nevada in the 1980s and other BLM radio collar projects. The EA/EIS must disclose and analyze that BLM-sourced data – including the resulting harm that occurred to the collared horses, deaths, euthanasia, etc.	
56.	AWHPC	Page 12 of the Draft EA indicates that pre-treatment behavior data will be collected on 16 tagged males and 4 collared females and their associates between March and September 2017. Please provide additional details regarding the collection of data. How often will horses be observed and for how long?	
57.	AWHPC	Page 12 of the Draft EA indicates that in "consultation with BLM specialists" a decision to geld 50-75% of all adult males in the	

No.	Commenter	Comment	BLM Response
		population was made. However, there is no evidence or information in the Draft EA or associated appendices that provides the public with an opportunity to review this "consultation" and provide feedback.	introduces bias into the study design and ultimately, the resulting data.
58.	AWHPC	Page 13 of the Draft EA indicates that males will be randomly selected for gelding. What procedure is the BLM utilizing to ensure that genetically valuable males are not gelded?	
59.	Individual	The BLM also fails to provide specific information concerning the disastrous attempt to conduct studies using radio collars on wild horses from Nevada in the 1980s which resulted in great harm to the collared "research" subjects including deaths and euthanasia. The data resulting from this botched "research" endeavor must be provided and thoroughly analyzed to ensure no such debacle endangers or kills federally-protected equines in any future "research studies" including the present proposed action.	
60.	Individual	Furthermore, prior to initiating this research study, I must insist that the results of radio collar research recently conducted by USGS at the BLM's Paul's Valley holding facility in Oklahoma be reviewed and disclosed as part of any Environmental Assessment (EA). Also, the results of the radio collar research conducted in the 1980's on Nevada wild horses should be included in any EA: injuries, death, foals orphaned, all pertinent data; so those mistakes will not be repeated in any research going forward.	
61.	Individual	Present your plan - how are you going to separate the stallions from their bands, transport them, treat their injuries from being transported together, and how are you going to tag them.	
GENERAL			
62.	Several	Several comments were received which expressed general support or opposition to the gather, but did not contain substantive information.	Several comments recommended changes in policy or actions which are outside of the scope of this EA.

No.	Commenter	Comment	BLM Response
63.	Individual	As required by regulation [43 CFR 4740.1(b)], a public hearing was held in Price, Utah on December 8, 2015 and will be held in subsequent years to discuss the use of helicopters and motorized vehicles in the management of Utah BLM's wild horses and burros. ... Comments received from the Preliminary Environmental Assessment (EA) and at those public meetings will be considered and, if applicable, will be addressed in management actions, NEPA documents, and decision documents using the most current direction from the National Wild Horse and Burro Program. EA pdf-page 44 I submitted detailed, substantive comments for the hearing. By now, BLM should have acted upon them and made reforms.	These comments were considered but will not be addressed specifically in this document. The comments and reforms received at the public meeting in Price on December 8, 2015 had to do with national policies and actions. They have been passed onto the national program by the Price Field Office.
64.	AWHPC	The first two paragraphs of the Draft EA at page 31 appear to be contradictory. The first sentence of the first paragraph states that BLM Standards and Guidelines for Healthy Rangelands "were not being met," while the first sentence of the next paragraph states these same guidelines are being met. Please explain this contradiction.	The first paragraph is referring to the Frisco HMA and the second is referring to the Conger HMA.
65.	AWHPC	It does not appear that the BLM has read or responded to the expert declarations of Dr. Anne Perkins, Dr. Allen Rutberg, Dr. Jay Kirkpatrick, and Dr. Bruce Nock, submitted with our scoping comments. The BLM must review these declarations and incorporate this information into the analysis for this project.	These individuals comments and opinions contained in these declarations were reviewed and address where appropriate within the impact analysis section of the EA.
66.	Individual	Questions about Procedures a) it isn't clear if you are comparing frisco to conger with frisco as the control group b) in frisco you tag 20 adult males, 40 adult mares, and 15 yearling; in conger, on the other hand, you tag 30 adult males and 30 adult mares; in conger you also tag 8 treated, 8 untreated males and 4 mares to do your gelding studies c) observations on animals targeted for gelding studies are unspecified but later in your ea you speak to observing if geldings form bachelor bands or intermix with harem bands, band sizes, gelding distribution in the habitat, and	See Section 1.4 Purpose of the Proposed Action in Final EA.

No.	Commenter	Comment	BLM Response
		utilization and activities around water sources.	
67.	Individual	The EA states that the study-results will also be used to update WinEquus, a population-projection software program. However, WinEquus evidently has internal programming errors that yield contradictory and even impossible predictions. A product that is broken must be fixed before it can be updated; but fixing WinEquus is not part of the study.	Out of scope of this document.
68.	Individual	This proposal includes releasing the wild horses back to the herd areas, but you have not especially addressed the mares and foal pairs. Will those be returned?	See Proposed Action in Final EA.
69.	Individual	You also do not make provisions for how close the horses will be returned to their home areas and in what band configuration.	See Proposed Action in Final EA.
70.	Individual	The public must be provided with information showing a complete and detailed breakdown of water allocations, which include multiple use projects such as: % of water allocated to mining projects % of water allocated to gas/oil explorations/ extractions % of water allocated to wind projects % of water allocated to solar projects % of water allocated to geothermal projects % of water allocated to other multiple use projects % water allocated to Livestock % water allocated to Wildlife % water allocated to Wild Horses and/or Burros	This information is not necessary for the analysis of environment impacts included in this document.

APPENDIX H

Procedure for Affixing Radio Collars on Wild Horse Mares and Burro Jennies

Introduction

The purpose of this document is to provide detailed methods that will be used for fitting radio collars on wild horse mares and burro jennies. This document does not include methods for chemical immobilization, care and maintenance of horses during gathers, while in captivity, or for any other handling procedures beyond those needed for fitting a radio collar.

The study of animal behavior and ecology requires understanding the daily life of the focal species (King 2013). It is now common to use radio collars fitted with VHF transmitters, GPS recorders, or satellite transmitters to obtain and record data on movement and other activities. While most radio collars are considered to be minimally invasive, they can impose a cost on the animal carrying them. Thus guidelines have been developed for a weight ratio (a collar should not exceed 5% of the animal's body weight) and best practice in their use (Ministry of Environment, Lands and Parks Resources Inventory Branch for the Terrestrial Ecosystems Task Force Resources Inventory Committee 1998, Sikes et al. 2011). Collars have the potential to cause injury to the animal wearing them. However when the collar is fitted correctly and monitored regularly it can provide invaluable data without any measureable impact on the study animal.

Telemetry collars have been used extensively on carnivores (Germain et al. 2008, Creel and Christianson 2009, Hunter et al. 2010, e.g. Broekhuis et al. 2013, Cozzi et al. 2013, Dellinger et al. 2013), rodents (Chambers et al. 2000, Solomon et al. 2001, Koprowski et al. 2007), and some ungulates (Johnson et al. 2000, Creel et al. 2005, Ito et al. 2005, Allred et al. 2013, Buuveibaatar et al. 2013, Latombe et al. 2013), however they have not been commonly used on equids. A few studies have used this tool to examine habitat use, movements, and behavior of zebra (Fischhoff et al. 2007, Sundaresan et al. 2007, Brooks and Harris 2008) and Asiatic wild asses (Kaczensky et al. 2006, 2008, 2011). Even fewer published studies have used telemetry collars on feral horses (Committee on Wild Horse and Burro Research 1991, Asa 1999, Goodloe et al. 2000, Hampson et al. 2010).

Although some research has been conducted on wild horse use of vegetation and habitat (e.g. Beever and Brussard 2000), little has been done recently, and long-term, fine-scale data on habitat use has never been gathered. Yet it is important that resource managers have a scientifically based understanding of wild equid seasonal habitat use and movements on public lands. Due to the scale of some of the Herd Management Areas (HMAs) it is logistically challenging to collect habitat use data via direct observation. Utilization of GPS and VHF collars for marking and locating individuals will provide fine-scale data about where wild horses spend their time and how they use their habitat.

From March 2015 through March 2016 researchers at the U.S. Geological Survey conducted a year-long preliminary study on captive wild horses and burro jennies to determine proper fit and wear of radio collars (Schoenecker et al. 2014). The condition of wild horses wearing radio collars was compared to non-collared controls and documented with photographs. In addition, the behavior of both collared individuals and controls was recorded for one hour daily, in order to quantify any impact of the collar on their behavior and health. At the end of the study period (March 2016) the collars were removed. At this time data are being analyzed and written up for submission to a peer reviewed journal (Schoenecker et al. 2016 *in prep*).

Radio collars consist of a 2-inch wide strap/belt made of soft pliable plastic-like material (Figure 1). Some are oval shaped with adjustments on both sides of the collar, and others are teardrop shaped with adjustments at the top of the collar so it can be fitted to different neck sizes. This is the most optimal

shape for the neck of equids. Attached to the belt of the collar is a battery pack and transmitter module. These may either be combined in the same unit, or placed at the top and bottom of the collar to counterbalance each other. The size of the battery is determined by the amount of power needed, both in terms of length of deployment, and how much data will be recorded by the collar. The type of transmitter used will depend on the study, but all principles stated here for collar fitting and use apply regardless of communication systems used.

Collars can be placed on horses' necks when wild horses are in a padded squeeze chute during a gather. It takes between 7 and 12 minutes to fit a collar on the animal. The transmitter should be functioning and turned on before the collar is fitted, then checked that it is working correctly before the animal is released.

Fitting of the collar

Fitting a collar on an equid requires an understanding of the neck circumference and shape; that is, when the head of the animal is raised the collar should be tight, and when the head is down grazing the collar will become looser (Figures 2, 3). The collar should rest just behind the ears of the equid and be tight enough so it does not slip down the neck, yet loose enough that it does not interfere with movement when the neck is flexed. The collar must fit snugly to minimize rubbing. USGS researchers used 0-1 finger between collar and neck, depending on season collar is deployed to give consideration to the potential for weight gain. Other studies (e.g. Committee on Wild Horse and Burro Research 1991) have had problems with the fitting of collars due to animals gaining weight in spring, or losing weight in winter, causing collars to become too tight or too loose. In the USGS study, researchers did notice collars were looser or tighter at different times during the year, but it did not affect the behavior of collared mares or jennies, or cause sores or wounds on mares or jennies. Whenever collars are deployed they should be fitted by experienced personnel who can attach the collar quickly but proficiently to minimize handling stress on the animal.

Figure 1. Two collar designs to use on wild horses and burros; one is teardrop shaped, and the other is oval shaped from Collins et al. (2014).

Figure 2. Burro jenny fitted with a radio collar in the USGS study showing appropriate placement of collars higher on the neck, behind ears.

Figure 3.Wild horse mares fitted with radio collars in the USGS study showing head up and head down, and demonstrating appropriate placement of collars higher on the neck just behind the ears.

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APPENDIX I

M E M O R A N D U M

To: Paul Griffin, Chad Hunter, Eric Reid, Gus Warr (BLM), Kate Schoenecker (USGS)
CC: Bea Wade, Dean Bolstad (BLM)
From: Bruce Lubow, IIF Data Solutions
Date: 20 May 2016
RE: Statistical analysis for 2016 horse population surveys in Utah

I. Summary Table

HMAs and Dates*	February 8, 2016	Frisco HMA	(UT0445)
	February 9, 2016	Conger HMA	(UT0553)
	February 10, 2016	Sulphur HMA	(UT0448)
	February 11, 2016	Sulphur HMA	(UT0448)
	February 12, 2016	Sulphur HMA	(UT0448)
Type of Survey	Simultaneous Double-observer		
Aviation Company	Cody J (pilot), El Aero Services, Bell 206B3 (N8052G)		
Agency Personnel	Chad Hunter, Eric Reid (BLM), Kate Schoenecker (USGS), Levi Anleers (BLM helicopter manager)		

*HMAs are listed for any day on which they were surveyed, and any day on which an animal found outside the HMA boundaries was closest to that HMA.

Table 1. Estimated population sizes (Estimate) are for the numbers of horses in each surveyed area at the time of survey. 90% confidence intervals are shown in terms of the lower limit (LCL) and upper limit (UCL). The coefficient of variation (CV) is a measure of precision; it is the standard error as a percentage of the estimated population. Number of horses seen (No. Seen) leads to the estimated percentage of animals that were present in the surveyed area, but that were not recorded by any observer (% Missed). The estimated number of horses outside each HMA, is already included in the total estimate for that HMA.

Area	Age Class	Estimate (No. Horses)	LCL [a]	UCL	Std Err	CV	No. Horses Seen	% Missed	Estimated # of Groups	Estimated Group Size	Foals per 100 Adults	Est. No. Horses Outside HMA
Frisco HMA	Total	288	257	373	34.8	12.1%	245	14.9%	69	4.2	11.2	136
	Foals	29	24	39	4.1	14.1%						
	Adults	259	233	334	31.7	12.2%						
Conger HMA	Total	310	270	347	23.5	7.6%	285	8.0%	44	7.1	19.1	0
	Foals	50	42	58	4.5	9.0%						
	Adults	260	229	292	19.8	7.6%						
Sulphur HMA	Total	1097	1025	1269	71.5	6.5%	958	12.7%	191	5.7	22.0	340
	Foals	198	182	232	14.6	7.4%						
	Adults	899	837	1034	58.4	6.5%						

[a] 90% confidence interval based on percentiles of bootstrap simulation results. The lower 90% confidence interval limit (LCL) is actually less than the number of animals sighted during the survey for some estimates. This is a normal statistical result and reflects the fact that a confidence interval expresses what would likely happen if the survey were repeated. If repeated many times, some surveys would miss more animals and produce lower estimates, even after corrections, than were actually observed during this survey. Clearly, I conclude that there are at least as many animals as were observed during this survey, rather than using the lower confidence limit as a minimum number.

II. Narrative

In February 2016, Bureau of Land Management (BLM) personnel conducted simultaneous double-observer aerial surveys of the wild horse populations in the Frisco, Conger, and Sulphur herd management areas (HMA) in Utah (Figure 1). Surveys were conducted using survey methods recommended by BLM policy (BLM 2010) and a recent National Academy of Sciences review (NRC 2013). I analyzed these data to estimate sighting probabilities for horses, which I then used to correct the raw counts for systematic biases (undercounts) that are known to occur in aerial surveys, and to provide confidence intervals (which are measures of uncertainty) associated with the estimated population sizes.

Population Results

The estimated total horse populations (Table 1) within or associated with the HMAs that were the focus of the surveys were relatively small at Conger HMA and Frisco HMA, but higher at Sulphur HMA, resulting in a sample size of 269 horse groups (Table 2, Figure 1) of which 216 horse groups had data recorded in a way so that they were suitable to be used in estimating statistical estimates of sighting probability. All 269 observations made during 2016 aerial surveys were used to inform the total estimates of population size. Confidence intervals and coefficients of variation are within acceptable levels of precision for management purposes (Table 1).

I estimated the mean size of detected horse groups, after correcting for missed groups, to be 5.6 horses/group across surveyed areas with a median of 4 horses/group. I note that the detected groups may have been composed of more than one social band. I estimated a composition of 19.5 foal horses per 100 adults at the time of these surveys, but this number is a reflection of the winter season of the surveys and should not be interpreted as an indication of foaling rate for 2015 or 2016.

Sighting Probability Results

The front observer saw 77.2% of the horse groups (81.0% of the horses) seen by any observer, whereas the back seat observers saw 72.6% of all horse groups (76.5% of horses) seen (Table 2). These results demonstrate that simple raw counts do not fully reflect true population size, without statistical corrections for missed groups made possible by the double observer method and reported here.

Informed by *a priori* reasoning, past analyses, and preliminary analyses I considered 64 alternative models. In all of these alternatives, I included an intercept and 5 additional parameters for the effects of: (1) front seat observers when a horse group was visible on both sides of the flight path, (2) back seat observers when a horse group was located directly on the flight path and not visible to either of them, (3) horse group activity (movement), (4) percent vegetation cover, and (5) observer position in the back seat. All of these parameters received overwhelming support in preliminary analyses. In addition to these 5 parameters included in all models, I considered the 64 models with all possible combinations of 6 additional covariates believed *a priori* to be likely predictors of sighting probability: (1) an additive effect for front observers' sighting probability for groups located on the pilot's side of the flight line, (2) horse group size, (3) rugged terrain, (4) snow cover percent and snow cover percent squared (both covariates or neither), (5) distance from the flight transect to the group, and (6) presence of high contrast lighting.

Of the 6 covariates tested, support (% of AIC_c model weight) was highest for the effect of

distance (77.2%). The other 5 effects tested all had weak support ranging from 27.1%-31.5% AIC_c model weight. As expected, estimated sighting probability was higher for groups that were: visible to both front seat observers, larger, active, or closer. Horses groups on the pilot's side and in the back for groups on the centerline were less visible. Also as expected, vegetation cover and high contrast lighting conditions reduced visibility. Sighting probability was also slightly higher for back-seat observers for groups on the primary observer's side of the aircraft (i.e., not the pilot's side, center, or both sides). The effect of snow cover was reduced visibility for intermediate levels of snow cover than for none or complete cover; in other words, patchy snow conditions reduced visibility as expected (Table 3).

The estimated sighting probabilities for the combined observers ranged across horse groups from 41.2%-100%. Sighting probability was lower than desirable for many horse groups. There were 25 groups (9.7% of all observed groups) with sighting probability <70% for the combined front and back observers and another 25 with sighting probability between 70-80%. Vegetation cover and patchy snow conditions were the primary factors resulting in these lower estimates. Note that the baseline case in Table 3 is for horses that were not moving and were ¼ - ½ mile from the observer, leading to the relatively low sighting probabilities indicated. However, most groups (151 groups, 69.9%) were moving and some (26 groups, 12%) were closer to the observers, so the average sighting probability (88.3%) was, in fact, much higher.

Comparing actual horses seen to the estimated population size computed from the estimated sighting probabilities, I estimate that 12.2% of the horses in these surveys were never seen by any of the observers (Table 1). The wide range of sighting probabilities were a result of diverse conditions with vegetation cover contributing the most to variability, followed by group activity, distance, and snow cover. Group size was as high as 33 horses. There were 32 observed horse groups with >10 horses (12.4% of groups containing 33.0% of the horses), however the effect of group size in sighting probability was small relative to other effects.

Assumptions and Caveats

Given several potential sources of bias, listed below, it is more likely that the estimates are somewhat lower, rather than higher, than the true population. The high sighting probabilities and precision estimated for these surveys, the population estimates I present here appear to provide a sound and reliable basis for management decisions. Although the sample size available for this analysis was adequate, a larger survey would provide additional information about sighting probability and increase confidence in the results.

The reliability of results from any population survey that is based on the simultaneous double-observer method rests on several important assumptions. First, the results obtained from these surveys are estimates of the horses present in the areas surveyed at the time of the survey and should not be used to make inferences beyond this context. I must presume that pre-flight planning by the district specialist and the BLM aerial survey coordinator led to the surveyed areas including as much as possible of the areas used by each population of horses using the surveyed HMAs. Although fences and topographic barriers can provide deterrents to animal movement that help to contain them within the areas surveyed, these barriers may not present either a continuous, unbroken barrier or an impenetrable one. However, the surveys did not necessarily extend as far beyond the boundary as horses might move. Consequently, it is possible that temporary emigration from the surveyed areas may have contributed to some animals of a

given population not being present in the surveyed areas and the numbers of animals found within the survey areas at another time could differ substantially.

Second, the validity of the analysis rests on the assumption that all groups of animals are flown over once during a survey period, and thus have exactly one chance to be counted by the front and back seat observers, or that groups flown over more than once are identified and considered only once in the analysis. Groups counted more than once would constitute 'double counting,' which would lead to estimates that are biased higher than the true number of groups present. The Conger and Frisco surveys were each completed on a single day, which should have helped to reduce the risk of double counting for these locations. The identification of 'marker' horses (horses with unusual coloration) in each group was recorded on paper, and variation in group sizes probably helped the observers to reduce the risk of double counting during aerial surveys. Observers also took photographs of almost every group, and used those photos after landing to identify any groups that were inadvertently recorded twice. Additionally, groups that were never available to be seen (for example, due to temporary emigration from the study area or due to moving, undetected, from an unsurveyed area to one already surveyed) can lead to estimates that are negatively biased compared to the true population size. Given the tendency of horses in these populations to run from the approaching helicopter, this is a significant concern.

Third, this method assumes that all horse groups with identical sighting covariate values have equal sighting probability. If there is additional variability in sighting probability not accounted for in the sighting models, such heterogeneity could lead to a negative bias (underestimate) of the population. Low sighting probabilities for horses in some situation leave open the possibility that other factors not considered could result in variation in actual sighting probabilities that are not fully modeled. Horses that were unavailable because they ran from the approaching helicopter before they could be seen would certainly contribute to heterogeneity and a negative bias in the estimates.

A fourth assumption is that the number of animals in each group is counted accurately. In very large groups it may be common to miss a few animals unless photographs are taken and scrutinized after the flight. Relying on raw counts made from a helicopter could lead to biased low estimates of population size. Observers in this survey, though, circled over large groups to get as accurate a count as possible and used photography on nearly all groups, thereby minimizing the risk of undercounting group size.

Recommendations for Future Surveys

This survey was well designed and generally followed the specified protocols. Nevertheless, several observations about the data may offer opportunities to improve future surveys.

1. Increasing sighting probability increases precision of the population estimates. To achieve higher sighting probabilities, it is best to time surveys so that snow cover is either absent or nearly 100%, to the extent possible. Flying more slowly over vegetated areas and patchy snow would also help address this problem, as would tighter transects in areas with these conditions (see below). In addition, slowing or circling while observers photograph groups and record data could also contribute to higher sighting probabilities.
2. Predefined transect spacing varied from approximate 0.5-1.0 miles. The pilot flew these as planned most of the time, although the flight path deviated from the plan in a few locations, leaving larger gaps between transects than desired. In most portions of the survey area the spacing was appropriate for the conditions, however, there were a few

gaps in the vegetated mountains that were >0.5 miles. Spacing in the unvegetated, flat valleys was probably tighter than necessary, given the high estimates sighting probabilities for these conditions. Increased spacing in open areas to 1.5 miles and better coverage with consistent 0.5 mile spacing in more rugged, vegetated terrain would improve sighting probability and reduce error at roughly the same cost.

3. Observers reported (Kate Schoenecker and Chad Hunter, pers. comm.) that horses were frequently running in response to the approaching helicopter. This makes it more likely that some groups will run into areas not yet surveyed and be double counted, while other run before detection into areas already surveyed and have no chance of being detected. Although photographs were used to effectively reduce or eliminate the double counting problem, there is no effective way after the survey to correct for the converse problem of horses fleeing and evading observations entirely. Participants in this survey have already conferred and made changes to the planned transects for future surveys that are intended to reduce the likelihood that horses will run toward areas already surveyed and thus never be available to observers.

4. In 18 instances, a horse group was observed by both back seat observers even though the side of ship was not recorded as "both". Of these, 8 instances were marked as not following protocol, leaving 10 cases with this anomaly in the analysis. Observers reported (Kate Schoenecker, pers. comm.) that these groups were first seen on one side and then ran across the flight path and became visible on the other side as well. The side of ship where they were first seen was recorded. In the future, groups like this that could be visible to observers on both sides should be recorded as "both"; however it is vital that this be done for ***all*** **groups that could have been seen by observers on both sides and not *only* those that actually were *actually* seen on both sides**. This requires that the single observer who sees a group and knows it could have been seen on the other side but was not, report this to the data recorder.

5. The number and ability of the observers was good and seat positions were rotated correctly, with a limited number (2) of back seat observers. This should be continued on future flights, preferably with the same front seat observer and rear seat observers.

6. I emphasize the importance of continuing to use photography for large horse groups (>10) to ensure that such groups are counted accurately. The current draft of the standard operating procedures for aerial surveys requires use of photography for all groups of >20 horses; however I advise that it be used for groups of >10 horses. There were 32 groups with >10 horses in this survey, only 3 of which were >20 horses. Surveys should continue to use a reliable, high-resolution camera with an adequate telephoto or zoom lens for the distance between observer and horses for this purpose.

Table 2. Tally of raw counts of horses and horse groups by observer (front, back, and both) for combined data from the Frisco HMA, Conger HMA, and Sulphur HMA surveyed in February, 2016.

Observer	Groups Seen (Raw Count)	Horses Seen (Raw Count)	Actual Sighting Rate[a] (groups)	Actual Sighting Rate[a] (horses)
Front	200	1,205	77.2%	81.0%
Back	188	1,138	72.6%	76.5%
Both	129	855	49.8%	57.5%
Combined	259	1,488		

[a] Percentage of all groups seen that were seen by each observer.

Table 3. Effect of observers and sighting condition covariates on estimated sighting probability of horse groups for both front and rear observers. Baseline case (**bold**) for horses presents the predicted sighting probability a group of 4 horses (the median group size observed) that are not moving, in smooth (not rugged) topography, ¼-½ miles from the transect (the most common distance recorded), 0% vegetation and snow cover, and not in high contrast lighting conditions. Other example cases vary a covariate or observer, one effect at a time, as indicated in the left-most column, to illustrate the relative magnitude of each effect. Sighting probabilities for each row should be compared to the baseline (first row) to see the effect of the change in each observer or condition. Baseline values are shown in bold wherever they occur. Sighting probabilities are weighted averages across all 64 models considered (Burnham and Anderson 2002).

	Sighting Probability, Front Observer	Sighting Probability, Back Observer
Baseline	**61.6%**	**71.0%**
Effect of group on centerline	**100%**	0.0%
Effect of group on both sides	100.%	91.6%
Effect of Pilot's Side	59.5%	**71.0%**
Effect of group size (N=1)	61.1%	70.6%
Effect of active group	86.7%	90.9%
Effect of rugged topography	62.7%	72.0%
Effect of vegetation cover (50%)	10.7%	15.4%
Effect of vegetation cover (100%)	0.9%	1.3%
Effect of snow (50%)	56.5%	66.5%
Effect of snow (100%)	61.9%	71.3%
Effect of distance (0-1/4 mile)	86.9%	91.0%
Effect of high contrast lighting	58.3%	68.1%

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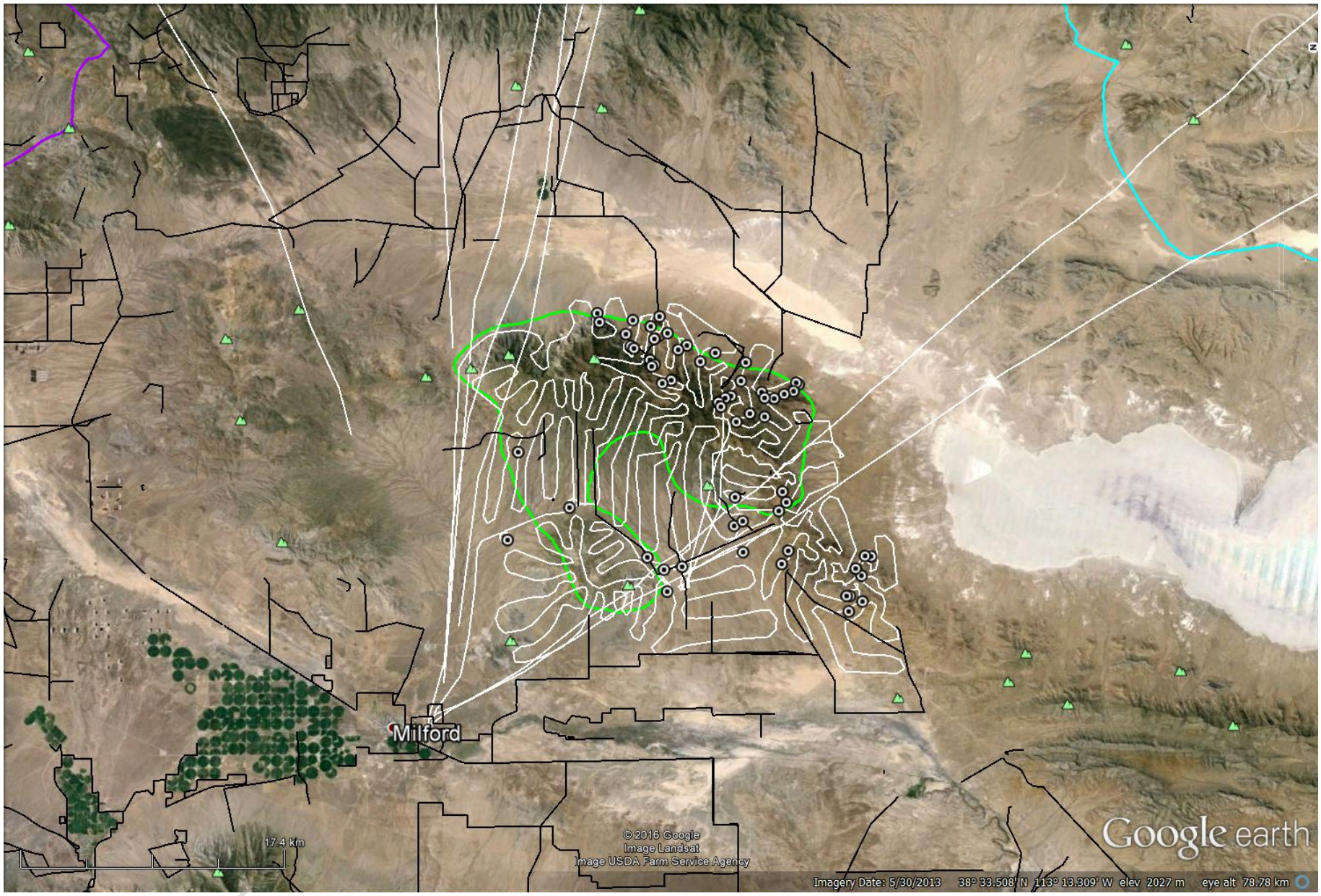
Figure 1 (following page). Map of survey tracks flown (white lines), locations of horse groups (black and white circles), and surveyed HMA boundaries: (A) Frisco HMA (bright green), (B) Conger HMA (dark blue), and (C) Sulphur HMA (red). Other HMAs near those surveyed but not surveyed at the same time that are visible in these panels: Eagle, NV (dark green), Bible Springs, UT (orange), Four Mile, UT (lavender), Kingtop, UT (turquoise), Confusion, UT (yellow), Swasey, UT (maroon).

A


Milford
17.4 km
© 2016 Google
Image Landsat
Image USDA Farm Service Agency
Google earth
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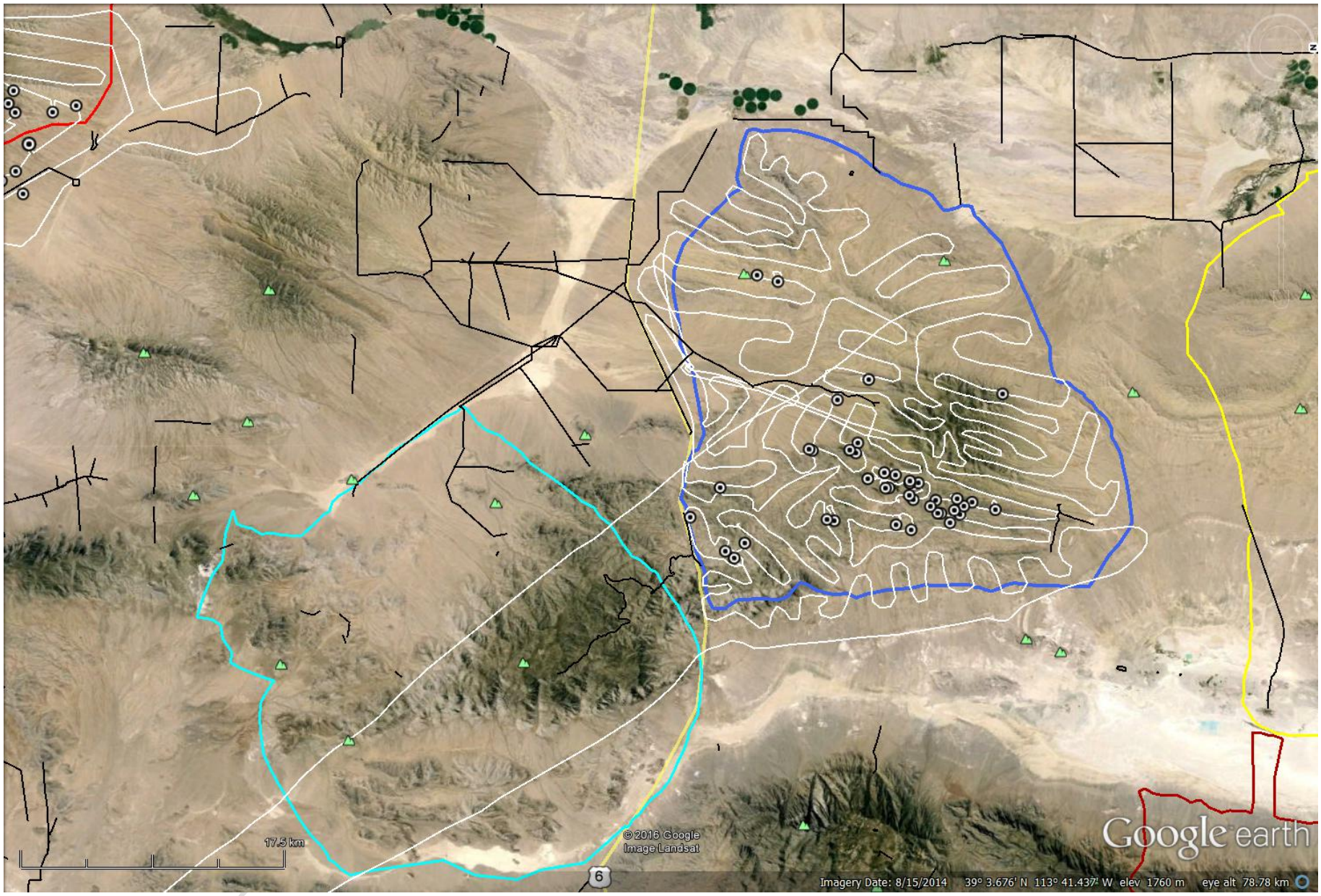
B


17.5 km
© 2016 Google
Image Landsat
6
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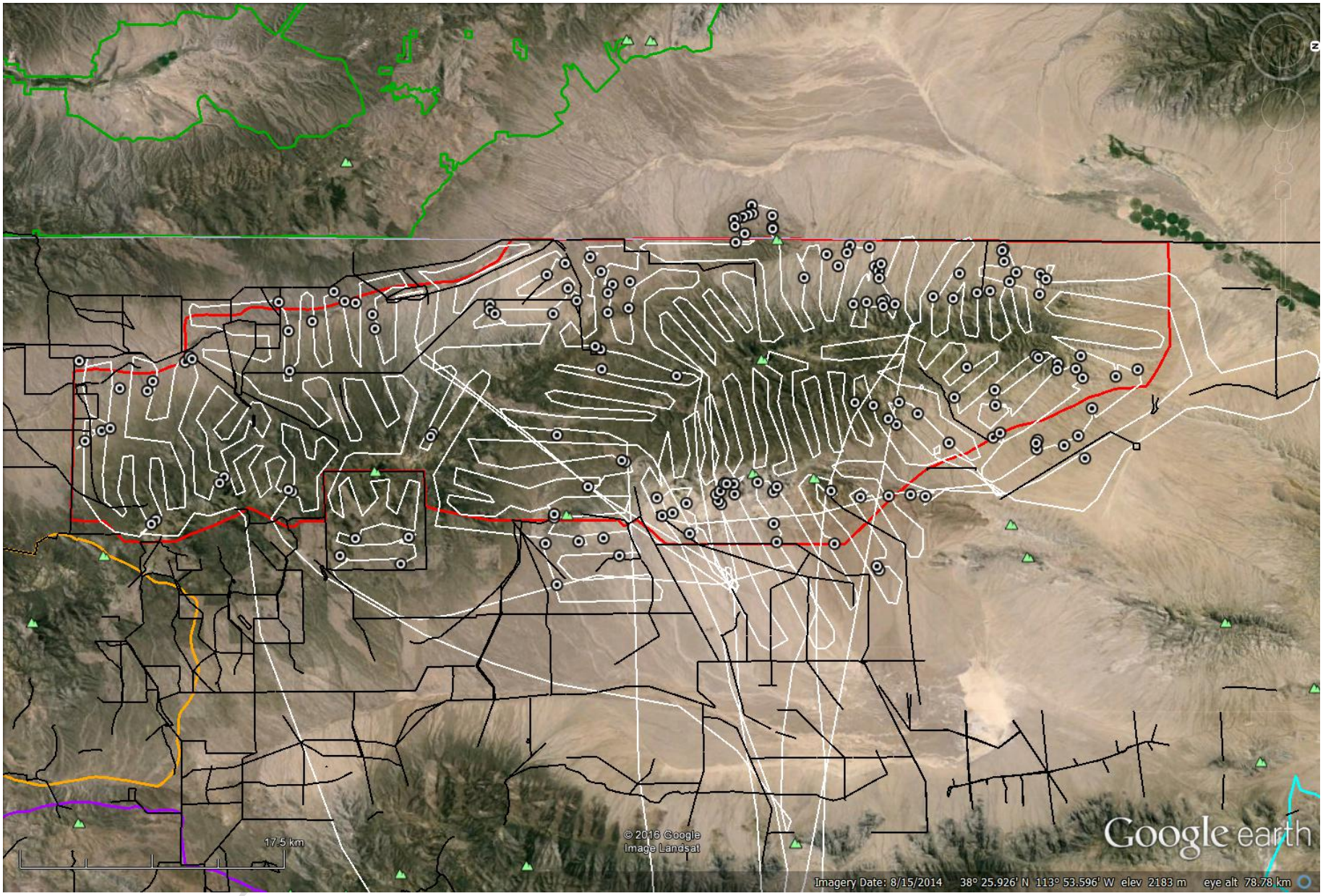
C


17.5 km
© 2016 Google
Image Landsat
Google earth
Imagery Date: 8/15/2014 38° 25.926' N 113° 53.596' W elev 2183 m eye alt 78.78 km

United States Department of the Interior
Bureau of Land Management

Final Environmental Assessment
DOI-BLM-NV-0000-2020-0001-EA

Oocyte Growth Factor Vaccine Study

U.S. Department of the Interior
Bureau of Land Management
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BLM

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1.0 Purpose and Need for Action

1.1 Introduction

This environmental assessment (EA) has been prepared to disclose and analyze the environmental impacts of a proposed Population Growth Suppression study that would test the contraceptive effects of an oocyte growth factor vaccine. The Bureau of Land Management (BLM), Nevada State Office (NSO) in cooperation with United States Department of Agriculture, Animal and Plant Health Inspection Services National Wildlife Research Center (NWRC) would conduct the research with previously-gathered wild horses that BLM has removed from the range and determined to be excess. *See* 16 U.S.C. § 1533(b).

This EA analyzes the potential impacts that could result with the implementation of either the Proposed Action or a No-Action alternative to the Proposed Action. BLM also considered other alternatives but has dismissed them from a more detailed analysis as they do not fulfill the purpose and need identified in the EA. Preparation of an EA assists the BLM authorized officer to determine whether to prepare an Environmental Impact Statement (EIS) if significant impacts could result, or a Finding of No Significant Impact (FONSI) if no significant impacts are expected.

1.2 Background

The Wild Free-Roaming Horses and Burros Act (WFRHBA) of 1971 directs BLM to "protect and manage wild free-roaming horses and burros as components of the public lands." 16 U.S.C. § 1333(a). BLM is required to manage the horses and burros in a manner that is "designed to achieve and maintain a thriving natural ecological balance on the public lands." *Id.* The Interior Board of Land Appeals defined the goal for managing wild horse (or burro) populations in a thriving natural ecological balance as follows: "As the court stated in Dahl vs. Clark, supra at 594, the 'benchmark test' for determining the suitable number of wild horses on the public range is 'thriving natural ecological balance.' In the words of the conference committee which adopted this standard: 'The goal of WH&B management should be to maintain a thriving ecological balance (TNEB) between WH&B populations, wildlife, livestock and vegetation, and to protect the range from the deterioration associated with overpopulation of wild horses and burros.'"

The WFRHBA states that BLM must "make determinations as to whether and where an overpopulation exists and whether action should be taken to remove excess animals." *Id.* § 1333(b)(1). To make that determination, BLM has established appropriate management levels (AML) for public lands. *Id.* If the number of wild horses and burros exceeds the AML and action is necessary to remove the animals, BLM must "immediately remove excess animals from the range to achieve" AML. *Id.* § (b)(2). While stating that BLM's management activities should be "at the minimal feasible level," the WFRHBA also mandates that BLM determine whether AML can be achieved "by the removal or destruction of excess animals, or other options (such as sterilization, or natural controls on population levels)." *Id.*; *id.* § 1333(a).

Wild horses and burros tend to have exceptionally high reproductive and survival rates. BLM has determined that the high end of AML for wild horses and burros on the public range across ten western states is 26,690. However, as of March 1, 2019, there was an estimated 88,090 wild horses and burros on the range, 47,468 of which are in the State of Nevada. Therefore, the population on the range exceeds AML by more than 61,400 animals in total – with almost half of those animals in Nevada. In addition to those animals on the range, as of January 17, 2020, there are over 49,500 wild horses and burros that BLM houses in short- and long-term care facilities. BLM gathered and removed these animals from the range after it determined that an overpopulation existed in areas on the range. There is limited funding

and space available for the care of additional animals in short- and long-term care facilities, and insufficient adoption demand for the excess horses removed from the range.

BLM has attempted to address both the high population growth rate of wild horses and burros and the overpopulation on the range by using a variety of methods, including periodic gathers and removals, sex-ratio adjustments, and temporary fertility control vaccines like PZP Zonastat-H, PZP-22 vaccine pellets, and GonaCon. However, these methods have not proven effective at managing the population of wild horses and burros in the long-term or at all herd management areas and herd areas. For example, gathers and removals do not limit the ability of wild horses and burros to reproduce. And, for wild horses there is no currently available fertility control vaccine from which one dose reliably leads to substantial effects that last longer than two years (Rutberg et al. 2017, Baker et al. 2018, Carey et al. 2019). To cause long-lasting physiological effects, existing fertility control vaccines require repeated vaccine applications, which require sufficient budgetary and logistical resources (such as contractors qualified to conduct gathers) and can cause repeated stress to the animals as this generally involves capturing the animals. Capture and vaccination can be difficult for BLM to repeatedly perform at certain herd management areas and herd areas due to the areas' topography, size, skittishness of the horses, etc., on top of budgetary and logistical challenges.

In 2013, the National Research Council of the National Academies of Sciences (NAS) released a report titled "Using science to improve the BLM Wild Horse and Burro Program, a way forward." The NAS Report concluded that it could be useful to have a long-lasting fertility control method available for some situations for wild horse and burro management.

With regards to the duration of a fertility treatment's efficacy, the Report said:

> "Duration of fertility inhibition has major practical importance. Shorter-acting methods require substantially more effort and financial resources to implement even if the cost of the contraceptive itself is low. Longer-acting methods are preferable to minimize requirements for personnel and financial resources and to decrease the frequency of animal handling … In cases in which reversibility is important and repeated treatment is practical, one of the [existing] vaccines would be preferable, with the caution that treatment for more than a few years may prolong recovery of fertility. A single treatment that induces lifetime infertility could be preferable in other situations."

Since 2013, BLM has funded the development and testing of methods that could lead to long-lasting fertility control[1]. BLM's goal is to develop contraceptive treatments that achieve and maintain wild horse population sizes within the established AML, so as to reduce population growth rates in the short term, allow for increased time between gathers, and decrease the number of excess horses that must be removed from the range. The Proposed Action in this EA is designed to determine whether a one-dose oocyte growth factor (OGF) vaccine could be a reliable, long-lasting fertility control method. Oocyte growth factors are proteins that influence the development of oocytes (egg cells) in the ovary. If the one-dose OGF vaccine proves effective, it could be considered for use in future BLM wild horse and burro herd management actions, which management decisions would be subject to additional NEPA compliance.

Decreasing the number of excess wild horses on the range is consistent with findings and recommendations from the National Academy of Sciences (NAS), American Horse Protection Association (AHPA), The American Association of Equine Practitioners (AAEP), Humane Society of the United States (HSUS), Government Accountability Office (GAO), Office of Inspector General (OIG) and

[1] These include, but are not limited to, testing booster doses of GonaCon vaccine, intrauterine devices (IUDs), and new encapsulated formulations of PZP vaccine.

BLM policy; BLM notes that some of those non-governmental groups appear to advocate that removals should take place as part of a broader program that includes fertility control measures. The preface to the NAS report (2013) stated that "It is … evident that the consequences of simply letting horse populations, which increase at a mean annual rate approaching 20 percent, expand to the level of "self-limitation"—bringing suffering and death due to disease, dehydration, and starvation accompanied by degradation of the land—are … unacceptable." The GAO (2008) noted that "Populations that exceed AML can harm the health of the range….The overpopulation of wild horses and burros on the range may negatively impact herd health, rangeland health, and livestock and wildlife that depend on the range."

Contraception Vaccines in Wild Horse Management

As mentioned above, the WFRHBA specifically contemplates BLM using long-lasting fertility control, which could include sterilization, as a population control tool. 16 U.S.C. § 1333(b). No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros. *Id.*

In recent years, BLM has prioritized expanding the use of fertility control methods to slow population growth rates, reduce the overpopulation of wild horses on public lands, decrease the number of animals that BLM must gather and remove from the range, and protect rangeland ecosystem health. BLM has identified fertility control vaccines as a promising method of contraception. Contraception has been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used with other techniques, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). A number of humane contraceptive methods exist, including but not limited to the use of fertility control vaccines (also called immunocontraception), intrauterine devices (IUDs), and humane sterilization techniques; each method has advantages and limitations. Contraception by itself does not remove excess horses from the range. Instead, successful contraception reduces future reproduction. So if a wild horse population is currently above AML, then further action may still be required to remove excess horses in order to achieve a thriving natural ecological balance.

Research to test the efficacy of contraceptive methods in wild horses is valuable to BLM because successful contraception that requires a minimum number of handling occasions would be expected to, among other things,: (1) slow the population growth rate; (2) reduce the frequency of horse gathers; (3) provide alternatives to the current fertility control vaccines which have limited duration of efficacy from one dose; and (4) lower wild horse management costs to taxpayers by reducing the frequency of gathers and lowering the number of horses that BLM must permanently remove and send to short- and long-term care facilities. Bartholow (2007) concluded that the application of 2 or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs. Similarly, Hobbs et al. (2000) concluded that population suppression becomes more cost-effective if fertility control is long-lasting. Bartholow (2007) also concluded that contraceptive treatment would likely reduce the number of horses that must be removed in total, with associated reductions in the number of private placements and total holding costs.

Although fertility control treatments on individual wild horses may be associated with some potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient (other than the temporary or long-lasting loss of reproductive potential for the treated individuals, which is the intended effect), do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013). The use of the oocyte growth factor vaccine for management of wild horses on the range, would be subject to a separate decision-making process and site-specific NEPA compliance, as informed by scientific information available at that time.

Development of a new fertility control vaccine is important as single doses of the existing vaccines are only reliably effective for up to two years. Rutberg et al. 2017, Baker et al. 2018, Carey et al. 2019. Two of the three fertility control treatments that the NAS identified in 2013 as being the "most promising" for immediate use were the porcine zona pellucida (PZP) and gonadotropin releasing hormone (GnRH) fertility control vaccines. Those vaccines were deemed promising partly because of the ease of their delivery method by injection, their availability, their relative efficacy, their lack of effects on scavengers that may eat carcasses of treated animals, and the fact that physiological side effects outside of the reproductive system are limited. Development of a new fertility control vaccine that confers long-lasting effects from a single injected dose would be another, potentially promising, tool for use in wild horse management. The OGF vaccine did not exist when the NAS reviewed fertility control methods available in 2013.

An additional reason that it is prudent to test new types of fertility control vaccines that can be used to manage wild horse populations is the small, but measurable, risk that PZP vaccine availability could be limited in the future, as a result of the growing global incidence of African swine fever. If that disease reaches the United States, then the supply of pig ovaries could be affected, and there may be restrictions on the movements of swine-derived tissues. African swine fever is not known to affect horses. An alternative to PZP would be recombinant zona pellucida (rZP) proteins, which are ZP molecules produced from genetically engineered microbes. Those could be used in vaccines but, as far as BLM is aware, the contraceptive effectiveness of any fertility control vaccines using rZP proteins has not yet been tested on mares in the United States.

Oocyte Growth Factor Vaccine

In 2019, BLM received a proposal from the USDA Animal and Plant Health Inspection Services National Wildlife Research Center (NWRC), for a study designed to test a one-injection formulation of a fertility control vaccine that had preliminary success when used in an earlier formulation that used four injections. If the one-dose formulation of the OGF vaccine is effective, then it could at least cause short-term infertility, and may be able to cause more long-term sterility in treated mares. If the effect lasts more than two or three years, then such a one-dose fertility control vaccine could be critically useful in reducing wild horse growth rates in BLM-administered herd management areas across the west. The research study proposed is responsive to the search for a long-lasting, "…single treatment that induces lifetime infertility…" as was alluded to by the 2013 NAS report.

The proposed study would assess the efficacy and contraceptive effectiveness of the one-dose OGF vaccine, but is not designed to assess behavioral effects of vaccination in free-roaming wild horses (since the study will be conducted on already gathered horses in a more confined environment). The previous four-injection version of the vaccine that would be used in this research has already been tested and appeared safe and successful (Appendix A). The one-treatment vaccine formulation in this research study would induce an immune response to the same oocyte growth factor proteins (called GDF9 and BMP15), but would use a different adjuvant (AdjuVac) and antigen packaging preparation (liposomes) to determine whether a single dose could have the same effect.

Fertility control vaccines meet BLM requirements for safety to mares and the environment (i.e., EPA 2009, 2012, 2013). The oocyte growth factor vaccine would constitute a new form of immunocontraceptive, but would have some of the same advantages that have been identified in other vaccines, including: because they work by causing an immune response in treated animals, there is no risk of hormones or toxins being taken into the food chain when a treated mare dies.

1.3 Purpose and Need for Action

The purpose of the Proposed Action is to test the efficacy of a vaccine against two oocyte growth factors, where the vaccine has been formulated to cause long-lasting contraception from a single dose, on

approximately 16 wild horse mares that have already been permanently removed from the range as excess animals. An equal number of untreated, control mares would live side by side with treated mares. A previously tested version of the vaccine caused contraception for at least 2 years, but it used a weak vaccine adjuvant that required four doses to be effective (results of that study are described in Appendix A).

This action is needed in order to determine whether the one-dose oocyte growth factor vaccine could be a reliable, longer- or long-lasting fertility control method for future BLM wild horse and burro herd management actions, that are subject to separate decisions by BLM and that are covered under separate NEPA documents. Conducting nondestructive research and seeking the recommendations of qualified scientists on matters related to wild horse and burro management is consistent with the provisions of the WFRHBA § 1333(b)(1) and 43 CFR § 46.210 (e).

1.4 Land Use Plan Conformance

The Proposed Action is in conformance with the Carson City Consolidated Resource Management Plan (2001) because it is consistent with national policy listed there to "…preserve and maintain a thriving natural ecological balance…" and with Standard Operating Procedure # 3, "*…contraceptive techniques may be used to slow the rate of an increase of wild horses within the HMAs."*

1.5 Relationship to Laws, Regulations, and Other Plans

Statutes and Regulations

The Proposed Action has been designed to conform with Federal regulations and other authorities that provide official and suggested guidance for management of wild horses by BLM.

1. ***Wild Free-Roaming Horses and Burros Act*** (WFRHBA) of 1971 (Pub. L. 92-195), as amended through the ***Public Rangelands Improvement Act*** of 1978 (Pub. L. 95-514). The Proposed Action is consistent with the WFRHBA specifically, but not limited to, the following:

"§ 1332. Definitions

(f) "excess animals" means wild free-roaming horses or burros (1) which have been removed from an area by the Secretary pursuant to application law…

§ 1333. Powers and duties of the Secretary.

(b) Inventory and determinations; consultation; overpopulations; research study; submittal to Congress.

(1) The Secretary shall … determine whether appropriate management levels should be achieved by the removal or destruction of excess animals, or other options (such as sterilization, or natural controls on population levels). In making such determinations the Secretary shall consult with the United States Fish and Wildlife Service, wildlife agencies of the State or States wherein wild free-roaming horses and burros are located, such individuals independent of Federal and State government as have been recommended by the National Academy of Sciences, and such other individuals whom he determines have scientific expertise and special knowledge of wild horse and burro protection, wildlife management and animal husbandry as related to rangeland management.

(3) For the purpose of furthering knowledge of wild horse and burro population dynamics and their interrelationship with wildlife, forage and water resources, and assisting him in making his determination as to what constitutes excess animals, the Secretary shall contract for a research study of such animals with such individuals independent of Federal and State government as may be recommended by the National Academy of Sciences for having scientific expertise and special knowledge of wild horse and burro protection, wildlife management and animal husbandry as related to rangeland management."

The Proposed Action includes the use of a fertility control vaccine that may cause short-term contraception, long-term-contraception, or sterilization. The wild horses that will take part in the study have been previously determined to be excess animals and were already removed from the wild, as a

result of previous BLM NEPA determinations. The proposed action makes use of USDA APHIS NWRC expertise in animal fertility control methods.

2. ***Federal Land Policy and Management Act*** (Pub. L. 94-579), as amended
The Proposed Action studies a fertility control method that should slow wild horse population growth rates and, therefore, aid BLM in management on the basis of multiple use and sustained yield as described in the FLPMA.

3. ***Wild Free-Roaming Horse and Burro Management*** (43 C.F.R. § 4700)
The Proposed Action is consistent with 43 CFR 4700 specifically, but not limited to, the following:
"4710.4. Constraints on management: Management shall be at the minimum level necessary to attain the objectives identified in approved land use plans and herd management area plans."

The Proposed Action would determine whether a one-dose vaccine could lead to long-term fertility control in wild horses. Currently available fertility control vaccines require more than one dose to have long-lasting effects. Therefore, if the one-dose OGF vaccine is effective in causing long-term infertility, its use could reduce the amount of animal handling that would be required, compared to available vaccines.

4. ***BLM Wild Horses and Burros Management Handbook*** (BLM H-4700-1)
Guidelines in the BLM management Considerations handbook are not legally binding policy, but they do provide suggestions for BLM management. The Proposed Action is consistent with handbook H-4700-1 specifically, but not limited to, the following:
"4.5.3. Reduce Population Growth Rates: "Additional management alternatives (tools) may be considered in the future, pending further research."
"8.1 Other research projects may be initiated as needed to support the management of WH&B."
"8.2 A National Research Advisory Team exists to review research proposals, monitor research project progress, update the Strategy as necessary, and provide recommendations to the WH&B Division Chief…All requests for research and/or research proposals pertaining to the Wild Horse and Burro program, whether generated from the field or through field-related contacts, are to be directed to the Research Advisory Team for review."

The Proposed Action includes the testing of a new fertility control method (one-dose OGF vaccine), which would be responsive to current wild horse management needs, which still include long-lasting fertility control methods that require only one handling occasion. Since the time that handbook H-4700-1 was written, a study at the Northern Nevada Correctional Center (NNCC) revealed that pelleted PZP did not provide adequate long-lasting contraceptive effect. The research proposal from NWRC was reviewed by the BLM WHB Research Advisory Team, which provided a recommendation to the WHB Division Chief.

5. ***BLM Management Considerations*** (BLM H-4710-1)
Guidelines in the BLM management Considerations handbook are not legally binding policy, but they do provide suggestions for BLM management. The Proposed Action is consistent with handbook H-4710-1 specifically, but not limited to, the following:
".5 Research.
.51 Strategic Research Plan. The BLM shall conduct wild horse and burro research in accordance with the priorities identified in the Strategic Research Plan for Wild Horse and Burro Management."

Handbook H-4710-1 refers to the BLM Wild Horse and Burro Strategic Research Plan (BLM 2005), which includes the following guidance: "…the BLM seeks a contraceptive agent that lasts three to five

years following a single injection….Reproduction in wild horses may differ in a number of subtle ways from domestic horses, for example, in the season of estrus cycles. The explanation for these observed differences may be merely environmental, or there may be some minor physiological differences that are inherited. Until the differences can be defined, the BLM has determined that non-invasive research into fertility control could be conducted on captured wild horses held in BLM facilities…" If it is successful, the one-dose OGF vaccine may deliver a form of fertility control that may last three to five years, or longer. The Proposed Action would test the one-dose OGF vaccine in wild horses, in a BLM-contracted facility (the NNCC).

6. ***Comprehensive Animal Welfare Program*** (BLM IM 2015-151)
The Proposed Action is consistent with this policy. Animals in the study will be maintained and cared for in keeping with BLM Comprehensive Animal Welfare Program (Appendix C).

7. ***Animal Health, Maintenance, Evaluation, and Response*** (BLM IM 2015-070)
The Proposed Action is consistent with this policy. Any decision to humanely euthanize animals would be made in conformance with this policy. Euthanasia of otherwise-healthy animals is not a planned part of the Proposed Action.

In addition to the above, the Proposed Action is also consistent with 43 C.F.R. § 46.210, and does not meet any extraordinary circumstance that would require further NEPA review. Despite BLM's conclusion that a Categorical Exclusion could have been an appropriate vehicle for NEPA compliance, BLM Nevada chose to analyze the Action Alternative in an EA to allow for additional public input after publication of a preliminary EA.

1.6 Decision to be Made

The authorized officer would determine whether or not to approve the Proposed Action. The authorized officer's decision is limited to whether to conduct the study. The decision would not set any precedent for use of the vaccine in any future BLM wild horse and burro herd management actions, though preliminary or final results of the proposed study could be taken into consideration in future BLM NEPA determinations.

1.7 Scoping and Identification of Issues

The following issues were identified as a result of internal scoping relative to the BLM's management of wild horses in the planning area:

Impacts to individual wild horses included in the Proposed Action. Measurement indicators for this issue include:

- Expected impacts of handling stress to individual wild horses
- Expected effects of proposed fertility control vaccination
- Potential impacts to animal health and condition
- Potential impacts to pregnant mares and foals
- Potential impacts of vaccination on adoptability

2.0 Description of the Alternatives

2.1 Introduction

This section of the EA describes the Proposed Action and alternatives, including any that were considered but eliminated from detailed analysis. Two alternatives are considered:

- Alternative 1: Proposed Action – Treat approximately 16 mares with oocyte growth factor vaccine and determine efficacy during a three-year study.
- Alternative 2: No Action — Do not conduct the proposed study.

The Proposed Action Alternative was developed to respond to the Purpose and Need. The No Action Alternative would not achieve the identified Purpose and Need. However, it is analyzed in this EA to provide a basis for comparison with the Action Alternative, and to assess the effects of not conducting the proposed study at this time.

2.2 Description of Alternatives Considered in Detail

2.2.1 Management Actions Common to Alternatives 1-2

Whether or not the Proposed Action is taken, the wild horses in the study will be housed and maintained at the Northern Nevada Correctional Center (NNCC) until they are adopted or sold according to existing BLM policy, or are moved to a different off-range facility. BLM contracts with NNCC for the maintenance and care of excess wild horses removed from the range.

2.2.2 Proposed Action

The Proposed Action would include a study designed to test the efficacy of a modified fertility control vaccine in excess wild mares, over a three-year period. This study research is needed to improve the understanding of the effects of a new vaccine that is specific to two ovarian proteins, and which has been designed to be effective after just one dose. The vaccine would be administered as a single injection to the mares in the treatment group.

The one-dose formulation of ovarian growth factor vaccine would include two antigens, BMP15 and GDF9, that have been identified as having a role in oocyte development in the ovary (Galloway et al. 2000, Eckery et al. 2002, Appendix A). These protein antigens would be conjugated to a carrier protein, keyhole limpet hemocyanin (KLH; Curtis et al. 2008), to increase the presentation and longevity of antigen epitopes to the vaccinated mares' immune system. To prolong the immune response further, some or all of the antigen and carrier molecules will be enclosed in spherical, phospholipid bilayer molecules known as liposomes; liposome molecules are a feature of the SpayVac vaccine (Bechert et al. 2013). The adjuvant used to enhance immune response would be one that has been tested and used as a component in other NWRC vaccines, AdjuVac, which contains a small amount of *Mycobacterium avium* as an immunostimulant (Miller et al. 2004). Vaccine success would be measured in terms of the response including at least short-term infertility, with longer-term sterility in treated mares also being a desirable outcome.

Notwithstanding the stated start dates in Appendix A, the timing of the start of the study may depend on administrative factors. The NNCC was selected as a suitable site for the study because it houses and works with excess wild horses removed from the range and has pen designs that are ideal for fertility control studies. The pens are constructed with sturdy materials and high walls, appropriate for housing stallions. These pens have been successfully used in a BLM-funded 2011-2013 pen trial of pelleted versions of a PZP vaccine that also included wild horse mares and stallions in breeding groups that would be similar to the proposed OGF vaccine study design. The same pens at NNCC used for the previous study would be used for the proposed OGF vaccine study.

Research done in coordination with US Department of Agriculture (USDA) Animal Plant Health Inspection Services (APHIS) National Wildlife Research Center (NWRC) would include:

- Treatment of approximately 16 mares with the fertility control vaccine;
- Non-treatment of approximately 16 mares to serve as untreated, 'control' animals;
- Maintenance of mares, in stable groups of 4 treated mares and 4 control mares, with one fertile stallion at a time;

- Periodic blood sampling for hormone and antibody characterization;
- Periodic pregnancy checking via transrectal palpation or ultrasound examination, conducted by a qualified reproductive professional.

Animal care would meet BLM standards identified in the Comprehensive Animal Welfare Program (Appendix C). Holding pens would be approximately 200 feet by 100 feet, with 8 foot tall solid panel fencing. Fertile stallions would be rotated in with mare groups approximately every 3-4 months. To increase the probability that any potentially fertile mares (whether vaccinated or untreated) become pregnant, fertile stallions will be periodically rotated in and out of the bands of stable mares. It is expected that this would happen approximately every 3-4 months. In cases where a mare becomes pregnant, the BLM will take appropriate measures for the safety, welfare, care and handling of pregnant mares and their foals. Approximately 16 mares will receive the one-dose OGF vaccine, and will be monitored throughout the three-year study. The same number of non-pregnant control animals (approximately 16) will be present at the start of each year of the study; to accommodate this need, additional 'control' animals could be added to the study each year as necessary to maintain the sample size of 'control' mares at the start of each year.

The following animal selection stipulations would apply:

- The starting target score for Henneke body condition will be at least 4 for both mares and stallions;
- Mare and stallion ages at the start of the study will be between 3-10 years old;
- Random selection will be used to place mares in either the vaccine-treated group or the untreated control group.

Other Stipulations:

- Guidelines as set forth in the Comprehensive Animal Welfare Program (Welfare Assessment Standards for Gathers) will be followed.
- Individuals handling the vaccine will take standard precautionary steps used when handling any vaccine, to minimize the risk of needle sticks. Out of an abundance of caution, for this study no female humans of childbearing age or younger will be involved with the handling or injection of vaccine.
- During operations, the Lead Contracting Officers Representative (COR) for the BLM contract with the NNCC would be present, or would delegate supervision duties to another BLM employee.
- The BLM contract Veterinarian associated with the NNCC facility, Animal and Plant Health Inspection Service (APHIS) Veterinarian or other licensed Veterinarian would be on call or on site.
- Decisions to humanely euthanize animals would be made in conformance with BLM policy (BLM Washington Office Instruction Memorandum 2015-070).

2.2.3 Alternative 2: No Action

Alternative 2 would be to not test the efficacy of the oocyte growth factor vaccine. This Alternative would not meet the Purpose and Need identified in section 1.3. BLM would not be able to gather information about the long-term efficacy of the oocyte growth factor vaccine that has been formulated for a single injection. Available information about effects of oocyte growth factor vaccines would remain limited to the previous studies that demonstrated the effectiveness of a vaccine administered over four injections, rather than for the single injection formulation.

2.3 Alternatives Considered but Dismissed from Detailed Analysis

2.3.1 PZP Vaccine Study

An alternative in which a PZP vaccine would be tested in a pen trial at NNCC was not considered for detailed analysis. This alternative was not analyzed because BLM has already supported pen trials for two types of PZP vaccine formulations that were intended to cause long-lasting effects from a single dose of vaccine. BLM supported pen trials testing fertility rates of wild mares inoculated with SpayVac vaccine; the mares and fertile stallions were housed at the Pauls Valley, Oklahoma, BLM corral (Roelle et al. 2017). Similarly, nominally long-lasting formulations of PZP vaccine pellets were tested in pen trials at the NNCC from 2011 to 2014, in the same pens that would be used in the Proposed Action, and using similar methods for rotation of fertile stallions with bands of treated and non-treated mares. Results of the most recent trials for both SpayVac (in Oklahoma) and the nominally long-lasting PZP vaccine pellet formulations (at NNCC) did not cause an extended period of infertility, and were discontinued after higher than expected pregnancy rates were observed in treated mares after a short time period (Roelle unpublished results, Turner unpublished results). An additional PZP vaccine trial would not be responsive to the purpose and need identified in this EA, and would not provide BLM with information about a new type of fertility control vaccine that would be free of risk from possible disruptions that could be caused by any future outbreak of African swine fever.

2.3.2 GonaCon Vaccine Study

An alternative in which the GonaCon vaccine would be tested in a pen trial at NNCC was not considered for detailed analysis. An additional GonaCon vaccine trial would not be responsive to the purpose and need identified in this EA. GonaCon is approved for use as a fertility control vaccine in wild horses and burros (EPA 2013). However, this alternative was not analyzed because field trials have already demonstrated that GonaCon is an effective vaccine, with potential for long-lasting efficacy after a second hand-injected dose is administered (Baker et al. 2018). BLM has been supporting an ongoing field trial in Theodore Roosevelt National Park, North Dakota, that aims to determine optimal dose timing and long-term efficacy of GonaCon booster doses. The physiological and contraceptive effects of GonaCon are fairly well understood, and another pen trial with wild horses is not needed for BLM to use this vaccine in wild horse management.

2.3.3 Oocyte Growth Factor Vaccine Study in Domestic Mares

An alternative where the proposed study would take place with domestic horses outside of BLM custody was dismissed from detailed study for several reasons. Most importantly, wild horses are the intended population of use for the vaccine. The vaccine should work in wild horses; it is possible that some phenotypic or physiological traits that are idiosyncratic to wild horses may influence vaccine effectiveness, as noted in the WHB Strategic Research Plan (BLM 2005). Unlike domestic horses, most wild horses have not typically been exposed to numerous vaccines over the course of their lives. There is some possibility that the oocyte growth factor vaccine could interact with the immune system of wild horses in a way that is different from how it interacts with the immune system of domestic horses.

3.0 Affected Environment

This section of the EA briefly discusses the relevant components of the human environment which would be either affected or potentially affected by the Proposed Action or No Action Alternative (refer to Table 2). Direct impacts are those that result from the management actions while indirect impacts are those that exist once the management action has occurred.

3.1 General Description of the Affected Environment

The NNCC is a Nevada state prison facility located on non-federal lands, within Carson City County, Nevada. The topography at NNCC is flat, at an elevation of approximately 4,680 feet. Precipitation in the region of Carson City municipality averages 9.6 inches. Temperatures also vary, from average high

temperatures around 90 degrees Fahrenheit in July to average low temperatures around 22 degrees Fahrenheit in December.

The NNCC grounds include equine facilities suitable for wild horse care, maintenance, and handling by inmates. The facilities include a number of large pens, including eight 100 foot by 200 foot pens that have 8 foot high, smooth steel walls made of highway-grade collision railing. There are runways between all pens that lead to sorting pens. Animals can be humanely moved into runways leading to a padded hydraulic squeeze chute that is suitable for inspections and veterinary care. The nearest public lands are approximately a half mile east of the animal maintenance and handling areas on the NNCC.

There is no public access to the areas of the NNCC where the study would take place, as this area is accessible to inmates and with limited visibility from the security watchtower. Because these are areas with inmate presence, there is no photography, recording or videography allowed in the wild horse maintenance and handling areas of the NNCC. When BLM staff periodically visit the animal maintenance and handling areas of the NNCC, the NNCC requires that any cameras and/or cell phones be left behind and that they be accompanied by a special security escort. Although there are scheduled wild horse and burro adoption events held on NNCC grounds that are open to the public, those events are held in an area where public safety can be ensured. The adoption area has additional security staff are present and are either in uniform or plain clothing, visitors pass through a security check point, the area is apart from where the inmates work, and is separate and distinct from the area where the horses in the study would be housed, cared for by the inmates, vaccinated, and checked for pregnancy.

3.2 Description of Affected Resources/Issues

Table 2 lists the elements of the human environment subject to requirements in statute, regulation, or executive order which must be considered.

Table 2: Supplemental Authorities (Critical Elements of the Human Environment)

Supplemental Authorities	Present	Affected	Rationale
ACECs	**NO**	**NO**	Not present.
Air Quality	**NO**	**NO**	The planning area is within an established prison facility where horses are already housed. The Proposed Action would have no measurable influence on air quality.
Cultural Resources	**NO**	**NO**	Not present.
Environmental Justice	**NO**	**NO**	Not present.
Fish Habitat	**NO**	**NO**	Not present.
Floodplains	**NO**	**NO**	Not present.
Forest and Rangelands	**NO**	**NO**	Not present.
Migratory Birds	**YES**	**NO**	The planning area is within an established prison facility where horses are already housed. Although some migratory birds may occasionally be present on the grounds, the Proposed Action would have no measurable influence on migratory birds.
Native American Religious Concerns	**NO**	**NO**	None known.
Noxious Weeds	**YES**	**NO**	The planning area is within an established prison facility where horses are already housed. The proposed action would have no measurable influence on noxious weeds.
Prime or Unique Farmlands	**NO**	**NO**	Not present.

Riparian-Wetland Zones	**NO**	**NO**	Not present.
T&E Species	**NO**	**NO**	Not present.
Water Quality	**YES**	**NO**	The planning area is within an established prison facility where horses are already housed. The Proposed Action would have no measurable influence on water quality.
Waste (Hazardous or Solid)	**NO**	**NO**	Not present.
Wild and Scenic Rivers	**NO**	**NO**	Not present.
Wilderness and Wilderness Study Area	**NO**	**NO**	Not present.

There are no critical elements of the human environment identified as present and potentially affected by the Proposed Action or by the No Action Alternative. The Proposed Action would take place on the grounds of the Northern Nevada Correctional Center, in an area that does not include any federal lands. In addition to the critical elements listed in Table 2, the only resources that may be affected by the Proposed Action are wild horses that would be included in the proposed action. The affected environment, relative to wild horse resources, is described below.

3.2.1 Wild Horses

Any wild horses included in the Proposed Action would have been previously gathered and removed from the public range as excess animals. They would have arrived at the NNCC as a part of their off-range care. The length of time since the horses were removed from the range may vary by individual animal. Their ages would be between 3-10 years old at the time of the start of the Proposed Action, and their Henneke body condition scores would be at least 4. Any stallion that would be included in the study would still be fertile. To the extent possible, potentially fertile mares would be identified as candidates to be included in the study; this determination could be from BLM records about whether the mare had a foal at her side when she was initially gathered, or if information is available about the mare's current pregnancy status. All animals at the NNCC, whether included in the proposed study or not, are properly fed and watered, consistent with the standards for their maintenance and care.

4.0 Environmental Consequences

4.1 Introduction

This section of the EA documents the potential environmental impacts which would be expected with implementation of the Proposed Action and/or the No Action Alternative. These include the direct impacts (those that result from the management actions) and indirect impacts (those that exist once the management action has occurred).

4.2 Predicted Effects of Alternatives

The direct and indirect impacts to these resources which would be expected to result with implementation of the Proposed Action or No Action Alternative are discussed in detail below.

4.2.1 Wild Horses

Impacts of Proposed Action Alternative

Under the Proposed Action Alternative, impacts to wild horses could be both direct and indirect, occurring to individual horses included in the study. All the wild horses that would be included in the study would have been previously gathered and removed from the public range as excess, and the impacts of gathering, transporting, holding, and adopting horses would have been considered in the NEPA analyses supporting the BLM decisions that led to their gather. The impacts analysis that follows is limited to potential impacts that could result from the Proposed Action; it does not include the impacts of

already-analyzed BLM actions that led the horses being housed at the NNCC, or actions that resulted directly from their prior gathering.

Animal Handling

There is the potential for direct impacts to occur, associated with having wild horses living in a corral setting. Under past management practices, captured mares experienced elevated stress from handling (Ashley and Holcombe 2001), but BLM has instituted guidelines to reduce the sources of handling stress (BLM 2015). The CAWP (Appendix C) would be implemented to ensure that conditions for animal care and welfare are safe and humane, and to minimize potential stress and injury to wild horses. Handling may include freeze-marking, for the purpose of easily identifying individual animals.

It is possible that there could be conflict between individual animals that live together in pens. Such impacts are known to occur intermittently in holding facilities. An example of an indirect individual impact could be a brief 1-2 minute skirmish between animals; usually such conflicts are between studs, and would not be expected in bands with only one stud and eight mares. Injuries typically involve a bite or a kick that bruises, but does not break, the skin. The frequency of this type of minor impact would vary across individual animals. There is the potential for conflict after introduction of new stallions to a group of mares, but the expectation is that the level of that conflict will be low, based on previous experience at the NNCC. The same pens at NNCC that would be used for the proposed study were previously used in a 2011-2013 pen trial of pelleted versions of a PZP vaccine. In that trial, up to 18 mares and one stallion at a time were held per pen, and with stallions rotated to ensure that mares were exposed to fertile stallions. Despite the density of animals in that study being about twice as high as proposed for the OGF vaccine study, no injuries to mares, foals, or stallions were mentioned in progress reports or the final report from that work (Turner, unpublished reports to BLM).

Mares in the Proposed Action that are not treated with the oocyte growth factor vaccine would remain fertile, and would likely become pregnant and produce foals. With 16 untreated mares in the Proposed Action, it is possible that as many as 16 foals could be born per year to these mares, over the course of the 3-year study. Some number of foals may also be born to mares treated with the vaccine.

Foals born to wild mares in the NNCC would have wild horse legal status, and would stay with their mother through the age of weaning (typically 6 months or older). BLM would seek to place these foals into private care through adoption. Researchers may examine or draw blood from foals to determine their reproductive development. It is not expected that either a vaccine-treated or an untreated mare would reject a foal; mares would be living in bands with the same set of other females throughout the study. If a mare dies or must be humanely euthanized during the study, every effort would be made to provide appropriate care to orphaned foals. Veterinarians may administer electrolyte solutions or orphan foals may be fed milk replacer as needed to support their nutritional needs. Orphan foals may be placed in a foster home in order to receive additional care.

In the event of injury or health concerns, any decisions to humanely euthanize animals would be made in conformance with BLM policy. BLM Euthanasia Policy IM-2015-070 is used as a guide to determine if animals meet the criteria and should be euthanized.

Fertility Control Vaccine Direct Effects

All fertility control methods in wild animals are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). In any vaccine, the antigen molecules are a stimulant to which the body responds with a primary immune response, including the generation of antigen-specific antibodies. Those antibodies can later lead to an immune response that reduces the relative bioavailability of the antigen molecules in the body. Adjuvants are additional substances that are included in vaccines to

elevate the level of immune response. Adjuvants help to strengthen immune responses, and can foster a long-lasting immune response that is specific to the antigen.

Each treated mare would receive a single dose of the oocyte growth factor vaccine. The intended purpose of this vaccine is to cause long-term infertility. Vaccination in this study would be given as a one-time intramuscular injection, by hand. Because the vaccine is designed to impair female fertility, the oocyte growth factor vaccine will not be handled by human females during this study. The vaccine formulation would include GDF9 and BMP 15 proteins conjugated to KLH molecules, encapsulated within liposomes. The vaccine also would include the Adjuvac adjuvant (Miller et al. 2004). Treated mares could experience immune responses to any of those vaccine components. There may be transiently elevated body temperature and injection site soreness.

Most mares recover quickly from the stress of handling, and none is expected to suffer serious long term effects from the injections, other than the direct consequence of temporary or permanent infertility, which is the desired outcome of the vaccination. The oocyte growth factor vaccine should stimulate a persistent immune response resulting in prolonged antibody production against the oocyte growth factors BMP15 and GDF9, the KLH carrier protein, and the adjuvant (Appendix A). Elevated levels of antibodies that are specific against BMP15 and GDF9 should follow vaccination with oocyte growth factor vaccine, which in turn attenuates and disrupts oocyte and / or follicular development (Appendix A). Changes associated with ovarian growth factor vaccination should lead to measurable changes in ovarian structure and function, with the result that ovulation ceases. Specifically, a combined vaccination against both GDF9 and BMP15 appears to cause premature, but incomplete development of follicles in such a way that they do not develop into fully competent ovulatory follicles (Appendix A). Another specific result of vaccination is a lack of observed estrous cycling (Appendix A). There may also be associated changes in hormone concentrations and related physiological effects, consistent with a lack of ovulation and estrus. Preliminary data from a combined oocyte growth factor vaccine against BLM15 and GDF9 indicated that there was a prolonged lack of estrus in mares, even after the level of these antibodies decreased (Appendix A). The effects of the one-dose OGF vaccine, whether temporary or long-lasting, are expected to be comparable to what is observable in mares' seasonal anestrous period, in that during shorter days of fall, winter and early spring, the ovaries are minimally active. Often during that time period small follicles develop but none ovulate. Hormonal changes (gonadal estrogen and progesterone) resulting from OGF vaccination are expected to be consistent with changes that occur naturally both annually, during the shorter days of the non-breeding season, and those that take place in older mares that have reached reproductive senescence (and which, therefore, are no longer cycling). In principle, it is possible that the effects of vaccination may lead to a lifelong dysregulation of oocytes or their growth factors from the ovaries; this would be a form of vaccine-induced sterility.

Treated mares may become only temporarily infertile, or may remain infertile for many years. Of the 10 mares treated with the multi-injection oocyte growth factor vaccine against GDF9 and BMP15 (Appendix A), all 10 were infertile in the first year after vaccination, and 9 of 10 were infertile in the second year (NWRC, unpublished data). Over the course of the proposed 3-year study, it may not be possible to determine whether the one-dose version of the oocyte growth factor vaccine would cause permanent sterility, because mares can live well over 20 years.

If the oocyte growth factor vaccine causes permanent infertility in some or all of treated mares, then that would be consistent with the desired contraceptive effect, and would be similar in outcome to mares treated multiple times with existing PZP vaccines to suppress reproduction, where some horses can become sterile from repeated multiple PZP doses.

Injection site reactions associated with immunocontraceptive treatments are possible in treated mares, as has been seen in mares treated with PZP vaccines (Roelle and Ransom 2009, Joonè et al. 2017) or with

GnRH vaccines (Goodloe 1991, Miller et al. 2008. Roelle and Ransom 2009). The one-dose oocyte growth factor vaccine that would be used in the Proposed Action would include the Adjuvac adjuvant, which is also a component of GonaCon vaccine. As associated with its use in GonaCon, the Adjuvac adjuvant is known to have the potential to cause injection site reactions whether injection is by hand or via darting. Reactions may include some minor degree of inflammation, swelling, and the potential for subcutaneous sterile abscesses, or purulent abscesses, at the injection site (Powers et al. 2011, Baker et al. 2018). Miller et al. (2008) noted that the water and oil emulsion in GonaCon will often cause cysts, granulomas, or sterile subcutaneous abscesses at injection sites; in some cases, a sterile abscess may develop into a draining abscess. In elk treated with GonaCon, Powers et al. (2011) noted up to 35% of treated elk had an abscess, despite the injection sites first being clipped and swabbed with alcohol. Even in studies where swelling and visible abscesses followed GonaCon immunization, the longer term nodules observed did not appear to change any animal's range of movement or locomotor patterns (Powers et al. 2013, Baker et al. 2017, Baker et al. 2018).

It is not expected that the oocyte growth factor vaccine would cause miscarriage in pregnant mares, because the oocyte growth factors that are included in the vaccine are not thought to play a role in fetal development, other than perhaps in ovarian development. A comparison of any observed miscarriage rates in treated and untreated mares could be used to quantify such an effect, if it occurs. Observations following gather operations indicate that the rate of miscarriage varies, but can occur in about 1 to 5% of the captured mares, particularly if the mares are in very thin body condition or in poor health. However, as any mares included in this study would not have been immediately gathered, and their body condition would be at least 4, any miscarriage rate would be expected to be lower. There is some possibility that the fetuses of treated mares would have compromised ovarian function, but this is unlikely because maternal antibodies do not typically cross the placental barrier. Nursing foals may be exposed to oocyte growth factor antibodies in colostrum but antibodies in colostrum do not typically lead to lifelong innate immune responses in nursing offspring.

Infanticide is a rare but natural behavior that has been observed in wild equids (Feh and Munktuya 2008, Gray 2009), but there are no published accounts of infanticide rates increasing as a direct result of fertility control vaccine application in wild horse or burro herds. "Foal stealing," where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including fertility control vaccine treated mares in a wild horse herd. Any suggestion that there would be a connection between fertility control treatment and "foal stealing" would be speculative, as there has not been any published record of that in a peer-reviewed study. McDonnell (2012) noted that "foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling," such as in horse feed lots with extremely high density. Those conditions are not present at NNCC, where pregnant mares will be in large pens with a small number of other animals present, and where the expectation is that any parturition dates would be distributed across the normal foaling season. Killian et al. (2008) conducted a pen trial of several fertility control methods at the NNCC and did not report any signs of infanticide or "foal stealing."

Fertility Control Vaccine Indirect Effects

One expected long-term, indirect effect on wild horses treated with a successful fertility control vaccine would be an improvement in the mares' overall health. This result has been observed in mares treated with PZP vaccine (Turner and Kirkpatrick 2002). Many treated mares would not experience the biological stress of pregnancy, foaling and lactation. An observable measure of improved health is high body condition scores (i.e., Nuñez et al. 2010). If a treated mare returns to fertility, her future foals would be expected to be healthier overall, and would be expected to benefit from improved nutritional quality in the mare's milk. Past application of fertility control vaccines has shown that mares' overall health and body condition remains improved even after fertility resumes. Other types of fertility control vaccine treatment may increase mare survival rates, leading to longer potential lifespan (Turner and Kirkpatrick 2002,

Ransom et al. 2014a). Anecdotal BLM observations of mares treated in past gathers showed that many of the treated mares were larger than, maintained higher body condition than, and had larger healthy foals than untreated mares.

Vaccination with ovarian growth factor vaccine may lead to behavioral effects that are consistent with a lack of estrus. Davis et al. (2018) observed reduced estrus behavior in mares vaccinated with either a GDF9 vaccine or a BMP15 vaccine. However, lack of estrus behaviors does not imply that treated mares will not maintain social relations with other horses. A near lack of estrus behaviors has also been observed in mares vaccinated with a different fertility control vaccine, GonaCon; such a lack of estrus behaviors is very similar to what can be observed in pregnant mares (Ransom et al. 2014b, Baker et al. 2018). In those studies, treated free-roaming mares continued to maintain social relationships with other horses in established social bands. Similarly, free-roaming mares that lacked ovaries appeared to maintain social activity insofar as those mares were always observed with bands of other horses (Collins and Kasbohm 2017).

Mares treated with the oocyte growth factor vaccine may have long-lasting granulomas at the vaccine injection site. This outcome has been observed in mares treated with GonaCon, which is another vaccine with the Adjuvac adjuvant. It is conceivable that mares with granulomas or small visible scars may be more difficult to place into private care in the future. However, the magnitude of any such effect would likely be small, considering that wild horses often have a number of other scars and marks resulting from earlier life events.

The proposed study would not lead to the loss of ovaries in any treated animals. Nonetheless, based on the analogy that removal of ovaries would lead to complete loss of ovarian function, it is possible to make some inferences from literature related to ovariectomy. However, considering that the ovaries would remain in OGF vaccine treated horses, the analogous physiological effects in OGF treated mares would likely be less pronounced than in ovariectomized mares. Even under removal of ovaries – which would not happen in the proposed study – substantial bone density loss would not be expected in wild horses living in conditions such as in the proposed study, or in off-range pastures. The BLM knows of no scientific, peer-reviewed literature that documents bone density loss in mares following ovary loss. A concern has been raised in an opinion article (Nock 2013) that ovary removal in mares could lead to bone density loss. That paper was not peer reviewed nor was it based on research in wild or domestic horses, so it does not meet the BLM's standard for "best available science" on which to base decisions (Kitchell et al. 2015). Hypotheses that are forwarded in Nock (2013) appear to be based on inferences from modern humans leading sedentary lives. Post-menopausal women have a greater chance of osteoporosis (Scholz-Ahrens et al. 1996), though some estrogen is also produced in tissues outside the ovaries. Bone loss in humans has been linked to reduced circulating estrogen. There have been conflicting results when researchers have attempted to test for an effect of reduced estrogen on animal bone loss rates in animal models; all experiments have been on laboratory animals rather than free-ranging wild animals. While some studies found changes in bone cell activity after ovariectomy leading to decreased bone strength (Jerome et al. 1997, Baldock et al. 1998, Huang et al. 2002, Sigrist et al. 2007), others found that changes were moderate and transient or minimal (Scholz-Ahrens et al. 1996, Lundon et al. 1994, Zhang et al. 2007) and even returned to normal after 4 months (Sigrist et al. 2007). Consistent and strenuous use of bones, for instance using jaw bones by eating hard feed, or using leg bones by travelling large distances, appears to limit the negative effects of estrogen deficiency on micro-architecture (Mavropoulos et al. 2014). The effect of exercise on bone strength in animals has been known for many years and has been shown experimentally (Rubin et al. 2001). Dr. Simon Turner, Professor Emeritus of the Small Ruminant Comparative Orthopaedic Laboratory at CSU, conducted extensive bone density studies on ovariectomized sheep, as a model for human osteoporosis. During these studies, he did observe bone density loss on ovariectomized sheep, but those sheep were confined in captive conditions, fed twice a day, had shelter from inclement weather, and had very little distance to travel to get food and water

(Simon Turner, CSU Emeritus, written comm., 2015). Dr. Turner indicated that an estrogen deficiency (i.e., such as might happen if a mare had no ovaries) could potentially affect a horse's bone metabolism, just as it does in sheep and human females when they lead a sedentary lifestyle, but indicated that the constant weight bearing exercise, coupled with high exposure to sunlight ensuring high vitamin D levels, are expected to prevent bone density loss (Simon Turner, CSU Emeritus, written comm., 2015). A horse would have to stay on stall rest for years after removal of the ovaries in order to develop osteoporosis (Simon Turner, CSU Emeritus, written comm. 2015; a stall is a small enclosed space in which an animal cannot move more than a few feet in any given direction). Wild horses that would be involved with the proposed study would neither lose their ovaries, nor be on stall rest.

Two studies raise the concern that treatment with immunocontraceptives could possibly lead to an evolutionary increase in the frequency of individuals whose genetic composition fosters weak immune responses (Cooper and Larson 2006, Ransom et al. 2014a). Many factors influence the strength of a vaccinated individual's immune response, potentially including genetics, but also nutrition, body condition, and prior immune responses to pathogens or other antigens (Powers et al. 2013). The concern is based on an assumption that lack of response to any given fertility control vaccine is a highly heritable trait, and that the frequency of that trait will increase over time in a population with a high fraction of vaccine-treated animals. Cooper and Herbert (2001) reviewed the topic, in the context of concerns about the long-term effectiveness of immunocontraceptives as a control agent for exotic species in Australia. They posit that imunocontraception could be a strong selective pressure, and that selecting for reproduction in individuals with poor immune response could lead to a general decline in immune function in populations where such evolution takes place. Other authors have also speculated that differences in antibody titer responses could be partially due to genetic differences between animals (Curtis et al. 2001, Herbert and Trigg 2005). However, Magiafolou et al. (2013) clarify that if the variation in immune response is due to environmental factors (i.e., body condition, social rank) and not due to genetic factors, then there will be no expected effect of the immune phenotype on future generations. It is possible that general health, as measured by body condition, can have a causal role in determining immune response, with animals in poor condition demonstrating poor immune reactions (NAS 2013). Correlations between physical factors and immune response would not preclude, though, that there could also be some degree of a heritable response to immunocontraception. In studies not directly related to immunocontraception, immune response has been shown to be heritable (Kean et al. 1994, Sarker et al. 1999). Unfortunately, predictions about the long-term, population-level evolutionary response to immunocontraceptive treatments are speculative at this time, with results likely to depend on several factors, including: the strength of the genetic predisposition to not respond to the fertility control vaccine; the heritability of the responsible gene or genes; the initial prevalence of that gene or genes; the number of mares treated with one or more doses of the vaccine; and the actual size of the genetically-interacting metapopulation of horses within which the vaccine treatment takes place. BLM is not aware of any studies that have quantified the heritability of a lack of response to immunocontraception (such as PZP vaccine or GonaCon-Equine) in horses or burros. At this time, there are no studies available from which one could make conclusions about the long-term effects of sustained and widespread immunocontraception treatments on population-wide immune function. Although a few, generally isolated, feral horse populations have been treated with high fractions of mares receiving PZP immunocontraception over long time periods (e.g., Assateague Island National Park, Cape Lookout National Seashore, Pryor Mountains Herd Management Area), no studies have tested for changes in immune competence in those areas. Relative to the large number of free-roaming feral horses in the western United States, immunocontraception has not been, and is not expected to be used in the type of widespread or prolonged manner that might be required to cause a detectable evolutionary response.

In summary, there are few uncertainties about the outcomes of the Proposed Action. The study is designed to determine whether or not individual vaccine-treated mares do, or do not, become infertile and for how long, over the course of the three-year study. There is good reason to quantify those effects and

test the duration of efficacy of the vaccine over three years, but the set of possible outcomes is clearly identified. The initial study conducted with a different formulation of the OGF vaccine required four doses to be effective, but the outcome was infertility, disruption of ovarian follicle development, and a cessation of estrus cycling (Appendix A). Those were the intended consequences, and those are the expected and desired consequences of the one-dose OGF vaccine formulation. The action is not controversial, in the sense that there is no scientifically-based reason to expect general outcomes of vaccination other than either vaccine success or failure (as defined above) in individual treated mares.

Impacts after Study Conclusion
There are potential impacts on wild horses after they are no longer included in the study, or after the study has concluded. If treatment with oocyte growth factor vaccine leads to long-term infertility, it may be of interest to determine whether there are effects on female foals born to treated mares. It is conceivable that a future study could monitor ovarian function in female offspring born to vaccine-treated mares, beyond the 3-year duration of the Proposed Action.

At the end of the study, animals could be placed in private care through adoption or sale with limitations, or could go to long-term care in an off-range pasture (ORP), as already analyzed in the gather decisions that led to their removal from the public range.

Impacts of Alternative 2 (No Action)
Under the No Action Alternative, no elements of the proposed study would affect wild horses at the NNCC. However, wild horses at NNCC would continue to be maintained, housed, fed, and trained at the NNCC, consistent with BLM policy and with the existing BLM-NNCC contract.

4.3 Cumulative Effects

The NEPA regulations define cumulative impacts as impacts on the environment that result from the incremental impact of the Proposed Action when added to other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions (40 C.F.R. § 1508.7). Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time. The cumulative impacts study area (CSA) for the purposes of evaluating cumulative impacts is limited to the NNCC, and specifically the wild horses that would be included in the Proposed Action.

According to the 1994 BLM *Guidelines for Assessing and Documenting Cumulative Impacts*, the cumulative analysis should be focused on those issues and resource values identified during scoping that are of major importance. Accordingly, the issues of major importance to be analyzed are limited to Wild Horses.

4.3.1 Past and Present Actions

4.3.1.1 Wild Horses

Management actions that have influenced the wild horses at the NNCC are primarily wild horse gathers, which led to the removal of those horses from the range and their designation as excess animals, as a result of previous BLM gather decisions. The Proposed Action Alternative would cause an incremental change in potentially preventing future reproduction for mares treated with the oocyte growth factor vaccine. However, under current management, mares that have been removed from the range and are placed in BLM long-term care facilities do not breed with stallions. Unless they go into private care, wild mares live exclusively with other mares or with gelded (infertile) males. In the context of over 45,000

wild horses living in some form of off-range holding, the loss of reproductive capacity for up to approximately 16 mares treated with the one-dose OGF vaccine would not represent a substantial change.

4.4 Reasonably Foreseeable Future Actions

4.4.1 Wild Horses

It is not expected that genetic health of any wild horse herd in the wild would be impacted by the Proposed Action Alternative, because the animals that would be included in the action would have already been removed from the public lands as excess animals, and would not be expected to be returned to the wild. Any future wild horse management action that could include returning mares from the proposed study back to the wild would be subject to NEPA compliance, following site-specific planning.

Various forms of fertility control can be used in future wild horse and burro herd management. These can help with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 § 3(b)(1)). An extensive body of peer-reviewed scientific literature details the expected impacts of various fertility control methods on wild horses and burros.

The effects of the one-dose oocyte growth factor vaccine may lead to short-term or long-term mare infertility, or to sterility. Regardless, any future BLM herd management actions using the one-dose OGF vaccine in on-range management would also require additional NEPA compliance. The results of the proposed study could help to inform BLM in future decisions about the potential use of the one-dose OGF vaccine. However, a few topics are worth mentioning in the context of reasonably foreseeable future actions.

Additional studies of the short-term and longer-term effects of the single-dose OGF vaccine could be warranted in the future. It is conceivable that the animals in the study could be observed further into the future, either as part of an additional study, or through non-experimental monitoring activities. If that occurs, BLM would ensure that any long-term (i.e., 10-year) study of the same animals is in compliance with NEPA. Although it is possible that the OGF vaccine could be used in a future field trial with wild, free-roaming horses on BLM-managed lands in the future, or as part of management activities, any such future action would be analyzed appropriately with site-specific planning with public involvement, and would not be part of the cumulative effects to the wild horses in the proposed action.

Fertility control that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single herd management areas (HMAs), in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The NAS (2013) encouraged BLM to manage wild horses and burros at the spatial scale of "metapopulations" – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to influence local herd phenotypes and genotypes, and maintain high genetic diversity. Some HMAs could also be managed as non-reproducing, in whole or in part, depending on the specific circumstances and on a case-by-case basis. Thus, although fertility control vaccine-treated individuals may experience long-lasting effects, such as sterility, that does not of itself cause negative demographic or genetic impacts at the level of wild horse populations, which are the object of BLM management for decisions that address wild horse herd management.

Site-specific NEPA analyses for herds that are 'non-reproducing' in whole or in part should be considered in the context of this 'metapopulation' structure, where self-sustaining herds are not necessarily at the

scale of single HMAs. So long as the definition of what constitutes a self-sustaining population includes the larger set of HMAs that have past or ongoing demographic and genetic connections – as is recommended by the NAS 2013 report – it is clear that single HMAs could potentially be managed as nonreproducing in whole or in part while still allowing for a self-sustaining population of wild horses or burros at the broader spatial scale. Wild horses are not an endangered species (USFWS 2015), nor are they rare. Nearly 72,000 adult wild horses and over 16,000 adult wild burros roamed public lands as of March 1, 2019. Those numbers do not include at least 7,500 WH&B on US Forest Service lands, and at least 50,000 feral horses on tribal lands in the Western United States (Wallace et al. 2017, Beever et al. 2018).

Because any future herd management actions that include long-lasting mare contraception, including sterilization, via oocyte growth factor vaccine injection would likely require capturing, handling, and some form of marking, the risks and costs associated with animal capture and handling may be comparable to those of gathering for removal, but would be anticipated to lead to lower subsequent gather, adoption and long-term holding costs and a reduction in excess wild horses that have to be removed from the range. As noted previously, treating mares with a fertility control vaccine may lead to those mares having a longer lifespan. If this were to happen in a herd of horses living on the range, then, changes in lifespan and decreased foaling rates could combine to cause changes in overall age structure in a treated herd (i.e., Turner and Kirkpatrick 2002, Roelle et al. 2010), with a greater prevalence of older mares in the herd (Gross 2000).

If the oocyte growth factor vaccine leads to successful outcomes, in terms of causing long-lasting contraceptive effects, then it is reasonable to expect that BLM might develop guidelines for oocyte growth factor vaccine use in the future, similar to those for other immunocontraceptive vaccines. Existing, non-binding guidelines for fertility vaccine use in BLM wild horse herd management (BLM IM 2009-090) suggest that herds selected for fertility control vaccine use should have background annual growth rates over 5%, have a herd size over 50 animals, and have a target rate of treatment of between 50% and 90% of female wild horses or burros. Guidelines suggest that treated mares should be identifiable via a visible freeze brand or individual color markings, so that their vaccination history is known, and that follow-up population surveys should be used to determine the realized annual growth rate in herds treated with fertility control vaccines.

5.0 Monitoring and Mitigation Measures

The BLM COR assigned to the NNCC contract would be responsible for ensuring contract personnel abide by the contract specifications and the CAWP (Appendix C). Animal care will also be in keeping with protocols approved by the NWRC IACUC (Appendix B). Out of an abundance of caution, the oocyte growth factor vaccine will not be handled by female during this study. Ongoing monitoring of animal health would continue. BLM would continue to contract with an attending veterinarian, to be available for animal care, as needed. NWRC researchers would monitor the immune response and pregnancy status of treated and untreated mares.

6.0 List of Preparers

The following list identifies the interdisciplinary team member's area of responsibility.

Name	Title	Area of Responsibility
Ruth Thompson	Wild Horse Specialist	Project Lead/Wild Horses

Paul Griffin	Research Coordinator	Study Review
David Pritchett	Planning/NEPA/CADR Program Lead	Study Review

7.0 Consultation and Coordination

The NWRC proposal was reviewed by the BLM WHB program research advisory team. The team includes four BLM employees, and one USDA veterinarian not affiliated with NWRC, and has input from the WHB Advisory Board member whose purview is research.

8.0 Public Involvement

A preliminary environmental assessment was made available to interested individuals, agencies, and groups for a 15-day public review and comment period that opened on December 5, 2019 and closed on December 20, 2019 . Forty four comments were received from individuals, organizations, and county or state government agencies. Many of these comments contained overlapping issues/ concerns which were consolidated into 61 distinct topics. Below is a detailed summary of the comments received, BLM's responses, and how BLM used these comments in preparing the final environmental assessment. Comments and Responses can be found in Appendix E.

9.0 Appendices

Appendix A – USDA Research Proposal
Appendix B – USDA Animal Care and Use Protocol
Appendix C – Comprehensive Animal Welfare Program
Appendix D – Literature Cited
Appendix E – Comments and Responses

COMPREHENSIVE ANIMAL WELFARE PROGRAM FOR WILD HORSE AND BURRO GATHERS

IM 2015-151
Instruction Memorandum

UNITED STATES DEPARTMENT OF THE INTERIOR

BUREAU OF LAND MANAGEMENT

WASHINGTON, D.C. 20240-0036

http://www.blm.gov (http://www.blm.gov)

September 25, 2015

In Reply Refer To:

4720 (260) P

EMS TRANSMISSION 09/29/2015

Instruction Memorandum No. 2015-151

Expires: 09/30/2018

To: All Field Office Officials (except Alaska)

From: Assistant Director, Resources and Planning

Subject: Comprehensive Animal Welfare Program for Wild Horse and Burro Gathers

Program Area: Wild Horse and Burro (WH&B) Program

Purpose: The purpose of this Instruction Memorandum (IM) is to establish policy for the Wild Horse and Burro (WH&B) Gather component of the Comprehensive Animal Welfare Program (CAWP). It defines standards, training and monitoring for conducting safe, efficient and successful WH&B gather operations

while ensuring humane care and handling of animals gathered.

Policy/Action: The Bureau of Land Management (BLM) is committed to the well-being and responsible care of WH&B we manage. At all times, the care and treatment provided by the BLM and its contractors will be characterized by *compassion and concern* for WH&B well-being and welfare needs.

All State, District and Field Offices are required to comply with the CAWP policy for all gathers within their jurisdiction. The CAWP for WH&B gathers includes three components:

1. Comprehensive Animal Welfare Program Standards for Wild Horse and Burro Gathers (Attachment 1): These standards include requirements for trap and temporary holding facility design; capture and handling; transportation; and appropriate care after capture. The standards have been incorporated into helicopter gather contracts as specifications for performance.
2. Training: All Incident Commanders (IC), Contracting Officer Representatives (COR), Project Inspectors (PI) and contractors must complete a mandatory training course. The training is available online via DOI Learn: Course Title: BLM's Comprehensive Animal Welfare Program (CAWP) – gathers; Course Number: 4700-13.
3. CAWP Gather Assessment Tool (Attachment 2): The Gather Assessment Tool will be used during FY2016 for evaluating the effectiveness of mandatory training and adequacy of the Standards for CAWP for WH&B Gathers. The WO-260 Division is responsible for overseeing implementation of assessments as well as providing the necessary access to the assessment tool for those gathers selected for internal assessment during FY2016.
4. Starting in FY2017, the Assessment Tool will be used to evaluate compliance by the BLM and its contractors with the Standards for CAWP for WH&B Gathers. The WO-260 Division will oversee the completion of all assessments as well as providing the necessary access to the assessment tool for those gathers identified for both internal and external assessment by internal and external personnel during FY2017.

This IM supersedes Interim IM No. 2013-059, Wild Horse and Burro Gathers: Comprehensive Animal Welfare Policy which was issued as part of a package of IMs covering various aspects of the management of WH&B gathers, including:

- IM No. 2013-058, Wild Horse and Burro Gathers: Public and Media Management.
- IM No. 2013-060, Wild Horse and Burro Gathers: Management by Incident Command System
- IM No. 2013-061, Wild Horse and Burro Gathers: Internal and External Communicating and Reporting

The goal of this IM is to ensure that the responsibility for humane care and treatment of WH&Bs remains a high priority for the BLM and its contractors at all times. The Bureau's objective is to use the best available science, husbandry and handling practices applicable for WH&Bs and to make improvements whenever possible, while also meeting our overall gather goals and objectives in accordance with current BLM policy, standard operating procedures and contract requirements. The CAWP and its associated components will be reviewed regularly and modified as necessary to enhance its transparency and effectiveness in assuring the humane care and treatment of the WH&Bs.

The Lead COR is the primary party responsible for promptly addressing any actions that are inconsistent with the Standards set forth in the CAWP. The Lead COR may delegate responsibility to an alternate COR. The Lead COR will promptly notify the contractor if any improper or unsafe actions are observed and will ensure that they are promptly rectified. If issues are left unresolved or immediate action is required, the Lead COR has the authority to suspend gather operations. Through coordination with the Contracting Officer, the Lead COR shall, if necessary, ensure that corrective measures have been taken to prevent such actions from reoccurring and all follow-up and corrective measures shall be reported as a component of the Lead COR's daily reports.

Timeframe: All portions of this policy are effective as of October 1st, 2015.

Budget Impact: This IM is implementing new policy and guidance with additional training and reporting requirements for personnel and contractors. The cost for the required training is about $250 per person. CAWP program implementation, oversight, data compilation and reporting requirements will require an additional 12 to 15 work months per year.

Background: The authority for a Comprehensive Animal Welfare Program for WH&B Gathers is provided by Public Law 92-195, Wild Free-Roaming Horses and Burros Act of 1971 (as amended) and 43 CFR 4700.0-2.

The Comprehensive Animal Welfare Program for WH&B gathers consolidates and highlights the BLM's policies, procedures and ongoing commitment to protect animal welfare; provide training for employees and contractors on animal care and handling; and implement a gather assessment tool which will be used to evaluate the agency's and contractor's adherence to standards for the handling and care of animals during gather operations.

Manual/Handbook Sections Affected: None

Coordination: This IM was coordinated among WO-100, WO-200, WO-260, WO-600, WH&B State Leads and WH&B Specialists.

Contact: Bryan Fuell, On-Range Branch Chief, Wild Horse and Burro Program, at 775-861-6611.

Signed by:

Michael H. Tupper

Acting, Assistant Director

Resources and Planning

Authenticated by:

Robert M. Williams

Division of IRM Governance,WO-860

2 Attachments

1 - Comprehensive Animal Welfare Program Standards for Wild Horse and Burro Gathers (20 pp) (/sites/blm.gov/files/uploads/IM2015-151_att1.pdf)

2 - CAWP Gather Assessment Tool screen shots (26 pp) (/sites/blm.gov/files/uploads/IM2015-151_att2.pdf)

VIEW ALL POLICIES

Return to the Policies (/media/blm-policy/Instruction%20Memorandum)

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WILD HORSE AND BURRO COMPREHENSIVE ANIMAL WELFARE PROGRAM

PIM 2021-002
Permanent Instruction Memorandum

December 18, 2020

In Reply Refer To:
4700 (260) P

EMS TRANSMISSION 12/18/2020
Permanent Instruction Memorandum No. 2020- 002

To: All Headquarters Officials and Field Office Officials

Attn: State and Center Directors

From: Assistant Director, Resources and Planning

Subject: Wild Horse and Burro Comprehensive Animal Welfare Program

Program Area: Wild Horse and Burro Program

Purpose: The purpose of this Permanent Instruction Memorandum (PIM) is to re-affirm the Bureau of Land Management's (BLM's) longstanding commitment to ensure the humane treatment of wild horses and burros (WHB) in all on-range and off-range management activities through the Comprehensive Animal Welfare Program (CAWP). The humane treatment of wild horses and burros outlined in this policy reiterates the BLM's longstanding practices but also provides additional clarification to BLM employees. This PIM consolidates current humane practices, incorporates the existing Standard Operating Procedures (SOPs) outlined in Attachments 1 and 2 to ensure humane care and handling of the animals, and increases transparency concerning the humane treatment of wild horses and burros.

Policy/Action: The BLM is committed to continue protecting animal welfare and providing humane care and treatment to WHBs both on and off the range. Consistent with long-standing policies and practices, the handling and treatment provided by the BLM, its contractors, and its partners is based on compassion and concern for the animals' well-being at all times. Animals are managed and cared for to optimize animal condition, health, and safety.

All state, district, and field offices will continue to comply with the WHB CAWP policy within their jurisdiction at all times. The WHB CAWP policy includes:

1. CAWP Standards for Wild Horse and Burro Gathers (Attachment 1): These standards include requirements for trap and temporary holding facility design, capture and handling, transportation, and care after capture. The standards are also incorporated into helicopter gather contracts as specifications for performance.
2. Training for gather operations: All BLM staff associated with gather operations (incident commanders, contracting officer representatives, project inspectors, public affairs officials, and law enforcement officers) and contractor crews are required to complete online training annually for CAWP – Gathers (BLM-TC-4700-13), which is available through DOI Talent.
3. CAWP Standards for Off-Range Corral Facilities, Transportation, and Adoption/Sale Events (Attachment 2): This includes requirements for holding of animals in off-range corrals, transportation, and adoptions/sales events. These SOPs are incorporated into off-range corral contracts as specifications for performance.
4. Training for the Care, Handling, and Transportation of Wild Horses and Burros at off-range corrals and adoption/sales events: All personnel, including contractors and partners involved in the care, handling, and transportation of wild horses and burros at off-range

VIEW ALL POLICIES

ATTACHMENTS

COMPREHENSIVE ANIMAL WELFARE PROGRAM FOR WILD HORSE AND BURRO GATHERS (PDF / 409 KB)
CAWP STANDARDS OFF RANGE CORRAL TRANSPORT ADOPTION SALE EVENTS (PDF / 364 KB)

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corrals and adoption/sale events are required to complete online training annually. Development of this training will begin following issuance of this PIM.

5. CAWP Standards and SOPs (to be developed) for animals on the range, in off-range pastures, and public off-range pastures: All personnel, contractors, and partners involved in management of WHBs on the range, in off-range pastures, or public off-range pastures are required to complete online training annually following the development of these guidelines and SOPs.

The purpose of this PIM is to continue to ensure that the humane care and treatment of WHBs remains the priority for the BLM, its contractors, and its partners. The BLM will continue to use the best available science, husbandry, and handling practices applicable for WHBs. Whenever possible, the BLM will make improvements to its practices while also meeting planned objectives in accordance with current BLM policy, SOPs, as well as contractual and cooperative agreement requirements. The CAWP will be reviewed periodically by WHB program leadership and modified as necessary to strengthen public trust, transparency, and effectiveness in assuring the humane care and treatment of the WHBs.

The authorized officer is the primary party responsible for immediately addressing any actions that are inconsistent with the CAWP. Consistent with applicable BLM delegation of authority manuals, the authorized officer may delegate responsibility to an alternate individual. The authorized officer should promptly notify the employees, contractors, and partners of actions that are not compliant with the CAWP and correct all issues immediately. It is the responsibility of all individuals engaged in actions throughout the WHB program to implement all aspects of the CAWP.

The WHB CAWP Coordinator will lead the implementation, development, and modification of all aspects of the CAWP, including those developed to date as well as future components of the CAWP.

Timeframe: This policy is effective immediately.

Budget Impact: This PIM codifies long-standing policy, practice, and guidance to include mandatory annual training and reporting requirements for BLM personnel, contractors, and partners associated with gather operations. The cost for the required training is approximately $100 per person per year. This PIM also formalizes SOPs for off-range holding, adoptions, sales, and transportation of excess WHB. When fully developed, the CAWP program implementation, oversight, data compilation, and reporting requirements will require an additional 12 to 18 work months per year.

Background: Public Law-195, Wild Free-Roaming Horses and Burros Act of 1971 (as amended) and 43 CFR 4700.0-2 provide the authority for a CAWP for WHB.

This CAWP policy provides a comprehensive approach to long-standing policies and practices within the BLM WHB program. Despite these policies and practices, the BLM has been criticized by some animal protection organizations on its handling and care of wild horses and burros, especially during the gathering activities and transitioning of the horses from public lands to off-range facilities. Some of the criticisms include concerns as to the transportation conditions of the horses, facility design, environmental conditions, handling practices, and nutrition. Similar to policies adopted by other organizations in agricultural and animal research industries, this PIM establishes a framework to ensure there are programs in place to verify humane care and handling of animals. It provides transparency on how the animals are assured humane care and proper handling. This framework seeks to confirm that the WHB program is conducted in a humane and caring manner.

The CAWP for WHB consolidates and highlights the BLM policies, procedures, and ongoing commitment to protect animal welfare; provides training for employees, contractors, and partners on animal care and handling, and implements assessment tools to evaluate adherence to the CAWP SOP by the agency, contractors, and partners.

Manual/Handbook Sections Affected: This PIM affects policies in BLM Manual/Handbooks H-4700-1 “Wild Horse and Burro Management” and H-4750-2 “Adoption of Wild Horses and Burros”.

Coordination: This PIM was coordinated among HQ-100, HQ-200, HQ-260, HQ-600, Deputy State Directors, WHB State Leads, APHIS Veterinarian, WHB Facility Managers, and WHB Specialists.

Contact: If you have any questions, please contact the Wild Horse and Burro Division Chief, at 866-468-7826 or wildhorse@blm.gov.

Signed by:	Authenticated By:
David Jenkins	Ambyr Fowler
Assistant Director	Division of Regulatory Affairs (HQ-630)

Resources and Planning

2 Attachments

1. - CAWP Gather Standards (20 pp)
2. - Comprehensive Animal Welfare Program Standards for Off-Range Corrals, Transportation and Adoption/Sale Events (24 pp)

ATTACHMENT 1: COMPREHENSIVE ANIMAL WELFARE PROGRAM FOR WILD HORSE AND BURRO GATHERS

STANDARDS

Developed by

The Bureau of Land Management
Wild Horse and Burro Program

in collaboration with

Carolyn L. Stull, PhD
Kathryn E. Holcomb, PhD
University of California, Davis
School of Veterinary Medicine

June 30, 2015

WELFARE ASSESSMENT STANDARDS for GATHERS

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STANDARDS

Standard Definitions

Major Standard: Impacts the health or welfare of WH&Bs. Relates to an alterable equipment or facility standard or procedure. Appropriate wording is "must," "unacceptable," "prohibited."

Minor Standard: unlikely to affect WH&Bs health or welfare or involves an uncontrollable situation. Appropriate wording is "should."

Lead COR = Lead Contracting Officer's Representative

COR = Contracting Officer's Representative

PI = Project Inspector

WH&Bs = Wild horses and burros

I. FACILITY DESIGN

A. Trap Site and Temporary Holding Facility

1. The trap site and temporary holding facility must be constructed of stout materials and must be maintained in proper working condition, including gates that swing freely and latch or tie easily. (**major**)
2. The trap site should be moved close to WH&B locations whenever possible to minimize the distance the animals need to travel.(minor)
3. If jute is hung on the fence posts of an existing wire fence in the trap wing, the wire should be either be rolled up or let down for the entire length of the jute in such a way that minimizes the possibility of entanglement by WH&Bs unless otherwise approved by the Lead COR/COR/PI. (minor)
4. Fence panels in pens and alleys must be not less than 6 feet high for horses, 5 feet high for burros, and the bottom rail must not be more than 12 inches from ground level. (**major**)

5. The temporary holding facility must have a sufficient number of pens available to sort WH&Bs according to gender, age, number, temperament, or physical condition. (**major**)
    a. All pens must be assembled with capability for expansion. (**major**)
    b. Alternate pens must be made available for the following: (**major**)
        1) WH&Bs that are weak or debilitated
        2) Mares/jennies with dependent foals
    c. WH&Bs in pens at the temporary holding facility should be maintained at a proper stocking density such that when at rest all WH&Bs occupy no more than half the pen area. (minor)
6. An appropriate chute designed for restraining WH&Bs must be available for necessary procedures at the temporary holding facility. This does not apply to bait trapping operations unless directed by the Lead COR/COR/PI. (**major**)
7. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may cause escape or possible injury. (**major**)
8. Padding must be installed on the overhead bars of all gates and chutes used in single file alleys. (**major**)
9. Hinged, self-latching gates must be used in all pens and alleys except for entry gates into the trap, which may be secured with tie ropes. (**major**)
10. Finger gates (one-way funnel gates) used in bait trapping must be constructed of materials approved by the Lead COR/COR/PI. Finger gates must not be constructed of materials that have sharp ends that may cause injuries to WH&Bs, such as "T" posts, sharpened willows, etc. (**major**)
11. Water must be provided at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals, and environmental conditions, with each trough placed in a separate location of the pen (i.e. troughs at opposite ends of the pen). Water must be refilled at least every morning and evening. (**major**)
12. The design of pens at the trap site and temporary holding facility should be constructed with rounded corners. (minor)

13. All gates and panels in the animal holding and handling pens and alleys of the trap site must be covered with materials such as plywood, snow fence, tarps, burlap, etc. approximately 48" in height to provide a visual barrier for the animals. All materials must be secured in place.(**major**)

    These guidelines apply:

    a. For exterior fences, material covering panels and gates must extend from the top of the panel or gate toward the ground.(**major**)
    b. For alleys and small internal handling pens, material covering panels and gates should extend from no more than 12 inches below the top of the panel or gate toward the ground to facilitate visibility of animals and the use of flags and paddles during sorting. (minor)
    c. The initial capture pen may be left uncovered as necessary to encourage animals to enter the first pen of the trap. (minor)

14. Non-essential personnel and equipment must be located to minimize disturbance of WH&Bs. (**major**)
15. Trash, debris, and reflective or noisy objects should be eliminated from the trap site and temporary holding facility. (minor)

B. Loading and Unloading Areas

1. Facilities in areas for loading and unloading WH&Bs at the trap site or temporary holding facility must be maintained in a safe and proper working condition, including gates that swing freely and latch or tie easily. (**major**)
2. The side panels of the loading chute must be a minimum of 6 feet high and fully covered with materials such as plywood or metal without holes that may cause injury. (**major**)
3. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may cause escape or possible injury. (**major**)
4. All gates and doors must open and close easily and latch securely. (**major**)

5. Loading and unloading ramps must have a non-slip surface and be maintained in a safe and proper working condition to prevent slips and falls. Examples of non-slip flooring would include, but not be limited to, rubber mats, sand, shavings, and steel reinforcement rods built into ramp. There must be no holes in the flooring or items that can cause an animal to trip. (**major**)
6. Trailers must be properly aligned with loading and unloading chutes and panels such that no gaps exist between the chute/panel and floor or sides of the trailer creating a situation where a WH&B could injure itself. (**major**)
7. Stock trailers should be positioned for loading or unloading such that there is no more than 12" clearance between the ground and floor of the trailer for burros and 18" for horses. (minor)

II. CAPTURE TECHNIQUE

A. Capture Techniques

1. WH&Bs gathered on a routine basis for removal or return to range must be captured by the following approved procedures under direction of the Lead COR/COR/PI. (**major**)
   a. Helicopter
   b. Bait trapping
2. WH&Bs must not be captured by snares or net gunning. (**major**)
3. Chemical immobilization must only be used for capture under exceptional circumstances and under the direct supervision of an on-site veterinarian experienced with the technique. (**major**)

B. Helicopter Drive Trapping

1. The helicopter must be operated using pressure and release methods to herd the animals in a desired direction and should not repeatedly evoke erratic behavior in the WH&Bs causing injury or exhaustion. Animals must not be pursued to a point of exhaustion; the on-site veterinarian must examine WH&Bs for signs of exhaustion. (**major**)

2. The rate of movement and distance the animals travel must not exceed limitations set by the Lead COR/COR/PI who will consider terrain, physical barriers, access limitations, weather, condition of the animals, urgency of the operation (animals facing drought, starvation, fire, etc.) and other factors. (**major**)
    a. WH&Bs that are weak or debilitated must be identified by BLM staff or the contractors. Appropriate gather and handling methods should be used according to the direction of the Lead COR/COR/PI. (**major**)
    b. The appropriate herding distance and rate of movement must be determined on a case-by-case basis considering the weakest or smallest animal in the group (e.g., foals, pregnant mares, or horses that are weakened by body condition, age, or poor health) and the range and environmental conditions present. (**major**)
    c. Rate of movement and distance travelled must not result in exhaustion at the trap site, with the exception of animals requiring capture that have an existing severely compromised condition prior to gather. Where compromised animals cannot be left on the range or where doing so would only serve to prolong their suffering, euthanasia will be performed in accordance with BLM policy. (**major**)
3. WH&Bs must not be pursued repeatedly by the helicopter such that the rate of movement and distance travelled exceeds the limitation set by the Lead COR/COR/PI. Abandoning the pursuit or alternative capture methods may be considered by the Lead COR/COR/PI in these cases. (**major**)
4. When WH&Bs are herded through a fence line en route to the trap, the Lead COR/COR/PI must be notified by the contractor. The Lead COR/COR/PI must determine the appropriate width of the opening that the fence is let down to allow for safe passage through the opening. The Lead COR/COR/PI must decide if existing fence lines require marking to increase visibility to WH&Bs. (**major**)
5. The helicopter must not come into physical contact with any WH&B. The physical contact of any WH&B by helicopter must be documented by Lead COR/COR/PI along with the circumstances. (**major**)
6. WH&Bs may escape or evade the gather site while being moved by the helicopter. If there are mare/dependent foal pairs in a group being brought to a trap and half of an identified pair is thought to have evaded capture, multiple attempts by helicopter may

be used to bring the missing half of the pair to the trap or to facilitate capture by roping. In these instances, animal condition and fatigue must be evaluated by the Lead COR/COR/PI or on-site veterinarian on a case-by-case basis to determine the number of attempts that can be made to capture an animal.(**major**)

7. Horse captures must not be conducted when ambient temperature at the trap site is below 10ºF or above 95ºF without approval of the Lead COR/COR/PI. Burro captures must not be conducted when ambient temperature is below 10ºF or above 100ºF without approval of the Lead COR/COR/PI. The Lead COR/COR/PI will not approve captures when the ambient temperature exceeds 105 ºF. (**major**)

C. Roping

1. The roping of any WH&B must be approved prior to the procedure by the Lead COR/COR/PI. (**major**).
2. The roping of any WH&B must be documented by the Lead COR/COR/PI along with the circumstances. WH&Bs may be roped under circumstances which include but are not limited to the following: reunite a mare or jenny and her dependent foal; capture nuisance, injured or sick WH&Bs or those that require euthanasia; environmental reasons such as deep snow or traps that cannot be set up due to location or environmentally sensitive designation; and public and animal safety or legal mandates for removal. (**major**)
3. Ropers should dally the rope to their saddle horn such that animals can be brought to a stop as slowly as possible and must not tie the rope hard and fast to the saddle so as to intentionally jerk animals off their feet. (**major**)
4. WH&Bs that are roped and tied down in recumbency must be continuously observed and monitored by an attendant at a maximum of 100 feet from the animal. (**major**)
5. WH&Bs that are roped and tied down in recumbency must be untied within 30 minutes. (**major**)
6. If the animal is tied down within the wings of the trap, helicopter drive trapping within the wings will cease until the tied-down animal is removed. (**major**)
7. Sleds, slide boards, or slip sheets must be placed underneath the animal's body to move and/or load recumbent WH&Bs. (**major**)

8. Halters and ropes tied to a WH&B may be used to roll, turn, position or load a recumbent animal, but a WH&B must not be dragged across the ground by a halter or rope attached to its body while in a recumbent position. (**major**)
9. Animals captured by roping must be evaluated by the on-site/on-call veterinarian within four hours after capture, marked for identification at the trap site, and be re-evaluated periodically as deemed necessary by the on-site/on-call veterinarian. (**major**)

D. Bait Trapping

1. WH&Bs may be lured into a temporary trap using bait (feed, mineral supplement, water) or sexual attractants (mares/jennies in heat) with the following requirements:
   a. The period of time water sources other than in the trap site are inaccessible must not adversely affect the wellbeing of WH&Bs, wildlife or livestock, as determined by the Lead COR/COR/PI. (**major**)
   b. Unattended traps must not be left unobserved for more than 12 hours. (**major**)
   c. Mares/jennies and their dependent foals must not be separated unless for safe transport. (**major**)
   d. WH&Bs held for more than 12 hours must be provided with accessible clean water at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals and environmental conditions. (**major**)
   e. WH&Bs held for more than 12 hours must be provided good quality hay at a minimum rate of 20 pounds per 1000 pound adult animal per day, adjusted accordingly for larger or smaller horses, burros and foals. (**major**)
      1) Hay must not contain poisonous weeds, debris, or toxic substances. (**major**)
      2) Hay placement must allow all WH&Bs to eat simultaneously. (**major**)

III. WILD HORSE AND BURRO CARE

A. Veterinarian

1. On-site veterinary support must be provided for all helicopter gathers and on-site or on-call support must be provided for bait trapping. (**major**)

2. Veterinary support must be under the direction of the Lead COR/COR/PI. The on-site/on-call veterinarian will provide consultation on matters related to WH&B health, handling, welfare, and euthanasia at the request of the Lead COR/COR/PI. All decisions regarding medical treatment or euthanasia will be made by the on-site Lead COR/COR/PI. (**major**)

B. Care

1. Feeding and Watering
    a. Adult WH&Bs held in traps or temporary holding pens for longer than 12 hours must be fed every morning and evening with water available at all times other than when animals are being sorted or worked. (**major)**
    b. Water must be provided at a minimum rate of ten gallons per 1000 pound animal per day, adjusted accordingly for larger or smaller horses, burros and foals, and environmental conditions, with each trough placed in a separate location of the pen (i.e. troughs at opposite ends of the pen). . (**major**)
    c. Good quality hay must be fed at a minimum rate of 20 pounds per 1000 pound adult animal per day, adjusted accordingly for larger or smaller horses, burros and foals. (**major**)
        i. Hay must not contain poisonous weeds or toxic substances. (**majo**r)
        ii. Hay placement must allow all WH&Bs to eat simultaneously. (**major**)
    d. When water or feed deprivation conditions exist on the range prior to the gather, the Lead COR/COR/PI should adjust the watering and feeding arrangements in consultation with the onsite veterinarian as necessary to provide for the needs of the animals. (minor)
2. Dust abatement
    a. Dust abatement by spraying the ground with water must be employed when necessary at the trap site and temporary holding facility. (**major**)

3. Trap Site
    a. Dependent foals or weak/debilitated animals must be separated from other WH&Bs at the trap site to avoid injuries during transportation to the temporary holding facility. Separation of dependent foals from mares must not exceed four hours unless the Lead COR/COR/PI authorizes a longer time or a decision is made to wean the foals. (**major**)
4. Temporary Holding Facility
    a. All WH&Bs in confinement must be observed at least once daily to identify sick or injured WH&Bs and ensure adequate food and water. (**major**)
    b. Foals must be reunited with their mares/jennies at the temporary holding facility within four hours of capture unless the Lead COR/COR/PI authorizes a longer time or foals are old enough to be weaned during the gather. (**major**)
    c. Non-ambulatory WH&Bs must be located in a pen separate from the general population and must be examined by the BLM horse specialist and/or on-call or on-site veterinarian as soon as possible, no more than four hours after recumbency is observed. Unless otherwise directed by a veterinarian, hay and water must be accessible to an animal within six hours after recumbency.(**major**)
    d. Alternate pens must be made available for the following: (**major**)
        1) WH&Bs that are weak or debilitated
        2) Mares/jennies with dependent foals
    e. Aggressive WH&Bs causing serious injury to other animals should be identified and relocated into alternate pens when possible. (minor)
    f. WH&Bs in pens at the temporary holding facility should be maintained at a proper stocking density such that when at rest all WH&Bs occupy no more than half the pen area. (minor)

C. Biosecurity

1. Health records for all saddle and pilot horses used on WH&B gathers must be provided to the Lead COR/COR/PI prior to joining a gather, including: (**major**)
   a. Certificate of Veterinary Inspection (Health Certificate, within 30 days).
   b. Proof of:
      1) A negative test for equine infectious anemia (Coggins or EIA ELISA test) within 12 months.
      2) Vaccination for tetanus, eastern and western equine encephalomyelitis, West Nile virus, equine herpes virus, influenza, *Streptococcus equi*, and rabies within 12 months.
2. Saddle horses, pilot horses and mares used for bait trapping lures must not be removed from the gather operation (such as for an equestrian event) and allowed to return unless they have been observed to be free from signs of infectious disease for a period of at least three weeks and a new Certificate of Veterinary Examination is obtained after three weeks and prior to returning to the gather. (**major**)
3. WH&Bs, saddle horses, and pilot horses showing signs of infectious disease must be examined by the on-site/on-call veterinarian. (**major**)
   a. Any saddle or pilot horses showing signs of infectious disease (fever, nasal discharge, or illness) must be removed from service and isolated from other animals on the gather until such time as the horse is free from signs of infectious disease and approved by the on-site/on-call veterinarian to return to the gather. (**major**)
   b. Groups of WH&Bs showing signs of infectious disease should not be mixed with groups of healthy WH&Bs at the temporary holding facility, or during transport. (minor)
4. Horses not involved with gather operations should remain at least 300 yards from WH&Bs, saddle horses, and pilot horses being actively used on a gather. (minor)

IV. HANDLING

A. Willful Acts of Abuse

1. Hitting, kicking, striking, or beating any WH&B in an abusive manner is prohibited. (**major**)
2. Dragging a recumbent WH&B without a sled, slide board or slip sheet is prohibited. Ropes used for moving the recumbent animal must be attached to the sled, slide board or slip sheet unless being loaded as specified in Section II. C. 8. (**major**)
3. There should be no deliberate driving of WH&Bs into other animals, closed gates, panels, or other equipment. (minor)
4. There should be no deliberate slamming of gates and doors on WH&Bs. (minor)
5. There should be no excessive noise (e.g., constant yelling) or sudden activity causing WH&Bs to become unnecessarily flighty, disturbed or agitated. (minor)

B. General Handling

1. All sorting, loading or unloading of WH&Bs during gathers must be performed during daylight hours except when unforeseen circumstances develop and the Lead COR/CO/PI approves the use of supplemental light. (**major**)
2. WH&Bs should be handled to enter runways or chutes in a forward direction. (minor)
3. WH&Bs should not remain in single-file alleyways, runways, or chutes longer than 30 minutes. (minor)
4. Equipment except for helicopters should be operated and located in a manner to minimize flighty behavior . (minor)

C. Handling Aids

1. Handling aids such as flags and shaker paddles must be the primary tools for driving and moving WH&Bs during handling and transport procedures. Contact of the flag or paddle end of primary handling aids with a WH&B is allowed. Ropes looped around the hindquarters may be used from horseback or on foot to assist in moving an animal forward or during loading. (**major**)

2. Electric prods must not be used routinely as a driving aid or handling tool. Electric prods may be used in limited circumstances only if the following guidelines are followed:
    a. Electric prods must only be a commercially available make and model that uses DC battery power and batteries should be fully charged at all times. (**major**)
    b. The electric prod device must never be disguised or concealed. (**major**)
    c. Electric prods must only be used after three attempts using other handling aids (flag, shaker paddle, voice or body position) have been tried unsuccessfully to move the WH&Bs. (**major**)
    d. Electric prods must only be picked up when intended to deliver a stimulus; these devices must not be constantly carried by the handlers. (**major**)
    e. Space in front of an animal must be available to move the WH&B forward prior to application of the electric prod. (**major**)
    f. Electric prods must never be applied to the face, genitals, anus, or underside of the tail of a WH&B. (**major**)
    g. Electric prods must not be applied to any one WH&B more than three times during a procedure (e.g., sorting, loading) except in extreme cases with approval of the Lead COR/COR/PI. Each exception must be approved at the time by the Lead COR/COR/PI. (**majo**r)
    h. Any electric prod use that may be necessary must be documented daily by the Lead COR/COR/PI including time of day, circumstances, handler, location (trap site or temporary holding facility), and any injuries (to WH&B or human). (**major**)

V. TRANSPORTATION

A. General

1. All sorting, loading, or unloading of WH&Bs during gathers must be performed during daylight hours except when unforeseen circumstances develop and the Lead COR/CO/PI approves the use of supplemental light. (**major**)

2. WH&Bs identified for removal should be shipped from the temporary holding facility to a BLM facility within 48 hours. (minor)
    a. Shipping delays for animals that are being held for release to range or potential on-site adoption must be approved by the Lead COR/COR/PI. (**major**)
3. Shipping should occur in the following order of priority; 1) debilitated animals, 2) pairs, 3) weanlings, 4) dry mares and 5) studs. (minor)
4. Planned
5. transport time to the BLM preparation facility from the trap site or temporary holding facility must not exceed 10 hours. (**major**)
6. WH&Bs should not wait in stock trailers and/or semi-trailers at a standstill for more than a combined period of three hours during the entire journey. (minor)

B. Vehicles

1. Straight-deck trailers and stock trailers must be used for transporting WH&Bs. (**major**)
    a. Two-tiered or double deck trailers are prohibited. (**major**)
    b. Transport vehicles for WH&Bs must have a covered roof or overhead bars containing them such that WH&Bs cannot escape. (**major**)
2. WH&Bs must have adequate headroom during loading and unloading and must be able to maintain a normal posture with all four feet on the floor during transport without contacting the roof or overhead bars. (**major**)
3. The width and height of all gates and doors must allow WH&Bs to move through freely. (**major**)
4. All gates and doors must open and close easily and be able to be secured in a closed position. (**major**)
5. The rear door(s) of the trailers must be capable of opening the full width of the trailer. (**major**)
6. Loading and unloading ramps must have a non-slip surface and be maintained in proper working condition to prevent slips and falls. (**major**)

7. Transport vehicles more than 18 feet and less than 40 feet in length must have a minimum of one partition gate providing two compartments; transport vehicles 40 feet or longer must have at least two partition gates to provide a minimum of three compartments. (**major**)
8. All partitions and panels inside of trailers must be free of sharp edges or holes that could cause injury to WH&Bs. (**major**)
9. The inner lining of all trailers must be strong enough to withstand failure by kicking that would lead to injuries. (**major**)
10. Partition gates in transport vehicles should be used to distribute the load into compartments during travel. (minor)
11. Surfaces and floors of trailers must be cleaned of dirt, manure and other organic matter prior to the beginning of a gather. (**major**)

C. Care of WH&Bs during Transport Procedures

1. WH&Bs that are loaded and transported from the temporary holding facility to the BLM preparation facility must be fit to endure travel. (**major**)
    a. WH&Bs that are non-ambulatory, blind in both eyes, or severely injured must not be loaded and shipped unless it is to receive immediate veterinary care or euthanasia. (**major**)
    b. WH&Bs that are weak or debilitated must not be transported without approval of the Lead COR/COR/PI in consultation with the on-site veterinarian. Appropriate actions for their care during transport must be taken according to direction of the Lead COR/COR/PI. (**major**)
2. WH&Bs should be sorted prior to transport to ensure compatibility and minimize aggressive behavior that may cause injury. (minor)
3. Trailers must be loaded using the minimum space allowance in all compartments as follows: (**major**)
    a. 12 square feet per adult horse.
    b. 6.0 square feet per dependent horse foal.
    c. 8.0 square feet per adult burro.
    d. 4.0 square feet per dependent burro foal.

4. The Lead COR/COR/PI in consultation with the receiving Facility Manager must document any WH&B that is recumbent or dead upon arrival at the destination. (**major**)
    a. Non-ambulatory or recumbent WH&Bs must be evaluated on the trailer and either euthanized or removed from the trailers using a sled, slide board or slip sheet. (**major**)
5. Saddle horses must not be transported in the same compartment with WH&Bs. (**major**)

VI. EUTHANASIA OR DEATH

A. Euthanasia Procedure during Gather Operations

1. An authorized, properly trained, and experienced person as well as a firearm appropriate for the circumstances must be available at all times during gather operations. When the travel time between the trap site and temporary holding facility exceeds one hour or if radio or cellular communication is not reliable, provisions for euthanasia must be in place at both the trap site and temporary holding facility during the gather operation. (**major**)
2. Euthanasia must be performed according to American Veterinary Medical Association euthanasia guidelines (2013) using methods of gunshot or injection of an approved euthanasia agent. (**major**)
3. The decision to euthanize and method of euthanasia must be directed by the Authorized Officer or their Authorized Representative(s) that include but are not limited to the Lead COR/COR/PI who must be on site and may consult with the on-site/on-call veterinarian. (**major**)
4. Photos needed to document an animal's condition should be taken prior to the animal being euthanized. No photos of animals that have been euthanized should be taken. An exception is when a veterinarian or the Lead COR/COR/PI may want to document certain findings discovered during a postmortem examination or necropsy. (minor)
5. Any WH&B that dies or is euthanized must be documented by the Lead COR/COR/PI including time of day, circumstances, euthanasia method, location, a

description of the age, gender, and color of the animal and the reason the animal was euthanized. (**major**)

6. The on-site/on-call veterinarian should review the history and conduct a postmortem physical examination of any WH&B that dies or is euthanized during the gather operation. A necropsy should be performed whenever feasible if the cause of death is unknown. (minor)

B. Carcass Disposal

1. The Lead COR/COR/PI must ensure that appropriate equipment is available for the timely disposal of carcasses when necessary on the range, at the trap site, and temporary holding facility. (**major**)
2. Disposal of carcasses must be in accordance with state and local laws. (**major**)
3. WH&Bs euthanized with a barbiturate euthanasia agent must be buried or otherwise disposed of properly. (**major**)
4. Carcasses left on the range should not be placed in washes or riparian areas where future runoff may carry debris into ponds or waterways. Trenches or holes for buried animals should be dug so the bottom of the hole is at least 6 feet above the water table and 4-6 feet of level earth covers the top of the carcass with additional dirt mounded on top where possible. (minor)

CAWP

REQUIRED DOCUMENTATION AND RESPONSIBILITIES OF LEAD COR/COR/PI

Required Documentation

Section	Documentation
II.B.5	Helicopter contact with any WH&B.
II.C.2	Roping of any WH&B.
III.B.3.a and III.B.4.b	Reason for allowing longer than four hours to reunite foals with mares/jennies. Does not apply if foals are being weaned.
III.C.1	Health status of all saddle and pilot horses.
IV.C.2.h	All uses of electric prod.
V.C.4	Any WH&B that is recumbent or dead upon arrival at destination following transport.
VI.A.5	Any WH&B that dies or is euthanized during gather operation.

Responsibilities

Section	Responsibility
I.A.10	Approve materials used in construction of finger gates in bait trapping
II.A.1	Direct gather procedures using approved gather technique.
II.B. 2	Determine rate of movement and distance limitations for WH&B helicopter gather.
II.B.2.a	Direct appropriate gather/handling methods for weak or debilitated WH&B.
II.B.3	Determine whether to abandon pursuit or use other capture method in order to avoid repeated pursuit of WH&B.
II.B.4	Determine width and need for visibility marking when using opening in fence en route to trap.
II.B.6	Determine number of attempts that can be made to capture the missing half of a mare/foal pair that has become separated.
II.B.7	Determine whether to proceed with gather when ambient temperature is outside the range of 10°F to 95°F for horses or 10°F to 100°F for burros.
II.C.1	Approve roping of any WH&B.
II.D.1.a	Determine period of time that water outside a bait trap is inaccessible such that wellbeing of WH&Bs, wildlife, or livestock is not adversely affected.
III.A.2	Direct and consult with on-site/on-call veterinarian on any matters related to WH&B health, handling, welfare and euthanasia.

III.B.1.e	Adjust feed/water as necessary, in consultation with onsite/on call veterinarian, to provide for needs of animals when water or feed deprivation conditions exist on range.
III.B.4.c	Determine provision of water and hay to non-ambulatory animals.
IV.C.2.g	Approve use of electric prod more than three times, for exceptional cases only.
V.A.1	Approve sorting, loading, or unloading at night with use of supplemental light.
V.A.2.a	Approve shipping delays of greater than 48 hours from temporary holding facility to BLM facility.
V.C.1.b	Approve of transport and care during transport for weak or debilitated WH&B.
VI.A.3	Direct decision regarding euthanasia and method of euthanasia for any WH&B; may consult with on-site/on-call veterinarian.
VI.B.1	Ensure that appropriate equipment is available for carcass disposal.

COMPREHENSIVE ANIMAL WELFARE PROGRAM

STANDARDS

OFF-RANGE CORRAL FACILITIES

TRANSPORTATION

ADOPTION EVENTS

Developed by

The Bureau of Land Management
Wild Horse and Burro Program

in collaboration with

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PREFACE

The Bureau of Land Management (BLM) estimates that 58,150 wild horses and burros (WH&Bs) are roaming on BLM-managed rangelands in 10 Western states, based on the latest data available, compiled as of March 1, 2015. Wild horses and burros have virtually no natural predators and their herd sizes can double about every four years. As a result, the agency must remove thousands of animals from the range each year to control herd sizes.

The Bureau of Land Management manages many off-range corral facilities throughout the U.S. The primary mission of the off-range corral facilities is to act as a holding and preparation facility that accepts WH&Bs from gather/removal operations and prepares them for adoption to the public or placement into off-range pastures. As of November 2015, there are 16,182 WH&Bs in off-range corral facilities.

The design of each facility is organized to handle large numbers of WH&Bs with pens, corrals, alleys, and loading/unloading areas that facilitate animal movement. There are multiple pens and corrals to sort animals for compatibility and care upon arrival to the facility. Each pen or corral offers all animals continuous water accessibility and quality feed on a daily basis.

The BLM strives to place horses removed from the range into private homes that provide quality care. Horses placed in off-range corral facilities are made available to the public for adoption or sale at adoption events held throughout the U.S. and through the BLM's Adopt or Sales Program (http://www.blm.gov/wo/st/en/prog/whbprogram/adoption_program/schedule.html). The off-range corral facility transitions WH&Bs to hay diets, performs necessary vaccinations and deworming procedures, provides hoof care and may train WH&Bs. Other WH&Bs, due to age, temperament, or other factors, may not be adopted or sold and subsequently are placed in off-range pastures. These facilities are designed to provide unadoptable wild horses with humane life-long care in a natural setting off the public rangelands. There are approximately 46,016 WH&Bs being cared for in off-range pastures (November 2015). All WH&Bs residing in both off-range corral facilities and off-range pastures, like those roaming Western public rangelands, are protected by the BLM under the 1971 Wild Free-Roaming Horses and Burros Act, as amended.

The BLM has been criticized by the public and animal protection organizations on their handling and care of wild horses and burros especially during the gathering activities and transitioning of the horses from public lands to off-range facilities. Some of the issues include the transportation conditions of the horses, facility design, environmental conditions, handling practices, and nutrition. Agricultural and research animal industries have developed assessment or auditing programs to verify humane care and handling of their industry's animals. These industries are performing both internal and 3rd-party audits for animal welfare to assure their stakeholders and provide transparency on how these animals are provided care and proper handling. Assessment programs based on standards have multifaceted benefits in providing education to employees and assurance to societal entities that their programs are responsible for properly handling and providing care for the animals. An assessment welfare program reported routinely to the public will increase transparency concerning the humane treatment of BLM's WH&Bs, as recommended by the 2008 US Government Accountability Office.

STANDARDS

Deficiency Definitions

Major Deficiency: impacts welfare of WH&Bs; usually is a procedure. Appropriate wording is "must," "unacceptable," "prohibited."

Minor Deficiency: a reporting deficiency (lack of, not enough detail) or involves an uncontrollable situation. Appropriate wording is "should."

WH&Bs = Wild horses and burros

SECTION 1.

OFF-RANGE CORRAL FACILITY STANDARDS

I. FACILITY PERSONNEL

A. Facility management establishes and implements standards of care, treatment, and handling of WH&Bs, and communicates expectations to all personnel to ensure the humane care and treatment of all WH&Bs at the facility. (**major**)

B. The facility must have employees with training, skills, and experience to observe, move, and handle the WH&Bs on the facility. (**major**)

C. The facility must have personnel that can properly maintain the working chute systems and facility infrastructure to provide for the safe housing, movement, and processing of the WH&Bs. (**major**)

D. The facility should be staffed by appropriate office staff for record maintenance and recording. (minor)

II. FACILITY DESIGN

A. Facilities must be operated in a manner to provide a safe, clean and supportive environment for all WH&Bs. (major)

B. WH&Bs must be allowed to exist in an undisturbed environment unless being prepared for health procedures, identification, adoption events, or transportation. (major)
C. All WH&Bs must have adequate space to move freely within a pen enclosure. (major)
D. The facility must have a sufficient number of pens available to sort WH&Bs according to sex, age, temperament, health status, or physical condition as needed. (major)
E. Facility fences, gates, alleys, tubs, and working chutes must be constructed of stout materials and must be maintained in proper working condition. (major)
F. Fences in pens, alleys, and working chute systems must be not less than 6 feet high for horses, 5 feet high for burros, and the bottom rail must not be more than 12 inches from ground level. (major)
G. Fences must be of stout design and be maintained in proper condition with no holes, gaps, or sharp edges which could result in WH&Bs being injured. (major)
H. All WH&Bs within a pen must have adequate dry space to rest. (major)
I. Ground surfaces in pens must be maintained to promote drainage, reduce wet ground conditions, and allow for routine manure removal. (major)
J. Watering systems in WH&B holding pens must provide unlimited access to clean water appropriate for livestock at all times. (major)
K. Feeding areas must be accessible to all WH&Bs in the enclosure and maintained as a dry area without excessive manure accumulation. (major)
L. An appropriate squeeze chute maintained in proper working order must be available for safely restraining WH&Bs for necessary procedures at the facility including hoof trimming. (major)
M. Facilities must provide access to shade and shelter (wind breaks) in pens designated for compromised animals needing special care (i.e., injured or weak animals). Additional provisions for shade and shelter (wind breaks) will be evaluated and determined by managers as appropriate for their region, the function of their facility and the condition of the animals under their care. (major)

N. Shelter structures should be designed or constructed to reduce the risk for injury of the WH&Bs and maintained to avoid excessive manure and mud. (minor)

III. RECEIVING WILD HORSES AND BURROS

A. Loading and Unloading Facilities

1. Facilities in areas for loading and unloading WH&Bs must be maintained in a safe and proper working condition, including gates and doors that swing freely and latch or tie easily as designed. (**major**)
2. The side panels of the loading chute must be a minimum of 6 feet high and covered with materials such as plywood or metal without holes that may cause injury. (**major**)
3. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may cause escape or possible injury. (**major**)
4. Loading and unloading ramps must have a non-slip surface and be maintained in a safe and proper working condition to prevent slips and falls. Examples of non-slip floors would include, but not be limited to, rubber mats, sand, shavings, and steel reinforcement rods or rough surfaces built into the ramp. There must be no holes in flooring or items that cause an animal to trip. (**major**)
5. Trailers must be properly aligned with loading and unloading chutes and panels with no gaps between the chute/panel and floor or sides of the trailer creating a situation where a WH&B could injure itself. (**major**)
6. Stock trailers should be positioned for loading and unloading such that there is no more than 12" clearance between the ground and floor of the trailer for burros and 18" for horses. (minor)

B. Receiving Procedures

1. All unloading of WH&Bs must be performed during daylight hours except when unforeseen circumstances develop and the Authorized Officer approves of the use of supplemental light. (**major**)

2. At the time of unloading, WH&Bs must be inspected by facility personnel to look for signs of infectious disease, sickness or injury and take appropriate steps if these conditions are observed. (**major**)
3. Any WH&B needing immediate veterinary attention must be identified and separated as necessary for examination, and a consultation with a veterinarian must be conducted within 4 hours. (**major**)
4. An authorized, properly trained, and experienced person, as well as euthanasia equipment and supplies, must be available immediately following unloading in the event that the emergency euthanasia of an animal is required. Euthanasia must be done in compliance with BLM Euthanasia policy. (**major**)
5. The receiving Authorized Officer must document any WH&B that is recumbent or dead upon arrival at the destination. (**major**)
6. Reporting of animals received from gather operations or from another BLM facility must be done in compliance with BLM policy. (**major**)
7. WH&Bs received from a gather must remain segregated from resident animals at the facility until a negative test for Equine Infectious Anemia (EIA) can be confirmed. (**major**)
8. Newly arrived WH&Bs must immediately be provided hay and water upon unloading. (**major**)
9. When WH&Bs are received from gather operations or from another facility, the receiving facility should unload them in a manner to minimize stress. (minor)
10. WH&Bs should be penned with WH&Bs of like age, sex, and temperament. (minor)
11. Newly arrived WH&Bs should be observed for signs that they may have difficulty transitioning to hay in a domestic setting. (minor)

IV. CARE OF WILD HORSE AND BURROS

A. Veterinarian

1. Routine presence by an on-site or on-call veterinarian must be provided at each facility with records of those visits maintained at the facility. (**major**)
2. A veterinarian must be available to collect and submit blood samples for Equine Infectious Anemia (EIA) testing and rabies immunization. (**major**)
3. Health care protocols must be in accordance with Program guidelines and accepted by the facility veterinarian for preventive health care procedures (vaccinations, deworming, hoof trimming, freeze marking). Medical treatments will be prescribed by or performed under the supervision of a veterinarian. Surgical procedures will only be performed by a veterinarian. (**major**)
4. WH&B deaths in a facility should be discussed with the facility veterinarian to review the probable causes of death. (minor)
5. If multiple animals die due to a related cause or unknown cause(s), a post-mortem examination or necropsy should be performed by the facility's on-call or on-site veterinarian to identify the cause of death where possible. Laboratory testing should be performed when possible to aid in determining the cause of death or related underlying factors that may be present when these are not readily apparent during the gross examination. (minor)
6. If a series of unexplained deaths occurs, facility quarantine measures must be considered with input from the attending veterinarian until the cause has been determined or when indicated until adequate bio-security measures are in place to prevent the spread of disease. Coordination with State and/or Federal Animal Health Officials in consultation with the attending veterinarian must be done if this occurs. (**major**)

B. Bio-Security

1. Authorized Officers must consult with the on-site or on-call veterinarian(s) to establish and review biosecurity and health care decisions. (**major**)

2. Newly arrived WH&Bs from gather operations must not be co-mingled with resident animals at the facility. (**major**)
3. Pens should be provided to segregate sick, infectious, injured, or weak WH&Bs from other healthy individuals in the facility when possible. (minor)
4. Hazardous or infectious biological waste (i.e., sharps, needles, blood samples, etc.) must be properly disposed in labeled containers. (**major**)
5. Working chute and tub systems should be routinely cleaned and disinfected to reduce the risk of disease transmission, especially after moving contagious WH&Bs through the system. (minor)

C. Feed and Water

1. Quality hay must be provided daily to WH&Bs in the amount of 2-3% of their body weight per day. **(major)**
2. Fresh, clean water appropriate for livestock must be accessible to WH&Bs located in holding pens at all times. (**major**)
3. Water troughs and watering systems must be checked daily to ensure they are clean and operating correctly. (**major**)
4. Feeding sites must allow all WH&Bs within a pen simultaneous access to hay. (**major**)
5. Newly arrived WH&Bs from gather operations may be fed grass hay and transitioned to alfalfa. (minor)
6. Salt and/or mineral blocks should be provided in holding pens at all times. (minor)
7. Supplemental feeds should be provided to address the nutritional needs of the WH&Bs, if needed. (minor)

D. Preparation Procedures

1. Facilities must identify individual WH&Bs and maintain inventories in accordance with BLM policy. This includes unmarked WH&Bs as well as those provided a freeze-mark. **(major)**
2. Facilities must conduct Equine Infectious Anemia (EIA) testing and apply freeze-marks within 30 days of receiving WH&Bs, unless directed by the facility veterinarian when age or physical condition requires a delay. (**major**)

3. WH&Bs receiving a freeze-mark must be recorded on the animal preparation record using the approved Signalment Key. (**major**)
4. Foals born in the facility should be freeze-marked no earlier than 3 months of age. (minor)
5. WH&Bs must be evaluated daily by facility personnel to identify animals in poor body condition, poor hoof condition, injured, or in need of veterinary evaluation/treatment, and/or supplemental feeding. (**major**)
6. Facilities must adhere to the current BLM vaccination policy. (**major**)
7. Foals must be tested for EIA and vaccinated when weaned or at 6 months of age. (**major**)
8. Hoof trimming must be performed twice per year at minimum or as necessary to maintain hooves in a proper condition. (**major**)
9. Castration of stallions and jack burros must be performed by a veterinarian using general anesthesia. If a surgical plane of anesthesia is not reached initially animals must be re-dosed or the procedure postponed until the problem can be resolved. (**major**)
10. Stallions and jack burros should be castrated as soon as approved by the on-site veterinarian for the procedure in accordance with BLM policy. (minor)
11. WH&Bs being prepared for freeze-marking, blood collection, vaccination, hoof trimming, sorting, and other care treatments should be handled to minimize stress and injuries (minor).

E. Euthanasia Procedures

1. The decision to euthanize and method of euthanasia must be directed by the Authorized Officer(s) who may consult with the on-site/on-call veterinarian. (**major)**
2. Euthanasia must be done in compliance with BLM Euthanasia policy. (**major**)
3. An authorized, properly trained, and experienced person, as well as euthanasia equipment and supplies, must be available at off-range corral facilities in the event that the emergency euthanasia of an animal is required. (**major**)

4. Any WH&B that dies or is euthanized must be documented including the circumstances, freeze brand ID if present, a description of the age, sex, color of the animal and the reason the animal was euthanized. A death record must be entered into the WHBPS. (**major**)
5. If an animal is euthanized due to an unknown cause, a post-mortem examination or necropsy should be performed by the facility's on-call or on-site veterinarian to identify the cause of death where possible. Laboratory testing should be performed when possible to aid in determining the cause of death or related underlying factors that may be present when these are not readily apparent during the gross examination. (minor)

F. Carcass Disposal

1. The Authorized Officer must ensure that appropriate procedures are in place for the timely disposal of carcasses when necessary at off-range corral facilities. (**major**)
2. Disposal of carcasses must be in accordance with applicable state and local laws. (**major**)

V. HANDLING WILD HORSES AND BURROS

A. Willful Acts of Abuse

1. Hitting, kicking, or beating any WH&Bs in an abusive manner is prohibited. **(major)**
2. Dragging a recumbent WH&Bs without a sled, side board or slip sheet is prohibited. Ropes used for moving the recumbent animal must be attached to the sled, slide board, or slip sheet. (**major**)
3. There should be no deliberate driving of WH&Bs into other animals, closed gates, panels, or other equipment. (minor)
4. There should be no deliberate slamming of gates and doors on WH&Bs. (minor)
5. There should be no excessive noise (e.g., constant yelling) or sudden activity causing WH&Bs to become unnecessarily flighty, disturbed or agitated. (minor)

B. General Handling

1. All sorting, loading or unloading of WH&Bs must be performed during daylight hours except when unforeseen circumstances develop and the Authorized Officer approves of the use of supplemental light. (**major**)
2. Halters and ropes tied to a WH&B may be used to roll, turn, position, or load a recumbent animal, but a WH&B must not be dragged across the ground by a halter or rope attached to its body while in a recumbent position. (**major**)
3. WH&Bs should be moved into and out of pens in a manner that will minimize stress and injury. (minor)
4. When possible, WH&Bs should be allowed to move at their own pace to new pens or sorting/handling locations. (minor)
5. WH&Bs should be handled to enter runways or chutes in a forward direction. (minor)
6. Any unattended WH&Bs or a single WH&B should not remain in single-file alleyways, runways or chutes longer than 30 minutes. (minor)
7. No equipment should be operated in such a manner as to cause flighty behavior or injury to WH&Bs. (minor)

B. Handling Aids

1. Handling aids such as flags and shaker paddles must be the primary tools for driving and moving WH&Bs during handling and transport procedures. Contact of the flag or paddle end of primary handling aids with a WH&B is allowed. Ropes looped around the hindquarters may be used from horseback or on foot to assist in moving an animal forward or during loading. **(major)**
2. Electric prods must not be used routinely as a driving aid or handling tool. Electric prods may be used in limited circumstances only if the following guidelines are followed:
    a. Electric prods must only be a commercially available make and model that uses DC battery power with batteries fully charged at all times. (**major**)
    b. The electric prod device must never be disguised or concealed. (**major**)

c. Electric prods must only be used after three attempts using other handling aids (flag, shaker paddle, voice or body position) have been tried unsuccessfully to move the WH&Bs. (**major**)
d. Electric prods must only be picked up when intended to deliver a stimulus; these devices are not constantly carried by the handlers. (**major**)
e. Space in front of an animal should be available for the WH&B to move forward prior to application of the electric prod. (**major**)
f. Electric prods must never be applied to the face, genitals, anus, or underside of the tail of a WH&B. (**major**)
g. Electric prods must not be applied to any one WH&B more than three times during a procedure (e.g., sorting, loading) except in extreme cases with the approval of the Authorized Officer. Each exception must be approved at the time by the Authorized Officer. (**major**)

SECTION 2.

TRANSPORTATION STANDARDS TO ANOTHER OFF-RANGE CORRAL FACILITY, OFF-RANGE PASTURE, ECO-SANCTUARY OR ADOPTION EVENT

I. LOADING AND UNLOADING FACILITIES

A. Facilities in areas for loading and unloading WH&Bs must be maintained in a safe and proper working condition, including gates and doors that swing freely and latch or tie easily as designed. (**major**)

B. The side panels of the loading chute must be a minimum of 6 feet high and covered with materials such as plywood or metal without holes that may cause injury. (**major**)

C. There must be no holes, gaps or openings, protruding surfaces, or sharp edges present in fence panels or other structures that may allow escape or cause possible injury. (**major**)

D. Loading and unloading ramps must have a non-slip surface and be maintained in a safe and proper working condition to prevent slips and falls. Examples of non-slip floors would include, but not be limited to, rubber mats, sand, shavings, and steel reinforcement rods or rough surfaces built into the ramp. There must be no holes in flooring or items that cause an animal to trip. (**major**)

E. Trailers must be properly aligned with loading and unloading chutes and panels with no gaps between the chute/panel and floor or sides of the trailer creating a situation where a WH&B could injure itself. (**major**)

F. Stock trailers should be positioned for loading and unloading such that there is no more than 12” clearance between the ground and floor of the trailer for burros and 18” for horses. (minor)

II.CARE OF WILD HORSES AND BURROS DURING TRANSPORT PREPARATION PROCEDURES

A. WH&Bs being transferred to another off-range corral facility, off-range pasture, eco-sanctuary or adoption event must have a current negative EIA test, be in a state of health appropriate for a Certificate of Veterinary Inspection (CVI), and be accompanied by appropriate paperwork as required by the laws of the receiving state. **(major)**

B. WH&Bs being transferred to another off-range corral facility, off-range pasture, eco-sanctuary or adoption event must be fit to endure the travel. (**major**)

    1. WH&Bs that are non-ambulatory, blind in both eyes, or severely injured must not be loaded and shipped unless it is to receive immediate veterinary care or euthanasia (**major**)

    2. WH&Bs that are weak or debilitated must not be transported without the approval of Authorized Officer in consultation with the on-site veterinarian. Appropriate actions for their care during transport must be taken according to the Authorized Officer. (**major**)

C. WH&Bs being transferred to another off-range corral facility, off-range pasture, eco-sanctuary, or adoption event must be sorted prior to shipping to ensure compatibility and minimize aggressive behavior that may cause injury during transport. (**major**)

D. Except for exceptional circumstances including but not limited to trap site adoptions, WH&Bs being offered for adoption should have received primary and booster vaccinations as well as deworming medications prior to adoption. (minor)

II. VEHICLES

A. Straight deck trailers or stock trailers must be used for transporting WH&Bs to another off-range corral facility, off-range pasture, eco-sanctuary or adoption event. **(major)**

    1. Two-tiered or double deck trailers are prohibited. **(major)**
    2. Transport vehicles for WH&Bs must have a covered roof containing them such that WH&Bs cannot escape. **(major)**

B. WH&Bs must have adequate headroom during loading and unloading and must be able to maintain a normal posture with all four feet on the floor during transport without contacting the roof. **(major)**

C. The width and height of all gates and doors must allow WH&Bs to move through freely. **(major)**

D. All gates and doors must open and close easily and be able to be secured in a closed position. **(major)**

E. Loading and unloading ramps of vehicles must have a non-slip surface and be maintained in a safe and proper working condition to prevent slips and falls. Examples of non-slip flooring would include, but not be limited to, rubber mats, sand, shavings, and steel reinforcement rods built into ramp. There must be no holes in the flooring or items that can cause an animal to trip. **(major)**

F. Transport vehicles more than 18 feet and less than 40 feet in length must have a minimum of one partition gate providing two compartments; transport vehicles 40 feet or longer must have at least two partition gates to provide a minimum of three compartments. **(major)**

G. Partition gates in transport vehicles should be used to distribute the load into compartments during travel. (minor)

H. All partitions and panels inside of trailers must be free of sharp edges or holes that could cause injury to WH&Bs. **(major)**

I. The inner lining of all trailers must be strong enough to withstand failure by kicking that would lead to injuries. **(major)**

J. Surfaces and floors of trailers must be cleaned of dirt, manure and other organic matter prior to the beginning of a shipping event, and must have non-skid material on the trailer floor, such as, wood shavings, rubber non-skid mats etc. **(major)**

III. TRANSPORT PROCEDURES

A. WH&Bs must be loaded at the following rates: **(major)**

1. 12 square feet per adult horse.
2. 6.0 square feet per dependent horse foal.
3. 8.0 square feet per adult burro or yearling horse.

4. 4.0 square feet per dependent burro foal

B. Planned drive time from off-range corral facility to another off-range corral facility, off-range pasture, eco-sanctuary, or adoption must not exceed 24 hours without unloading. (**major**)

C. Trucking companies or haulers must be provided directions and contact phone numbers for the facility or adoption location. **(major**)

D. A transportation event that is longer than 24 hours will require unloading and a minimum rest period of 8 hours with access to hay and water prior to being re-loaded and sent to the final destination. **(major**)

E. While in transit, WH&Bs must be observed by the transport driver a minimum of once every 8 hours. **(major)**

F. Non-ambulatory or recumbent WH&Bs must be evaluated on the trailer and either euthanized or removed from the trailers using a sled, slide board or slip sheet. (**major**)

G. WH&Bs should not be allowed to remain standing on straight-deck and stock trailers while not in transport for a combined period of greater than three (3) hours. (minor)

IV. RECEIVING PROCEDURES

See Section 1. Off-range Corral Standards, Part III. B. 1-5 and 8-11.

SECTION 3.

ADOPTION EVENT STANDARDS

I. ADOPTION EVENT PERSONNEL

A. BLM establishes and implements standards of care, treatment, and handling of WH&Bs, and communicates expectations to all personnel to ensure the humane care and treatment of all WH&Bs at the adoption event. **(major)**

B. The adoption event must have employees with training, skills, and experience to observe, move, and handle the WH&Bs at the event. **(major)**

C. The adoption event must have maintenance staff that can properly maintain the facilities to provide for the safe housing, movement, and processing of the WH&Bs. **(major)**

II. SATELLITE FACILITY DESIGN

A. All satellite facilities must be operated in a manner to provide a safe, clean and supportive environment for all WH&Bs. **(major)**

B. The satellite facility must have a sufficient number of pens available to sort WH&Bs according to sex, temperament, or physical condition as needed. (**major**)

C. The satellite facility's fences, gates, alleys, and working chutes must be constructed of stout materials and must be maintained in proper working condition. (**major**)

D. Fences in adoption pens, alleys, and working chute systems must not be less than 6 feet high for horses and 5 feet high for burros. (**major**)

E. Adoption pen fences must be of stout design and must be maintained in proper condition with no holes, gaps, or sharp edges that could result in WH&Bs being injured. (**major**)

F. Watering systems in WH&B adoption holding pens must provide unlimited access to clean water appropriate for livestock at all times. (**major**)

G. Adoption pen feeding areas must be accessible to all WH&Bs in the pen at the same time. (**major**)

H. Ground surfaces in adoption pens should promote drainage to reduce wet ground conditions. (minor)

I. WH&Bs in pens at the satellite facility should be maintained at a stocking density such that when at rest all WH&Bs occupy no more than half the pen area. (minor)

III. CARE OF WILD HORSES AND BURROS RECEIVED AT SATELLITE FACILITIES

A. Provisions to provide care by a veterinarian in a timely manner on-site, on call, or by transporting an animal to a veterinarian must be made in advance**. (major)**

B. Provisions for medical care (treatments including euthanasia if necessary) at adoption events will be made in accordance with BLM policy and carried out as described above for preparation facilities (Section 1. Off-range Corral Facility Standards, Part IV. A. 1-6). (**major**)

C. WH&Bs must be sorted upon arrival at adoption events into pens with animals of appropriate age and temperament such that animals can promptly begin a period of rest, eating, and drinking with limited disturbance. **(major)**

D. All WH&Bs must have adequate space to move freely within an adoption facility pen. **(major)**

E. WH&Bs, to the greatest extent possible, must be allowed to exist in an undisturbed environment after arriving at an adoption facility until the adoption event begins. **(major)**

F. Quality hay must be provided to WH&Bs in the amount of 2-3% of their body weight per day upon unloading following transport and daily during the adoption event. **(major)**

G. WH&Bs at adoption events may be supplemented with feed or water supplements to address the stress of transport. (minor)

H. WH&Bs will be sorted during the adoption event in a manner to reduce stress and opportunity for injury. (minor)

I. WH&Bs transported for more than 12 hours to an adoption event should be allowed a rest period of 5 hours or more prior to formal viewing by the adopting public. (minor)

IV. EUTHANASIA PROCEDURES AT SATELLITE FACILITIES

See Section 1. Off-range Corral Facility Standards, Part IV. E. 1-4.

V. CARCASS DISPOSAL AT SATELLITE FACILITIES

See Section 1. Off-range Corral Facility Standards, Part IV. F. 1-2.

VI. HANDLING WILD HORSES AND BURROS AT SATELLITE FACILITIES

A. Willful Actus of Abuse

See Section 1. Off-range Corral Facility Standards, Part V. A. 1-5

B. General Handling

See Section 1. Off-range Corral Facility Standards, Part V. B. 1-7

C. Handling Aids

See Section 1. Off-range Corral Facility Standards, Part V. C. 1-2

REQUIRED DOCUMENTATION AND RESPONSIBILITIES OF AUTHORIZED OFFICERS

REQUIRED DOCUMENTATION

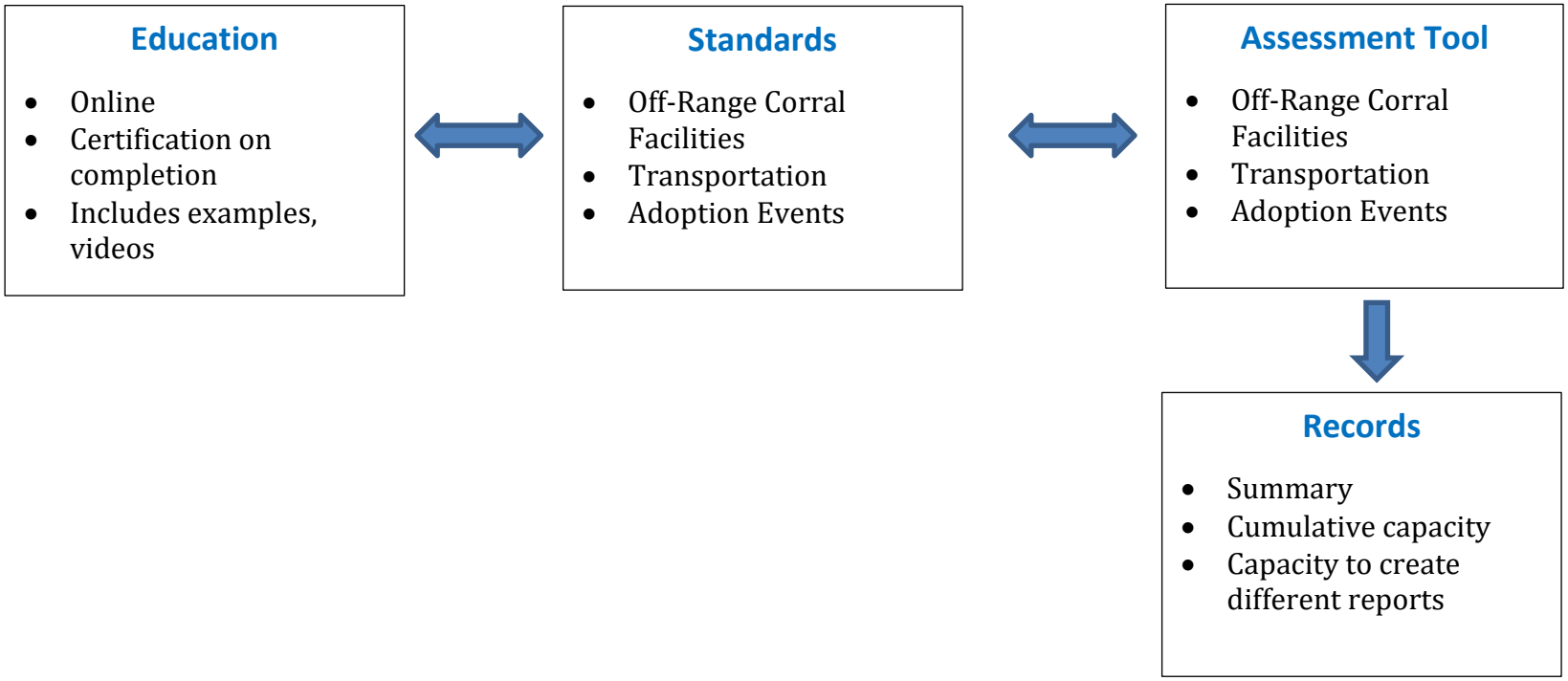
STANDARD	DOCUMENTATION
Section 1	**Off-Range Corral Facility Standards**
Part III. B. 5	Any WH&B that is recumbent or dead upon arrival at the destination
Part IV. A. 1	Record of visits by on-site/on-call veterinarian
Part IV. E. 4	Any WH&B that dies or is euthanized at facility, including the circumstances, freeze brand ID if present, a description of the age, sex, color of the animal and the reason the animal was euthanized. A death record must be entered into the WHBPS.
Section 2	**Transportation Standards**
Part V	Refer to Section 1. Off-range Corral Facility Standards, Part III. B. 5, as above.
Section 3	**Adoption Event Standards**
Part IV	Refer to Section 1. Off-range Corral Facility Standards, Part IV. E. 4, as above

RESPONSIBILITIES

STANDARD	RESPONSIBILITY
Section 1	**Off-Range Corral Facility Standards**
Parts III. B. 1 & V.B.1	Approve use of supplemental lighting when sorting, loading, or unloading WH&Bs
Part III. B. 5	Document any WH&B that is recumbent or dead upon arrival at the destination
Part IV. B. 1	Consult with on-site/on-call veterinarian to establish and review biosecurity and health care decisions
Part IV. E. 1	Direct decision regarding euthanasia and method of euthanasia for any WH&B; may consult with on-site/on-call veterinarian
Part IV. F. 1	Ensure that appropriate procedures are in place for carcass disposal
Part V. C. 2. g	Approve use of electric prod more than three times, for exceptional cases only

STANDARD	RESPONSIBILITY
Section 2	**Transportation Standards**
Part II. D	Approve of transport and care during transport for weak or debilitated WH&B
Section 3	**Adoption Event Standards**
Part VI. B	Refer to Section 1. Off-range Corral Facility Standard, Part IV.B.1, above.
Part VI. C	Refer to Section 1. Off-range Corral Facility Standard, Part V.C.2.g, above.

PROPOSED CAWP COMPONENTS FOR OFF-RANGE CORRAL FACILITIES, TRANSPORTATION, AND ADOPTION EVENTS



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Harvesting antler velvet

From Mr D. J. Thomas

SIR,—With regard to the harvesting of antler velvet, I feel that the veterinary profession should once and for all condemn this practice, which is, most importantly, of no advantage to the animal concerned and the product harvested of unproven medical value. The only advantage which I can deduce is a financial one to the harvesters and marketers of this commodity.

I object to being told by people who are out to make a financial gain from this practice what we as a profession "must" do to further this unnecessary mutilation. I am confident that the profession in this country will not support this unnecessary practice.

D. J. THOMAS
170 Llandaff Road,
Penhill,
Cardiff

B bronchiseptica vaccine

From Mr J. M. Phillips

SIR, The paper by C. J. Giles and others (Veterinary Record, January 12, 1980) provides an excellent insight into the role of *Bordetella bronchiseptica* in cases of infectious atrophic rhinitis (IAR) of pigs. I would like, through the courtesy of your columns, to comment on certain aspects of this work as they relate to the recently authorised *B bronchiseptica* vaccine (AR-PAC: Mycofarm).

I fully agree with the authors that the introduction of this vaccine will not provide an automatic answer to the problem and it most certainly should not be considered as obviating regimes of good management and hygiene. However, I feel that the author's comments regarding medication should be clarified. The pattern of *B bronchiseptica* infection is confirmed as occurring, in general, early in life (day one) and persisting for several weeks although "suppression" may delay overt infection. The most efficacious antibiotic medication programmes are those which provide therapeutic levels respective to the resistance of *B bronchiseptica* throughout this danger period. The full vaccination programme recommended for AR-PAC provides immunity to *B bronchiseptica* during this same period and indeed up until slaughter. In addition, a suppression of the carrier state of infected pigs is observed.

I consider, therefore, that AR-PAC provides a rational alternative to continuous medication programmes and satisfies the observation of Giles and others that continued control of IAR is best served by immunological rather than chemoprophylactic measures.

J. M. PHILLIPS
Mycofarm Ltd
London Road
Braintree, Essex

Price increase

From Mr D. C. North

SIR, — A lot has been written and said recently about the effects of the Medicines Act in removing old established products from the veterinary surgeon's shelves. It now appears that some companies are pricing themselves off the shelves. I have just received an invoice from my wholesaler which shows that the price of Norderm (Smith Kline) has increased by 400 per cent since I last ordered some three months ago. The selling price now works out about the same as a very good brandy — I just hope that the average dog fully appreciates this nectar.

D. C. NORTH
211 High Street,
Sheerness, Kent

A spokesman from Smith Kline comments: "From 1972 to 1978, although manufacturing and raw material costs of Norderm increased more than background inflation, the professional price in no way reflected this. In fact, price rises were very much less than inflation. By 1979 we were faced with a product being sold at a loss and suddenly increasing manufacturing and raw material costs. A very difficult decision had to be made whether to discontinue the product altogether or revise its price to an economic level. It now costs 5p a day to give Norderm to a 50 lb dog which we feel still represents very good value and we hope that this action will enable us to continue providing Norderm at good value."

RCVS appeal: The "Hundred Club"

From Miss W. M. Brancker and others

SIR,—Members will, by now, have read the letter from the President of the Royal College which was included as an insert in the Veterinary Record of January 19. We would like to commend to all our colleagues in the profession his plea that an increase in the staff and accommodation at Belgrave Square is urgently needed and that it should be the responsibility of the profession to raise the greater part, if not indeed all, of the capital sum required.

With a membership of 10,000 the Royal College could have raised the necessary money by a levy on each member of £20—but clearly this would be unfair on the new graduate and the retired. The College therefore, by implication, places its trust in the generosity of those of its members who can afford to make contributions of a substantial nature.

We believe that many senior members could make a donation of £100 towards the necessary extension of their Royal College and we ourselves have already made such a contribution. Can we appeal to as many of our colleagues as possible to join us in participating in membership of an informal "Hundred Club" so that we can meet the necessary target and meet it quickly? There are something of the order of 1,600 practices in this country and over 4,000 principals—so the requisite number of Hundred Club cheques ought not to be impossible to find; they should be sent to the President of the Royal College.

W. M. BRANCKER
63 Lichfield Road,
Sutton Coldfield,
West Midlands

D. L. HAXBY
Candent House, Upton,
Newark, Notts

J. A. PARRY
Watergate Mill,
Brecon, Powys

MSc in meat science (Bristol)

From Dr R. N. Smith

SIR,—The remarks attributed to me on p41 of the Veterinary Record of January 12 do not accord with what I believe I said at the meeting of Council on November 28. Certainly I would not have referred to the full-time MSc course at this university as "modular". Further, I expressed disappointment at the poor support from the profession, not from practitioners.

Of the 12 participants in the two years the course has now been in exisence only two are members of the Royal College and two others have veterinary qualifications from the Middle East. There are now many non-veterinary graduates with an interest in meat who are eligible for this type of course. Three of the remaining eight participants quote work with local authorities as part of their postgraduate experience (authorised meat inspector, environmental warden, public health inspector).

R. N. SMITH
School of Veterinary Science,
University of Bristol,
Park Row, Bristol

Libidinous behaviour in a gelding

From Mr S. Borsberry

SIR,—Some time ago I was presented with a 10-year-old pony gelding which had experienced a change in behaviour. During a period of 11 months the pony had been moved four times: (a) mixed grazing, mares and geldings, (b) only geldings present, (c) only geldings present and (d) mixed grazing, mares with geldings.

The pony had been at (a) for about six years and there had been no complaints as regards its behaviour. It was easy to ride and catch. For the sake of convenience it was moved to (b) on September 6, 1976 but unfortunately on January 15, 1977 the gelding started to break out and visit neighbouring mares. The owners of the field asked the owner to move him so she did to (c) on February 26, 1977. The situation eased but in April 1977 he began to visit mares again.

On May 12, 1977 he was moved to (d) where he became very difficult to catch, protective towards "his" mares and somewhat dangerous to ride. During this period he began to serve the mares. Palpation of the scrotal region revealed no abnormalities so it was decided to implant a 500 mg stilboestrol pellet. The rationale behind this was to reduce the male sexual behaviour with the female hormone. Unfortunately the opposite occurred; after about six days his sexual appetite increased considerably. It was then decided to explore surgically the inguinal region but there were no signs of cystic cords, testes or anything to suggest that he had not been gelded correctly as a yearling. The stilboestrol implant was removed at the same time.

Somewhat perplexed I rang John Cox at Liverpool University who suggested that this change in behaviour might be psychological and advised, if possible, to return the animal to (a). This was duly carried out and within three to four days the gelding had settled down, showed no interest in mares and was easy to catch, and has remained so.

S. BORSBERRY
608 Warwick Road,
Solihull,
Warwickshire

BSAVA "fund run"

From Mr C. P. DeVile

SIR, — The BSAVA has entered the 1980s confident of its ability to provide monies for small animal research in a decade in which it is not difficult to forecast that the universities will be increasingly short of funds.

The fund receives magnificent support from members of the profession and on behalf of the management committee I thank all those colleagues who give time and energy in the organisation of fund raising events, those who donate fees due to them for lecturing and journalism, and our collecting box holders.

Collecting boxes are now available again, on application to Mr D. R. Highet, 410 Eaton Road, Liverpool L12 3HG.

Finally, sir, I would like to invite you and your readers to participate in a unique fund raising "exercise" that is planned to take place at BSAVA Congress in April. A sponsored "fun-run" (or Fund-run) will take place at the White City Stadium at a time that is guaranteed not to interfere with the other pleasures of congress. Sponsorship forms and full details will be sent to BSAVA members with their congress mailing, and to all non-members who register to attend. I give this advance notice of the event so that readers may have an opportunity to get into training. By carrying out their domiciliary visits by jog between now and April they will not only improve their performance at the White City but will make in addition a positive contribution to the energy crisis, though possibly creating one of their own.

C. P. DeVILE
Chairman, Management Committee,
BSAVA Clinical Studies Trust Fund

Letters may be shortened for publication

Postscript

Welfare motives

THE welfare of animals has become a matter of increasing concern in recent years, yet this has always been a subject of intense human interest; and so it should be. It is instructive to contemplate the three historic reasons why animal welfare is important to us.

The first is the pragmatic knowledge that, if animals are not cared for, they do not produce the results which their owners and beneficiaries require from them.

The second is the feeling of guilt, that we are debasing our own characters by association with neglectful or harmful actions leading to criticism by our fellow citizens. This view is epitomised by the lines of William Blake: "A robin redbreast in a cage puts all heaven in a rage; a dog starved at his master's gate predicts the ruin of the state".

The feeling of guilt is cousin to that of fear which forms the third major motive for our concern. Many people are fearful of retribution should they "do unto animals that which they would not have done to themselves".

The modern attitude to human welfare has, of course, always been a mixture of these motives. Further, the current tendency to exaggerate all aspects of human activity has made welfare something of a religion in itself, rather than part of a wider spiritual undertaking. Thus, we encounter priests and pastors who are, themselves, unsure of whether or not animals have souls and cannot, therefore, be expected to advise we lesser mortals on the subject. How can we decide on these matters when we have little basis for our own conclusions and "Like the Indian, in another life, expect our dog, our bottle and our wife".

The veterinary concern for animal welfare has no less a basis than pragmatism, guilt and fear. It is none the less proper for this; nor indeed, would failure be less improper in ourselves than in others, but rather more so. There are those who hold the view, as does the present writer, that the most important aspect of human attitude to animals—and to children or matters of race, religion and human behaviour—reflects more on the individual's qualities and character than on an absolute criteria of right and wrong, good or evil. No-one knows, for sure, what is good and evil, right or wrong, because they are, surely, relative values and changing determinants of human thought and, to some extent, mirror images of the other. The killing of an animal for pleasure must be put in a different light to that of killing for necessity—however tenuous the necessity may be. Deliberate or inadvertent ill-treatment of an animal is a pastime that must be condemned depending on the motives of the perpetrator. But if the motives are justified, such as where ill-treatment is a consequence of defence from attack, the spread of disease, the benefit of human beings or fellow creatures, the case rests on a definition of ill-treatment. And this, in turn, brings us to the impossible position of judgement in these matters, because what constitutes ill-treatment cannot be defined objectively. And as soon as it is defined subjectively the three motives for the concern of welfare are bound to operate to a greater or lesser degree.

Spectator sports, of course, by their very nature, come under close scrutiny by the public conscience, that amalgam of pragmatism, guilt and fear. I received, recently, a letter from a colleague telling me that a client of his had been concerned at what had taken place on the racecourse at Newmarket when the 311th running of the Town Plate occurred this year. The scene, to quote my correspondent, was more reminiscent of a battle field than of a competitive sporting event. A number of horses finished dead, injured or exhausted; and many riders in a similar, or near similar, state. Protests, and these were numerous, have met with a sympathetic response from the Jockey Club as to the conduct of the race in future, to avoid similar sufferings by horses, riders and spectators. However, one suggestion, which would need careful analysis by our profession, is that competitors should be required to produce a veterinary certificate stating that their horse was in a fit condition to compete over three and three quarter miles, carrying 12 stone under racing conditions. I do not know if there is any clinician who would be prepared to make such a pronouncement, until he had seen the horse compete under similar conditions, when it would, presumably, be too late to prevent the event.

"TOTALISER"

U.S. Geological Survey
Fort Collins Science Center
2150 Centre Avenue, Building C
Fort Collins, Colorado 80526-8118

November 24, 2015

Dean Bolstad
Acting Division Chief,
Wild Horse and Burro Program
Bureau of Land Management, WO-260
20 M Street,
Washington, DC 20003

Dear Mr. Bolstad,

Attached please find a summary table and notes resulting from expert panel discussions on September 24, 2015, exploring several alternative methods for wild horse spaying. In addition to veterinary and equine experts, several USGS, BLM, USDA-APHIS, and Colorado State University staff also observed and contributed to discussions.

The materials reflect professional opinions about the current state of understanding of four spay methods currently used on domestic horses, as represented and discussed by panel members during and after the day-long meeting. These materials do not provide BLM with a recommendation, but hopefully provide useful information for BLM to consider.

Sincerely,

Zack Bowen
Branch Chief, Ecosystem Dynamics

Attachments.

Sarah R.B. King, Ph.D.
Research Scientist
Coordinator of the Equid Red List Authority, IUCN

Department of Ecosystem Science & Sustainability
A242 NESB - Campus Delivery 1476
Colorado State University, Fort Collins, CO 80523
Phone: (720) 587-9890; Fax: (970) 491-1965
Email: sarah.king@colostate.edu

November 24, 2014

Dean Bolstad
Acting Division Chief
Wild Horse and Burro Program
Bureau of Land Management, WO-260
20 M Street
Washington, DC 20003

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These materials do not provide BLM with recommendations, but provide information for BLM to consider. The materials reflect the professional opinions on the current state of understanding about the pros and cons of four spay methods currently used on domestic horses, as represented and discussed by panel members during and after the day-long meeting.

Sincerely,

Sarah R. B. King
Research Scientist, CSU

Attachments.

Assessment of spay techniques for mares in field conditions

Panel meeting held at USGS Fort Collins Science Center

September 24, 2015

Summary of panel expert responses on four potential spay methods

	Colpotomy	**Ventral midline**	**Flank incision**	**Flank laparoscopy**
Facilities needed	Squeeze chute with a kick panel and access to the perineum.	Squeeze chute, table fitted on a forklift.	Squeeze chute (may require access to both sides).	Squeeze chute (may require access to both sides), sling.
Equipment needed	Ecraseur, scalpel or bistury, blunt scissors, gauze sponges tied with umbilical tape.	Complete surgical pack, surgical drapes, gauze sponges.	Complete surgical pack, surgical drapes, gauze sponges.	Laparoscope, CO_2 for insufflation, surgical pack, surgical drapes, gauze sponges.
Equipment preparation	Ecraseur autoclaved or cold-sterilized in ortho-phthalaldehyde (OPA/28) >10 minutes then rinsed in sterile water, or use chlorhexidine for sterilization.	Autoclave or cold-sterilize instruments.	Autoclave or cold-sterilize instruments.	Prepare laparoscopic equipment – cleaned and cold-sterilized.
Sedation	IV injection to the jugular of xylazine + butorphanol + detomadine.	Horses are placed in dorsal recumbency following an induction dose of xylazine/ butophenol/diazepam and ketamine.	Local lidocaine infiltration of flank and IV butorphanol.	IV jugular catheter continuous sedation drip - 20 mg detomidine in 1 liter fluid for standing sedation.
Anesthetic /analgesic protocol	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine . Add low dose ketamine as needed.	Anesthesia may be maintained by IV administration of triple drip (IV-ketamine, xylazine and guaifenesin 5%) or using inhalant anesthesia.	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine. Injection of lidocaine in line or L-block (~100-200 ml) at incision site.	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine. Epidural for analgesia of the reproductive tract, local anesthesia at portal sites.
Procedure	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
	days. Wrap tail and tie up. Evacuate rectum/bowel, prep perineum. Make 1 cm incision in vaginal fornix. Expand incision via blunt dissection. Locate ovaries. Sterile 4x4 gauze soaked with 30 ml 2% lidocaine compressed over ovarian pedicle 3-5 minutes or lidocaine injected into pedicle. Remove ovaries via ecraseur. Repeat for other ovary through same incision.	days (or given 40 ml procaine penicillin and 10 ml flunixin meglumine post surgery). Surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Incision into peritoneum made on ventral midline. Ovaries exteriorized through ventral midline incision. An emasculator is applied to the ovarian pedicle. #6 MSA used to ligate the ovarian stump proximal to the emasculator. The emasculator is removed and the ligated stump allowed to retract into the abdomen. Closure is accomplished in three layers, the outermost being a subcuticular layer using #6 MSA absorbable.	days. Surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Line or L-block injections are administered. Wait until block has effect, then single incision in left flank through skin and fascia followed by blunt dissection into the peritoneum. 9-inch burdizzo (or emasculator) for removal of ovaries. Closure of the underlying layers of muscle and fascia such that only the skin requires suturing. Very bottom of the skin suture line is left open to prevent seroma formation.	days. Approach both flanks: surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Make incision at flank. Insert cannula for instruments. Insufflate with CO_2. Lidocaine injected to ovary and pedicle. Ovary is removed through incision; incision may have to be enlarged to remove ovary. Suture incision. Repeat on other side.
Incision	5 cm in anterior vagina.	9-15 cm incision made just cranial to the udder.	10-15 cm in flank (on one or both sides).	One 1 cm incision and one 6-10 cm incision in the left flank, and three 1 cm incisions on the right side.
Standing or recumbent surgery?	Standing	Recumbent	Standing	Standing
Surgery time per	15-20 minutes	20-30 minutes	45 minutes	40-60 minutes

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
horse				
Complications	1-2% seen within 2 days.	Infection of open wound; potential for evisceration; potential for injury upon recovering from anesthesia.	5% incisional complications under sterile conditions (more likely 10-20% under field conditions).	1-2% incisional complications (in sterile environment), 10% subcutaneous edema; puncturing a bowel; dropping ovaries in abdomen.
Recovery time (before release)	3 days	2-3 weeks	2-4 weeks	1-2 weeks
Contra-indications	Uterine infection/pyometra. Enlarged (>6 cm) ovary; pelvic or ovarian abnormalities. Heavy late gestation may prevent access to ovaries. Surgery may not be possible if the mare cannot be sufficiently sedated.	Very dirty animal; old/multiparous; any animal that is contraindicated for general anesthesia. Contraindicated in later term gestation due to risk of initiating labor and abdominal wall rupture during parturition. Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.	Very dirty animal; any animal that is contraindicated for general anesthesia. Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.	Dependent on technology; abnormal ovary.
Effect on pregnant/lactating mares	Late gestation may challenge access to ovaries. Pregnancy no issue following first ±70 days. No foal abandonment issues. No issues with lactation.	Late gestation may be challenging. May affect nursing (pain when the foal tries to nurse).	Unknown. May affect nursing (pain when the foal tries to nurse).	Unknown, but likely easily done.
Effect of breeding post-surgery	Vaginal incision usually healed by 7-10 days post-	Breeding may cause injury prior to recovery.	Breeding may cause injury to the incision line.	Breeding may cause injury to the incision line.

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
	surgery. If open or <100 days pregnant administer long acting progesterone which should suppress receptivity/save pregnancy.			
Operator safety	Strapping the back of the mare helps prevent kicking, kickboard and tail tied dorsally aids operator safety. Short time with scalpel.	Animal fully anesthetized.	Operator protected due to small window of access on side of animal. Longer time with scalpel.	Operator protected when coming from the flank, but injuries can occur due to equipment and two people in a restricted space.
Cost per horse	$250-$300[1] Long-acting progesterone ($7/mare)	$350[2] includes all drugs and supplies. Plus long-acting progesterone ($7/mare).	$350[2] Includes all drugs and supplies. Plus long-acting progesterone ($7/mare).	$450-$500[3] Long-acting progesterone ($7/mare).
Pro of method	Fast healing and recovery, quick surgery, can be done on pregnant mares.	Low risk to operator, common surgery for companion animals.	Low risk to operator, common surgery.	Direct visualization, low morbidity, good public opinion.
Con of method[4]	Higher risk to operator, need for trained surgeons.	Risk of evisceration, risk of incision infection.	Risk of incision infection and pain.	Most expensive and time-consuming approach.

Notes:

[1] Colpotomy cost per mare: $100-1500 initial equipment cost (chain ecraseur Jorgensen J-37E $450 buy 2 or 3, replacement chain MidWest 350.01254.2 J37ED1 $95). Then $80-100/mare: OPA/28 4 gal $99.56 (estimate 50-80 mares: $2/mare), suture 2-0 monocryl 36/box $205 ($6/mare), #10 or #21 scalpel blade 100/$25.52 ($3/mare), Lidocaine 100ml $8 ($4/mare), Xylazine $1/100 mg ($4/mare), Butorphanol 10mg/ml 50 ml $250 ($5/mare), Detomadine $16/mare, Ceftiofur - excede 15 ml $30/mare. Once the ecraseur and scalpels have been purchased, the expense via colpotomy for each mare is the drugs, the lidocaine and gauze for the pedicles, and the sterilizing of the ecraseur.

[2] Ventral midline and flank incision – there will be a cost of drapes, gowns, sutures, etc., as for all abdominal surgeries, and a cost for the surgical equipment. Drug and other equipment cost will be as in [1].

[3] Laparoscopy equipment costs: $25,000 for camera, light core, light source, monitor, Thorston telepack, $5,000 – insufflator, $3,000 – microscope, $750 x 6 for hand instruments (need two sets): $40,000 total new, or could buy used. Likely need to replace 5 hand instruments per 100 mares. Drug and other equipment cost will be as in [1].

[4] It should be noted that all surgeries are associated with a risk of death. There are no published data available to assess the mortality risk of any spay surgery in wild horses, although preliminary data on domestic and wild equids were discussed by the panel.

Assessment of spay techniques for mares in field conditions

Panel meeting held at USGS Fort Collins Science Center
September 24, 2015

Transcript of Comments

Questions or topics are in **bold**.
The speaker is in *italics*. If the person introducing the topic made the comment it is indented with a bullet. If the speaker is not known the comment is indented with a hyphen. All attendees and invitees have been given the opportunity to edit this document. As these are notes taken from a discussion, what is written here may not capture the exact intended meaning of a given statement.

Attendees

In person: Zach Bowen (USGS), Jason Bruemmer (CSU), Doug Eckery (USDA/APHIS), Paul Griffin (BLM), Al Kane (USDA/APHIS), Sarah King (CSU), Joanna Ruffino (USGS), Kate Schoenecker (USGS).
By WebEx/Phone: Cheryl Asa (St. Louis Zoo), Gail Collins (NPS), Robert Cope, Jay D'Ewart (BLM), Bryan Fuell (BLM), Dean Hendrickson (CSU), Katrin Hinrichs (Texas A&M), Sue McDonnell (U. Penn.), Leon Pielstick (DVM), Patricia Sertich (U. Penn.), Mark Stetter (CSU), Regina Turner (U. Penn.), Julie Weikel (DVM).
Information provided after the panel: Paul Zancanella (DVM)

Introduction

Paul Griffin

- Purpose of the meeting is to discuss different procedures for spaying wild horse mares.
- We will discuss pros and cons of the various methods that could potentially be used for spaying wild horse mares.
- This is not a definitive decision making meeting for BLM, but to get the opinions of experts.

Background of study to be conducted by USGS/CSU

Sarah King

- Aim of the study is to look at the short-term impact of spaying on health and behavior of individual mares, specifically any effects on band fidelity, spatial ecology and population demography.
- Location: White Mountain HMA, Wyoming.
- We have proposed to spay 60% of adult mares (adult mares are 3 years old and older), which will probably be 36-48 mares, depending on the age structure, leaving 24-32 untreated controls plus juveniles and foals.

- We will collect 1 year of pre-treatment data, and then 3 years of post-treatment data.

Facilities at Rock Springs BLM Adoption Facility

Al Kane:

- It's a typical BLM facility.
- Two hydraulic squeeze chutes. Most facilities do not have a split tailgate. At Rock Springs, the door would need to be re-fitted to have a split tailgate that would allow access to perform a colpotomy. It's an open question about whether BLM would have the funding to get a new tailgate (that is split).
- Squeeze chutes give access to the left side and hindquarters of the animal. They are - padded and compress the animal front to back and side to side.
- For recumbent surgery the mare can be rolled out of the chute onto the ground; no access to a table.
- No hospital and no indoor facility.
- Transportation to and from the HMA: gooseneck, stock type trailer, or semi-trailers.

Zack Bowen:

- We will compile notes and comments and put them in a briefing paper. This will not be published. It will be a statement to BLM compiling information, not making a recommendation.
- Information will be compiled based on considerations that will be asked of each technique.

Kane: Today we will discuss some considerations for what technique may be most appropriate for this study, but another technique may be more appropriate in the future for spaying on a wider scale across HMAs. But it may be the same.

King: The method chosen by the BLM for this study should be the same as what is used in the future elsewhere, as this will have been the method we gathered data on.

Discussion of Colpotomy - *Leon Pielstick*

History:

- Leon is a veterinarian who has worked with the BLM since 1975, and has also been involved with the management of horses at Sheldon-Hart. At Sheldon he spayed horses which had been placed on a private pasture for the trial.
- Spayed mares in the field successfully: Out of 34 mares spayed, 31 were open, 3 were pregnant and successfully foaled. At Sheldon they used spaying as a management tool – they vasectomized males and spayed females that were considered unadoptable, then turned them back out to the range. The majority of such spayed mares were pregnant.
- He has spayed 188 mares by colpotomy, including 16 spayed at a wild horse sanctuary in California, 16 spayed as part of a PZP safety study in Oregon.

- Out of the 188 mares there were 2 fatalities: one bled to death internally due to a clotting abnormality, and one got sick, aborted her foal and died (anecdotal evidence indicated that she had a peritoneal infection). We can expect a 1-2% complication rate with colpotomy. For any given choice of spay method, the BLM must be prepared to accept some level of loss.
- 70-75% of mares are likely to be pregnant in late summer. Pregnant mares can still be sterilized by colpotomy because of the way the foal drops in the uterus: the ovaries are still at the top of the uterus so can be reached. At 7-8 months pregnant it gets harder to move the intestines to reach the ovaries, so it is more difficult to keep the intestine out of the ecraseur tool. The only mare that had an abortion was the mare that died.
- To do the surgery, give heavy sedation/analgesia (butorphenol + xylazine or detomidine + Dormosedan) and heavy analgesics (banamine + butorphenol); the surgery is performed with the mares standing. Banamine was added to eliminate mild post surgical colic which had occurred in a few of the mares the first year in which the procedures were done.
- Mares held off feed for 24 hours before surgery seemed to have good recovery after. Holding off feed means that fecal balls are reduced, which can resemble ovaries on palpation and thus take time to sort out, and reduces abdominal fill. Depending on the horse there was a little colic within the first few hours post surgery until Banamine was added to the procedure. Mares generally walked out of the chute and started to eat, some would raise their tail and act as if they were defecating, however in most you could not notice signs of discomfort.
- There was no squeeze chute at Sheldon, but the mares could be held at the back of the chute where there was an access window. Some mares needed additional sedation as they could not be squeezed. It should be possible to make any facility functional for this surgery.

Facilities:

- Needs a kick panel and an access window, so that there is access to the perineal area. Use a strap above the rump, to help prevent jumping and kicking. Most facilities can be made functional. At the BLM Burns facility, for example, there is enough room to slide in a 3 foot tall plywood kick panel.

Behavior:

- In the first group of 33 spayed mares with 10 intact mares and 2 stallions (on private land) – the group all stayed together as a herd.
- In another situation two groups of 8 spayed mares formed their own bachelorette band. They were new to the facility.
- In Sheldon it is assumed that they returned to their band.

Recovery time:

- The only complications were seen within two days of surgery. After 2 days there were no visual problems so they could be released to the range.

- It is typically recommended in domestic mares that they not be ridden for a month, but they seem okay after 2 days.
- The incision in the anterior vagina is not sutured; this heals rapidly (within days, cannot be identified easily on speculum examination). Evisceration through the vaginal incision is often brought up as a possible complication of colpotomy, but none of the panel participants had had this occur nor had heard of it actually occurring. In addition, being held off feed would reduce the chance of evisceration.
- Being held off of feed before-hand is important according to Pielstick, although others said it was less necessary.

Gail Collins - At Sheldon they had the opportunity to recapture spayed mares that were released (3yr, 5yr, 6yr later) to monitor progress.

- They were given a dose of antibiotic (Excede -- ceftiofur) that lasts for 4 days within the mare (single injection).

Procedure:

- Cold sterilize ecraseur. Give antibiotic.
- Wrap tail and tie up for procedure.
- Evacuate bowel, surgically scrub peritoneum and flush out vagina. Clean vagina with iodine. Others perform procedure without vaginal flush (vagina should be essentially sterile).
- Put on sterile sleeves, introduced hand into vagina, and make the incision in the anterior vagina.
- Mares have no nerve receptors in that area, so they only feel pressure/stretching. They show no outward sign of discomfort. Mares feel the pull on and compression of the ovarian pedicle, but lidocaine administered to the pedicle to minimizes this. This analgesic lasts a couple / few hours. The pain afterwards is similar to that of castration.
- Timeline: 15 minutes for the whole procedure. Speed is often necessary due to volume of horses. Can do 30-35 horses a day as it is not physically taxing.
- Controlling the level of dust is very important, but otherwise it is possible to keep the area 'field sterile'.
- Can make a portable chute for this procedure out of a hydraulic cattle chute; this would have an adequate tail gate.
- Mares walk out of the surgery.

Comments:

Julie Weikel served as an observer for Leon's procedure at Sheldon; wrote a review:

- Some mares would walk out from surgery and immediately want to eat (hunger pain worse than spay pain due to keeping them off food).
- Some (a few individuals) showed minor signs of colic for about a half hour, such as getting up and down repeatedly. These were collected so that they could be watched more closely for up to an hour, then turned out to join a bigger group with feed and

water. For these horses they did not use lidocaine, which might alleviate the colic symptoms. Leon now uses liodocaine in every spay.

Katrin Hinrichs: **Did you give any antibiotics before the procedure?**

- Antibiotics are given after sedation, so not as long before surgery as ideal. There isn't a large opportunity to give it long before surgery due to the circumstances of these being wild horses.

How is the Lidocaine injected in to the pedicle?

- Via needle, an assistant pumps the drug, but the surgeon guides the needle.
- Dr. Pielstick is trying to modify the ecraseur to also hold a needle, thereby reducing the overall risk of contamination (and needle stick); having the needle on the ecraseur would mean one less trip in and out of the abdomen.

Are the mares in pain after the drugs wear off? Are they observed at night?

Hinrichs: has seen mares in pain at night after the surgery; she now gives butorphanol for 24 h after surgery, or morphine + detomidine epidural at the time of surgery.

Pielstick: They are observed for several hours after. They seem fairly comfortable. Pain is at an acceptable level; the banamine helps.

- Domestic mares are given banamine and sent home.
- They seem more comfortable than castrated stallions.

Weikel: I walked pens every morning and evening at the gather observing behavior. Mares were mostly involved in social status behaviors.

Pielstick: Finished surgery at 4pm on a good day, so not a lot of light for observations after surgeries.

Regina Turner: **What is the effect of operator experience?**

- This is a practical technique. Leon taught 9 vets in Arizona when spaying 5 donkeys. Any vet who generally works with equine reproduction can pick it up, however there is a learning curve. On one donkey they had trouble getting the left ovary out, after they finally succeeded the donkey bled to death. They did a flank incision on two donkeys and this seemed better on that species.

Are there enough trained people?

- Plenty of vets would be interested in learning the technique. If there is a complication it can be used as a learning experience to avoid future complications. There were complications with the donkeys as multiple people were learning how to do it. The chance of complications increases with the number of times you go in and out of the animal.

Cheryl Asa: **Could we have more follow up on the AZ procedures?**

- Concerns about the Arizona project include the fact that one died, one lost a 50-70 day old fetus, and others had post-operative infections.

Pielstick: The burro that died had a left ovary with a membrane around it; there was no post mortem exam to discern exact cause of death. If a female has an unusual ovary it would be best to abort surgery and not proceed.

It seems as though the training contributed significantly to the mortality there?

- If there are unusual situations then generally complications arise, and this is used as a learning curve to create a better procedure.
- It is also worth noting that the number of learning surgeons entering the body cavity of those burros could have contributed to the one in five mortality rate for those burros. Dr. Asa noted that one of the major complications could have been the training itself.

Kane: **What are the contra-indications of this technique? Does the condition of the horse affect the procedure?**

- Generally body condition will affect the choice made to do the procedure. Best to pass on very old mares or mares in poor condition.
- Late gestation will increase the complication rate, but pregnancy will not be an issue in the first or second trimester.
- Surgeons should also pass on mares with pelvic abnormalities.
- 1-2% of mares could not be sedated heavily enough to do the procedure. 1cc of ketamine would help. If a mare fails to sedate the issue is that she moves, so the procedure is not done.

Of the big group of mares that you spayed, what percentage were pregnant? Which were checked before-hand?

- Of the first 33 mares that had the procedure, 3 were pregnant (about 60-70 days). All 3 foaled.
- The 16 spayed in CA were all open.
- At Sheldon 70-80% were pregnant - up to 7 or 8½ months gestation. Ovaries were easy to reach, but it was generally a little harder with the foal in the uterus.

Did it affect pregnant or lactating mares to keep them off feed?

Pielstick: They were only held off feed for 24 hours, and were given water. Mares were released with their foals. There were no abandonment issues.

Hinrichs: does not hold off of food and has not had an issue.

Pielstick: feels holding off food is important; the less abdominal fill, the better.

Hinrichs: **How long do you take to close the ecraseur?**

Pielstick: A few seconds.

Griffin: **Could we release into the wild and worry about potential breeding?**

Hinrichs: The result of ecraseur is a very clean pedicle, as she has had the opportunity to observe via flank incision during removal of ovaries with the ecraseur. Additionally, after 2 or 3 days you can barely see the incision in the vagina, but if she is mated at that time it could open up the incision.

- There is a chance they could be in heat, but if the procedure is in fall many will be pregnant.

- There is a concern that, if they are bred shortly after the procedure, the vaginal incision could be opened by the stallion's penis. This could result in peritonitis and death of the mare.

Patricia Sertich: Not necessary to hold off feed. Instead rely on careful palpation of the uterus and ovary. 32 years experience doing ovariectomy by colpotomy. The initial incision (<1cm) is made in the vaginal fornix with a no.10 scalpel and the incision is enlarged by blunt dissection. This method separates rather than transects the muscle fibers so the incision decreases in length when the vaginal muscles contract after the tranquilization wanes post-surgery. Three days post-op the incision edges are adhered, and healed after 7-10 days. If the mare is not pregnant or less than 60-80 days pregnant she will likely tolerate copulation by a stallion in 3-7 days due to the decrease in ovarian progesterone.

Hinrichs: They could be given an injection of a long-lasting progesterone (e.g. Altrenogest) to stop the mare tolerating stallion advances.

Asa: Estrus behavior is seen in pregnant mares, but in our study they did not not allow copulation.

Pielstick: could do a study to monitor how often mares show heat after spaying.

Sue McDonnell: Spayed mares can be receptive all year round. Typically can not even put them with geldings as they would be mounted. If given the opportunity a spayed mare would tolerate the sexual advance of an amorous gelding. If over 100 days pregnant at the time of ovariectomy, and don't immediately lose the pregnancy, the feto-placental unit has taken over from the ovary for progesterone to support the pregnancy, and without the ovary will likely suppress attractivity and receptivity.

Kane: **What are the long-term complications of spaying? What is the incidence of pyometras in spayed mares?**

Gail Collins: At Sheldon 85% of mares that had been spayed and released (plus 30 mares not spayed) were recaptured. The survival rate of spayed and non-spayed mares was not different.

Hinrichs: Breeding is not a problem if the incision is elsewhere than the vagina.

- There may be a long-term risk of vaginitis and pyometra in spayed mares if bred repeatedly after spaying. There is risk of penetration into the abdomen and peritonitis if mares are allowed to breed before the vaginal incision has healed.

Hinrichs: This is unlikely as the cervix will be open due to lack of progesterone (no ovaries).

Griffin: **How long should we keep mares in captivity?**

Collins: At Sheldon they kept them in for 7-8 days before release.

Hinrichs: Has just visited a lab that does a lumbosacral epidural on mares for oocyte recovery. This would eliminate the need for lidocaine and make sure the mare does not move during the procedure. May not be feasible in wild mares. The lumbosacral was given while the perineum was being prepared; done at the level of the tuber coxae. Lab she visited has used this on client mares over 300 times successfully.

Pielstick: It may be faster to just block the ovarian pedicle.

Discussion of ventral midline and flank incision approach - *Julie Weikel*

History

- Julie has spayed about 100,000 cows by either flank or vaginal approach. The vast majority of these were spayed by the vaginal approach, however several hundred were spayed through the flank at all stages of pregnancy. These experiences are the ones that inform our discussion about spaying mares as there are many similarities in the issues faced and potential consequences. The adult cattle spays were all performed as part of federal Brucellosis eradication efforts. Under the Brucellosis control program neither sexually intact nor pregnant animals could leave a quarantined premises, hence the need for both spays and C-sections, frequently in the same individual animals. Rarely were the conditions under which these surgeries occurred ideal in any way; cleanliness, weather, adequate manpower, etc. In other words, true field conditions. While Julie always tried to obtain follow-up information about complications and survival, she did not always get that feedback. Some of the problems in cattle might inform the discussion.
- In cows you cannot reach the ovary through the vagina when they are pregnant, so had to go through the flank.
- All the mares (domestic) Julie spayed were via a single flank approach, probably less than a dozen. They were all decades ago and were for either granulosa cell tumor removal or attempts to control "nymphomania." All were done in horses used to being handled and in a clinical environment.
- Entry for flank spays utilized a skin and fascia incision followed by blunt dissection that results in a "closure" of the underlying layers of muscle and fascia such that only the skin requires suturing. Only the very bottom of that skin suture line is left open. Julie came to this procedure as a result of dealing with seromas as a not uncommon sequence in fully closed suture lines. Seromas are not a serious post surgical complication, but in field situations where any secondary handling poses additional risk to the animal, they should be avoided if possible.
- In mares always used a 9 inch burdizzo rather than an ecraseur when doing flank incisions. Has never had a hemorrhage issue with castration using the burdizzo.
- P. 869 in Loesch and Rodgerson (2003) article is very thorough in regards to complications from any non-colpotomy approach in a horse.
- Always better to do surgery with a horse standing if possible – want to avoid lying horses down. Surgical vasectomies are done recumbent.
- Surgical recovery in a wild horse is already an extraordinary event (i.e., presenting unusual circumstances).

Conditions:

- Dust control is crucial (not completely sterile by any means).
- Heifer corrals mirror field conditions.
- Any surgery poses an issue for infection at the incision site.

Training:

- Julie has taught numerous vets to spay heifers. Only a small percentage are still spaying. All found it to be quite difficult. Adopting colpotomy for wild mares means that we need to find people who have gone through the steep learning curve. Some people can develop a good feel to correctly assess what tissue you have hold of, but it takes experience to learn.
- In the west, many dude ranches do not like cycling mares. Julie works with a veterinarian who spays these mares. Other vets with this experience are a potential for pool of people to choose from for the colpotomy method.
- While Julie has usually required 100 heifers to "train" a veterinarian to spay heifers, 100 mares may not be necessary to train an already accomplished equine surgeon to perform colpotomies. Training will vary with the individuals involved and hopefully could be accomplished with many fewer animals, maybe 5-10, with time to rest between surgeries for reflection during training.

Comments:

Hinrichs: Feels there is not such a problem with training veterinarians for equine ovariectomies via colpotomy. Did ovariectomies by colpotomy for PhD work – everyone wanted to learn the technique. Reproduction vets are best because they can tell if the ovary is covered by omentum, and recognize the anatomy better via palpation than surgeons. They are more familiar with the feel of it. Katrin has trained many veterinarians on ovariectomy via colpotomy, and they have been successful. They have conducted colpotomy on 22-30 year old mares with no complications. Reproductive specialists are used to palpating mares, so the colpotomy method is not difficult to teach.

Weikel: The biggest mistake is that trainees are too eager to jump right in and because the initial entrance into the abdomen is extremely important this causes an issue. During autopsies of spayed heifers the vaginal wall penetration site was difficult to find. This was observed in heifers only because she has never lost a mare.

Griffin: **Is there a minimum number of mares to use to teach people how to do the procedure?**

Hinrichs: 3-5 colpotomies should be done under supervision.

Weikel: People who were good had it after 1-2 animals. There are others who still wouldn't get it after 100. You need people with experience or competence.

- Dust control is critical – spray pens down every day, and re-spray during the day. In her opinion, field conditions make colpotomy an attractive option.

Kane: **Is there one incision or both flanks?**

- Single incision (left flank) and does the left ovary first. Dr. Weickel uses the burdizzo to access the second ovary, carrying the tool though the abdomen.

Chemical immobilization?

- Lidocaine and adequate sedation (butorphenol). Some mares respond differently – can't settle even when sedated. These are generally released without surgery.
- Sedation is an important variable.

Issues with flank incisions?

- It's an open wound. An external wound can become an abscess.

Recovery time? Or effects on pregnant mare?

- Cannot speak first hand on the flank approach in pregnant mares.
- In heifers Weikel would attempt vaginally or go in via flank. She would C-section (after 120 days pregnancy) therefore could see the ovary and proceed.

Turner: It will be harder to find people who are good at colpotomies. Some people prefer flank incisions, and more people are trained to do it. However flank incisions can take longer to heal and be more painful.

Weickel concurred that colpotomy appears less painful.

Kane: **Can anyone comment on the access to the ovaries on pregnant mares via flank incisions?**

[No response from the panel]

Kane: **Recovery time on flank incision?**

Weikel: There is a prolonged recovery time (2-3 weeks) due to the risk of abscesses developing after surgery.

Asa: Following up with data from the burro project: 2 were spayed via flank incision. These got infected and opened up within a week. They took a month to heal. These animals were closely monitored.

Turner: You will likely be able to find more veterinarians who are trained in and comfortable with flank incisions for ovariectomy. However, there are many complications in flank incisions even under sterile conditions. Most of these are related to healing of the incision line and discomfort. On the other hand, it will be hard to find people who are good at colpotomy. When untrained people perform colpotomies there is in an increased risk that things will go wrong and sometimes things can go very wrong.

Weikel: We need to be conscious of trained personnel who are good at colpotomy to teach others to do this procedure so they can take over if there are complications.

Hinrichs: has not had issues training people, however, she is very detailed in her training methods. She goes through every complication she has experienced with each new trainee. Reiterated that she prefers reproductive vets over surgeons.

Kane: **Safety of the people involved during the surgery?**

- Colpotomy – strapping the back of the mare helped stopped the mare from kicking, plus there is a kickboard and the tail is tied up.
- Flank incision – operator can be more protected. Small window of access. Weikel does not believe that this outweighs the consideration of the colpotomy.
- Hinrichs noted that the time with the scalpel in colpotomy is only a couple of seconds. This time is longer for flank incision, which means more opportunity for fractiousness and infection.

Ventral midline incision approach: *Jay D'Ewart*

Procedure

- 8 mares were spayed using the ventral midline approach at Rock Springs, WY. All survived. It was not a blind procedure. Done under anesthetic with the surgery similar to a dog or cat. Given three-layer sutures. After surgery the mare got up and wandered to the recovery pen. The mares were watched for 2-3 weeks at Rock Springs and then sent to long-term holding facilities.

Comments:

Kane: **Were the mares pregnant? How long after were they turned out?**

- Unsure if any mares were pregnant, but it is unlikely as they had been in holding for some time prior to surgery.
- These mares were never released to the range (they are in long-term holding somewhere).

Griffin: **How would this affect pregnant mares?**

Hinrichs: It would be difficult to access ovaries via ventral midline in a pregnant mare.

Kane: **How would this affect lactating mares?**

D'Ewart: Might be able to select mares that are close to weaning.

Weikel: It is likely to be more of a problem if the mare is still sore. In heifers there is soreness due to the calf poking around to nurse. The relationship between dam and foal could be compromised. Edema could affect lactation. Flank incision might also make some mares resistant to nursing.

Griffin: **With the ventral midline procedure is there a potential for evisceration?**

Weickel: Yes, definitely. This is a primary possible complication of this procedure, and it is an awful outcome.

Hinrichs: what is the incision size?

D'Ewart: 5 inches

[Referred to Loesch and Rodgerson (2003): 25-35 cm]

Weikel: depends on the size of the ovary and size of the operator's hand.

Doug Eckery: close to the mammary gland, so may affect nursing.

- Evisceration is a horrific consequence so we want to be careful of a method in which evisceration is a complication, as in ventral midline.

Hinrichs: Although evisceration is said to be a risk of colpotomy, she has not known of any evisceration post-colpotomy.

[Neither had people at U. Penn.]

Sertich: Domestic horses that have had a ventral midline incision are usually restricted to stall rest for one month, and then only hand walked for the second month. These horses are kept with very limited activity. No data on mares that had free access to exercise.

D'Ewart: Had a successful experience in Wyoming. After anesthesia the horses are turned out in a holding pen where they don't get a lot of exercise. No eviscerations. The vet thinks it is a very teachable procedure.

King: We will be sending the briefing statement to Paul Zancanella who conducts and advocates for the ventral midline approach. He can add comments.

Ventral midline procedure - Paul Zancanella (written comment submitted after the panel)

- Mares are restrained in a padded chute and administered an induction dose of xylazine and ketamine. Upon induction an indwelling catheter was installed in the jugular vein. Anesthesia was maintained using intravenous administration of triple drip (IV-ketamine, zylazine and Guiafensin 5%) to effect. Horses are placed in dorsal recumbency. Surgical area is clipped, pre-surgical preparation was done with chlorhex scrub followed by chorhex solution swabs. Seven-inch incisions were made just cranial to the udder. Ovaries are exteriorized through the incision. A serra emasculator is applied to the ovarian stump. Number 6 MSA is used to ligate the ovarian stump proximal to the emasculator. The emasculator is removed and the ligated stump allowed to retract into the abdomen.
- Closure is accomplished in three layers. The outermost being a subcuticular layer using #6 MSA absorbable. The surgical time, induction to completion, is twenty to thirty minutes.
- Mares all received 40 ml procaine penicillin and 10 ml flunixin meglumine post surgery.
- Mares are standing within thirty minutes of surgery and eating within two hours of surgery.
- In conclusion, a ventral midline ovariectomy is a viable field surgical procedure for fertility control in mares. The surgery is accomplished relatively easily with less risk and expense than other fertility control methods.
- The ventral midline can be performed with little or no modifications to the existing facilities.
- Postoperative pain is much less than colpotomy and is easily managed with intraoperative IV flunixin meglumine.
- Operator and assistants safety is much better than standing procedures.
- Some concerns expressed for mares being anesthetized is exaggerated or naïve. Equine practitioners anesthetize thousands of horses with minimal problems.
- Ventral midline surgeries are accomplished routinely on horses without complications.
- Having performed both the colpotomy and ventral midline I much prefer the ventral midline approach.

Kane: We need sedation and anesthetic protocols for the three incision approaches.
Griffin: **Is there something additional about lying horses down that is an issue?**
Weikel: There is a lot of weight on the tissues if on its back, pressure on the aorta (cardiovascular effects), and recovery issues; these are mentioned in the review article [Loesch and Rodgerson (2003)]. When a domestic horse that trusts you is recovering you can keep them calm and help them. With wild horses this is not an option. The goal is to get away from them (and get them

away from people) as soon as possible. They can sometimes run and buck/get acrobatic; level ground is a must for recovery.
[*Hinrichs* and *Turner* agree with not laying horses down for surgery if at all possible.]
Hinrichs: We always do ovariectomy surgery standing. Injectable sedation for ventral midline seems risky, as compared to gas anesthesia. People are moving away from abdominal incisions in humans; for example in women they are doing hysterectomy etc. via colpotomy and this is associated with less pain and much faster recovery.
Weikel: There is potential for adhesions to the ovary, for example due to a history of inflammation. Those are problematic. Leon will not take such ovaries out of mares. Individuals with these inflammations are high risk - heifers walked around with the hump in their back even if they lived. Would be skeptical if there are adhesions.
King: **Is this similar to cryptorchid stallions?**

- If a mare has an ovary left she will still cycle, even if one ovary is infertile. Generally in cryptorchids they do not have adhesions, so not really comparable.

Kane: **What are contra-indications for flank or midline methods?**
Weickel: No external surgical incisions should be done on any mare that is very dirty. Any mare that is especially dirty we need to ask why is she laying down and rolling a lot. Will she be doing this post-surgical procedure? How dirty are the field conditions? At Sheldon, the corral where the surgeries took place was clean, and not used at all for many months per year.
Sertich: Would avoid ventral midline incisions in late gestation in mares (in last month before parturition) as they may go into labor. Some mares have delivered foals after midline surgery for colic, but they were watched carefully. The concern is that there may be a breakdown of the abdominal wall during delivery.
Kane: **Is there an age effect for these approaches?**
Sertich: No specific numbers, but as mares get older they are more at risk for abdominal wall rupture.
Turner: Older/multiparous mares have an increased risk under general anesthesia because they may be at increased risk for catastrophic fracture during the recovery process. Also, many physical conditions would preclude the use of general anesthesia and subsequent recovery (e.g. neurologic horses, horses with heart conditions, etc.) Any of these conditions would be contraindications for general anesthesia and therefore the ventral midline approach. Mares would need to have a full physical examination prior to anesthesia, which is unlikely in the field.
Sertich: Mares that lactate year round every year probably have lower bone density, so are more at risk of catastrophic fractures during recovery from general anesthesia. These risks are inherent to the recovery process following general anesthesia (not the method of surgery chosen). But since the ventral midline approach can only be done under general anesthesia, these potential complications would be unavoidable with ventral midline (vs. other procedures that could be done standing).

Weikel: The Sheldon mares (colpotomy) spayed were skewed towards older mares. The recovery was the same as the mares that did not have the procedure, but all of these mares were done standing and did not have general anesthesia.
Hinrichs: 8 mares 21-30 years old were given ovariectomies by (standing) colpotomy without complication.
Kate Schoenecker: **How does an ovariectomy affect the fetus?**
Hinrichs: [will be sending the research paper cited to Joanna] Mares ovariectomized earlier than 50 days – all lost their fetus. At 50-70 days about half lost their pregnancy. >140 days none lost their foal. In her own research, took ovariectomized mares off progesterone after 100 days of gestation and then had no problem.
Sertich: When mares get abdominal surgery of any kind they are also administered progesterone/progestin before and for a few weeks after surgery to prevent pregnancy loss. There is less risk of losing a foal with a colpotomy. General anesthesia for a ventral midline incision requires that the horse is off its feed, which means more muscle trauma and more stress.
Turner: Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.
Griffin: **Due to colpotomy sometimes early stage pregnancy can be lost. Can we give them long term progesterone injection to save the pregnancy?**
Hinrichs: It could be possible to keep ovariectomized mares pregnant by injecting progesterone. At <50 days of gestation won't find evidence of fetal loss. Pregnancies of 50-70 days might be helped by a long-lasting progesterone shot. Pregnancies over 70 days are likely to be maintained even without exogenous progesterone.
Kane: **What product would you recommend?**
Hinrichs: Not sure on commercial availability of certain products. BET had a long-lasting progesterone and long-acting altrenogest that was available. (Checked subsequent to the panel: 30-day altrenogest injection still available).
Kane: **Potential for a feed through on mares that die due to this procedure in the wild?**
Hinrichs: Main issue is if an animal were to eat the area of the mare where the progesterone is injected, but progesterone is a natural hormone and the consumer would just receive an oral dose. (Note added later: Altrenogest does have the potential for feed-through).
Kane: If gestational stage is <50 days, we should consider accepting fetal loss. If stage is 50-70 days, one could consider injecting a drug to maintain the pregnancy.
Hinrichs: **Does anyone know what time in gestation an abortion is visible?**
Kane: It is very difficult to find aborted fetuses, even in a stall. Most that are found are close to term (size of a beagle). There are behavioral signs preceding abortion. We want to avoid abortions to the extent possible.

- Beagle size is 150 or more days (5-6 months) gestation.

Collins: We rarely found aborted fetuses in corrals. Only a few times did we find beagle size fetuses.

How often is the fetus reabsorbed?

Sertich: It is thought to be expelled/passed out through the cervix and not found, rather than resorbed.
Weikel: When it's hormonal abortion we are less likely to see discharge from the mare. When it is infection one sees a messier discharge.
Kane: Horses are corralled for testing purposes and held for 30-90 days. We vary rarely see secondary complications in mares that abort a fetus. The complications with abortion of foals are minimal. The mares do not show signs of illness, pyometra, or founder. The mares are generally farther along when we see complications, if any.
Hinrichs: In Argentina the polo industry is aborting hundreds of male foals, then within a few weeks transferring another embryo to the same mare with no ill effects.

Discussion of Laparoscopy: *Dean Hendrickson*

Procedure:

- Done a lot in horses at CSU. Performed in domestic horses with standing sedation. Typically approach both flanks. Can do it through one flank but this takes more time. Typically it takes 40 mins to remove both ovaries (doesn't include time for preparing/sterilizing horses for surgery, which brings the total to 50-60 minutes per animal).
- Equipment needed is a laparoscopic tower, light source and monitor, camera box, CO_2 canister, insufflator, and one standard surgery pack per animal (scalpel, blades, clamps, etc.).

Recovery time:

- Some are sent home the same day or some stay over night. There is minimal postoperative care.
- Has done the surgery in a field setting (laparoscopic vasectomy of elephants in Africa). Can keep the animals/instruments clean and sterile enough with cold sterilization.
- Standing sedation may not be possible in a wild horse for this surgery.
- The effect on the fetus if the hormones are not maintained (at less than 50 days gestation) is that the mares will generally abort. It is most likely that there is the same level of risk as for other procedures.

Comments:

Hinrichs: **What is the sterilization time for the instruments?**

- About 20 minutes to sterilize between animals (would work better with 2 sets of instruments to avoid wasting time).

Jason Bruemmer: **If you were to do it from only one side does it affect your time of completion?**

- Yes, it can take up to an hour standing sedated in a squeeze chute if surgery is from one side.

Sertich: When we do ovariectomies by laparoscopy they allow 1 to 1.5 hours, which includes scrubbing the flanks. They make incisions from both sides to get both ovaries.

Collins: **How is sedation maintained?**

- IV jugular catheter, continuous sedation drip. More consistent than with a bolus.
- Have done epidural (caudal) which also works well, but not sure this will work for wild mares.
- With this procedure in elephants they are under general anesthetic and hung in a sling from a crane so there is access to the flanks. Not sure if this is possible for horses.

What is the size of the incision?

- Ovaries are removed through the flank. Incision size is dependent on the size of the ovary (just enough to pop the ovary through), normally 4-10 cm.
- In 2% of cases there is incisional drainage, and in 10% there is subcutaneous emphysema from the CO_2 insufflation.

How are they managed post operatively?

- 1 dose of antibiotics pre-op and 3-5 days of NSAID post-op.
- Kept in a pen for 1 week, and then normal work after 2 weeks. But would not have a problem waking the mares up and sending them off.

Is it possible to use the laparoscope vaginally?

- Yes, there have been two reports using it to aid in colpotomy.

King: **Have you ever carried out this procedure on a pregnant mare?**

- No.

Hinrichs: **What type of epidural do you use?**

- Caudal epidural as it reduces movement. But there is no flexibility in that method of sedation as if you put in too much it's hard to keep them standing, and too little means they aren't sufficiently sedated.
- 40 µg/kg detomidine is used. Lidocaine would require too much volume to be used as an epidural.

Do you use lidocaine on the ovary?

- Yes – injected into the ovarian pedicle (15 ml local anesthetic injected to the pedicle).Give an epidural for analgesia of the reproductive tract, rather than the flank.

Kane: **Can you comment on the ventral approach?**

- It makes laparoscopy more difficult. When dorsal it is harder to get to the ovaries and find them. Ovaries present better when standing from the flank.
- In the small animal world they put the animal head down tail up and tip them from side to side. It might be possible to roll horses one side to the other to get to the ovaries.

Kane: **What are the general equipment costs?**

- $25,000 for camera, light core, light source, monitor, Thorston telepacs (sp.?)
- $5,000 - insufflator
- $3,000 - microscope

- $750 x 6 for hand instruments
- $40,000 total new, or could buy used.
- You would need two sets of instruments to be efficient, so that one set could be cold sterilizing while the other was in use.

Kane: **Contra-indications for this method?**

- You are very dependent upon technology – if something breaks and you don't have a duplicate then you need to stop. It takes time to get used to the approach and do it.
- Worst-case complications would be puncturing a bowel. Withholding feed tends to reduce this risk.
- It is possible to drop ovaries in the abdomen. There is no proof that this is an issue but could be.

Durability of equipment?

- Not a huge worry – machinery is not that delicate. The monitor is most delicate - if kicked it could break the screen.

What do you recommend for pre-op?

- 12 hours off feed but not critical. The more comfortable you are with the tech the less you need to worry about feed – 3 hours would be fine. Time off food possibly upsets them. Elephants were never held off feed and they have similar digestive tracts.

Hinrichs: **How long does it take to learn the procedure?**

- With 3 days and 5 or 6 mares you can get someone up to speed. Doing it regularly helps. Has learned how to teach it well. Ovaries in a standing mare are easy to access. It is not difficult to do an ovariectomy.

Kane: **Can you guess what would happen in a pregnant mare?**

- Pregnant mares present ovaries well if standing. It could be easily done.

Do you have any ideas about slinging them under anesthesia in a chute?

- Can use the same concept as hoisting horses up and out of ditches. You can use this to keep them into position.
- You could potentially have two teams working on either side of the abdomen and cut your time shorter.

Hinrichs: **What is the infection rate?**

- It's about 1/10 less when the instruments go in and out of the body cavity through a cannula, rather than flank laparotomy where they go repeatedly in and out through an incision. Instead of 5 in 100 you'll get 1 in 100.
- We don't see them commonly in open flank procedure.

Kane: **How safe is this procedure for the operator?**

- Coming from the flank you can avoid the feet, but he has still ended up with bruises and a broken toe.
- The difficulty is the amount of equipment and two people puts you a little more at risk than standing flank ecraseur, but slightly safer than a colpotomy.

How do you feel about training on a large scale?

- He feels good about training people fully. Colpotomy is rough because you cannot see what you are doing. One could use the laparoscope to train colpotomy.
- Won't walk away from someone until comfortable that they could do it.

Hinrichs: When training students on colpotomy, the student will find the ovary and place it in the loop of the ecraseur, then she will put her hand in and make sure it's an ovary before anything is cut.

Griffin: **Can you comment on the approximate cost the procedure will be per animal?**

- CSU charge is $1400 to do mare ovariectomy at the hospital (including technician time).
- Could do it in the field for $250/ horse for procedure alone (not including salaries).
- Did ovariectomies on dude ranch mares in Durango – 11 horses in 3 days. The real cost was $450-$500 per horse.

What is the cost per colpotomy and ventral midline?

Collins: Colpotomy was $250-$300 per horse at Sheldon NWR.

- Ventral midline is approximately $350/horse at Rock Springs, WY.

Fragility of the equipment? Is the jostling of the wild mares going to be an issue for the equipment?

- Wise choice is per 100 mares look to replace 5 instruments (this is the rate with people who are not fiscally responsible for the equipment).
- More likely to break hand instruments than the scope.
- Laparoscopy is the most expensive approach, but there is direct visualization, low morbidity, and better public opinion.

Schoenecker: **Do you cold sterilize?**

- Yes. Clean with soap and then water, rinse with sterile water.

What happens with dust?

- Blowing dust is less of a problem as the instruments are solid. Has been able to brush dust off during elephant procedures.

Sertich: **Infection issues or seromas?**

- One does get some seromas but no big problems with them. 1-2% incisional complications, but those rates are for mares in a clean and controlled environment. Opening and draining generally fixes this.
- No long-term negative or infection rates. In a field setting there may be a 2-3 x higher risk, but this is still small and corrects on its own.

D' Ewart: **Is there a difference in laparoscopy vs just through the flank?**

- You can see what you are doing with lap.
- You can ligate the pedicle with two ($10) ligatures to cut sharply, rather than putting the ecraseur through with a flank incision.
- Two ligating loops is faster than the radiofrequency "Ligasure".

- There is a radio frequency device for cauterizing the ligated tissue, but it is expensive and finicky, and is just one more piece of equipment to worry about that may halt surgeries.

Bruemmer: **Can you use umbilical clamps?**

- Seems like you could. Thicker pedicle in the horse than cattle.

D'Ewart: **What is the infection rate difference in flank than scope?**

- 5% incisional infection for flank.
- 2% with laparoscopy.

Hinrichs: **Is the opening for the ovary a straight incision? How is it closed?**

- Incision of 5-10 cm modified grid. Close fascia and skin in continuous pattern.

D'Ewart: **How do you manage pain?**

- A lot of the pain comes from the flank incision. It is helpful to anesthetize the pedicle (with carbocaine).
- Since laparoscopy incision is smaller than with the flank laparotomy method, it is mildly less painful but not a huge difference.

Behavioral estrus post spaying: *Cheryl Asa*

History

- 10 ovariectomized mares and 10 anovulatory mares were put with stallions and monitored for 15 days in January. The study was published in 1980.
- Of the 20 mares all showed at least one day of weak estrus. One mare showed full estrus all 15 days. Most had at least some mounting and were accepting of a stallion to full copulation.
- Most notable is that there was nothing cyclic (it was on and off day to day).
- Considering our various studies, only when a mare had elevated progesterone (e.g., diestrus, pregnancy) was there an absence of estrus and copulation. The adrenal cortex produces sex steroids. Estrus was not shown in mares with adrenal cortical hormone production was blocked with dexamethasone, suggesting that it is adrenal sex steroids that support estrus in mares that are ovariectomized and during the non-breeding season.
- It would be interesting to know whether this represents what mares would do in a naturalistic circumstance.

Comments:

Weikel: **What was the relationship between stallion and exposure? And relationship to spay?**

- Mares and stallions were kept separate and introduced (several mares and a stallion in a paddock) and allowed to interact freely.

Weikel: The increase in breeding activity may have been because the mares were new to the stallion. In Asa's study, the stallions were not with the mares except during the study.

Collins: Didn't monitor for sexual behavior at Sheldon but did analyze band association. Spayed mares maintained their band associations, and were in mixed groups. They were not found in bachelorette bands, or as solitary animals.

Is there a more natural setting?

Asa: Horses stay together year round (unlike most animals), so behavioral estrus may be a mechanism for the band to stay together outside of the breeding season.

Collins: Mares that were not coming into estrus were not driven off or kicked out the band.

Hinrichs: **In an intact band full of intact mares what portion of those mares would not be pregnant?**

Collins: 45-50% recruitment in Sheldon.

Asa: Reviewed horse literature for the NAS report. It looked like there was a trend where mares on BLM range were reproducing about every other year.

Kane: varies from HMA to HMA. 60-75% of mares are coming up pregnant based on fecal testing or foaling. There is 75-80% pregnancy rate based on fecal testing or foaling, observed at Teddy Roosevelt NP.

Collins: Most foal deaths were within the first 2 months of life.

Griffin: If a foal survives to fall, then typically BLM would consider it part of the population. "Recruitment" is survival to nearly a year old.

King: **Any other observations of behavioral differences in mares post ovariectomy?**

Sertich: Teaser mares (stimulus mares for semen collection) are ovarectomized. They are sexually receptive to stallion advances every day for the rest of their lives. Sometimes ovariectomized mares are administered estrogen to encourage proceptivity, but most of the time they are not. Ovariectomy to prepare a stimulus mare is common in the horse breeding industry for semen collection to be used for artificial insemination.

Griffin: The main reason we are asking USGS to conduct this study is because this behavior is not well known, so part of the purpose of this study is to discover that.

Griffin: **An injection of progesterone would create more time for the mare to heal by making her less receptive to being bred?**

Hinrichs: Yes. A long-acting progesterone given at a concentration of 1-2 ng/ml could suffice. They should reject the stallion for about 7-10 days.

Kane: **Any concern about exogenous progesterone affecting post-operative uterine infection rates?**

Hinrichs- There could be concern, but because the cervix may open shortly thereafter, when the progesterone level falls, this should be self-limiting. Some contamination could occur during the procedure, but because of antibiotics the likelihood of infection would be low.

Collins: **If you are doing the surgery when is the injection being administered?**

Collins: At Sheldon we held animals for a week, and restricted the number of times they handled the animals.

Hinrichs: If you give injection at the time of ovariectomy it should last 7-10 days, which would be OK if the mares are released in 2-3 days. Otherwise would need to be given later for a later release, and it would be better not to handle the horses again.

Were the stallions held?

Collins: Depended on the studs (they were doing vasectomies). Some were turned out sooner than the mares, but not always.

Did the mares get back to the same harem group?

Collins: They were not released all at once and this was not monitored as some were adopted.

Griffin: **Is a week enough to heal?**

Weikel: Yes, the healing rate is very quick with colpotomy.

Hinrichs: To be safe maybe one should allow 2 weeks before the mare is repeatedly bred in the wild in order to avoid catastrophe. A stallion can rupture even an intact vagina. It takes a surgical incision 7-10 days to fully heal.

Risk is running through chute once or keeping them in captivity?

Weickel: Checking the BET website, there is a 30 day progestin injection, Altrenogest, that could also be given. Could it promote uterine infection?

Hinrichs: if the uterus was contaminated at surgery, but as noted before, this is unlikely.

King: **Could progesterone injections also be given to control animals** (to reduce any confounding effects of progesterone injection on comparisons of observed animal behavior)?

Hinrichs: This drug has been thoroughly tested in pregnant mares and shown not to be detrimental to the mare or foal.

Mare menopause approach: *Doug Eckery*

- The aim is to develop a vaccine. GonaCon and PZP are currently available and both have been shown to be effective, but for a limited time. PZP prevents fertilization; GonaCon prevents ovulation. A vaccine is only good as long as antibodies can be maintained. PZP, in particular, requires many booster shots. Follicles are constantly replenishing, so mares need a booster throughout their lifetime.
- The idea is to target something earlier in ovarian function. Successful reproduction in female mammals depends on an adequate supply of eggs that are found in primordial follicles. However, there is only a finite supply of eggs in the ovaries, that if destroyed would cause permanent sterilization. Every day a certain number of primordial follicles begins to grow, and only a very few ever reach the final stages of maturation and go to ovulation. The initiation of growth is a committed step and controlled largely by local growth factors. Oocyte-specific growth factors are involved in the early stages of follicular growth. A research team in New Zealand found that a certain group of infertile sheep had a mutation causing infertility. The research goal is to come up with a vaccine to mimic this mutation but for horses. If the primordial follicles do not mature then the animal is sterile. For sterility you need to deplete the ovaries of eggs by directly killing the eggs/primordial follicles or by

causing a mass activation of the primordial follicles (once growth is started it cannot be stopped).

- We are going to test vaccines against oocyte specific growth factors in horses. This will prevent follicular growth and ovulation in the short term, but hopefully will eventually deplete eggs leading to mare menopause, but this is a hypothesis.

Timeline:

- Start research in November by vaccinating mares against the growth factors. After 1 year will conduct a unilateral ovariectomy and look at the ovaries under the microscope. Throughout the year will conduct hormone testing. AMH levels will be measured, as this can be one index of follicular reserve. The project will run for 2 years. Also will track ovarian function through ultrasound and behavior through teasing.

Comments:

Kane: **When would you expect them to be sterile? Are you planning a fertility challenge?**

- We do not expect animals to continue to cycle. The follicles will not produce any steroids so behavior will be changed. Eventually we will conduct fertility testing. It is unknown how long it will take to deplete the ovaries. This is a test to see if the mechanism will work.
- Vaccine and booster will be 6 weeks apart. Next step is to develop a vaccine.

Kane: single shot vaccination is a lofty goal.

Schoenecker: **What happens if given to a pregnant mare and depletes her eggs?**

- The vaccine should have no effect on an existing pregnancy as it is not affecting the corpus luteum.
- The time frame of egg depletion would take longer than the term of a pregnancy, so therefore no effect on the pregnancy.

Kane: **What about the effect on the fetus?**

- Antibodies would probably not pass through the placenta to affect the foal, and probably not in the colostrum long enough to affect the foal's antibodies. But it is unknown whether there would be a long-term effect on the offspring.

How to Perform Hysteroscopy in the Mare

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1. Introduction

Hysteroscopy is not a common procedure in broodmare practice. A thorough breeding soundness examination that includes transrectal palpation and ultrasonography, endometrial cytology, endometrial biopsy, endometrial culture, and vaginal/cervical speculum examination will detect many reproductive maladies frequently encountered in the mare. However, in some circumstances, these more commonly employed techniques are insufficient to clearly identify or ameliorate the problem, and hysteroscopy has been used to achieve this end. In an early study, 17 of 40 mares admitted to a veterinary teaching hospital with a history of infertility had reproductive tracts that were considered normal on palpation, yet hysteroscopy revealed abnormalities in 26 of these 40 mares.[1] Since that time, abnormalities observed through the use of transrectal ultrasonography have either obviated the need for or alternatively prompted the use of hysteroscopy to render a more definitive diagnosis or treatment.

The use of hysteroscopy in the mare was described as early as the 1960s.[2] Subsequently, many authors have published articles on the use of hysteroscopy in the mare for a variety of purposes.[1,3–9] While the term hysteroscopy will be used throughout this manuscript, it is also called uteroscopy and has been referred to as examination of the uterus via fibroscopy, endoscopy, and videoendoscopy in other publications.

2. The Equipment

A flexible videoendoscope, 100 cm in length and 1 to 1.4 cm in diameter, is commonly used for hysteroscopy. The unit should have sufficient light intensity and image quality to optimize visualization of the uterine lumen. Most modern units are equipped with a video monitor, which enhances the examination. The use of recording equipment to capture still and video images is highly recommended to enable review of the procedure and to provide a record of the examination. Ideally, the insertion tube should have a working channel that accommodates ancillary equipment such as catheters, grasping forceps, biopsy forceps, electrocautery loops, and laser fibers.

Since endoscopy equipment is used for examination of a variety of body cavities, it is important that it be thoroughly cleaned and sterilized according to the manufacturer's instructions between uses. Particular attention should be paid to areas where moisture may accumulate, as these sites provide an environment that facilitates the growth of *Pseudomonas aeruginosa*. Persistent endometritis with *Pseudomonas* as well as growth of other organisms has been reported following hysteroscopic proce-

NOTES

dures.[10] If the equipment has undergone chemical sterilization, it is also imperative that the insertion tube, including all of its channels and the water reservoir, be thoroughly rinsed to remove the disinfecting solutions as these chemicals can be very caustic to mucosal tissues.

3. Preparation of the Mare

The mare should be placed in stocks with the tail wrapped and deviated away from the perineal area. Feces should be evacuated from the rectum and the perineum of the mare should be cleansed in an aseptic manner. Sedation and analgesia should be administered. Most practitioners have a favorite sedation protocol, and the following is the author's preferred method. Depending on the size and temperament of the mare, she is administered detomidine HCL[a] (0.006–0.01 mg/kg, IV), and a catheter is placed in the jugular vein. Depending on the anticipated length of the procedure, 5 or 10 mg of detomidine HCL is added to a 500 or 1000 mL bag of saline, respectively, which is then connected to the catheter in a routine manner. The detomidine/saline mixture is then administered by slow drip until the desired level of sedation is achieved. If necessary, more can be administered during the procedure as needed. The author prefers this method because it maintains a more even level of sedation and avoids the wide fluctuations in sedation that can occur over time with repeated larger boluses of sedative. After the mare is sedated and just prior to the start of the procedure, butorphanol tartrate[b] (0.01–0.02 mg/kg) is administered intravenously thought the catheter.

4. Basic Procedure

Under ideal circumstances, at least three people should be present to perform the hysteroscopic procedure; one person remains at the head of the mare for restraint as well as monitoring and administering sedation, one person inserts and maintains the position of the insertion tube through the cervix, and one person operates the steering controls of the insertion tube and directs the overall procedure.

After the insertion tube has been rinsed thoroughly, the image should be "white balanced" to optimize visualization. It is easy to become disoriented once the insertion tube enters the uterus, especially if rotation of the tube has occurred. Therefore it is helpful, prior to inserting the instrument into the mare, for the operator to select a point of reference and become familiar with the directional controls of the steering tube.

A small amount of sterile lubricant is applied to the end of the insertion tube, avoiding contact with the lens, and the assistant wearing a sterile glove and sleeve carries the insertion tube through the vulva and vestibule into the vagina so that the cervix can be visualized (Fig. 1). The cervix is then penetrated and slightly dilated with the index finger, and the insertion tube is passed through the cervix into the uterus. Once inside the uterus, the lumen needs to be distended to allow visualization. While the hysteroscopic procedure can be performed at any time in the mare's cycle, diestrus is preferable because, at that time, the cervix is of sufficient length to be grasped around the insertion tube and maintain uterine distention. Administering progestogens to estrous or anestrous mares for 5 to 7 days prior to the procedure can also accomplish this. In periovulatory mares, the cervix may be sufficiently relaxed that the entire hand carrying the insertion tube can be placed though the cervix into the uterine body and then the hand pulled back against the internal cervical os to act as a plug to maintain distention.

Fig. 1. Endoscopic view of the cervix.

Distention of the uterine lumen can be accomplished by insufflation with filtered air administered via the endoscopic equipment or by a sterile, isotonic fluid such as saline or lactated Ringer's solution (LRS). Note that insufflation with air can cause endometrial hyperemia, which should not be misinterpreted as inflammation. The method of distention depends on clinician preference and the purpose of the procedure. Regardless of which method is chosen, over distention of the uterus should be avoided so as not to cause significant discomfort to the patient or damage to the uterus. One study determined that a hysteroscopy can be performed safely and efficiently within a pressure range of 12.8 to 28.6 mm Hg and that clinical or cardiac signs of discomfort or effects on the circulatory system were only registered after pressures significantly exceeded 100 mm Hg.[11] Precise monitoring of intraluminal pressures is not practical in most clinical environments, so it is recommended to use only the amount of distention necessary to optimize visualization and to continuously monitor the mare for signs of discomfort.

Insufflation allows visualization of normal structures as well as the relative amount and location of

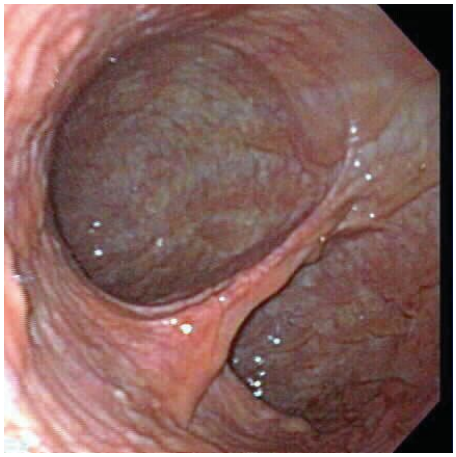

Fig. 2. Bifurcation of the uterine horns viewed after insufflation.

Fig. 3. Uterotubal papilla at the uterotubal junction.

foreign materials such as purulent exudate or blood, etc. Samples of exudate, foreign material, or other abnormalities can be obtained via instruments introduced through the working channel. However, when using insufflation, the lens of the insertion tube may require repeated rinsing to remove mucus or other debris during the procedure. Distention with fluid tends to keep the insertion tube lens free of direct contaminants, but visualization can be compromised if significant amounts of exudate, blood, or other materials become suspended in the distention fluid. If this occurs, drainage or aspiration of the cloudy fluid followed by redistention is recommended prior to proceeding with the procedure. Fluid distention of the uterine lumen can be accomplished using the fluid reservoir or the working channel of the endoscope, but this can take an inordinate amount of time. The author prefers to attach a stallion catheter to the insertion tube with waterproof tape so that the two can be passed through the cervix simultaneously and maintained in proximity. The distal end of a typical IV extension set is then inserted into the belled end of the stallion catheter, and this system is connected to a 5 L bag of LRS or other sterile, isotonic fluid. If drainage of the distention fluid becomes necessary, the extension set can be disconnected and the fluid allowed to escape via gravity flow, or a suction device can be attached to the stallion catheter to facilitate drainage.

Once the uterine lumen is sufficiently dilated, the uterine body and bifurcation of the uterine horns should be visualized (Fig. 2). The insertion tube is directed toward the uterine horn of choice and slowly advanced proximally through the entire length of the horn until the uterotubal papilla at the uterotubal junction (UTJ) is observed. This structure can vary somewhat in appearance being relatively flat and somewhat difficult to visualize in some mares to very prominent in others (Fig. 3). The instrument is then slowly withdrawn to the level of the bifurcation and then the opposite uterine horn is similarly examined. The character and color of the endometrium is observed throughout the examination; it should be relatively uniform in its entirety. The color is typically a pale pink, but the extent of endometrial folds observed can vary with the degree of luminal distention. If the examination is performed during estrus, the surface of the endometrium will typically glisten with mucus, and the endometrial folds will be visibly edematous (Fig. 4).[9] Any abnormalities such as discoloration, ulceration, plaque-like lesions, biofilm, cystic structures, luminal protuberances, adhesions, foreign bodies, or accumulations of blood or exudate should be digitally recorded and sampled as necessary. When the examination is completed and the insertion tube is being removed, the majority of air or fluid used for luminal distention should be removed either passively or by gentle suction. If the fluid distention

Fig. 4. Endometrial folds of the uterus prior to optimal distension.

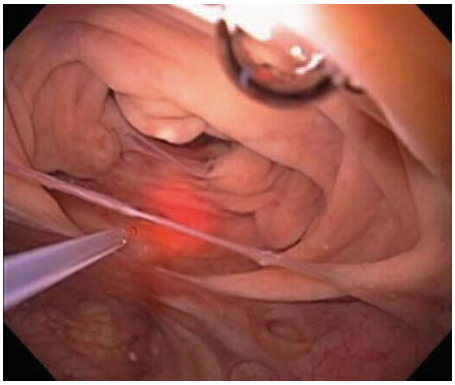

Fig. 5. Laser fiber approaching a thin transluminal adhesion prior to photoablation.

Fig. 7. Multiple transluminal adhesions viewed by hysteroscopy after multilocular fluid filled structures were detected via transrectal ultrasound.

technique was employed, fluid remaining in the bag can then be used to further lavage the uterus.

5. Common Applications

The most common use of hysteroscopy in our practice is for laser ablation of endometrial lymphatic cysts using an Nd:YAG or diode laser. Various methods of cyst ablation using hysteroscopy have been previously described in more detail and improvements in pregnancy rates have been reported following these procedures.[12–16] The cysts are first identified using transrectal ultrasonography, and their size and location are recorded in the mare's record. The mare is prepared as described above, and the entire uterus is examined noting the location of any visible cysts prior to initiating cyst ablation. Either air insufflation or fluid distention can be used. The author prefers to use fluid when possible because, in addition to keeping the lens clean, no smoke is produced while ablating the cyst in the fluid environment as can occur with insufflation, which results in temporary obscurement of the visual field. Another advantage of fluid distention is the beneficial effect of concurrent uterine lavage. Laser ablation can also be used for removal of small transluminal adhesions (Fig. 5). Note, however, that when fluid is used for luminal distention, more wattage will be necessary to achieve the same degree of cauterization as would occur if air insufflation were used.

Hysteroscopy has also been used for direct examination of the endometrium when suspicious, nondefinitive findings are observed via transrectal ultrasonography. Examples include foreign bodies appearing as hyperechoic structures, differentiating of large multiloculated structures as cysts or transluminal adhesions (Figs. 6–8), or suspected uterine tumors.[17–20] Retrieval of foreign bodies and endometrial biopsy procurement via hysteroscopy has recently been described.[8] Attempts to suppress estrus with marbles has been attempted by

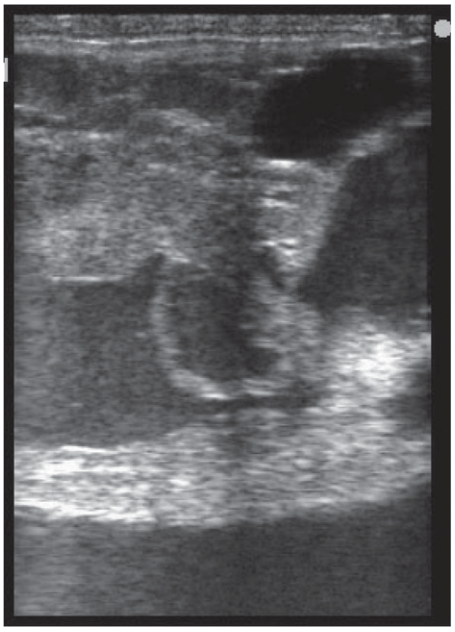

Fig. 6. Ultrasonographic image of the uterus of a mare with large multilocular fluid filled structures.

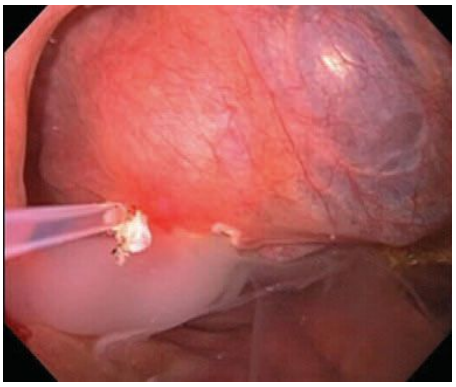

Fig. 8. Purulent fluid being released during laser photoablation of a large transluminal adhesion.

a number of practitioners, sometimes with disastrous results, when multicolored marbles are used and they disintegrate within the uterine lumen causing significant damage. Although large fragments could be retrieved via hysteroscopy, the disintegration often results in numerous minute glass fragments that become embedded in the endometrium and are virtually impossible to remove. While biopsies can be obtained via flexible instruments passed through the working channel, samples obtained are typically small and shallow whereas using a standard biopsy instrument passed along the side of the insertion tube will provide superior samples.[8,21]

Two manuscripts describing direct hysteroscopic insemination of the UTJ with low numbers of sperm were presented at the AAEP meeting in 1998.[22,23] However, achieving direct cannulation of the UTJ is too difficult for routine use and, subsequently, deposition of semen on or near the UTJ was shown to be an effective method of low dose, deep horn insemination.[24] Comprehensive summaries of hysteroscopic insemination studies have been published.[25–27] While hysteroscopy insemination allows direct visualization of the UTJ, semen deposition at the tip of the uterine horn using a manual, transrectally guided technique has been shown to be equally effective and is more economical and efficient for most practitioners.[28]

Recently, Inoue described direct cannulation of the UTJ and deposition of dye into the oviduct as a method of determining tubal patency.[29] He proposed that the difficulty others have experienced in cannulating the UTJ was a result of over insufflation of the uterus, which makes it difficult to center the UTJ in the center of the endoscopic image. Therefore, it was recommended that the endoscope be passed up the uterine horn to the level of the UTJ with minimal insufflation of the uterus.

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Effect of ovariectomy on serum adiponectin levels and visceral fat in rats

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Summary

This study was aimed to examine the effect of ovariectomy on visceral fat, serum adiponectin levels and lipid profile. Forty-five female Sprague Dawley rats were divided into three groups (n=15 each): ovariectomized group (OVX), ovariectomized plus estrogen-treated group (OVX+E_2), and sham-operated group (SHAM). Body weight, abdominal adipose tissues, serum adiponectin and lipid profile were measured and compared among the groups after three-month feeding post-surgery. Significant increases in body weight and visceral fat were found in ovariectomized rats when compared with sham-operated ones and significant increases were also observed in serum adiponectin, triglyceride and very low density lipoprotein cholesterol levels in ovariectomized rats. Body weight, visceral fat and serum adiponectin levels were profoundly reduced in OVX+E_2 group as compared with OVX group. It was concluded that ovarian hormone deficiency induced by ovariectomy leads to significant increases in body weight and visceral fat, along with increased serum adiponectin, triglyceride and very low density lipoprotein cholesterol levels in rats. Attenuation in these changes can be achieved by estrogen supplementation.

Key words

ovariectomy estrogen post menopause serum adiponectin visceral fat

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Urinary androgen concentrations and social status in two species of free ranging zebra (*Equus burchelli* and *E. grevyi*)*

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Summary. In both species of zebra, breeding males had higher urinary androgen concentrations (ng androgens/mg Cr) than did non-breeding bachelor males (30·0 ± 5·0 (N = 9) versus 11·4 ± 2·8, (N = 7) in the plains zebra; 19·0 ± 2·2 (N = 17) versus 10·7 ± 1·2 (N = 14) in the Grevy's zebra). In the more stable family structure of the plains zebra (single male non-territorial groups) variations in androgen concentrations could not be ascribed to any measured variable. In the Grevy's zebra, androgen values were significantly lower in samples taken from territorial (breeding) males which had temporarily abandoned their territories (N = 4) and the urinary androgen concentration for a male on his territory was negatively correlated with the time since females last visited the territory.

Keywords: zebra; testosterone; androgens; territoriality; social behaviour; dominance

Introduction

Members of the family Equidae show two patterns of social organization (Klingel, 1975). In the first type, typified by the plains zebra, *Equus burchelli*, a single breeding male is found in constant association with a fixed group of 1–6 unrelated females and their offspring. These groups, or harems, show little sight fidelity. Mating activity shows seasonal peaks coincident with periods of food abundance, but mating may occur at any time throughout the year (Klingel, 1969). Bachelor males remain in fixed membership groups separate from harems and have never been observed mating with adult females (Klingel, 1969). In the second type of social organization, represented in this study by the Grevy's zebra, *Equus grevyi*, there are no permanent bonds between any two individuals nor is there any seasonality in breeding (Klingel, 1974; Ginsberg, 1987, 1989). Breeding males defend territories in which they retain exclusive access to oestrous females when present. Klingel (1974) never observed bachelor males mating. Of 94 copulations observed by Ginsberg (1987), 87 (93%) were made by territorial males while on their territories.

For this study, data were collected from a free ranging population of zebras in their natural habitat to examine whether there is a potential hormonal basis for both intra- and inter-specific differences in male behaviour. Data from other studies suggest that it is likely that there is an endocrinological basis to the different behaviours observed in the various groups of males. In the congeneric domestic horse, *E. caballus*, the secretion of luteinizing hormone and testosterone show seasonal variations (Berndtson *et al.*, 1974, 1983) as do seminal and behavioural characteristics

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(Pickett *et al.*, 1970; Thompson *et al.*, 1977) suggesting an endocrine mechanism. The seasonality of these changes coincides with the breeding cycles of females.

Materials and Methods

Study area. Data were collected in the Buffalo Springs Reserve, in the region around the town of Archer's Post, Kenya (37°30′ E,0°40′ N). The study area, while climatologically homogeneous (dry and hot), is vegetationally diverse. This diversity is apparent even on a small scale (Barkham & Rainy, 1976). Average rainfall for the Archer's Post Station is 365 mm (Government of Kenya, 1984) with the maximum rainfall occurring in two seasons, March/April and October/November.

Definition of behavioural status of males. Urine samples were collected from known individuals identified by their unique stripe patterns (Peterson, 1972; Klingel, 1974). In both species, males were placed into one of two categories, breeding or bachelor, according to differences in behaviour. In the plains zebra, an adult male was considered to be a breeding, or harem, male if he was seen in association with a particular group of females and juveniles on at least 90% of all observations. In contrast, bachelor males were nearly always observed in fixed membership groups with other bachelor males.

In the Grevy's zebra, neither bachelor males nor territorial males were seen in consistent association with other individuals. Territorial males make frequent forays off their territories and were usually seen in association with bachelor males at these times (Ginsberg, 1987). While on territory, the breeding male exhibited typical patterns of behaviour (calling, marking), a distinctive posture, and dominance over all other individuals (Klingel, 1974).

Behavioural measurements were taken on 1084 individual Grevy's zebra identified by their stripe patterns. Zebra stripes are individually distinct (Klingel, 1974) and identifications were made with an accuracy of at least 98% (Ginsberg, 1987). Information was collected daily on the distribution and identity of the zebra in the study area. Point-sample data, collected for 9795 sightings (5061 males; 4734 females) during the entire study, provide records of associations of individual zebra. Of course, not all zebra were identified on all days nor, given the scope of the study, were animals observed continuously.

Urine collection. In both species, urinary testosterone concentrations of potentially breeding males (territorial for the Grevy's zebra, and harem breeding for the plains zebra) were compared to those of non-breeding males. Within the sub-sample of breeding males, variations in androgen concentrations were examined to determine the effect of time of day, season, and association with both bachelor males and females. Urine was collected from zebras in April, May and June of 1985. Males were observed urinating and the urine was aspirated from the ground using a sterile 10-ml syringe. Samples were collected within 5 min of urination and were stored in glass jars, packed on ice and frozen on return to camp at −5°C. Specimens were transferred to Nairobi on a monthly basis and stored at −20° C. At the time of collection, the following data were recorded: time of day; individual identification; group composition (no. of males, no. of females, presence/absence of oestrous females); breeding male or bachelor male; if territorial, on or off territory. In addition, for territorial male Grevy's zebra, the time since last association with either bachelor males or females was calculated from data collected on daily surveys of all individuals within the study area.

During the period in which urine samples were collected, none of the plains zebra harem males were observed in association with oestrous females. Because the duration of oestrus in the plains zebra is brief ($\simeq$ 1 day, J. R. Ginsberg, unpublished data), the possibility that a harem male was in association with an oestrous female the day preceding collection of a sample cannot be eliminated.

Androgen assay. All androgen values are expressed relative to urinary creatinine (Cr) concentrations to allow for correction of variation in urinary water content. The procedure of Taussky (1954) was used to measure Cr and the sensitivity of the assay was 0·1 mg/ml urine.

The urine samples were subjected to acid hydrolysis. An aliquant of each urine sample (0·4 ml) was diluted 1:2·5 with water and 0·2 ml 12 N-HCl; 1500 c.p.m. [^{3}H]testosterone/0·1 ml were added for recovery calculations. This mixture was incubated in a boiling water bath for 15 min. After neutralization with sodium hydroxide, an aliquant of this hydrolysed urine (0·05 ml) was extracted twice with 5 ml freshly opened diethyl ether. The extract was dried under a stream of nitrogen and then reconstituted in 1·0 ml assay buffer (0·1 M-phosphate, pH 7·0, containing 0·9% (w/v) sodium chloride, 0·01% sodium azide and 1% gelatin). A 0·5 ml sample was counted for recovery while 0·05 ml was assayed for androgens by radioimmunoassay as described by Janne *et al.* (1974). Two samples were run from each sample and no inter-assay differences were found. The antiserum was raised in rabbits against testosterone-17-hemisuccinate–BSA. The major cross-reactions were 18·2% with 5α-dihydrotestosterone and 12·9% with 5β-dihydrotestosterone. The assay tubes containing the sample, antibody, and [1,2,6,7,16,17-^{3}H]testosterone (sp. act. 135 Ci/mmol: New England Nuclear, Boston, MA, USA) were incubated for 2 h at 4°C. The separation of antibody-bound and free steroid was achieved by addition of 0·5 ml charcoal–dextran (0·5% charcoal Norit A, 0·05% dextran in the assay buffer) during a 15-min incubation at 4°C. After centrifugation for 15 min, the supernatant was decanted and mixed with scintillation cocktail and counted for 5 min. The standard curve ranged from 3 to 1000 pg/tube and the lowest detectable sample measured was 3 ng/ml urine. Serial dilutions of hydrolysed zebra urine showed parallelism with the standard curve. All the samples were run in a single assay and the mean ± s.e.m. recovery of titrated testosterone added to the hydrolysed urine was 72·9 ± 1·8%, n = 53). Various known amounts of unlabelled

testosterone were also added to a pool of zebra urine before hydrolysis. The mean ± s.e.m. recovery was 77·8 ± 8·9% (n = 8).

Body size. Body size was estimated using photometric analysis for the Grevy's zebra. Complete details of the method are given by Ginsberg (1987). Linear girth, the distance from the withers to the belly, was estimated and used as the measure of body size. For any particular individual, estimates of linear girth had an error of ≃8%. Given that arid-zone adapted equids can vary in weight up to 20% due to dehydration (Maloiy, 1970), linear girth measurements appear to provide an accurate measure of relative size.

Statistical analysis. Statistical analyses were performed on an Apple Macintosh S.E. running Statview software (Abacus Concepts, 1986). As data were not found to deviate from a normal distribution, parametric statistics were used throughout. Results are expressed as the mean of all values ± the standard error of the mean (s.e.m.).

Results

Plains zebra

Harem (breeding) males had significantly higher androgen concentrations than did bachelor males (Fig. 1: 30·0 ± 5·9 ng/mg Cr, N = 9, versus 11·4 ± 2·8 ng/mg Cr, N = 7; d.f. = 13, t = 3·23, $P < 0·01$). Among harem males, neither the time of day at which samples were collected (r^2 = 0·01, $P > 0·50$ nor the number of females in a male's harem (r^2 = 0·16, $P > 0·30$) had any significant effect on androgen concentrations. No data on body size were available for plains zebra.

Grevy's zebra

Territorial males had higher concentrations of androgens than did bachelor males (Fig. 1: 19·0 ± 2·2 ng/mg Cr, N = 17, versus 10·7 ± 1·2 ng/mg Cr, N = 14; d.f. = 29, t = 3·13, $P < 0·01$). Samples were collected from 4 territorial males who were on forays off their territories. These results show that when a territorial male is off his territory he is likely to have reduced androgen values compared to concentrations in the same male when he is on territory (Fig. 1: paired Student's t test, d.f. = 3, t = 2·49, $P < 0·05$). There was no significant difference in androgen concentrations between off-territory males and bachelor males (d.f. = 16, t = 0·93, $P > 0·30$).

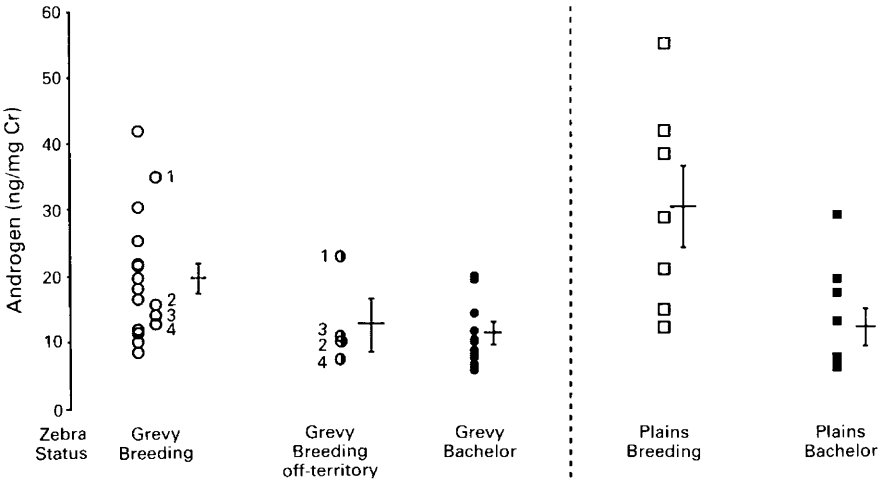

Fig. 1. Urinary androgen concentrations (mean ± s.e.m.) for two species of zebras. For Grevy's zebra, samples were collected from 4 breeding individuals both on and off their territories. In the first column (open circles), these samples are numbered and are slightly off-set to the right. Samples in column two for corresponding males are similarly numbered.

In samples taken from territorial males, neither the presence of females (N = 4) at the time of sampling (d.f. = 15, $t = 0{\cdot}77$, $P > 0{\cdot}40$), nor the presence of bachelor males (N = 3) at the time of sampling (d.f. = 15, $t = 0{\cdot}64$, $P > 0{\cdot}50$), or the time of day at which samples were collected ($r^2 = 0{\cdot}14$, $P > 0{\cdot}10$) affected androgen concentrations.

There was no correlation of urinary androgen concentrations with the time since last association with bachelor males (d.f. = 16, $P > 0{\cdot}10$) but there was a negative correlation between urinary androgen concentrations and the time since a territorial male was last seen in association with at least one female (Fig. 2: $y = -0{\cdot}43x + 22{\cdot}2$, $r^2 = 0{\cdot}26$, $P < 0{\cdot}05$).

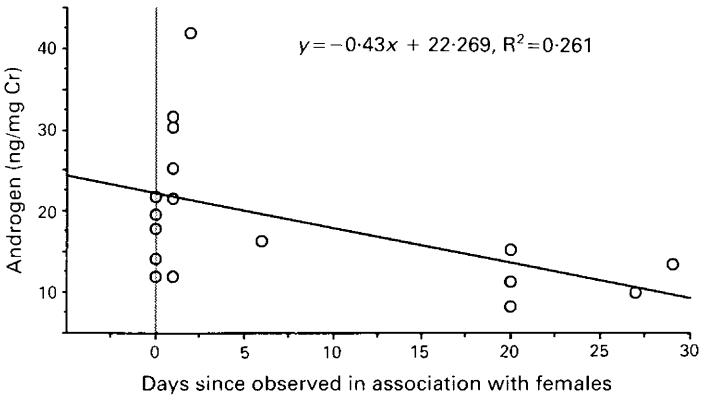

Fig. 2. The effect of absence of females on testosterone concentration in territorial Grevy's zebra males.

Among bachelor males and territorial males, body size had no effect on urinary androgen concentrations. When males of both types were simultaneously included in the analysis, there was a strong correlation between body size and urinary androgen concentrations (d.f. = 27, $y = 0{\cdot}951x - 48{\cdot}5$, $r^2 = 0{\cdot}25$, $P < 0{\cdot}01$).

Discussion

The results of this study indicate that, despite marked differences in social organization, breeding males of both the Grevy's and plains zebra exhibit similar endocrine changes. In both species, males that have access to females (i.e. potentially breeding males) excrete more androgens than do non-breeding males. Hence, there is an endocrinological correlation with social dominance, as measured by access to sexually receptive females, which appears to be consistent across breeding systems.

The use of a urinary assay for androgens had several advantages. The method allowed samples to be collected, without immobilizing animals, from a large number of individuals in free-ranging wild populations of two sympatric species of zebra. One of the species, the Grevy's zebra, is classified as endangered (Thornback, 1977). As immobilization of wild equids is often difficult (King & Klingel, 1965), collection of samples on the scale required would have been impossible. Furthermore, the fluctuations observed in plasma androgen measurements (Katangole *et al.*, 1971; Bartke *et al.*, 1973) are minimized using urinary samples since they presumably reflect androgen secretion over a period of several hours. It was assumed that most of the androgens were of testicular origin as in other species the contribution of the adrenals is minimal.

The correlation between dominance and androgen concentrations has been observed in a wide variety of other ungulate families including bovids (impala, *Aepyceros melampus*: Bramley &

Neaves, 1972), cervids (red deer, *Cervus elephus*: Lincoln *et al.*, 1972; white-tailed deer, *Odocoileus virginianus*: Miller *et al.*, 1987) and elephants (African elephant, *Loxodonta africana*: Poole *et al.*, 1984). In experiments with antelopes, territorial males had higher plasma testosterone values than did bachelor males in response to gonadotrophin-releasing hormone (Illius *et al.*, 1983).

In the Grevy's zebra, the results suggested further endocrine–behavioural interrelationships. That the concentration of androgen appeared to decline when females had not recently been present suggests that social stimuli may be a factor influencing androgen secretion. When females have not visited a territory for some time, males will temporarily abandon their territories (Ginsberg, 1987), and so the fact that males which have temporarily abandoned their territories have lower concentrations of androgens is not surprising.

That association with other males does not affect androgen concentrations may be due to small sample sizes. A territorial male may spend up to several weeks on territory without being in association with either males or females (Ginsberg, 1987). Hence, another way to evaluate the effect of conspecifics on androgen concentrations is to examine the relationship between urinary androgen concentrations in samples taken from territorial males as a function of the time since their last association with conspecifics.

While these data demonstrate that there is an endocrinological basis to dominance in zebra, the direction of causality cannot be determined from these observations. In a variety of other species, sexual activity (rabbit: Ågmo, 1976; ram: Sanford *et al.*, 1974; Gonzalez *et al.*, 1988; boar: Liptrap & Raeside, 1978; bull: Katangole *et al.*, 1971; mouse: Coquelin & Bronson, 1980; rat: Kamal & Frankel, 1978; rhesus monkey: Herndon *et al.*, 1981) or merely exposure to females (ram: Gonzalez *et al.*, 1988) or their scent (hamster: Macrides *et al.*, 1974) led to elevated concentrations of serum testosterone. In boars, aggressive encounters also led to increased concentrations of testosterone in the blood (Liptrap & Raeside, 1978); however, in the lesser mouse lemur, only dominant males showed higher levels of testosterone after aggressive encounters (Peret, 1985). In other studies sexual activity (bull: Price *et al.*, 1986; ram: Moore *et al.*, 1978) and aggressive encounters (bull: Price *et al.*, 1986) had little or no effect on serum testosterone concentrations.

It remains to be determined whether, in zebra, increasing concentrations of androgens cause an animal to be dominant, or whether high androgen concentrations are characteristic of dominant males. It is tempting to suggest that proximity to females raises androgen concentrations which, in turn, increases aggression enabling the dominant male to defend his mate. However, further research is required to confirm this hypothesis.

Physical factors could also have an effect on androgen concentrations. As all samples were collected at the same time of the year, differences observed cannot be ascribed to seasonality. In red deer stags, testosterone was correlated with aggressive behaviour, while dominance position was affected by other variables including weight, antler size, age and experience in addition to testosterone concentrations (Lincoln *et al.*, 1972). In this study, within a reproductive class (territorial or bachelor), there was no correlation between size and androgen concentrations. When reproductive classes were pooled, there was a significant effect of body size on androgen level. However, as territorial males are larger than bachelor males (Ginsberg, 1989), the size and reproductive class are highly auto-correlated. Furthermore, among territorial males, male reproductive success is not correlated to body size (Ginsberg, 1989). Given the intra-individual variation in urinary androgen concentrations among males on and off their territories, it seems unlikely that body size has an independent significant effect.

The results of this study demonstrate that, in zebras, androgen values and the reproductive status of individual males are inter-related. In the more stable family structure of the plains zebra, in which association of a breeding male and his females is essentially constant, little can be concluded other than that breeding males have higher androgen concentrations than do bachelor males. The fluid social organization of the Grevy's zebra allows a more detailed analysis of how different social variables affect androgen values. For this species we show that the hormone concentrations changed with the breeding status, and social situation, of the male being studied.

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Neutering affects mRNA expression levels for the LH- and GnRH-receptors in the canine urinary bladder

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Abstract

Neutering a bitch increases the incidence of acquired urinary incontinence (AUI) 20-fold. Mechanistically this effect is thought to be related to altered steroid/reproductive hormone concentrations and a recent study showed that gonadotrophin releasing hormone (GnRH) analogue treatment improved continence in bitches with AUI. The aim of this study was to examine mRNA expression levels for luteinizing hormone (LH)- and GnRH-receptors in the canine bladder and the correlation between these and *in vitro* contractility of the bladder using age matched entire and neutered, male and female canines and canines with AUI.

Biopsies from the dome of the bladder were dissected post mortem with informed owner consent. mRNA expression for LH- and GnRH-receptor was quantified by rtPCR (relative to β-actin). Contractility was assessed (cumulative concentration response curve for carbachol) in strips of bladder muscle using standard protocols.

Analysis of variance (Tukey post-test) demonstrated thet neutering was associated with significantly increased levels of expression of LH- and GnRH-receptor mRNA in both sexes ($P < 0.01$). mRNA expression for both receptors was significantly higher in female versus male canines. Neither effect was affected by animals' age and/or weight. A significant inverse correlation (Spearman's test) was found between bladder contractility and mRNA expression for both receptors. This effect was most pronounced in canines with AUI which demonstrated the highest mRNA expression levels yet had the lowest contractility of all animals studied. This suggests that increased LH- and GnRH-receptor mRNA expression is associated with changes in bladder function that increase an animal's predisposition to develop AUI.
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Keywords: Urinary incontinence; Bladder; Bitch; LH; GnRH; mRNA

1. Introduction

Acquired urinary incontinence (AUI) is a debilitating and so far incurable condition that causes significant welfare problems. Two diverse populations have been identified that are particularly susceptible to AUI, post-menopausal women and spayed female canines. Information relating to the exact pathophysiology of the condition in these two groups is not understood well enough to allow determination of commonality of cause; however, both groups are subject to a reduction in gonadal steroids and associated endocrine changes.

Urinary incontinence (UI) is defined as the involuntary leakage of urine [1] and, in the bitch, is an increasingly recognised clinical problem with severe welfare implications that can lead to the ultimate

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euthanasia of the animal. Interestingly, AUI is reported to affect up to 20% of neutered bitches [2] but is seen in less than 1% of intact bitches [3] and is rarely reported in male canines regardless of gonadal status. A direct relationship between neutering and AUI has been reported [4] which is proposed to occur as a consequence of hormonal, vascular or neurological changes [5], rather than mechanical damage of the lower urinary tract, sustained during surgery [6]. Incontinence in the neutered bitch is frequently associated with a decrease in maximal urethral closure pressure [7,8], however, as this is not a defining characteristic of AUI, multiple causative factors are likely to be involved, not all of which will be a consequence of gonadectomy. It has thus been hypothesized that factors in addition to those that decrease urethral closure pressure, lead to a bitch's developing urinary incontinence after neutering. Previous *in vitro* studies have shown that neutering a canine of either gender leads to a decrease in maximal contractile response of the detrusor muscle to both muscarinic and electrical field stimulation as well as a decreased sensitivity to muscarinic stimulation [9]. These decreases in contractile function *in vitro* are similar to those reported in post-menopausal women who suffer from UI due to impaired contractility of the bladder [10].

Given the commonality of endocrine change in the two populations identified as being susceptible to AUI, it is now a widely held hypothesis that the condition may be due to, or be exacerbated by, hormone-mediated effects. This view is supported by the fact that a pharmacological treatment for AUI in the bitch, is steroid replacement [11]. In both spayed bitches and post-menopausal women there is a deficiency of endogenous gonadal steroid hormones which decreases or removes the normal negative feedback to the hypothalamo-pituitary axis, and results in greatly increased production and secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus, and increased secretion of the pituitary gonadotrophins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) [12–18]. This increase in plasma concentrations of LH and FSH post-neutering has been demonstrated to have a direct relationship with the development of urinary incontinence in the bitch [19]. A recent paper has described the clinical use of GnRH analogues to decrease LH and FSH concentrations in neutered female canines suffering from AUI. The results of this study also indicated that GnRH analogue treatment resulted in clinical continence, or a decrease in incontinence, during the treatment period [12,13]. This result would suggest that the increase in the concentrations of either GnRH and/or the pituitary gonadotrophins could be a causative factor for AUI in the bitch. Whilst GnRH and the gonadotrophins are classically thought of as hormones of the hypothalamo-pituitary-gonadal axis, the presence of their receptors in tissues such as skin [20], reproductive tract, prostate and mammary gland [21,22] as well as the urinary bladder [23–25] would suggest more widespread actions. In particular the presence of receptors within the urinary tract would indicate that altered concentrations of gonadotrophins could induce local effects within the bladder which could affect function. As specific tissue action and function rely upon the presence of specific receptors in the tissue it is possible that changes in either absolute receptor numbers or receptor–ligand concentration could influence the action of a tissue. In this regard a study in women has reported a decrease in LH-receptor numbers in the bladder of post- compared to pre-menopausal women [25], and a number of recent studies in the canine have reported either a decrease [24,26] or no effect on LH-receptor mRNA concentrations after neutering [17]. However, there are no published reports of mRNA levels for LH-, FSH- or GnRH-receptors in the bladder of bitches known to be suffering from AUI or the correlation between detrusor contractility, a factor known to be involved in UI [10,27], and receptor mRNA expression levels within the urinary bladder.

The aim of this study was to determine the effects of gonadal status and gender on the expression levels of GnRH-, LH- and FSH-receptor mRNA in the canine urinary bladder and to investigate whether these levels are altered in individuals suffering from AUI. A further aim was to see if there is a correlation between the expression of the mRNAs for these receptors and maximal contractility of the bladder, using age matched groups of entire and neutered, male and female canines.

2. Materials and methods

2.1. Animals and tissues

The study was approved by The University of Glasgow Veterinary School's ethical review committee. A total of 78 canines were included in the study, with a mean age of 6.0 years (range 1–16 years) and a mean weight of 22.7 kg (range 8–50 kg). The majority of canines were cross bred, with no pedigree breeds appearing more than once.

In all cases tissue was collected from canines euthanized (intravenous overdose of pentobarbatone),

with full informed owner consent, for reasons other than scientific investigation. The majority of animals were destroyed for severe behavioural problems, the remainder for a number of different complaints, none of which involved the urinary system. In all cases a detailed history of each animal was taken and, within 2 h of death, a gross post mortem study was performed. None of the animals used in the study were receiving medical treatment in the 7 days prior to euthanasia and there was no evidence of previous medical therapy in at least the month prior to this. Biopsies were collected in the area of the bladder dome and samples either stored at 4 °C in Krebs solution for tissue bath studies or immediately frozen and stored at −80 °C until required for mRNA studies. Any animal with a history or signs of gross pathological urinary, neurological or reproductive tract disease (e.g. tumours, cystitis, pyometra) were excluded from the study (except for those suffering from AUI—see below). The remaining animals were split into five groups depending on sex and gonadal status: entire males (ME), entire females (FE), neutered males (MN), neutered females (FN) and neutered females known to be suffering from AUI (FN AUI). All of the entire bitches were considered to be in anoestrous, as the ovaries showed no signs of follicular activity or of an active corpus luteum and all of the neutered animals were at least 6 months post-gonadectomy (determined by history and clinical examination). A diagnosis of AUI was made on the basis of history and clinical signs at time of euthanasia, coupled with full gross post mortem results that ruled out other causes of UI in the bitch such as ectopic ureters, kidney disease, uroliths, cystitis and gross abnormalities of the urinary system.

Ovarian tissue as well as skin biopsies, from the shoulder and groin regions were collected from a subset of animals included in the study, as a control for FSH-receptor mRNA expression [28]. Ovarian and skin samples were handled and stored as described above.

2.2. mRNA extraction and quantitative reverse transcription PCR

Biopsies where placed in 1 ml ice cold TRIzol reagent (Invitrogen, Paisley, UK), homogenised and RNA extracted according to the manufacturers protocol. After extraction, RNA was suspended in 40 μl RNase-free water and the concentration of RNA determined using a NanoDrop ND-1000 spectrophotometer. The purity of RNA (A_{260}/A_{280}) was greater than 1.9 in all samples. 1 μg of each RNA sample was reverse transcribed into cDNA using random hexamers (Promega, Southampton UK), Moloney-Murine Leukaemia virus (M-MLV) reverse transcriptase (Invitrogen, UK) and Rnasin (Promega, UK), as described previously [28]; final reaction volume of 24 μl. Quantitative real time taqman PCR (qPCR) was performed on cDNA for GnRH-, LH- and FSH-receptor with β-actin as the housekeeping gene using the Amplitaq Gold kit (Applied Biosystems, Warrington, UK) and a Strategene MX 3000P thermal cycler. The primers and probes for the three receptor mRNAs were as described by Welle et al. [20], sourced from Eurogentech (Southampton, UK). Prior to use in the current experiment the concentration of primers and florescent FAM-labelled probes against LH-, GnRH- and FSH-receptor mRNAs [20] were optimised relative to the housekeeping gene β-actin, to ensure that amplification of each of the genes of interest matched that of the housekeeping gene. All probes used were at a final concentration of 530 nM, with forward (f) and reverse (r) primers at: LH- and FSH-receptor mRNAs 1000 nM (f), 800 nM (r), GnRH-receptor mRNA 660 nM (f), 800 nM (r), β-actin mRNA 800 nM (f and r). All samples were run in duplicate, PCR conditions used were an initial step of 95 °C for 10 min followed by 45 cycles of 95 °C for 30 s and 60 °C for 1 min. Non-template controls were included for each primer pair to check for significant levels of any contaminants and in all cases were negative.

2.3. Tissue preparation for tissue bath studies

Tissue was used within 48 h of collection. Following removal of the lining urothelium strips of smooth muscle (2 mm × 10 mm) were dissected from the dome of the bladder. The strips were mounted under 4 g resting tension (the optimum tension demonstrated in preliminary previous length-tension studies) on a fixed hook in a 15 ml organ bath bathed at 37 °C in Krebs solution (NaCl 118 mM, KCl 4.8 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 24 mM and glucose 11 mM) gassed with 95% O_2/5% CO_2. Tension was measured with a BIOPAC TSD125C isometric force transducer and displayed using the AcqKnowledge 3.8.1. Biopac data acquisition system (Linton Instruments). Tissues were allowed to equilibrate for 60 min before experiments were begun, during which time the resting tension was re-adjusted to 4 g if required [9]. For all studies, a minimum of 2 muscle strips per animal were included.

2.4. Assessment of carbachol-induced contraction

To determine the response to the non-specific muscarinic agonist carbachol, cumulative concentration

Table 1
Age and weight of animals, by group.

	ME ($n = 27$)	MN ($n = 17$)	FE ($n = 18$)	FN ($n = 13$)	FN AUI ($n = 3$)
Age (years)	5.6 ± 0.7	5.8 ± 0.9	6.1 ± 1.0	6.5 ± 1.0	4.3 ± 2.0
Weight (kg)	23.7 ± 1.8	27.4 ± 3.2	21.9 ± 2.2	21.1 ± 3.1	17. ± 5.0

Mean ± S.E.M. values for age and weight in groups of entire and neutered, male and female canines, and neutered bitches identified as suffering from AUI (ME, MN, FE, FN and FN AUI, respectively). n = number of animals.

response curves to carbachol (1 nm–30 μM) were generated in strips of bladder smooth muscle. Maximal tension was recorded and data calculated as grams tension produced per milligram of wet tissue.

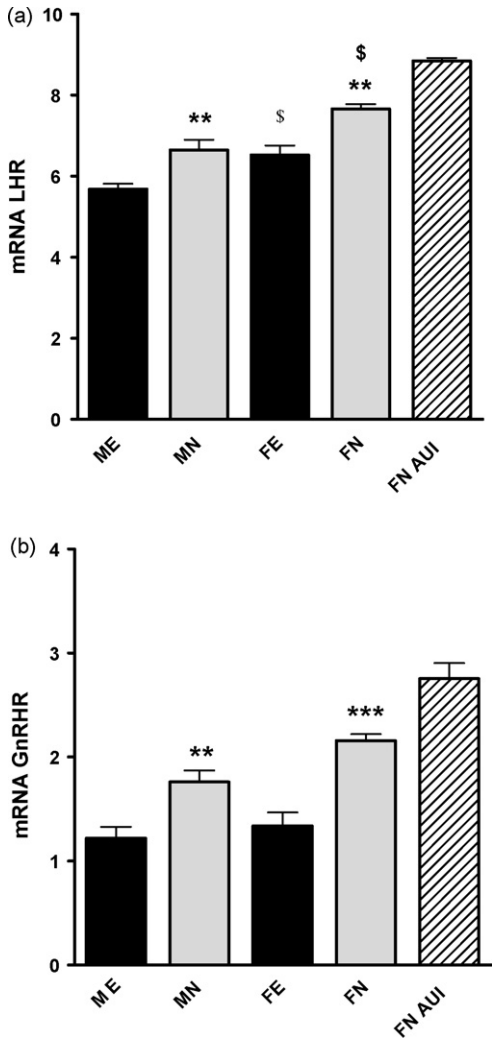

Fig. 1. Mean (±S.E.M.) mRNA expression for: (a) LH-receptor and (b) GnRH-receptor, in entire and neutered male and female canines and neutered female canines identified as suffering from AUI. mRNA values are logarithmically transformed. $^{***}P < 0.001$, $^{**}P < 0.01$ compared entire of same gender. $^{\$}P < 0.05$ compared to male animals of same gonadal status.

2.5. Statistical analysis

Results for both mRNA expression levels and contraction to carbachol were analysed against both age and weight using multivariant analysis with $P < 0.05$ considered significant.

Results are given as mRNA expression levels relative to β-actin using the comparative C_T method (User Bulletin no. 2, PE Biosystems, UK). Data was ln-transformed prior to statistical analysis to equalise the variance between groups and data compared by two-way ANOVA for effects of gender and gonadal status. Post hoc comparisons were conducted using Tukey's test with a significance threshold of $P < 0.05$.

To examine relationships between the levels of mRNA expression of the hormone receptors studied and the ability of the detrusor to contract when stimulated with carbachol, mRNA expression levels of each hormone receptor were plotted against maximal contraction (as grams of tension produced per milligram of wet tissue) and tested for a correlation using Spearman's test with a significance threshold for P (two tailed) <0.05.

3. Results

The mean age and weight of the 78 canines included in this study, 27 entire males (ME), 17 neutered males (MN), 18 entire females (FE), 13 neutered females (FN), and 3 neutered females known to suffering from AUI (FN AUI) are presented in Table 1. There was no statistical difference between the mean ages and weights for the five groups.

The mean levels of mRNA expression for LH- and GnRH-receptor are shown in Fig. 1a and 1b, respectively. Two-way ANOVA indicated significant effects of gonadal status on the expression of the mRNAs for both LH- and GnRH-receptor, whilst there was an additional effect of gender on the expression of LH-receptor mRNA. There was no interaction between the effects of gonadal status and gender. Neutering was associated with significantly ($P < 0.01$) higher levels of mRNA expression for both LH- and GnRH-receptor in

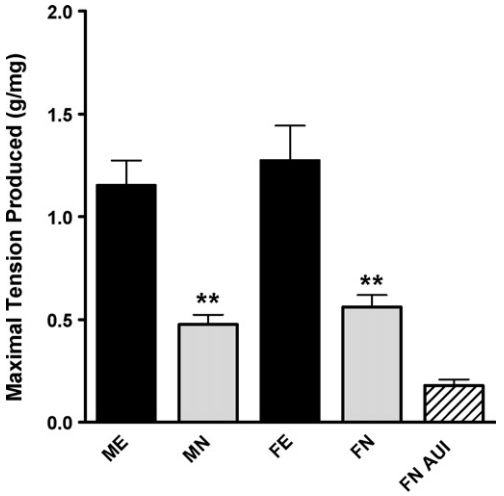

Fig. 2. Mean (±S.E.M.) maximal tension values (g/mg wet tissue) in response to carbachol in entire and neutered male and female canines and neutered female canines identified as suffering from AUI. $^{**}P < 0.01$ compared entire of same gender.

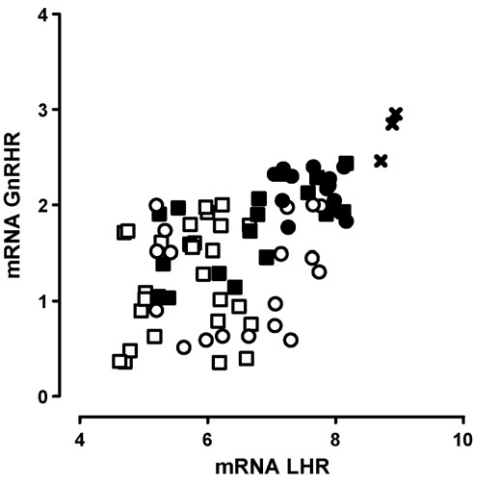

Fig. 3. Correlation between mRNA expression levels of LH- and GnRH-receptor ($P < 0.001$, $r = 607$). (□) Male entire, (■) male neuter, (○) female entire, (●) female neuter, and (×) AUI female neuter.

both genders. Furthermore, female animals showed significantly ($P < 0.05$) greater levels of mRNA expression of LH-receptor, regardless of gonadal status, than their male counterparts (Fig. 1a).

The mRNA expression levels for both LH- and GnRH-receptor for the females identified as AUI were the highest of all the animals in the study and fell above the 95% CI for the continent FN group, although this increase was not considered statistically significant using ANOVA analysis.

Real time rt-PCR was performed for the quantification of FSH-receptor mRNA expression within samples of urinary bladder. In all cases, expression was found to be below the limits of detection of the assay system. To ensure validity of the primers and probes used, they were tested against samples of canine ovary and skin as described in Welle et al. [20]. mRNA for the FSH-receptor was found at very low levels ($3.22E^{-15}$, $n = 2$) in samples of canine skin but was not found in canine ovary.

Carbachol-induced dose dependant contraction of the isolated strips of detrusor muscle from all animals. As in our previous study [9], maximal response was significantly decreased ($P < 0.01$) in neutered compared to entire canines of either gender (Fig. 2). Sensitivity of strips to carbachol, as measured by log EC_{50} values, was also decreased ($P < 0.01$) in neutered compared to entire animals (Table 2). Two-way ANOVA revealed that there was no effect of gender and no interaction between gender and gonadal status. The group of animals identified as suffering from AUI had the lowest maximal contraction of all animals studied, although this was not considered significant using ANOVA.

Statistical analysis revealed a significant positive correlation ($P < 0.001$, $r = 0.607$) between LH- and GnRH-receptor mRNA expression (Fig. 3) and significant negative correlations between the maximal tension produced by detrusor muscle strips and both LH-receptor ($P < 0.001$, $r = -0.671$) and GnRH-

Table 2
Maximum and log EC_{50} values for Carbachol.

	ME ($n = 11$)	MN ($n = 10$)	FE ($n = 10$)	FN ($n = 8$)	FN AUI ($n = 3$)
E_{max}	1.15 ± 0.12	0.49 ± 0.05*	1.27 ± 0.17	0.56 ± 0.06*	0.18 ± 0.03
log EC_{50}	−6.98 ± 0.05	−6.39 ± 0.02*	−7.09 ± 0.06	−6.51 ± 0.07*	−6.26 ± 0.10

Maximum (E_{max}) and log EC_{50} values for carbachol in isolated strips of detrusor muscle from entire and neutered, male and female dogs and neutered females identified as suffering from AUI (ME, MN, FE, FN and FN AUI, respectively). Data are expressed as g/mg of wet tissue with results given as mean ± S.E.M. where n: number of animals.

* $P < 0.01$, indicates different from entire of same gender.

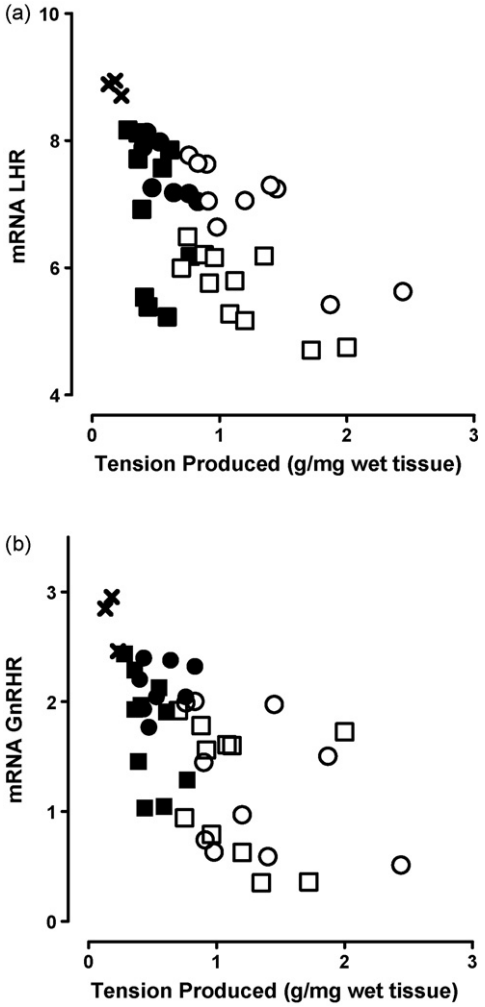

Fig. 4. Correlation between maximal tensions produced by detrusor muscle strips *in vitro* and mRNA expression levels for: (a) LH-receptor ($P < 0.001$, $r = -0.671$) and (b) GnRH-receptor ($P < 0.001$, $r = -0.612$). (□) Male entire, (■) male neuter, (○) female entire, (●) female neuter, and (×) AUI female neuter.

receptor ($P < 0.001$, $r = -0.612$) mRNA expression (Fig. 4a and b, respectively).

Multivariant analysis of the levels of expression of mRNA for both GnRH- or LH-receptor, and the maximum tension produced by detrusor muscle strips indicated no significant effect of age or weight of the animal on the results.

4. Discussion

This is the largest study of the expression levels of the mRNAs for LH- and GnRH-receptors in the canine urinary bladder, and the only study to correlate levels of receptor mRNA expression to bladder contractility (*in vitro*). In addition, although numbers are low, this is the only study to report bladder LH/GnRH-receptor mRNA expression in neutered bitches known to be suffering from AUI.

Bladder contractility is thought to play a significant role in the development of clinical signs of UI [29]. We have previously shown that the maximal contractile response of isolated strips of detrusor muscle to muscarinic and neurogenic stimulation was significantly decreased in neutered compared to entire canines of either gender [9]. We have also demonstrated that sensitivity of the detrusor to muscarinic stimulation is decreased in neutered compared to entire animals of either gender. These results are similar to those reported for studies conducted with tissues collected from women who suffer from UI due to impaired contractility of the bladder [10], a condition known to cause decreased speed and magnitude of detrusor contractions during the emptying phase of micturition. This leads to residual urine retention, a shortened storage phase and predisposes the patient to cystitis [30]. That decreased contractility and sensitivity of the detrusor to muscarinic stimulation in neutered canines may be involved in AUI is supported by the results of this study, looking at animals known to be suffering from AUI, as these had the lowest contractility of all the animals studied. Although impaired bladder contractility has been implicated in UI, the exact mechanisms responsible for its development have not been reported and we hypothesise that the muscarinic receptor effector pathway is involved, its function being altered due to effects in reproductive hormones and the expression of their receptors in the bladder post-neutering/menopause.

It was first suggested that gonadotrophins could influence bladder function in a study which showed that post-menopausal women, who are most likely to suffer from UI, had lower numbers of LH mRNA transcripts and receptors in their bladders than their premenopausal counterparts [25]. As post-menopausal women have a similar hormonal profile to neutered canines, and as both post-menopausal and post-neutering UI are thought to be hormonally related, it follows that a similar aetiology and pathophysiology may link both conditions. Our results clearly show that neutering a canine, regardless of gender, significantly increases levels of mRNA expression for both LH- and GnRH-receptors, and that in general, female animals, regardless of gonadal status, have higher levels of mRNA expression for these receptors than their male counterparts. Although our results, for female canines, are in contrast with those of Tao et al. [25] it should be noted

that in addition to the species difference our study include age matched groups of healthy entire and neutered animals as well as those neutered female canine suffering from AUI, whereas the study by Tao et al. compared women of two distinct age groups, with a number of patients in both groups suffering from UI and genital prolapse, therefore an additional explanation for the difference in the two studies could be an effect of age and mechanical stress on the lower urinary tract. Interestingly, the results of the FN animals in this study known to be suffering from AUI suggest that incontinent neutered bitches may have higher levels of LH-receptor mRNA than their continent counterparts. These findings support the hypothesis that altered gonadotrophins have a role in the pathophysiological mechanism of post-neutering UI as suggested by the efficacy of GnRH analogues in the treatment of canine AUI [13].

In addition to being different from the study reported in humans our results also contradicts with two recent canine studies that reported either no change [24] or a decrease [26] in mRNA expression for these receptors, respectively, in neutered canines. The differences between the three canine studies may again reflect differences in the study populations and conditions. In both of the studies by Reichler et al. [24] and Ponglowhapan et al. [26] a substantially smaller number of animals were used. In addition, the study by Reichler et al. included entire bitches in varying stages of oestrus, thus making comparisons between the groups and studies more complex. Whereas the study by Ponglowhapen et al. looked at relatively acute effects of neutering (24–40 weeks), and thus it is possible as plasma concentrations of FSH and LH continue to increase until week 42 post-neutering [16] and some structural changes may take even longer [31], that there will be significant effects of time post-neutering on the observed mRNA changes.

A further difference between this study and those of Tao et al. and Ponglowhapan et al. is methodological, in that the current study used real time PCR with fresh frozen tissue to quantify mRNA expression levels, whilst the former two studies used in situ hybridisation with formalin fixed, paraffin-embedded tissue. It is known that degradation of target nucleic acids [32,33] and artificial mutations [34] can occur during formalin fixation and paraffin-embedding procedures, which again could have influenced the results, relative to those seen from fresh frozen tissue [35].

The changes in expression of mRNA for LH- and GnRH-receptors observed in our study could support a role for altered expression of GnRH/LH-receptors in the development of AUI in the neutered bitch. Although it is worthy of note that increases in LH- and GnRH-receptor mRNA expression also occurred in the bladders of neutered male canines, which do not typically become incontinent, the magnitude of increase was smaller and the final levels of expression were only marginally above that of entire female canines which also rarely become incontinent. The sex difference in the propensity to develop AUI may also reflect an interaction between post-neutering effects on smooth muscle function and anatomical differences in the urethra of a male canine as has been previously hypothesized [36]. It may be the case however that there is a threshold level of LH- and GnRH-receptor expression, above which UI is more likely to develop. This possibility is supported by the fact that the three animals known to be suffering from AUI had the highest LH- and GnRH-receptor mRNA expression levels observed in this study.

The exact role of the LH-receptor in the urinary bladder has not yet been described. It is known that the plasma concentrations of LH increase in both post-menopausal women and in neutered dogs [16,37], although it is still released in a pulsatile manner. It is also known that this subset of the population is most prone to developing UI. In this study we have shown that neutering causes an increase in mRNA expression levels for the LH-receptor in the canine urinary bladder, and that these expression levels are highest in bitches known to be suffering from AUI. We have also shown that maximal bladder contractility is decreased in neutered animals and is lowest in bitches suffering from AUI and that there is a direct inverse correlation between bladder contractility and mRNA expression levels for LH. It therefore follows that the increase in mRNA for the LH-receptor may be implicated in the decrease in contractility of the detrusor muscle in the canine, either directly or via interactions with another mechanism, possibly involving the muscarinic receptor effector pathway, the primary pathway involved in bladder contraction [38].

The exact role of the GnRH-receptor within the urinary bladder has not been characterised in any species. However, based on a recent study that reported increases in bladder threshold volume following administration of GnRH analogues to neutered continent bitches it has been hypothesized that it may have a role in determining bladder function [12]. This could occur if GnRH-receptor expression modulated the bladders sensitivity to muscarinic stimulation to affect the bladder threshold volume. This hypothesis is supported by the data contained within our study,

where a decrease in *in vitro* detrusor muscle sensitivity to muscarinic stimulation was seen in neutered canines when GnRH-receptor mRNA expression levels were found to be high. Thus, the increase in GnRH-receptor expression could be associated with a decreased sensitivity of the detrusor which would allow further relaxation of the bladder and increased capacity.

Although our study did not identify mRNA for FSH-receptor in the canine bladder two other studies [20,23] have reported its expression within this tissue. The reason for these reported differences studying FSH-receptor mRNA expression, could be methodological, as Ponglowhapan et al. [23] used in situ hybridization whilst our study used real time PCR techniques. Alternatively it could be related to differences in the FSH mRNA sequences used, as our results were similar to those described by Welle et al. [20], who used the same primers and probes and reported expression in canine skin, and inconsistent expression within the canine bladder [20] whilst Ponglowhapan et al. used a different molecular sequence as the basis for their probe. As FSH is known to express multiple isoforms [39] it is a possibility that the primers and probes used in this study and that of Welle et al. [20] were designed against part of the mRNA sequence of an FSH-receptor isoform that is expressed in skin but not in the bladder. As part of the validation of the probe in the current study, in addition to testing the primer and probes against bladder and skin, ovarian tissue was also included as the ovary is a key target organ for the actions of FSH [40]. The ovary, however, was also found to be devoid of significant quantities of the FSH-receptor mRNA expression that could be identified using these primers and probes. Thus, at this point, we are unable to make any definitive conclusions with regard to the effects of neutering on FSH-receptor mRNA expression within the bladder wall.

In conclusion we have shown that neutering a canine, regardless of gender, is associated with an increase in the levels of mRNA for LH- and GnRH-receptors in the canine urinary bladder, and that female animals, regardless of gonadal status have higher LH-/GnRH-receptor mRNA expression levels than males. A further novel observation shown by this study is the positive correlation between increased mRNA expression for LH- and GnRH-receptors and the negative correlation between expression of these receptors and contractility of the detrusor to muscarinic stimulation *in vitro*. It is hypothesized that the changes in mRNA expression for both GnRH- and LH-receptor may have a role to play in the development of post-neutering AUI and this hypothesis is supported by data from a limited number of neutered female canines identified as suffering from AUI which had the highest levels of LH- and GnRH-receptor mRNA expression and the lowest contractility to muscarinic stimulation of all the animals studied.

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Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings

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RESPONSES OF CORTISOL AND PROLACTIN TO SEXUAL EXCITEMENT AND STRESS IN STALLIONS AND GELDINGS[1]

D. R. Colborn[2], D. L. Thompson, Jr.[3], T. L. Roth, J. S. Capehart and K. L. White

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ABSTRACT

Sexual stimulation induces rapid secretion of cortisol and prolactin (PRL) in stallions. Experiment 1 was designed to determine whether stallions associated location and(or) procedure with previous sexual stimulation in that location. After a control period on d 1, four stallions were exposed to an estrous mare for 5 min on d 2. On d 3, 4, 5, and 6, the same procedure was followed with no mare present. Concentrations of PRL and cortisol increased ($P < .05$) after mare exposure on d 2 but did not vary ($P > .05$) on d 1, 3, 4, 5, or 6. In Exp. 2, six stallions were used to determine the short-term effects of 1) sexual stimulation, 2) acute physical exercise, 3) restraint via a twitch (twitching), 4) epinephrine administration, and 5) no stimulation on plasma concentrations of PRL and cortisol. Stallions received one treatment per day separated by 2 d of no treatment. Concentrations of cortisol increased ($P < .05$) within 10 min after sexual stimulation, exercise, twitching, and epinephrine administration but not during control bleedings. Concentrations of PRL increased ($P < .05$) immediately after sexual stimulation, exercise, and twitching but not after epinephrine administration or during control bleeding. In Exp. 3, the same five treatments were administered to six geldings. Concentrations of cortisol increased ($P < .05$) after epinephrine administration, exercise, and twitching but not after sexual stimulation or during control bleedings. Concentrations of PRL increased ($P < .05$) after exercise and sexual stimulation. It is concluded that 1) a single episode of sexual excitement in stallions does not lead to a memory-mediated rise in either cortisol or PRL, 2) in stallions, cortisol and PRL are secreted in response to sexual stimulation, exercise, and twitching, whereas only cortisol is secreted in response to epinephrine administration, and 3) in geldings, PRL is secreted in response to exercise, and cortisol is released in response to epinephrine administration, exercise, and twitching.

Key Words: Stallions, Cortisol, Prolactin, Stress, Sexual Behavior

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Introduction

Concentrations of cortisol in horses increase after exercise (Snow and Munro, 1975; Dybdal et al., 1980; Snow et al., 1982; Church et al., 1987; Hower and Wickler, 1989), during isolation stress (Alexander et al., 1988), after induction of hypoglycemia and after surgery (James et al., 1970), after restraint via a twitch (Thompson et al., 1988), and after sexual activity (Tamanini et al., 1983; Rabb et al., 1989).

Prolactin (PRL) concentrations also increase after various stressful stimuli in rats (Neill, 1970; Euker et al., 1975; Dohler et al., 1977) and man (Noel et al., 1972). Thompson

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et al. (1988) found no effect of restraint via a twitch on PRL secretion in ovariectomized pony mares although ketamine administration briefly increased concentrations of PRL. Sexual stimulation increases PRL secretion in rats (Kamel et al., 1975, 1977; Kamel and Frankel, 1978) and bulls (Convey et al., 1971), as well as in stallions (Rabb et al., 1989).

Rabb et al. (1989) reported that stallions that had previously been exposed to sexual stimulation had increases in cortisol secretion and, to a lesser degree, PRL secretion in the absence of any visual or physical contact with mares. The authors suggested that the sound of other stallions being stimulated was sufficient to cause a memory-mediated rise in cortisol and PRL secretion.

The experiments described herein were designed 1) to determine whether stallions exhibited an increase in cortisol and(or) PRL secretion when returned to the site of a previous sexual encounter and, if so, to determine how long such a response would persist, 2) to compare the cortisol and PRL responses in stallions after sexual excitement to those occurring after various physical and chemical stimuli, and 3) to determine whether the responses to these stimuli in stallions were dependent on the presence of the testes.

Materials and Methods

Experiment 1. Four lighthorse stallions aged 6 to 14 yr were used. All stallions were sexually experienced and were given at least 7 d of sexual rest before the onset of the experiment. Stallions were housed in outdoor lots and were fed sufficient hay and grain to maintain good body condition. At approximately 0700 on the mornings of treatment, each stallion was fitted with an 18-gauge, polyethylene jugular catheter that was flushed with heparinized .15 *M* saline. Stallions were kept loosely tethered in their lots and had ad libitum access to hay and water.

Beginning at approximately 0800 on d 1, 3, 4, 5, and 6, two 5-ml blood samples were drawn via the catheter 10 min apart (–10 and 0 min) and were placed in heparinized tubes. Immediately after the 0-min sample, each stallion was led to a specified site along the fence of his lot and held there for 5 min. On d 2, the same procedure was followed except that an estrous mare was also brought to the site (across the fence) such that the stallion could make visual and limited physical contact for a total of 5 min. On all days, additional blood samples were drawn at 10, 20, and 30 min after time 0.

Experiment 2. Six sexually experienced lighthorse stallions aged 6 to 14 yr were managed as described for Exp. 1. Three of these stallions had been used in Exp. 1. After at least 7 d of sexual rest, all stallions were exposed to each of five treatments: 1) sexual stimulation (5-min exposure to an estrous mare), 2) acute physical exercise (running for 5 min), 3) restraint via a twitch (twitching; 5 min), 4) epinephrine[5] administration (3 mg i.v.), and 5) no stimulation (control). Stallions received one treatment per day on five separate days, with 2 d of sexual rest between treatments (based on results of Exp. 1). The order of treatments for each stallion was unique and was randomly selected from the 120 possible combinations. Also, stallions were started on treatment on a staggered schedule such that only two stallions were treated on any given day.

Blood sampling and other procedures were the same as those described for Exp. 1. Immediately after the 0-min blood sample, the appropriate treatment was administered. Subsequent samples were taken at 10, 20, and 30 min after time 0. To minimize the amount of activity seen and heard by a stallion on his treatment day, the two treated stallions for each day were housed approximately 100 m apart.

Experiment 3. Six long-term (> 1 yr) geldings aged 5 to 15 yr and of unknown sexual experience were exposed to the same five treatments described for Exp. 2. Geldings were managed in the same manner as the stallions. Each gelding also received a unique sequence of treatments with 2 d of rest between treatments. Procedures and frequency for blood collection were identical to those described for Exp. 2.

Radioimmunoassay and Statistical Analyses. All blood samples were kept on ice and were centrifuged within 1 h after collection. Heparinized plasma was harvested and stored

[5]The Butler Co., Columbus, OH.

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at −15°C. Concentrations of cortisol in plasma were measured by RIA as described previously (Thompson et al., 1988). Sensitivity of the cortisol assay was 37 pg/tube, and inter- and intra-assay CV were 12 and 6%, respectively. Concentrations of PRL in plasma were measured by double-antibody RIA similar to that described by Thompson et al. (1986). Antiserum (R4PRL) generated in a rabbit against porcine PRL (USDA-pPRL-B-1) was diluted 1:15,000 and was used at 200 μl/tube. Highly purified equine PRL (AFP-2451B[6]) was radioiodinated by the chloramine-T method and was isolated on Sephadex G-25. Inhibition curves in the assay generated with serial dilutions of equine plasma and pituitary homogenates were all parallel to those produced by purified equine prolactin. Cross-reactivities in the prolactin assay of equine LH, equine FSH, equine thyrotropin-stimulating hormone, porcine and canine growth hormone, synthetic adrenocorticotropic hormone and pregnant mare's serum gonadotropin were < .145, < .07, .53, .19, .06, < .0001, and < .03%, respectively. Moreover, fractionation of a pool of pituitary extracts via PAGE yielded a single peak of immunoreactivity coincident with radiolabeled equine prolactin. For 20 randomly selected samples of plasma, regression of PRL estimates in this assay with estimates from our previous assay (Thompson et al., 1986) resulted in a correlation coefficient of .95. Sensitivity of the PRL assay averaged .04 ng. Intra- and interassay CV averaged 7 and 12%, respectively. For each hormone, all samples from a given stallion were included in a single assay.

In all experiments, data for each hormone were analyzed by split-plot ANOVA with stallions as the random effect (Steel and Torrie, 1980). Fixed main effects were days and sampling times in Exp. 1 and treatments and sampling times in Exp. 2 and 3. Due to heterogeneity of variances, data were also analyzed after log transformation, but this was found not to affect the overall results. Before analysis, data from each stallion were placed on a percentage of pretreatment (average of −10 and 0 min samples) basis to eliminate pretreatment variation among stallions; only posttreatment samples (10, 20, and 30 min) were used in the ANOVA. The lsd test was used to determine whether treatment means differed from 100% (Steel and Torrie, 1980).

[6]Supplied by A. F. Parlow, Harbor-UCLA Medical Center, Torrance, CA.

Results

Experiment 1. Concentrations of cortisol and PRL in plasma of stallions increased ($P < .05$) only on the day of exposure to an estrous mare (d 2; Figure 1). This increase occurred within 10 min of PRL, which remained elevated through 30 min. Concentrations of cortisol increased by 30 min. On the pre-exposure (d 1) and all post-exposure days (d 3, 4, 5, and 6), none of the means for either cortisol or PRL differed from 100%.

Experiment 2. Concentrations of cortisol in plasma of stallions during the control period did not vary ($P > .05$) relative to pretreatment (Figure 2). Concentrations of cortisol for all other treatments increased ($P < .05$) rapidly after the onset of treatment. These concentra-

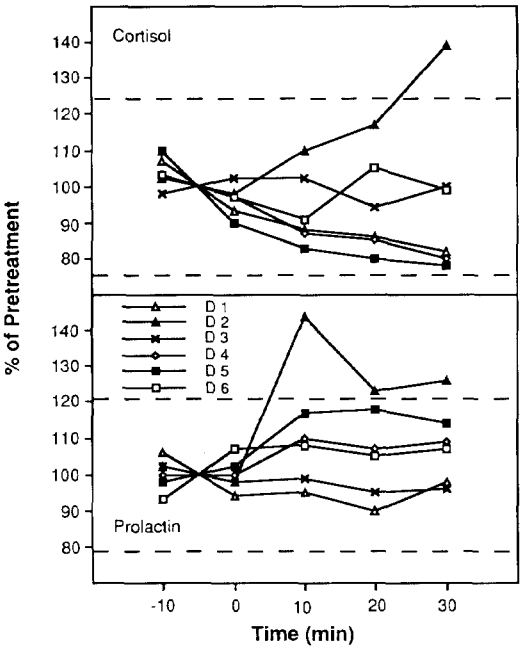

Figure 1. Concentrations of cortisol and prolactin in plasma of stallions exposed to an estrous mare for 5 min on d 2 and returned to the site of exposure on d 3, 4, 5, and 6. Day 1 was a control period (no mare present). Data are expressed as percentage of pretreatment. Horizontal dashed lines bracketing 100% indicate ± the lsd ($P < .05$). Pooled SE for cortisol and prolactin were 8.6 and 7.4%, respectively. Actual pretreatment concentrations of cortisol and prolactin averaged 28.4 and 9.0 ng/ml, respectively.

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tions remained elevated ($P < .05$) through 30 min for all treatments.

Concentrations of PRL increased ($P < .05$) in stallions in response to sexual stimulation and exercise (Figure 2) at 10 min and remained elevated through 30 min. Concentrations of PRL increased ($P < .05$) in response to twitching only at 10 and 30 min. Means for the control period and epinephrine administration did not vary ($P > .05$) relative to time 0.

Experiment 3. Concentrations of cortisol in plasma of geldings did not vary ($P > .05$) relative to pretreatment for the control period or during sexual stimulation. Concentrations of cortisol increased ($P < .05$) within 10 min after the onset of exercise and within 20 min after epinephrine administration and twitching (Figure 3) and remained elevated through 30 min.

Concentrations of PRL in plasma of geldings did not vary ($P < .05$) relative to pretreatment during the control period or after epinephrine administration or twitching (Figure 3). Concentrations of PRL increased ($P < .05$) an average of 3.5-fold within 10 min after exercise and remained elevated through 30 min. There was a minor (50%) increase ($P < .05$) in concentrations of PRL in plasma of geldings after exposure to an estrous mare.

Discussion

The results of Exp. 1 and 2 were similar to those reported by Rabb et al. (1989) for concentrations of cortisol and PRL in stallions after sexual stimulation with or without ejaculation. In our experiments and in that of Rabb et al. (1989), concentrations of PRL increased rapidly after sexual stimulation and then declined. Similar results have been reported for the rat (Kamel et al., 1975, 1977; Kamel and Frankel, 1978) and bull (Convey et al., 1971), in which concentrations of PRL peaked rapidly

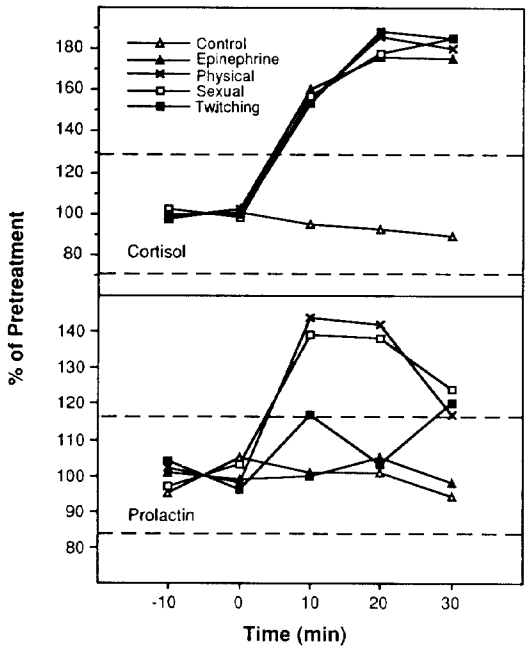

Figure 2. Concentrations of cortisol and prolactin in plasma of stallions exposed to sexual stimulation, physical exercise, restraint via a twitch, epinephrine administration, or no stimulation (control) beginning immediately after time 0. Data are expressed as percentage of pretreatment. Horizontal dashed lines bracketing 100% indicate ± the lsd ($P < .05$). Pooled SE for cortisol and prolactin were 10.4 and 5.7%, respectively. Actual pretreatment concentrations of cortisol and prolactin averaged 18.3 and 5.9 ng/ml, respectively.

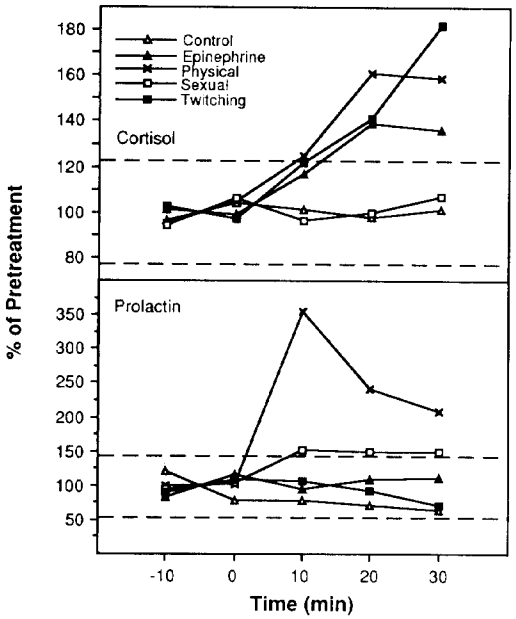

Figure 3. Concentrations of cortisol and prolactin in plasma of geldings exposed to sexual stimulation, physical exercise, restraint via a twitch, epinephrine administration, or no stimulation (control) beginning immediately after time 0. Data are expressed as percentage of pretreatment. Horizontal dashed lines bracketing 100% indicate ± the lsd ($P < .05$). Pooled SE for cortisol and prolactin were 7.7 and 16.4%, respectively. Actual pretreatment concentrations of cortisol and prolactin averaged 21.6 and 1.8 ng/ml, respectively.

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after mating. However, it has been reported that concentrations of PRL vary considerably in humans after sexual intercourse (Noel et al., 1972; Stearns et al., 1973).

There was no increase in secretion of cortisol or PRL on the days after return of the stallions to the site of their previous sexual stimulation. These data support the contention of Rabb et al. (1989) that the cortisol and PRL responses during control periods in their stallions previously exposed to sexual stimulation were not induced by the location of the control treatments, but rather by the sounds of other stallions being sexually stimulated at the same time. We also concluded from Exp. 1 that 2 d of rest between treatments would be sufficient to avoid carry-over effects from one treatment to the next, assuming the sounds and other potential stimuli were absent.

Concentrations of cortisol in stallions after sexual stimulation in Exp. 1 increased at a slower rate than those in Exp. 2 or those reported previously (Tamanini et al., 1983; Rabb et al., 1989). In the latter three experiments, concentrations of cortisol increased within 10 min or less relative to treatment. In Exp. 1, concentrations of cortisol did not rise until 30 min after treatment. The reason for this delayed response is not known.

It is likely that a general stimulation (discharge) of the sympathetic nervous system is the common event mediating the cortisol response to physical exercise, twitching, and other similar physical stimuli. Because sexual stimulation in stallions usually involves physical activity, the cortisol rise in response to sexual excitement might be attributed solely to this physical component. However, Rabb et al. (1989) reported that previously exposed stallions seem to be able to respond to the sounds of sexual activity with a cortisol rise in the absence of any noticeable physical activity, indicating a strictly psychic mediation (conditioned response). As expected, geldings showed only casual interest in mares during the exposure period. Because there was no sexual excitement, there was no associated physical activity and apparently no psychic stimulation, given the lack of a cortisol response.

Although there was a significant PRL response to sexual stimulation in geldings, the response was minimal and was present in only three of the six animals. Whether or not this increase was truly due to mare exposure needs to be studied further. Given that there was no simultaneous response in concentrations of cortisol, we believe that the PRL response may be of little physiologic significance. The only other treatment in which geldings differed from stallions was twitching, which itself was variable among stallions. If the cortisol and PRL responses observed in these experiments are mediated through discharge of the sympathetic nervous system in both stallions and geldings, these responses would not be expected to be gonadally dependent. However, it is possible that stallions are generally more excitable because of the presence of testicular androgens and estrogens in their blood, and thus may respond to stimuli of intermediate intensity, such as twitching, more readily than geldings.

In stallions as well as geldings, bolus administration of epinephrine resulted in a rapid increase in concentrations of cortisol in all animals. James et al. (1970) injected similar amounts of epinephrine into two ponies over a 2- to 5-min period and found no significant increase in concentrations of cortisol, even though lacrimation, profuse sweating, trembling, and rapid respiration were observed. The slower rate of administration used by James et al. (1970) apparently accounted for the lack of cortisol rise in their ponies. The lack of a PRL response to epinephrine injection indicates that the PRL responses to other forms of physical and sexual stimuli are via neural pathways that are not directly affected by catecholamines or cortisol. In contrast, the epinephrine effect on cortisol secretion may indicate an interaction of epinephrine with nervous pathways leading to secretion of corticotropin-releasing hormone and(or) adrenocorticotropic hormone.

Physical activity for 5 min caused a rapid increase in concentrations of cortisol in both stallions and geldings. These results are in agreement with others concerning concentrations of cortisol in horses after exercise of various intensities and durations (Snow and Munro, 1975; Dybdal et al., 1980; Snow et al., 1982; Church et al., 1987; Hower and Wickler, 1989). It was reported that physical training does not affect this increase in cortisol (Church et al., 1987), although trained horses do have a more rapid recovery rate of concentrations of cortisol, decreasing back to normal with 30 min (Hower and Wickler, 1989). In Exp. 2 and

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3, all stallions and geldings were untrained, which likely accounts for the continued elevation of concentrations of cortisol at 30 min after treatment.

Restraint via a twitch caused a rapid increase in concentrations of cortisol both in stallions and geldings. Thompson et al. (1988) reported a similar increase in cortisol secretion in ovariectomized pony mares after twitching, with peak concentrations occurring within 20 min. In contrast to ovariectomized pony mares and geldings, stallions also exhibited a transient increase in plasma concentrations of PRL after twitching. Given the diversity in these three experiments and the variability in response within Exp. 2, it is apparent that the PRL response to twitching is not as consistent as the cortisol response.

The increased secretion of PRL in stallions after sexual excitement has been speculated as being part of a generalized stress response rather than a result of any specific reproductive function (Rabb et al., 1989). The results of this study are consistent with this idea, given that similar PRL and cortisol responses occurred in stallions and geldings after various types of stimuli. However, the fact that twitching produced varied results indicates that all apparently stressful stimuli may not have the same impact on PRL secretion. Because the role of PRL in the horse has yet to be defined, the reason for its secretion in response to these stimuli is unknown.

Implications

These experiments provide further evidence that cortisol and prolactin are secreted in response to various sexual and physical stimuli in stallions and geldings. The cortisol and prolactin responses to acute exercise do not seem to be gonadally dependent, because they occur similarly in stallions and geldings. The cortisol and prolactin responses to sexual stimulation in stallions do not differ from those after acute exercise, indicating that the physical component of sexual excitement may be the main factor responsible for their occurrence.

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Research Article

Population Dynamics and Fertility Control of Feral Horses

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ABSTRACT The management of free-roaming, feral horse (*Equus caballus*) populations in the western United States is a contentious and challenging issue. Between 2008 and 2014, 1,873 individual horses from a closed population in the northern Great Basin, USA were captured, uniquely marked, and were either removed for private adoption or had permanent fertility control treatments (i.e., vasectomy or ovariectomy) applied prior to release. We derived the annual population size, growth rate, and the number of horses by sex and age by using the horse's estimated age at first capture to infer its age during each year it was part of the population. Estimates of population size from 2 aerial survey techniques averaged within 5.3%–9.6% of derived population estimates. The overall survival (i.e., recapture) rate for individual horses was 88% for individuals released back into the population between 2009 and 2012. Treated horses maintained group associations and there were no differences between survival rates for released females or males that received fertility control treatment, compared to animals released without treatment. The application of combined fertility control efforts resulted in a decline in the population-level annual foaling rate from >20% to <4% within 4 years. Maintaining a proportion of a population as permanently non-reproducing has the potential to safely maintain free-roaming horse herds at desired management levels. © 2016 The Wildlife Society.

KEY WORDS *Equus caballus*, feral horse, fertility control, foaling, ovariectomy, population dynamics, vasectomy.

Management of free-roaming, feral horses (*Equus caballus*) in the western United States is an increasingly controversial and contentious issue. The species is viewed as a symbol of freedom and an icon of the American West, but horses also are a non-native, feral grazer that present significant land management challenges. Unmanaged or poorly managed non-native grazers, including horses, can have substantial impacts on ecosystem integrity, influencing a wide array of native flora (Smith 1986, Levin et al. 2002, Zalba and Cozzani 2004, Beever et al. 2008, Davies et al. 2014), fauna (Beever 2003, Beever and Brussard 2004, Beever and Herrick 2006, Hall et al. 2016, Gooch et al. 2017), and ecosystem processes (Beever and Brussard 2000, Zeigenfuss et al. 2014). On lands managed by the Bureau of Land Management in the western states alone, there are an estimated >67,000 free-roaming horses and burros (*Equus asinus*) occupying 12.8 million ha, more than twice the maximum management level that can exist in balance with other public rangeland resources and uses (Bureau of Land Management 2016*a*).

Free-roaming female horses typically have their first foals at 2–3 years of age, often produce foals in consecutive years, and remain reproductively active throughout their lives with only a small reduction in foaling rates in the oldest age classes (Speelman et al. 1944, Berger 1986, Siniff et al. 1986, Wolfe et al. 1989, Garrott et al. 1991). In addition, mortality rates for free-roaming horses are low as or lower than other similarly sized, native mammals (Berger 1986); annual survival estimates for adult horses have ranged from 70–80% (Wolfe 1980) to 95% and higher (National Research Council 1980, Berger 1986, Garrott and Taylor 1990). The result is that free-roaming horse populations can grow at annual rates of 15–20% or more per year (Eberhardt et al. 1982, Berger 1986, Garrott and Taylor 1990) and it is expected that most populations approach this biological potential because current management generally ensures that horses are not resource- or self-limited (National Research Council 2013).

Western land managers have traditionally relied on the practice of capture, removal, and public adoption to manage free-roaming horse abundance. This process is costly and unsustainable because the number of adopted animals represent only a fraction of animals removed, which has resulted in almost as many horses now held for the remainder of their lives in long-term, federally subsidized private pastures as there are on the range (Garrott and Oli 2013, Bureau of Land Management 2016*a*). Consequently, despite previously aggressive programs to control the number of horses on public rangelands, current management strategies have not been able to reduce free-roaming horse populations to target levels (National Research Council 2013). For a segment of the public, neither capturing and removing horses nor letting horses perish on the range as a result of limited resources is

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acceptable. Methods to control population growth (e.g., fertility control, contraception) may reduce the need for intensive and controversial removals while ensuring that free-roaming horse populations do not become self-limiting (National Research Council 2013). Controlling population growth would also provide significant cost-savings to the American taxpayer (Bartholow 2007, de Seve and Griffin 2013) by affecting the ability to attain free-roaming horse management goals (National Research Council 2013).

Fertility control has been an attractive alternative to managing wildlife and other animal populations that have become overabundant (Ransom et al. 2014*b*), including free-roaming horses, white-tailed deer (*Odocoileus virginianus*), and elk (*Cervus canadensis*; Hobbs et al. 2000, Ransom et al. 2010). Contraception that persists for the lifetime of the animal may be the most efficient method for maintaining a population at target densities once an adequate number of animals have been sterilized (Hobbs et al. 2000). However, little empirical data are available on how such efforts affect population-level rates of change (Ransom et al. 2014*b*) or have demonstrated meaningful reductions in population growth rates in the field (Hobbs et al. 2000). Although a wide variety of fertility control agents have been and are being investigated for free-roaming horses (Bureau of Land Management 2016*b*), many of the management tools examined thus far are costly or impractical for large-scale use (Ransom et al. 2014*a*). In addition, managers are generally constrained by a lack of data including scientifically defensible horse population estimates, population growth rates, demographics, and subsequent effects of these proposed management actions (National Research Council 2013). One contraceptive technique, permanent sterilization of males via vasectomy, has had limited field application and previously targeted only socially dominant individuals (Eagle et al. 1993); although it has been recognized that the strategy of targeting only dominant males may not be successful in limiting population growth (Garrott and Siniff 1992, Bowling and Touchberry 2001). A second technique, targeting female horses for contraceptive control via permanent sterilization (i.e., ovariectomy) has not been tested within free-roaming horse populations to date.

We report on an 8-year study of the population dynamics of a free-roaming, feral horse herd in the northern Great Basin, USA. Our objectives were to evaluate the effectiveness of 2 permanent sterilization techniques used to control population growth while targeting individuals of both sexes regardless of social status.

STUDY AREA

Sheldon National Wildlife Refuge (Refuge), managed by the United States Fish and Wildlife Service, consisted of approximately 234,703 ha in northwest Nevada, USA, in the northern Great Basin. The Refuge was established in 1936 for the conservation of American pronghorn (*Antilocapra americana*) and other native sagebrush-obligate species, including greater sage-grouse (*Centrocercus urophasianus*). Elevations ranged from 1,326 m to 2,183 m. Recent summer temperatures ranged from 0°C to 34°C and winter temperatures between −29°C and 14°C; annual precipitation rarely amounted to more than 30 cm. Dominant vegetation consisted of shrubs, primarily sagebrush (*Artemisia* spp.) and associated sagebrush-steppe habitats. Scattered open woodlands consisting of western juniper (*Juniper occidentalis*) or curl-leaf mountain mahogany (*Cercocarpus ledifolius*) occupied ridgelines and some slopes. Aspen (*Populus* spp.) and willows (*Salix* spp.) were found in dispersed snowpockets and in areas of persistent water.

The feral horse population occupying Sheldon National Wildlife Refuge dated back to prior to the establishment of the Refuge as privately owned animals escaped or were released from ranches and grazing allotments (U.S. Fish and Wildlife Service 2013). During the early 1990s, the horse population averaged 200–300 animals as a result of a proactive population control (removal) program; however, without continued active management, the population substantially increased by the mid-to-late 2000s necessitating management action. Since the early 2000s, feral horses were reported to affect the Refuge's natural resources and native wildlife (Barnett 2002, Davies et al. 2014, Zeigenfuss et al. 2014, Gooch et al. 2017) and were targeted for removal in 2013–2014 (U.S. Fish and Wildlife Service 2013). Small numbers of feral burros also occurred on the Refuge but were not included in this study. The area surrounding the Refuge was predominantly federal land administered by the Bureau of Land Management, and included areas designated as wild horse Herd Management Areas (HMAs) and Herd Areas (HAs); recent surveys estimated over 3,200 horses occupying the surrounding areas (Lubow 2015). A fence along the exterior boundary of the Refuge geographically restricted the movement of livestock, horses, and burros between the Refuge and adjacent lands.

METHODS

Between 2008 and 2014, each fall we used a helicopter to herd feral horses into a trap corral and then transported them by trailer to an on-site facility for handling (U.S. Government Accountability Office 2008). An equine veterinarian estimated the age of each captured horse using teeth characteristics (e.g., eruption date, rate of growth, tooth wear; McMullan 1983). Accurately aging animals by teeth characteristics can be challenging because of individual rates of tooth growth and wear; therefore, the same person aged all horses to ensure consistency in estimates over the duration of the study. We determined the sex of each horse, and paired females with their dependent foals. From 2008 to 2012, we removed approximately 10–20% of the total population each year (predominantly younger horses estimated at <5 yr) from the Refuge and transported them to contractors for private adoption; no removals occurred in 2007. We treated the majority of the remaining captured animals (i.e., those not transported for adoption) with permanent fertility control and released them back at the site of capture. Horses established as previously captured and marked (see below) were identified and recorded, and assessed for removal or re-release back onto the Refuge (prior to 2013). We surgically vasectomized (2008–2009) or chemically epididymectomized (2010–2011; Scully et al. 2015) males and surgically ovariectomized females via colpotomy (2009–2011; McKinnon et al. 1991, Hooper et al.

1993, Auer and Stick 1999). Costs associated with treatments ranged from approximately $125/horse for vasectomies to $150/horse for ovariectomies, and included veterinarian time and veterinary supplies (L. G. Pielstick, HCVC, personal communication).

Veterinarians experienced in handling domestic and non-domestic horses performed all procedures. All procedures followed taxon-specific guidelines approved by the American Society of Mammalogists (Sikes et al. 2016), consistent with recommendations for wild taxa (Sikes et al. 2012). In addition, we contracted with an independent veterinarian to provide protocol and program review and animal welfare oversight during all phases and for the duration of the study. We held and observed treated horses in captivity on average 3–8 days before release, as per veterinarian recommendations, and the losses attributed to treatment complications pre-release was 2% ($n = 8$). We implanted all horses with a uniquely identifying microchip in a passive integrated transponder (PIT) tag. We injected the microchip into the nuchal ligament along the base of the horse's mane to reduce the potential for tag migration (Stein et al. 2003); tag retention was 100%. We also marked individuals that received fertility control treatments with a visible freeze-brand signifying treatment type. In 2013 and 2014, all remaining horses in the population were targeted for removal and transportation to contractors for private adoption, including those which had been previously captured and released; approximately half of the animals were removed in 2013 ($n = 415$) and the remainder in 2014 ($n = 397$). As of fall 2015, an estimated <30 horses remained on the Refuge.

We compared 2 aerial survey methods used to monitor temporal trends in the feral horse population. Each July between 2007 and 2014, as part of routine wildlife composition surveys, a pilot and 2 experienced observers made uncorrected direct (i.e., raw) counts of horses from a helicopter flying linear transects at approximately 91 m above ground level (AGL) and 0.4-km spacing, dependent upon visibility, terrain, and habitat. We recorded the global positioning system (GPS) location of and the number of individuals in each group of horses, classified each animal as an adult or foal (<1 yr), and noted the number of freeze-branded individuals present by treatment type. We used the July helicopter survey data to evaluate differences in group size over time and group association of treated individuals, and to calculate observed foaling rates (foals:100 adults). We used foals:100 adults rather than foals:100 females because the sexes are not distinguishable from a distance (i.e., during aerial surveys), and to be comparable to foaling rates commonly derived from aerial survey estimates for adjacent and other free-ranging horse populations.

In addition to annual helicopter surveys, we also surveyed horses on the Refuge in June 2008, 2010–2012, and 2014 as part of a larger, interagency landscape-level effort, which included surveying 14 surrounding wild horse HMAs and HAs administered by the Bureau of Land Management. This double-observer count technique, detailed by Ransom (2012) and which includes an analysis of detection probabilities, used a fixed-wing aircraft (Cessna 185, 206, or 210) to fly parallel transects spaced 2.4 km apart at approximately 152 m AGL at approximately 100–140 knots. A pilot and 3 observers independently recorded GPS locations of horse groups when sighted, number of horses in each group, and age classification (foal vs. adult; 2010, 2011, and 2014 only). We used the observed June foaling rate (i.e., foals:100 adults) obtained from fixed-wing surveys to compare annual rates across populations within the larger landscape.

Because the recapture rate for individuals was 88% for the entire study period (i.e., 88% of individual horses captured, marked, and released back onto the study area were physically recaptured ≥1 yr later), we assumed this to be an essentially closed population, with minimal exchange of horses moving between the fenced study area and surrounding lands in any given year. This was further corroborated by data collected between 2009 and 2012 from horses marked with collars outfitted with GPS receivers (Collins et al. 2014), all of whom remained entirely within the study area (G. H. Collins, U.S. Fish and Wildlife Service, unpublished data). We derived the annual population size and number of horses by sex (sex ratio) and age class (foal [<1 yr], 1-yr-old, 2-yr-old, adult [≥3 yr]) each year by using the horse's estimated age at first capture to infer its age during each year it was part of the population (Table 1). We assumed all horses ≥3 years

Table 1. Number of feral horses by year and reproductive status (treated vs. untreated) derived by using the horse's estimated age at first capture to infer its age during each year it was part of the population, Sheldon National Wildlife Refuge, Nevada, USA, 2007–2014.

Age/sex class	2007	2008	2009	2010	2011	2012	2013	2014
Foal (<1 yr)	169	185	155	115	85	48	23	33
1-yr-old	141	169	127	96	67	56	27	7
2-yr-old	123	141	126	95	59	39	47	18
Adult F (≥3 yr)	353	428	402	402	347	320	253	113
Treated adult F	0	0	0	32	70	97	73	28
Untreated adult F	353	428	402	370	277	223	180	85
Adult M (≥3 yr)	424	471	512	501	461	474	426	226
Treated adult M	5[a]	5[a]	99	117	165	225	202	108
Untreated adult M	419	467	413	384	296	249	224	118
Other[b]	6	7	7	7	7	7	4	0

[a] Includes surgically castrated males (i.e., geldings), which were treated and released prior to this study.
[b] Horse/burro hybrids (i.e., mule or hinny), which are sterile. Not included in reproductive calculations, only in population estimates.

Table 2. Estimates of feral horse annual population sizes and growth rates, Sheldon National Wildlife Refuge, Nevada, USA, 2007–2014.

Year	Double observer estimate[a] (95% CI)	Direct count estimate[b]	Derived estimate[c]	Derived annual growth rate (λ)[d]
2007		786	1,216	—
2008	1,341 (1,215–2,043)	1,148	1,402	15.3% (1.15)
2009		1,210	1,329	−5.2% (0.95)
2010	1,236 (1,215–1,263)	1,053	1,216	−8.5% (0.91)
2011	1,070 (998–1,118)	1,104	1,026	−15.6% (0.84)
2012	908 (865–934)	973	944	−8.0% (0.92)
2013		831	780	−17.4% (0.83)
2014	452 (394–505)	411	397	−49.1% (0.51)

[a] Fixed-wing survey with corrected population estimate using sighting probabilities; no fixed-wing surveys conducted in 2007, 2009, or 2013. Individual survey results can be found in Lubow (2009), Lubow et al. (2010), Lubow (2011), Manning (2013), and Lubow (2015).
[b] Helicopter survey with uncorrected direct (i.e., raw) count.
[c] Derived by using the horse's estimated age at first capture to infer its age during each year it was part of the population.
[d] From derived population estimate. Declining values for 2008–2014 represent the combined result of removals and fertility control treatments.

old to be capable of reproducing (Garrott and Taylor 1990) unless treated with fertility control.

Foal mortality in free-roaming horse populations is highest during the first 1–2 months of life (Welsh 1975, Boyd 1979, Berger 1986, Turner and Morrison 2001). As such, we defined foal recruitment as foals that survived to October each year. We derived the actual number of foals and actual foaling rate (foals/adults) for each year. We calculated estimated annual foal recruitment as the difference between observed (Jul) and actual (Oct) foaling rates. In 2007 and 2008, prior to fertility control treatments, the derived ratio of recruited foals to adult females averaged 45% (47.9% and 43.2%, respectively), that is, an average of 45% of all potentially reproductive adult females (≥3 yr) recruited foals each year. Using that average, for each year, we estimated the number of recruited foals that would have been expected had no fertility control treatments been applied (adult females × 0.45) and the expected foaling rate without treatment (expected foals/adults).

To infer the relative effectiveness of each treatment type, we calculated the expected number of recruited foals if only females were treated ([adult F × 0.45] − [treated F × 0.45]) and divided by the number of adults to calculate the foaling rate considering treated females only for each year. We calculated the fecundity of reproductive females exposed to treated males present in the population as the derived ratio of number of foals to untreated adult females. We performed statistical analyses following Wilkinson (1990). We used Pearson's chi-square to test for differences in survival rates (i.e., recapture rates) between treated and untreated horses. We used multiple regression analysis of variance to determine whether 1) the number of treated males or females was correlated with group size as observed during aerial surveys; and 2) the number of foals was correlated with group size, the number of treated males, or number of treated females; $P \leq 0.05$ was considered statistically significant.

RESULTS

Between 2008 and 2014, we captured 1,873 individual feral horses. The population occupying the Refuge was estimated to have reached a high of around 1,400 animals in 2008 and, with the exception of 2007, both aerial survey techniques used to estimate the size of the feral horse population were in close agreement with derived estimates of population size (Table 2). The population's adult sex ratio averaged from 127.2 males:100 females (2007–2012) to 172.2 males:100 females (2013–2014; Table 1). For recruited foals, the sex ratio averaged 90.2 males:100 females for 2007–2014 (range = 68.2–120.0 M:100 F).

Estimated ages of captured horses ranged from dependent foals at 2–3 months old to 30 years old for males and 26 years old for females. Estimated ages of untreated females with dependent foals ranged from 2–22 years old, with foal recruitment peaking for females at 3–11 years old (79.1%; Fig. 1). Estimated ages of fertility control treated males ranged from 1–27 years old (83% were ≥7 yr old) and for fertility control treated females 2–26 years old (84% were ≥7 yr old). Recapture rates (i.e., survival rates) for individual horses were 85.3% for released females ($n = 150$) and 89.2% ($n = 278$) for released males across the period of study. Survival rates for released females that received fertility control treatment (85.1%, $n = 114$) did not differ from females that were released without treatment (86.1%, $n = 36$; $\chi^2 = 0.023$, $P = 0.880$). Also, survival rates for released males that received fertility control treatment (89.2%, $n = 268$) did

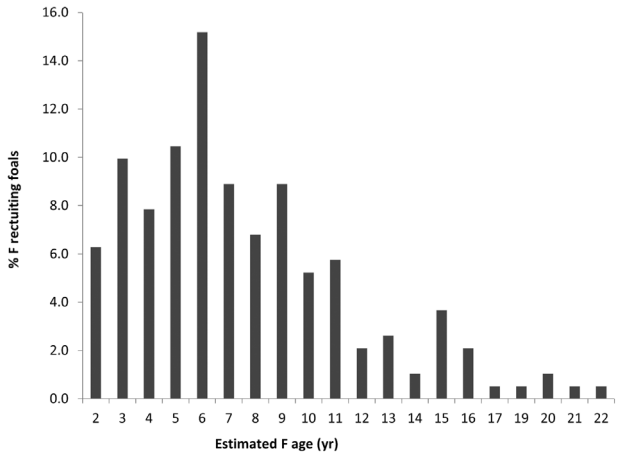

Figure 1. Estimated ages of untreated female feral horses recruiting foals (accompanied by dependent foals in the fall each year), Sheldon National Wildlife Refuge, Nevada, USA, 2008–2014.

not differ from those that were released untreated (90.0%, $n = 10$; $\chi^2 = 0.007$, $P = 0.935$). Of the treatments applied to released males, 51% received surgical vasectomies ($n = 137$) and 47% were chemically epididymectomized ($n = 126$); we also recaptured 5 males that were surgically castrated and released prior to this study (~2005). The percent of treated individuals in the population ranged from 7.4–35.3% each year, with 8.0–30.3% for females and 19.3–47.8% for males (Table 1).

Based on helicopter surveys, horse group sizes in July did not differ for 2008–2011, averaging 9.9 individuals/group (range = 1–115 ± 13.9 [SD], $n = 446$ groups). Group sizes were smaller in 2012–2013 ($\bar{x} = 6.2$ horses/group, range = 1–63 ± 7.6, $n = 291$ groups). We were able to determine the sex composition for 61% of the groups we observed during helicopter survey efforts ($n = 737$ groups) using the combined presence of foals and marked individuals. Of those, 48.4% were mixed-sex groups (≥1 M and ≥1 F; $n = 357$) and 13.1% were solitary animals ($n = 97$); the sex composition of the remaining groups ($n = 283$) could not be determined (i.e., no foals present or combination of marked individuals). Of the solitary animals documented, 23.7% were treated males ($n = 23$ observations); there were no observations of solitary treated females. Because they were not visibly marked, it is unknown how many of the remaining solitary individuals were untreated males versus untreated females. However, during captures in 2013 and 2014, the number of horses captured alone was predominantly untreated males; therefore, it is reasonable to assume that the majority of the remaining solitary animals observed during helicopter surveys were also untreated males.

The number of treated males in a group was positively correlated to group size based on helicopter surveys (i.e., larger groups had more treated M; $R^2 = 0.765$, $F_2 = 1{,}197.785$, $P < 0.001$), although the number of treated females was not ($R^2 = 0.000$, $F_2 = 0.348$, $P = 0.706$). Of groups containing multiple treated males (≥2 treated males; $n = 106$ groups), 70.0% were mixed-sex groups ($n = 71$) and 33.0% ($n = 35$) were groups of undetermined sex composition. As an example, 1 mixed-sex group of 34 horses observed in 2013 comprised 16 treated males, 4 treated females, 13 untreated (i.e., sex unknown) individuals, and 1 foal.

Overall, each horse group (>1 horse) averaged 1.5 ± 2.7 foals/group ($n = 396$ groups) for 2008–2011 and 0.50 ± 0.81 foals/group ($n = 244$ groups) for 2012–2013 based on helicopter surveys. For groups with no fertility control treated horses, the average number of foals/group was 1.2 ± 2.3 ($n = 402$ groups). Not surprisingly, the number of foals in a group was positively correlated to group size; larger groups had more foals ($R^2 = 0.702$, $F_1 = 1{,}731.924$, $P < 0.001$). For groups with ≥1 treated males, the average number of foals/group decreased as the number of treated males within the group increased (1.05, 0.68, and 0.65 foals/group for 1, 2, or 3 treated males, respectively). A similar pattern was observed for groups with ≥1 treated females (0.67 and 0.56 foals/group for 1 or 2 treated females, respectively). However, the best predictor for the number of foals within a group was the interaction between group size and both treatments (group size × no. treated females × no. treated males; $R^2 = 0.769$, $F_3 = 816.733$, $P < 0.001$). Larger groups had more foals (ß = 0.921, $t_3 = 49.469$, $P < 0.001$), but the number of foals decreased correspondingly with increasing numbers of treated females (ß = −0.071, $t_3 = -3.685$, $P < 0.001$) and treated males (ß = −0.238, $t_3 = -11.960$, $P < 0.001$) within a group.

The foaling season on the Refuge ranged from February to September, as estimated by ages of foals captured in the fall. The peak of foaling season was between April and June (83.2% of foals born), with on average 90.8% of foals on Sheldon Refuge born by the end of June each year. In comparing peak foaling rates across the larger landscape based from fixed-wing surveys in June 2010, 2011, and 2014, values within adjacent HMAs averaged annually between 7.6 foals:100 adults and 21.0 foals:100 adults compared to the Refuge's average of 7.4 foals:100 adults; the highest observed June foaling rate was 32.2 foals:100 adults documented in the Carter Reservoir HMA in 2014 (Table 3). Observed foaling rates based on helicopter surveys between 2008 and 2014 for the Refuge population ranged from 6.0 foals:100 adults to 18.0 foals:100 adults (Table 4). Actual foaling rates for the Refuge population derived from captured individuals ranged annually from 3.4 foals:100 adults to 21.8 foals:100 adults and were comparable to annual observed estimates from July helicopter surveys (Table 4). Estimates of annual foal recruitment (post-Jul) averaged 100.1% (range 97.4–103.0%); values >100% represent the small number of foals born after June.

The application of combined fertility control treatments resulted in a decline in the population-level actual foaling rate from >20.0 foals:100 adults to <4.0 foals:100 adults within 4 years (Table 4). In our population, a greater proportion of the males received treatment compared to the number of treated females. We were able to infer that treating approximately 30% of the female population had the potential to result in substantial declines in the expected foaling rate (>20 foals:100 adults with no treatment vs. $\bar{x} = 12.0$ foals:100 adults with only

Table 3. Comparative peak foaling rates of feral horses (foals:100 adult horses) within Sheldon National Wildlife Refuge, and adjacent Bureau of Land Management Herd Management Areas (HMA) and 1 Herd Area (HA) as estimated from fixed-wing aerial survey efforts in June, 2010, 2011, and 2014, Oregon and Nevada, USA.

	Comparative peak foaling rate			
Survey area	**2010**	**2011**	**2014**	$\bar{x}$
Sheldon National Wildlife Refuge, OR	11.4	5.4	5.4	7.4
Beaty Butte HMA, OR	21.6	21.6	19.8	21.0
Bitner HMA, NV	17.5	9.8	11.4	12.9
Carter Reservoir HMA, NV	—[a]	4.7	32.2	18.4
FoxHog HMA, NV	17.2	14.7	8.6	13.5
HighRock HMA, NV	28.8	14.5	7.6	17.0
Massacre Lakes HMA, NV	13.0	3.3	6.5	7.6
NewYears Lake HA, NV	12.4	11.9	16.2	13.5
NutMtn HMA, NV	—[a]	12.1	8.3	10.2
WallCanyon HMA, NV	20.8	19.0	2.8	14.2
Black Rock Range East HMA, NV	16.9	4.0	13.7	11.5
Black Rock Range West HMA, NV	17.6	—[a]	12.3	15.0
Calico Mtns HMA, NV	15.6	13.1	14.9	14.5
Granite Range HMA, NV	30.5	8.3	5.9	14.9
Warm Springs Canyon HMA, NV	16.3	17.3	8.5	14.0

[a] Sample size too small for estimate.

Table 4. Feral horse foaling rates for observed (from helicopter surveys), actual (derived from known captured individuals), expected without treatments, and expected with treated females only. We also provide fecundity for untreated females exposed to treated males (no. foals/untreated F) and percent of the population treated by sex (% treated), Sheldon National Wildlife Refuge, Nevada, USA, 2007–2014.

	Foaling rates (foals:100 adults)				
Year	Observed	Actual[a]	Expected without treatment	Expected with treated F only (% treated F)	F fecundity with treated M (% treated M)
2007		21.8	20.4		0.48 (1.2)
2008	18.0	20.6	21.4		0.43 (1.1)
2009	14.0	17.0	19.8		0.39 (19.3)
2010	13.0	12.7	20.0	18.4 (8.0)	0.31 (23.4)
2011	11.0	10.5	19.3	15.4 (20.2)	0.31 (35.8)
2012	8.0	6.0	18.1	12.6 (30.3)	0.22 (47.5)
2013	6.0	3.4	16.8	11.9 (28.9)	0.13 (47.4)
2014	9.0	9.7	15.0	11.3 (24.8)	0.39 (47.8)

[a] Includes the combined effects of both treatment groups.

females treated, $\bar{x} = 28\%$ treated). In addition, treating approximately 23–47% of the male population also likely contributed to substantial declines (30–72%) in untreated female fecundity after 2007–2008 (Table 4).

DISCUSSION

The fertility control treatments we applied did not affect the survival of horses post-release. In addition, fertility control efforts were effective in reducing the population-level annual foaling rate within a short period of time (from >20% to <4% within 4 years). Annual foaling rates across the larger area peaked in June but were variable between years and across the larger landscape. Our estimates of annual foal survival in the Refuge population were high (>97%) and although we were unable to account for potential overwinter mortality during a foal's first year, we suspect it was a small influence on the Refuge, particularly given the high recapture rates over the course of this study. This indicates that free-roaming horse composition counts from aerial surveys conducted in July and later can be used to reasonably approximate annual foal recruitment in populations where annual recruitment is generally high. Although not directly tested for, we did not detect any noticeable shifts in the timing of the foaling season in response to fertility control treatments as postulated by others (Garrott and Siniff 1992, Asa 1999).

Within the relatively open habitats of the Refuge, our population estimates generated from the fixed-wing double-count technique tended to underestimate population size, although the method provided the ability to calculate confidence intervals and account for animals not seen, and the derived population size generally fell within those ranges. In contrast, uncorrected direct counts via helicopter generally tended to overestimate population size, particularly as the population began to decline as a result of increasing management activities between 2011 and 2014, and did not provide the ability to estimate confidence intervals. Helicopters were used to conduct direct count surveys and to capture the horses, and as a result individuals became increasingly familiar with and reactive to the presence of the helicopter over time; we did not observe the same degree of reactivity to the fixed-wing platform used during double-count surveys. We suspect the overestimation from the direct counts were the result of inadvertently double-counting a proportion of increasingly reactive individuals that were moving between areas and between days in response to the presence of the survey helicopter.

Limiting fertility through the use of immunocontraceptives and other techniques is becoming a more commonly considered tool for controlling free-roaming horse abundance; although the physiological changes that may accompany some of these tools have the potential to influence individual behavioral changes and alter family group structure and population dynamics (Nuñez et al 2010, Ransom et al. 2014*b*). Fertility control treated males in our study showed no difference in measurements of normal testicular endocrine function compared to untreated individuals (Scully et al. 2015), suggesting there should be no change in male breeding behaviors or social status (Asa 1999). We documented that all treated individuals appeared to maintain group associations and there were no groups consisting only of treated males or only treated females. We documented mixed-sex groups consisting of multiple treated males in all years. The social structure of free-roaming horse groups generally consists of a harem stallion, occasionally a satellite stallion, and females and offspring (Roelle et al. 2010). The duration of multiple-male bands has been thought to be short-lived, from several hours to a year (Berger 1986, Roelle et al. 2010), although Bowling and Touchberry (2001) reported that 39% of their harem bands contained multiple males; differences between populations relative to multiple male bands may be associated more with sex ratio and population density (Kirkpatrick and Turner 1986). Further, male offspring may remain with their maternal band for up to 4 years (Berger 1986) and this also could account for some of our observations. Regardless, the complexity of social behaviors among free-roaming horses is not entirely centered on reproductive receptivity, and fertility control treatments that suppress the reproductive system and reproductive behaviors should contribute to minimal changes to social behavior (Ransom et al. 2014*a*).

Cost is another factor for consideration, and there are 2 categories associated with fertility control in free-roaming horses: cost of the treatments themselves and costs associated with treatment application. The 2 commonly used temporary agents are formulations of porcine zona pellucida (PZP; ZonaStat-H and PZP-22; Science and Conservation Center, Billings, MT, USA) and are approximately $35.00/dose and $325.00/dose, respectively (A. B. Shepherd, Bureau of Land Management, personal communication). In comparison, the permanent techniques we used ranged from approximately $125/horse to $150/horse (i.e., vasectomy and ovariectomy, respectively). However, both temporary agents require repeated dosing approximately every 12 months to maintain reproductive control. Thus, assuming the average free-roaming horse lives to be at least 20 years old, estimated costs of re-dosing would range from $590/horse to $5,500/horse over the individual's lifetime. In addition are the costs associated with application; of the currently available techniques, all require the

animals to be captured and handled. Even in the case of temporary agents being used by the Bureau of Land Management, the horses are typically captured, transported to holding facilities (both temporary and permanent), and then held in captivity for days to months while individuals slated for treatment and release are selected (A. B. Shepherd, personal communication). Thus, although capture, transport, and holding would be a one-time expense in the case of permanent treatments, the repeated dosing required for temporary treatments also would require repeated handling and substantially increase the cost per horse (i.e., >$1,000/horse/capture). Further, each additional capture event also increases the risk of injury or death to individuals. In very rare cases, some free-roaming horse herds have been sufficiently habituated to humans and are able to be approached (<36 m) and remotely darted with a temporary contraceptive agent (A. B. Shepherd, personal communication). In those specific circumstances, long-term reproductive control could potentially be maintained without repeated capture events; however, although this approach is gaining popularity with some members of the public, this scenario is not realistic for the majority of free-roaming horse herds occupying public rangelands in the western United States.

As discussed, social structure may be an important consideration in the efficacy of fertility control in free-roaming horses. For example, previous efforts to control fertility in male free-roaming horses reported that permanent sterilization limited to socially dominant males may be effective in reducing foaling rates in individual, stable groups. However, it was postulated that it might not effectively suppress overall population growth unless a large proportion of the males were treated (Garrott and Siniff 1992, Eagle et al. 1993, Ransom et al. 2014*b*). Hence, our efforts did not target only socially dominant individuals but sought to saturate the study area with permanently treated animals, both males and females, and as such, we saw an almost immediate reduction in foaling rates at the group- and population-levels. Our results indicate that a combination of effective measures are currently available that can reduce annual foaling rates without the majority of individuals maintained as non-reproductive, and that these measures can be used to successfully control population growth in large populations of free-roaming horses.

MANAGEMENT IMPLICATIONS

One primary advantage of permanent fertility control is that treated animals do not require repeated exposure to potentially risky capture and handling to maintain reproductive control. Our findings demonstrate that maintaining a proportion of a population as permanently non-reproducing has the potential to safely reduce annual foaling rates within a short period of time, and provide managers the ability to start to attain and then maintain free-roaming horse herds at desired management levels. Regardless of the technique selected, foregoing any measures to control annual foaling rates will require the continuation of frequent, high-profile, controversial capture operations, with the consequent, unsustainable management of free-roaming horses and associated declines in rangeland ecological health, unless even more drastic measures are undertaken.

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Post-castration retention of reproductive behavior and olfactory preferences in male Siberian hamsters: Role of prior experience

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Abstract

Reproductive behavior of virtually all adult male rodents is dependent on concurrent availability of gonadal steroids. The ejaculatory reflex is incompatible with long-term absence of testicular steroids and typically disappears within 3 weeks after castration. Male Siberian hamsters are an exception to this rule; mating culminating in the ejaculatory reflex occurs as many as 6 months after castration (persistent copulation). The emergence of persistent copulation many weeks after gonadectomy is here shown not to require repeated post-castration sexual experience. Preoperative sexual experience, on the other hand, significantly increases the percent of males that copulate after gonadectomy, but is not required for the emergence of this trait in 25% of males. Castration prior to puberty prevents persistent copulation in all individuals in adulthood. Persistent copulators, unlike males that cease mating activity after castration, prefer the odors of estrous over non-estrous females when tested 4 months after castration and 7 weeks after the last mating test. Neural circuits of persistent copulators retain the ability to mediate male sex behavior and preferences for female odors in the complete absence of gonadal steroids; they are influenced by preoperative sexual experience and organizational effects of gonadal hormones at the time of puberty.
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Keywords: Siberian hamster; Male copulatory behavior; Olfactory preferences; Sexual experience

Introduction

Copulatory behavior of most adult male mammals is dependent on concurrent availability of gonadal hormones. In the most well studied animal models, castration of adults leads to a rapid decline in male sexual behavior that is reversible with testosterone treatment (Young, 1961; Sachs and Meisel, 1988). Testicular hormones affect both appetitive and consummatory components of male copulation (Beach, 1956; Davidson, 1972). Beginning a few weeks after castration male rodents paired with estrous females no longer display the distinct intromission and ejaculatory behaviors characteristic of intact animals (rats, Davidson, 1966; guinea pigs, Grunt and Young, 1952; Syrian hamsters, Beach and Pauker, 1949; mice, McGill and Tucker, 1964). Only rarely do males of these species sustain ejaculatory behavior for more than a few weeks after castration (e.g., Davidson, 1966). B6D2F-1 hybrid mice, 30–80% of which retain the ejaculatory reflex 5–6 months after orchidectomy (Manning and Thompson, 1976; Clemens et al., 1988; Wee and Clemens, 1989), and Siberian hamsters, approximately one third of which maintain the complete copulation pattern for 4–6 months after castration (Park et al., 2004), are exceptions to this rule. Furthermore, 50% of Siberian hamsters that undergo testicular regression in short winter photoperiods also continue to display the ejaculation reflex despite low to undetectable blood testosterone concentrations (Park et al., 2004). The long-term persistence of copulation in inbred mice does not require the presence of adrenal hormones (Thompson et al., 1976).

Preoperative sexual experience facilitates retention of male sex behavior in mice (Manning and Thompson, 1976), cats (Rosenblatt and Aronson, 1958) and Syrian hamsters (Bunnell and Kimmel, 1965; Lisk and Heimann, 1980) castrated in

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adulthood as well as Syrian hamsters subjected to ablation of the vomeronasal organ (Meredith, 1986). In contrast, prior sexual experience does not promote retention of sex behavior in orchidectomized rats (Rabedeau and Whalen, 1959; Bloch and Davidson, 1968) and dogs (Hart, 1968; Beach, 1970).

In a previous study, male Siberian hamsters that continued to copulate many weeks after castration (persistent copulators) were sexually experienced at the time of orchidectomy, and were tested bi-weekly beginning 1 week after castration (Park et al., 2004). The present study, which assesses the role of pre- and postoperative sexual experiences in the retention of mating behavior after castration, parallels earlier investigations of B6D2F-1 mice (Manning and Thompson, 1976; Wee and Clemens, 1989). Because pubertal testicular hormones organize sex behavior in hamsters (reviewed in Schulz and Sisk, 2006), we also determined whether prepubertal castration was compatible with persistent copulation in adulthood.

Copulation in rodents is conceived as completely dependent on a combination of chemosensory cues and hormones (Wood, 1997; Wood and Newman, 1995). Sexually experienced male rats (Stern, 1970; Paredes et al., 1998) and Syrian hamsters (Fiber and Swann, 1996; Swann, 1997) prefer the odors of estrous females over those of non-estrous females or males. This preference disappears a few weeks after castration, and is restored by testosterone treatment (e.g., Stern, 1970 for rats; Gregory et al., 1975 for Syrian hamsters). The loss of this olfactory preference may contribute to the males' reduced mating activity after castration.

The present investigation further characterizes the post-castration persistent copulator phenotype of Siberian hamsters by extending observations to another behavior: the male's preference for estrous female odors. Males that displayed the ejaculation reflex 7 or more weeks after castration were designated persistent copulators. To test the hypothesis that persistent copulators would remain attracted to odors of estrous females, we assessed olfactory preferences of castrated male Siberian hamsters that either had stopped copulating or continued to mate for several months after castration. Testing was conducted 4 months after castration and approximately 7 weeks after the most recent mating test.

Materials and methods

Animals

Siberian hamsters (*Phodopus sungorus sungorus*) were from our local breeding colony, descended from stock originally supplied by Bruce Goldman (University of Connecticut, Storrs, CT) and later outbred to hamsters supplied by Katherine Wynne-Edwards (Queen's University, Kingston, ON). All procedures were approved by the Animal Care and Use Committee of the University of California at Berkeley.

To facilitate behavioral testing during the daytime, hamsters entrained to an LD 14:10 photocycle (14 h light/day with onset of darkness at 1300 h PST) were mated. Males weaned at 18 days of age were housed individually in polypropylene cages (25×14×12 cm). Food (Purina rodent chow 5015) and water were provided *ad libitum*, and the ambient temperature was 22±2°C.

Female hamsters used in behavioral testing were ovariectomized at approximately 60 days of age and were treated with 4 mm Silastic capsules implanted subcutaneously (Dow Corning, Midland, MI, USA; ID 1.98 mm, OD 3.18 mm). Capsules filled with crystalline estradiol benzoate (EB; Sigma Chemical, St. Louis, MO, USA) were soaked in sterile saline solution for 24 h prior to implantation. To induce behavioral estrus each female was injected with progesterone (0.6 mg/0.1 ml peanut oil, sc; Sigma) 6 h prior to testing. Stimulus females were maintained in the same 14 L photoperiod as the males.

Mating test

Testing, conducted under dim red light between 1400 and 1700 h in the male's home cage, began with the introduction of a hormonally-treated stimulus female. If the female failed to display receptive behavior in response to male approaches within 10 min, she was replaced with another female. Otherwise the male's behavior was recorded for 30 min, or until 2 ejaculation reflexes were observed, whichever occurred first. Observation was terminated after 20 min if the male failed to mount the second female.

Data were collected as described by Park et al. (2004). The information recorded included: the number of mounts not accompanied by an intromission that preceded an ejaculation, the number of intromissions that preceded each ejaculation, latencies to the first mount, first intromission, and first ejaculation, and the duration of the post-ejaculatory interval, which is the time between an ejaculation and the next intromission. Males that failed to mount, intromit, or ejaculate were assigned the maximum copulation latency of 1800 s. Males were considered persistent copulators when mating culminating in the ejaculation reflex was recorded on one or more post-castration tests at least 7 weeks after gonadectomy.

Olfactory preference testing

Feces were collected from hormonally primed, sexually receptive females and from untreated ovariectomized females. Females were housed without bedding for 4–6 h during fecal collection. Feces were removed just before the onset of the dark phase (1300 h), 4–6 h after hormonally-treated females had been injected with progesterone. Five feces of each type, placed into separate Petri dishes (5 cm in diameter), were inserted to opposite ends of the male's home cage, and his behavior recorded for 10 min. The positions of the two dishes (A and B) were randomized. The amount of time the males spent investigating each dish in which the male was in direct physical contact with the feces was recorded. Groups were considered to prefer odors from dish A if the mean of investigatory time spent in contact with feces from dish A was significantly higher than that spent investigating feces from dish B.

Morphological parameters

Hamsters were anesthetized with isoflurane vapors, and the testes were weighed (±0.1 mg) within 10 min of extraction. Body weights were obtained at the time of surgery (±0.1 g).

Statistical analysis

The Statview program (Statview 5; SAS Institute, Cary, NC, USA) was used for all analyses. Differences in copulatory behavior between groups at certain weeks were assessed with the Chi square measure. Repeated measures ANOVAs were used to analyze changes in the number of mounts and intromissions, and mount, intromission and ejaculation latencies over time. When an effect of time was present, paired *t*-tests were used to analyze differences between two time points within groups. Post-hoc comparisons were conducted using the Fisher Protected Least Significant Difference test where appropriate. Observed differences were considered significant if $p<0.05$.

Experiment 1: Effects of post-castration experience on persistent copulation

After successfully completing three of four mating tests ending in ejaculation, 20 hamsters were castrated as described in Park et al. (2003); 9 of these males were tested for copulatory behavior every other week for 7 weeks, beginning 1 week after castration. The remaining 11 animals were first re-tested 7 weeks after castration.

Experiment 2: Effects of preoperative sexual experience and puberty on copulation in adulthood

Twelve sexually naive male hamsters were castrated at 18 days of age, approximately 2–3 weeks before puberty (Hoffmann, 1978). Twenty-four hamsters were castrated postpubertally at ~70 days of age. Twelve of the latter animals had been given sexual experience culminating in ejaculation once a week for 3 weeks prior to castration, and 12 were sexually naive at the time of castration. All castrated males were tested with sexually receptive females once a week for 3 weeks, beginning at approximately 4 months of age. The interval between castration and the first mating test was approximately 13, 7, and 7 weeks for the prepubertal, sexually naive and sexually experienced males, respectively. Seven weeks after completion of the mating behavior tests and 4 months after the hamsters were orchidectomized, male attraction to female odors was assessed.

Results

Experiment 1: Effects of post-castration experience on persistent copulation

Males first re-tested with estrous females 7 weeks after castration were at least as likely to be persistent copulators as were males tested with females every 2 weeks post-castration (91% vs. 67%, respectively; $p>0.05$; Fig. 1A). Repeated post-operative sexual experience at bi-weekly intervals is not a prerequisite for the emergence of persistent copulation.

There were no significant differences between the two groups of persistent copulators with respect to mount, intromission, or ejaculation latencies or the duration of the post-ejaculatory interval during the postoperative test 7 weeks after castration ($p>0.05$), although a trend toward shorter latencies was apparent in the males given their first postoperative test 7 weeks after castration (Figs. 1B–E; only males that ejaculated on post-operative test 7 were included in this analysis).

During preoperative testing, mount latency (ML), intromission latency (IL), ejaculation latency (EL), and the post-ejaculatory interval (PEI) were progressively shorter on successive mating tests for both groups of hamsters (Figs. 1B–E). The difference was significant for 3 of the 4 measures for each group ($p<0.05$ for week −4 versus week −1 values; Figs. 1B–E).

The EL and the PEI were significantly longer during the first two postoperative tests of males tested bi-weekly beginning 1 week after castration ($p<0.05$ in each case), but by the fourth test 7 weeks after castration, both measures returned to values that did not differ significantly from preoperative baseline values 1 week prior to castration. ML, IL, and EL values of males first

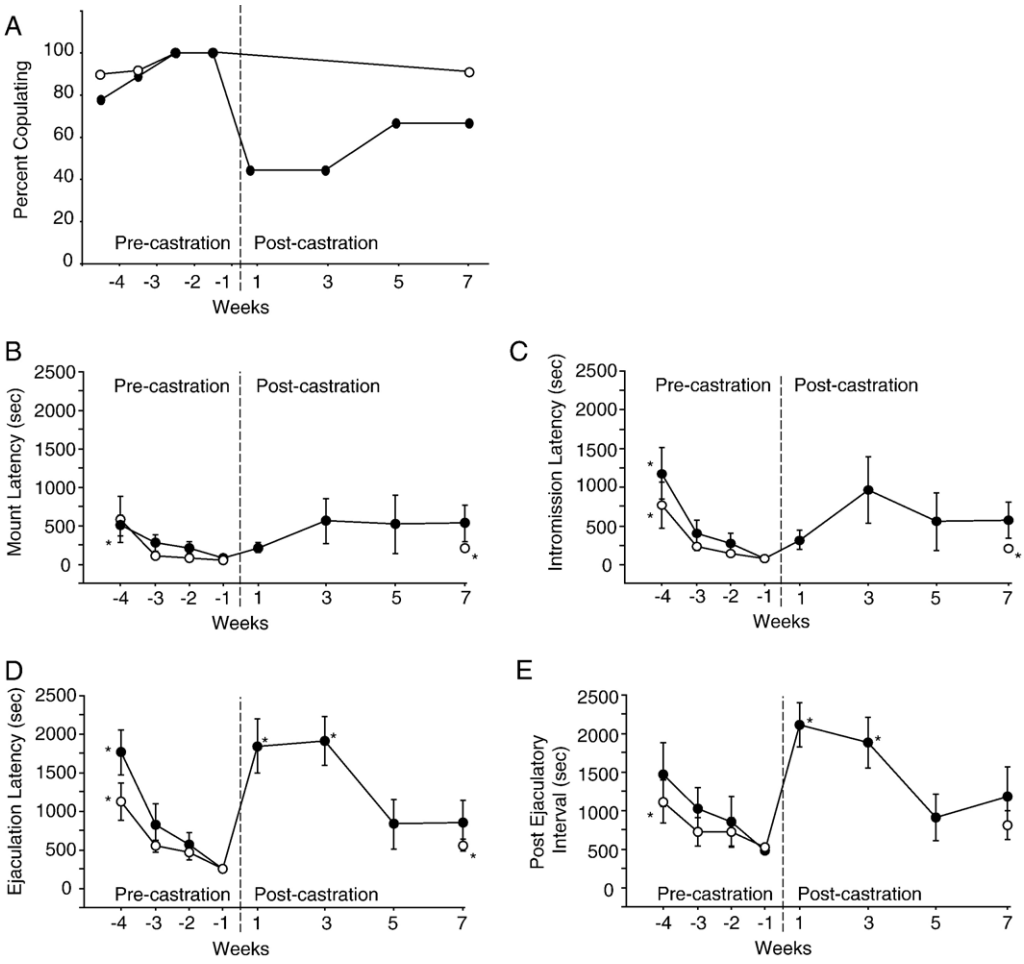

Fig. 1. (A) Percent of males copulating each week to a criterion of behavioral ejaculation. One group of males was tested bi-weekly beginning 1 week after castration (filled circles; $N=9$) and the other was first tested 7 weeks after castration (open circles; $N=11$). Mean±SEM (B) mount, (C) intromission, and (D) ejaculation latencies and (E) post ejaculatory interval of persistent copulators (those that that ejaculated 7 weeks after castration) tested bi-weekly beginning 1 week after castration (filled circles; $N=6$) and first tested 7 weeks after castration (open circles; $N=10$). *Significantly different from respective pre-castration values at week −1 ($p<0.05$).

retested 7 weeks after castration were significantly higher than preoperative week−1 baseline values (Fig. 1B–D), whereas their PEI values were not (Fig. 1E). Although the latency measures at week 7 of the animals tested bi-weekly were also higher than their week−1 baseline values, these comparisons failed to reach significance (p values ranged from 0.08 to 0.13).

Experiment 2: Effects of preoperative sexual experience and puberty on copulation in adulthood

Copulatory behavior

As in experiment 1, a majority (58%) of the adult sexually experienced males copulated to a criterion of behavioral ejaculation 7 or more weeks post-castration, compared to 25% of sexually naive males ($p<0.05$, Fig. 2A). None of the males castrated prior to puberty mounted the females, and thus never successfully intromitted or ejaculated.

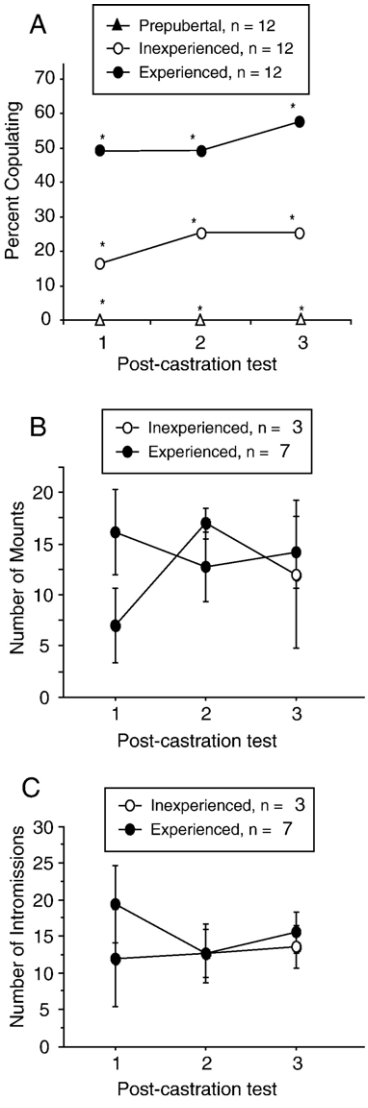

Fig. 2. (A) Percent of males copulating to a criterion of behavioral ejaculation after castration. The interval between castration and the subsequent mating test was ~13, 7, and 7 weeks for the prepubertal, adult sexually naive, and adult sexually experienced males, respectively. *The three groups were significantly different from each other ($p<0.05$). Mean±SEM number of (B) mounts and (C) intromissions by persistent copulators preceding the first behavioral ejaculation in each of the postoperative tests.

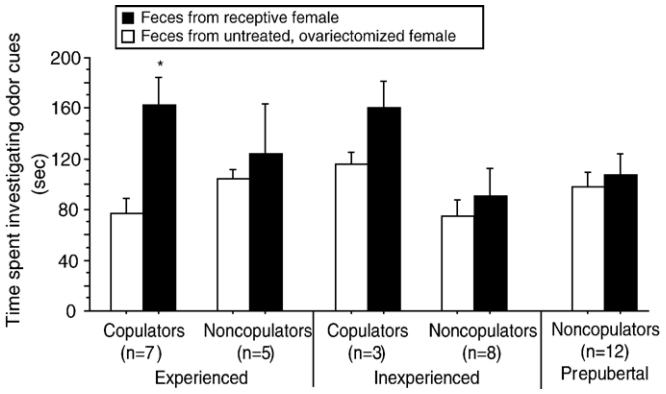

Fig. 3. Mean±SEM investigatory time directed toward feces from sexually receptive females or untreated ovariectomized females. Only sexually experienced persistent copulators ($n=7$) spent significantly more time investigating feces from estrous than non-estrous females (*$p<0.05$).

There were no differences in either the number of mounts or intromissions ($p>0.05$; Figs. 2B, C) or latencies to mount or intromission ($p>0.05$; data not shown) between the sexually experienced and sexually naive males once they displayed these behaviors.

Morphology

Body weights were significantly different between all three treatment groups at the time of castration; sexually naive males were heaviest and prepubertal males lightest ($p<0.05$, not illustrated). The experienced and inexperienced males were castrated at approximately the same age, but the prepubertal males were about 7 weeks younger at the time of gonadectomy.

Testes mass

At the time of castration sexually-experienced adult males had heavier testes than inexperienced males of the same age, 660±17 mg and 514±50 mg, respectively, as previously reported for male rats (Taylor et al., 1983). The testes removed from prepubertal hamsters were undeveloped (106±6 mg).

Olfactory preference tests

Sexually experienced persistent copulators that were castrated after puberty spent a significantly greater portion of time investigating feces from sexually receptive than non-receptive females ($p<0.05$; Fig. 3). Although inexperienced persistent copulators also spent more time investigating feces from estrous females, this comparison was not statistically significant ($p>0.05$), likely because of small sample size. None of the non-copulator groups showed a preference for either type of feces ($p>0.05$).

Discussion

Sexually experienced males successfully bridged a long gap without sexual activity and manifested the complete sex behavior repertoire when first re-tested 7 weeks after castration. The

persistence of copulatory behavior in long-term castrated Siberian hamsters was not dependent on intermittent post-operative sexual experience or priming provided by such tests. Weekly testing after castration also was not necessary for the emergence of persistent copulation in B6D2F1 mice (Wee and Clemens, 1989). Pre-operative sexual experience did, however, increase the incidence of post-castration sex behavior, perhaps in part by inducing long-term changes in penile morphology (Taylor et al., 1983); in the present study 58% of sexually-experienced hamsters, compared to 25% of sexually-naive hamsters, continued to copulate postoperatively.

The decreased incidence of gonad-independent sex behavior in naive hamsters was not unexpected. Prior sexual experience renders neural substrates less dependent on androgens (Hart, 1974). The benefits of sexual experience for retention of mating behavior after castration have been documented in cats and Syrian hamsters (Rosenblatt and Aronson, 1958; Bunnell and Kimmel, 1965; Bunnell and Flesher, 1965; Lisk and Heimann, 1980). Remarkably, 25% of sexually naive males copulated to a criterion of behavioral ejaculation when first tested 7 weeks after castration. Concurrent availability of gonadal hormones is required by most sexually naive adult Siberian hamsters for activation of copulatory behavior in adulthood, but a minority of naive males is not so constrained. The neural substrate of the latter hamsters was able to support sex behavior many weeks after the withdrawal of gonadal steroids. The extent to which activation of neural mating circuits is similar in castrated persistent copulators and gonadally-intact males, is unknown.

Persistent copulation was absent in males castrated prepubertally and tested in adulthood. None of these males even attempted to mount females. The combined absence of precastration sexual experience and pubertal hormone secretion was incompatible with persistent copulation in adulthood; the absence of mounting behavior reflects a lack of sexual arousal and motivation (Sachs and Meisel, 1988). The significantly lighter penises of prepubertal castrates (Park et al., 2003) may also prevent normal copulation. The experimental design we employed controlled for age at the time of testing, but the interval between castration and the first postoperative mating test was 13 weeks for the prepubertal and 7 weeks for the postpubertal males and may have contributed to the mating deficit in the former animals. The extended interval after castration is unlikely to explain the complete absence of copulatory behaviors in this group, as 40% of adult Siberian hamsters continue to display the complete repertoire of mating behaviors 13 weeks after castration (Park et al., 2004). Differences in methodologies between the two studies, however, prevent definitive conclusions. Pubertal testicular hormone secretion is required for normal adult mating behavior of Syrian hamster (reviewed in Schulz and Sisk, 2006) and here is implicated in the emergence of gonad-independent persistent copulation of Siberian hamsters.

Emancipation from strict hormonal control of mating activity in male mammals was formerly thought to be restricted to carnivores and primates. Some dogs continue to copulate for years after gonadectomy (Hart, 1968) and some untreated men reportedly engage in sexual intercourse a decade after bilateral orchidectomy (Heim and Hursch, 1979; Weinberger et al., 2005). Increased cerebral cortical influences were the proposed mediators of long-term maintenance of mating behavior in gonadectomized males (Ford and Beach, 1951) but subsequent analyses failed to document a correspondence between degree of brain development and corticalization on the one hand, and post-castration retention of male sex behavior, on the other (Aronson, 1959; Hart, 1974; Manning and Thompson, 1976; McGill and Manning, 1976; Clemens et al., 1988; Park et al., 2004, present study).

Persistence of olfactory preferences in castrated males has not to our knowledge been reported previously for any rodent (see Paredes et al., 1998 for a review of the older literature). We demonstrated that sexually experienced persistent copulators prefer the odors of estrous females, but such preferences were absent in experienced and inexperienced non-persistent copulators, as well as males castrated prior to puberty. Male rat preferences for odors of estrous females disappear 3 weeks after castration and are restored by testosterone treatment (Stern, 1970). The robust preference of sexually-experienced persistent copulators for the odors of sexually receptive females is therefore remarkable, considering that tests were conducted 7 weeks after the last mating test and 4 months after castration. Responsiveness to sexually salient odors may be causally related to post-castration emergence of persistence copulation.

Our results suggest but do not definitively establish that the amount of prior sexual experience may influence post-castration preference for estrous odors. It is unknown whether gonadal steroids are required for initial establishment of the male's preference for estrous odors or if this preference subsequently becomes steroid-independent. Alternatively, a subset of sexually naive castrated male hamsters may have strong preferences for the odors of estrous females prior to the first mating test; perhaps only those males subsequently develop persistent copulation.

During odor tests, fos-like immunoreactivity is detected in more brain areas throughout the vomeronasal pathway and the nucleus accumbens of sexually experienced than sexually naive male rats. Sexual experience may be necessary for full activation of this pathway (Hosokawa and Chiba, 2005). It remains to be specified whether the olfactory mating circuit of persistent copulators responds differently than that of non-persistent copulators to salient female odors (cf. Kollack-Walker and Newman, 1997; Wood and Newman, 1995), although we consider this likely. Retention of mating and olfactory behaviors in persistent copulators may be dependent on neurosteroids (Frye, 2001), or constitutively activated steroid receptors (Blaustein, 2004), but is unlikely to depend on adrenal estrogens or androgens, as evidenced by the persistence of male sexual behavior in castrated hybrid B6D2F1 hybrid mice after adrenalectomy (Thompson et al., 1976).

It is also unknown whether all gonadally-mediated traits are retained to a greater extent in persistent copulators than in males that cease mating activity after castration. Persistent copulators may fail to manifest the increased aggressive behavior that follows castration in Siberian hamsters (Jasnow et al., 2000). Castration does not affect all androgen-dependent traits or all

genotypes equally; e.g., all Long–Evans rats failed to copulate several weeks after castration but ultrasonic vocalizations decreased markedly in half the animals and increased above preoperative values in the remaining rats (Matochik and Barfield, 1994). Similarly diverse responses were observed in meadow voles subject to castration; heavier voles decreased and lighter voles increased their body mass postoperatively (Dark et al., 1987).

Future studies of persistent copulators stand to increase our understanding of the mechanisms by which environmental factors, sensory systems, and neurotransmitters sustain male sex behavior in the absence of gonadal steroids.

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EQUINE VETERINARY EDUCATION
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Critically Appraised Topic

Can ovariectomy be justified on grounds of behaviour?

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Clinical scenario

In practice, clinicians are commonly presented with mares reportedly behaving 'badly' either persistently or at regular or irregular intervals. This behaviour is often presumed by the owners or riders to be due to their mares being in oestrous or their reproductive hormone balance. Undesirable behaviour is varied and includes an unwillingness to respond to rider instruction or signals, bucking, rearing, overt reproductive behaviours, aggression and stallion-like behaviour (Hedberg *et al*. 2007a; Kamm and Hendrickson 2007; Crabtree 2011). It is assumed that when considering these behavioural problems, the behaviour in question has been confirmed by the clinician to be temporarily associated with the oestrus cycle or with ovarian pain and not musculoskeletal, soft tissue, gastric or dental pain. The question has been refined to exclude those behavioural issues experienced due to the presence of granulosa cell tumour (GCT; Crabtree 2011). These criteria define the population of mares for the question, 'can ovariectomy on grounds of behaviour be justified?' The question is not an ethical one, rather whether or not behaviour can be improved with surgery.

Search strategy

Pubmed/Medline (http://www.ncbi.nlm.nih.gov/pubmed), the University of Liverpool electronic library search (http://www.liv.ac.uk.ezproxy.liv.ac.uk/library) and IVIS (http://www.ivis.org): ovariectomy AND mare AND behaviour. Hand searching of references in all articles identified and those within the author's knowledge was conducted. In addition a request for references and opinions was made via a private worldwide equine reproduction e-mail network.

Quantity of evidence

Sixteen, 41 and 18 papers were retrieved respectively; relevance screening was conducted based on abstracts identifying 3 papers applicable to the question in the last 30 years. Of the 3 papers there was one randomised controlled trial (Hedberg *et al*. 2007b) and 2 retrospective studies (Hooper *et al*. 1993; Kamm and Hendrickson 2007).

Quality of evidence

The study by Hooper and co-workers (1993) looked retrospectively at 23 cases of bilateral ovariectomy between 1984 and 1990 by client survey. Of the 23 cases, 16 were for the purpose of behaviour modification and 7 were for other reasons not including ovarian pathology. Kamm and Hendrickson (2007) reported the results of a retrospective study of 35 cases between 1996 and 2005 by client survey. The reported data included both bilateral and unilateral ovariectomy with the latter being due to ovarian pathology. Twenty-three mares had bilateral ovariectomy for behavioural problems. These retrospective observational studies, based on client opinion, represent relatively weak evidence; in addition they are inherently biased. The study by Hedberg and co-workers (2007b) primarily looked at the effect of ACTH (tetracosactide) on steroid hormone levels in 5 intact vs. 5 ovariectomised mares. The mares acted as their own controls and the study was conducted in successive years (2003–2004). Of the parameters measured, oestrous behaviour was assessed by daily teasing with a stallion. Teasing behaviour was assessed using a standardised protocol and scored. This prospective case–control design has greater evidentiary value than the retrospective study despite the low numbers. Teasing scores however, were not subject to statistical analysis and remained observational. This paper formed part of a greater work which looked at the adrenal response to ACTH between normal and 'problem' mares (Hedberg *et al*. 2007a).

Clinical implications

Hedberg and co-workers (2007b) demonstrated that ovariectomy resulted in continued displays of sexual receptiveness in all mares with a loss of normal cyclic activity. Ovariectomised mares showed more days of oestrus compared with when they were intact; 44 days of oestrus for all intact mares compared with 152 days for all ovariectomised mares during the study period. One ovariectomised mare showed 78 consecutive days of oestrus. This finding is supported by the author's experience and earlier published observations that ovaries are not essential for oestrus behaviour in the mare (Asa *et al*. 1980; Wesson and Ginther 1981).

Hooper and co-workers (1993) reported that 35% of mares demonstrated continued oestrus behaviour. In 9% of cases this was judged to be 'objectionable' by the owners. Of 12 performance mares, 10 were judged to be competing at greater than preoperative levels. In the report by Kamm and Hendrickson (2007) 22% of owners reported that they were either impartial or dissatisfied mainly due to a failure of behavioural change following surgery. More generalised behavioural problems were more likely to be corrected with an ovariectomy than more specific behavioural issues. Aggressive behaviour and a generalised disagreeable demeanour were most commonly corrected (86 and 81% respectively), followed by excitability (75%), kicking and biting (73%), and problems during training that related to oestrus-like behaviour (72%). Problems with other horses and frequent urination were less likely to be improved after surgery (64%). None of the studies addressed the concept of ovarian pain as a cause of subtle behavioural issues encountered in ridden competition horses.

Clinical message

The current data would suggest that if the undesirable behaviour which prompts presentation is normal sexual

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(oestrus) behaviour then the likelihood is that ovariectomy will not correct the problem. In fact ovariectomy may make the situation worse as oestrus behaviour may become irregular and/or persistent post surgery. If the behaviour is aggressive in character then ovariectomy is more likely to be a successful therapy but the evidence for this is weak. If ovarian pain is considered as a cause of abnormal behaviour then inducing ovarian inactivity via the use of a GnRH vaccine may be a viable option that does not require surgery, however, mares vaccinated against GnRH will also demonstrate oestrus behaviour (Dalin *et al*. 2002). Future studies with accurate definition of the cause, or characteristics of, the behaviour with matched controls may enable a more definitive answer to the question.

Author's declaration of interests

No conflicts of interests have been declared.

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Graham Munroe and Scott Weese

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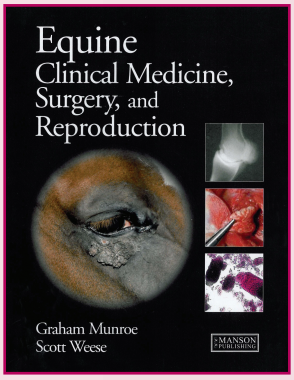

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THE ECOLOGY OF STRESS

The ecology of stress: effects of the social environment

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Summary

1. Many aspects of the social environment affect hypothalamic-pituitary-adrenal (HPA) axis function and increase circulating glucocorticoid concentrations. In this review, we examine the relationships between the social environment and the function of the HPA axis in vertebrates.

2. First, we explore the effects of the social environment on glucocorticoid secretion in territorial (primarily non-social) species, with an emphasis on the effects of variation in population density, as modified by environmental factors such as predation risk and food availability. In general, high population density or frequent territorial intrusions are associated with increased glucocorticoid secretion in a wide range of taxa, including mammals, birds, fish and reptiles, although there is considerable variability across species.

3. Second, we consider the effects of social interactions and dominance rank on glucocorticoid secretion in social species, mostly in birds and mammals. We review studies that have detected an association between social status and glucocorticoid levels – sometimes with higher glucocorticoid levels in low-ranking individuals, and sometimes with higher glucocorticoid levels in dominant individuals. The relationship between dominance and glucocorticoid levels varies among species, populations and years, in a manner that depends on the stability of the social hierarchy, environmental conditions, the type of breeding system, and the manner in which high rank is obtained and maintained.

4. Finally, we discuss the concept of allostasis and consider interactions between social effects and other environmental factors, noting that there is relatively little research on these interactions to date. For both non-social and social species, we identify priorities of future research. These priorities include more complete descriptions of HPA function that move beyond measurements of basal glucocorticoid concentrations (which will generally require field experiments), to studies that examine organizational effects of social stressors, that directly test the relationship between HPA function and fitness, and that examine how glucocorticoid responses affect population dynamics.

5. Although several lines of evidence suggest that glucocorticoid responses can affect the fitness of individuals and therefore can alter the dynamics of populations, the effect of glucocorticoid responses on population dynamics remains essentially unstudied.

Key-words: allostasis, dominance, glucocorticoid, hypothalamic-pituitary-adrenal axis, sociality, social behaviour, stress, territoriality

Introduction

Animals are faced with a constant flood of information from their external environment. Although much of this information can be ignored, some of it can be used in an adaptive fashion. In vertebrates, one of the primary mechanisms by which external environmental information elicits such an adaptive response is through initiating a physiological cascade of internal changes mediated in part through the hypothalamic-pituitary-adrenal (HPA) axis.

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The HPA axis is the neuroendocrine pathway by which stress hormones (glucocorticoids) are released from the adrenal cortex. The structure and function of the HPA axis are reviewed elsewhere (e.g. Sapolsky, Romero & Munck 2000; Sapolsky 2002). The HPA axis integrates environmental information and the downstream release of glucocorticoids can potentiate an adaptive behavioural response (Monclús *et al.* 2005; Schulkin, Morgan & Rosen 2005) or alteration in life-history strategy (Wingfield *et al.* 1998; Boonstra 2005).

The social environment is one of the primary sources of information that can induce a physiological stress response. In both social and non-social animals, the frequency and type of interaction with conspecifics as well as status in social species can affect HPA axis activity. In this review, we examine the relationships between the social environment and the function of the HPA axis in vertebrates. First, we explore the effects of the social environment on glucocorticoid secretion in territorial species, with an emphasis on the effects of variation in population density, as modified by environmental factors such as predation risk and food availability. Second, we consider the effects of social interactions and dominance rank on glucocorticoid secretion in social species. Many studies have detected an association between social status and glucocorticoid levels – sometimes with higher glucocorticoids in low-ranking individuals, and sometimes with higher glucocorticoids in dominant individuals. Many studies also consider the particular traits associated with dominance or subordination that might be causally related to variation in glucocorticoid secretion. Other studies suggest that the general social environment (e.g. the stability or instability of social relationships and the type of dominance hierarchy) can affect the association between glucocorticoids and rank. Finally, we discuss the concept of allostasis and consider interactions between social effects and other environmental factors, noting that there is relatively little research on these interactions to date. Thus, this review reveals relationships between glucocorticoid secretion, environmental variables such as predation risk, population variables such as density or the rate of territorial intrusion and social variables such as dominance rank, and behavioural variables such as the rate of aggression. Clearly the HPA response is a physiological mechanism that might mediate a wide range of fitness effects. The review also reveals considerable variability among species in the response of glucocorticoid levels to these factors and suggests new hypotheses that might explain this variation.

Effect of the social environment on the stress axis in territorial vertebrates

NON-SOCIAL SPECIES: TERRITORIALITY

The patchy distribution of limited but defendable resources such as food, mates or preferable habitats can favour the evolution of territoriality. Territoriality is the exclusive use of an area (i.e. 'ecological' territoriality) or the active defence of an area from conspecifics using various visual, acoustic, or chemical signals that indicate ownership (i.e. 'behavioural' territoriality; Brown & Orians 1970). Signals advertising ownership may deter intrusions by conspecifics of the same gender or both genders and can also attract opposite-sex conspecifics, which can allow for the monopolization of mates and other limited resources.

Many vertebrate species exhibit either temporary or extended periods of territoriality. Although territoriality implies exclusiveness from conspecifics of the same or opposite gender, the true degree of non-sociality (i.e. avoidance of conspecifics) exhibited by territorial vertebrate species is variable. For example, males of many avian species breeding in temperate climates establish breeding territories but aggregate in large flocks at times outside of the breeding season (Nice 1941). The breeding territories of male songbirds are often defended from male but not female conspecifics, which results in male birds spending a significant portion of each year exclusive of other males. Similarly, females of some small mammal species (e.g. *Microtus*, *Clethrionomys,* and *Peromyscus* spp.) defend nesting territories during the breeding season, but will live in groups of related or unrelated individuals during the non-breeding season (Ostfeld 1990; Wolff 1993). At the other end of the continuum of non-sociality are species that defend year-round territories from both intra- and inter-sexual intruders. For example, both female and male North American red squirrels (*Tamiasciurus hudsonicus*) defend exclusive food-based territories year-round (Smith 1968). Female red squirrels are spontaneous ovulators, and they only appear to allow male intruders on their territories when they are in behavioural oestrous, which is approximately 1 day per year (Smith 1968; Lane *et al.* 2008). Thus, the degree of non-sociality in territorial vertebrate species can range from highly non-social (red squirrels) to tolerance of both sexes for most of the year except during the breeding season (temperate breeding avian species and some small mammal species). There is similar variation in the degree of non-sociality in species that actively defend mates (mate-defence polygyny) rather than actively defending a territory (resource-defence polgyny or monogamy). In mate-defence polygyny (e.g. impala, *Aepyceros melampus*), males typically attack other males during the mating season, but aggregate in large herds with little aggression outside of the mating season (Jarman 1974). Because social interactions can strongly affect the activity of the HPA axis, variation in the degree of non-sociality may interact with stress physiology. Here, we focus on the effects of the social environment on the activity of the HPA axis in vertebrate species that exhibit some degree of non-sociality due to territoriality (Fig. 1).

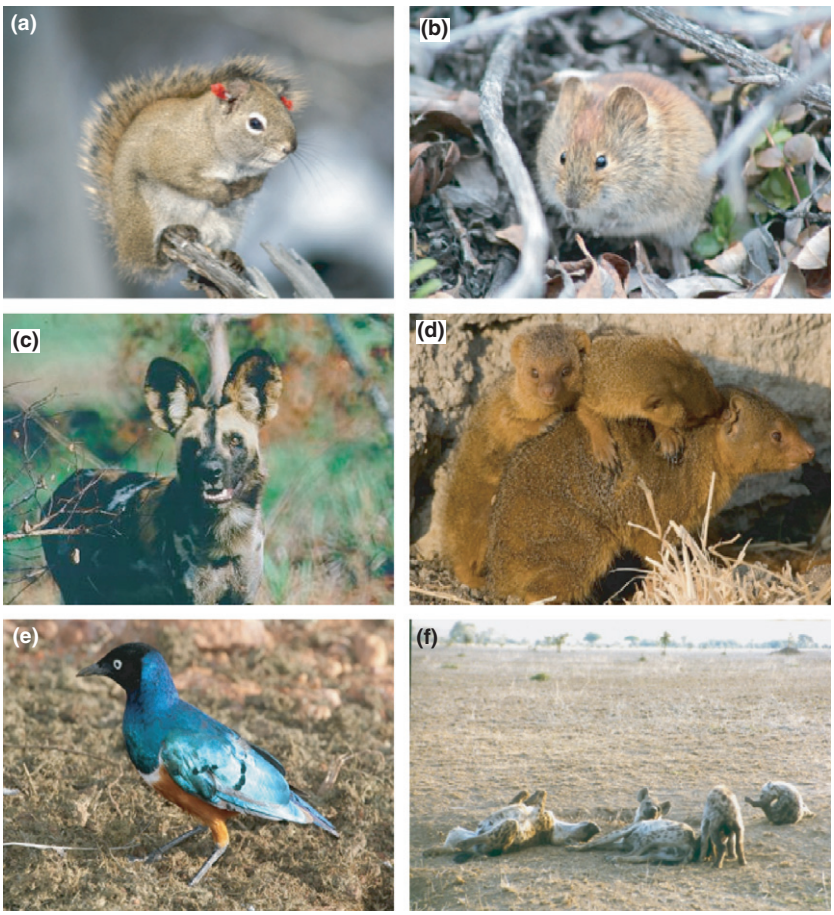

Fig. 1. (a, b) Population density and glucocorticoid levels are usually positively associated in non-social species, although the degree to which territorial vertebrate species are non-social is highly variable. For example, both male and female North American red squirrels (a) defend year-round food-based territories. In other small mammal species such as the northern red-backed voles (b), females only defend nesting territories during the breeding season (photos by R.W. Taylor). In many social species, behavioural dominance is strongly correlated with reproductive success but dominance is also associated with heightened glucocorticoid levels. For example, within groups of African wild dogs (c) and dwarf mongooses (d), only the behaviourally dominant individual of each gender is assured of breeding (photos by S. Creel). However, dominant wild dogs and dwarf mongoose also engage in more aggressive interactions and their glucocorticoid levels are higher than those of subordinates, which may impose fitness costs. In many social species, the relationship between social status and glucocorticoid levels can be complicated by both environmental variation and the method by which social status is acquired and maintained. (e) For example, in cooperatively breeding superb starlings, annual differences in rainfall and resource availability not only influence glucocorticoid levels, but they may do so differently in dominants and subordinates (photo by D. R. Rubenstein). (f) Unlike most other carnivores, social rank in spotted hyenas is inherited from the mother, which complicates the positive relationship between social status and age/size that is seen in many other species (photo by W. Goymann).

FACTORS AFFECTING ACTIVITY OF THE HPA AXIS IN TERRITORIAL SPECIES

In territorial vertebrates, population or breeding density is one of the best documented factors that influence the activity of the HPA axis. Christian (1950) first used Selye's description of stress (or general adaptation syndrome; Selye 1936) to describe how the adrenocortical response to population density might cause the density-dependent population cycles exhibited by some small mammal species. Christian (1950) hypothesized that increases in population density in small mammal species such as voles led to increased antagonistic social interactions, which caused an increase in adrenocortical activity. Christian further predicted that the consequences of this heightened adrenocortical activity were a decrease or suppression of reproduction and an increase in mortality due to decreased resistance to disease or other limiting factors (Christian 1950; Christian & Davis 1964). As a result, population growth ceased or declined during peak population densities due to heightened mortality and decreased fecundity. Christian and colleagues (Christian 1950, 1956, 1961, 1964, 1971; Christian & Davis 1964, 1966) provided extensive empirical documentation in territorial mammalian species of the relationship between adrenal gland mass (a proxy of adrenocortical activity: Bronson & Eleftheriou 1964) and population density and/or the frequency of antagonistic interactions.

Christian's pioneering studies were instrumental in connecting how environmental factors influence the HPA axis

activity of individuals, which in turn affected population dynamics. Groundbreaking studies in a variety of mammalian taxa (primarily rodents) found significant positive relationships between population density and adrenal mass (reviewed by Christian 1971; To & Tamarin 1977) or *in vitro* adrenal secretion of glucocorticoids (Andrews 1968). However, Christian's research has been criticized because it was largely performed on captive animals often in a laboratory setting (Krebs & Myers 1974; Lee & McDonald 1985). Nonetheless, more recent studies in other mammalian species have documented a similar positive relationship between population density and plasma glucocorticoids (Boonstra & Boag 1992; m 1998) or faecal glucocorticoid metabolite concentrations (Novikov & Moshkin 1998; Harper & Austad 2004; Bian *et al*. 2011) in free-living individuals. For example, Boonstra & Boag (1992) found that populations of meadow voles (*Microtus pennsylvanicus*) at the highest densities had the highest free plasma corticosterone concentrations and lowest corticosterone-binding globulin concentrations. Support for this positive relationship between population density and stress axis activity in territorial mammalian species is not universal (e.g. To & Tamarin 1977; Harper & Austad 2004; Kuznetsov *et al*. 2004; Charbonnel *et al*. 2008), and we have little information about the relationship between population density and stress axis activity in some mammalian orders that include many solitary species (e.g. carnivores, of which more than 80% of species are solitary: Gittleman 1984).

The relationship between population density and the activity of the stress axis is best known from studies of mammals. This is not surprising given the initial focus of Christian and colleagues (see above). Although less well-studied than mammals, birds often show similar relationships between density and HPA function. With regard to stress physiology, most of the well-studied avian species breed in temperate regions exhibit territoriality only during the breeding season, and only defend territories from same-gender conspecifics. Although there are fewer studies of birds than mammals, a positive relationship between population density and plasma (Silverin 1998a) or yolk glucocorticoids (Love *et al*. 2008) has been reported for several species, while others have detected no relationship (Beletsky, Orians & Wingfield 1990, 1992). However, further support for the predicted positive relationship between density and stress axis activity in avian species may come from simulated territorial intrusion (STI) experiments. In these experiments, a territorial male is presented with an unfamiliar caged male (or a stuffed male) within his territory, paired with an audio playback of the species' song. Several studies in temperate-breeding birds have found that STIs cause a pronounced increase in plasma glucocorticoids (e.g. Silverin1993, 1998b; Van Duyse *et al*. 2004; Canoine & Gwinner 2005; Landys *et al*. 2007), whereas others have not found such a relationship (e.g. Harding & Follett 1979; Wingfield 1985; Pärn *et al*. 2008; Scriba & Goymann 2010). Because antagonistic interactions and other territorial intrusions are predicted to increase as population density rises (e.g. Bretagnolle, Mougeot & Thibault 2008), we therefore would predict that population density is also positively correlated with stress axis activity in species that responded to STIs with elevated glucocorticoid production. To date, very few studies in avian species that exhibit year-round territoriality have directly tested the relationship between glucocorticoids and population density. However, some studies have found that STIs cause a significant increase in plasma glucocorticoids in bird species exhibiting year-round territoriality (e.g. Gill, Costa & Hau 2008; Landys *et al*. 2010), providing indirect evidence for a positive relationship between population density and stress axis activity.

Other taxa, including amphibians, reptiles and fish, also offer insights into the relationship between population density and HPA function. Amphibians and reptiles often defend resource-based territories and are non-social as adults. Many species only interact with conspecifics during the larval stage or during breeding as adults. Some studies in amphibians and reptiles also support the positive relationship between population or breeding density and stress axis activity. For example, increased population density during mating in anurans (Leary, Garcia & Knapp 2008) or nesting density in turtles (Jessop, Limpus & Whittier 1999) is associated with increased plasma glucocorticoids. High population density either were associated with increased or decreased plasma glucocorticoids in female side-blotched lizards (*Uta stansburiana*) depending upon the female reproductive tactic (Comendant *et al*. 2003). In contrast, experimentally increased densities in breeding salamanders did not elicit elevated plasma glucocorticoids (Cooperman, Reed & Romero 2004). It is important to note, however, that increased glucocorticoid concentrations during breeding aggregations might be driven by changes associated with reproduction that are not related to density (e.g. glucocorticoid concentrations rise during gestation in many mammals; Creel, Winnie & Christianson 2009; Dantzer *et al*. 2010; or glucocorticoid concentrations rise during intense mate competition in marsupials; Bradley, McDonald & Lee 1980; Bradley 1987). Evidence for positive effects of population density on stress axis activity in amphibians and reptiles also comes from laboratory studies. For example, heightened competition over burrows in captive spotted salamanders (*Ambystoma maculatum*; Cooperman, Reed & Romero 2004) or increased housing density in American alligators (*Alligator mississippiensis*; Elsey *et al*. 1990) was associated with increased plasma glucocorticoids (see also Hayes 1997; Glennemeier & Denver 2002). In some lizard species, forced antagonistic interactions with conspecifics in laboratory experiments can elevate plasma glucocorticoids (Klukowski & Nelson 1998; Schuett & Grober 2000; Yang & Wilczynski 2003), which suggests that stress axis activity would be elevated under high density conditions due to a heightened

frequency of antagonistic interactions. In many ways, such studies of forced interaction are similar to early studies of 'social stress' in captive animals that we discuss in the second part of this review.

Finally, it is well established that crowding increases stress axis activity in captive or hatchery reared fish (Barton & Iwama 1991), but we are aware of no studies examining the relationship between population or breeding densities and stress axis activity in free-ranging territorial fish. This is not surprising given the logistical difficulties of such a study. However, future studies in fish species in which males provide paternal care and actively defend brooding sites from conspecifics such as bluegill (*Leopomis macrochirus*; Neff & Knapp 2009) and smallmouth bass (*Micropterus dolomieu*; Hanson *et al*. 2009; Dey *et al*. 2010) should investigate how breeding or nesting density affects stress axis activity.

REMAINING QUESTIONS IN TERRITORIAL SPECIES

In the above section, we have detailed how population or breeding density increases HPA activity in vertebrate taxa and in some cases how forced antagonistic interactions between conspecifics can elevate stress axis activity. However, the positive relationship between population density or the frequency of antagonistic interactions and HPA axis activity is not universal across different taxa. Departures from the general pattern of increased density leading to increased HPA activity could be due to the confounding effects of reproductive condition, seasonality or recent extreme weather events on HPA axis activity or responsiveness (Kenagy & Place 2000; Romero 2002; Romero *et al*. 2008) or to the effects of trapping/handling stress on glucocorticoid levels (Kenagy & Place 2000; Sheriff *et al*. 2011a). As such, future studies examining relationships between population density and HPA axis activity should sample their study animals carefully to avoid these potentially confounding effects.

A central remaining question is to identify if the relationship between population density and HPA axis activity that has been found in many species is due to an increase in antagonistic interactions between conspecifics at high density, decreased per capita food abundance, or even increased attraction of predators. For example, food-abundance often co-varies with population density such that increased overall food abundance is associated with increased reproductive output and/or population density, but perhaps constant or decreased per capita food availability. As a result, determining the actual mechanism that induces heightened HPA axis activity under high population density conditions will require experimental manipulations of the potential causal variables, or careful sampling designs with multiple populations.

Few studies have experimentally manipulated population density, food-abundance or predator densities in free-living non-social territorial animals. In a recent study using a non-social species, the North American red squirrel, population density was positively correlated with faecal cortisol metabolite concentrations (B. Dantzer, unpublished data). Experimental increases in both actual (using long-term food supplementation) and perceived population density (using audio playbacks of territorial vocalizations to simulate high density conditions) also caused a significant increase in faecal corticosteroid metabolites (Dantzer *et al*. unpublished data). This suggests that, at least in this species, the actual variable causing changes in glucocorticoids (and also density-dependent behavioural responses; Dantzer *et al*. in press) is the perception of social competition and not food abundance or predator densities.

In addition to population density, predation risk may affect HPA axis activity in territorial species. For example, predator density affects plasma free glucocorticoid concentrations in snowshoe hares (*Lepus americanus*) (Boonstra *et al*. 1998; Sheriff, Krebs & Boonstra 2011b) and total plasma glucocorticoid concentrations in African stonechats (Scheuerlein, Van't Hof & Gwinner 2001). In elk, (*Cervus elaphus;* Creel, Winnie & Christianson 2009), predator density did not detectably affect faecal corticosteroid metabolites concentrations, although progesterone concentrations and calf production did decline in response to predation risk (Creel *et al*. 2007). To date, too few studies have examined the relationship between HPA activity and predation risk to infer general relationships with confidence. However, recently Zanette *et al*. (2011) simulated a strong increase in the perceived risk of predation using predator vocalization playbacks and observed behavioural changes in territorial female song sparrows, associated with a nearly 40% reduction in clutch size at fledgling. The impact of predation pressure in natural situations may be more subtle than in this experiment, because it simulated a situation with severe predation pressure by broadcasting predator vocalizations steadily for 130 days on a 4 day on/off basis (Zanette *et al*. 2011). This reduction in clutch size may be through a stress-mediated pathway because previous studies have found that glucocorticoids were elevated in response to increased predator abundance (Clinchy *et al*. 2004, 2011). This study experimentally confirms observational studies that have shown strong effects of predation risk on individual reproduction in wild populations, although these are not necessarily stress-mediated (Creel *et al*. 2007). We suggest that future studies examining relationships between population density and the activity of the stress axis should also consider how food abundance and predation risk co-vary with population density and HPA activity (see also McColl 1998).

As with studies of social species (see below), broader assessments of HPA function and tests for fitness effects in non-social species remain important. Although the initial focus of Christian and colleagues was to examine how population density or environmental variation influences the population dynamics of non-social animals through HPA axis activity, no studies that we are aware of have actually explored how HPA axis activity of individuals affects population dynamics as the focus of most field

studies to date has been on *individuals*. While it is reasonable to assume that effects on individual fitness often have consequences for population dynamics, it will be productive to return to the ideas of Christian and explore how the glucocorticoid responses of individuals to environmental variation can influence population or even community dynamics (Hawlena & Schmitz 2010). This will require careful experimentation of population densities and even HPA axis activity with tractable study species to examine if the glucocorticoid responses of individuals to environmental variation affect population density.

Effect of the social environment on the stress axis in group-living vertebrates

SOCIAL SPECIES: SOCIAL STATUS AND DOMINANCE RANK

Animals living in social groups often form dominance hierarchies. An individual's position within the dominance hierarchy can have strong effects on fitness, because social dominance often confers priority of access to mates, food and other resources (Fig. 1). In many cooperatively breeding birds and mammals, the probability of breeding is almost one for the socially dominant individual of each gender and almost zero for social subordinates. Given these clear benefits of social dominance, many early studies of aggression, rank and physiological stress responses were based on the hypothesis that low social status is stressful. Early research on captive rodents and primates supported this hypothesis (Bronson & Eleftheriou 1964; Louch & Higginbotham 1967; Manogue, Leshner & Candland 1975). In paired contests between laboratory rats, mice and monkeys, aggression increased glucocorticoid secretion in both winners and losers, but the effect was significantly stronger among losers. Thus, as field studies began to examine stress responses in the wild in the 1980s, a plausible hypothesis was that the 'stress of subordination' might underlie the reproductive suppression of social subordinates in many cooperatively breeding species.

However, social hierarchies and agonistic behaviour evolve to avoid the costs of overt aggression. This general explanation for the evolution of agonistic behaviour is predicated on the assumption that contests among group members can carry costs (Rubenstein & Shen 2009). The costs of engaging in agonistic or aggressive interactions (and more generally the costs of a given social position) are often not equal for both of the individuals involved, and this observation raises an interesting range of possibilities. If winning a direct contest provokes a weaker stress response than losing, but dominant individuals engage in more contests, then the total effect may be that dominants have higher mean circulating glucocorticoid levels than subordinates. In addition to direct contests, it is plausible that the maintenance of high rank requires more monitoring or 'policing' of the behaviour of others than is required for animals with low rank, simply due to differences in the number of group mates who are subordinate to individuals of different rank. Observations of wild groups commonly show that dominant animals do engage in higher rates of aggressive and agonistic behaviour in many species (e.g. most social carnivores: Creel *et al*. 1997; Sands & Creel 2004), so it is plausible to hypothesize that social stress is greater for dominants than for subordinates, but the fitness costs are offset by the other benefits of high rank.

Given that one might plausibly hypothesize either positive or negative correlations between social status and glucocorticoid secretion, empirical data from wild populations are necessary to advance the science. Robert Sapolsky, one of the pioneers of social stress research in free-living animals, found that subordinate male olive baboons (*Papio anubis*) are more likely to be physiologically stressed than dominants and show chronically elevated levels of glucocorticoids (Sapolsky 1982, 1983). As a consequence of chronically elevated glucocorticoids, subordinate baboons are more likely to develop stress-related pathologies than dominants (Sapolsky, Alberts & Altmann 1997; Sapolsky & Spencer 1997). In follow-up studies, Sapolsky and coworkers discovered that this rank-related pattern in glucocorticoid physiology may be modified by styles of dominance (Ray & Sapolsky 1992), or may be suspended by environmental impacts, such as during a severe drought during which all members of the social group devoted most of their time to foraging, leaving little opportunity for social harassment (Sapolsky 1986). Most notably, Sapolsky (1992a) suggested that the relationship of glucocorticoid concentrations to rank depended on the stability or instability of baboon hierarchies. In stable hierarchies, the glucocorticoid levels of subordinates were higher than the glucocorticoid levels of high-ranking baboons, but this pattern was reversed during periods of social instability. Sapolsky's early results from free-ranging baboons supported the prevailing view from laboratory studies (e.g. Manogue, Leshner & Candland 1975) that subordinates are generally more likely to suffer from social stress than dominants. More importantly, these studies highlighted the role that social stability has on glucocorticoid levels in some species.

With the availability of non-invasive methods to measure hormone metabolites from urine or faeces, studies on the relationship between social rank and glucocorticoid levels of free-living animals have become more common over the past two decades. Some of these studies challenged the traditional view of the relationship between glucocorticoids and social rank in free-living animals that subordinates tend to have higher levels than dominants, arguing instead that dominants experience more psychosocial stress and often express higher levels of glucocorticoids than subordinates (Creel 2001). For example, glucocorticoid levels were higher in dominants than in subordinates in free-living African wild dogs (*Lycaon pictus*) and dwarf mongoose (*Helogale parvula*) (Creel, Creel & Monfort 1996; Creel *et al*. 1997). Both species are cooperative breeders and live in groups with '*despotic hierarchies*'

(Sapolsky 2005). Similarly, in cooperatively breeding African cichlid fish (*Neolamprologus pulcher*) dominants had higher glucocorticoid levels than subordinates (Mileva, Gilmour & Balshine 2010). The same pattern has been reported for several wild populations of cooperatively breeding primates (reviewed in Creel 2001) and gray wolves (*Canis lupus*; Sands & Creel 2004). In cooperatively breeding birds, dominants and subordinates often show similar glucocorticoid levels (e.g. Schoech, Mumme & Moore 1991; Wingfield, Hegner & Lewis 1991; Mays, Vleck & Dawson 1991; Malueg, Walters & Moore 2009). Together, these studies demonstrate that social rank has important effects on glucocorticoids in social species, but that the patterns differ among taxa and may vary with the stability and type of dominance hierarchy. Even in closely related pairs of species, pronounced differences have been observed. For example, dominant dwarf mongooses largely monopolize reproduction (Creel & Waser 1991; Creel *et al.* 1992), although dominant individuals have higher mean glucocorticoid levels than subordinates. When subordinate females do breed, their offspring are often killed, but social interactions with the breeding subordinate do not detectably change. In contrast, glucocorticoid levels are higher in subordinate females of alpine marmots (*Marmota marmota*), which are frequently attacked by dominant females (Hackländer, Möstl & Arnold 2003) and slender meerkats (*Suricata surricata*), who are subject to violent eviction from their group if they become pregnant (Young *et al.* 2006). Sapolsky (2005) reviewed the conditions under which social stress is stronger for dominants or stronger for subordinates and suggested several social conditions or individual traits that promote differences in social status related glucocorticoid patterns (resource inequity, maintenance of dominance, breeding style, stability of social ranks, subordinate coping strategies, subordinate avoiding mechanisms of dominants, subordinates' use of alternative strategies, stress of dominating mating, atmosphere of culture and personality). While much has been learned, further work is needed to fully disentangle the relationships between glucocorticoid secretion, social status and the behavioural causes and consequences of rank in social vertebrates (Creel 2001; Goymann & Wingfield 2004; Sapolsky 2005; Rubenstein & Shen 2009).

Social status and social interactions can be described in many ways. Some studies treat social status as a dichotomy, pooling subordinates into one category and comparing them to the top-ranked (alpha) individual in each group. Other methods produce a rank-order, treating social status as an ordinal variable. Still other methods consider quantitative variation, treating rank as a continuous variable. Most studies to date have tested for a categorical difference in the glucocorticoid levels of alphas and subordinates, or for a linear relationship between glucocorticoid levels and rank. Empirical research is just beginning to evaluate the possibility that glucocorticoid secretion may relate to social rank in a more complex manner. A recent, long-term study of savannah baboons (*Papio cynocephalus*) illustrates this point well. Although the highest-ranking (alpha) males had elevated glucocorticoid levels, high-ranking males other than alphas tended to have lower glucocorticoid levels than low-ranking males (Gesquiere *et al.* 2011). Studies of other species have also reported that the glucocorticoid levels of alphas are elevated, with no detectable relationship between rank and glucocorticoids among non-alphas (e.g. in Yellowstone wolves; Sands & Creel 2004). Still other studies have found that the endocrine profiles of alphas and betas (second-ranked) animals are similar, with little relationship among lower ranks (for example in African wild dogs; Creel *et al.* 1997). Interestingly, the patterns that Gesquiere *et al.* (2011) observed for baboons were not affected by the stability or instability of the hierarchy, in contrast to the results of Sapolsky (1992a) for the same species.

LIVING IN SOCIAL GROUPS MAY REDUCE STRESS

An important, but often neglected, aspect in the discussion of the effect of the social environment on stress physiology is that the social environment may not only produce stress, but in fact it may reduce overall stress levels. For example, social support may buffer glucocorticoid levels in baboons (Alberts, Sapolsky & Altmann 1992; Ray & Sapolsky 1992; Virgin & Sapolsky 1997) and in greylag geese (*Anser anser*), the size of the family unit led to a decrease in the stress response during competitive feeding situations with other flock members (Scheiber *et al.* 2005; Scheiber, Kotrschal & Weiss 2009; see also a review on social buffering of the HPA-axis by Hennessy, Kaiser & Sachser 2009).

HOW SOCIAL STATUS IS ACQUIRED AND MAINTAINED MAY AFFECT THE RELATIONSHIP BETWEEN SOCIAL STATUS AND GLUCOCORTICOIDS

It is often assumed that success in aggressive interactions and the achievement of high dominance status are always a function of morphological and physiological characteristics. However, it has now become clear that social rank means different things in different species or even populations of the same species. Rank as a descriptor of social status does not specify the process by which social roles are obtained or maintained. Thus, behavioural strategies related to the acquisition and maintenance of rank, rather than the rank *per se*, are likely to determine the degree of social stress (and glucocorticoid levels) associated with a particular social status (von Holst 1998; Creel 2001; Goymann *et al.* 2001, 2003; Abbott *et al.* 2003; Goymann & Wingfield 2004; Sapolsky 2005).

In groups where dominance rank is inherited or dominance is achieved via queuing conventions social status is unlikely to have a major effect on glucocorticoid levels,

as long as there are no acute challenges (Goymann & Wingfield 2004; Sapolsky 2005). A similar scenario may occur in many 'high skew' cooperatively breeding species where a single dominant pair breeds and the other group members assist rearing the offspring (Vehrencamp 1984; Keller & Reeve 2004). In some species (e.g. many cooperatively breeding birds), adult subordinates are mainly offspring from prior years (e.g. Brown 1978). In other species, group structures are more complex and subordinates encompass a wide range of ages and degrees of genetic relatedness, including immigrants from other groups (e.g. in African wild dogs; Creel & Creel 2002). In cooperatively breeding species with high skew in reproductive success, reproductive suppression is experienced by most group members at some stage of their lives, and many individuals remain socially subordinate for their entire lives. Neuroendocrine mechanisms that operate mainly within the hypothalamic-pituitary-gondal axis are the prime mediators of reproductive suppression and glucocorticoid levels play only a minor role (e.g. Faulkes & Abbott 1997; Saltzman, Digby & Abbott 2009). Hence, similar to species with rank-inheritance or queuing conventions, rank-related patterns of glucocorticoid levels are unlikely to be the cause of reproductive suppression, and as long as there are no acute challenges, the correlation between rank and glucocorticoids could be positive, negative or neutral (Creel, Creel & Monfort 1996; Goymann & Wingfield 2004; Sapolsky 2005).

In contrast to stable social societies where rank is inherited or reproductive suppression is common, when there is conflict about reproduction in cooperative species, rank-related patterns of glucocorticoids may arise. For example, dominant pregnant female meerkats (*Suricata suricata*) aggressively target older and more distantly related subordinate females that represent a threat to their own reproduction. These subordinates show an impaired function of the hypothalamic pituitary gonadal axis, which may be caused by chronic elevation of glucocorticoid levels (Young *et al.* 2006). A similar situation occurs in many plural breeding species (where there are normally multiple breeding individuals within the group). Here, reproductive options exist for more than one breeding pair in a group and social dominance is acquired and maintained with low level or overt aggression, and subordinates are frequently threatened by dominants. Here, a social status related pattern of acute and chronic elevation of glucocorticoid levels is commonly observed in subordinates (Abbott *et al.* 2003; Goymann & Wingfield 2004; Sapolsky 2005). Such patterns are more likely to occur in societies with strong group cohesion as opposed to fission-fusion groups (Goymann & Wingfield 2004; Sapolsky 2005). In summary, current research suggests that it may not be the dominant or subordinate position itself, but rather the process by which social status is acquired and maintained that determines the physiological and psychosocial effects associated with a given social status (Sapolsky 1992b; Abbott *et al.* 2003; Goymann & Wingfield 2004).

INTERACTIONS BETWEEN SOCIAL AND ENVIRONMENTAL FACTORS

Social living is strongly influenced by ecological and environmental factors. In particular, resource distribution in time and/or space drives dispersal decisions, movement patterns and patterns of association in many taxa (Jarman 1974; Emlen & Oring 1977; Rubenstein & Lovette 2007; Jetz & Rubenstein 2010). These same ecological and environments factors also affect glucocorticoid levels in social species. In cooperatively breeding superb starlings (*Lamprotornis superbus*) (Fig. 1), annual differences in rainfall and resource availability not only influence glucocorticoid levels, but they may do so differently in dominant and subordinate individuals (Rubenstein 2007). However, these rank-related differences in glucocorticoid patterns may be more related to environmentally driven differences in levels of social conflict than to environmental factors directly (Rubenstein & Shen 2009). Similarly, in ring-tailed lemurs (*Lemur catta*), where social stressors and rank influenced glucocorticoid levels in captivity (Starling *et al.* 2010), socially-mediated glucocorticoid levels were also differentially impacted by food availability and environmental stress in the wild (Pride 2005). Although glucocorticoid levels are strongly influenced by social rank in savannah baboons (Gesquiere *et al.* 2011), environmental stressors also had strong effects on the patterns (Gesquiere *et al.* 2008). Thus, while social factors like rank and dominance interactions shape glucocorticoid levels in many species of birds and mammals, environmental factors like resource availability often interact with social factors to shape hormone profiles. One of the future goals in comparative studies of stress physiology in social species should be to disentangle the effects of social and environmental factors on hormone levels and social roles. So far, few studies have experimentally altered social (population density) or environmental factors (predator densities or food-abundance) in social species and investigated the consequences on demography, reproductive success and glucocorticoid secretion For example, food supplementation increased reproductive success in Florida scrub jays (*Aphelocoma coerulescens*). Breeding females supplemented with food started to reproduce earlier, laid larger clutches, and the survival of their offspring was increased (Schoech *et al.* 2008). Glucocorticoids possibly play a role in the mediation of this enhanced reproductive success, as jays supplemented with a high fat and high protein diet had lower glucocorticoid levels than controls and jays supplemented with a diet high in fats but low in proteins (Schoech, Bowman & Reynolds 2004; Schoech 2009). As a consequence, food supplementation may increase local population densities of this threatened species perhaps by lowering glucocorticoid levels and enhancing reproduction.

ALLOSTASIS, SOCIAL STATUS AND GLUCOCORTICOIDS

Separating the effects of social and ecological factors on glucocorticoid levels in free-living social animals may be difficult. The theoretical framework of allostasis has been proposed to help simultaneously study the effect of social and ecological factors on glucocorticoid levels in group-living species (Goymann & Wingfield 2004; Rubenstein & Shen 2009). Allostasis is a controversial theory that emerged from the biomedical literature (McEwen & Wingfield 2003) and has been applied more broadly to other biological disciplines, including studies of vertebrate social behaviour (Goymann & Wingfield 2004; Rubenstein 2007; Rubenstein & Shen 2009; Saltzman, Digby & Abbott 2009). Allostasis, which means achieving internal stability through bodily change of state, supports homeostasis by adjusting set-points to unpredictable stressors away from homeostatic baselines (Sterling & Eyer 1988). McEwen & Wingfield (2003) have used this allostasis framework to describe the physiological costs of coping with social and environmental challenges, and how physiological mediators such as glucocorticoids regulate responses (McEwen & Wingfield 2003). The allostasis framework has been extended by Romero, Dickens & Cyr (2009) in the reactive scope model to build from the allostasis model and address some of its criticisms. Together these models provide a starting place for exploring how predictable and unpredictable social and environmental stressors influence physiology and behaviour in social species.

Models of allostasis treat social interactions as a type of unpredictable social stressor (McEwen & Wingfield 2003; Goymann & Wingfield 2004; Romero, Dickens & Cyr 2009; Rubenstein & Shen 2009). Laboratory experiments have shown that unpredictable or uncontrollable stressors (such as electric shocks) provoke stronger glucocorticoid responses than the same stimuli when they are predictable or controllable (e.g. Weiss 1970). Although it is obvious that intense, short-term stressors like storms are unpredictable challenges that organisms cannot anticipate, many types of social interactions may also be relatively unpredictable. Animals that live in groups will experience affiliative social interactions daily (e.g. foraging, grooming, sleeping), and although these interactions may increase seasonally, they are still generally predictable events because animals can largely anticipate their occurrence. However, for social animals living within a dominance hierarchy that defines reproductive roles, changes in social status are often triggered by violent, escalated aggression. These escalated fights might be less predictable (although they are more likely under certain circumstances, such as the annual breeding period: Creel & Creel 2002; Young *et al.* 2006). In response to such interactions, an individual will rapidly shift its level of glucocorticoids to a new optimum in order to cope with the stressor. Thus, an acute social conflict might be analogous to a somewhat unpredictable environmental challenge (McEwen & Wingfield 2003; Goymann & Wingfield 2004; Romero, Dickens & Cyr 2009).

Allostasis models also specifically address the idea we described above that the physiological and psychosocial effects associated with a given social status are the result of the process by which social status is acquired and maintained rather than the resulting dominance rank *per se*. The process of rank acquisition is reflected in the level of allostatic load, or the cumulative physiological burden exacted on the body, as individuals adjust behaviour to aggressive social interactions (Goymann & Wingfield 2004). Allostatic load can be reflected in glucocorticoid levels (McEwen & Wingfield 2003; Goymann & Wingfield 2004; Rubenstein & Shen 2009). A comparative study by Goymann & Wingfield (2004) demonstrated that across a large sample of social bird and mammal species, the relative levels of glucocorticoids in dominants and subordinates are related to allostatic load and the way that rank is achieved and maintained in different societies. This work was extended theoretically and empirically by Rubenstein & Shen (2009) to predict the environmental conditions under which dominants or subordinates are expected to have higher allostatic load. This work also demonstrated that group structure (i.e. the ratio of breeders to non-breeders in a group) could also influence allostatic load and relative glucocorticoid levels. Together, these studies suggest that the allostatic load framework may help explain not only the different rank-related patterns seen in glucocorticoid patterns in vertebrates, but also the environmentally driven intra-specific variation in levels seen across years in long-term studies of individual species. The allostasis framework may also prove fruitful in studying the effects of factors on stress physiology in social species, such as the role of ontogeny and maternal effects during development.

SOCIAL ENVIRONMENT DURING ONTOGENY: PRENATAL AND POSTNATAL DEVELOPMENT

The effect that the social environment may have during the ontogeny of animals has received little attention in studies of free-living social species. The hormonal stress-axis of the mother and the offspring during early life may play a role in shaping the expression of phenotypic traits. Phenotypic plasticity, or the ability of an individual organism to alter its phenotype in response to changes in environmental conditions (Stearns 1989; Garland & Kelly 2006), is relatively understudied at both the functional and mechanistic levels in social species. In social species, individuals may adapt their phenotype to the social conditions encountered during ontogeny. A variety of laboratory studies have demonstrated that stressors acting on the mother during pregnancy or lactation (or during oogenesis in birds) may result in long-lasting effects on the offspring (reviewed in de Kloet *et al.* 2005; Catalani *et al.* 2011; Henriksen, Rettenbacher & Groothuis 2011). Whereas the mammalian literature generally takes a biomedical

perspective with the prevailing view that environmental stressors lead to pathologies, or deviations from the norm, the avian literature typically takes a more evolutionary perspective (Groothuis *et al.* 2005), with the prevailing view that environmental influences during early ontogeny (e.g. maternal effects) may help offspring to adapt to the current environment (Henriksen, Rettenbacher & Groothuis 2011). In captive animals, such as guinea pigs (Kemme, Kaiser & Sachser 2007, 2008), manipulations of the stability of social groups had no effect on HPA activity. Interestingly, however, male guinea pigs whose mothers lived in an unstable social environment during pregnancy and lactation had higher levels of testosterone later in life compared to males whose mothers lived in a stable social environment during this period. A study in spotted hyenas (Fig. 1) found that androgen metabolite levels are higher in dominant female spotted hyenas during pregnancy, which may affect offspring aggression (Dloniak, French & Holekamp 2006). However, the observed effect on aggressive behaviour was small, and potential effects on the HPA axis of the offspring were not investigated. A study of adoption in free-living spotted hyenas found that the influence of the social status of the surrogate mother on offspring behaviour was much stronger than the effects of the prenatal hormonal environment (East *et al.* 2009). In birds, development occurs in both the egg and during the nest stage (when direct hormonal effects from the mother are unlikely to occur). In cooperatively breeding Florida scrub-jays, parental provisioning rates directly influence baseline glucocorticoid levels in nestling (Rensel, Boughten & Schoech 2010b). The stress response in this species varies in different developmental stages (Rensel, Wilcoxen & Schoech 2010a) and is quite repeatable across different developmental periods (Rensel & Schoech 2011). Therefore, early life and developmental experiences in the nest may influence stress-physiology throughout adulthood.

Developmental effects on stress physiology in social species may not just be limited to circulating hormone levels. In studies of laboratory rats, variation in maternal care altered the expression of genes that regulate behavioural and hormonal stress responses, forming the basis of consistent individual differences in stress reactivity (Meaney *et al.* 1996; Meaney 2001). Similar programming may occur during adolescence. In guinea pigs, housing conditions from early adolescence through adulthood had profound effects on behaviour and hormone physiology of males: males that spent the adolescence period in mixed gender colonies showed a reduction in stress responsiveness compared to males that were housed with a single female during adolescence (Kaiser *et al.* 2007; Lürzel, Kaiser & Sachser 2010). It is unclear if these laboratory studies can be generalized to free-living animals, but it is possible that this kind of programming of the offspring stress responsiveness may be adaptive. For example, the social status of the mother, the stability of the social hierarchy she lives in, or the amount of alloparental care provided by helpers could influence offspring provisioning and/or care. This variation in offspring care may then transduce a social status related signal to the offspring, which could in turn, programme the offspring's reactivity to environmental stressors. Rank-related differences in maternal care that may potentially lead to such programming are known from wild populations (Pusey, Williams & Goodall 1987), and in wild baboons, persistent effects of female rank on offspring HPA activity have been demonstrated (Onyango *et al.* 2008). In summary, laboratory studies suggest that the social environment during embryonic development, in the nursing or nestling phase, and during adolescence may programme the hormonal stress response. Because it is currently unclear whether such programming may affect fitness or what consequences this programming may have on population demography, studies of free-living animals are needed to answer these questions.

Future directions

LINKING HPA FUNCTION AND FITNESS IN THE WILD

For both social and non-social species, much of our interest in relationships between social or environmental conditions and glucocorticoid concentrations is motivated by a desire to understand the physiological mechanisms by which the environment affects fitness. However, it is difficult to establish causal relationships between glucocorticoid levels and social behaviour or environmental conditions. Likewise, understanding the relationship between glucocorticoids and fitness is not straight-forward. Prolonged elevated baseline levels of glucocorticoids are commonly viewed to be detrimental, costly and decrease fitness (Sapolsky, Romero & Munck 2000). However, there is little consistent evidence for a negative association between elevated baseline glucocorticoids and fitness (Bonier *et al.* 2009a). Part of the variation among species in the glucocorticoid/fitness relationship could be attributed to a variety of demographic, social (e.g. social rank, age, group size) or environmental factors (e.g. resource availability). Additionally, seasonal patterns of glucocorticoid release could also obscure the relationship. For example, many of the studies of HPA-fitness relationships reviewed by Bonier *et al.* (2009a,b) were conducted in the breeding season. Although many species show elevated baseline levels of glucocorticoids during breeding (Romero 2002), this does not necessarily indicate that these breeders are chronically stressed. Rather, elevated levels of glucocorticoids during breeding may play a role in the regulation of enhanced metabolic needs during reproduction. During breeding, elevated baseline levels of glucocorticoids may actually be predictive for high reproductive success in some species (Bonier *et al.* 2009b; Ouyang *et al.* 2011). Just as the accumulation of studies across a range of species and conditions altered our understanding of relationship between HPA function and rank, we suggest that studying the relationship between HPA function and fitness across a range

of species and conditions will yield new insights. Studies along ecological or social gradients (e.g. variation in population density or variation in group size) will be of particular value.

HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION AT MULTIPLE LEVELS

In addition to looking at glucocorticoids in different life history stages, it may also be important to look at other components of the HPA-axis. Studies that incorporate a range of methods [baseline glucocorticoid measurements, glucocorticoid responses to acute stressors, adrenocorticotropic hormone (ACTH) challenge and dexamethasone suppression] will be of particular value. For example, baseline and maximal stress-induced glucocorticoid levels bind to different types of receptors (Romero 2004) and may have fundamentally different effects on behaviour. In cooperatively breeding, superb starlings, baseline, maximal levels and stress responses tended to show similar patterns in individuals of different social rank (Rubenstein 2007), but the same may not be true in all species. What may be most relevant to understanding the relationship between glucocorticoids and fitness in social species is the response to stress and the feedback regulation of glucocorticoid release (reviewed in Romero 2004), which has been demonstrated for baboons (Sapolsky & Altmann 1991; reviewed in Sapolsky, Romero & Munck 2000; Sapolsky 2002). Although dominant baboons typically expressed lower baseline glucocorticoid levels than subordinates, when faced with a stressor, glucocorticoid levels not only increase much faster in dominants than in subordinates, but dominants also terminated the release of glucocorticoids more quickly after cessation of the stressful event (Sapolsky & Altmann 1991; Sapolsky 1993). These differences between dominants and subordinates were neurally driven and could not have been elucidated simply with studies of circulating hormones levels. When injected with corticotropin-releasing factor (CRF), the hypothalamic peptide that induces release of ACTH from the anterior pituitary, dominants responded with a larger increase in ACTH than subordinates, which then led to a much faster increase in glucocorticoid production. Shutting down the adrenocortical stress response via negative feedback was more efficient in dominants. Sapolsky demonstrated this by administering dexamethasone, a synthetic glucocorticoid that artificially stimulates negative feedback of the HPA axis (Sapolsky & Altmann 1991; Sapolsky 1993). To our knowledge, these data are the only ones relating negative feedback regulation of the HPA-axis with fitness and social status in a free-living vertebrate. Data from marine iguanas (*Amblyrhynchus cristatus*) suggest that individuals with an effective negative feedback regulation were more likely to survive a severe environmental stressor than individuals with a less effective negative feedback regulation (Romero & Wikelski 2010). Thus, for baboons and marine iguanas, the effectiveness of negative feedback regulation of the HPA-axis can be a predictor of fitness. These studies demonstrate that field studies can examine not only circulating glucocorticoid levels, but also feedback mechanisms. Broad inferences will not be possible until more studies have been performed, because HPA sensitivity can vary with age, and despite their generally inhibitory effects on CRF, glucocorticoids can have locally positive feedbacks on CRF release (e.g. in the central amygdala, which is involved in social behaviour: Schulkin, Morgan & Rosen 2005).

Conclusion

In summary, the increase in the number of studies of glucocorticoids in free-living vertebrates in the past two decades has greatly influenced our understanding of stress physiology. However, many exciting avenues for future research exist. More complete descriptions of HPA function and a better understanding of the complex relationships between glucocorticoids and fitness will be fundamental. Determining these relationships will require long-term studies of organisms with known ages in different life history stages and demographic, social and environmental contexts, as well as comparisons among populations or experimental manipulations of the putative causal variables that affect HPA axis activity. Maternal and organizational effects on neuroendocrine and epigenetic components of stress physiology in free-living animals are also areas ripe for exploration. To achieve these and other goals, we must move beyond simply assaying plasma glucocorticoid or faecal glucocorticoid metabolite levels. Studies should simultaneously examine natural and experimentally induced stress responses and the associated negative feedbacks. Moreover, studies of glucocorticoid receptors and binding globulins are mostly lacking in field studies and would help to fully understand the complexities of stress physiology. Much of what we know about stress comes originally from the biomedical literature. For several decades, extending concepts from biomedical research to studies in the wild has been a productive way to learn how individuals respond to unpredictable social and environmental challenges, and how these responses influence fitness or population demography. While much has been learned, well-identified avenues for future research remain.

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Sexual behavior of mares

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Abstract

The mare is seasonally polyestrus, having an anovulatory period during the short light days of late fall and early winter, and beginning to ovulate as the days become longer during the winter. The complete estrus cycle is typically about 3 weeks, with 5 to 7 days of estrus and approximately 2 weeks of diestrus. When a mare lives within the natural social structure of the horse, i.e. a family band with several adult mares and one or more stallions, estrus is characterized by repeatedly approaching the stallion, frequent urination, deviating the tail away from the perineum, and standing still with the hind limbs spread apart. Diestrus is characterized by avoidance of an approaching stallion, and aggression toward the stallion, such as squealing, striking, and kicking, if he persists in attempting to court the diestrus mare. However, mares and stallions with long-term social relationships will often rest together, graze together and groom each other, all without sexual interactions. Hormonally, estrous behavior in the mare is initiated by estradiol that is secreted by the follicle, while estrous behavior is suppressed by progesterone, secreted by the corpus luteum. Mares are unusual among the ungulates in that they periodically exhibit estrous behavior during the anovulatory period. This is probably due to the release of estrogenic steroids secreted by the adrenal cortex. The display of sexual behavior by the mare throughout the year is thought to facilitate maintenance of the horse's social structure, in which the male remains with a group of females year round, in contrast with most ungulates in which the females and males only come together during the mating season.
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Introduction

The sexual behavior of the mare has evolved in the social context of family groups that typically consist of multiple adult mares and their young offspring, plus one or more adult stallions. This long-term cohesive family structure is probably an important factor in the evolution of the unusually long period of estrus in the mare, i.e. 5–7 days versus 1–2 days for other female ungulates. The long period of estrus in all the mares of the herd would facilitate maintenance of proximity to the mares by the herd's stallion. While strong social bonds develop between the mares and the stallion(s), the social core of the group is formed by the mares, and it is not necessary for the stallion to be present for the mares to remain as a cohesive group. Nevertheless, the stability of a mare's consort relationship with a given stallion significantly correlates with her lifetime reproductive success (Kaseda et al., 1995). In the context of sexual interactions with the stallion, however, the mares may become very competitive, and if more than one mare is in estrus at one time, the higher ranking mare will actively disrupt interactions between the subordinate mare and the stallion and drive the subordinate away from proximity to the stallion (Asa et al., 1979).

Normal estrus cycle

The mare is seasonally polyestrus, with estrus cycles beginning in mid to late winter and continuing through the early fall. Increasing daylight length plays a major role in the initiation of cycling, and the use of artificial light can induce a resumption of cycling earlier than would happen under natural conditions (Allen, 1977; Colquhoun et al., 1987; Hughes et al., 1972; Kooistra and Ginther, 1975; Palmer et al., 1982; Sharp, 1980). With an 11-month gestation period, this timing maximizes the likelihood that foals will be born and spend their first few months of life at a time when the weather is warm and food is plentiful. The exact months that a mare will ex-

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perience estrous cycles depend on the latitude at which she lives. On Assateague Island National Seashore, which lies off the east coast of the United States, most foals born to the feral ponies that live on this island are conceived in May, June, and July and born the following April, May, and June (Keiper and Houpt, 1984). A period of follicular growth and ovulation, accompanied by behavioral estrus, generally lasts 5–7 days. Insemination by a fertile stallion 48 to 72 h before ovulation will generally result in conception (Brinsko and Varner, 1993). While insemination 4–5 days before ovulation can potentially result in conception, rates are much lower. If the mare does not conceive during this period, a period of diestrus, with the development of corpora lutea and an absence of sexual activity, lasting approximately 2 weeks, will occur (Back et al., 1974; Fig. 1). Post-partum mares experience estrus, commonly called "foal heat", during the first week after birth and may conceive at this time. Mares become anovulatory sometime in the fall and remain so until increasing day length triggers a resumption of estrous cycles.

Behavior that identifies mares as being estrous or non-estrous status is arbitrarily grouped into three categories: attractivity, proceptivity, and receptivity (Beach, 1976). As applied to the mare, attractivity refers to the stimulus value of the mare that elicits appetitive sexual responses from the stallion. Thus, attractivity is actually measured by the behavior of the stallion, but is a consequence of various behavioral and non-behavioral attributes of the mare, such as posture and olfactory cues, respectively. Proceptivity refers to appetitive behaviors shown by the mare in response to stimuli received from the stallion. These would include such behaviors as approaching the stallion and rotating the body so that the hindquarters are in front of the stallion. The mare's proceptive behavior occurs as a response to the sexual attractivity of the stallion. Certain attributes of the stallion are more or less likely to elicit proceptive behavior from the mare. For example, stallions that have a high frequency of vocalizations are more attractive to mares than stallions with a low frequency of vocalizations (Pickerel et al., 1993). Finally, receptivity refers to behavior by the mare that facilitates copulation with the stallion, the most important of which is simply standing still so that the stallion can mount and intromit. These three categories are clearly not mutually exclusive. Nevertheless, they can be useful constructs when discussing various aspects of sexual behavior.

The estrous mare will approach the stallion more frequently than the diestrous mare and will turn her hindquarters toward him. The estrus mare will also frequently adopt a posture in which she lowers her pelvis, spreads her hind limbs, and deviates her tail either to the side or straight back from the body, exposing the perineal region (Fig. 2). This posture is accompanied by a rhythmic eversion of the clitoris, called clitoral winking (Asa et al., 1983; Back et al., 1974; Clayton et al., 1981). Estrous mares will also void small quantities of urine very frequently, while diestrous mares void larger quantities of urine less frequently. In a test situation, stallions show no difference in olfactory response, including flehmen, to the urine of estrous and non-estrous mares. Therefore, the stallion appears to be unable to distinguish the two. However, in the field situation, the frequent urination of estrous mares causes an increased frequency of showing flehmen in the stallion. Therefore, the frequent urination of estrous mares may function in chemosensory priming of the stallion for reproduction (Marinier et al., 1988; Stahlbaum and Houpt, 1989).

The estrous mare also has a unique facial expression, with relaxed facial muscles, slightly lowered head and ears turned to the side (Fig. 3). One case has been reported of a mare that exhibited an atypical facial expression when she was in estrus that was similar to snapping behavior, also referred to as champing or tooth-clapping, of immature horses. She exhibited the facial expression when she first noticed the stallion or heard him neighing. When the stallion approached, she would flex her

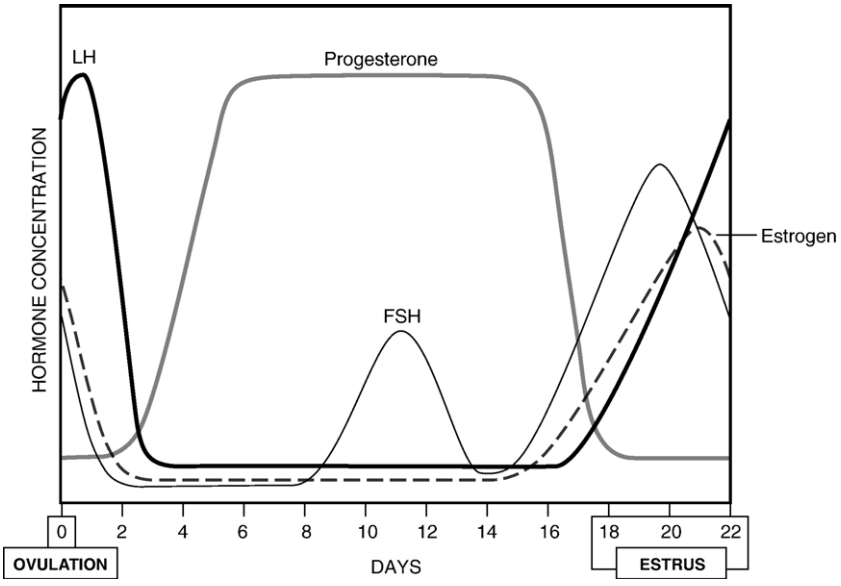

Fig. 1. Changes in the concentration of LH, FSH, estrogen and progesterone over the course of a typical estrous cycle. Behavior of estrus declines rapidly following ovulation and in conjunction with the rapidly rising levels of progesterone, so that the mare will actively reject the stallion 12–48 h after ovulation.

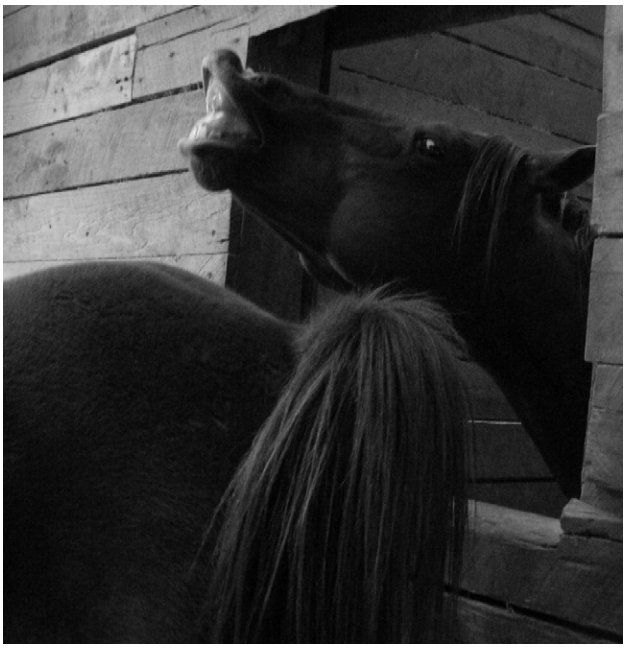

Fig. 2. An estrous mare that has presented her hindquarters to the stallion elevates her tail and deviates it to the side. The stallion is showing flehmen.

limbs, clamp her tail against her perineum, and open and close her mouth (Woods and Houpt, 1986). Snapping has been hypothesized to be a displacement behavior that occurs when foals or, in this case, a mare is in a conflict or approach–avoidance situation (Crowell-Davis et al., 1985). Therefore, in this situation, the mare may have been both attracted to and fearful of the stallion. It is likely that this phenomenon will occasionally be observed in other mares.

The proceptive behavior of the estrous mare is important in facilitating reproduction. If multiple mares are in estrus at the same time, mares that follow the stallion and repeatedly position themselves near the stallion are more likely to be mated repeatedly than estrous mares that follow the stallion less (Ginther et al., 1983). During copulation, the estrous mare demonstrates receptivity primarily by standing still. She may also lean into the stallion once he has mounted and intromitted and may look back at him during copulation.

The most visually notable behavior of the diestrous mare is the fact that, while the estrous mare will stand still when a stallion approaches, licks, and nibbles her, the diestrous mare will squeal, strike, and kick at a stallion that attempts to initiate courtship interactions with her. The relaxed facial expression of the estrous mare is replaced by tension of the facial muscles, turning the ears back and threatening to bite if the stallion is persistent (Fig. 4). Diestrous mares that have a long-term, stable social relationship with a stallion will only engage in these behaviors if he attempts courtship, e.g. prancing, nickering, nibbling, and licking. Stallions and diestrous mares will often graze and rest together and groom each other amicably so long as the stallion does not engage in any courting behaviors.

Yearling fillies (young females) may exhibit estrus, but are less attractive to the stallion at this time than are older mares (Asa, 1986). While the proceptive behavior of yearling fillies is often more exaggerated than that of older mares, they are also likely to run away if the stallion actually approaches. Long bouts of grooming are common between yearling fillies and stallions and may serve to habituate the filly to the proximity of the stallion (Tyler, 1972).

Hormonal control of estrus

During the ovulatory season, the developing follicle releases estradiol, which facilitates the estrous behavior. The corpus luteum releases progesterone which, in the mare, serves to inhibit estrous behavior, resulting in diestrus (Asa, 1986). Injection of 1 mg of estradiol induces estrous behavior in ovariectomized mares. Paradoxically, concurrent injection of 100 mg of progesterone causes more intense exhibition of

Fig. 3. An estrous mare interacts with a stallion. The ears are turned to the side and the facial muscles are relaxed.

Fig. 4. A diestrous mare being held in proximity to a stallion. The ears are back, the facial muscles are tense and the nostrils are flared. The mare also kicks at the stallion, who is protected by a stall wall.

estrous behavior on the first day of treatment, but subsequently causes inhibition of estrous behavior (Asa et al., 1984).

Estrus detection

In modern domestic management, there are three primary categories of techniques for horse breeding. In pasture breeding, the mare(s) and stallion are housed together on the same pasture. In this situation, the stallion repeatedly approaches the mares, often prancing and nickering. The diestrous mare will ignore or avoid him and will become aggressive if he persists in approaching, while the mare entering estrus will watch him and will often interact with him, e.g. engaging in mutual grooming. Regular courtship will occur over a period of days, progressing to the point where the mare stands still, adopting the mating posture described above while the stallion licks and nibbles her, especially in the withers (base of the crest of the neck) and in the perineal region. Once this occurs, the stallion will mount. Because estrus is readily detected both by dramatic behavioral changes and by rectal palpation (see below), measurements of hormone levels in urine or saliva are not used for routine detection of estrus. Mares and stallions kept in a pasture breeding situation will typically copulate multiple times and engage in long periods of courtship interactions. In Icelandhorse herds with a ratio of 1 stallion to 10–23 mares, each mare mated approximately 6 times (Steinbjörnsson and Kristjansson, 1999). Conception rates are highest in this context, as compared to herds with a higher mare to stallion ratio, in hand breeding or artificial insemination.

In "in hand" breeding, the mare and stallion are likely to meet only for copulation. The mare and stallion are both restrained via halter and lead rope. In addition, the mare may be twitched and/or hobbled, while a stallion that becomes aggressive in this context may be muzzled to prevent his injuring the mare. Twitching involves applying a chain or rope to the mare's upper lip and twisting it, while hobbling involves tying two or more of her limbs together with a length of rope in between so that her ability to move is limited. In hand breeding requires that some means be used in advance to determine if the mare is in estrus. Rectal palpation can be conducted to determine if the mare is developing follicles. However, there is always some degree of risk in conducting rectal palpation in this species, in which the rectum is very easy to tear. Rectal palpation is therefore not typically done as a routine, daily means of assessing a mare's estrous status. Instead, a technique called "teasing" is used in which the mare exposed to one or more stallions that are separated from the mare so that they cannot fully interact with her, i.e. they can only interact over a fence, a stall door, or a chute designed specifically for this purpose. The mare's behavioral responses to the stallion are then observed to assess her estrous status. If the mare attempts to avoid the stallion and shows aggressive responses, such as squealing and striking, she is presumed to be in diestrus. If the mare assumes the estrous posture, urinates, and/or develops the relaxed musculature of the mating face, she is probably in estrus.

Teasing is not 100% accurate in detecting estrus. Teasing one mare at a time is more accurate than teasing a group of mares, an arrangement in which the observer determines whether mares approach the stallion or not. Usually, 40 to 50% of estrous mares will not approach the stallion (Ginther, 1979, as reported in Asa, 1986). More intense aggression is exhibited by the diestrous mare when she is restrained near the stallion than if she is loose with the stallion in a paddock. In the latter case, the mare can simply avoid the stallion, and low intensity aggression is sufficient to signal the stallion to keep his distance. Depending on the standards of the particular farm, she may or may not be palpated per rectum at this point to determine her ovulatory status. While the conception rate is lower in hand breeding, it is a popular method because it minimizes the chance that the stallion will be injured by the mare kicking him.

While the normal mare does not exhibit stallion-like sexual behavior, mares with ovarian tumors that secrete androgen will do so (Fretz, 1977; Stabenfeldt et al., 1979). Stallion-like behavior can also be induced by injecting the anabolic androgenic steroid boldenone undecylenate at 0.55 mg/kg IM q7days. Such mares fail to elicit signs of behavioral estrus in fence line trials with estrous mares, but do sometimes elicit estrous behavior when allowed to interact with normally cycling mares at pasture (McDonnell et al., 1986).

Finally, artificial insemination is allowed in some breeds. In this case, a mare is teased as described above to determine if she is in estrus or not and will probably be palpated. If appropriate, she is then inseminated by intrauterine infusion of semen that has been collected in an artificial vagina. Stimulation that mimics the courtship behavior of the stallion may facilitate detection of estrus. Specifically, playing a recording of the vocalizations of a courting stallion, and manual tactile manipulations of the withers, the flank and the external genitalia has been shown to elicit estrous behavior in the majority of Belgian warm-blood mares (Veeckman and Odberg, 1978). However, playing recordings of stallion vocalization alone is not useful in detecting estrus in mares when a stallion is not nearby (McCall, 1991). Manual stimulation of the cervix and vagina during artificial insemination may facilitate uterine contractions and increase passive sperm transport (Campbell and England, 2004). However, this procedure has not been found to have any effect on conception rate (Valle et al., 1998).

Silent estrus

Mares may have follicular development and ovulate without showing any signs of behavioral estrus. Social factors, such as inhibition of proceptive behavior by subordinate mares in the presence of dominant mares, may be relevant, but they have not been empirically studied. Mares also show individual preferences for particular stallions and, conversely, non-preference for or avoidance of other stallions (Pickerel et al., 1993). Therefore, if a mare is evaluated only by exposure to one or two stallions, she may not show behavioral estrus because of a lack of attraction to those particular stallions. In one study in which cycling mares were able to freely interact with, or avoid, a stallion in a paddock with them, one in six showed silent estrus (Asa et al., 1979).

Sexual behavior in nonovulatory mares

During the winter months the mare's hypothalamic–pituitary axis becomes nonfunctional. GnRH secretion approaches zero, folliculogenesis ceases, and the ovary becomes nonfunctional (Sharp and Davis, 1993). Mares are unusual among ungulates in that they may exhibit estrous behavior during the winter, when they are anovulatory. Ovariectomized mares will also exhibit periods of estrous behavior (Asa et al., 1980a; Ginther, 1974). There is qualitative and quantitative individual variation in the estrous behavior. While some mares only exhibit mild estrous behavior, such as by occasionally approaching the stallion, others will exhibit the complete repertoire of estrous behavior, including standing for copulation.

Physiologically, this estrous behavior is probably due to hormonal stimulation of nonovarian origin. The adrenal cortex secretes androgens, estrogens, and progestins, which might influence the expression of sexual behavior (Nett, 1993). While much smaller quantities are secreted by the adrenal cortex than by the ovaries, the mare is likely more sensitive to small quantities of hormones than are other ungulates (Nishikawa, 1959). This possibility is supported by the fact that when ovariectomized mares are given dexamethasone (15 mg IM b.i. d.), which suppresses ACTH and therefore the production of adrenal steroids, they discontinue exhibiting estrous behavior. If they are given dexamethasone and estradiol, they continue to exhibit estrous behavior (Asa et al., 1980b).

From the ethological perspective of behavioral phylogeny, the development of this unusual behavior is probably significant for the maintenance of long term relationships between the mares and stallion of a family band. In most other ungulate species, the males and females come together only during the breeding seasons and separate during the non-breeding season. Family bands of horses remain together all year, and the stallion contributes to the defense of the mares and foals from predators. Sexual activity between the mares and stallion during the months when the mares are anovulatory probably facilitates the maintenance of this social organization.

Sexual behavior of pregnant mares

Five to ten percent of pregnant mares exhibit estrous behavior. Therefore, the display of sexual receptivity does not provide certain confirmation that a mare has not conceived (Asa et al., 1983; Satoh and Hosh, 1933; Tomasgard and Benjaminsen, 1975). The physiological cause of this phenomenon is not fully understood.

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AN ECONOMIC MODEL DEMONSTRATING THE LONG-TERM COST BENEFITS OF INCORPORATING FERTILITY CONTROL INTO WILD HORSE (*EQUUS CABALLUS*) MANAGEMENT PROGRAMS ON PUBLIC LANDS IN THE UNITED STATES

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AN ECONOMIC MODEL DEMONSTRATING THE LONG-TERM COST BENEFITS OF INCORPORATING FERTILITY CONTROL INTO WILD HORSE (*EQUUS CABALLUS*) MANAGEMENT PROGRAMS ON PUBLIC LANDS IN THE UNITED STATES

Charles W. de Seve, Ph.D., and Stephanie L. Boyles Griffin, M.S.

Abstract: In recent years, the Bureau of Land Management's (BLM) Wild Horse and Burro Management program costs have increased dramatically due to a rise in the number of animals removed from public lands coupled with significantly decreased adoption rates. To assist with development and implementation of effective, cost-containing management programs, a robust economic model to project the costs and optimize outcomes of various management scenarios was created. For example, preliminary demonstration model runs show that by gradually replacing "removal-only" programs with contraception-and-removal programs on one hypothetical Herd Management Area (HMA), the BLM could save about US$8 million over 12 years while maintaining an area target population of 874 horses. Because the BLM estimates that more than 38,000 wild horses roam on 179 HMAs in the United States, the use of this economic model could result in a cost-savings of tens of millions of dollars if applied broadly across all HMAs.

Key words: Bureau of Land Management, economic model, *Equus caballus*, fertility control, porcine zona pellucida, wild horses.

BRIEF COMMUNICATION

The Wild Free-Roaming Horses and Burros Act of 1971 (Public Law 92–195)[13], as amended, directs the Bureau of Land Management (BLM) to manage wild horse herds by setting appropriate management levels (AMLs). AMLs are estimates of the optimum number of wild horses which results in a "thriving natural ecologic balance and avoids deterioration of the range" for each Herd Management Area (HMA). As of February 2012, the BLM estimates that approximately 37,300 wild horses and burros (31,500 horses and 5,800 burros) exist on 179 HMAs that comprise 31.6 million acres of public lands, of which 26.9 million are under BLM management.[10]

Herds can grow at an average rate of 20% annually.[2,3,4] When herd populations exceed established AMLs, the BLM conducts roundups to remove excess animals from HMAs, and roundups occur every 3–5 yr to ensure BLM complies with the requirements of the amended act. Removed animals are transported to long-term government holding pastures in the Midwest where they are cared for in perpetuity or, to a lesser extent each year, adopted by private individuals.

From 1971 to 2012, the BLM has removed over 300,000 wild horses and burros from the range and adopted out more than 230,000 animals.[10,12] However, in recent years the number of animals removed from the range and placed in long-term holding has far exceeded the number adopted or sold; adoption demand has fallen in response to the U.S. economic recession and other factors. According to the BLM's data reports for 2000 through 2012, over 121,000 animals were removed from the range while only 62,000 were adopted or sold. In Fiscal Year (FY) 2012, 66% fewer animals (i.e., 2,598) were adopted compared to FY 2002 when the BLM adopted out 7,746 animals.[10]

The remaining 46,500 animals have been placed in government holding facilities and, as a result, off-the-range wild horse and burro management costs have increased dramatically.[10] In FY 2012 alone, the cost of holding and caring for these animals exceeded $43 million—59% of the $72.4 million in total annual expenditures for the entire wild horse and burro management program.[10]

BLM is committed to caring for these animals, but the costs associated with their care will continue to increase because mortality rates in captivity are low, the average life span of wild horse and burros in captivity is approximately 30 yr, and every year the agency continues to remove more animals from the range and place them in additional holding facilities. This situation will

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result in exponentially increasing holding costs that will continue to rise as holding facilities become scarce and operators increase their prices, a trend now evident. Unless the agency develops and implements long-term management strategies to reduce the number of removals by reducing population growth rates on-the-range, the current removal-only approach seems doomed by inevitable budget constraints off-the-range.

Fertility control is a viable alternative to the current wild horse management regime. In early 2012, the Environmental Protection Agency (EPA) registered the first contraceptive vaccine for wild horses commonly known as porcine zona pellucida (PZP) and registered under the brand name ZonaStat-H.[11] Fertility control techniques and immunocontraception vaccines have been shown to be safe, humane, reversible,[8] and capable of stabilizing and reducing wild horse populations over time, either when used alone or when combined with other wild horse population growth suppression methods.[5,6,9] A United States Geological Survey (USGS) economic study[1] also demonstrated that reducing wild horse population growth rates through the combined use of fertility control drugs and sex ratio adjustments may reduce the need and frequency of removals which, in turn, could lead to long-term reductions in off-the-range management costs. This study estimated that fertility control on-the-range could reduce total wild horse and burro management costs by 14%, saving $6.1 million per year. The question is how to implement a humane and efficient cost-saving management plan.

Currently the BLM uses WinEquus, a stochastic wild horse population model, to evaluate possible management actions and select preferred alternatives, but the model does not calculate any costs associated with various management options.[7] The model cannot optimize management options, compare the combined removal and holding costs of alternative management plans, loop through multiple ranges to help build an efficient overall strategy, or simulate multi-year planning.

To assist the BLM in developing and implementing a long-term plan to maintain healthy wild horse and burro herd populations, a robust economic model to project the costs and outcomes of various management regimes was created which can be used by the agency to plan effective, cost-beneficial programs. The management system is designed to simulate range populations and alternative BLM management interventions and costs in either a single HMA or over multiple HMAs. It employs not only simulation methods but also optimization. Thus, it can help make sound management decisions under uncertainty, including allocating resources and reducing costs. The modeling incorporates stochastic simulations, conventional optimization, and stochastic optimization. There are four sub-models in the overall model framework: The Dynamic Population Simulation Model, The Economic Costing Model, The Management Intervention and Optimization Model, and The Population and Range Database System.

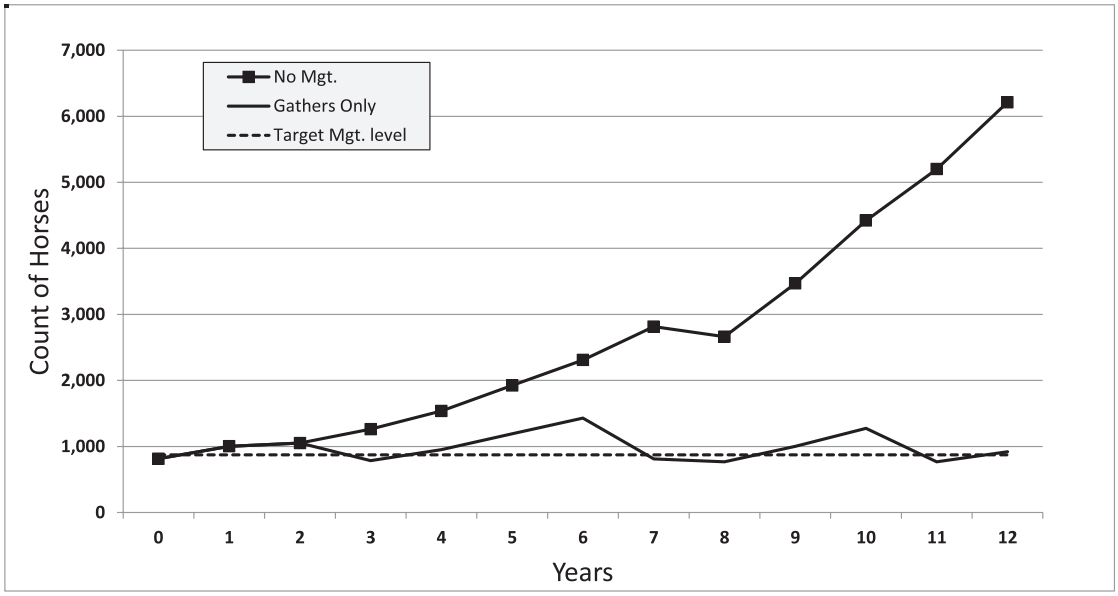
Recognizing the sparse data currently available for wild horse populations, the database system incorporates techniques for simulating missing data. It also guides the user to track range management results and enter additional data as the years pass, helping to initiate sound data collection procedures on the range and from contractors hired to conduct gathers. As with range data, missing cost items can be approximated or simulated using appropriate BLM assumptions.

BLM could use the model to decide on the optimum number and locations of round-ups, age–sex gather targets, fertility control elements, and remove:release ratios in order to allocate a fixed total budget, thus selecting the "best" limited set of gathers, or allocate to minimize cost in a single year or over multiple years to reach annual or overall population targets. The model might also optimize to control costs by suggesting adjustments to consider to HMA target populations or AMLs.

To demonstrate, using a hypothetical HMA with a target population of 874 animals, initial model runs over a 12-yr period demonstrate that the "no management" alternative, meaning no round-ups, removals, or contraception (or any combination thereof), is incapable of maintaining the target population and would lead to an exponential population growth rate that is not desirable or sustainable, as the population would rise from under 874 to over 6,000 in 12 yr (Fig. 1a, b). A model run of a typical "removal-only" management regime, where animals are removed from the HMA at years 3, 7, and 11, shows that such a scenario is capable of maintaining a population at or near the target population level (Fig. 1a.) but at a cost of over $13 million over 12 yr (Table 1).

A lower cost approach uses a contraception-and-removal management program where during a gather some animals are removed, selected by age and sex, but a high proportion of the mare population is treated with fertility control drugs and released. Population projections for years 3,

a.



b.

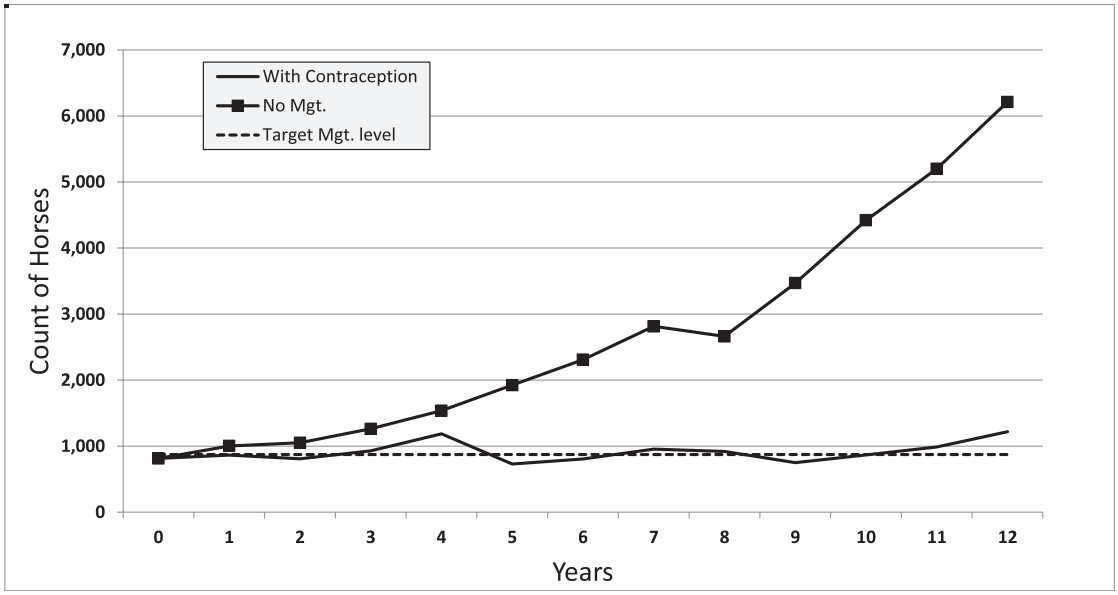


Figure 1. Example economic model output for a hypothetical Herd Management Area (HMA) with a target management population of 874 animals. The model simulates the difference in population growth rates over a 12-yr period when no management is conducted on the HMA versus (**a**) removal-only and (**b**) contraception-and-removals at years 3, 7, and 11.

7, and 11 show that such a management scheme will both maintain the target population level (Fig. 1b.) and reduce management costs by approximately $8 million over 12 yr—or $5.9 million present value compared to the "removal only" scenario (Table 1). Note that even a suboptimal effectiveness of PZP can achieve substantial population control and cost savings.

The model is flexible and, while these demonstration runs are based on realistic working assumptions provided by experts in the field, virtually all inputs can be adjusted to see the

Table I. Example of economic model output results of 12-yr contraception-and-removals versus removals-only scenarios.

	Contraception and removals	Removals only	Change (%)
Total horses to holding over 12 yr	798	1,790	−55.4
Average range pop. over 12 yr	910	982	−7.3
AML[a]	874	874	
Average difference from AML (%)	4.1	12.4	
Total gather and treatment costs	$2,435,523	$2,634,579	−7.6
Total holding costs	$5,560,426	$10,694,712	−48.0
Total cost (sum of years)	$7,995,949	$13,329,290	−40.0
Total cost (present value)	$5,852,135	$9,314,090	−37.2

[a] AML = appropriate management levels.

impact of other conditions, to test the limits of the model, the risks of other outcomes, or to use other assumptions. The examples here describe the population in an individual HMA while the gold-standard model would be a simultaneous collection of model runs for multiple, or all, HMAs in order to calculate the full population impact and the total cost to the agency's budget.

BLM estimates that there are approximately 38,000 wild horses and burros roaming on 179 HMAs located on public rangelands in 10 Western states. If about $8 million could be saved by implementing the proposed management scheme at one HMA over 12 yr, it stands to reason that such an approach could result in a cost-savings of tens of millions of dollars, if applied across all HMAs, recognizing the size differences among ranges.

Given current budget constraints imposed on federal and state wildlife and land management agencies, the hybrid population and economic model describe here could be used to develop and support the implementation of cost-effective and long-term wildlife and land management programs. It is clear to us that BLM and other agencies should invest more resources into both modeling and population growth suppression methods.

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The Varying Role of the Male in Feral Horses

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Abstract

For the past four years a study has been conducted on the Red Desert of Wyoming concerning the role of males in multi-male bands and multi-band herds. During a colt's life he is barely recognized as playing a male's role. From the age of two-three, his role begins to emerge, at which time he usually joins a bachelor group or becomes a loner. At the age of three-four, the male associates with a mixed-sex band without constant status. With age comes a decrease in dominant status. In contrast to other equid systems, the multi-male organization could possibly represent an R strategy, whereas the multi-band herd organizational scheme must be emergent and represent a new strategy for large numbers of equids.

Introduction

The ungulate traditionally shows a sexual dimorphism in behavior. The male shows much more agonism, intra- and interspecifically. His prototype might be the bull elk or ram bighorn sheep, where a large amount of the rut is spent in sexual combat. In both, threat, ritualization, dominance-subordination, and submission play well recognized roles. The dominant male is a harem master, rounding up, defending, and doing most of the breeding with his group of females--a male chauvinist par excellence.

So it has seemed to be with equids, and four years ago when we started to work with feral horses this is what we looked for (Denniston and Miller, 1978; Miller and Denniston, 1979). To some extent it is what we found, but two sorts of exceptions appeared: multi-male bands and multi-band herds. Multi-male bands may represent a trade-off by a dominant male for band defense by subordinate male band members. Multi-band herds are an exception because they may include or have attached to them single males or bachelor groups. Such herds are groups of bands with a common home range and common movement patterns, organized into a social structure with considerable mutual recognition and compatibility.

Agonism and Dominance

Male-male conflict occurs in many situations. Some typical encounters are presented in Table I. Most often the instigator is a band male or a satellite male of a multi-male band. More rarely it may be the male of a small band; and occasionally, under crowded conditions, it may be a dominant female. The resource competed for is often related to band integrity: a female or a foal. It shifts from such resources to water, in the latter part of the summer. The recipient is most often an interloping male, either a bachelor or one from another band. The whole

array of agonistic behaviors described by Feist and McCullough (1975) may be shown in male-male conflicts. If the recipient is other than an adult male, the herding posture is most often used.

It is possibly equally significant that, under apparently provocative conditions, conflict and agonistic behavior do not appear. In the case of the band male who mounted a band female three times and then allowed a male from a different band to breed her, no conflict resulted. These bands were part of the same herd and were familiar with each other. This is also the case with bands which pass through each other while grazing without conflict. One band passed through another in which a foal had been born within the hour. No agonism was shown.

Development of the Male Role

I would like to examine the ontogeny of this male role in both exceptional situations--the multi-male band and the multi-band herd--and then speculate briefly on possible evolutionary significance.

During the first two years of a colt's life, his role is hardly distinguishable as a male. He may attempt to mount his mother or a sib. His sister may be mounted by mature or immature males as a two- or three-year-old.

As a two- or three-year-old and rarely as a yearling, the colt's role begins to emerge. He will probably leave his family band and either become a loner or join a bachelor group.

1. If he remains with his parental band, his presence is largely ignored by the mature stallion(s). He certainly is not driven off.
2. If he becomes a loner, he may associate temporarily with other males of like or older age or he may attempt to associate with an established band.
3. If he joins a bachelor group, the association may last up to two and one-half years. A bachelor group which acquires a mare may be quite lasting, but should be classified as a mixed band. Intra-group agonism is frequent but much of it seems somewhat playful. The dominant male in a bachelor group may act in many ways like a harem master, herding his bunch and responding to challenges from males from mixed-sex bands, to whom he is almost always subordinate.
4. If a lone male attempts to associate with a well established band, he will be challenged, chased, and driven off repeatedly by the mature male(s). Often the bachelor persists in his attempts, trailing the band at some distance, grazing close to them and watering with or immediately after them. A two-year-old palomino first started his attempts at association with a band early last fall. He is still not completely accepted, but now is most active in driving off intruding males and often waters with the band (c.f. Welsh, 1973).
5. A lone male may acquire a single mare, perhaps with foal, and so start the nucleus of a band. Groups commonly encountered include: a male-female pair; such a pair with a foal; a pair with a foal and a yearling; even a pair with a foal, a yearling, and a two-year-old.

However a three- to five-year-old male comes to be associated with a mixed sex band: as a member of a bachelor group which acquires females; as a finally accepted interloper; or possibly as one who grows up in and remains with his family band, his status does not remain constant. He will be increasingly active in defending the band and in breeding. He

will utilize the scarce water supply with the band. As the mares of his band come on heat, he may share them with little friction from other band stallions, either during the same day or doing all the breeding for two or three days and then none for a similar period. He will be increasingly recognized by other band stallions in the herd. Almost imperceptibly he may gain a dominant position in his own band and in relation to other bands.

Just as gradually, he will lose his dominant status. If the other stallions in his band are much younger, the band may start losing mares and their foals. The band may break up completely and the previously dominant stallion become a loner, sometimes tolerated briefly by other bands in the herd.

Adaptiveness of the Male Role

Such a system does several things for the overall population: (1) it insures that most breeding is usually done by the most rugged males; (2) at the same time it is usually only distantly related females with whom they breed; (3) a given female may be bred by a variety of males -- all with a high degree of fitness. The efficacy of this system is demonstrated by the thriftiness, generally good configuration, variety of coloration of the horse population on the Red Desert of Wyoming.

Our findings as to how a male may acquire a band are similar to Welsh's. His Sable Island ponies were also exposed to rigorous winters when bands mingled. He reported no multi-male bands or multi-band herds. Keiper in 1976 reported some multi-male bands. What he describes as co-dominance in bands of young horses is like our shifting dominance in mixed bands.

Both Tyler's (1972) New Forest ponies and Imanishi's (1950) Toimisaki horses had the adult males culled from the group, leading to a very low male/female ratio and little male-male competition.

These horses do not fit either Klingel's (1969, 1974, 1979) harem category nor his territorial male type. In the Pleistocene, horses showed a great morphological radiation. Is it possible that we now have an equally great behavioral radiation?

Why is this system different from other equid systems? Klingel lists only two types: harems and territorial males (Klingel, 1969, 1974, 1979). Feral animals tend to revert toward the wild condition. Possibly Red Desert horses are only partly reverted toward a harem system. The multi-male organization may represent an R strategy in an expanding population. The relatively low foal loss reported by Miss Lee Boyd (Boyd, 1979) would be characteristic of R strategy. The super-band herd organization must be emergent and represent a new anti-agonistic strategy for large numbers of equids in contact with each other. In the somewhat parallel situation of zebra, different strategies have evolved.

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Table 1

Month	Instigator	Resource	Recipient	Special Circumstances	Agonistic Behaviors	Outcome
May	Band M	F (estrous)	Bachelor M	Bachelor approaches F in band.	Forefoot pawing, deep head-bowing, competition on stud pile	Interloper rejoins bachelor group.
May	Small band M	F from large band	Large band M		Arched neck threat, rearing	Small band M returns to its group.
May	Small band M	F from large band	F + 3-yr. M from large band	Herding by small band M of M and F for over one hour	Herding Head projecting	Small band M returns to own band.
May	Large band	Graze	Medium band	Move through each other grazing one-half hour	None	Separation of bands
May	Large band M	Estrous F (2-yr.-old)	M from other large band	M from other band mounts F. M from 3rd band interferes.	Rearing and striking	Breeding M driven off. F returns to own band.
May	Newly accepted M to band	Band integrity	Bachelor M, M from small band	New M goes out to incoming MM.	Challenges Arched neck threat Foreleg kick Neck bite Chase	Interlopers driven off.
May	Small band M	Band integrity	2 bachelors Newly accepted M from other band		Chase Fight	Interlopers driven off.
June	Band M	Band integrity	Foal	Foal wanders off.	"Snake" herding	Foal returns to band.
June	Band M	Band integrity	Former band MM	2 MM who lost FF over winter approach large band.	Chase	Interlopers driven off.
June	Band M	Estrous F	M from other band	Band M breeds F 3 times then strange band M breeds her.	None	Other band M returns to band.
June	Band M	Band integrity	Other band M	Strange M herds foal back to foal's band, is challenged by M of that band.	Rush Chase	Herding M returns to own band.

Table 1 (continued)

Month	Instigator	Resource	Recipient	Special Circumstances	Agonistic Behavior	Outcome
June	Large band M	Dominance	Small band M	Small band approaches large band.	Rear Challenge Small band M bows deeply, extends foreleg.	Small band M retreats.
July	Band M	Band integrity	2 FF from band	FF started away from water, trailing strange band.	Arched neck threat "Snake" herding	FF return to own band.
July*	Band M	Water	9-member band	Many bands attempting to drink	Arched neck threat Rearing Shouldering	Band remains on water.
July	2 Band MM (same band)	F, 2-yr.	M, 3-yr.	M and F herd foal back to original band. 2 MM from that band challenge 3-yr. M.	Chasing Arched neck threat Rearing	3-yr. M goes off with F.
July	Band M	Water	Bachelor M from 5 MM	Crowd at water	Arched neck threat	Band stays on water.
July	Dominant F	Dominance	Other FF in band	Crowding around water	Chasing	---
July	Band M	Band integrity	M who had lost band; M from other band		Separated M marks stud pile; band M marks then arches neck. Band M rears with M from other band.	Separated M leaves.
July	2 Band MM	Water	5 Bachelors	Crowd at water	Arched neck threat Chasing	Band stays on water.
August	Band M	Band integrity (Water?)	Bachelor		Bachelor marks stud pile. Band M marks on top then chases bachelor; LEADS him away from band.	Bachelor led off.
August	2 Bachelors	Dominance	2 Other bachelors		Alternate fighting on hind legs	Move off together in group.

*Repeated by different band males throughout dry period

Article

Evaluation of post-operative pain after active desufflation at completion of laparoscopy in mares undergoing ovariectomy

Ian F. Devick, Britta S. Leise, Sangeeta Rao, Dean A. Hendrickson

Abstract — The cause of transient post-operative pain in a subset of horses undergoing laparoscopy is unclear. The objective of this study was to evaluate if residual pneumoperitoneum is associated with transient post-operative pain in mares undergoing ovariectomy. Thirty-eight mares undergoing routine standing laparoscopic ovariectomy were randomly allocated into 2 groups. At the completion of laparoscopy, either the abdominal cavity was actively desufflated or the cannulas were opened to achieve ambient pressure before incisional closure. Assessments were performed for 18 hours after surgery using a validated visceral/somatic pain scale for horses. Overall, pain was minimal in both groups (median score 2/39) post-surgery. Active desufflation of the pneumoperitoneum at the completion of laparoscopy approached statistical significance (P = 0.07) in decreasing pain at 12 hours after laparoscopy. However, effects of active desuffation were not significant throughout the monitored 18-hour post-surgery period. We conclude that the decision to actively desufflate at the completion of laparoscopy should be based on surgeon's preference.

Résumé — Évaluation de la douleur postopératoire après un désoufflement actif à l'achèvement d'une laparoscopie chez les juments subissant une ovariectomie. La cause d'une douleur postopératoire transitoire dans un sous-groupe de chevaux subissant une laparoscopie est indéterminée. Cette étude avait pour objectif d'évaluer si le pneumopéritoine résiduel est associé à de la douleur postopératoire chez les juments subissant une ovariectomie. Trente-huit juments subissant une ovariectomie laparascopique debout de routine ont été réparties au hasard dans deux groupes. À l'achèvement de la laparoscopie, soit la cavité abdominale a été activement désoufflée ou les canules ont été ouvertes pour obtenir une pression ambiante avant la fermeture de l'incision. Des évaluations ont été réalisées pendant 18 heures après la chirurgie en utilisant une échelle de douleur viscérale/somatique validée pour les chevaux. Dans l'ensemble, la douleur a été minimale dans les deux groupes (note médiane de 2/39) après la chirurgie. La désoufflement actif du pneumopéritoine à l'achèvement de la laparoscopie s'approchait de l'importance statistique (valeur de P = 0,07) en réduisant la douleur à 12 heures après la laparoscopie. Cependant, les effets du désoufflement actif n'étaient pas significatifs pendant la période de surveillance 18 heures après la chirurgie. La décision de désouffler activement à l'achèvement de la laparoscopie est toujours sujette à la préférence du chirurgien.

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Introduction

Standing laparoscopic ovariectomy is performed in mares for behavior modification, removal of ovarian tumors, preparation of "jump" mares for breeding operations, prevention of pregnancy, and for elimination of estrus-related abdominal discomfort (1–4). Laparoscopic ovariectomy has several advantages compared with other surgical approaches and includes superior visual observation, tension-free hemostasis, observation of the ovarian pedicle for hemorrhage during the procedure, reduced surgical morbidity due to the minimally invasive nature of the procedure, and shortened hospitalization (3–8). Additionally, when laparoscopy is performed in a standing sedated mare, the complications associated with general anesthesia are eliminated (9).

Peritoneal insufflation during laparoscopic surgery improves visual observation and creates working space (10). Carbon

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dioxide is commonly used for insufflation because it can be used safely with electrosurgery, has a high diffusion coefficient which may reduce the risk of gas embolism, and is inexpensive (11–14). Pneumoperitoneum appears to be well-tolerated in healthy standing horses undergoing laparoscopy with few adverse cardiopulmonary, plasma chemistry variables, and hematology effects (15). However, it has been reported that horses can display signs of mild discomfort during a laparoscopic procedure at insufflation pressure of 15 mmHg or greater (15,16). Peritoneal insufflation and laparoscopic surgery result in a mild inflammatory response within the peritoneal cavity and a significant acute increase in peritoneal fluid total protein and nucleated cell count (3,15). The cause of the inflammatory response and post-operative discomfort has been suggested to be a result of carbonic acid formation from the carbon dioxide in contact with the peritoneal fluid (13,16).

Potential complications associated with laparoscopic surgery can be grouped as complications related to laparoscopy and those related to the specific procedure being conducted (12). In equine laparoscopic surgery, complications have been well-documented and include intestinal, splenic, or vascular puncture, retroperitoneal insufflation, and inadequate procedural hemostasis (17,18). Septic peritonitis, adhesions, ileus, and incisional complications can also occur (3). Subcutaneous emphysema can develop after surgery and is a result of the carbon dioxide escaping from the pneumoperitoneum through the incision and emanating subcutaneously (18). After peritoneal insufflation, the presence of the pneumoperitoneum remains for 24 h (19) or even a few days after surgery in humans (20). When the pneumoperitoneum is actively aspirated at the completion of laparoscopic surgery in humans, there is a decrease in post-operative pain (19). However, there are no reports on active peritoneal desufflation at the completion of equine laparoscopy and its effect on post-operative pain. Allowing carbon dioxide to escape at the completion of laparoscopic surgery has been reported as advantageous in reduction of development of subcutaneous emphysema in the horse (16). It has been suggested that actively desufflating the pneumoperitoneum at the end of the laparoscopic procedure would further reduce the incidence of post-operative subcutaneous emphysema (18). It has been reported that 18% to 20% of horses undergoing standing laparoscopic ovariectomy show signs of abdominal discomfort after surgery (17,21).

The objective of this study was to compare post-operative pain following active desufflation of carbon dioxide or no active desufflation at the completion of laparoscopic ovariectomies in mares.

Materials and methods

Cases and presurgical management

Mares admitted to Colorado State University Veterinary Teaching Hospital for a unilateral or bilateral ovariectomy between March 2015 and September 2016 and hospitalized for at least 18 h after surgery were included in this study. The study was approved by the Colorado State University Institutional Animal Care and Use Committee. Written client consent was obtained before participation in the study and clients received a financial incentive for enrollment in the study. Prior to initiation of the study, an online random list generator was used to create an order of group assignment for each mare to either the active desufflation or non-active desufflation (control) group. The author responsible for post-operative pain score assessment was blinded to the group assignment and the active or non-active desufflation procedure. Pre-operative vital parameters were recorded, and blood was collected for determination of packed cell volume and total protein in all mares. Feed was withheld for 12 h prior to surgery and mares were given access to water until the time of surgery.

Surgical procedure

An IV catheter was placed in the left or right jugular vein and the mare positioned in standing stocks. Presurgical antibiotic (penicillin G potassium; Pfizerpen, Pfizer, New York, New York, USA), 22 000 IU/kg body weight (BW), IV, and a nonsteroidal anti-inflammatory drug (flunixin meglumine; Prevail, VetOne, Boise, Idaho, USA), 1.1 mg/kg BW, IV, were administered approximately 30 min before the start of surgery. Sedation was initially achieved with detomidine hydrochloride (Dormosedan; Zoetis, Kalamazo, Michigan, USA), 0.01 mg/kg BW, IV, and butorphanol tartrate (Torbugesic; Zoetis), 0.01 mg/kg BW, IV. A level plane of sedation was maintained throughout the procedure by a continuous infusion of detomidine hydrochloride (20 mg) and butorphanol tartrate (10 mg) in 1 L 0.9% sodium chloride (Hospira, Lake Forest, Illinois, USA) at a rate adjusted to the desired effect depending on the individual mare's behavior and stimulation at any given time throughout the procedure.

Depending on previous determination (from history and clinical examination of the patient) and whether a unilateral or bilateral ovariectomy was to be performed, either one or both paralumbar fossae were clipped with wide margins, then aseptically prepared, and draped in a routine manner. Mepivacaine hydrochloride (Carbocaine-V; Zoetis), 0.01 to 0.05 mL/kg BW per site was injected subcutaneously and intramuscularly at 3 portal sites in one or both paralumbar fossae for local anesthesia. The middle portal site was at the level of the ventral aspect of the tuber coxae and halfway between the cranial aspect of the tuber coxae and the last rib. The dorsal portal was 4 to 5 cm dorsal and 4 to 5 cm cranial to the middle portal. The ventral portal was 5 to 6 cm ventral to the middle portal. A 1-cm incision was made through skin, subcutaneous tissue, and external abdominal oblique fascia at the middle portal site in the left paralumbar fossa (or right paralumbar fossa in the case of a right-sided unilateral ovariectomy). A laparoscopic cannula and blunt obturator (10 mm diameter, 20 cm long; Surgical Direct, DeLand, Florida, USA) were inserted into the abdomen at an angle perpendicular to the skin. A 30° forward viewing laparoscope (10 mm diameter, 56 cm long; Karl Storz Veterinary Endoscopy, Goleta, California, USA) was inserted into the cannula, confirming proper location in the peritoneal space. The peritoneal space was insufflated (Stryker 40L High-Performance Insufflator; Kalamazo, Michigan, USA) with carbon dioxide at a rate of 12 L/min and maintained at 10 to 12 mmHg throughout the procedure. Incisions were made in a similar fashion for the

remaining 2 instrument portals at the previously determined sites. Two additional cannulas were inserted into the abdominal cavity under direct visual observation and the laparoscope was transferred into the dorsal cannula for the remainder of the procedure. The left ovary in the case of bilateral ovariectomies or the ipsilateral ovary for unilateral ovariectomies was observed and mepivacaine hydrochloride (0.01 to 0.05 mL/kg BW) was injected into the mesovarium, mesosalpinx, and proper ligament of the ovary using a laparoscopic injection needle (5 mm diameter, 45 cm long, 19 gauge; Surgical Direct). The ovary was observed and grasped with acute claw laparoscopic grasping forceps (10 mm diameter, 45 cm long; Surgical Direct) through the ventral portal. A vessel-sealing device (LigaSure Atlas; Medtronic, Minneapolis, Minnesota, USA) was inserted through the middle portal and the jaws were applied to the cranial aspect of the mesovarium and proper ligament of the ovary. The device was then activated (setting 3 bars or 190 to 275 V) and once the tissue was sealed, it was transected using the integrated blade. This process was repeated across the mesovarium, proper ligament of the ovary, and the mesosalpinx, until the ovary was separated from the ovarian pedicle. For bilateral ovariectomies, once the ovary was transected, it was held by grasping forceps and remained in the left abdomen while a similar approach was carried out to the right ovary by approaching through the right paralumbar fossa. After transection of the ovary, the remaining ovarian pedicle(s) was assessed for hemorrhage and the ovaries were removed from their respective side of the abdomen in the case of unilateral ovariectomies. In cases of bilateral ovariectomy, the right ovary was passed to the left side of the abdomen using the acute claw graspers and sweeping the ovary over the bladder and under the uterus and small colon. The right ovary was then visualized and grasped with acute claw graspers from the left side of the abdomen. The 2 most ventral portal sites on the ipsilateral side for unilateral ovariectomies and the left side in cases of bilateral ovariectomies were connected by incising through the skin, subcutaneous tissue, and external abdominal oblique fascia. The muscle layers and peritoneum were separated bluntly with digital manipulation and the ovaries were then extracted through the incision with traction and manipulation. For the non-active desufflation group the external abdominal oblique muscle of the extended incision was closed with 0 polyglyconate (Maxon; Covidien, Mansfield, Massachusetts, USA) in a simple continuous pattern (10). The skin was sutured with 0 polypropylene (Surgipro; Covidien) with a simple continuous pattern for the extended incision and a cruciate pattern for the remaining portal incisions (10). For mares in the active desufflation group, the cannulas remained in the dorsal portal on each side and all other incisions were closed in the same fashion as described. The insufflation tubing was connected to suction and each side of the abdominal cavity was desufflated separately under laparoscopic visual observation until full peritoneal cavity collapse was achieved and no free gas was observed within the peritoneal cavity. The scope and cannula were then removed and the remaining portal incision(s) was/were routinely closed as described (10). The total surgery time and time of active desufflation, if performed, were recorded for each mare.

Post-operative management

After surgery the mares were monitored hourly while in the hospital. Complete physical examinations including evaluation of the incision sites for subcutaneous emphysema, swelling, heat, discharge, or pain on palpation were conducted every 6 h. Pain was assessed and scored every 6 h for the first 18 h after surgery using the composite pain scale described by Bussières et al (22).

The pain scale is a multifactorial rating scale with 13 assessment categories incorporating behavior, response to observer, and physiologic data. Pain is assessed with the patient in a box stall and each of the 13 categories is scored from 0 to 3 for a total pain scale range between 0 (no pain) and 39 (maximal pain) (22).

Patients received flunixin meglumine (1.1 mg/kg BW, IV) 12 h after surgery and then transitioned to a course of phenylbutazone (VetriBute; VetOne), 2.2 mg/kg BW, PO, q12h for 4 d. No post-operative antibiotics were administered.

Statistical analysis

Before initiation of the study, 30 mares were determined as the minimum number of mares to be included in the study for a power of 0.9. The association between active desufflation at the completion of standing laparoscopic ovariectomy and post-laparoscopic pain was evaluated using the Fisher's exact test separately for each time point (6, 12, and 18 h after surgery). The likelihood of post-laparoscopic pain was evaluated using logistic regression analysis to calculate odds ratios (OR) and 95% confidence limits (CI). Age, weight, and surgical time were evaluated for statistical difference between the 2 groups using a non-parametric Wilcoxon test and data were reported as the medians. A *P*-value of 0.05 was used to determine statistical significance and SAS v9.4 (SAS Institute, Cary, North Carolina, USA) was used for all data analysis.

Results

Cases

Thirty-nine mares were admitted to Colorado State University Teaching Hospital for a unilateral or bilateral ovariectomy between March 2015 and September 2016. One mare was excluded from the study group due to being discharged from the hospital shortly after surgery resulting in the inability for 18 h post-operative monitoring and pain score assessment. The remaining 38 mares fit the inclusion criteria and were included in the study for analysis. Breed was evenly distributed between the groups and was predominantly American Quarter Horse (n = 19), American Paint (n = 5), and Warmblood (n = 3) breeds with the remainder (n = 11) belonging to 8 different breed types. The median (range) in ages of the mares in the active and non-active desufflation groups were 11 y (range: 3 to 17 y) and 10 y (range: 3 to 24 y), respectively. The median (range) weights of the mares were 470 kg (range: 327 to 550 kg) and 490 kg (range: 390 to 650 kg), for the active desufflation and non-active desufflation groups, respectively. The median ages and weights were not significantly different between active desufflation and non-active desufflation groups. In 32/38 (84%) mares, a bilateral ovariectomy was performed and in the remaining 6/38 (16%) mares, a unilateral ovariectomy was performed (3 left-sided and 3 right-sided). Through randomization, 17/38 (45%)

Table 1. Distribution of no pain *versus* pain for the different post-operative time points of all mares.

Time after surgery	No pain score 0/39	Pain Score > 0/39	Odds ratio (95% Confidence interval)	*P*-value
6 h				
Active desufflation	4 (24%)	13 (76%)	1.30 (0.30 to 5.46)	0.73
Non-active desufflation	6 (29%)	15 (71%)		
12 h				
Active desufflation	7 (41%)	10 (59%)	0.24 (0.05 to 1.13)	0.07
Non-active desufflation	3 (14%)	18 (86%)		
18 h				
Active desufflation	3 (18%)	14 (82%)	0.49 (0.07 to 3.34)	0.47
Non-active desufflation	2 (10%)	19 (90%)		

Table 2. Distribution of no pain *versus* pain for bilateral ovariectomies.

Time after surgery	No pain score 0/39	Pain Score > 0/39	Odds ratio (95% Confidence interval)	*P*-value
6 h				
Active desufflation	4 (27%)	11 (73%)	0.85 (0.71 to 4.20)	0.84
Non-active desufflation	4 (24%)	13 (76%)		
12 h				
Active desufflation	7 (47%)	8 (53%)	0.24 (0.05 to 1.22)	0.09
Non-active desufflation	3 (18%)	14 (82%)		
18 h				
Active desufflation	3 (20%)	12 (80%)	0.25 (0.02 to 2.71)	0.25
Non-active desufflation	1 (6%)	16 (94%)		

of the mares were allocated to the active desufflation group and 21/38 (55%) to the non-active desufflation group. Of the unilateral ovariectomies performed, 2/6 (33%) were randomly allocated to the active desufflation group and 4/6 (67%) to the non-active desufflation group. Of the bilateral ovariectomies performed, 15/32 (47%) were randomly allocated to the active desufflation group and 17/32 (53%) to the non-active desufflation group.

Surgical procedure

The median (range) surgical times from first incision to complete closure of all flank incisions for the active desufflation group and non-active desufflation group were 55 min (range: 40 to 75 min) and 55 min (range: 35 to 85 min), respectively. The median (range) time for active desufflation when performed was 95 s (range: 40 to 300 s). In 1 mare (active desufflation group), the right circumflex iliac artery was lacerated while making the portal incisions. The artery was ligated and there were no resulting complications during surgery or during hospitalization.

Post-operative pain assessment

Patient pain was assessed and scored using a composite pain scale described by Bussières et al (22). The post-operative pain scores were mild with a median of 2/39 in each group. The likelihood of pain (score of 1 to 39) compared to no pain (score of 0) was evaluated. There were no statistically significant differences between the active desufflation and non-active desufflation groups at any of the post-operative time points (Table 1).

Unilateral and bilateral ovariectomies were then analyzed separately. The unilateral ovariectomy group (n = 6) was too small for meaningful statistics. The bilateral ovariectomy group (n = 32) showed a pattern similar to the overall study population. When the likelihood of pain (score of 1 to 39) compared to no pain (score of 0) was evaluated, there were no statistically significant differences between the active desufflation and non-active desufflation groups at any of the post-operative time points (Table 2).

Within the first 18 h after surgery, 7/17 (41%) and 11/21 (52%) mares had developed subcutaneous emphysema around the incisions for the active desufflation and non-active desufflation groups, respectively. There was 1 mare in the active desufflation group that experienced moderate discomfort and displayed signs of colic in the immediate post-operative period. The clinical signs resolved with medical therapy alone and the mare had no other complications after surgery. No other post-operative complications were observed within the defined study period of 18 h.

Discussion

Post-laparoscopic pain originates from 3 broad sources: the skin incisions and trocar sites in the abdominal wall, the pneumoperitoneum that is created, and the procedure itself (23,24). The stretching of the peritoneum that is caused by insufflation is thought to be one of the components of post-laparoscopic pain and may be exacerbated through secondary factors, such as peritoneal irritation and visceral hypoxia (23). Peritoneal irritation

Table 3. Pain scale median values and ranges for each post-operative time point.

Time after surgery	Pain score median (/39) All mares (n = 38)	Pain score median (/39) Bilateral ovariectomy (n = 32)	Pain score range (/39)
6 h			
Active desufflation	2	2	0–8
Non-active desufflation	2	2	0–4
12 h			
Active desufflation	2	1	0–5
Non-active desufflation	2	1	0–6
18 h Post-surgery			
Active desufflation	3	2	0–6
Non-active desufflation	2	3	0–6

may develop from carbonic acid formation as a result of the carbon dioxide in contact with the peritoneum which could result in post-operative discomfort (23,25). Visceral hypoxia can result from compression of the intra-abdominal pressure on the abdominal microvasculature (23). In human studies, the pneumoperitoneum can remain for 24 h (19) or even a few days after laparoscopy (20). There is an association between the volume of remaining post-laparoscopic pneumoperitoneum and the level of post-operative pain (26). Techniques previously used in humans to reduce the volume of the pneumoperitoneum include manual decompression of the abdominal wall to expel carbon dioxide through the trocar (25) or active aspiration at the completion of laparoscopic surgery to decrease post-operative pain (19).

More recently, low pressure pneumoperitoneum and intraperitoneal washout with saline at the end of the laparoscopic procedure have shown a reduction in post-operative pain and a superior outcome to low pressure alone in humans (23). In the standing equine patient, a complete saline washout at the end of a laparoscopic procedure could prove challenging to achieve due to the non-dependent nature of a gas and the mid-abdominal portal locations which would presumably result in gas remaining within the abdomen dorsal to the portal locations. If residual pneumoperitoneum is a component of post-laparoscopic pain and methods such as manual compression of the abdominal wall, active aspiration, or a saline washout improve post-operative pain in humans, then one would suspect that laparoscopic-guided active desufflation of the pneumoperitoneum would improve post-laparoscopic pain in horses.

Post-laparoscopic discomfort in horses has been reported (17,27), but is usually transient and resolves within 4 to 6 h after surgery (6,27). In this study the assessment of mare comfort was carried out over the first 18 h after surgery using a 39-point pain scale (22). The pain scale has been further validated for somatic and visceral pain in horses in a study by van Loon et al (28). Analysis of pain scores obtained in this subset of mares did not reveal a significant difference in post-operative pain between horses undergoing active abdominal desufflation or not. Throughout this study post-laparoscopic pain in mares was mild (Table 3). Due to the mild level of post-laparoscopic pain observed, the probability of pain (score of 1 to 39) compared to no pain (score of 0) was modeled (Table 1). The likelihood of pain did not reveal a statistically significant difference between the active desufflation and non-active desufflation groups at any of the post-operative time points. However, for the 12-hour post-operative time point, pain scores approached statistical significance (P = 0.07) with lower pain scores for the active desufflation compared to the non-active desufflated mares.

Other factors that may have contributed to post-operative pain that were not evaluated in this study include level and duration of anesthetic desensitization of the ovarian pedicle, length of the body wall incision created to extract the ovaries, and level of manipulation of the incision required to obtain successful ovarian extraction. Other limitations of this study include that multiple surgeons with different levels of laparoscopic experience were involved in the ovariectomies. As well, potentially a different pain scale assessment, such as a visual analogue scale, may have given different results. Although a power calculation was performed before the initiation of the study, the post-laparoscopic pain scores of the 38 mares were lower than anticipated and as a result a larger study population may have been necessary to show a significant difference between the 2 groups.

Lower pain in the active desufflated mares approached statistical significance, however, since a statistically significant difference was not achieved in this study, the cause of discomfort post-standing laparoscopic ovariectomy in a subset of mares still remains a question to be further investigated.

Subcutaneous emphysema surrounding the incision was observed after surgery and although fewer mares had emphysema (7/17; 41%) in the active desufflation group compared with the non-active desufflation group (11/21; 52%) there was no significant difference between groups. Pain was observed during peri-incisional palpation and was incorporated into the pain assessments. It is worth noting that in most cases in which pain was detected on palpation, it was located near the unilateral elongated incision used for ovary extraction. There was also 1 mare in the active desufflation group that experienced moderate discomfort and displayed signs of colic in the immediate post-operative period. The clinical signs resolved with medical therapy alone and the mare had no other complications post-surgery.

Results from this study demonstrate that pain assessed by a validated pain scoring system was very mild after laparoscopic ovariectomy. Active carbon dioxide desufflation of the pneumoperitoneum at the completion of standing laparoscopic ovariectomies in this group of mares approached statistical significance

(P = 0.07) towards decreasing post-laparoscopic pain at 12 h after laparoscopy. However, there were no statistically significant differences between the active desufflation and non-active desufflation groups at any post-operative time point. The lack of difference between the 2 groups could have resulted from the difficulty in detecting pain or from absence of moderate to severe pain observed in these patients. It is also possible that residual pneumoperitoneum is not a cause for post-operative pain in the horse as it is in humans. Therefore, the decision to actively desufflate or not at the end of laparoscopic surgery in horses to improve comfort should continue to be based on surgeon preference and experience.

Acknowledgment

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Sexual and Aggressive Behaviour of Adult Male Marmosets (*Callithrix jacchus*) Castrated Neonatally, Prepubertally, or in Adulthood

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DIXSON, A. F. *Sexual and aggressive behaviour of adult male marmosets (Callithrix jacchus) castrated neonatally, prepubertally, or in adulthood.* PHYSIOL BEHAV **54**(2) 301–307, 1993.—During early postnatal development, males of some primate species exhibit higher levels of plasma testosterone than females. The possible significance of this for behavioural development was examined in male marmosets castrated neonatally (NC), prepubertally (PC), or in adulthood (AC) and reared in their natal groups. Age-matched intact (AI) adult males served as controls. All males were removed from their natal groups as adults for pair testing with unfamiliar conspecific males and females. NC males did not mount, but were frequently aggressive during tests with females. NC males also elicited aggression from females but rarely from intact males. PC and AC males attempted copulation during the majority of tests with females; less aggression occurred but more so in tests with PC males than between females and AC males. Aggression between PC or AC males and intact males was much more frequent than when NC males met intacts in paired encounters. Results show that secretion of testicular hormones during infancy in male marmosets has important effects upon the development of sexual and aggressive behaviour. Castration in infancy has measurably different effects upon behavioural development than castration performed later in life (prepubertally or in adulthood).

Primates Behavioural development Testicular hormones Sex differences Marmoset

PRIMATES belonging to the South American family Callitrichidae (Marmosets and tamarins) are unusual among monkeys in that they normally produce twins. The twins are dizygotic and, during the presomite stages of foetal development, placental vascular anastomoses develop resulting in a shared blood supply throughout gestation. Haemopoietic chimaerism occurs in some species (4) but, despite this, there is no masculinization (freemartinism) of female foetuses which develop next a male cotwin (29). The role played by prenatal androgen secretion in masculinizing the neural substrates which control sexually dimorphic behavioural patterns in primates has been demonstrated in rhesus monkeys (14,16,22) and man (8,20). However, in both these species, elevated levels of plasma testosterone also occur in males during early postnatal life (13,23), and these may be implicated in behavioural and neuroendocrine development (18,24). Early postnatal increases in circulating testosterone have also been documented for one callitrichicd species, the common marmoset (1,5). Plasma testosterone is elevated in male marmosets until approximately 100 days of age, then declines before rising again at approximately 250 days, with the onset of puberty. Because of the unusual vascular arrangement that links twin foetuses in the Callitrichidae, it has been suggested that some aspects of neural sexual differentiation may be delayed until early postnatal development in these monkeys (1,26). Support for this hypothesis comes from the observation that early postnatal exposure to testosterone masculinizes some aspects of behaviour in female marmosets (1). Further, in a related species, the saddle-back tamarin (*Saguinus fuscicollis*), neonatal castration causes marked deficits in copulatory behaviour that are not reversible in adulthood by treatment with testosterone propionate (11,12). The purpose of the present study is to describe the effects of castration performed during three periods of life (neonatally, prepubertally, and adulthood), upon sexual and aggressive behaviour in male common marmosets. Behaviour of adult males that were castrated in infancy (between days 1–7) is compared to that shown by males castrated prepubertally (between days 140–180) or in adulthood (between 2.5–3.0 years) as well as to age-matched intact adult males, reared under similar conditions and observed during paired encounters with unfamiliar conspecifics.

METHOD

Animals and Husbandry Procedures

Seventy-eight marmosets were studied; eight males castrated neonatally, seven males castrated prepubertally, seven males castrated in adulthood, eight age-matched intact adult males, 24 nonage-matched sexually experienced adult males and 24 sexually experienced ovariectomized adult females. Females were

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each implanted subcutaneously with two silastic capsules containing oestradiol 17β in order to stimulate proceptivity (6). All castrated males had been reared in their natal family groups until 2–3 years of age and then caged singly prior to behavioral testing between 3–4 years of age. The eight age-matched intact males were reared under the same conditions as the castrated males. None of the males in these four experimental groups was allowed physical contact with other marmosets, except for their parents and siblings in the family group. However, visual, olfactory, and auditory communication with other animals in the breeding colony was possible throughout development. Details of the diet have been described previously (25) as have the caging and management procedures (17).

Surgical Procedures

Bilateral castrations were performed on eight infant males (on days 1, 1, 2, 5, 6, 6, and 7 and 7, respectively), seven prepubertal males (aged between 140–180 days), and eight adults (aged 2.5–3.0 years). Operations were performed under aseptic conditions, using ketamine hydrochloride (Vetalar, Parke–Davis, Gwent, UK) and Saffan (Glaxovet, Middlesex, UK) for anaesthesia. A small incision was made in the scrotum or in the inguinal region (the testes are inguinal in position in younger animals), the spermatic cord was ligated using chromic catgut, and the testes removed. Incision sites were dusted with powdered antibiotic and closed with chromic catgut. Animals were returned to their family groups on the day of the operation and kept under observation during the weeks which followed. Postoperative recovery was uneventful and healing of the incision sites occurred rapidly in all cases.

Behaviour Tests

Each male in the four experimental groups (neonatal castrates (NC), prepubertal castrates (PC), adult castrates (AC), and age-matched intact adults (AI) was observed twice weekly during 10-min encounter tests with unfamiliar conspecifics of the same or opposite sex. Experimental males were tested under four conditions:

1. Resident in their home cages: female introduced.
2. Resident in home cage: male introduced.
3. Removed from home cage and introduced as an intruder into a female's cage.
4. Removed from home cage and introduced as intruder into a male's cage.

Each of the NC, PC, AC, and AI males was pair tested twice under each of the above conditions (1–4) and never met the same animal more than once. The experimental males were maintained in a separate room from the unfamiliar males and females used in encounter tests. A Latin square design was used to balance the order of testing conditions across subjects.

The following behavioural patterns were recorded during encounter tests: noncontact aggression (slit-eyed staring, arching and arch-ruffle displays, lunges, chases, and vocal threats), contact aggression (hitting, grabbing, or biting), submissive behaviour (squealing vocalizations, facial grimaces, and fleeing from an approaching conspecific), and sexual behaviour (proceptive tongue flicks, stares, and immobile displays, attempted mounts, mounts, penile erection, pelvic thrusting, ejaculation, refusal or initiation of male's mount attempts, or termination of mounts). Descriptions and definitions of behavioural patterns and testing procedures have been reported previously (6,17,28).

Tests were divided into 40 × 15-s intervals, and frequencies of noncontact aggression, contact aggression, and submissive behaviour were scored in terms of the number of intervals in which the various patterns occurred (Hansen frequencies) (3). The latency to first occurrence of biting attack was timed, and tests were terminated (promptly) if attacks persisted and injurious aggression seemed likely. Aggression was rapid in onset and severe in some tests (e.g., between NC or PC males and female partners) and it was not possible to record each individual agonistic display accurately. In many cases, frequencies of agonistic behaviour were, therefore, calculated as the percentage of 15-s intervals completed, in which the patterns had occurred. Aggressive dominance was scored if one partner consistently outranked the other in agonistic encounters. The various sexual patterns were recorded whenever they occurred and data are expressed as frequencies per test for these measurements.

Statistics

Statistical analyses were performed using overall mean values for each of the behavioural patterns, calculated for each animal Friedman analysis of variance was used to test for significant variation in behaviour between the four experimental groups (NC, PC, AC, and AI male groups). Paired comparisons between behaviour shown by NC males and each of the other three experimental groups were then made using the Mann–Whitney U-test (two tailed) (27).

RESULTS

Sexual Behaviour With Female Partners

Data on copulatory behaviour for the four experimental male groups are shown in Fig. 1. Neonatally castrated (NC) males did not attempt to mount females during pair tests. Prepubertally castrated (PC) males, by contrast, mounted females, displayed penile erections, and made pelvic thrusting movements. None of the PC males achieved intromission. Adult castrates (AC) mounted females and achieved intromission during some tests. However, intromission frequencies were lower in AC males (mean ± SEM, 0.61 ± 0.17 per test) than in tests involving intact adult (AI) males (1.12 ± 0.18; $U = 12$, $N_1 = 7$, $N_2 = 8$, $p < 0.05$). Only one AC male achieved ejaculation, whereas all AI males ejaculated (0.84 ± 0.11 per test; $U = 0.5$, $N_1 = 7$, $N_2 = 8$, $p < 0.001$). Both PC and AC males sustained penile erections and made pelvic thrusting movements. Failure to intromit was not, therefore, due simply to erectile problems, although in the case of PC males, the penis was markedly smaller during erection than in intact male subjects (Fig. 2).

Frequencies of precopulatory tongue-flicking behaviour (an invitational display used by both sexes in the marmoset) are shown in Fig. 3. Males in the PC, AC, and AI experimental groups all tongue flicked at females more frequently than did NC males. Females tongue flicked more frequently at intact male partners (mean ± SEM, 26.16 ± 8.02 per test) than at AC males (6.18 ± 2.79; $U = 14$, $N_1 = 8$, $N_2 = 7$, $p = 0.06$) or PC males (9.03 ± 3.42; $U = 15$, $N_1 = 8$, $N_2 = 7$, $p = 0.076$). Females rarely directed proceptive tongue flicks at NC males, however, the frequencies (1.22 ± 0.52 per test) being significantly less than for the other groups (Fig. 3).

Also shown in Fig. 3 are the percentages of males mount attempts or mounts that females refused or terminated. This provides a measurement of sexual receptivity in the female. Because NC males never attempted to mount females, it was not possible to calculate refusal or termination rats. Females were maximally receptive with intact adult males, refusing or terminating only 6.62 ± 4.72% of mounts. Adult castrates were refused or terminated more frequently (13.6 ± 6.10%) but the difference between the two groups of males is not statistically significant. The major effect upon female receptivity concerned

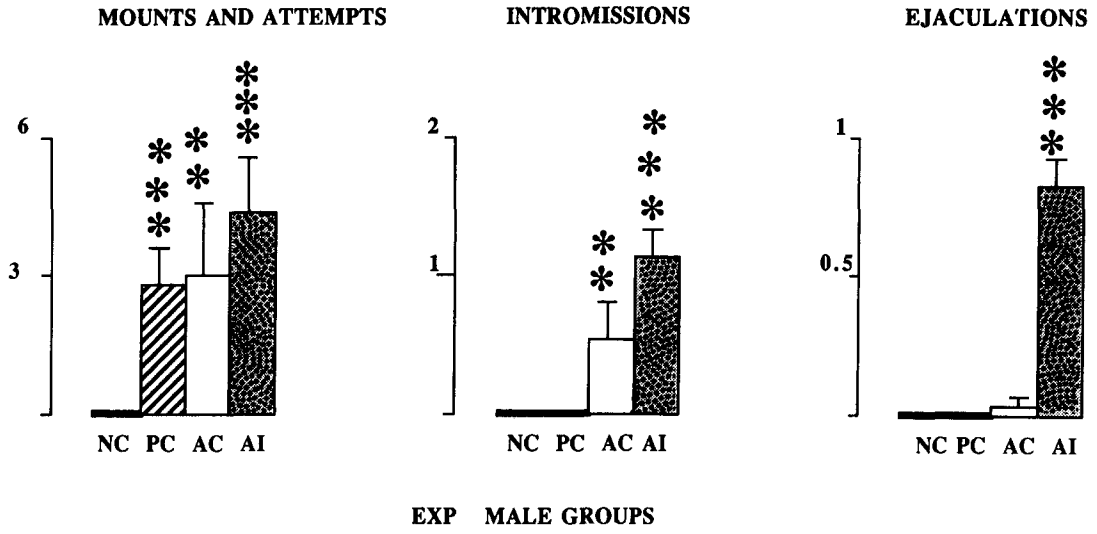

FIG. 1. Sexual behaviour by male marmosets in the four experimental groups during paired encounters with females. Data are individual means (+SEM) per test. Black bars = neonatally castrated males (NC); hatched bars = prepubertally castrated males (PC); open bars = adult castrates (Ac) and stippled bars = age-matched, intact, adult males (AI). Statistical comparisons are between NC males and those in each of the remaining groups. $^{**}p \leq 0.01$; $^{***}p \leq 0.001$ (Mann–Whitney *U*-test: two tailed).

tests with PC males. Mounts by PC male were refused or terminated, on average 60% of the time, significantly more so than those of adult castrates ($U = 0$, $N_1 = 7$ $N_2 = 7$, $p < 0.001$) or adult intact males ($U = 0.5$, $N_1 = 7$ $N_2 = 8$, $p < 0.001$). Many of the refusals or terminations by females occurred in response to repeated mounts and failures to intromit by their PC partners.

Sexual Behaviour With Male Partners

When males of the four experimental groups were pair tested with sexually experienced adult males, it was notable that the latter frequently attempted to mount males belonging to the three castrate groups (NC, PC, and AC groups) but only rarely behaved in this way towards AI partners (Fig. 4). Mount frequencies were highest with NC males, but did not differ statistically from those measured with either PC or AC males. Thus sexually experienced intact males attempted to mount, or mounted, NC males an average of 2.75 times per test; 88% of mounts were accompanied by penile erection and 65% by pelvic thrusting movements. Castrated males, by contrast, never attempted to mount sexually experienced intact males. Nor did the castrates behave in a proceptive or receptive manner during these tests. Thus, NC males rarely tongue flicked at intact males (mean ± SEM, 1.3 ± 0.7 per test), whereas intact males frequently directed this precopulatory display towards NC partners (13.7 ± 3.2 per test; $U = 7.5$ $N_1 = 8$, $N_2 = 8$, $p = 0.005$). The sexually experienced intact males initiated 96% of their mounts during encounters with NC males. The NC males were not proceptive by comparison with the other experimental groups and, because they refused or terminated 67 ± 16% of mounts by intact partners, there was little evidence of sexual receptivity (Fig. 4). Prepubertally castrated males refused or terminated 94% of mounting activity; AC males 93% and AI males 100%. Three of the NC males behaved passively and tolerated mounting by at least one of the four intact male partners. One NC male exhibited tongue flicking whilst mounted by an intact male. The behaviour observed appeared submissive rather than sexual and was in keeping with the virtual absence of aggression between NC and intact male partners (see the following paragraphs).

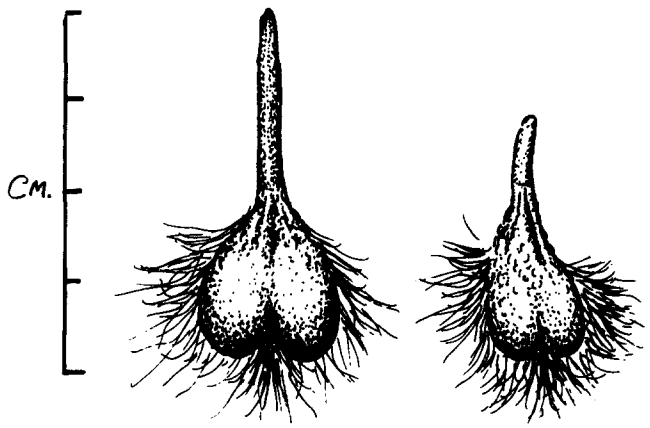

FIG. 2. External genitalia of adult male marmosets. Left: an intact male. Right: a male which was castrated prepubertally. Both males exhibit full penile erection. Fat deposits in the scrotum of the prepubertally castrated male give a false impression that small testes might be present.

Agonistic Behaviour With Female Partners

Encounters between NC males and females were characterized by much higher frequencies of mutual aggression than in tests with the other three male groups. It was necessary to terminate 66% of encounters between NC males and females in order to avoid possible injuries (Fig. 5A) due to frequent contact aggression by both sexes (Table 1). Each test was divided into 40 × 15-s time intervals; the average duration of tests between NC males and females was 19 ± 3 time intervals. Both sexes initiated aggression and fought vigorously, so that no clear-cut dominance–subordinance relationships were established for 75% of encounters (NC males were dominant in 6% and females in 19% of the tests). By contrast, no tests between females and AC or AI male partners were terminated; females never attacked males belonging to these experimental groups, and aggression

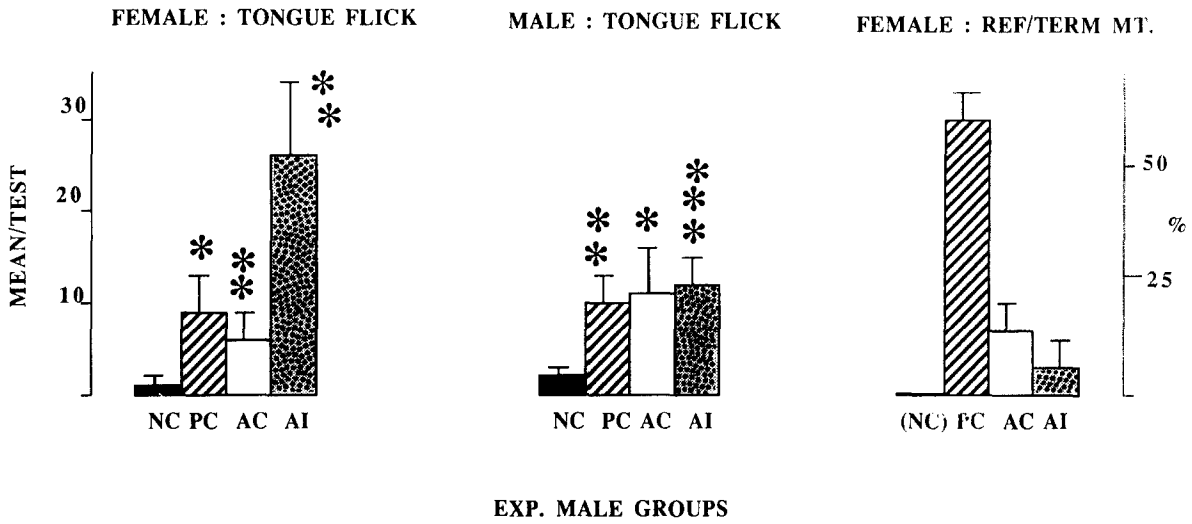

FIG. 3. Precopulatory tongue flicking displays by male and female marmosets and female's refusals or terminations of male's mounts during paired encounters. Data are individual means per test (+SEM) for precopulatory tongue flicking and mean percentages for female's refusals or terminations of male partner's mount attempts or mounts. Black bars = neonatally castrated males (NC); hatched bars = prepubertally castrated males (PC); open bars = adult castrates (AC) and stippled bars = age-matched, intact, adult males (AI). Statistical comparisons are between NC males and those in each of the remaining groups. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$ (Mann–Whitney *U*-test: two tailed).

by the males occurred at very low frequencies (Fig. 5A; Table 1). Encounters between females and PC males did involve high frequencies of mutual aggression, although significantly less so than during tests between females and NC partners. Thirty-two percent of tests with PC males were terminated to avoid potential injuries to the animals. Examination of Table 1 indicates that females were more aggressive than PC males during these tests. Aggression often occurred in association with the females' refusals and terminations of males mounts and repeated failures to attain intromission. The PC males were very persistent in their attempts at copulation, however, and in some cases this led to attacks by females.

Agonistic Behaviour With Male Partners

Data on aggressive interactions between males in the four experimental groups and intact adult males during paired encounters are shown in Fig. 5B and Table 1. As expected, encounters between intact adult males resulted in frequent aggression and 58% of tests were terminated to avoid possible injuries. Tests between intact males and castrates (NC, PC, and AC males) resulted in less aggression, and this effect was most pronounced in the case of intact vs. NC male encounters. The NC males rarely behaved aggressively towards intact males, no tests were terminated, and attacks (by either male) occurred only during 9% of encounters. Encounters between intact males and PC males resulted in more frequent aggression, but in only one case was this statistically significant by comparison with data from NC—intact male tests (intact males contact aggression vs. PC males; $p = 0.05$, Table 1). Encounters between intact and AC males produced consistently higher frequencies of contact aggression than occurred during tests with NC males. Intact males also

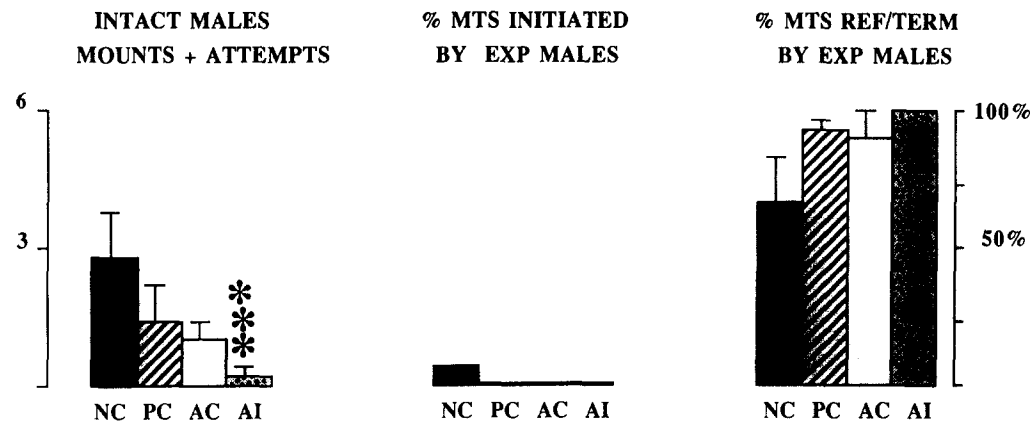

FIG. 4. Sexual behaviour between males in the four experimental groups and intact males during paired encounters. Data are individual means per test (+SEM) for mounting behaviour and mean percentages for mounts initiated or refused/terminated. Black bars = neonatally castrated males (NC); hatched bars = prepubertally castrated males (PC); open bars = adult castrates (AC) and stippled bars = age-matched, intact, adult males (AI). Statistical comparisons are between NC males and those in each of the remaining groups. $***p \leq 0.001$ (Mann–Whitney *U*-test: two tailed).

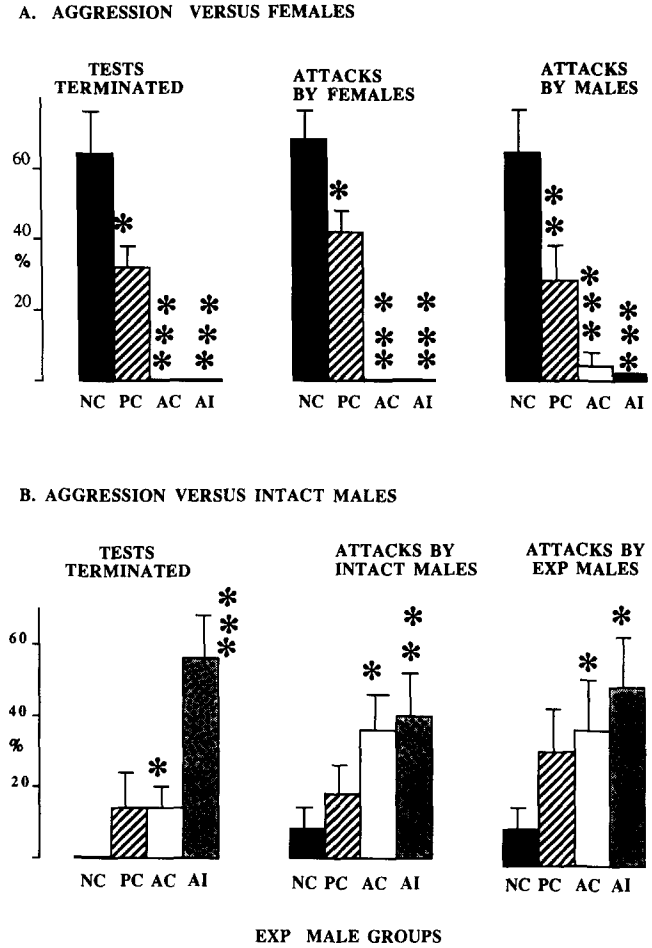

FIG. 5. Aggressive interactions between males in the four experimental groups A with female partners and B with intact male partners. Data are mean (+SEM) percentages per test. Black bars = neonatally castrated males (NC); hatched bars = prepubertally castrated males (PC); open bars = adult castrates (AC) and stippled bars = age-matched, intact, adult males (AI). Statistical comparisons are between NC males and those in each of the remaining groups. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$ (Mann–Whitney *U*-test: two tailed).

directed more noncontact aggression towards AC partners than towards NC males ($p = 0.01$; Table 1).

DISCUSSION

The effects of castration during neonatal life in male marmosets differed from those produced by castrating males prepubertally or in adulthood. As adults, males that had been neonatally castrated (NC males) did not attempt to mount females during paired encounters. Prepubertally castrated (PC) males, by contrast, are sexually very active in adulthood, frequently mounting females and making pelvic thrusting movements with the penis erect. However, PC males lacked full phallic development, which probably explains the absence of intromissions during their mounts. Male marmosets that were castrated in adulthood, between 2-1/2–3 years of age, and tested with females 6–14 months after the operation were also very active in mounting their partners. Mount frequencies did not differ from those exhibited by PC males. However, the adult castrates succeeded in attaining intromission, although intromission frequencies were significantly less than those displayed by age-matched intact adult males. Only one adult castrate ejaculated during tests with female partners. It appears that adult male marmosets retain a high degree of sexual activity after castration even when they are relatively inexperienced at copulation. In their studies of castrated male rhesus monkeys, Michael and Wilson (19) observed film records of mating tests which "showed the penis bending on contact with the female's perineum." The AC marmosets observed in the current study maintained full erections, however, and made well-oriented mounts with vigorous pelvic thrusting movements. It seems likely that deficits in sensory feedback from the penis or in the central processing and responsiveness to such stimuli influenced intromission and ejaculation in these males (7).

The effects of neonatal castration upon copulatory behaviour in male marmosets parallel those reported by Epple et al. (11) in experiments on a related callitrichid species, the saddle-back tamarin (*Saguinus fuscicollis*). In that study, four males were castrated on day 2 of postnatal life. As adults, these NC male tamarins failed to mount females, whereas prepubertally castrated males did exhibit mounting behaviour.

As adults, NC male marmosets did not behave in a proceptive or receptive manner when paired with intact males. Intact males frequently directed precopulatory tongue-flicking displays at NC males and initiated mounts. The NC males rarely tongue flicked at intact partners, they initiated less than 4% of mount attempts made by the latter and refused or terminated 67% of their mounts. Although NC males tended to behave submissively towards intact males, they were not more receptive sexually than PC or AC males, tested under the same conditions. These results do not support the view that early postnatal secretion of testicular androgens is required for behavioural defeminization in male marmosets. However, no experiments comparing effects of androgen and oestrogen treatments to NC, PC, and AC marmosets have been reported. It remains unknown, for instance, whether oestrogen treatment of NC males might stimulate proceptive or receptive behaviour.

Effects of neonatal castration upon the development of aggressive behaviour were more pronounced than those which occurred in the PC or AC male groups. Encounters between NC males and females resulted in intense mutual aggression; 66% of these tests were terminated to avoid possible injuries to the animals. Aggression also occurred, although less frequently, during tests between females and PC male partners, but rarely during their encounters with AC or AI males. To this extent, the behaviour of NC males in particular was socially feminine, because pair tests between adult female marmosets also involve frequent mutual aggression (21). However, it is possible that nonbehavioural cues from NC males, and to a lesser extent PC males, might have affected the reactions shown by female conspecifics. Thus, early castration may have affected the development of cutaneous scent marking glands or urinary cues; both of which are of proven importance for social communication in Callitrichid primates (9,10). Likewise the infantile appearance of the external genitalia of NC and PC male marmosets might have influenced the behaviour of conspecifics. It is unknown whether visual cues play any role in sexual recognition in these monkeys. The remarkable lack of aggression shown by intact adult male marmosets towards NC males and their frequent attempts to copulate might also have resulted from the absence of those chemical and morphological changes which are stimulated by androgens during puberty. Further experiments are required to examine these questions.

The effects of neonatal castration in male marmosets upon aggressive behaviour differ from those reported by Epple et al. (11) for saddle-back tamarins. Neonatally castrated saddle-back tamarins rarely behaved aggressively when pair tested, in

TABLE 1

FREQUENCIES OF AGGRESSION BETWEEN MALES IN THE FOUR EXPERIMENTAL GROUPS AND PARTNERS DURING ENCOUNTER TESTS

Behavioural Measurement	Experimental Male Groups			
	NC	PC	AC	AI
A. Aggression Between Experimental Males and Females				
Non-contact aggression				
Experimental males' aggression towards females	48.1 ± 8.4	11.5 ± 3.3*	1.8 ± 0.8*	0 ± 0*
Females' aggression towards experimental males	49.2 ± 5.8	17.2 ± 3.7*	4.3 ± 2.5*	0.6 ± 0.3*
Contact aggression				
Experimental males' aggression towards females	23.9 ± 3.2	4.7 ± 1.2*	0.07 ± 0.07*	0 ± 0*
Females' aggression towards experimental males	24.6 ± 3.0	9.0 ± 3.3*	0 ± 0*	0 ± 0*
B. Aggression Between Experimental Males and Intact Males				
Non-contact aggression				
Experimental males' aggression towards intact males	6.9 ± 2.0	11.9 ± 4.2	11.7 ± 4.2	22.2 ± 6.2†
Intact males' aggression towards experimental males	5.0 ± 2.5	13.1 ± 4.4‡	22.2 ± 3.2§	20.4 ± 4.8§
Contact aggression				
Experimental males' aggression towards intact males	1.2 ± 0.8	3.2 ± 1.0	3.75 ± 1.0‡	23.3 ± 10.9‡
Intact males' aggression towards experimental males	0.5 ± 0.3	0.71 ± 0.3	2.9 ± 1.0	18.0 ± 6.9*

Data are mean ± SEM percentages of 15-s intervals per test in which aggression occurred.
NC = neonatally castrated males; PC = prepubertally castrated males; AC males castrated in adulthood; AI = age-matched intact adult males.
* $p \leq 0.001$.
† $p = 0.08$.
‡ $p \leq 0.05$.
§ $p \leq 0.01$ (Mann–Whitney *U*-test; two-tailed).

adulthood, with females. However, pair tests involved familiar partners, rather than strange conspecifics as in the present experiment. Procedural differences, as well as interspecific variability may explain some of the differences in aggression observed in the two studies. An important point relates to the possible masking effects of aggression between NC male marmosets and females upon the expression of sexual behaviour during paired encounters. Females were pretreated with oestradiol 17β to maximise their proceptivity (6) yet they rarely tongue flicked at NC males. Females directed their proceptive tongue flicks more frequently towards PC or AC male partners and were most proceptive towards intact males. The relative absence of sexual initiating behaviour by females when tested with NC males, coupled with the intense aggression shown by both partners during the encounters, made it unlikely that males would display mounting behaviour. The possibility exists that the neural substrates that control mounting behaviour are organized by androgen primarily during foetal development in marmosets, as is the case in rhesus monkeys (15), but NC males fail to express the behaviour due to the negative effects of aggression. However, PC male marmosets also interacted aggressively with females, and yet they made persistent mounting attempts. Further, NC male tamarins failed to exhibit mounting behaviour in adulthood, even after treatment with testosterone propionate (11,12). Female common marmosets treated with testosterone during the first 50 days of postnatal life exhibited masculinization of behaviour (e.g., pursuit courtship and mounting) as adults (1). Taken together with the results of the present experiment on behavioural effects of castration neonatally, prepubertally, and in adulthood in the marmoset, these various studies indicate that testicular hormones secreted during early postnatal life play an important role in the development of sexual behaviour and aggression in callitrichid primates.

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Viewpoint

Behaviour of castrated animals

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Vet. Rec. (1975). **96.** *92-93*

THE main reason for castrating cats and dogs is to render them infertile. However, in clinical practice they are often castrated at the request of the owner, to hopefully reduce or abolish so-called "objectionable behaviour" such as mounting and onanism, urine scent marking, fighting and roaming. Often the veterinary surgeon is unsure of the outcome of the operation and the owner is disappointed when castration does not produce the required effect. In connection with a letter by J. Wharrier in your issue of July 27, 1974, concerning the "abnormal" mounting behaviour of castrated male dogs and the spraying behaviour of castrated tom cats, this note is an attempt to elucidate the behavioural sequelae of castration of dogs and cats, so that practising veterinary surgeons may be able to offer a fairly reliable prognosis regarding the effects of the operation.

For a long time it has been evident that testicular hormones contribute in some manner towards the male capacity for sexual arousal and performance. However, the relationships between androgens and mating behaviour are extremely complex and there appear to be many individual and species differences. In particular it has been shown that following the castration of some species of male mammals, various components of the copulatory pattern may persist for considerable lengths of time.

It has been proposed that with phylogenetic progression, the decline in mating behaviour following the castration of male mammals becomes more gradual and variable. This generalisation is reflected by a progressive emancipation of the males' sexual behaviour from dependence on testicular androgen. Furthermore, the extent to which male mating activity is dependent on testicular androgens is inversely proportional to the degree that the cerebral cortex is involved in the mediation of mating behaviour (Beach 1947, 1958). Accordingly, in rodents, the components of male mating behaviour begin to decline very soon after castration (Block and Davidson 1968), whereas male carnivores may retain the ability to copulate for a longer period. With monkeys, apes and man there is a very gradual decline in sexual behaviour and components of the copulatory pattern sometimes persist for a very long period following castration. Also, to some extent, this notion of 'phylogenetic progression' may be extended to scent marking and aggressive behaviour (Hart 1974b).

In dogs all elements of the mating pattern may survive for several years following castration (Hart 1968, Beach 1970). The main effects of castration after a period of six months are a slight decrease in the frequency of intromissions and a marked decrease in the duration of the copulatory lock or tie, presumably due to the failure to maintain full erection. There is, however, little effect on the mounting behaviour. If anything, immediately following castration there appears to be an initial increase in the incidence of mounting behaviour. Perhaps this is augmented by the decreased ability to achieve intromission yet the persistence on the part of the male to continually attempt to do so. There is usually no further decline in the mating ability for up to three to five years following the operation.

Adrenalectomised castrated dogs similarly continue to display the complete copulatory pattern, indicating that the retention of mating activity is not due to a compensatory increase in adrenal androgen (Beach 1970). Nevertheless, Beach has repeatedly demonstrated that the effects of castration are due to an androgen deficiency, since with replacement androgen therapy the mating activity is restored to preoperative levels.

In a study investigating the mating behaviour following the castration of sexually-experienced toms, there also appeared to be gross individual differences. For some toms the ability to achieve intromission persisted for several years and mounting behaviour continued indefinitely. In others intromission disappeared after only a few months, although mounting was still apparent many months thereafter. With some toms, however, there was a cessation of all mating activity shortly after castration (Rosenblatt and Aronson 1958a). The changes in mating behaviour were first evidenced by a decrease in the frequency of intromission and a prolongation of the mount and thrust response. The mounts became more prolonged and were often unsuccessful in achieving intromission, presumably since the ability to maintain a full erection was weakened. Eventually, only short mounts were apparent and these occured without the characteristic pelvic thrusting and treading responses. Gradually the mounting behaviour became infrequent and in some cases disappeared altogether. Overall, the toms retained the ability to achieve intromission for about three months, whereas mounting behaviour survived much longer and in some cases indefinitely.

The considerable individual variation in the effects of castration upon mating behaviour may be attributed to several factors: species differences, the degree of sexual experience prior to castration and the age at which the individual is castrated.

Dogs and cats seem to differ in the effects of sexual experience prior to castration. Compared with sexually-experienced toms, sexually inexperienced animals exhibit a lower frequency of mounting behaviour, and the ability to achieve intromission is considerably reduced and only

persists for a relatively short period following castratin (Rosenblatt and Aronson 1958a). However, with dgs, there is no evidence to suggest that sexually inexperiened castrates are in any way inferior to experienced castrates with regard to the many various components of appetitve and consumatory mating behaviour (Hart 1968). These data may reflect apparent species differences between cats and dogs or they may be the result of procedural differences between the separate experiments.

Compared with dogs castrated in adulthood, prepubrally castrated dogs show a much lower frequency of intmission, and the ejaculatory reflex and the ability o achieve a copulatory lock are markedly impaired. This may be related to insufficient development of the genial organs, in particular the bulbus glandis. The penes of prepuberally castrated animals are much smaller than those of dogs that have been castrated in adulthood. Nevertheles, prepuberally castrated dogs will mount an oestrous bith frequently and vigorously (LeBoeuf 1970). This is in contrast to prepuberally castrated male cats, which seldm exhibit mounting behaviour as adults (Rosenblatt nd Aronson 1958).

The castration of male dogs seems to have very lile effect on urinary scent marking behaviour apart from a generalised decrease in the frequency of urination (Hrt 1974a). Similarly, it has been reported that aggressie behaviours in male dogs are not readily reduced by castrtion (LeBouef 1970). These data, however, are in diret contrast to the effects of castration of adult toms. In a study to evaluate change in objectionable behaviours f male cats following castration in adulthood, it was estalished that there was a postoperative decline in fightig, roaming and urine spraying in 88 per cent, 94 per cent ad 88 per cent respectively. Moreover the greater proporton of these cases showed a rapid and prolonged decline within approximately two weeks following castration. This vas particularly so with the cases involving urine sprayng (Hart and Barret 1973). Often these behaviours declied at different rates in the same individual cat and furthrmore there was no apparent correlation between the ge of the cat at the time of castration and the subsequnt rate of decline of any behavioural pattern. Also it should be mentioned that a postcastrational decline in fightng and spraying in tom cats does not preclude the occasional display of these behaviours, perhaps in some cases precipitated by an environmental change.

Castrated animals behave very differently under est conditions than when unconfined, and one must be wry of direct extrapolation of experiment results to the domestic situation. For example, when a castrated dog is placed in a small pen in intentional proximity to an oestrous bitch, there is obviously a much greater likelihood that he will mate than casual observation of the free-ranging domestic situation might suggest. In summary, however, although there appear to be significant species differences between cats and dogs it would be fair to conclude that the major effect of castration of either species is reflected by an overall reduction in the frequency of intromissions sometimes followed by a decrease in mounting behaviour. Nevertheless, some individuals retain the ability to copulate for a substantial period of time. Castration is likely to have a more pronounced effect on the mating behaviour of male cats than on male dogs. Castration seems to have no reliable effect on either aggressive or urinary scent marking behaviour of dogs, whereas in male cats, castration usually proves to be quite effective in reducing urine spraying, fighting and roaming within a relatively short period.

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Conferences, lectures, meetings

FEBRUARY

Radiological safety Introductory course for supporting staff not possessing professional qualifications, February 5 and 6. Details from Mr C. Lewis, Medway and Maidstone College of Technology, Horsted, Maidstone Road, Chatham, Kent ME5 9UQ.
Current diseases of poultry Course arranged by the Kesteven Agricultural College, Brant Broughton House, Brant Broughton, nr Lincoln, February 17 to 19. Details from Mr J. L. Odell, Kesteven Agricultural College, Caythorpe Court, Grantham, Lincs NG32 3EP.
Pigs: How far integration? Meat and Livestock Commission-*Farmers Weekly* conference, Cambridge, February 17 to 19.
Conference on experimental medicine and surgery in primates Jerusalem, Israel. February 24 to 27.
Industrial radiological protection Four-day residential course on general radiological protection and the sealed sources regulations, February 24 to 27. Details from Head, School of Science, Ipswich Civic College, Rope Walk, Ipswich.
Man as his own experimental animal Lecture by Professor P. Turner, at The Pharmaceutical Society's House, 17 Bloomsbury Square, London. February 27 at 7 pm.
Some diseases of fish, rabbits and mink One-day lecture course, Department of Clinical Veterinary Medicine, Madingley Road, Cambridge. February 28.

MARCH

Endangered deer British Deer Society symposium, Edward Lewis lecture theatre, Middlesex Hospital Medical School, Cleveland Street, London W1. March 1 at 11.30 am.
The Nutrition Society Details of programme from Dr J. D. Sutton, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT. March 14.
European conference on equine nutrition Cambridge, March 18 to 21. Programme and registration forms from the Conference Secretary, 76 Kingswood Road, Tadworth, Surrey.
Association of Veterinary Anaesthetists of Great Britain and Ireland Spring meeting at University of Liverpool Veterinary Field Station, Leahurst, Neston, Wirral, Cheshire. March 25 and 26.

APRIL

Mastitis control International Dairy Federation Seminar, University of Reading. April 7 to 10.
History of veterinary medicine 12th International symposium, Rapperswil am Zürichsee (Switzerland). April 17 to 19.
Radiological safety Training course organised by the National Radiological Protection Board. Held in the evening, approximate duration two hours. At Bristol Polytechnic, April 10. Details from Mr D. T. Mullarkey, National Radiological Protection Board, Harwell, Didcot, Berks, OX11 0RQ.

MAY

European Society of Veterinary Surgery congress Copenhagen, Denmark. May 8 to 11. Subjects include: Abdominal surgery in cattle, orthopaedics, dental diseases of small animals, experimental surgery. Details from the Secretariat, ESVS, Institut for Kirurgi, Bülowsvej 13, 1870 Copenhagen V, Denmark.
Animal Models of Human Pathology VIth annual meeting of the French Society for Laboratory Animal Science, at the University of Paris-Val de Marne Medical School, 94000, Creteil. Details from Professor J. C. Friedman, Centre d'Etudes Biologiques I.F.M., BP 309, 27005 Evreux, France, May 31.

Efficacy of Dominant Male Sterilization to Reduce Reproduction in Feral Horses
Author(s): Thomas C. Eagle, Cheryl S. Asa, Robert A. Garrott, Edward D. Plotka, Donald B. Siniff and John R. Tester
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EFFICACY OF DOMINANT MALE STERILIZATION TO REDUCE REPRODUCTION IN FERAL HORSES

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Control of feral horse (*Equus caballus*) populations on federal lands in the western United States, since their protection by federal legislation in 1971, has been difficult and controversial. Constraints on management alternatives have been imposed by Congress and public interest groups (Wagner 1983, Boyles 1986). One management technique, advocated by Turner and Kirkpatrick (1986), is temporary sterilization of dominant males to reduce reproductive rates. Horses are polygynous, with dominant males maintaining a harem of females throughout the year (Berger 1986:129), so treating the dominant male might limit reproduction in many females. Kirkpatrick et al. (1982) treated 10 dominant males in Idaho with microencapsulated testosterone propionate. They reported reduced foal production for 1 year in 7 treated bands and suggested use of the technique to manage feral horse populations. But the effectiveness of the technique as a management tool has been questioned. Nelson (1978:64) and the National Research Council (1982) indicated that the technique is dependent on a stable harem structure and assumes that dominant males perform all breeding. Because of these conflicting opinions, the Bureau of Land Management (BLM), the primary agency responsible for managing feral horses, requested additional research on sterilizing dominant males. Our objective was to evaluate the efficacy of sterilizing dominant males to reduce foaling rates of feral horses for ≥ 2 years in Nevada and Oregon.

STUDY AREA AND METHODS

The Flanigan Herd Management Area (HMA) in northwestern Nevada and the Beaty Butte HMA in southeastern Oregon were chosen for study. The Flanigan HMA (66 km^2) primarily is mountainous with maximum relief of approximately 1,000 m. The Beaty Butte HMA (1,769 km^2) mainly consists of broad, flat plateaus with maximum relief of approximately 500 m. Both areas are managed by periodic removals of horses by the BLM. The most recent removals, before the study, occurred in September 1985 and November 1984 in the Flanigan and Beaty Butte areas, respectively. The BLM surveys conducted in the fall of 1985 resulted in counts of 320 horses (postremoval) in the Flanigan area and 167 horses in the Beaty Butte area.

Horses were trapped for the study between 5–10 December 1985 in the Flanigan area and 3–6 February 1986 in the Beaty Butte area. A helicopter was used to locate and herd individual bands into corral traps. Each horse was funneled into a squeeze chute, sex was determined, and age was estimated based on tooth eruption and wear (Am. Assoc. of Equine Practitioners 1981).

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All horses ≥3 years old were fitted with numbered collars. We photographed each collared horse and recorded coat color plus face and leg markings to assist in identification throughout the study.

If >1 male was present in a band, we observed interactions among band members in the corrals until the dominant male could be identified. Dominance was indicated by outcomes of confrontations between adult males. We sterilized the dominant male in each group and fitted it with a numbered collar carrying a radiotransmitter. Vasectomy was chosen for sterilization because its effect was assured for >1 breeding season.

Males were immobilized with etorphine hydrochloride and xylazine hydrochloride using procedures described by Plotka et al. (1987). Following immobilization, the rear legs were secured with a rope, and the scrotal area was washed with Betadine solution. During surgery, diazepam (20–40 mg) was administered intravenously, as required, to reduce muscular rigidity and tremors (Plotka et al. 1987). A small (approx 3 cm) incision was made along the midline of the scrotum and the vas deferens was isolated by blunt dissection. Following ligation of the vas deferens with a 2-0 silk suture at 2 locations approximately 2 cm apart, a 1-cm segment was excised. The procedure then was repeated on the other side through the same incision. The incision was not sutured to allow drainage and minimize swelling. After intramuscular administration of 10.5×10^6 units of benzathine penicillin, immobilization was reversed with diprenorphine and yohimbine as described by Plotka et al. (1987). After all horses from a band had been processed, they were released as a group.

We attempted to locate each vasectomized male by aerial surveys from a helicopter 3–4 times annually between June and August 1986 and April–July 1987 and 1988. However, the April survey in Flanigan was omitted from the analysis because it occurred before the peak of foaling for that year. Baseline data collection by aerial survey began in spring 1986, before treatment could be effective (females were already pregnant for that season at the time of surgery). We searched each study area systematically, recording the number of adults and foals in each band. When collared horses were seen, we flew close enough to record collar numbers of marked animals in each band. By 1987, many collar numbers were unreadable, and vasectomized males sometimes had to be identified by radio frequency. If a vasectomized male was not observed during a survey, telemetry was used to try to locate the horse.

Approach by the observation helicopter frequently caused bands of horses to coalesce or split temporarily. When feasible, data were not recorded until large groups of horses split into smaller groups, presumably individual bands. Sometimes, however, we recorded numbers of adults and foals in the large groups. Thus, our data consisted of numbers of adults and foals in groups containing a male identified as vasectomized (treated groups) or groups without a male identified as vasectomized (untreated groups). Occasionally, foals from bands without treated males were recorded in groups with vasectomized males because intermingling of bands occurred during surveys.

We assumed that each aerial survey provided 1 independent sample of the foal/adult ratios in treated and untreated groups for a specific short time interval. We tested the efficacy of our sterilizations by contingency table analysis of number of adults and foals in treated and untreated groups of horses for each survey. We recognize that such an analysis likely underestimates efficacy of the treatment because foals from bands where the male was untreated occasionally joined treated groups as described above.

Data from aerial surveys were augmented by ground observations made during behavioral studies conducted from April through November 1987 (72 observation days) and in January plus May through August 1988 (39 observation days) in the Flanigan area. Observations in the Beaty Butte area (15 observation days) occurred in July and October 1987. When numbered collars were missing or unreadable, horses were positively identified using the descriptions and photographs taken at capture.

Data from ground observations were analyzed differently from aerial observations because ground surveys resulted in many observations of particular bands and few of others; thus, it was impossible to obtain a pool of observations reflecting foal/adult ratios for a specific short interval.

Vasectomized males were recaptured to replace damaged collars when needed. Horses were immobilized using darts containing etorphine and lyophilized xylazine following procedures described by Plotka et al. (1987).

RESULTS

In the Flanigan area, we captured 175 horses (77 male, 98 female) in 30 bands. In 25 bands with both males and females, 12 contained 1 adult male (≥3 yr old) and 13 had >1 adult male. Twenty males were vasectomized, radiocollared, and released with their band. One 17-year-old male died during handling, apparently from a complication with anesthesia, and the other adult male (8 yr old) in the band was vasectomized.

In the Beaty Butte area, we captured 148 horses (71 male, 77 female) in 25 bands. We collared horses from 19 distinct bands, including 2 bachelor bands and 2 harem bands from which the dominant male apparently had escaped. Among bands containing both males and females, 9 had 1 adult male, and 6 had >1 adult male. During 1 drive, the pilot de-

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Table 1. Number of adults and foals counted in groups of ≥2 horses with (treated) and without (untreated) vasectomized dominant males in the Flanigan unit, Nevada, and the Beaty Butte unit, Oregon, 1986–1988.

Year[a]/ Area	Date	Treated groups			Untreated groups		
		Number adults[b]	Number foals	Foals/ 100 adults	Number adults	Number foals	Foals/ 100 adults
1986							
Flanigan	8 Jun	52 (9)	7	13	232	28	12
	17 Jul	36 (5)	3	8	172	18	10
	13 Aug	25 (5)	6	24	136	16	12
Total		113 (11)	16	14	540	62	11
Beaty Butte	14 Jun	69 (13)	17	25	127	20	16
	18 Jul	65 (10)	14	22	118	20	17
	14 Aug	60 (12)	14	23	115	20	17
Total		194 (18)	45	23	360	60	17
1987							
Flanigan	9 May	35 (7)	1	3	226	39	17
	3 Jun	31 (3)	1	3	197	34	17
	17 Jun	63 (9)	2	3	143	26	18
Total		129 (9)	4	3	566	99	17
Beaty Butte	10 May	53 (8)	7	13	159	17	11
	4 Jun	57 (9)	6	10	139	22	16
	18 Jun	40 (7)	1	2	169	28	16
	17 Jul	36 (8)	3	8	173	32	18
Total		186 (12)	17	9	640	99	15
1988							
Flanigan	21 Apr[c]	47 (7)	3	6	197	12	6
	5 May	30 (5)	1	3	243	28	12
	19 May	36 (5)	1	3	240	27	11
	16 Jun	65 (5)	2	3	180	24	13
Total		131 (7)	4	3	663	79	12
Beaty Butte	9 May	44 (8)	2	4	192	27	14
	20 May	45 (8)	2	4	170	27	16
	20 Jun	38 (8)	5	13	178	37	21
Total		127 (11)	9	7	540	91	17

[a] Females were pregnant when males were treated in December 1985 and January 1986; hence, foaling in 1986 was unaffected by treatments.
[b] Number of treated males counted in the survey is in parentheses.
[c] The 21 April survey was conducted before the peak of foaling and is not included in annual total or analyses.

viated from the normal procedure of capturing individual bands when he consolidated 32 animals for capture. We held the horses overnight anticipating that the dominant males would isolate their bands from the others. They sorted into 6 groups from which 5 dominant males could be discerned; these 5 were vasectomized. Twenty dominant males were treated, but only 19 were released because 1 showed signs of radial nerve damage after surgery and was euthanized when no improvement in his condition was noted after 3 days in captivity.

Numbers of treated males seen in aerial surveys decreased each year (Table 1). This decrease was primarily attributed to collars lost or removed from treated males during the study. In the Flanigan area, 10 collars were retrieved from the ground before data collection in 1986. Three males were immobilized in January 1986 and 2 collars were replaced; 1 male died from injury during the recapture effort. Three additional collars were retrieved in 1987, and 2 in 1988 before data collection, and 2 collars were lost during the 1988 spring flights.

In Beaty Butte, 2 males were lost from the

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study before data collection in 1986; 1 collar was lost and 1 male died (cause of death was undetermined). Six collars were lost between surveys in 1986 and 1987, and 1 additional collar was lost between 1987 and surveys in 1988. Damaged collars were replaced on 8 horses in 1987 and 1988.

Examination of retrieved collars revealed numerous rips in the collar material and tooth marks on the transmitter container; 6 transmitters had been torn from the collars. This extensive collar loss and damage was attributed to fighting among harem males.

Data collection in 1986 allowed assessment of the effect of capturing and handling horses on foaling rates, improvement of flight procedures, and observer familiarity with the study areas. In 1986 foaling rates (foals/100 adults) in both study areas seemed higher among treated groups than untreated groups (Table 1). This result was expected because all treated males were dominant males in harem bands, but untreated groups, which usually consisted of uncaptured, thus unmarked, horses, contained both harem bands and bachelor bands. Five of 30 bands we captured in Flanigan and 2 of 19 bands in Beaty Butte were bachelor groups, and we avoided capture of bachelor groups in both study areas when feasible.

Foaling rates seemed reduced in treated groups in both Flanigan and Beaty Butte study areas in 1987 and 1988 (Table 1). Coalescing of groups of horses was more of a problem in the Beaty Butte area than in Flanigan. This mixing of groups in Beaty Butte was attributed to the flat terrain combined with few places where water was available; on each survey in Beaty Butte we encountered 30–75 horses near each of 2 wells. As the helicopter approached, the horses ran in 3–5 large groups that subsequently split into smaller groups as we searched for collars. In the Flanigan area, where water sources were dispersed, bands of horses tended to remain separated, and we encountered only 1 group of >30 horses in 3 years of surveys.

Our analyses of aerial surveys (Fig. 1) indicated that treated groups of horses had fewer foals than untreated groups in the Flanigan area in 1987; treatment effectiveness was marginal in 1988. In Beaty Butte, efficacy of treatments was less clear; 1 of 7 flights provided results with a difference ($P < 0.05$) in foaling rates between treated and untreated groups. Trends in results were apparent, however. Treatments seemed more effective in Flanigan than Beaty Butte and more effective in 1987 than 1988.

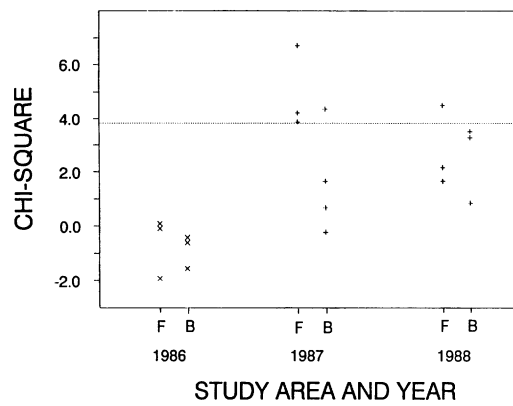

Fig. 1. Chi-square values of contingency tables comparing number of adults and foals in treated versus untreated groups of horses in the Flanigan, Nevada (F) and Beaty Butte, Oregon (B) study areas, 1986–1988. Females were pregnant when males were treated in December 1985 and January 1986; hence, foaling in 1986 was unaffected by treatments. Sign indicates the direction in which deviations occurred; positive indicates fewer foals in treated than untreated groups (positive treatment effect), and negative indicates more foals in treated than untreated groups. The dashed line shows the χ^2 value at which $P = 0.05$ with 1 df.

DISCUSSION

Although numbered collars with radiotransmitters attached have been used to identify and locate large numbers of female feral horses (Siniff et al. 1986, Eagle et al. 1992), their use for marking feral males seems to be of limited value. Berger (1986:134) noted that biting was a favored form of aggression between males, and the damage received by collars in our study indicated that radiocollars were a target of that biting. Therefore, we recommend alternative

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techniques, such as freeze brands or implantable radiotransmitters, to identify and locate feral males in future research.

Foaling rates in 1986 were unaffected by male sterilization because females already were pregnant when surgeries were performed. In both study areas, the proportion of foals in treated groups did not differ from that in untreated groups (Table 1, Fig. 1), and these were similar to foal/adult ratios in 3 other herds in the Great Basin Desert (Eagle et al. 1992). We concluded that despite the lack of sham-treated controls, the reduction of foaling rates in our study was caused by the treatment rather than stress during capture and handling procedures or disturbance during data collection.

There was a reduction in foaling within bands of horses when dominant males were sterilized, a conclusion consistent with that of Kirkpatrick et al. (1982); they selected bands with 1 adult male and found their treatment reduced foaling by about 80% for 1 year. We saw a reduction in foaling among treated groups in a herd area where approximately half of the bands captured contained >1 adult male, and the reduction in foaling among treated groups was noted for 2 years. We could not calculate the extent to which foal production was reduced.

Some foaling occurred in bands of horses with vasectomized dominant males, and ground observations provided insights into the apparent failure of our treatment to eliminate all foaling in bands with treated males. Band composition changed because of dispersal of juvenile horses of both sexes and acquisition of new females, possibly already pregnant, by dominant males. The most unstable band in our study contained 2 males (1 vasectomized) that alternated dominance every 2–3 months. Females sometimes left the band during exchanges of dominance between the 2 males. During 1988, foals were produced in 2 bands with treated dominant males. Both bands contained subordinate untreated males that had challenged for dominance and access to females. Although both vasectomized males remained dominant, the presence of foals in these bands suggested that subordinates succeeded in inseminating some of the females.

Although sterilization of dominant males may be an effective treatment to reduce foaling in a small sample of bands selected from a population, this treatment might not limit population growth. Enough animals must be treated so that foal production is reduced in the entire population (Garrott 1991). The success of male sterilization for limiting feral horse populations primarily depends on stable bands and the ability of the dominant male to prevent copulations by subordinate or bachelor males (Natl. Res. Counc. 1982). Although stable harem bands seem to be common (Kirkpatrick and Turner 1986), instability occurs in rapidly expanding populations (Berger 1986:77, 129) and when conditions are harsh (Miller 1981). Mean annual growth rate of 13 feral horse herds in the western United States was estimated to be about 20% (Eberhardt et al. 1982, Garrott et al. 1991), so many populations of horses may be rapidly expanding between periodic removals by the BLM.

Eberhardt et al. (1982) studied the Beaty Butte herd and estimated the instantaneous annual rate of increase from 1972–1977 as 0.20; they projected this rate from 1978–1980 and found it compatible with observations. Thus, the Beaty Butte herd is experiencing rapid growth and is subject to instability of bands. In their study of 5 feral horse herds (including Flanigan and Beaty Butte) in Nevada and Oregon, Bowling and Touchberry (1990) found that nearly one-third of 121 foals from 69 intact bands were sired by males that were unassociated with the band at the time of capture.

A further complication for male contraception has been suggested by Garrott and Siniff (1992). They demonstrated through modelling that in a polyestrous species with reproductive characteristics like those of feral horses, if the probability of breeding with a sterile male was significant (0.5–0.9 at each estrous period), the foaling dates for the population would be seasonally shifted. Obviously, this possibility should be considered if male contraception becomes part of management plans.

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Ground observations in our study and findings by Bowling and Touchberry (1990) indicate that the bands in our study populations and others in the Great Basin are somewhat unstable and that breeding by subordinate or bachelor males occurs; thus, sterilization of dominant males, although efficacious in small samples, may be of limited use as a tool for population management. We therefore recommend that future research include studies in which both male-oriented (Kirkpatrick et al. 1982, this study) and female-oriented fertility control (Kirkpatrick et al. 1990, Eagle et al. 1992) are tested as tools for limiting growth of feral horse populations.

SUMMARY

We studied the efficacy of dominant male sterilization for reducing reproduction in feral horses on western rangelands by vasectomizing harem males in 20 bands of horses in each of 2 study areas. Reduction in foaling occurred for 2 years in groups of horses containing sterilized dominant males, thereby indicating the treatment was efficacious. However, the treatment may be unable to reduce foaling sufficiently to effect population control because females may move between bands and subordinate or bachelor males may breed.

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ORIGINAL ARTICLE

Vessel sealer and divider instrument temperature during laparoscopic ovariectomy in horses

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Abstract

Objective: To determine the temperature of a vessel sealer and divider device during unilateral paralumbar laparoscopic ovariectomy in standing, sedated mares.

Study Design: Prospective study.

Animals: Fifteen healthy research mares.

Methods: Healthy mares with normal ovarian palpation and ultrasonographic appearance were enrolled. Horses were restrained in standing stocks and sedated. A right or left paralumbar ovariectomy was performed with a laparoscopic portal and 2 instrument portals. Ovaries were excised with traumatic forceps and a blunt tip vessel sealer and divider. Temperatures of the vessel sealer and divider were recorded with a thermocouple device adhered to the tip of the instrument. Variables were reported as median and interquartile range (IQR).

Results: Surgical time was 30 minutes (IQR, 25-32) including use of the vessel sealer and the divider for 4.1 minutes (IQR, 3.2-5.8). The tip of the instrument reached temperatures of 77°C (IQR, 72-85) during activation and 64°C (IQR, 61-67) at end cycle. The median increase in end-cycle instrument tip temperature per activation cycle was 2°C (IQR, −1-6). All mares returned to their intended use.

Conclusion: Despite the instrument temperatures observed during unilateral laparoscopic ovariectomy, surgical complications were minimal. The clinical relevance of the increase in instrument tip temperature of the vessel sealer and divider is presently unclear, but surgeons should use the instrument with caution, especially in close proximity to viscera. The increase in temperature observed at the tip of the vessel sealer and divider during unilateral ovariectomy could be associated with morbidity. The clinical relevance of instrument tip heating during other procedures, such as adhesiolysis and intestinal resection, is unknown and should be evaluated.

1 | INTRODUCTION

Minimally invasive standing laparoscopy is a common technique used for performing ovariectomy in the mare.[1,2] The advent of energy devices for laparoscopic surgery has resulted in reduced blood loss and shorter operative times in both people and animals. Numerous reports describe the

The results of this study were presented in part at the 12th International Equine Colic Research Symposium; July 18th, 2017, Lexington, Kentucky.

advantages of vessel sealing devices, but disadvantages may be overlooked. Heating of the vessel sealer and divider instrument tip could increase surgical morbidity in surgical patients if the instrument inadvertently comes in contact with adjacent viscera.[3] Electrosurgery has been associated with inadvertent trauma to nearby tissues through the lateral spread of thermal energy.[4-6] Thermal injury, including denaturation of proteins and necrosis, can occur when tissues adjacent to surgical devices are heated at least 4°C. Thermal injury may have negative consequences, such as tissue dysfunction or perforation of vulnerable structures.[4,7] However, thermal heating during serial activation of the vessel sealer and divider instrument has not yet been investigated in the horse.

The present study sought to determine the temperature at the tip of the vessel sealer and divider when applied to unilateral paralumbar laparoscopic ovariectomy in the standing, sedated mare. We hypothesized that this temperature would increase beyond a safe threshold for visceral tissue during serial activation of the device.

2 | MATERIALS AND METHODS

Fifteen grade mares participating in a research project requiring unilateral laparoscopic ovariectomy were enrolled in a study protocol approved by the Colorado State University Institutional Animal Care and Use Committee (IACUC 15-5984A) in compliance with the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act, and the Federation of Animal Science Societies *Guide for the Care and Use of Agricultural Animals in Research and Teaching*. Mare age was recorded.

All mares were stalled the evening prior to surgery, and food was withheld for approximately 12 hours prior to the procedure. Mares were restrained in standing stocks for surgical preparation. Procaine penicillin (22,000 IU/kg IM) and flunixin meglumine (1.1 mg/kg IV) were administered 30 minutes prior to surgery. A dose of detomidine hydrochloride (0.01 mg/kg IV) and a dose of butorphanol tartrate (0.01 mg/kg IV) were administered immediately prior to the surgical procedure.

2.1 | Surgical procedure

The left or right paralumbar fossa was clipped and aseptically prepared. Laparoscopic portal sites were each infiltrated with 5 mL of 2% mepivacaine hydrochloride local anesthetic. After routine draping, 3 portals were created as previously described.[1] For each portal site, a scalpel was used to create a 1 to 1.5-cm incision in the skin and external muscular fascia, after which a blunt obturator and an 11-mm diameter, 20-cm length laparoscopic cannula were inserted into the abdomen. The first portal was created midway between the tuber coxa and the last rib, at the level of the ventral aspect of the tuber coxa. Position within the abdomen was confirmed by using a 10-mm diameter, 30°, 57-cm length rigid laparoscopic telescope (Hopkins Telescope 62032BP; Karl Storz Veterinary Endoscopy-America, Goleta, California) prior to insufflation. Carbon dioxide was then insufflated at a rate of 12 L/min to a maximum pressure of 10 mmHg. The second and third portals were 5-10 cm craniodorsal and caudoventral to the first portal.

FIGURE 1 A thermocouple has been attached to the vessel sealer and divider instrument tip to allow temperature measurement of the instrument tip during the laparoscopic procedure. The wire connection between the thermocouple and microprocessor-based, digital thermometer is held to the instrument with a blue electric shrink-wrap sleeve

The laparoscopic telescope was moved to the craniodorsal portal to facilitate observation and instrument use during ovarian manipulation. When the ovary was visible, a laparoscopic needle (SD161919; Surgical Direct, Deland, Florida) was inserted through a portal, and 10-20 mL of mepivicaine hydrochloride was injected into the mesovarium through multiple injections and applied topically to the ovary and mesovarium. After the ovary was anesthetized, it was grasped with traumatic forceps and a 10-mm diameter, 37-cm length blunt tip vessel sealer and divider (LS1037 Ligasure; Covidien-Medtronic, Mansfield, Massachusetts) with an attached thermocouple connected to a microprocessor-based, digital thermometer with 7 Hz data recording (54IIB; Fluke Corporation, Everett, Washington), which was attached to an energy platform (Force Triad; Covidien-Medtronic) at the intermediate power setting, and was used to transect the mesovarium, mesosalpinx, and proper ligament of the ovary, avoiding contact with surrounding abdominal structures (Figure 1). The wire connection between the thermocouple and thermometer was held to the instrument with a blue electric shrink-wrap sleeve. Because of the resulting increased diameter, insertion of the vessel sealer and divider with attached thermocouple required removal of the laparoscopic cannula. Temperature readings were taken outside of the abdomen approximately 10 seconds after insertion into the

abdomen, upon initial clamping of soft tissues, immediately after each sealing cycle, and prior to removal of the instrument from the abdomen. The vessel sealer and divider device was removed from the abdomen after recording of the final temperature, without delay or other regard to tip temperature. Position of the thermocouple was observed during vessel sealing and dividing (Supporting Information Video). Vessel sealer and divider activation time and number of activations were recorded. The traumatic forceps remained attached to the amputated ovary in the abdomen, and the ovarian pedicle was observed to ensure adequate hemostasis had occurred.

Abdominal insufflation was discontinued, and positive abdominal pressure was released. The ovary was removed by enlarging the ventral incision. For incisions >4 cm, the external abdominal oblique muscle fascia was opposed with 2-0 polyester in a simple continuous pattern. Skin was closed with 2-0 nylon in a simple continuous (n = 7) or a continuous horizontal mattress (n = 8) pattern. Surgical time was recorded as the duration from the first incision to completion of incisional closure.

2.2 | Postoperative care

Postoperatively, mares received flunixin meglumine (1.1 mg/kg IV every 12 hours) for 5 days. Feed was gradually reintroduced, and monitoring consisted of twice daily examination including incisional inspection, behavior, appetite, urination, and defecation. Mares were confined to a stall until suture removal 10-14 days after surgery. After suture removal, horses were allowed small paddock turnout for an additional 2 weeks, prior to gradual return to routine exercise.

All continuous variables were reported as median and interquartile range (IQR). A Friedman test of multiple comparisons was used to identify changes in vessel sealer and divider tip temperature.

3 | RESULTS

Mare median age was 13 years (IQR, 10-14). Ovaries were removed unilaterally in all mares by using the described technique with no operative complications. Median surgical time was 30 minutes (IQR, 25-32), and median surgical time during which the vessel sealer and divider instrument was used was 4.1 minutes (IQR, 3.2-5.8). Median number of vessel sealer and divider activations was 12 (IQR, 10-12). Median temperature outside the abdomen prior to surgery was 23°C (IQR, 22-25). Median temperature inside the abdomen following initial insufflation was 32°C (IQR, 31-33). The thermocouple became dislodged from the instrument tip in 2 mares upon insertion into the abdomen and was located within the abdominal cavity approximately 1 cm from the instrument tip (n = 1) or along the instrument shaft and 2 cm

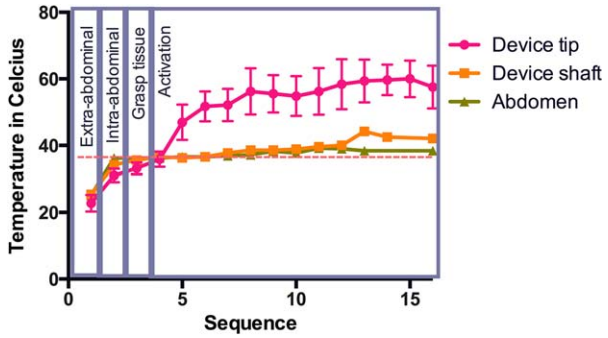

FIGURE 2 Temperature measurements (mean ± SD) recorded during unilateral laparoscopic ovariectomy performed with a vessel sealer and divider device

from the instrument tip (n = 1); therefore, data from these 2 mares were not included in instrument tip temperature calculations. Median temperature of the instrument tip after initial clamping of ovarian pedicle tissues was 34°C (IQR, 33-34). Sequential end-cycle temperature increases were observed with instrument activation. The vessel sealer and divider end-cycle tip temperature increased significantly from the preabdominal and postabdominal insertion temperature after the third instrument activation cycle. The vessel sealer and divider end-cycle tip temperature increased significantly from the initial soft tissue clamping temperature after the fifth instrument activation cycle. Median maximum instrument tip temperature during activation was 77°C (IQR, 72-85). Median end-cycle maximum instrument tip temperature was 64°C (IQR, 61-67). The median increase in end-cycle instrument tip temperature per activation cycle was 2°C (IQR, −1-6). After the initial activation of the vessel sealer and divider, instrument tip temperature did not return to preactivation values during the recording period in any of the mares. The ratio of the duration of instrument activation to the inactive interval was approximately 1:3, with a median activation time of 4.0 seconds (IQR, 4-5) and an inactive time of 10.5 seconds (IQR, 8-15) per activation. All temperature measurements are summarized in Figure 2.

The majority of mares had normal examination parameters postoperatively. One mare had mild colic that resolved after flunixin meglumine administration. Another mare developed serous incisional drainage and crusting postoperatively that resolved by the time of suture removal. No mares were prevented from returning to their intended activity due to complications resulting from ovariectomy.

4 | DISCUSSION

The main finding of our study is that end-cycle temperatures at the tip of the vessel sealer and divider increased with each sequential activation, reaching up to 77°C during activation, in mares undergoing unilateral ovariectomy. Temperature

increases in our study exceeded those described in a previous report, in which temperatures at the tip of a vessel sealer and divider reached 45.8°C during the activation cycle and were associated with tissue temperature increases exceeding 15°C for up to 20 seconds after activation.[3] Thermal tissue damage can occur when contact with surgical devices increases collateral tissue temperatures by 4°C or more. Inadvertent contact between the vessel sealer and divider and adjacent tissues during or following activation could, therefore, result in necrosis and other indicators of thermal damage in horses during ovariectomy.[4] Caution is also likely indicated to prevent surgeon injury during open or hand-assisted procedures.[8]

Endoscopic electrosurgical technology, specifically, electrothermal vessel sealing devices, have contributed to the rapid advancement of veterinary minimally invasive surgery. These devices have essentially eliminated the requirement for suture loops, intracorporeal suturing, or emasculation in many surgical procedures. Instead, these instruments allow hemostasis but remain easy to use and require fewer technical skills.[9-12] Although vessel sealing and dividing devices might be considered less technically challenging and a mainstay of equine minimally invasive procedures, including ovariectomy,[9,10] the present study suggests that their application may be associated with risks of iatrogenic tissue damage. The present study justifies caution when using a vessel sealer and divider during excision of the ovarian pedicle. Endoscopic control is required to check the location of adjacent viscera and to minimize the risk of lateral thermal energy spread and inadvertent contact between the instrument tip and tissues. Local erythema has been reported in the descending colon mesentery 3 days after ovarian excision with a harmonic scalpel, although gross changes were not noticed at the time of surgery and occurred in mares without detectable clinical effects.[13] Fibrosis of the descending colon mesentery has also been observed 3 weeks after ovariectomy in horses that underwent operations with the same vessel sealing and dividing device applied in the present report.[14] However, this instrument creates minimal collateral thermal spread, and its tip cools rapidly compared with other devices.[15] Activation of this device during equine ovariectomy in which the tip was in contact with the duodenum cranial to the ovarian pedicle for approximately 1 second has resulted in intraoperative blanching of the serosa and serosal fibrosis 3 weeks postoperatively, without associated clinical signs.[14] No complications directly related to the proximity of the electrosurgical devices were observed in the present study.

Multiple investigators have evaluated the safety of vessel sealing and dividing devices in close proximity to vital structures as well as the temperature of sealed tissues.[16,17] Previous articles describing clinical trials have reported device-related thermal injuries with vessel sealing and dividing devices, such as ureter injury, small bowel perforations, colonic perforations, and vascular injury.[18-21] Similar to the findings in the present study, Dunay et al[17] reported that the surface temperature at the sealing site of a vessel sealing and dividing device reached 69.25°C ± 0.98°C. Others report temperatures of the vessel sealer and divider reaching means of 88.9°C, 96.4°C, and 76°C during in vitro transection of porcine peritoneum, mesentery, and liver, respectively.[8] Mean maximum temperatures of 55.5°C have been reported 2 mm lateral to the vessel sealer and divider activation site during a single activation in vitro.[15] Furthermore, Chikamoto et al[16] evaluated thermal injury to the inferior vena cava after contact with a vessel sealing and dividing device during activation. Significant increases in temperature occurred within tissues as the activation duration increased from 5 to 15 seconds.[16] Muscular layers of the inferior vena cava sustained histological damage when placed in contact with the device for 10 seconds.[16] Activation time in the present study lasted approximately 5 seconds, with sequential increases in device tip temperatures observed over time. Investigators have previously reported that the vessel sealer and divider tip temperature exceeded 42°C for approximately 10-15 seconds after 15 seconds of application.[22] On the basis of these findings, care should be taken to avoid contact, especially during or immediately after activation and in applications requiring serial activation over several minutes, such as in the present study. Maintaining appropriate spacing between the device tip and adjacent organs can be especially challenging in the horse, depending on the amount of intestinal distention or relative migration of the viscera. Fasting horses prior to minimally invasive surgery is critical for maintaining safe distances between viscera and reducing the risk of accidental contact with the vessel sealer and divider.

Our study focused strictly on temperatures at the tip of the vessel sealer and divider during laparoscopic surgery in horses. Measuring tissue temperatures adjacent to the device would have determined collateral thermal variations relative to instrument tip temperature and improved the clinical relevance of our findings. Additional studies are warranted to determine the influence of bilateral ovariectomy or other common equine procedures, such as those requiring a longer duration of activation or additional activations. Real-time thermography could be considered to evaluate instrument and tissue temperatures and regional or collateral heating.[23] Furthermore, because thermal injury at the cell or tissue level is influenced by temperature and duration, evaluation of the duration of tissue and instrument temperature increases would further elucidate the risk of injury.[3,7] An ex vivo study measuring tissue temperatures at varying sites relative to the activation site of the vessel sealer and divider could further elucidate thermal spread and provide more information relative to the clinical application of this device. Finally, the time required for the instrument to return to preactivation

temperature and duration of thermal risk could have been determined if temperatures at the tip of the device had been monitored beyond final activation in the present study.

The present study emphasizes the importance of surgical technique and safety when using a vessel sealer and divider in minimally invasive surgery. In contrast to small animal and human surgery, equine tissues can be thicker and larger and may, therefore, accommodate longer periods of device activation before heat generation increases the risk of inadvertent thermal injury. In addition, the size of the equine abdomen provides adequate working space for the vessel sealer and divider, further reducing the risk of iatrogenic damage. The benefits of vessel sealing and dividing technology in minimally invasive surgery far outweigh the potential risks. Furthermore, lateral thermal injury can be mitigated by minimizing device activation times, increasing the activation interval, using the lowest effective device settings, and irrigating the region with cooled saline following device activation.[24,25] However, surgeons should be aware of the safety margin of these devices and understand that inadvertent contact between the device tip and surrounding tissue during or immediately following activation could result in thermal injury. Such damage could lead to surgical complications, especially in operations requiring multiple, sequential activations in close proximity to bowel or other viscera in sedated patients.[26] This study shows that unintentional contact between the tip of the vessel sealer and divider and abdominal structures is likely to result in thermal tissue damage. The present study highlights the importance of surgical safety, specifically the requirement to avoid contact with tissues and organs during or following activation of the vessel sealer and divider in equine laparoscopic surgery.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Celebrating 50 Years

AMERICAN COLLEGE OF THERIOGENOLOGISTS

Newsletter

Spring 2021

Reminder

Ballot deadline is June 15

VOTE HERE!

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Dear Diplomates,

With the Annual SFT Meeting just around the corner on July 21 to 24 in Omaha, NE, I would like to note (again) that 2021 marks the 50th anniversary of the founding of the ACT as a Recognized Veterinary Specialty Organization, and as such, the occasion will be a featured component of the SFT meeting. A major highlight will be a keynote address by former ACT President, Dr. John Kastelic, in which he will share an historical overview of the first 50 years of the ACT. Dr. Kastelic's presentation will be given during the Plenary Session on Friday morning, July 23rd. Additional recognition events are slated for our annual ACT business meeting on Thursday, July 22nd, and a social reception later that same day (details tbd). Given the importance of the ACT milestone we will be celebrating in Omaha, I hope to see many of you there.

One group we certainly plan to acknowledge this summer are the Charter Diplomates of the ACT who are listed on the ACT website at https://www.theriogenology.org/page/History. Over the years, I have occasionally perused the list of Charter Diplomates, and each time I have done that I have been struck by the sheer eminence of those individuals (most of whom are now deceased). Not surprisingly, it's a "Who's Who" of Theriogenologists! Although I only had the pleasure of meeting a few of the Charter Diplomates, those I did meet made a lasting impression on me. And on that note, I would like to recount here a fond memory I have of sharing breakfast at an SFT meeting with ACT Charter Diplomate, Dr. Stephen J. Roberts of "Veterinary Obstetrics and Genital Diseases Theriogenology" (i.e., "big red book") fame. To set the stage, Dr. Roberts and I share our Alma Mater, Cornell University, so my knowledge of (more so knowing the Legend of) Dr. Roberts goes back to my undergraduate days at Cornell in the early 1980's. In addition to being a renowned clinical theriogenologist at Cornell (through his "retirement" in 1972, after which he practiced with his brother in Vermont for many more years), Dr. Roberts was an accomplished collegiate polo player having served as the Captain of the Cornell Polo Team, and then later the Team Coach during his time on the faculty. Collectively therefore, Dr. Roberts was a "larger than life" individual to me. Now back to the SFT meeting in San Antonio, TX in late November/early December of 2000 (in conjunction with the Annual AAEP meeting). One morning before the program started, I walked into the hotel restaurant to have breakfast, and who did I see sitting alone at a table, none other than Dr. Roberts! Now, I am not one to generally be particularly assertive, but I walked over to Dr. Roberts and introduced myself and asked if I could join him … and he graciously invited me to do exactly that! I can't say that I recall any of the details of our interaction at breakfast that day, but I have no doubt our conversation included some discussion of Cornell and his book. Given the fact that I am recounting what was likely no more than a 45-minute to one-hour interaction with Dr. Roberts that morning in San Antonio nearly 21 years ago, I hope you can sense the lasting effect it had on me. Quite simply, it's a memory I will cherish forever.

In closing, I hope that by sharing my recollection of a fairly brief encounter with Dr. Roberts, some of you will be prompted to reflect on similar experiences you have had with ACT Diplomates who have touched your lives in one manner or another. Doing so would seem to be a fitting way for each of us to begin getting into the celebratory spirit as we prepare to mark our 50th anniversary as a specialty college this summer in Omaha.

Sincerely,
Dirk Vanderwall
ACT President 2020-2021

Species	Sat exam	Passed exam		
Multi-species	19	7		
Equine	7	3		
Bovine	0	0		
Small Animal	3	2		
TOTAL	29	12		
Route				
Standard (Resident)	21	10		
Alternate (Mentorship)	8	2		
TOTAL	29	12	41.4%	

COUNTRY	ACTIVE DIPLOMATE	EMERITIS DIPLOMATE	HONORARY MEMBER	TOTAL
Argentina	1		2	3
Australia	27	1		28
Brazil	1			1
Canada	35	8	2	45
Colombia	2			2
Egypt	1			1
France	3			3
Germany	2			2
Grenada	1			1
Ireland	2			2
Israel	3			3
Jordan	2			2
Malaysia	1			1
Mexico	1			1
Netherlands	3			3
New Zealand	6	1		7
Saint Kitts and Nevis	2			2
South Africa	6			6
Sweden			1	1
Switzerland		1		1
United Kingdom	7		1	8
United States	284	92	22	398
Venezuela		1		1
TOTALS	390	104	28	522

Welcome New Diplomates!

Dr. Luis Henrique da Aguiar

Dr. Rory Gormley

Dr. Viviane Cristine Leite Gomes

Dr. Daniela Orellana Guerrero

Dr. Melissa Prell

Dr. Lacey Rosenberg

Dr. Agustin Javier Ruiz Olivieri

Dr. Julie Storme

Dr. Katie Withowski

Congratulations to the following candidates that passed the 2020 exam: Drs. James Barrett, Tessa Fiamengo, and Vanessa Peixoto de Souza.

Breakdown by Employment Type:

Academia	197
Government	9
Industry	27
Private Practice	169
Retired	43
Other	77

Breakdown by Primary Species:

Mixed	57
Bovine	124
Canine	17
Equine	176
Feline	2
Porcine	6
Small Animal	43
Small Ruminant	9
Wildlife	1
Other	87

The American College of Theriogenologists fondly remember the following members that have passed away since the 2020 spring newsletter.

Clarence John Bierschwal

Clarence John “Bush” Bierschwal, Jr., D.V.M., M.S. born 29 June 1923 in Carroll, Carroll County, Iowa to Ida Dankel Bierschwal and Clarence John Bierschwal. Bush was raised in Nevada, Iowa with his older sister, Virginia D. Bierschwal, who predeceased him 8 July 2012, in Springfield, Missouri.

Bush attended Iowa State University in Ames, Iowa briefly before World War II led him to San Diego as a Radioman in the Signal Battalion of the U.S. Marine Corps. Ultimately based in Hawaii, Bush fought in the Pacific Theater of the War (1942-45). He landed on Iwo Jima, but never talked about it. If asked he would just say he carried the radio. Bush was honorably discharged 28 September 1945, continuing his military service in the Army Reserves until he retired a Colonel, U.S. Army Veterinary Corp. (1950-1988). He received the Distinguished Service Medal, 3 Army Meritorious Service Medals and the Army Achievement Medal.

After the war, Bush returned to Iowa State where he met and married the love of his life, Lorette Beryl Nelson on 27 December 1948. Iowa State is where Bush received his doctorate in Veterinary Medicine in 1950. Bush and Beryl moved to Excelsior Springs where he first practiced. About 1952 they moved to Columbia, Missouri when Bush joined the faculty as a Professor of Veterinary Medicine and Surgery at the University of Missouri College of Veterinary Medicine. Columbia is where they raised their two daughters, Beverly B. Sherman D.V.M. (Paul) now of Springfield, Missouri and Betsy A. McQueary, D.D.S. of Rogersville, Missouri. Football Saturday at their home in Columbia was pure black and gold. In those days, all walked through campus to Faurot Field as the Autumn leaves fell, picking up the pace when Marching Mizzou was heard to play in the distance. Rain or shine, win or lose, Bush would not leave the game until the end.

Bush taught veterinary medicine at the University for 34 years (1952-1986). There he established research and clinical programs in his specialty, large animal reproduction (Charter Diplomate and former President of the American College of Theriogenologists). At MU he was twice awarded the Distinguished Teacher Award. He also received the Alumni Faculty Award, Distinguished Professor Award, Distinguished Faculty Award, Dr. David Bartlett Lecture Award and the Iowa State University Stange Award. His professional career took Bush and Beryl around the world, they loved to travel and dance. Bush loved his big band and swing music, Katy Trail, fishing, Jekyll, computers, photography, Missouri Tigers, Kansas City Chiefs, a great scotch and a fine cigar. He and his family spent a year in the Netherlands, (about 1968) on sabbatical while he taught and co-authored with Dr. C.H. W. de Bois the textbook “The Technique of Frenotomy in Large Animals”. It was in the Netherlands that Dr. de Bois introduced Bush to cycling. Bush was hooked on bicycling from then on. Veterinary students of his fondly recall making farm calls in a clinic truck with a bicycle tucked away in the back. After their farm calls were finished, Dr. Bierschwal would say “just let me off here” and then he bicycled the 30 miles or more back to the Vet School. They also report his classroom instruction was made more interesting because he loved to instruct using movies, he had made of actual procedures performed. Bush thoroughly enjoyed teaching, his students, his colleagues and photography, then along came computer digital photography. All along, he kept current, built his own computer, and could often be found late at night in his office editing film. Bush retired from the MU Vet School a Professor Emeritus. His portrait hangs in a place of honor at the Vet School to this day.

Truly, Bush and Beryl most enjoyed each other, family, and their many friends. Betsy has one son, Matt (Alex) of Ozark, and they have a young son James. Betsy has two daughters, Dr. Melissa Broaddus (J.T.) of Springfield (their son Sam) and Lindsey (Cody) of Charleston, South Carolina. Bev and Paul have two sons, Luke (Springfield) and Sam (Kansas City, engaged to Taryn Nash), and two daughters, Elise (Columbia) and Hayley (Iowa City). Bush leaves behind his cat, Tebow, now a new member of the Sherman household. All animals loved Bush, and he them.

Always active, always thoughtful of others, always positive, and always supportive, Bush taught us all many wonderful lessons, real life lessons including humility, hard work, responsibility and the importance of family and friends. Much loved, we miss him deeply but smile knowing Bush and Beryl are together again. Bush passed away Saturday, October 17, 2020 in Springfield.

Dr. Bierschwal was a founding member of the ACT.

C. H. W. de Bois

Prof. dr. C.H.W. (Cornelis Hendrik Willem) de Bois was born on 10 July 1927 and passed away on 23 January, 2021. He received his veterinary degree from the University of Utrecht and was a Professor of Veterinary Obstetrics and Gynaecology at the University of Utrecht, from 4 March 1964 to 1 January 1985. On 29 November 1965, he gave an inaugural lecture on “The development of instrumental and surgical obstetrics in horse and bovine” and he was co-author (1972) of the classic manual on fetotomy (Bierschwal CJ and de Bois CHW, "The Technique of Fetotomy in Large Animals”).

William Cates

The family of William F. Cates announces his passing on June 13, 2020 in Vancouver.

William Francis Cates was born to James Earl and Catherine Elizabeth (Simpkins) Cates on July 18, 1926 in Prior Lake Minnesota. He was the second child and oldest son in a family of seven children. As a child of the Depression, Bill learned to hunt and fish at an early age and these became life long passions. His parents farmed and worked a dairy operation in the town, and Bill helped with this as he grew. He attended elementary school in Prior Lake and finished his high school

in nearby Shakopee.

Bill was 15 years old when Pearl Harbour was attacked in December of 1941, and as soon as he finished high school in 1944, he enlisted in the U.S. Navy, serving as a Gunner's Mate on a minesweeper in the Pacific theater.

When the war was over and his enlistment done, Bill returned to Minnesota. He helped briefly with the family business, which by then had turned to home construction, and then began undergraduate work at St. John's University near St. Cloud. After earning a Bachelor of Science, he was accepted into the University of Minnesota's school of veterinary medicine, graduating with his DVM in 1954. This was the same year he married his sweetheart Ladonna Marie Scharf.

Bill worked a short time in the area while he and Donna started their family, but in 1955 they headed west to Mitchell, South Dakota, where he tried his hand at private veterinary practice. They were there a couple of years, and then returned to Minnesota, where he started on his PhD. He finished this in the early 1960s, working as an instructor at the U of M veterinary college at the same time.

All this time, the family was growing, and Bill and Donna now had five children to chase after. By the mid sixties, big changes were in store. The University of Saskatchewan was opening a veterinary college, the first in Western Canada. In 1965, Bill was offered a professorship there and the family moved to Saskatoon, where his youngest child was born, making it a family of six for Bill and Donna.

Bill enjoyed taking his family on weekend outings to places like Beaver Creek and the Forestry Farm. Sundays were special days that often involved a visit to the Victoria Ice Cream Shoppe and ended with the family gathered around TV to watch Walt Disney or Jacques Cousteau. His son Jim commented he knew Dad never played hooky from work because he always came home smelling like a cow pasture. When his children were young, it was common to see one or more of them in tow when Bill was called out to attend a sick animal.

Throughout their marriage Bill and Donna were inseparable. Their passion for dancing included several decades as members of The Happy Wanderers Square Dance Club. Bill was a quietly committed Catholic. When the family first moved to Saskatoon St Phillips Neri Church was still an unrealized vision and masses were held in a school gymnasium. Bill and Donna contributed towards building the church, supported the church's projects in South America and were among the first lay people to dispense communion.

Bill played a pivotal part in getting the Western College of Veterinary Medicine started. He taught at the U of S and worked his practice on the surrounding farms and ranches. His specialty was bovine theriogenology - herd medicine and reproduction. He was very active in research and much respected in his field, and worked at the college until he retired in 1994. He was professor emeritus for several years after that, but he and Donna enjoyed retirement and made a number of trips to Arizona and Texas, always trying to fit in a little bird watching wherever they went. They bought property at a lake north of Saskatoon, and enjoyed their time there as well.

He and Donna moved to Vancouver in 2010, where their daughter Kitty helped them out as they grew older. Bill suffered a stroke in 2011, and moved into a care home, and the family was glad that Donna was able to join him in the same home shortly thereafter.

Bill is predeceased by his beloved Donna (2015), and his siblings Jack, Anne Mae, Rosie, and Jim. He is survived his older sister and her husband (Kate and Duke Schrader), his sister in law (Pat Cates), his youngest brother and wife (Larry and Betty Cates), his six children (Steve, Cindy, Kitty, Jim, Barb, Michelle) and by his 4 grandchildren (Joel, Ryan, Zoë, and Connor).

Dr. Cates was a charter member of the ACT.

John Melancon

John Joseph Melancon, age 77 of St. Cloud passed away on Friday at the St. Cloud Hospital.

John was born September 12th, 1943 in St. Paul, MN to Joseph and Phyllis Melancon. He was known for his bright smile, generous spirit, and ability to easily make friends. He and wife Nancy visited many corners of the world making numerous friends along the way. When not travelling the globe, John enjoyed spending time with his adored children and grandchildren, playing bridge, fishing, and hunting. Always quick with a "Dad" joke, he relished time with family and friends playing parlor games, sitting by an open fire, or watching a movie with homemade popcorn.

While calling Stearns County home for more than 30 years, John grew up in the Highland Park neighborhood in St. Paul. He completed undergraduate studies at St. Thomas and went on to obtain a doctorate from the College of Veterinary Medicine at the University of Minnesota. John owned a veterinary clinic in Sauk Center for many years and then went on to work in veterinary pharmaceuticals. He retired from Merck/Merial in 2003.

He is survived by his wife, Nancy Gilbert; children, Maggie (Erin) Daugherty, Mike (Emily) Melancon, Toni Melancon, Jay (Mary) Melancon, Nathan Gilbert; siblings, Tom (Elizabeth Dichter) Melancon, Mary (Tom Spencer) Melancon, Jeanne (Bob) Dolan; and seven grandchildren.

He is preceded in death by his parents; and wife Carol.

Willis Parker

Dr. Willis G. Parker of Clayton, IN died peacefully on April 15, 2021 at Life's Journey Hospice of Avon. Willis, son of the late Roy and Doris (Kelly) Parker was born on June 10, 1936 in Greencastle, IN. Willis and his twin sister Willa Dean spent their early years growing up on the family farm near Coatesville. As a young boy he was active in both 4-H and FFA and graduated from Amo High School in 1954. He volunteered for the draft and served in the U.S. Army from 1954 to 1956.

Following his military service, Willis attended Purdue University where he earned his Bachelor of Science Degree in Agriculture in 1960. As an undergraduate student he resided in Rochdale Co-op House and participated in Purdue Reamer Club, Baptist Student Foundation, and Purdue Men's Varsity Glee Club. Upon graduation he married Linda Owens on August 14, 1960. They started their life together in West Lafayette, IN where Willis entered the Purdue University School of Veterinary Medicine. He received his Doctor of Veterinary Medicine Degree with the second graduating class in 1964. After graduation Willis and Linda, along with their son Eric, returned to their roots near Coatesville where Willis established a mixed veterinary practice. After the birth of their daughter Lisa, he made the decision to pursue his interest in animal reproduction and in 1965 accepted the position of staff veterinarian at American Breeders Service (ABS) located in DeForest, WI where he worked for 26 years. The family's primary residence was in Poynette, WI where Willis served several years on the Poynette Public Schools' Board of

Education.

Throughout his life, Willis was a humanitarian who was committed to his profession as well as service to others. He was a member of the American Veterinary Medical Association (AVMA) as well as the IVMA and WVMA, the American Association of Bovine Practitioners, the Society of Theriogenology, and the American College of Theriogenologists which he served as a diplomate and president from 1983-84. In 1990, Willis represented ABS on a trade mission to

Lithuania. This experience spurred his consulting and educational programing trips to the Baltic countries and to Ethiopia with Project Mercy. In addition, he served several weeks in the United Kingdom for the U.S. Department of Agriculture's Animal Disease Eradication Program. For his professional contributions, Willis was recognized by the WVMA as the 1991 Wisconsin Veterinarian of the Year and in 2000 was recognized by the IVMA as the Indiana Veterinarian of the Year. In addition, Willis was presented the Purdue University School of Veterinary Medicine's 2000 Distinguished Alumnus Award.

Willis's faith and values were an important aspect of his life. While a member of the Congregational United Church of Christ in Windsor, WI he served as moderator and on various church boards. During his several years of service on the Corporate Board for World Ministries of the United Church of Christ, he traveled to Sri Lanka on a mission trip. At the time of his death, he was a member of Hope United Presbyterian Church in Plainfield, IN where he served as an elder and contributed greatly to their mission projects.

Following his retirement in 1991, Willis and Linda returned home to Indiana where he established the Mill Creek Christmas Tree Farm on the family farm near Coatesville. He also continued his passion for historic preservation of agricultural implements, especially those related to the horse. He was a member of the Horse Brass Society of England and the Belgian Draft Horse Corporation of America. His interest in history included collecting postcards and he was a member of the Indianapolis Postcard Club. Willis also served his home county by helping to establish the White Lick Heritage Community Foundation, now the Hendricks County Community Foundation, and was a past president of its board of directors.

It is often said you can take the boy off the farm, but you cannot take the farm out of the boy. Willis often shared his love of the family farm with his granddaughters. He delighted in taking them there to share the joy of nature and his appreciation for agriculture.

In addition to his parents, Willis was preceded in death by his son Eric and nephew Christopher Yeager. He is survived by his wife Linda, his daughter Lisa Parker-Gummere and son-in-law Jeff Gummere, and granddaughters Erica and Anna. He is also survived by his sister Willa Dean Yeager, nephew Alan (Abby) Yeager, and great nephews Logan and Adam. In addition, he is survived by a large and beloved extended family.

From the President of the Theriogenology Foundation

Wow! What a year! I hope this finds all of you healthy. One year ago I was learning to negotiate curbside service, trying to understand what type of a/c filters would trap viruses, and figuring out which masks would not leave my ears raw. All this while dealing with an unexplainable increase in workload. It seems like January, 2020 was a very long time ago.

Along with learning how to practice in a pandemic, it didn't take much reflection to realize the other important aspects of life. Faith has always been a priority for me. Time with family became precious, particularly those family members that the pandemic made more difficult to see. The same goes for friends and our therio family. I am very excited to have an "in person" conference in Omaha this July. There are so many of you that I ache to see, and as great as zoom is, it is not the same.

The TF board has been busy during the last year. We have worked to continue providing benefits to students, with your help, to fund externships, research, student chapter events and speakers. The AKC/AKC CHF/TF residency program is still going strong. Although the Working Dog Project is not directly affiliated with TF, we are still supporting the important work that WDP is doing. And our newest project, the Nandi Scholars Program, is up and running. We will be presenting four scholarships to some incredible fourth year veterinary students in Omaha this year.

None of this would be possible without the hard work of many. Importantly, many of the individuals responsible for the success of these programs are not TF board members. When Dr. Jim Floyd, who heads up the Nandi Scholars Program, put together a team of applicant reviewers, I was pleased (but not surprised) at how quickly our SFT/ACT membership stepped up to the difficult task of reviewing the applications. A huge thanks to all who helped make this possible

We are able to continue to help so many thanks to the generosity of all of you. Thanks for your support. I hope that you will be able to attend the awards banquet this year in Omaha and get to meet our inaugural group of scholarship recipients.

Please stay well. Please don't hesitate to contact me if I can help you in any way. I'm looking forward to seeing everyone sans the computer screen.

Mike

Future Theriogenology Conference Dates

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and
The American College of Theriogenologists

MAKE PLANS NOW TO JOIN US AT
THE FOLLOWING LOCATIONS:

2021
July 21-July 24
Omaha, Nebraska

2022
July 20-July 23
Bellevue, Washington

2023
July 19-July 22
Birmingham, Alabama

2021 Certifying Exam Committee Report

The ACT Certifying Exam Committee has been very active in the past year. Not only has the committee reviewed, updated, and set 2021 examination, there have been multiple efforts to update the examination process due to the electronic nature of our examination. Many thanks to the committee for their hard work and dedication to this committee: Brian Whitlock, Past-Chair; Julie Cecere, Chair; Ahmed Tibary, Vice-Chair; Kara Kolster, ACT Secretary; Jo Randall, Soon Hon Cheong and Jacobo Rodriguez. A summary of the committee's efforts, outside of examination preparation and review, are below:

1. A thorough question bank cleanup is underway. Each essay question in the bank was reviewed, assigned knowledge domain(s) and inventoried. Multiple choice questions will be reviewed over the next year. This is in an effort to maintain alignment with the current Job Task Analysis (JTA), which is the basis for the species and knowledge breakdown of the certifying examination as well as training skill sets. The JTA sets these parameters based on membership demographics and responses, so you as an ACT member are actively involved with the process. Please stay tuned for future requests of the ACT membership to submit new questions for use by the Examination Committee as well as an updated JTA survey, which is essential to creating a balanced and appropriate certifying examination for the ACT.
2. A completed movement towards a fully electronic examination has occurred. ExamSoft has been used as the examination platform since 2017. Due to COVID, the 2020 examination was administered remotely with live proctors. This electronic process was well received and this year we are adding the use of artificial intelligence (AI) proctor software. It is likely that the examination will follow the remote format moving forward with the use of AI proctoring software for ease of candidate and mentor travel as well as time zone and work constraints.
3. All forms related to the examination were revised to reflect the use of electronic software and AI proctors. This was essential to encapsulate this historic change for the ACT certifying examination process.
4. Dr. Réjean Lefebvre was selected as the new member of the Exam Committee, to replace Dr. Brian Whitlock. Dr. Lefebvre has accepted this nomination and will start his duties on the committee after the Annual Conference at the end of July 2021.

Dr. Brian Whitlock is finishing his term and will be leaving the committee this year. We are grateful for all his contributions to the committee, his friendship, and his stories. If you have not had the chance to sit around a dinner table with Brian I highly suggest it, you will not walk away the same person. All of us will miss him.

Lastly, the 2021 Certifying Examination will be administered July 13th and 14th electronically via Examsoft and AI proctor software. All successful candidates will be announced at the ACT Business meeting July 22 as well as new ACT members from 2020.

Sincerely,

Julie T. Cecere

ACT Certifying Examination Committee Chair

Thank you!

A huge thank you to the following individuals for all their efforts in planning the 2021 conference!

Annual Conference Program Chair
Dr. Ahmed Tibary

Equine Track Program Chair
Dr. Karen Wolfsdorf

Production Animal Track Program Chair
Dr. Juan Romano

Small Animal Track Program Chairs
Drs. Julie Cecere and Orsolya Balogh

Reproductive Pathology Symposium Chair
Dr. Christopher Premanandan

Zoological Species Theriogenlogy Symposium Chair
Dr. Anneke Moresco

Student Seminar Chair
Dr. Juan Samper

Silent Auction Chairs
Drs. Jim Floyd and Will Schultz

Live Auction Chair
Dr. Jim Floyd

Theriogenologist of the Year
Dr. Gary Althouse

Dr. Gary Althouse received his BS degree from Sul Ross State University, his MS from Texas A&M University, and he completed a combined program leading to both DVM and PhD degrees at Iowa State University. Althouse became board certified with the American College of Theriogenologists in 1995. He joined the faculty at the School of Veterinary Medicine at the University of Pennsylvania (Penn Vet) in 2001. In 2011, he was named to the Marion Dilley and David George Jones Endowed Chair in Animal Reproduction. In 2019, Althouse was appointed Associate Dean of Sustainable Agriculture and Veterinary Practices. Additional leadership roles have included serving as president for both the Society for Theriogenology, and for the American College of Theriogenologists.

Althouse's efforts focus on global food supply and food security. He is the founder and director of Penn Vet's Reference Andrology Laboratory, which provides both critical research and clinical services in food animal production. He currently provides services to clientele throughout North America, with his current work having a direct impact on ~34% of the U.S. swine breeding herd. Globally, he provides veterinary consultation services in the remaining Americas, Europe, Asia, and Australia. Collectively, these experiences are woven into Dr. Althouse's teaching efforts in the areas of theriogenology and swine production medicine.

Althouse has been fortunate to have had many influential mentors over his career, including L. Evans, S. Hopkins, W. Hsu, M. Pineda and S. Seager. Over the past 27 years, he has enjoyed mentoring numerous talented veterinary and graduate students, faculty, post-doctoral fellows, interns and residents.

Nominees for officers and directors of the College-Vote Now!

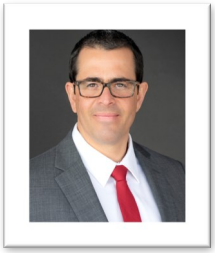
The newly elected vice-president will assume the offices of president-elect and president in 2022 and 2023, respectively. Beginning in 2014, the decision was made by the ACT Board of Directors to initiate online voting. Please complete the ballot by June 15, 2021.

Vote and you could win!

Sign in to the ACT site, then click the link below or place the ballot link in your browser (**https://www.theriogenology.org/surveys/default.asp?id=1602791**). You will be allowed to vote one time only. Your vote is completely confidential. There will be a random drawing of the individuals who vote in the officer/board elections for a non-transferable prize of a complimentary Therio meeting registration within three years of the award.

Vote today for your chance to win!

Vice President nominees



Leonardo Brito

Dr. Brito, DVM, MSc, MVetSc, PhD is the Director of Global Quality Assurance at STgenetics and an associate editor of the journal Theriogenology. He became a diplomate of the ACT in 2004, served on the Certifying Examination Committee from 2009 to 2015, served on the ACT Executive Board as Treasurer from 2017 to 2020, and is currently serving on the newly created Standard Setting Committee. More details of education and professional experience can be found at https://www.linkedin.com/in/lbrito/.

If given the opportunity to serve the ACT, Dr. Brito would focus on initiatives to promote the specialty and increase the College membership. Student marketing: promote the College to veterinary students by participating in national events as an exhibitor, providing speakers, and supporting other scholar activities. STF Student Chapters: promote organization of mentor network to exchange experiences and practices, support new mentors, and stimulate creation of new chapters; organize network of diplomates willing to support chapters through presentations and other instructional activities; promote cross-chapter student interactions; support industry lobbying efforts to secure financial support for student chapters. Residency training opportunities: support food-animal and equine industry lobbying efforts to secure financial support for residencies building on the successful AKC experience. Alternate Training Route: facilitate mentorship by organizing support network of prospective mentors, and sharing study plans and instructional materials; facilitate completion of clinical training requirements by organizing network of clinical trainers to include diverse geographical distribution and species representation; facilitate completion of scientific publication requirement by organizing network of specialists to provide expert advice on experimental design or case report planning, data analysis, manuscript writing, and peer-review process. Graduate Training Route: charge the credentialing committee with studying the re-institution of this training route in ways that would ensure minimal clinical competency and at the same time tap into the enormous benefits and diversity expert scientists would bring to the College.

Karen Wolfsdorf

Dr. Wolfsdorf obtained her DVM from the University of Florida, College of Veterinary Medicine in 1992. She completed an internship in equine field service at North Carolina State University and a Theriogenology residency at the University of Florida. Dr. Wolfsdorf became a Diplomate of the American College of Theriogenologists in 1995 then entered equine private practice as a specialist in reproduction first in Australia then at Hagyard Equine Medicinal Institute where she is presently a partner and specialist in reproduction and field care. Dr. Wolfsdorf served on several committees in SFT, AAEP and ISER. She served ACT as a board member and as a member of the training and credentialing committee. Dr. Wolfsdorf has authored several peer-reviewed articles and book chapters in the field of equine reproduction. She has contributed numerous presentations and proceedings at national and international conferences. She enjoys interaction with practitioners and veterinary students. She has lectured to several student chapters of the Society for Theriogenology.

Dr. Wolfsdorf is committed to advancement of the specialty of theriogenology in practice and veterinary education.

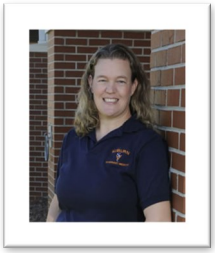
Board of Director nominees

C. Scott Bailey

I am C. Scott Bailey and I would be honored to serve as an executive board member. I graduated from Kansas State University in 2003 and pursued a theriogenology residency at the University of Florida 2005-2008, achieving diplomate status in 2008. I subsequently completed an MS at the University of Florida under the guidance of Dr. Macpherson and then moved to North Carolina to serve as a faculty member at NCSU from 2009-2019. I had a multi-species interest with clinical programs focused primarily on equine and canine reproduction and research projects that stretched across genera. In 2019, I left academic practice and moved to central KY to serve as resident veterinarian of Claiborne Farm, where I supervise the general and reproductive care of approximately 500 horses and continue to pursue clinical and collaborative research projects through NCSU and the University of Kentucky.

Like many members of our college, I have a broad interest in veterinary reproductive physiology and medicine, and varied clinical experience to match that. I have provided instruction to veterinary students at four different institutions and mentored both house officers and graduate students to successfully develop a career in theriogenology. I am passionate about the mission of fostering the development of clinician scientists who will aid our (collective) patients and clients, and further the knowledge in our field. I am also passionate about the need and opportunity for research arising from clinicians in private practice and hope to emulate some of the great members of our college who precede me in this endeavor. Our college has an ongoing important role to play, both through its mentorship of prospective members and through its outreach to veterinarians, students, breeders and owners. I very much look forward to participating in this important work.

Thank you once again for giving me the opportunity to run for the position of Executive Board member.



Aime Johnson

I entered private practice for 5 years following graduation from vet school (equine and mixed animal), but decided I wanted to specialize. I returned to "school" and completed my residency training at Texas A&M. This perspective allowed me to be a better specialist as I was able to gain experience in a large volume repro clinic, but also be in the world of general practice. I joined the faculty at Auburn University in 2007 and have enjoyed my career here. I was certainly strongest in equine after my residency, but due to clinical needs at Auburn, I have expanded to companion animal therio and am now comfortable in both. I am one of the few theriogenologists comfortable with feline reproduction. I became involved in Auburn's research cattery in 2008 when the fertility of the colony had declined to the point that they were unable to obtain animals for research. Since that time, I have learned through experience and now consult on catteries all over the US.

My research experience is concentrated in the horse and cat. Most notably, I have collaborated with several researchers using methods of non-surgical castration in small animals and estrus suppression in mares. Outside of theriogenology, I am also involved heavily with viral vectored gene therapy for degenerative neurologic disease in the cat as a model for a neurodegenerative, fatal disease in children. Through this research, clinical trials began last year and children with these diseases are showing improvement.

I look forward to serving my specialty though this board position. My children are older and I want to dedicate time to my fellow ACT diplomates. I admire those currently serving and have learned from their leadership. I can benefit the Board through my varied experiences from private practice and academia and from my experience in a variety of species. I am also the student theriogenology advisor at Auburn and hope to have a part in encouraging students to pursue theriogenology and board certification to the betterment of our specialty.

Honorary Member nominees

Jon Oatley

Jon M. Oatley, Ph.D. is the Director of the Center for Reproductive Biology and a full Professor in the School of Molecular Biosciences at Washington State University. Dr. Oatley received a Bachelor of Sciences degree in Animal Sciences from the University of Nevada-Reno, Master of Science and Doctor of Philosophy degrees from Washington State University, and was a postdoctoral fellow in the laboratory of Dr. Ralph Brinster at the University of Pennsylvania. Over the course of two decades, Dr. Oatley's research has focused on understanding the biology of germ cells which are the eternal cellular link between generations. Dr. Oatley's research also focuses on engineering the genetics of farm animals to develop more efficient avenues for feeding the expanding global human population. Dr. Oatley has authored more than 80 scientific research papers, book chapters, and news articles on the areas of germ cell biology and animal genetic engineering. As an independent investigator, his research program has been funded by multiple grants from the National Institutes of Health, the United States Department of Agriculture, and industry contracts.

Gary Warner

Dr. Warner grew up in north Louisiana in a family run cow/calf and stocker operation. His dad moved the family to Ferriday, Louisiana in 1965 where his dad operated an auction barn and continued in the cow/calf business and was also a procurer of replacement cows. Dr. Warner received a BS from the University of Louisiana at Monroe in 1977 and received his DVM degree from the Louisiana State University School of Veterinary Medicine in 1980. Immediately after graduation, Dr. Warner entered clinical practice as a mixed animal associate at Elgin Veterinary Hospital. After 2 years of employment, he became a partner and developed a primary interest in large animals and eventually, just beef cattle practice. Today, the practice is largely referral based, focusing primarily on lameness and reproductive problems in both the male and female. Dr. Warner has been actively involved with the Professional Bull Riders as a consultant and practitioner for many years, providing for animal welfare and the well-being of the animals involved in their sport. He sustains a very active presence within the industry.

Other practice interests involve the purebred and show cattle industries and servicing the needs of individual producers. He has been actively involved in the show steer industry in Texas, helping many children with their projects and even raising a few of his own. All three of his children grew up showing steers with many championships. Two of his children, Dr. Jenna Beyer and Dr. Kelly Warner, now practice veterinary medicine and the youngest, Jacob, is a 2nd year veterinary student at Texas A&M University College of Veterinary Medicine. Dr. Warner is a career-long member of AVMA, TVMA, (served as longtime member and chair of The Beef Practice Committee), AVC, AABP, PAC and the Society for Theriogenology, where he served a long tenue on the executive board culminating in becoming president of that organization in 2011-2012. Dr. Warner, along with his wife Terry, remain actively involved in the cattle industry at their ranch north of Elgin; producing show steers and high-quality replacement females. He continues to support the beef cattle production community and is always available to colleagues for consultation.

2021 Scientific Information/Abstract Committee Report

We want to thank all authors for their support and contributions to the scientific program of the 2021 Theriogenology Conference to be held in Omaha, Nebraska. We received 61 abstracts for consideration in the following categories: 19 Competitive Abstracts, 27 Non-competitive Abstracts, 14 Case Report Abstracts, and 1 Poster Abstract. Abstracts submitted to the Therio Conference are scored blindly by all members of the Scientific Committee. The eight competitive submissions with the highest scores were selected for oral presentations at the Dr. Jerry Rains Memorial Abstract Competition session. The next eight abstracts with the highest scores will be presented during the Opening Session on July 21, 2021. The remaining abstracts were assigned to their respective species-specific sessions: Equine - 8 abstracts; Mixed Species - 9 abstracts; Production Animal – 8 abstracts; Case Reports – 8 abstracts; and Posters – 12 abstracts. All abstracts will be included in the conference proceedings published in Clinical Theriogenology. The current members of the Scientific Abstract Committee are Drs. Candace Lyman (Chair), Julie Gard Schnuelle, Orsolya Balogh, Jennifer Roberts, and Julia Baldrighi. Drs. Gard Schnuelle and Roberts will end their terms in August 2021. Drs. Roberto Palomares (4-year term) and Jamie Stewart (2-year term) are the new members of the Abstract Committee. We would like to extend a warm invitation to everyone to attend the oral and poster sessions, interact with the authors and learn about their exciting work. We also ask you to actively participate in the Dr. Jerry Rains Memorial Abstract Competition session by asking questions to the presenters as answering your questions are also calculated in the final presentation score. We hope to see you in Omaha!

Sincerely,

Candace Lyman
Therio Conference Scientific Abstract Committee Chair

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2021 Annual Conference Agenda

Wednesday, July 21

Opening Session Abstracts
Opening Session Reception
Reproductive Pathology Symposium (additional registration required)
Zoological Species Theriogenology Symposium (additional registration required)
Student Seminar: Critical Thinking in Practice: What does it look like?

Reproductive Pathology Symposium
(additional registration required)
8:00 am - 5:00 pm

Introduction
Christopher Premanandan, DVM, PhD, DACVP, DACT

General concepts in failure of pregnancy
Christopher Premanandan, DVM, PhD, DACVP, DACT

Equine/Camelid failure of pregnancy
Robert A. Foster, BVSc, PhD, MANZCVS, DACVP

Ruminant failure of pregnancy
Dalen Agnew, DVM, PhD, DACVP

Canine/Feline failure of pregnancy
Robert A. Foster, BVSc, PhD, MANZCVS, DACVP

Round table Q/A session
Drs. Agnew, Foster, and Premanandan

General concepts in perinatal mortality
Christopher Premanandan, DVM, PhD, DACVP, DACT

Ruminant perinatal mortality
Robert A. Foster, BVSc, PhD, MANZCVS, DACVP

Equine/Camelid perinatal mortality
Dalen Agnew, DVM, PhD, DACVP

Canine/Feline perinatal mortality
Drs. Agnew and Premanandan

Case based roundtable/discussion
Drs. Agnew, Foster, and Premanandan

Zoological Species Theriogenology Symposium
(additional registration required)
7:30 am - 4:30 pm

Welcome

Overview of traditional and novel approaches to amphibian reproduction in zoological institution
Jessi Krebs, OHDZA Curator of Reptiles and Amphibians

Birds-General husbandry as it pertains to reproduction
M. Scott Echols, DVM, DABVP (Avian Practice)

Birds- Common reproduction problems and diseases
M. Scott Echols, DVM, DABVP (Avian Practice)

Overcoming reproductive disorders in female greater one-horned rhinoceros (Rhinoceros unicornis) to improve artificial insemination and natural breeding success
Monica Stoops, MS, PhD Lead Scientist, Comparative Endocrinology OHDZA

Assisted reproductive technologies for endangered felids
Jason Herrick, PhD, Director of Reproductive Services

Zoo Clinical Challenge- Gorilla infertility
Anneke Moresco, DVM, PhD, Zoo Reproductive Specialist

Current understanding of elephant reproduction
Dennis Schmitt, DVM, PhD, DACT

Application of domestic dog theriogenology practice in wild canids
Bruce Christensen, DVM, MS, DACT

Avian reproductive tract diseases
M. Scott Echols, DVM, DABVP (Avian Practice)

Developing assisted reproduction for reptiles, what's next?
Sean Perry, DVM, PhD

Attendees will have the options of attending a seminar by Drs. Echols and Perry discussing "*Advanced topics in reptile and avian reproduction*" or traveling to the Henry Doorly Zoo and Aquarium for a behind the scenes tour.

Student Seminar
1:00 pm - 5:00 pm

Critical Thinking in Practice: What does it look like?
Juan Samper, DVM, PhD, DACT

Opening Session Abstracts

Luteinizing hormone receptor activation stimulates endothelial adhesion of neoplastic canine T-lymphocytes
Alexa Dietz

Suitability of non-cycling recipient mares for in vitro produced equine embryos
Charles Scoggin, DVM, MS, DACT

The temporal associations of B-mode, Power doppler, and ovarian steroid changes of the periovulatory follicle and corpus luteum during luteogenesis and luteolysis in jennies
Humberto Magalhaes, DVM, MSc

Cholesterol-loaded cyclodextrin improves cooling and fertility of donkey semen
Lorenzo Segabinazzi, DVM, MS

Characterization of reproductive parameters from Pennsylvania bull elk (Cervus canadensis)
Luisa Ramirez-Agamez,, DVM

Serum prostaglandin E metabolite (PGEM) in diestrus and pregnant mares
Mariana Diel de Amorim, DVM, DVSc, PhD, DACT

Expression and abundance of prostaglandins in the healthy and fibrotic mare endometrium
Michael Byron, MSc

Umbilical vein catheterization as an option for intravenous access in neonatal puppies
Soon Hon Cheong, DVM, PhD

Thursday, July 22

Bartlett Award and Address
Theriogenologist of the Year Award and Address
Dr. Jerry Rains Memorial Abstract Competition
sponsored by Merck Animal Health
SFT Business Meeting
ACT Business Meeting
Student Case Presentations
Student Research Presentations
Student/Mentor Reception
Opening Reception in the Exhibit Hall

Dr. Jerry Rains Memorial Abstract Competition
sponsored by Merck Animal Health

Comparison of nanoparticles and single-layer centrifugation for separation of dead from live stallion spermatozoa
Christian Bisiau, DVM

No adverse effect of air exposure on stallion sperm motility after 48 hours of cooled storage
Emily May, DVM

Epididymal sperm granulomas are associated with antisperm antibodies in frozen-thawed donkey semen
Giorgia Podico, DVM, MSc

Laser ablation of the equine oviductal papilla as a novel contraceptive technique
Grace Edwards

Clinical and physiological ultrasonography of normal and abnormal donkey pregnancies
Humberto Magalhaes, DVM, MSc

Comparing serum progesterone measurements by a point-of-care analyzer with a chemiluminescent immunoassay in breeding management of the bitch
Julia Zuercher

Mammary gland electrolytes and pH to detect impending parturition in jennies
Lucas Canuto, DVM, MSc

The effects of Kisspeptin-10 on equine chorionic girdle trophoblast cell in vitro migration
Viviane Gomes, DVM, DACT

Student Case and Research Presentations *sponsored by Bovine Services, LLC.*

Incidence of and factors affecting congenital defects in miniature Dachshunds
Colleen Kutzler

Electroejaculation and breeding soundness exam on a clouded leopard
Taylor Lashlee

Preputial prolapse and injury in a Brahman bull
Maryanna Hudson

Atypical mammary mass in an intact geriatric female Labrador retriever
Colleen Kutzler

Abnormal mobility in neonatal Labrador Retrievers
Jordan Farrell

Recurrent uterine torsion in an Arabian mare
Vaiva Palunas

Dystocia and diaphragmatic hernia in a Quarter Horse mare carrying twins
Vaiva Palunas

Vulvar discharge associated with exogenous estrogen exposure in a spayed Weimaraner bitch
Camille Ogdon

Phimosis and preputial abscessation with draining tract in an Angus bull
Rachael Doenges

Spermatic cord torsion in an Arabian stallion
Hannah Neer

Friday, July 23

Plenary Session
Scientific Abstract Presentations
Scientific Abstract Poster Presentations
Species Tracks
Awards Dinner Reception
Awards Dinner, Veterinary Story Night, & Live Auction
sponsored by Merck Animal Health

Plenary Session
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History of the ACT: Celebrating 50 Years
John Kastelic, DVM, MS, PhD, DACT

Surrogate sires: A next generation breeding tool in livestock production
Jon Oatley, MS, PhD

Case Abstracts

Reduction of equine monozygotic twins using craniocervical dislocation via colpotomy
Adam Bassett, DVM, MS

Proteomic analysis of sperm from a subfertile stallion with impaired acrosomal exocytosis
Camilo Hernandez-Aviles, DVM

Anaphylactic reaction following intrauterine application of misoprostol in a Friesian mare
Jordan Kiviniemi-Moore, DVM

Polled intersex syndrome in a four month old Finnish Landrace lamb
Katelyn Waters, DVM

Chronic seminal vesiculitis and blocked ampullae in a Gypsy Vanner stallion
Lauren Huggins, VMD, MS

Thoraco-omphalopagus conjoined twins in a Standardbred mare
Matthew Cochran, DVM

Next-Generation Sequencing to aid decision making for when to discontinue antibiotic treatment in a Stallion with Actinomyces spp. in semen
Soon Hon Cheong, DVM PhD

Thromboembolic disorder in a post-cesarean section bitch
Yamilka Lago-Alvarez, DVM

Equine Abstracts

Low volume uterine lavage: advantages for use in problem mares
Christina Divine, DVM

Pre-partum amniotic rupture in a Thoroughbred mare
David Alexander, DVM

Daily sperm out, spermatogenic efficiency, and sexual behavior of donkey jacks mounting jennies in estrus
Humberto Magalhaes, DVM, MSc

Pregnancy rates and ovarian activity following uterotubal infusion of n-butyl cyanoacrylate via a hysteroscopic approach in mares: a pilot study
Irwin Liu, BSc, DVM, PhD

Oocyte collection rate and in vitro embryonic development in the mare with administration of low-dose deslorelin
Jennifer Hatzel, DVM, MS, DACT

Evaluation of passively acquired rabies antibody titers and immune responses in healthy foals vaccinated against at 4 or 6 months of age
Karen Wolfsdorf, DVM, DACT

Prospective ultrasonographic evaluation of the caudal placenta and cervix in pregnant mares in relationship to foaling outcomes and placental abnormalities
Kristina Lu, VMD, DACT

The use of progesterone and estradiol-17-beta prior to transvaginal aspiration of oocytes
Peyton Draheim, DVM

Mixed Species Abstracts

Using reflection to optimise student engagement during theriogenology practical classes; the benefits of mentoring and peer support in teaching
Allan Gunn, BVM&S, DACT, FRCVS

Screening canine sera for smooth Brucella strain antibodies via Brucella abortus Fluorescent Polarization Assay
Alyssa Helms, DVM

Sperm acrosome associated 3 protein expression in the feline ovary decreases with age
Ann Ramsey

Infertility, pregnancy, and concurrent pyometra in a Greater Swiss Mountain Dog
Brittany Shumack, DVM

Fetal loss at time of elective cesarean section in a Greater Swiss Mountain Dog
Brittany Shumack, DVM

The agreement level among four techniques for analysis of stallion sperm morphology
Camilo Hernandez-Aviles, DVM

Factors affecting survival and future foaling rates in thoroughbred mares with hydrops
Louise Lemonnier, DVM

Vaginal vault diverticulum causing functional urinary obstruction in a maiden bitch
Marthina Greer, DVM

Comparison of 2D and 3D ultrasonography for gestational aging in dogs
Sabina Pestelacci, DVM

Production Animal Abstracts

Melatonin and l-arginine mitigate heat stress-induced reductions in quality of frozen-thawed ram sperm
Abdallah Mohamed, BVSc, MSc

An example of incorrect storage of bull semen samples on spermiogram assessment
Allan Gunn, BVM&S, DACT, FRCVS

Association of metabolic status with uterine diseases and reproductive outcomes in lactating Holstein dairy cows
Andrew Hubner, DVM

Effect of GnRH administered at the time of AI for dairy cows detected in estrus by an activity monitoring system or by conventional estrus detection
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Evaluation of ovarian response to PG600 in alpacas
Eduardo Arroyo, DVM

Morphometric characteristics, testicular histology, and semen parameters of two mature hybrid bucks born from white-tailed deer dams sired by a mule deer buck
Giorgia Podico, DVM, MSc

A preliminary study on bovine prenatal sex determination using PCR on maternal peripheral blood
Navgeet Singh, BVSc & AH, MVSc

Obstructive urolithiasis in a Dromedary camel
Yatta Boakari, DVM, MS, PhD

Abstract Poster Presentations

Ovine male pseudohermaphrodite (Katahdin) with testes adjacent to the mammary gland
Alyssa Ball, DVM, MS

Reproductive tract findings in a Thoroughbred filly with non-patent uterus, considered congenital failure of a normal uterine lumen
Christine Bartley, DVM

Identification of fetal bones in the uterus of a Thoroughbred mare
Ethan Sutherland, DVM

Respective pregnancy rates and subsequent pregnancy losses of in-vitro produced embryos from oocytes aspirated at two facilities, transported to 3 commercial ICSI labs and resulting embryos transported and transferred at two commercial recipient herds
Etta Bradecamp, DVM, DACT, DABVP

Luteal blood flow and side effects of luteolytic doses of dinoprost tromethamine and cloprostenol sodium in jennies
Humberto Magalhaes, DVM, MSc

Induction of parturition in a late gestation Thoroughbred broodmare with colon displacement
Lauren Pasch, DVM, DACT

Methods to prepare platelet-rich plasma for use in mare practice
Lorenzo Segabinazzi, DVM, MS

Sperm-filter enhanced semen parameters and fertility of stallion poor cooled semen
Lorenzo Segabinazzi, DVM, MS

Intra-uterine injection of amnion-derived acellular bioscaffold product in mares, a description of systemic and intra-uterine effects over 21 days
Nathaniel Newton, DVM,

Incidence rate of reproductive problems in non-pregnant mares
Patrick McCue, DVM, PhD, DACT

Effect of Hytryl on fresh-cooled semen motility in two semen extenders when stored at 5 degrees Celsius for 72 hours
Rachel Fladung, DVM

Uterus unicornis in a maiden Standardbred mare
Stephanie Walbornn, DVM, DACT

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Reproductive biology and assisted reproductive technologies in felids
Nucharin Songsasen, DVM, PhD

Current and future perspective of reproductive technologies in domestic and wild canids
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Comparison of OFA and PennHIP results for canine hip dysplasia screening
Robyn Wilborn, DVM, DACT
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Single cell transcriptomics to understand germ cell function in livestock
Jon Oatley, MS, PhD

Pregnancy loss in ruminants
Andres de la Concha, DVM, PhD

Bovine trichomoniasis
Jeff Ondrak, DVM, MS

Equine Track
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Examination of fresh, cooled and frozen semen
sponsored by the Michelle LeBlanc Equine Memorial Lectures
Charles Love, DVM, PhD

The use of immunomodulators in equine reproduction: How do they work and when should they be used
Carleigh Fedorka, PhD

Use of nonantibiotic therapies for endometritis: What, when, and why
Kristina Lu, VMD, DACT

Saturday, July 24

So you want to become a Diplomate?

Species Tracks
Small Animal Track

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Medical control of reproduction in the bitch: an update
Alain Fontbonne, DVM, PhD, DECAR

Medical control of reproduction in the male dog: the experience of Alfort Veterinary College
Alain Fontbonne, DVM, PhD, DECAR

Medical control of reproduction in the cat: old and new drugs
Cindy Maenhoudt, DVM, DACT, DECAR

Use of anti-progestins in Europe
Cindy Maenhoudt, DVM, DACT, DECAR

Is there a future for surgical contraception in the dog
Alain Fontbonne, DVM, PhD, DECAR

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Pregnancy loss in cattle
Andres de la Concha, DVM, PhD

Pregnancy loss in small ruminants
Andres de la Concha, DVM, PhD

The use of laboratory in pregnancy loss
Andres de la Concha, DVM, PhD

A review of bovine trichomoniasis
Jeff Ondrak, DVM, MS

Diagnosis in bovine trichomoniasis
Jeff Ondrak, DVM, MS

Management and control of bovine trichomoniasis
Jeff Ondrak, DVM, MS

Pharmacokinetic of antibiotics on plasma and semen in beef bulls
Juan Romano, DVM, MS, PhD

Equine Track
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Breeding the aged mare: Which management plans are best and what are the downfalls
David Scofield, DVM, MS, DACT

Management and considerations of the post-partum mare and foal
Justin McNaughten, BVMS, DACT

Prebreeding season/prepurchase examination of the stallion: U/S testes, semen evaluation and considerations
sponsored by the Michelle LeBlanc Equine Memorial Lectures
Charles Love, DVM, PhD

Extending cooled semen: How and what?
David Scofield, DVM, MS, DACT

Reinforced breeding: What and How?
David Scofield, DVM, MS, DACT

Use of progestins in the mare
Carleigh Fedorka, PhD

Reproductive surgeries: Why are they necessary and which ones are best for the specific problem
Justin McNaughten, BVMS, DACT

Acupuncture and Chinese herbal medicine in equine reproduction
Kristina Lu, VMD, DACT

To learn more about transportation, places to dine, upcoming events, and more, please click the Visit Omaha link below.

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TF SILENT AUCTION 25.0

The passing along of boutique items from colleague-to-colleague through the silent auction has become a signature event of our annual conference. We are limiting the number of items to 25 again this year as it worked out well last year! Here are some suggestions for items:

*Educational items (text books, etc.)
*Arts and crafts
*Liquor/wine
*Sporting events
*Veterinary equipment

Once you decide on what your priceless creation or generous gift will be, please e-mail the attached form along with pictures of the item(s) by June 15th, 2021 to: jgfloydjr@gmail.com.

Get your donation into the spotlight by submitting early…….the final item list will be posted on the website by July 1 so the membership has a month to start saving their dollars to bid!

After 25 items have been selected, we will respectfully ask you to hold your gift until 2022!

No on-site donations can be accepted this year.

Thank you for your continued support of the Theriogenology Foundation Silent Auction!!!

We look forward to seeing you in Omaha!!

Click here for the Silent Auction Donation Form.

Awards Dinner and Story Night

This year's live auction will be held in conjunction with the Veterinary Story Night. Captivating stories will be paired with unique auction items. This year's event will feature stories by Dr. Isaac Bott, Dr. Kara Kolster, Dr. Andrew Lovelady, Dr. Cliff Shipley, and Mr. Jody Wade.

The 2021 Nandi Scholars will also be announced during the Awards Dinner.

Purchase your ticket at: therio.org.

Sealed bids for live and silent auction items are accepted until July 23. Items will be posted online and updated as submitted. Please complete the sealed form on the TF website at: http://www.theriofoundation.org/?page=sealedbidform.

Craft Beer Tasting Fundraiser for the Theriogenology Foundation

During the Opening Session Reception Wednesday evening, July 21 or during the Exhibit Hall Opening Reception, Thursday, July 22, attendees will have the chance to experience some locally brewed craft beer and help the Theriogenology Foundation at the same time. For $40 you will receive four samples of beer. Once you have sampled and chosen your favorite, you will get a full serving of that beer. Proceeds will benefit the Theriogenology Foundation. Tickets may be used at the event of your choice.

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CHAPTER ELEVEN *Anatomy and Physiology of Reproduction in the Mare*

Traditionally, the mare has been regarded as having a low reproductive efficiency because many mares that had successful performance records were kept as broodmares without consideration of their existing reproductive problems. Reproduction in the mare is a complex process, and she is unique in several ways, including anatomical site of ovulation, follicular growth patterns, spontaneously prolonged corpora lutea, differentiation of fertile and nonfertile ova in the Fallopian tubes, and serum gonadotropin secretion (in pregnant mares). To be a successful horse breeder and to induce the difficult broodmare to conceive and foal, one must have a working knowledge of the anatomy of the reproductive tract and of the physiology of reproduction in the mare.

11.1 Reproductive Tract

The mare's reproductive tract is shown in Figure 11-1. The tract consists of 2 ovaries, 2 Fallopian tubes, uterus, cervix, vagina, clitoris, and vulva.

Ovaries

The ovaries function as endocrine and exocrine glands and are the essential organs of reproduction. As endocrine glands, they produce the estrogenic and progestational hormones whose functions are described in Chapter 12. As exocrine glands, they produce and release ova. The anatomical structure of the mare's ovaries has been described by Sisson and

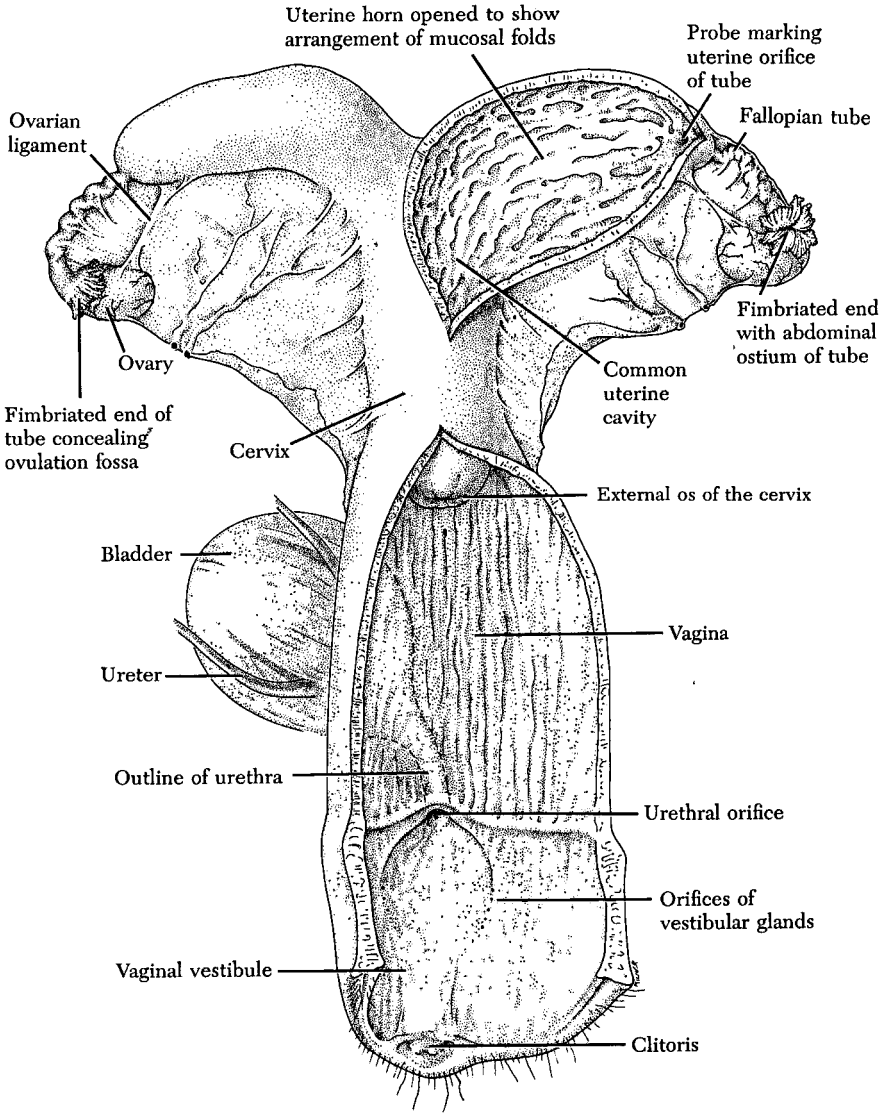


FIGURE 11-1
Reproductive organs of the mare (*posterior view*) showing vagina and right half of uterus opened. From Eckstein and Zuckerman. 1956.

Grossman (1953) as bean- or kidney-shaped because of the presence of a definite *ovulation fossa* (Figure 11-2). The size of the ovaries varies according to the age, breed, size, and reproductive state of the mare. In the mature mare, each ovary weighs approximately 50–75 g and is 6–7 cm long and 3–4 cm wide. Hammond and Wodzicki (1941) have reported that the mature ovary reaches maximum size at 3–4 years of age and then decreases in size. Although one ovary is frequently larger than the other,

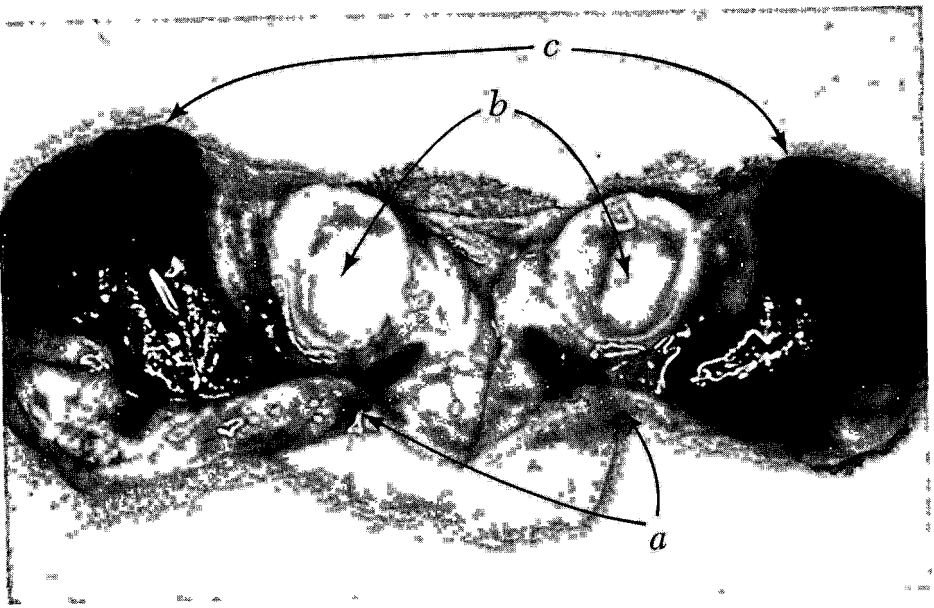


FIGURE 11-2
Cross section of a mare's ovary: *a*, ovulation fossa; *b*, Graafian follicles; *c*, corpus hemorraghicum. Photograph courtesy of John Hughes.

these researchers found that the average weights for each side from several mares were not significantly different. Nishikawa (1959) has studied a Japanese sample and reported that the ovaries of the jenny ass are similar in size and shape to those of the mare, whereas Berliner (1959) has observed that in the United States the ovaries of the jenny ass are larger than mare ovaries.

A *serous coat* that is part of the *peritoneum* covers the outer surface of the ovaries except at the *hilus*, where the blood vessels and nerves enter the ovaries, and at the ovulation fossa, which is covered by a layer of primitive germinal epithelium. The inside of the ovaries is composed of connective tissue and numerous follicles that are in varying stages of development during the breeding season. The *follicles* are not confined to a particular area or outer layer, as they are in other animals, but are distributed throughout the ovary.

The follicles are classified as being either primordial, growing, or Graafian. Primordial follicles consist of an *oocyte* surrounded by a flattened layer of cells known as the *zona pellucida*. As the primordial follicle starts to develop, it becomes surrounded by several layers of granulosa cells and is referred to as the growing follicle. The growing follicle continues to develop and forms the Graafian follicle. The Graafian follicle (Figure 11-3) is surrounded by the granulosa cells and by the *theca folliculi*, which has developed from the connective tissue surrounding the follicle. The theca folliculi differentiates into two layers called the *theca externa* and *theca interna*. The theca externa is a fibrous network

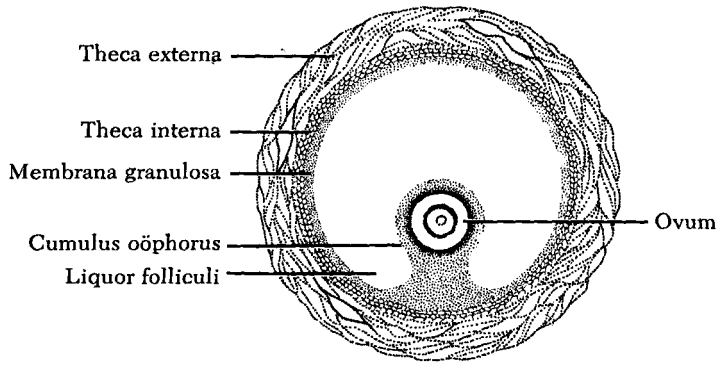


FIGURE 11-3
A Graafian follicle. Diagram courtesy of M. Morris.

that forms a supporting structure for the Graafian follicle. The theca interna, the inside layer of the theca folliculi, contains the vascular supply, and the cells that compose it are responsible for secreting the estrogenic hormones. The granulosa cells continue to increase in number. A cavity or antrum forms within the granulosa cells. At maturity, the cavity or antrum of the Graafian follicle is surrounded by the granulosa membrane and is filled with follicular fluid. Granulosa cells forming the *cumulus oöphorus* surround the ovum and form a stalk that projects the ovum toward the center of the antrum. As the follicle grows and develops, it migrates toward the ovulation fossa. Ovulation (rupture of the follicle) is spontaneous, and extrusion of the egg occurs in the ovulation fossa.

The development of the *corpus luteum* has been described in detail by Harrison (1946) and by many other investigators. Upon ovulation, the follicle walls collapse and fold inward. A blood clot forms in the cavity as a result of hemorrhage of the thecal capillaries (Figure 11-2). The granulosa cells undergo *lutealization,* and the theca interna cells invade the granulosa wall. The folds of the wall take on the appearance of *trabeculae,* which consist of theca externa cells and degenerating theca interna cells. The theca externa cells and degenerating theca interna cells invade the lutealizing granulosa cells. The trabeculae are extensively vascularized, and thecal capillaries exist between the luteal cells. By the sixth day, the whole corpus luteum is well vascularized, the theca cells are evenly distributed, the trabeculae are decreased in size and serve as a support for blood vessels, and the theca externa cells begin to pass inward. After approximately 14 days, the corpus luteum decreases in size, and its progestin-synthesizing capabilities decline quite rapidly if the mare is not pregnant.

Blood is supplied to the ovaries via the ovarian artery (Figure 11-4). The ovaries are supplied with an extensive arterial network that forms

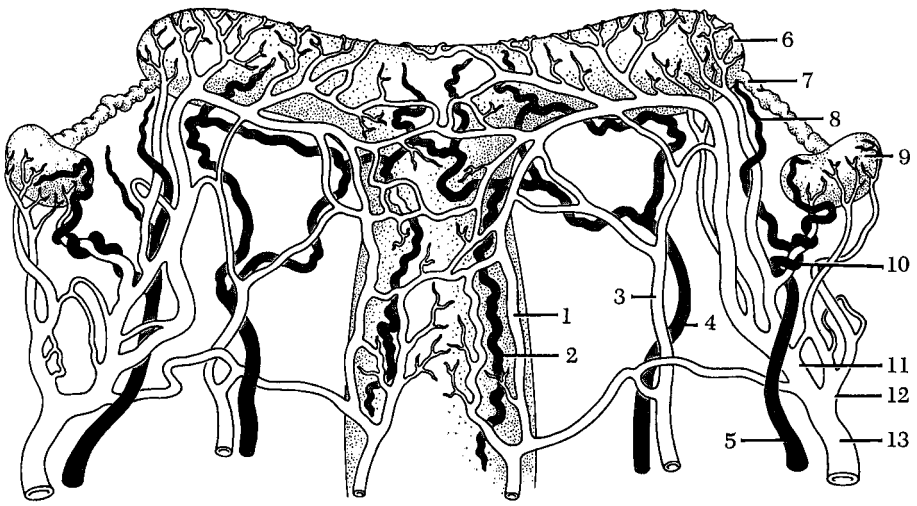


FIGURE 11-4
Composite diagram of dorsal view of arteries (*cross-marked*) and veins (*shaded*) of uterus and ovaries of the mare: (1) uterine branch of vaginal vein; (2) uterine branch of vaginal artery; (3) uterine vein; (4) uterine artery; (5) ovarian artery; (6) uterine horn; (7) uterine tube; (8) uterine branch of ovarian artery; (9) ovary; (10) ovarian branch of ovarian artery; (11) uterine branch of ovarian vein; (12) ovarian branch of ovarian vein; (13) ovarian vein. From Ginther, Garcia, Squires, and Steffenhagen. 1972.

anastomoses with tributaries of the uterine branch of the ovarian artery. Several veins drain the ovaries and then unite to form the ovarian branch of the ovarian vein. The drainage is such that there is an extensive utero-ovarian plexus. The nerve supply is derived from the renal and aortic plexus of the sympathetic system.

Fallopian Tubes

The Fallopian tubes or oviducts conduct the ova from the ovary to the uterus and are the usual site of fertilization. They are approximately 30–70 mm long and 4–8 mm thick and have an inside tubal diameter of 2–3 mm. The end adjacent to the ovaries is expanded to form the *ampulla* and the *infundibulum,* which partially surrounds the ovary and directs the ova into the Fallopian tube. The edge of the infundibulum is split so as to form fingerlike projections, or fimbriae. The opening of the oviduct into the peritoneal cavity is called the *ostium abdominale,* and the uterine opening is called the *ostium uterinum.* Three layers of tissue compose the Fallopian tubes: tunica serosa, muscularis (longitudinal and circular), and mucosa.

Uterus

The uterus of the mare consists of a body and 2 horns. The horns, which are located in the abdominal cavity, are approximately 25 cm in length and cylindrical. The body of the uterus is located partly in the abdominal cavity and partly in the pelvic cavity. The body is approximately 18–20 cm long and 10 cm in diameter. The broad ligament of the uterus attaches it to the abdominal and pelvic walls.

Three coats of tissue similar to the ones forming the oviducts form the uterine wall. The serous coat is continuous with the broad ligament. The muscular coat consists of a muscularis longitudinalis, muscularis vascularis, and muscularis circularis. The muscularis longitudinalis and muscularis vascularis form one inseparable layer of muscle that can be separated from the muscularis circularis. The third layer of the uterus or mucosa is composed of a loose connective-tissue network (tunica propria), a deeper layer containing the long, tubular-shaped uterine glands, and the stratum epithelial.

Uterine blood supply is derived on both sides from 3 arteries: the uterine branch of the vaginal artery, the uterine artery, and the uterine branch of the ovarian artery. Three veins drain the uterus and correspond to the 3 arteries. Nerves derived from the uterine and pelvic plexus provide the nerve supply to the uterus.

Cervix

The cervix separates, anatomically and physiologically, the body of the uterus from the vagina. It is approximately 5–7.5 cm in length and consists of a powerful, well-developed muscular layer. Extensive folding of the muscle layer leaves no well-defined cervical canal. The mucosa of the cervix contains mucosal cells that secrete the mucus found in the vagina. The cervix protrudes into the vagina, forming a well-defined angle between the cervix and vagina known as the fornix.

Vagina

The vagina extends from the cervix to the vulva. It is approximately 15–20 cm in length and 10–12 cm in diameter. The vagina is divided into the vagina proper and the vestibulum vaginae. The hymen separates the two parts and is just anterior to the area where the urethra enters the reproductive tract. The mucous coat contains no glands, is composed of a layer of loose connective tissue, and is covered with a stratified epithelium. Longitudinal and circular muscle fibers are surrounded by a *fibrous adventitia.*

Blood supply to the vagina is via the internal pudic arteries and drainage is via the internal pudic veins. The nerve supply is from the pelvic plexus of the sympathetic nervous system.

Vulva

The vulva is the terminal part of the reproductive tract. It is 10–12 cm in length and the external orifice is a vertical split approximately 12–15 cm long. The external urethral orifice is located 10–12 cm inside the vulva, and the glans clitoris is located approximately 5 cm within the vulva. The clitoris is the homologue of the male penis.

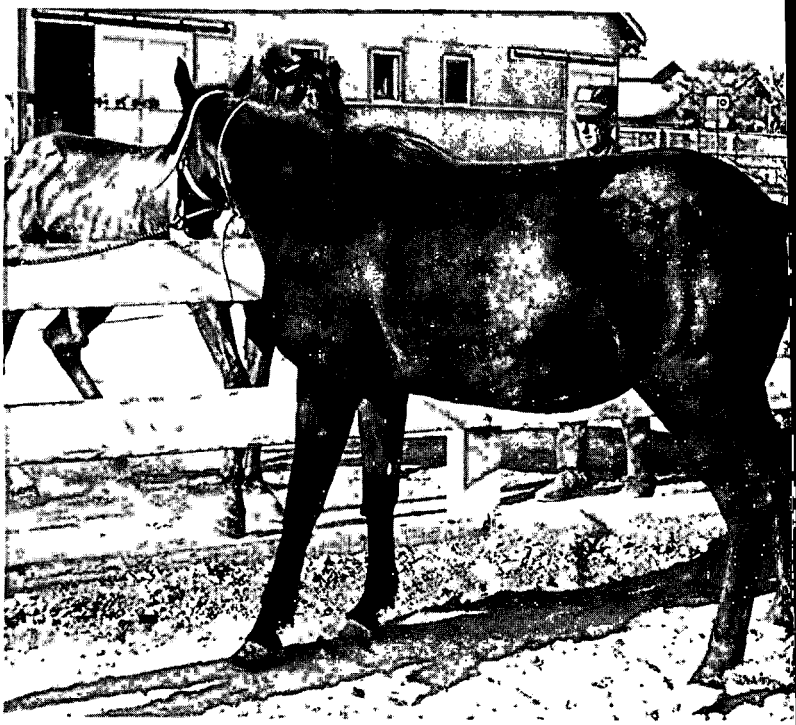
Mammary Gland

The udder is located in the inguinal region and is composed of 2 gland complexes. The 2 teats are broad and flat. Each teat is canalized by at least 2 streak canals that lead from separate teat cisterns. Each teat cistern is connected to a gland cistern that has a system of ducts leading into it from a secretory gland. The udder is served by the external pudendal artery and is drained by the external pudendal and subcutaneous abdominal veins. The nerve supply is derived from the inguinal nerve.

11.2 Physiology of Reproduction

Estrous Cycle

The average age of the filly at puberty is 12–15 months. Russian researchers have reported that crossbred Russian trotter mares reach sexual maturity at 10–11 months. Once puberty is reached, the filly begins to come into estrus (heat) in a rhythmic cycle. The estrous cycle is the interval from the onset of estrus until the onset of the next estrus. In the mare, it is commonly divided into 2 periods for practical purposes. The follicular phase or estrous period of the cycle is the period during which the mare shows behavioral signs of estrus, has rapid follicular growth, and finally ovulates. During the estrous period, the mare is interested in and is receptive to a stallion. Most mares in estrus display several characteristic behavioral signs when teased with a stallion (Figure 11-5). Initially, a mare may be agitated but will respond to the stallion and allow him to bite her on the neck and flank. Her stance is characterized by a raised tail without switching, hind legs spread apart, and pelvis flexed. The labia



a

b

FIGURE 11-5
Behavioral display of estrus (heat) by a mare: *a*, elevated tail, flexed pelvis, and acceptance of stallion; *b*, eversion of clitoris (winking).

of the vulva contracts and relaxes, and there is eversion of the clitoris, which is commonly referred to as "winking." Estrual winking, alone, should not be confused with winking after urination. Mares in estrus usually urinate quite frequently when teased.

Sexual receptivity is influenced by season. The behavioral signs of mares coming out of anestrus are rather variable, that is, some mares

have a normal estrous period whereas others are in and out of estrus for several days.

The behavioral signs of estrus in the jenny are quite different from those in the mare. Jennies will stand for the jackass with their hind legs spread apart. The head is lowered, the jaws display a chewing motion, and the labia repetitively contracts, exposing the clitoris. Jennies urinate frequently, and quite often a slimy mucus is discharged from the vagina.

In both the mare and jenny, the intensity of estrus manifestations increases from weak signs at the start of estrus to a peak at ovulation and then quickly declines to the diestrous state.

The diestrous period is the luteal phase of the cycle and begins with ovulation. During *diestrus,* the corpus luteum is formed and secretes progestins. The behavioral response of a diestrous mare to teasing with a stallion varies from a passive, noninterested attitude to complete resentment, often manifested by violent attempts to kick and bite the stallion.

The change from behavioral estrus to diestrus occurs quite rapidly after ovulation. Hughes *et al.* (1972b) have reported that approximately 50 percent of the mares they studied were in diestrus within 24 hours after ovulation and 80 percent were in diestrus within 48 hours. This finding emphasizes the close relationship between ovulation and the end of sexual receptivity. Occasionally, a mare will ovulate after she has ceased showing signs of estrus.

Estrous cycles of the mare are usually 21–23 days in length (Table 11-1). A wide range of estrous cycle lengths has been reported in the literature. Cycles vary from 7–175 days, but generally, approximately 50–60 percent of all mares have a cycle of 21, 22, or 23 days.

There are many causes of prolonged estrous cycles. Season of the year is the most common cause of irregularity. In California, the average estrous cycle length is shortest between April and October and longest between November and March. In South Africa, the average estrous cycle is shortest during the summer months of November through February and longest during the winter months of May to August. Winter *anestrus* (period of sexual inactivity) is responsible for the longer average length of time from one estrus to the next between November and March (Figure 11-6) (Hughes *et al.*, 1972b). During the winter months, ovulation often occurs without estrus. The estrous cycles can also be interrupted by spontaneous prolongation of the corpus luteum life-span (Figure 11-7). A spontaneously prolonged corpus luteum is a corpus luteum that continues to secrete enough progestins to maintain plasma levels above 3–5 ng/ml plasma, which suppresses signs of estrus. The spontaneous prolongation may be due to a failure of the uterus to produce or release the "luteolysin" required to lyse the corpus luteum at the proper stage of the cycle. An interruption of regular cyclicity may occur during very hot weather and was observed by Hughes *et al.* (1972a) in a 3-year study of a group of mares. Prolonged cycles are often merely a result of the stud

TABLE 11-1
Length of estrous cycle and estrous period of mares with 1 or 2 ovulations per cycle

No. of Ovulations per Estrous Cycle	Length of Estrous Cycle (days)	Length of Estrus (days)	Length of Diestrus (days)	Corpus Luteu Life-span (days)
One	20.7 ± 0.2 (234) (range 13–34)	5.4 ± 3.0 (225) (range 1–24)	15.4 ± 3.1 (201) (range 6–25)	12.6 ± 2.9 (1 (range 8–18)
Two	20.2 ± 4.5 (76) (range 13–27)	6.1 ± 2.7 (69) (range 1–21)	14.6 ± 3.5 (59) (range 8–25)	12.2 ± 3.3 (1 (range 5–17)

SOURCE: Figures in last column from Stabenfeldt, Hughes, and Evans. 1970. All other figures from Hughes, Stabe feldt, and Evans, 1972b.

NOTE: All deviations are standard deviations. Same 11 mares were studied to obtain data for Tables 11-1 through 11-

manager's failure to observe estrus. Another problem that is frequently encountered is that lactating mares that are extremely possessive of their foals may not show estrus.

The seasonal variability of the estrous cycle, excluding anestrus, seems mainly to be due to variability of the estrous period (Table 11-2). The normal length of estrus is usually 5–7 days, but from February to May (late winter and early spring in California), it is 7.6 days. The longest estrous periods are observed when mares are either going into or coming out of anestrus. Throughout the year, the diestrous period seems to average 14–16 days in length when anestrous periods are excluded.

Loy (1970) has classified mares into 3 groups according to seasonal variability. There is considerable overlapping of his classifications because of the variability of mares. In the first category is the polyestrous mare, which cycles regularly throughout the year even though she has seasonal variations that fall within the normal range. The second group includes the seasonally polyestrous mare, which has a definite breeding season and a definite anestrous period (Figure 11-6). The third classification is the seasonally polyestrous mare (Table 11-2), which has erratic reproductive patterns. "Silent heat," the failure to show behavioral signs of estrus, occurs infrequently in some mares, although other mares seldom show behavioral signs (Figure 11-8). Usually these mares ovulate and their plasma progestin profile is normal. Approximately 50 percent of mares ovulate at least once without estrus during a 2-year period. The opposite condition, estrus without ovulation, is less frequently observed—it occurred in approximately 3 percent of estrous periods during a 2-year period (Hughes *et al.*, 1972b). Nonovulatory estrus is usually observed immediately preceding the onset of winter anestrus. Split estrous periods, in which the mare is in estrus for a few days, out of estrus for a few days,

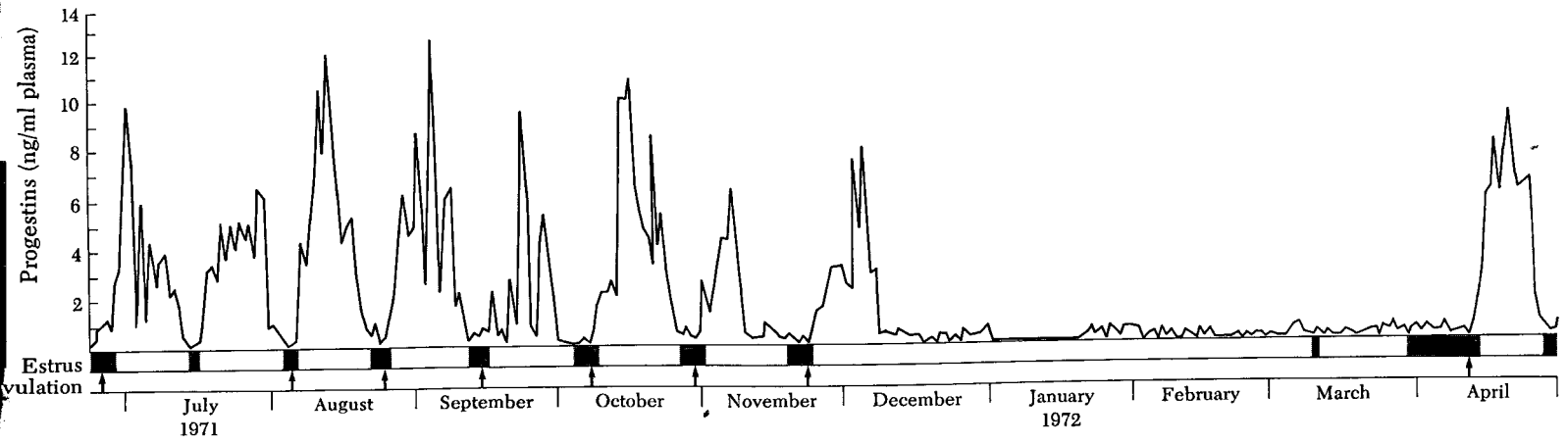
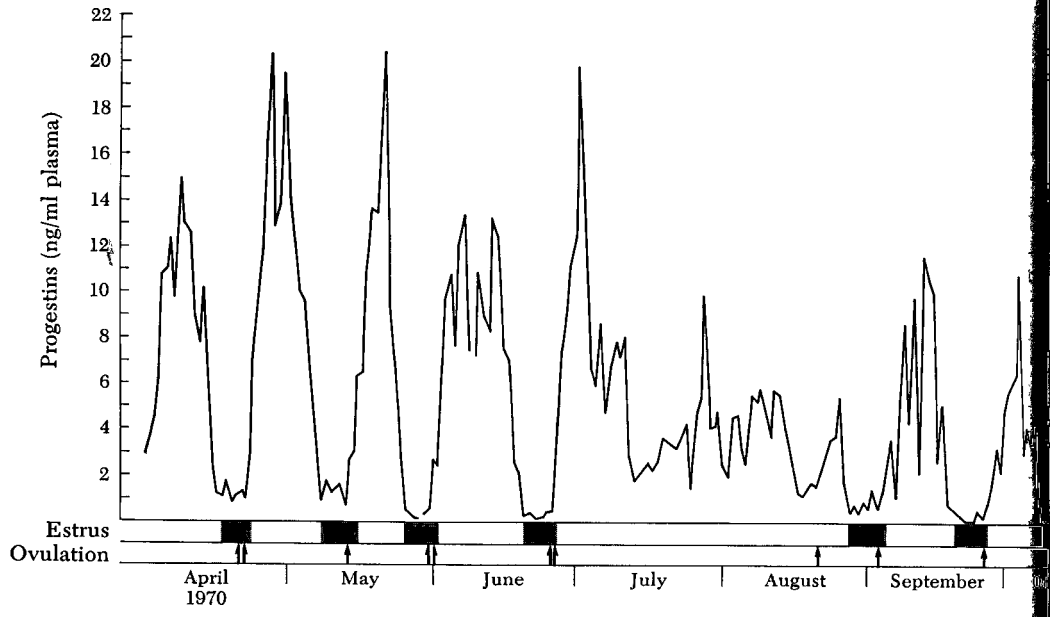
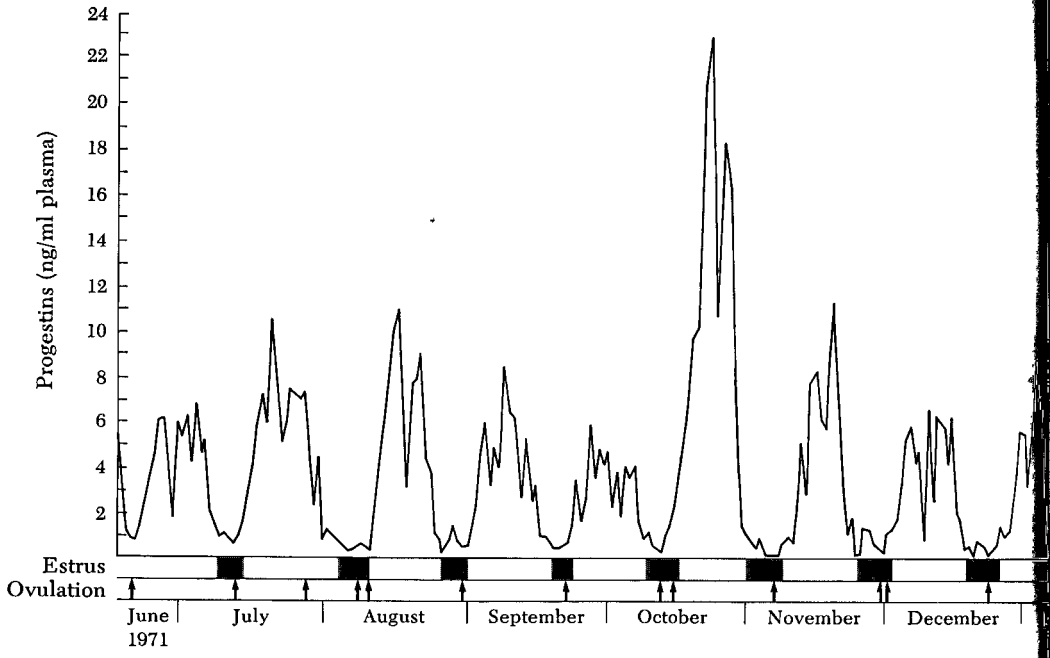


FIGURE 11-6
Reproductive history of Hi Aggie (■ = estrus; ↑ = ovulation). From Hughes, Stabenfeldt, and Evans. 1972b.


Progestins (ng/ml plasma)
22
20
18
16
14
12
10
8
6
4
2
Estrus
Ovulation
April
1970
May
June
July
August
September


Progestins (ng/ml plasma)
24
22
20
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July
August
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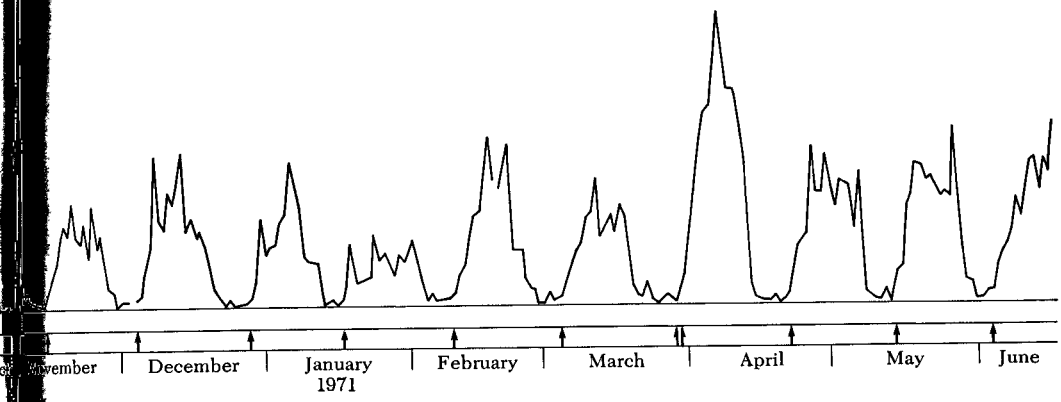
October
November
December



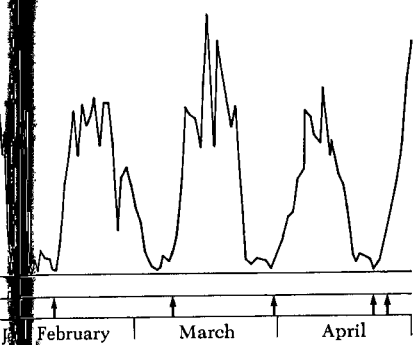


FIGURE 11-7
Reproductive history of Paisley Star (■ = estrus; ↑ = ovulation). From Hughes, Stabenfeldt, and Evans. 1972b. p. 119.

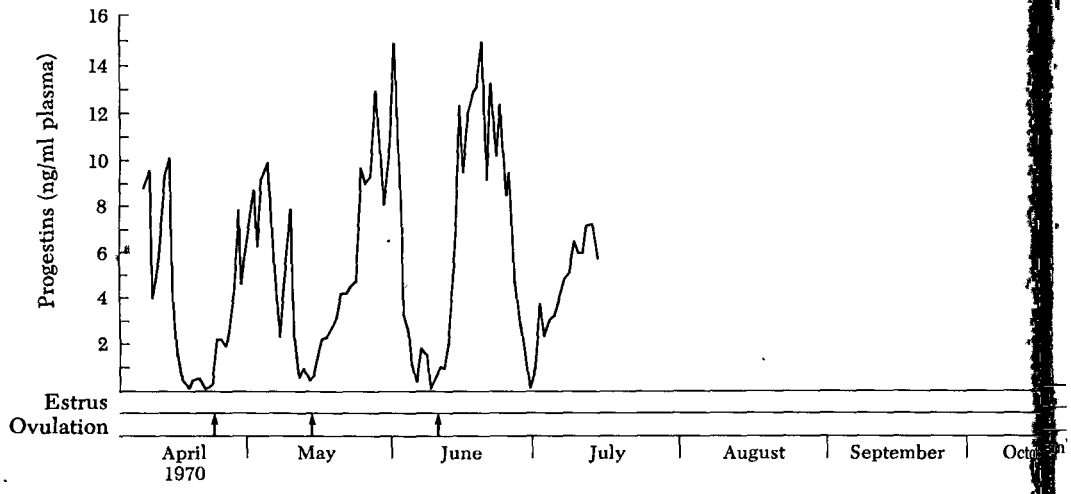


FIGURE 11-8
Reproductive history of Bashful Boots (▬ = estrus; ↑ = ovulation). Notice the "silent heat" periods and double ovulations. Graph courtesy of G. H. Stabenfeldt, J. P. Hughes, and J. W. Evans.

and then returns to estrus, are observed during approximately 5 percent of the cycles. Split estrous periods average 12 days in length.

Since most mares are seasonally polyestrus, periods of anestrus are observed in most mares that are observed for approximately 2 years. The winter anestrous period is usually 50–70 days in length but may be 4 or 5 months long. During the anestrous period, approximately 50 percent of mares ovulate at regular cyclic intervals. Other anestrous periods are accompanied by insignificant ovarian activity or the persistent presence of a corpus luteum. Even though anestrus is primarily a winter phenomenon, cyclic ovulation without estrus has been observed at other times of the year.

Diestrous ovulations without signs of estrus occur in most mares some time during a 2-year period (Figure 11-9, Table 11-1). The ovaries are the only part of the reproductive tract that is changed when ovulation occurs during diestrus. If the reproductive history of a mare is not known, the diestrous ovulation may be assumed to occur during a silent heat.

The mare is generally assumed to have one ovulation per estrous period; however, several follicles start to grow during each estrous cycle. Just before estrus, follicles destined to ovulate rapidly increase in size until they are to 35–60 mm long. When a group of Thoroughbred and Quarter Horse mares were observed at the University of California at Davis (Hughes *et al.*, 1972b), twin ovulations occurred approximately 21 percent of the time during a 2-year period (Figures 11-6 and 11-9 and

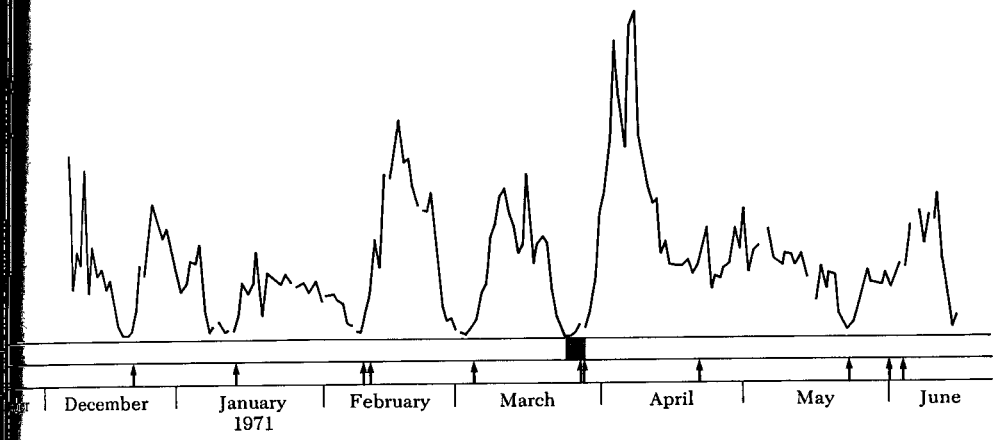


Table 11-3), and several other researchers have reported similar percentages. The percentage of twin conception is lower (1–5 percent). The percentage of live foals is 0.5–1.5 percent (Berliner, 1959). Some mares have a tendency to have multiple ovulations, and one mare had 25 twin ovulations in 24 months (Figure 11-9). Multiple ovulations do not appear to affect the duration of estrus, the length of the estrous cycle, or the level of plasma progestins (Table 11-1). A seasonal influence was indicated since few multiple ovulations were observed early in the breeding season (Table 11-4). Double ovulations are rare in pony mares.

Changes in the Reproductive Tract During the Estrous Cycle

The reproductive tract of the mare undergoes several characteristic changes during the estrous cycle. These changes are useful as a guide in determining the optimal time to breed a mare during estrus—especially mares with silent heats. The changes have been discussed by Warszawsky *et al.* (1972) and are summarized in Table 11-5.

During anestrus, the ovaries may be small and inactive, although some mares have considerable ovarian activity. As the mare comes out of anestrus, the ovaries begin to enlarge and soften as the follicles begin to develop. Although several follicles may start to increase in size, usu-

TABLE 11-2
Effects of seasonal changes on reproduction in the mare

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average estrous cycle length (days)	32.3	34.4	26.1	21.9	19.5	21.1	24.6	20.4	20.6	20.5	24.8	30.4
Number of cycles observed	22	20	25	28	39	31	28	27	32	35	25	21
Number of periods of estrus	18	13	26	29	28	28	20	24	27	31	27	22
Average length of estrus (days)	5.06	6.69	8.38	7.76	5.72	4.47	4.70	4.75	4.44	4.53	5.20	7.23
Total ovulations for each month for all 11 mares	20	23	46	45	44	54	48	41	42	44	43	27
Average number of ovulations per month	0.9	1.1	2.1	2.1	2.0	2.5	2.2	1.9	1.9	2.0	2.0	1.2
Total number of diestrous periods without anestrus	16	13	21	27	24	24	21	21	26	27	22	20
Average length of diestrus without anestrus	16.3	16.3	14.2	11.8	14.0	15.8	16.2	15.4	16.1	15.3	16.0	14.3

SOURCE: Adapted from Hughes, Stabenfeldt, and Evans. 1972b. p. 119.

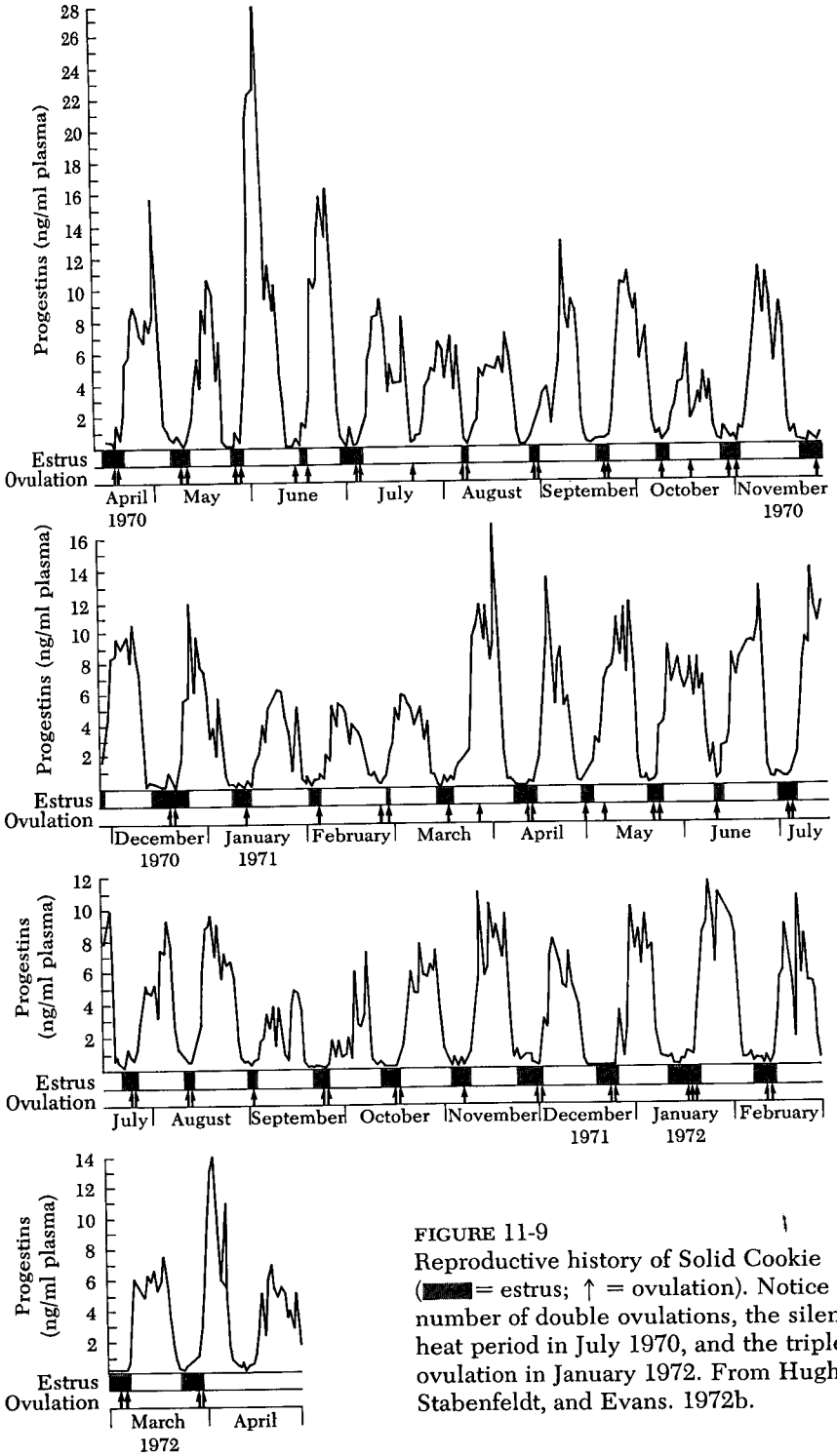


FIGURE 11-9
Reproductive history of Solid Cookie (■ = estrus; ↑ = ovulation). Notice the number of double ovulations, the silent heat period in July 1970, and the triple ovulation in January 1972. From Hughes, Stabenfeldt, and Evans. 1972b.

TABLE 11-3
Incidence of multiple ovulation by mare and by ovaries, during 2-year period

Mare	No. of Triple Ovulations	No. of Double Ovulations	Ovulations				Average Interval between Ovulations (days)
			No. LL	No. RR	No. LR[a]	No. RL[a]	
Aggie Tess	0	7	1	1	2	3	0.7
Bashful Boots	0	9	2	2	1	3	1.5
Double Dark	0	5	2	2	1	0	1.6
Hi Aggie	0	1	0	0	1	0	0.0
Mr. Bruce's Miss	0	2	1	1	0	0	1.0
Paisley Star	0	6	2	1	3	0	1.2
Psychedelic Miss	0	4	0	2	0	2	1.2
Solid Charm	0	4	2	0	1	1	1.0
Solid Cookie	1	25	3	9	10	3	0.7
Solimistic	0	10	2	2	3	3	1.5
Tule Goose	1	11	1	3	6	1	0.5
Total	2	83	16	23	28	16	1.0

SOURCE: Hughes, Stabenfeldt, and Evans. 1972b. p. 119.

[a]First letter indicates ovary in which first ovulation occurred.

TABLE 11-4
Multiple ovulations, by month

Month	Number per Month	Percentage per Month
January	3	23.5
February	4	22.2
March	10	40.6
April	9	25.7
May	10	32.3
June	10	25.5
July	9	25.0
August	7	21.2
September	8	24.2
October	7	20.0
November	6	17.1
December	4	18.2

SOURCE: Hughes, Stabenfeldt, and Evans. 1972b. p. 119.

TABLE 11-5
Changes in mare's reproductive tract during the estrous cycle

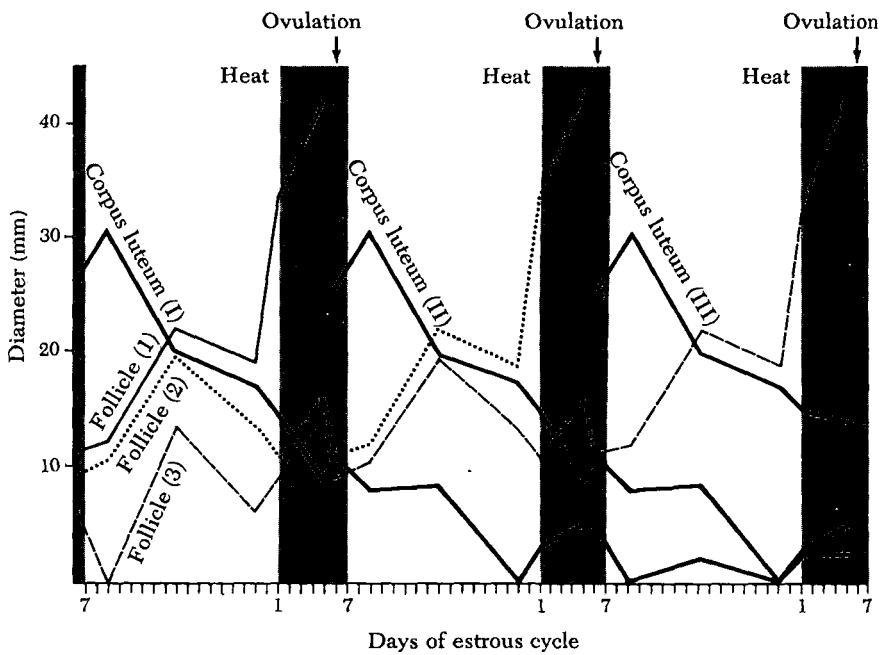
Structure	Day of Estrous Cycle				
	2	4	7	11	17
Anterior portion of vagina (g)	188	216	173	194	230
Cervix (g)	141	168	153	133	164
Uterus (g)	696	674	558	669	849
Uterine horn (left)					
Diameter (mm)	68	55	61	58	62
Length (mm)	151	146	152	154	137
Uterine horn (right)					
Diameter (mm)	58	60	44	61	64
Length (mm)	148	155	144	160	152
Oviduct (left)					
Weight (g)	5.57	5.72	4.85	6.17	5.20
Length (mm)	147	140	149	162	152
Oviduct (right)					
Weight (g)	7.12	6.83	6.02	6.61	6.61
Length (mm)	154	155	147	166	143
Ovaries	136.7	128.4	122.0	111.7	116.4
Extraluteal tissue (g)	71.9	69.6	70.5	69.0	81.8
Extraluteal fluid (g)	62.7	54.4	42.9	25.6	22.4
Follicles 10–30 mm (no.)	6.3	6.1	7.6	4.6	3.8
Follicles 20–30 mm (no.)	3.4	2.0	1.4	1.7	1.3
Follicles > 30 mm (no.)	1.3	0.9	0.5	0.2	0.1

SOURCE: Adapted from Warszawsky, L. F., *et al.* 1972. pp. 19–26, 172.

ally only 1 or 2 reach ovulatory size. The follicle destined to ovulate usually becomes quite prominent just before estrus and starts the rapid growth phase approximately 5 days before ovulation. Generally, maximum follicle size (40–50 mm) is reached the day before ovulation, but it may occur 3–4 days before ovulation. Some follicles soften just before ovulation, whereas others do not. Because of the variations in the occurrence and degree of softening, softening of the follicle cannot be used to predict ovulation accurately. Hughes *et al.* (1972b) observed that follicles that were 25–50 mm in size appeared in addition to the ovulatory follicle in 57.3 percent of the estrous periods of the 11 mares studied. These follicles may continue to increase in size before they regress. Smaller follicles may remain for a couple of cycles and then develop into the ovulatory follicle (Figure 11-10).

Definite changes occur in the cervix during the estrous cycle, and they are quite useful in predicting approaching ovulation. During diestrus, the cervix is covered by a small amount of sticky mucus. The quantity increases during estrus until a considerable amount of slimy mucus is present. During diestrus, the cervix is tightly constricted and pale pink in color. During estrus, the vascularity increases and the cervix becomes

FIGURE 11-10
Curves of growth of follicle and corpus luteum during the estrous cycle. From Hammond and Wodzicki. 1941.



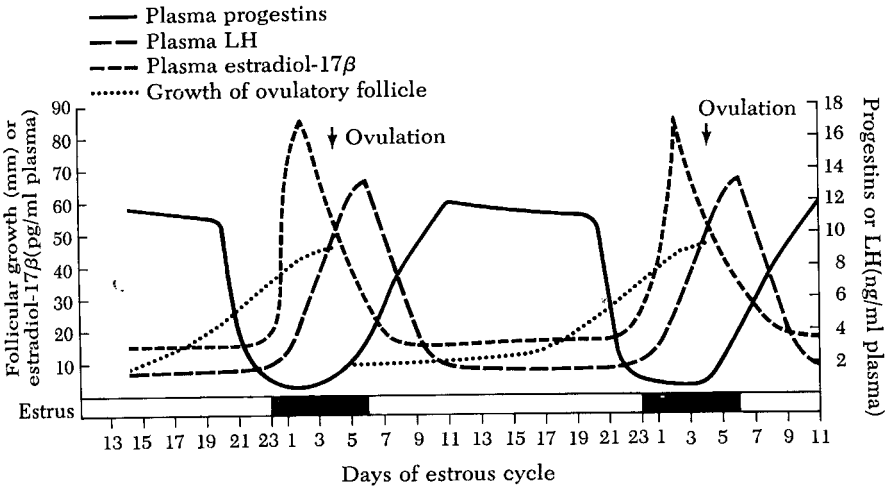


FIGURE 11-11
Endocrine patterns during the estrous cycle of a mare.

reddish in color. A complete relaxation occurs during estrus to the extent that the cervix may lie on the floor of the vagina. Palpation reveals the tactile sensitivity and muscular contractions of the estrual cervix.

The changes that occur in the vagina are more difficult to interpret than the cervical changes. The most obvious changes that occur in the vagina during estrus are an increase in vascularity resulting in a change from a pale to a rosy color and an increase in the size and number of secondary mucosal folds. The walls are dry and sticky during diestrus and become slimy during estrus as a result of changes in cervical mucus secretion.

Hughes *et al.* (1972b) have discussed the changes in uterine form that occur during the estrous cycle. From a practical standpoint, these changes vary in interpretation and their pattern is not sufficiently uniform to be useful in determining the optimal time to breed. The uterus has only slight muscle tone and is turgid during estrus; it may even be termed flaccid. During diestrus, an increase in tone and "tubularity" (contracted state that closely resembles a tube) is observed. However, during late diestrus, tone and tubularity may decrease.

During anestrus, the cervix and uterus lose muscle tone and usually become completely flaccid in most mares that have no follicular activity. In mares that have considerable ovarian activity, the uterus may have some muscle tone and the cervix may be firm and tightly constricted.

Endocrine Control

Endocrine control of the estrous cycle is not completely understood. Figure 11-11 is a generalized diagram of the sequence of events. The

hormones known to control the estrous cycle originate in the pituitary gland, the ovaries, and possibly the uterus.

The pituitary hormones are the *follicle stimulating hormone* (FSH) and the *luteinizing hormone* (LH). They are produced in the basophil cells of the medullary area of the anterior pituitary gland. FSH causes ovarian follicles to grow and to produce increasing amounts of estrogenic substances as they grow. FSH has been purified and its properties characterized by Braselton and McShan (1970) and by Nuti *et al.* (1972). It is a glycoprotein consisting of 74.9 percent protein, 9.3 percent hexose, 9.1 percent hexosamine, 4.7 percent sialic acid, and 1.3 percent fucose, and it has a molecular weight of approximately 33,200–47,900. The amino acid composition is characterized by high levels of cysteine (half-cystine), threonine, lysine, and aspartic and glutamic acids, and a low level of methionine. The NH_2-terminal amino acids are phenylalanine and aspartic acids, and the COOH-terminal residues are leucine and glutamic acid. The isoelectric point is pH 4.1.

The luteinizing hormone causes ovulation and initiates the formation of the corpus luteum. Equine LH has a number of LH components and an FSH component. Four LH components have isoelectric points at pH 7.5, 5.9, 6.6, and 7.3, and the FSH component isoelectric point is pH 4.8. The molecular weight is 44,500–63,800. LH concentration starts to increase in the plasma 2–3 days before ovulation, reaches a peak 1–2 days after ovulation, and declines to diestrus levels 6 days after ovulation (Geschwind *et al.*, 1975). FSH and LH work in a synergistic manner in the processes of follicular growth, maturation, and ovulation.

The ovarian hormones that control the estrous cycle are estrogens and progestins. Estradiol-17β is produced by the granulosa cells and by the theca interna of the Graafian follicles. The amount of estradiol-17β in the plasma begins to increase at the onset of estrus, reaches a peak 12–27 hours before ovulation, and declines to diestrus levels 5–8 days after ovulation (Figure 11-11). Because estrogen reaches a peak before LH peaks, it is possible that the estrogen surge may facilitate the ovulatory surge of LH. Within 24 hours after ovulation, the corpus luteum begins to secrete progestins. By the sixth day after ovulation, maximum levels of plasma progestins have been attained. The corpus luteum remains active for approximately 12–14 days, and then rapidly undergoes regression. The roles of the pituitary gland and the uterus in the maintenance and regression of the corpus luteum function are not fully understood. Injection of antibodies against FSH and LH during days 3–7 of diestrus causes a significant decrease in the weight of the corpus luteum, indicating that the mare's pituitary contains a substance that is necessary for maintenance of the corpus luteum. Evidence for a so-called uterine luteolysin has been provided by hysterectomy studies conducted by Stabenfeldt *et al.* (1974), in which functional corpora lutea were main-

tained for as long as 175 days postovulation after hysterectomy. It is believed that prostaglandin is the luteolysin.

The interval between the cessation of corpus luteum function and estrus is approximately 3 days. Estrus normally does not occur until the progestin concentration is less than 1 ng/ml in the plasma.

11.3 *Gestation*

Length of Gestation Period

Knowledge of the length of gestation and the ability to predict the mare's foaling date are important to the successful management of the pregnant mare. The average gestation period usually ranges from 335 to 340 days. There is considerable variation in the length of gestation, as evidenced by Paul's (1973) observations of Morgan Horses. The range was 300–385 days, the median was 342.5 days, the mean was 339.6 days.

Sex of the foal, month of conception, and the individual traits of the mare significantly affect the duration of pregnancy, although size of foal, sire of dam, age of sire, age of dam, and year apparently have no effect. Colts are usually carried 2–7 days longer than fillies, indicating that the sire does exert an influence on gestation length through the genotype. Season of the year seems to have an effect, in that the gestation length is shorter for foals born during warm weather. Mares on a high plane of nutrition foal approximately 4 days before mares on a low plane of nutrition. Therefore, the rate of development of the fetus could be affected by the nutritional plane of the mare. Fetal genotype has an effect; the gestation period of mares carrying a mule fetus (jack × mare) is approximately 10 days longer than the gestation period of the same mare when carrying a horse fetus.

The ovum migrates to the uterus 4–6 days after fertilization. Implantation does not occur for approximately 8 weeks. During the first 8 weeks of gestation, fetal fluid pressure holds the chorion (Figure 11-12) next to the uterine mucosa. By the tenth week, penetration of the uterine mucosa by the chorionic villi is quite extensive, and by 14 weeks the attachment is complete. The type of attachment is epitheliochorial, that is, the chorion of the fetus is in contact with the epithelium of the mare's uterus.

In the mare and donkey, the fetal placenta is formed by three membranes (Figure 11-12): chorion, allantois, and amnion. Villi, or fingerlike projections, are scattered over the surface of the chorion (outermost membrane), and for this reason the attachment is frequently said to be

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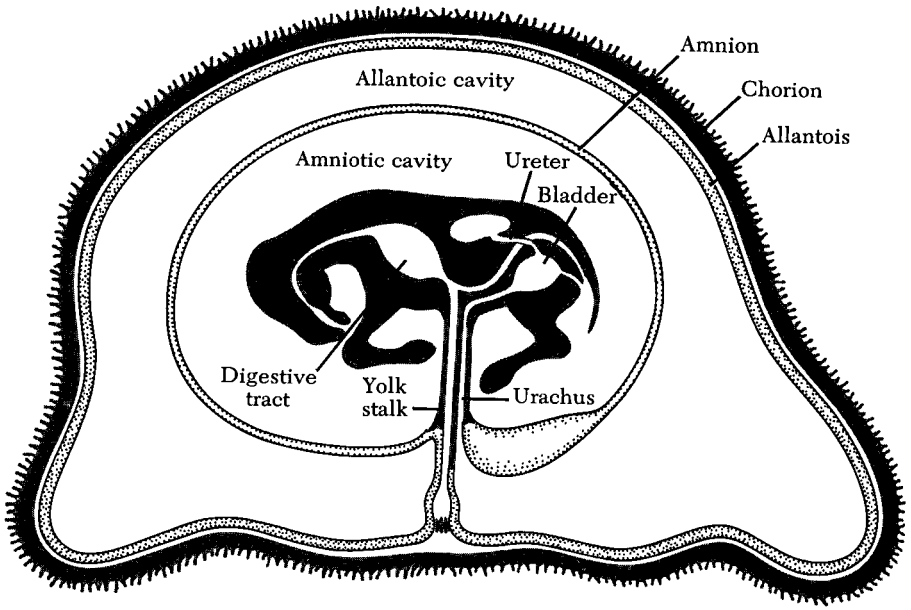

FIGURE 11-12
Fetus of horse within the placenta. The chorion and allantois make up the chorioallantois, often called the chorion. From Frandson, R. D., ed. 1974. p. 388. Originally in Witchi. 1956. *Development of Vertebrates.* Philadelphia: W. B. Saunders.

"diffuse." The allantois (second membrane) lines the inside of the chorion and is fused with the amnion, thus forming the first water-bag or allantoic cavity. The allantoic cavity is continuous with the bladder by way of the urachus, which passes through the umbilical cord. The amnion is the innermost membrane and forms the second water-bag or amnionic cavity.

The umbilical arteries and veins are located in the connective tissue between the allantois and chorion. The umbilical arteries and their tributaries carry unoxygenated blood and waste products from the fetus to the mare. In general, the blood of the fetus does not mix with maternal blood. However, the two circulations are very close at the junction of the chorion and endometrium. At least 6 layers of tissue separate the maternal and fetal blood (Figure 11-13): fetal vein, stroma between fetal vein and cytotrophoblast, cytotrophoblast, uterine epithelium, uterine stroma, and maternal artery.

The mare has a peculiar specialization, in that *endometrial cups* begin to form on the thirty-sixth day of pregnancy opposite a transitory, though well-defined, circumferential thickening of the chorion called the allantochorionic girdle. Allen (1970a) has shown that the endometrial cups of the mare are composed of fetal cells and are not of maternal origin, as was thought previously. The endometrial cups are a discrete and densely packed mass of large, epithelioid, decidual-like cells, that develop before implantation. Upon development of the cups, a central depressed area

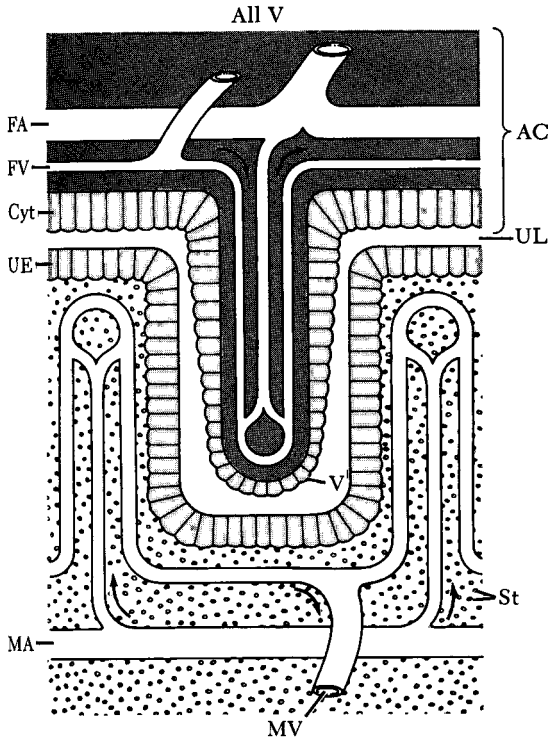

FIGURE 11-13
Diagrammatic representation of relationship between maternal and fetal tissues in the epitheliochorial type of placenta: FA, fetal artery; FV, fetal vein; AC, allantochorion; UL, uterine lumen; MA, maternal artery; MV, maternal vein; UE, uterine epithelium; Cyt, cytotrophoblast; V, villous; St, stroma; All V, allantoic vesicle. From Harvey, E. B. 1959, p. 433.

filled with coagulum is formed. The accumulation of coagulum, consisting of degenerate epithelial cells, erythrocytes, polymorphonuclear leucocytes, and pregnant mare's serum gonadotropin, causes a pouch to form in the allantochorion. The importance of the secretion of gonadotropins is discussed in the next section on hormonal changes that occur during pregnancy. Figure 11-14 illustrates the growth and development of the fetus.

Hormonal Changes that Occur during Pregnancy

Large quantities of pregnant mare's serum (gonadotropin), or PMSG, are present in the serum of mares between days 40 and 130 of gestation (Figure 11-15). The rate of secretion and/or absorption is sufficient by 35–40 days for it to be detected in the blood. Maximum levels are attained at 55–60 days, and the concentration declines until, after 130 days, it is undetectable in the blood. The amount of PMSG produced by the mare is related to the genotype of the fetus. Mares carrying a mule fetus (jack × mare) produce less PMSG than mares carrying a horse fetus. A jenny carrying a hinny fetus (stallion × jenny) produces more PMSG than the same jenny when carrying a donkey fetus.

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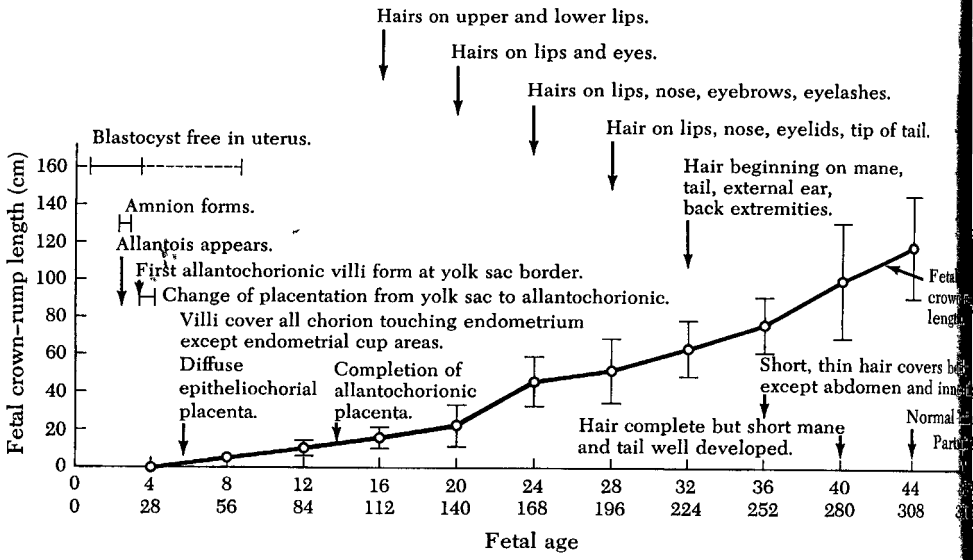


FIGURE 11-14
Fetal growth. From Bitteridge and Laing. 1970. p. 98.

PMSG is predominately follicle-stimulating in effect, but it also pos sesses a luteinizing fraction. The chemical characterization of PMSG ha been reviewed by Papkoff (1969). The molecular weight is approximatel 28,000. PMSG has a polypeptide content of 30–40 percent and a carbo hydrate content of 45 percent.

Nett *et al.* (1972) have shown that estrogen starts to increase at approx mately 45 days of gestation and increases at a rapid rate beginning at 8 days of gestation. Maximum levels are reached at 200–210 days, and the a gradual decline occurs. At parturition, a rapid decline occurs. Lovel *et al.* (1975) have observed that a rapid decline in progesterone level occurs on the day of parturition.

Ovarian activity, including formation and ovulation of many Graafia follicles and luteinization of follicles, reaches a maximum during the se ond and third month of pregnancy. It is during this period that accesso corpora lutea are formed and secrete progestins to maintain pregnanc Between 150 and 200 days, the corpora lutea undergo degeneration an no large follicles are present. By 200 days, the secretion of progesteron by the corpora lutea is insignificant since removal of the ovaries do not result in abortion.

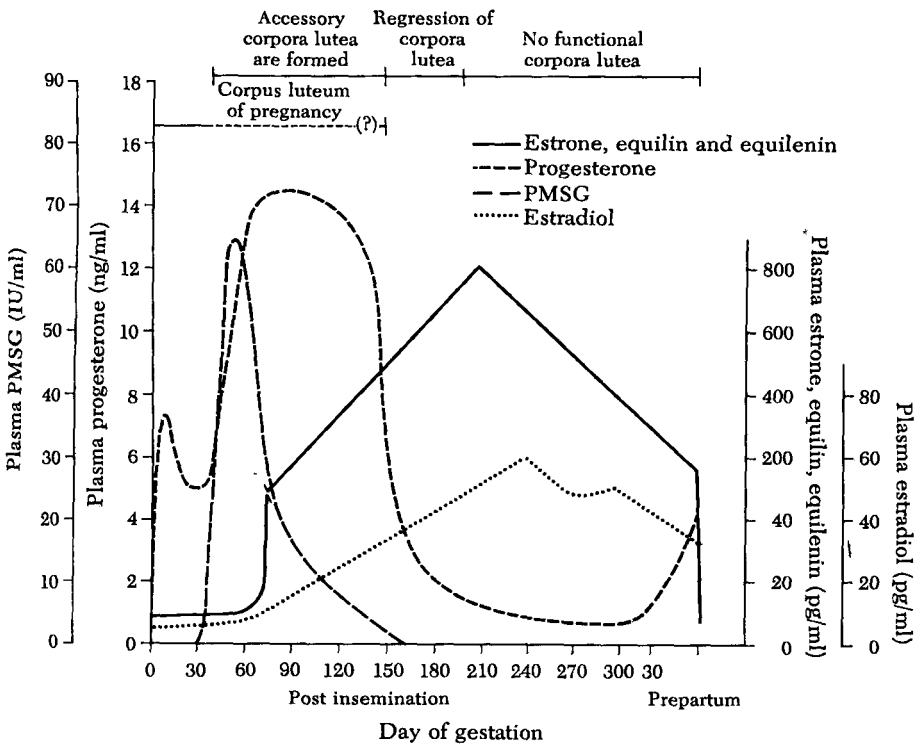
There are several methods to diagnose pregnancy in the mare, althoug under normal conditions the first indication of pregnancy is failure to com into estrus 16–18 days after being bred. The most popular method is dia nosis by rectal palpation. This method has a decided advantage in that th result is immediately available. A skilled clinician can detect pregnanc in most mares at 25–30 days of gestation and be sure of accuracy after 3

days. After 45 days and until approximately 140 days, the detection of PMSG in the blood is a positive pregnancy diagnosis. Commercially prepared kits to test for PMSG are available to the horse owner who is without veterinary service. The detection of urinary estrogen by chemical means after 120 days of gestation is an accurate method. Fetal movements can often be observed in the mare's flank after the seventh month.

Foal Heat

A mare should come into "foal heat" between 1 and 12 days post-foaling. Even though a wide range of 1–50 days post-foaling has been observed, most mares are in estrus on the seventh to ninth day. The foal heat period may be as long as 12 days, but normally it is approximately 3 days in length.

FIGURE 11-15
Endocrine patterns during gestation of a mare.



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Alliances and reproductive success in Camargue stallions

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A study of a herd of Camargue horses, *Equus caballus*, showed that while the majority of high-ranking stallions held single-male harems, some sons of low-ranking mares, being low ranking themselves, formed alliances that could last a lifetime. The two stallions were each other's closest associate and preferential grooming partner. Alliances were based on coalitions in which either both partners confronted an intruder synchronously or the dominant of the pair tended the female(s) while the subordinate simultaneously displayed towards the rival. Alliance partners were of similar age but were not more closely related to each other than to other stallions in the herd. Long-term paternity data revealed that subordinates sired close to a quarter of the foals born into the alliance group, and significantly more foals than low-ranking stallions in the herd adopting a 'sneak'-mating strategy. The dominant appeared to benefit from the presence of his subordinate partner. Fights occurred all year round, and the subordinate stallion of each alliance pair fought outside competitors more than twice as often as the dominant. Forming short-term alliances before defending mares on their own may enhance long-term reproductive success for both partners. Other benefits to both partners include higher survivorship of their foals and increased access to proven reproductive mares. These results suggest that the relationship between alliance partners is based on mutualism, but several conditions for reciprocity seem to be fulfilled: the benefit to the dominant (assistance in fights), and the benefit to the subordinate (access to reproduction), are both costly to the other partner and delayed in time.

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Alliances are long-term cooperative coalitions (de Waal & Harcourt 1992), cooperation often being provided in the form of support during competitive encounters (e.g. van Hooff & van Schaik 1992). Alliances between males are generally rare in mammals (Clutton-Brock 1989). As low-ranking males are disadvantaged over access to resources or reproduction (Dunbar 1988), they may be able partly to compensate by forming alliances based on coalitions. The value of male alliances with regard to mating success has been reported in a variety of primates (Smuts 1987), but seldom in nonprimate species, where they have been observed only in lions, *Panthera leo* (Packer et al. 1991), wild dogs, *Lycaon pictus* (Moehlman 1986), hyaenas, *Crocuta crocuta* (Zabel et al. 1992), dolphins, *Tursiops* sp. (Connor et al. 1992), captive plains zebras, *Equus burchelli* (Schilder 1990) and feral horses, *Equus caballus* (Berger 1986). Only the study on lions was able to measure individual reproductive success. Packer et al.'s (1991) results indicate not only that male lions form coalitions, but also that paternity in coalitions of unrelated males is more evenly shared than in coalitions of related males. Therefore, whereas the immediate benefits of cooperative behaviour have been widely demonstrated (e.g. Smuts 1987), few studies have been able to show the effect of alliances on survival or reproduction (Harcourt & de Waal 1992) or to measure trade-offs between the partners.

Horses and plains zebras provide an unusual opportunity to investigate the effects of alliances on male reproductive success. Adults form small, long-term groups, usually consisting of one stallion, one to two mares and their offspring. As is typical for a species in which males have long tenure of a group, both sexes disperse from their natal family (Clutton-Brock 1989). Young mares usually join reproductive units directly (Monard & Duncan 1996), whereas young males first spend 1–2 years in a stallion group before forming their own family (this study). Apart from the typical one-male family, groups with more than one adult stallion have been reported by all authors who have studied free-living horses. Berger (1986) noted that 12% of the families that persisted longer than 7 months had two or more stallions, and Stevens (1990) reported that half of the 12 groups she watched for 2 years were multimale. With regard to these different types of groups in horse societies (one-male, multimale and all-male), the following mating strategies are observed in stallions: exclusive access to females in one-male families; shared reproduction in multimale

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groups, including preferential male–female relationships in terms of proximity and exchanged interactions over several years (Feh 1987); and sneak matings by stallions living in all-male groups, these matings mainly occurring at night and without a social bond between partners (personal observation).

Individual reproductive success in Camargue stallions is positively correlated with their mother's rank and their adult rank, but not with their weight (Feh 1990). Stallions may therefore vary in 'quality' (e.g. rank), and the high-ranking individuals may be better able to hold groups on their own than others. The alternative for low-ranking individuals might be to team up in alliances in order to be able to defend females.

In this paper, I describe alliance behaviour based on coalitions between Camargue stallions living under semi-natural conditions. I then investigate the influence of genetic relatedness, rank and age on alliance formation and describe costs and benefits to the two partners with regard to fighting and reproductive success, based on paternity analyses. I finally examine mutual benefits to both alliance partners in terms of overall reproductive success, foal survival and access to specific females.

METHODS

Study Animals

The study herd started in 1974 with 14 horses of known genetic relatedness. They were released in a Camargue pasture of 300 ha composed of freshwater marshes, salt steppes and halophyte grassland. The number of horses peaked at 94 in 1981, with 11 family groups and one bachelor group. From 1980 on, horses were removed regularly to avoid overgrazing. No supplementary food or veterinary care was given. From 1979 on, all individuals were weighed twice a week. At the same time, blood samples were taken for paternity tests. All individuals were easily approached and identified and their exact dates of birth are known. From 1977 on, several family groups existed and from 1978, these family groups lived together in one herd. This meant that the horses were in sight of each other most of the time, so interactions between stallions were relatively frequent. This analysis considers 13 stallions, for which long-term reproductive success and social behaviour from birth to the respective ages of 11–14 years were known. These ages are beyond the peak of reproductive success in stallions (Berger 1986; Feh 1990).

Paternity, Relatedness and Foal Survival

Paternity exclusion tests were based on electrophoresis on a number of proteins at 28 loci (for further procedural details see Scott 1978). The combined reproductive success was calculated up to 11 years, when the paternity analyses stopped. A total of 154 foals were born to the groups under consideration; 10 foals were found dead shortly after birth (<48 h) and therefore were not analysed for paternity as the analyses needed fresh blood samples. As the adult sex ratio of the herd changed over the study years, stallions of different ages did not have the same opportunity for siring foals in each year. To standardize data for each stallion, I calculated the number of 'available' mares for each stallion at each age in the following way. Nearly all the mares had a foal every year and gestation lasts 11 months. The age at first breeding is taken as 2 for the mares and 4 for the stallions. The number of 'available' breeding mares was thus the ratio of the number of mares 2 or more years old divided by the number of stallions 4 or more years old. So the relative reproductive success of a stallion at a given age was the number of foals produced at that age divided by the number of available breeding mares the year before.

I calculated the relatedness of each pair of horses (e.g. H1 × K6) in the herd measured as the additive relationship (a) as follows:

$$a\text{H1} \times \text{K6} = 0.5(a\text{H1} \times \text{G3} + a\text{H1} \times \text{G4})$$

where G3 and G4 are, respectively, the mother and father of K6. For the full description of the method, see Evans et al. (1977).

For the comparison of foal survival between one-male and alliance families, I considered all 154 foals found shortly after birth (<48 h). Under such circumstances, identification of the mother, and therefore of the group into which the foal was born, was easy even for dead foals.

Assessment of Ranks

From 1976 to 1987, I established the dominance hierarchy in the field using observations of headthreats and bites, followed by a clear avoidance reaction by the recipient (Wells & von Goldschmidt-Rothschild 1979). Both focal animal and all-occurrences sampling were used. Observation hours varied from 564 to 1872 per stallion. Contexts were food, water or wind shelter. The horses were then ranked according to the classical method (Martin & Bateson 1993), where the individual that is never supplanted is at the top and the one that is always supplanted is at the bottom. As it was sometimes difficult to observe a resource-based hierarchy in the field, the herd was brought to a 1-ha enclosure where access to water could be controlled. Every 48 h, I stopped their otherwise free water supply for 24 h, so the horses were induced to show agonistic behaviour at a measurable rate (1.4 headthreats and 0.17 bites per horse in the first hour after having access to water). I repeated the experiment 10 times over a 3-week period in 1988 and 1989, and compared the results with data obtained in the field. There was no difference between the two data sets, and a stable linear dominance hierarchy could thus be determined among the stallions.

Relationships, Confrontations and Fights

Observation hours were evenly distributed over daylight hours in all seasons. The nearest horse to any other

horse of the herd was determined every 15 min. Single linkage cluster analysis based on these data was used to define groups and establish preferential individual relationships (Duncan 1992). For the same purpose, I used focal animal sampling, following each stallion for 60 min per day, 200 days per year from 1976 to 1980. I recorded 30 different types of interaction with the aid of a tape recorder and used them to calculate frequencies per h of 11 behavioural categories. A detailed description of observational methods is given in Feh (1987). For 2 years, the herd was observed for 48 h consecutively every 2 weeks in order to establish activity budgets. These observations also allowed sneak matings to be detected, although not quantitatively for all stallions. As all the families stayed close together during the study period and fights are conspicuous, I used all occurrence sampling to calculate the fighting frequency for each stallion. Fights in equids typically consist of rearings, attempts to bite or bites, and kicks with the fore or hind legs (Feist & McCullough, 1976; Berger 1986). Before the stallions start to fight, they usually go through a confrontation (Tschanz 1985; Berger 1986), where one stallion defecates on a dung pile while the other approaches. They then smell the dung together, and most often raise their heads rapidly while squealing. These actions are typically followed by kicks with the forelegs, and the dominant stallion of the pair then defecates over the dung while the subordinate leaves (Feh 1987). If the subordinate stallion is reluctant to leave, the confrontation usually ends in a fight.

Statistical Analysis

The statistical analysis is based on two categories of stallions. The first takes into account the stallions that adopted only one reproductive strategy during their life. They were treated as independent samples and compared with the Wilcoxon test (Siegel & Castellan 1988). For stallions that changed reproductive strategies during their life, I compared these using the Wilcoxon signed-ranks test or the permutation test for paired replicates (Siegel & Castellan 1988). For comparisons between alliance partners, these same pairwise tests were used.

RESULTS

Proportion, Duration and Stability of Two-male Groups

Detailed descriptions of the herd history, including group composition, genealogy and births are given in Duncan et al. (1984) and in Duncan (1992).

The majority of stallions first tried to monopolize mares on their own, but few succeeded at the first attempt. Stallions that failed to do so seem to have had two options: either they joined another stallion in a two-male group or they stayed in a bachelor group and tried to mate mares as 'sneaks'. Forming a two-male group with another stallion was a temporary situation for the majority of individuals but permanent for four out of 10 males that formed a two-male group.

Seven out of 13 stallions attempted to form a family on their own when 2–3 years old (corresponding to sexual maturity in males), whereas the other six joined an all-male group (Fig. 1). Half of the latter had been chased out of their original group by the dominant stallion. Attempts by young stallions to defend at least one mare against rivals never lasted for more than one breeding season (2 months), when they were forced by other stallions to abandon the mare(s) after numerous fights. The stallions then became integrated into a stallion group for up to 2 years. Only three males out of the 13 were subsequently successful in forming a one-male family group with a tenure length of up to 18 years. The other 10, on their own accord, formed a two-male group with a partner. Four of them stayed together over many years: one of these subsequently died while two stallions were still living in the same group after 16 years at the time of writing. Six left their partners after 2–3 years to form families on their own. Once they had succeeded, they never reverted to a two-male group until they died (9–17 years later) or were taken out of the herd (two stallions, 4 years after they formed a one-male group).

Anecdotal evidence suggests that from a certain age, the strategies adopted by stallions are fixed. In one case, I was able to test the stability of reproductive strategies in four 13-year-old stallions followed since birth. Each stallion had adopted a different strategy between the ages of 4 and 6 years. The dominant had gained control over three mares, the second-ranking stallion was a bachelor, and the third- and fourth-ranking stallions had formed a two-male group. The four stallions were separated from their mares and kept together on a different pasture for 2 months. They were then introduced to a group of six mares unknown to them. Initial fights (N=16 for all stallions) after the release lasted for 21 min in total. By the end of this period, the dominant stallion had monopolized three mares on his own, the second-ranking was a bachelor, and the two last-ranking stallions had teamed up in a two-male group and controlled the other three mares. The group composition remained stable over the next 3 months. Therefore, 7–9 years after initially forming their families with different mares, the stallions had adopted the same reproductive strategy and kept the same rank position. Three months later, when the stallions were reintegrated into the herd together with their old mares, there was again no change detectable in their strategies.

Relationship between Partners of Two-male Groups

Apart from the low-intensity agonistic interactions (bites and headthreats) maintaining the relative rank position seen in all pairs (Table 1), stallion partners in two-male groups were each other's closest associate. Proximity between the partners was maintained by the subordinate. Their relationship was further based on a high frequency of mutual groomings mainly initiated by the subordinate, smellings or body contacts as well as lightly agonistic rank displays that caused no physical harm (Tschanz 1985). The relationship with the mare(s)

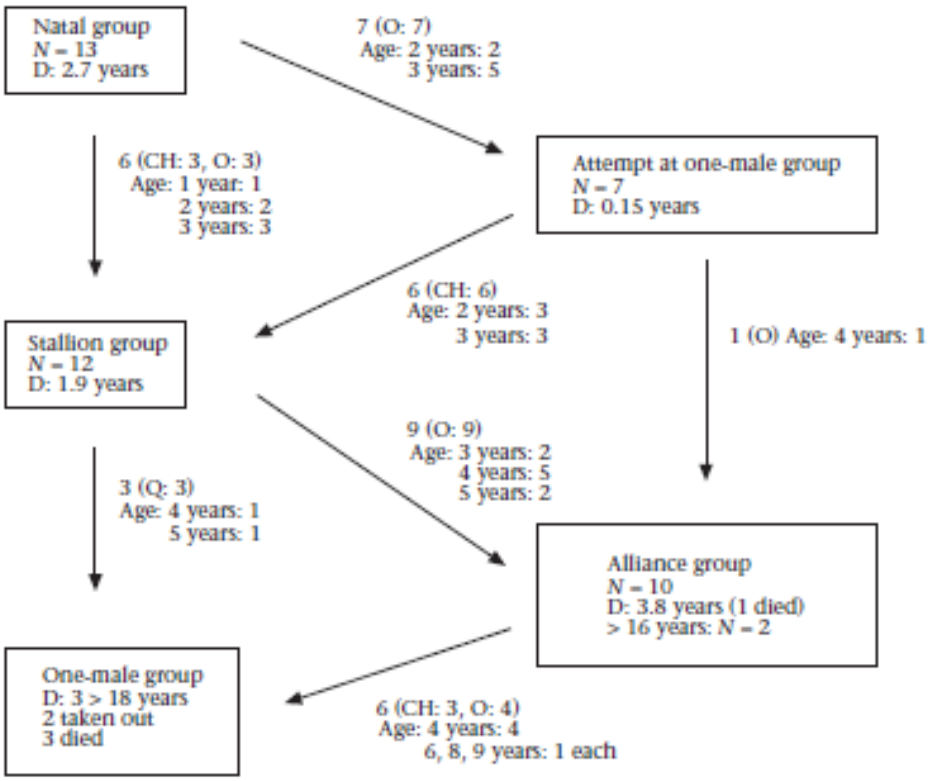

Figure 1. Succession of group changes showing alternative reproductive strategies adopted by the different stallions over their lifetime. D: mean duration of stay in the respective group. Age: respective age when the stallions changed groups or reproductive strategies. Of the 13 stallions studied, seven were still alive at the time of writing and in the herd. Four died and two were removed; all were more than 11 years old so were still included in the analysis. CH: number of stallions changing groups or reproductive strategies after being aggressively chased by a dominant stallion or subsequent to losing a fight. O: number of stallions changing groups or reproductive strategies on their own accord, that is, without being chased or attacked by other stallions.

differed between the dominant and the subordinate, the former being closer, engaging in more mutual grooming, and driving her (them) more often than the latter. The relationships of dominants and subordinates with both foals and yearlings of their group were similar, characterized by an exchange of mutual groomings, and submissive behaviour from the young horses towards the adults.

Alliance Behaviour Based on Coalitions

When an outside stallion approached the two-male group, the partners took one of two options. They either confronted their rival together, with aggression mostly initiated by the subordinate (20 times versus 4 times by the dominant; binomial test: N=24, P=0.002, two-tailed; in 10 cases initiator not determined), or the subordinate usually displayed to the rival stallion while the dominant drove away the mares (Fisher's exact test: P=0.001; Table 2).

Identity of Alliance Partners

Partners teaming up for alliances were low-ranking unrelated stallions of the same or similar age who had developed strong bonds during the time they lived together in a stallion group.

Of the individuals that formed alliances at some stage in their lives, seven out of 10 were part of the low-ranking half of the male dominance hierarchy (Table 3), and were sons of mares in the lower-ranking half of the female dominance hierarchy, whereas the three one-male family holders that never participated in alliances were sons of the higher-ranking mothers (Fisher's exact test: P=0.035) and were themselves in the high-ranking half of the dominance hierarchy (Fisher's exact test: P=0.02).

There was no consistent tendency for stallions to form alliances with close relatives. Thus, there was no significant difference between the genetic relatedness (additive relationship) of an alliance member with its partner and the relatedness of the same individual with any other stallion of the herd (paired t test: t_9=1.91, NS) or with another member of the same stallion group (Fig. 1) before the pair formed an alliance (paired t test: t_9=1.85, NS), although the mean additive relatedness between low-ranking mothers was higher ($\bar{X}$=0.239, N=4) than that between dominant mothers (0.083, N=3). It cannot therefore be concluded that close genetic relatedness was at the origin of alliances. Moreover, six pairs of maternal half-siblings (same mother, different fathers) from five different mothers knew each other well, having spent up to 2 years in the same group during their youth. None of these formed an alliance pair. The effect of paternal half-siblings could not be investigated. The fathers of six

Table 1. Spatial relationships and interactions exchanged between alliance partners and between alliance partners and all other herd members

Partners	Approach	Smell	Light agonistic	Strong agonistic	Mutual grooming	Stallion display	Greeting	Driving	Submissive gesture	Play	Interposition	Nearest neighbour	All interactions
Dom-Sub	0.46	0.29	0.22	0.04	0.15	0.38	0	0	0	0.02	0.04	168	1.58
Sub-Dom	1.82	0.35	0.01	0	0.21	0.34	0	0	0	0.01	0	122	2.74
Dom-mare													
Initiated	2.29	0.17	0.13	0	0.67	0	0.04	0.57	0	0	0.14	107	3.99
Received	0.21	0	0.23	0	0.12	0	0	0	0	0	0.03		0.59
Sub-mare													
Initiated	1.15	0.16	0	0	0.07	0	0.1	0.07	0	0	0.12	46	1.66
Received	0.12	0	0.23	0	0.05	0	0	0	0	0	0		0.39
Dom-yearling													
Initiated	0.13	0.12	0.05	0.03	0.24	0	0	0.01	0.12	0	0	16	0.67
Received	0.09	0.09	0	0	0.09	0	0	0	0.07	0	0		0.34
Sub-yearling													
Initiated	0.18	0.08	0	0.03	0.32	0	0	0	0.06	0	0	9	0.66
Received	0.08	0.03	0	0	0.09	0	0	0	0.03	0	0		0.22
Dom-foal													
Initiated	0.09	0.06	0.06	0	0.05	0	0	0.04	0	0	0	23	0.28
Received	0.01	0.04	0	0	0	0	0	0	0.03	0	0		0.07
Sub-foal													
Initiated	0.06	0.05	0	0	0.03	0	0	0	0	0	0	28	0.13
Received	0.08	0	0	0	0	0	0	0	0	0	0		0.08
Dom-rival													
Initiated	0	0.01	0.01	0.003	0.003	0.06	0	0	0	0.003	0	0	0.09
Received	0.01	0.04	0.14	0.02	0.003	0.09	0	0	0	0.01	0		0.32
Sub-rival													
Initiated	0.003	0.02	0.02	0	0.003	0.23	0	0	0	0	0	2	0.36
Received	0.01	0.03	0.09	0.01	0.01	0.30	0	0	0	0.01	0		0.46

Dom: dominant stallion in an alliance; Sub: subdominant stallion in an alliance. The mean frequencies of interactions per h for four pairs of alliance partners observed during 44 h each over 6 months are shown. Light agonistic: head threats and bites. Strong agonistic: attacks and chases. Interposition: one horse moves in between two interacting horses. The nearest neighbour data were recorded every 15 min at the same time as the interactions. The frequency indicates how many times the second of the 'partners' was seen closest to one of the alliance partners, e.g. 'Sub was closest to Dom 168 times'.

Table 2. Number of occurrences of display by one alliance partner to a rival while the other drives away their mare(s)

Actor	Display	Driving mare(s)	Total
Dominant alliance partner	2	33	35
Subordinate alliance partner	32	3	35
Total	34	36	70

See text for test. The total number of observation hours: 564 for each of the five alliance pairs.

of the stallions were absent from the herd, having sired their sons before the start of the study. The fathers of the other four were present, but the sons did not grow up in the same group as their fathers.

Stallions usually formed alliances with partners of the same age. Six stallions formed alliances with partners born in the same year, the difference in age for the other two pairs being 1 and 2 years, respectively. All but one of the pairs formed while they were together in a stallion group. Two such groups existed during the study, and all stallions forming alliances after leaving the stallion group had preferred social relationships with their future partner during their time together in this group. Future alliance partners were always the most frequent nearest neighbours and exchanged more interactions than with all other members of the herd (Wilcoxon signed-ranks test: $T=55$, $N=10$, $P=0.002$, two-tailed, for both nearest neighbours and interactions).

Benefit of Alliance to Subordinate Stallion

Subordinate alliance stallions had a higher relative reproductive success than sneaks (Table 3, Fig. 2). The sneak strategy was the least successful reproductive strategy (sneak strategy versus subordinate alliance strategy: permutation test for paired replicates: $N=5$, $P=0.02$, two-tailed; sneak strategy versus dominant alliance strategy: permutation test for paired replicates: $N=5$, $P=0.02$, two-tailed; sneak strategy versus one-male family holders: permutation test for paired replicates: $N=8$, $P=0.01$, two-tailed). Although in all of the five alliance pairs, dominants sired more foals than subordinates (permutation test for paired replicates: $N=5$, $P=0.02$, two-tailed) and dominants had a higher relative reproductive success than subordinates in individuals that never changed strategies (Wilcoxon test: $W_s=15$, $N_1=N_2=3$, $P=0.05$, one-tailed), stallions adopting a subordinate alliance strategy had a higher relative reproductive success than when remaining as sneaks (see tests above).

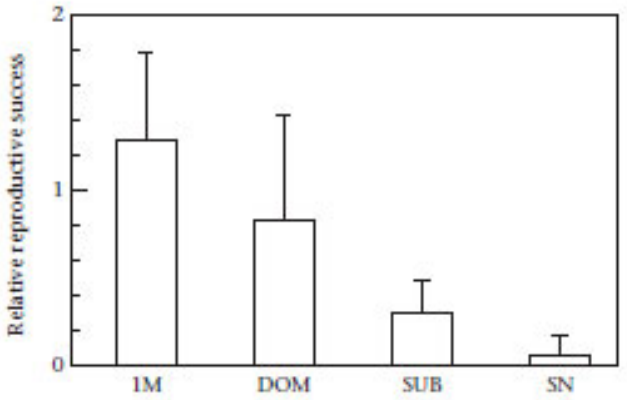

Figure 2. Relative reproductive success in relation to the four reproductive strategies (mean number of foals per year+SD). Data cover the stallions' relative reproductive success up to 11 years of age and correspond to the 10 years of analysis on fighting frequency. The data for this figure are pooled, but the statistical tests are based on independent samples or pairwise comparisons (see text). 1M: one-male family holders, $N=8$; DOM: dominant alliance stallions, $N=5$; SUB: subordinate alliance stallions, $N=5$; SN: sneaks (bachelors), $N=12$.

Cost of Alliance to Dominant Stallion

Apparently as a result of conceding foals to the subordinate, dominant stallions in alliances had a lower

Table 3. Relative individual reproductive success* and rank for the different reproductive strategies

Individuals	Rank	One-male harem	Dominant alliance	Subordinate alliance	Bachelor	Combined over all years
G4	1	1.89±0.27	1.88±0.59		0.29±0.40	0.99
H1	7	1.36±0.42	0.50±0.71		0	0.72
H4	8	0.57±0.17		0.56±0.42	0	0.37
I3	3	0.77±0.18		0.25	0.30	0.56
I4	2	1.66±0.77			0	0.92
I5	5	1.85±0.48		0.38±0.54	0	0.97
I6	11			0.17±0.71	0	0.10
J1	9		0.67±0.52		0	0.50
J7	6	1.07±1.05			0	0.63
K3	12		0.58±0.59			0.42
K6	13			0.10±0.28	0	0.08
K8	4	1.04±0.64			0	0.76
O11	10		0.50±0.29		0	0.50
$N=13$	13	8	5	5	12	13

*Mean±SE number of foals fathered per year from birth to 11 years, corrected for the number of available mares in the herd the year before.

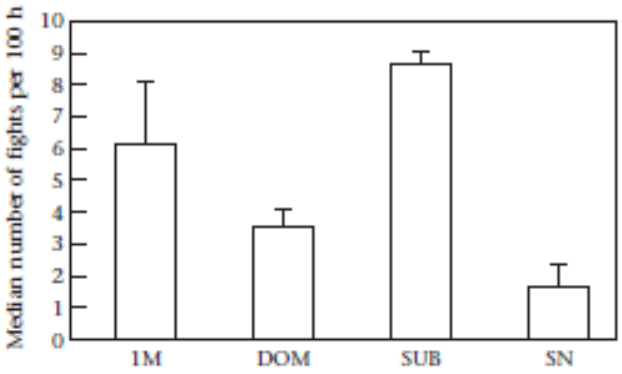

Figure 3. The stallions' fighting frequency with outside competitors in relation to the four reproductive strategies (number of fights per 100 h, median and interquartile range for all stallions). Data were collected from 1977 to 1986, at the same time as the paternity analyses. Observation hours for each stallion varied from 1240 to 1872, distributed over the 10 years. The data for the figure are pooled, but the statistical tests are based on pairwise comparisons (see text). 1M: one-male family holders, N=8; DOM: dominant alliance stallions, N=5; SUB: subordinate alliance stallions, N=5; SN: sneaks (bachelors), N=12.

relative reproductive success than males controlling mares on their own. Excluding individuals that changed strategies (Table 3, Fig. 2), stallions in one-male groups were more successful than dominant alliance stallions (Wilcoxon test: W_x=15, N_1=N_2=3, P=0.05, one-tailed). Stallions changing from alliances to one-male family holders improved their relative reproductive success (permutation test for paired replicates: N=5, P=0.02, two-tailed). As they changed strategies after the age of 4, fertility linked to age could not act as a confounding factor (Evans et al. 1977).

Cost of Alliance to Subordinate Stallion

Subordinate stallions took more risks and probably expended more energy because they fought more often than dominants and sneaks (Fig. 3). In all pairs, the fighting frequency of subordinate alliance stallions with other stallions of the herd was significantly higher than that of the dominants (permutation test for paired replicates: N=5, P=0.02, two-tailed). Comparisons between reproductive strategies show that subordinate stallions fought more often than sneaks (permutation test for paired replicates: N=5, P=0.02). There was no significant difference between the frequency of fights for the other categories.

Benefit of Alliance to Dominant Stallion

Dominant alliance stallions benefited from the presence of the subordinate because he assisted in fights against outside rivals. Excluding stallions that changed reproductive strategies, the total fighting frequency for both alliance stallions combined was significantly higher (Wilcoxon test: W_x=15, N_1=N_2=3, P=0.05, one-tailed) than the frequency for one-male strategists, probably because alliance stallions were lower ranking so had to defend their mares more often. The sample size was inadequate to test for tenure length and/or survival in order to see whether there was a long-term benefit to the dominant from the presence of his subordinate ally. Again, only anecdotal evidence suggests that this may be the case. At the time of writing, of the same four stallions tested for the stability of reproductive strategies, the bachelor stallion died 3 years ago, the one-male family holder now controls two mares whereas the two alliance partners share five mares between them.

Mutual Benefits to Alliance Partners

Short-term alliance partners, dominants and subordinates alike may have the same long-term reproductive success as one-male group holders that never formed alliances. The stallions that lived in alliances for 2–3 years only and later changed to form a one-male group did not have a lower overall reproductive success than stallions adopting a one-male strategy from the start: when comparing the combined reproductive success up to 11 years (Table 3), there was no significant difference between stallions that had never formed an alliance and those that were temporarily dominant (Wilcoxon test: W_x=8, N_1=N_2=3, P>0.90) or even temporarily subordinate in an alliance (Wilcoxon test: W_x=12, N_1=N_2=3, P=0.35) that had later changed to a one-male group strategy.

Stallions in alliances were more successful than those in one-male groups in rearing their foals to weaning. There was significantly less neonatal (0–1 week) mortality in foals born into alliance families than in foals born into one-male groups. Only one foal out of 31 (3.2%) died in a family with two stallions, whereas 27 out of 123 (22%) were found dead in families with only one male (Fisher's exact test: P=0.02). As the overall foal mortality in this herd was highest during the first week and close to zero thereafter (Duncan 1992), foals born into alliance groups had a higher chance of survival.

Alliance stallions had increased access to proven reproductive mares. All young females of breeding age leave their natal family to integrate into a reproductive unit (Monard & Duncan 1996). They therefore become 'available' to stallions outside their natal group. The majority of mares (72.5%, Monard & Duncan 1996) leave at puberty before having a foal (gestation length is 11 months), but some mares wait until shortly after the birth of their first foal before they change groups. When two allies start their group, or integrate a new female into their already existing group, they associate preferentially with mares with very young foals of less than 1 week of age. Nine (50%) out of 18 available mares with very young foals were acquired subsequent to fights by allied partners compared to only four (16%) out of 25 taken over by single males (chi-square test: χ^2_1=4.24, P<0.05).

DISCUSSION

In this herd of horses, low-ranking unrelated stallions of similar age formed alliances based on coalitions where

both partners participated in defending their mares against rivals. Why should some stallions engage in alliances at all? As Dunbar (1988) stated, low-ranking males in general are at a disadvantage with regard to access to females. Alliance stallions both gained substantial benefits from their cooperation. Some individuals seemed to engage in short-term alliances in order to enhance their long-term reproductive success so as to catch up, in the long term, with the most successful strategy, holding mares alone, but the mechanisms are unclear. Other stallions seemingly engaged in long-term alliances because this represented the best option or because they might have had an advantage in controlling more mares in the distant future, but only anecdotal evidence suggests the latter. Another benefit was higher foal survival in alliance groups than in one-male groups. Again, the reason is not entirely clear, but a number of newborn foals were wounded accidentally during fights between adults shortly after birth, although we never observed infanticide. The young foals during their first critical hours after birth may benefit from the protection of two stallions against harassment from other horses. The third advantage of alliances was increased access to proven reproductive females that already had a foal. Mares are usually on heat from 7 to 10 days after the birth of their foal. However, since the majority of young mares, most of them without foals, change groups when they are on heat anyway (Monard & Duncan 1996), this may well indicate that oestrus alone was not the key factor in the alliance stallions' preference for proven breeding mares. Ninety-five per cent of the mares from the herd had a foal every year (Duncan 1992), so it was not possible to investigate the long-term consequences for this preference of stallions in alliances.

In conclusion, the benefits for both alliance partners seem to be straightforward. Mutual benefits to male alliances have been widely reported in mammals, although nonprimate species are poorly represented (for a review, see Clutton-Brock 1989), and benefits are usually discussed in terms of access to resources or enhancement of the common rank position rather than direct measurements of the consequences for an individual or its offspring (Harcourt & de Waal 1992). Nevertheless, mutualism or its synonym by-product mutualism (Mesterton-Gibbons & Dugatkin 1997) is defined as 'cooperation regardless of the opponent's behaviour, cooperation being merely a by-product of following the best strategy' (West-Eberhard 1989). My results suggest that alliance stallions both benefit from their association and follow the best strategy for both of them but not without costs to each of the partners.

Two main conditions are required for a cooperative behaviour to be reciprocal, first that the initiator behaves altruistically, which implies at a cost to him, and second that his reward, costly to the recipient, is delayed in time (Axelrod & Hamilton 1981). Moreover, if reciprocal altruism exists not only to enhance inclusive fitness, it should be observed between unrelated individuals and not just between kin. This seems to be the case in stallions. In a herd with many groups and multiple reproductive males, there is a dilemma for any stallion controlling females when challenged by a rival. He has to keep the rival at bay while making sure his mare(s) are not approached by other males. Stallions overcome this difficulty by forming coalitions where two males support each other. They either confront the intruder(s) together or share the task: while the dominant of the pair drives away the mare(s), the subordinate engages in an encounter with the other stallion(s). This is consistent with Berger's (1986) data on three alliance pairs, where the subordinate on average fought three times more often than the dominant. Guarding the females is less risky than fighting, thus the subordinate has a higher cost.

During a fight both stallions rear up, kicking with their forelegs or trying to bite each other at the throat, neck or legs. The risks incurred during these fights are infected wounds or damage to the legs which may lead to severe limping and can incapacitate a stallion to the point of his not being able to follow his group and permanently losing it as a consequence (Berger 1986; this study). During Berger's 5-year study, he estimated that 3% of all stallions died as a result of fighting. In our herd, no stallion was killed or mortally wounded, but fights clearly are risky and costly in terms of an individual's fitness. Two stallions, a one-male family holder and a subordinate alliance stallion, had reverse melanism (their usually white coat-hair grew black on scars); at the age of 20 in May 1996, the first had 35 permanent scars whereas the second had 146. Besides the risks, fights and displays are energetically costly. I do not have data on direct energy expenditure stemming from the higher frequency of displays or fights for subordinates, but Berger (1986) calculated that one of his dominant alliance stallions spent 7.1 MJ/day less than the subordinate. In domestic horses, the basal rate (digestible energy without work) is 65 MJ/day (National Research Council 1978), so the difference is considerable.

In exchange for his investment in defending their mares, the subordinate stallion gains paternity. Alliance stallions of one family almost never lose sight of each other. Mating needs a minimum of 3 min if the mare is receptive. The position of the stallion during mating is unbalanced, and therefore makes it easy for another stallion to intervene and prevent it. For example, a one-male family holder immediately interferes when another stallion tries to make body contact with one of his mares. The benefit granted by the dominant alliance partner to the subordinate is thus a passive one.

Finally, fights are not immediately related to matings. Although fights are most frequent during the reproductive season, they occur all year round (Feh 1987), whereas the majority of mares are on heat for only a few days per year (Duncan 1992). The reward to the subordinate partner, siring foals, is therefore delayed in time.

These results suggest that the relationship between the two allies contains elements of reciprocity. Although I could not test the Prisoner's Dilemma model or any strategy such as 'Tit for Tat' experimentally, in one case I was able to observe a dominant alliance male punishing noncooperation by the subordinate. In the experiment on the stability of reproductive strategies (see above), the four stallions were filmed individually and continuously

after their release with the new mares. For the first 15 min, the subordinate alliance stallion tried to monopolize a mare on his own without participating in the defence of the dominant's mares against the other stallions. In the 16th minute, the dominant attacked the subordinate repeatedly, tearing out part of his lip. From that moment on, the subordinate cooperated in the defence of the mares. This may imply that in some situations, the dominant may coerce the subordinate into cooperation (Clutton-Brock & Parker 1995).

Authors mostly agree that reciprocal altruism between unrelated mammals is difficult to measure and examples are rare (Harcourt & de Waal 1992; Slater 1994). This study, like Packer et al's (1991) on lions, and Connor et al.'s (1992) on dolphins, shows the existence of male alliances in a nonprimate species, the alliance being based on coalitions against rivals with reproductive benefits to both partners.

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Behavior Patterns and Communication in Feral Horses

By JAMES D. FEIST[1]) and DALE R. MCCULLOUGH

With 15 figures

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Abstract

The social behavior of feral horses was studied in the western United States. Stable harem groups with a dominant stallion and bachelor ♂♂ groups occupied overlapping home ranges. Groups spacing, but not territoriality, was expressed. Harem group stability resulted from strong dominance by dominant stallions, and fidelity of group members. Eliminations of group members were usually marked by urine of the dominant stallion. ♂-♂ aggression involved spacing between harems and dominance in bachelor groups. Marking with feces was important in ♂-♂ interactions. Foaling occurred in May and early June, following the post-partum estrous. All breeding was done by harem stallions. Young were commonly nursed through yearling age. These horses showed social organizations similar to other feral horses and plains zebras.

Introduction

A study of the behavior and ecology of a population of feral horses (*Equus caballus*) was conducted on the Pryor Mountain Wild Horse Range between May 1 and November 1, 1970. The study area (Fig. 1) encompasses 13,598 ha of public land administrated by the Bureau of Land Management (BLM) on the Wyoming-Montana border approximately 16 km north of Lovell, Wyoming. Complete information on the composition and social structure of the population is given by FEIST (1971) and FEIST and MCCULLOUGH (1975). All 270 horses on the Range were individually identified. Many showed evidence of the Barb and Andalesian blood lines which were originally introduced to the American west by Spanish explorers in the 16th century. During the period of the study there was no human manipulation of the population, with the exception of completion of a Range boundary fence

[1]) JAMES DEAN FEIST, age 27, was killed on September 12, 1973 in the crash of a light plane while conducting a count of caribou in Yukon Territory and adjacent Alaska. This study was originally done for a master's degree (FEIST 1971) under the direction of the junior author who prepared this manuscript.

during the study which excluded 40 horses from the Range. Pryor Mount horses were in naturally occurring social groups, and can be considered have been a natural population at the time of the study. Subsequent to t study some horses have been removed from the range.

Methods

Travel on the Range was primarily by a half-ton truck on three main access roads. T usual procedure was to drive to an access point and work from there on foot. A saddle ho was used on the extreme western side of the Range where there were no roads. The horse w not used when behavior notes were being taken because of interactions between the sad horse and the wild horses.

When horses were first sighted, the color, sex, age, and other markings were record for each individual on prepared silhouettes. These were further documented with photograp Individuals were classified to age as foal (born that spring), yearling, immature (two or thr years old), and adult. In many cases it was difficult to distinguish immatures with certain since mature body size and length of mane and tail are usually attained at this age.

Locations of horses were recorded on a map with quarter-mile square grids. Each i dividual was given a name, and the name of the group usually was that of the domina stallion. Data on members of a social group were filed together. Each time the group was sight again date, location, and group changes were recorded.

Detailed quantitative notes were obtained by direct observation over extended perio of time. Frequency of behavioral interactions was recorded continuously and reported minute intervals. This usually consisted of watching one band at a time and noting the tim of occurrence of behavior events for all individuals in the band. Stereotyped behavior ac were described and photographed. Cloud cover, wind, and temperature were described in qualitative manner.

Charts on group cohesion were kept simultaneously with the quantitative notes. The charts were based on a determination every 15 min of estimated distances between (1 mares, (2) mares and their foals or yearlings, (3) mares and the dominant stallion, and (4) t group and the next closest group, if one was in sight. Extended periods also were spe observing water holes to obtain information on frequency of use and behavior at this resourc When it was possible to observe more than one group at once, sketch maps were kept t diagram the groups movements and their relative positions throughout that period of no taking. Behavior observations were aided by 16 × 50 binoculars. To avoid biases all of th quantitative notes were taken while the horses were unaware of the presence of the observe

In addition, six bands were habituated to presence of the observer in order to ga observations at close range. By approaching groups in a slow, calm, and relaxed manner, nev moving directly towards them or staring at them, these groups came to tolerate presenc within 8 to 30 m for extended periods of time. There was considerable variation in excitabilit of horse groups. All horses were excitable during strong winds, as previously reported fo elk (MCCULLOUGH 1969), white-tailed deer (NEWHOUSE 1973) and pronghorn antelope (KIT CHEN 1972). Similar excitability occurred during stormy weather.

Results and Discussion

Population Characteristics

To summarize, a total of 270 horses were censused on the Range by individual identification. They included 78 mature stallions, 79 mature mares 21 immature stallions, 17 immature mares, 11 yearling stallions, 19 yearlin mares, 19 colts and 16 fillies.

Horses were organized into 44 harem groups consisting of a dominan stallion, 1—3 mares, and their immature offspring, and 23 bachelor stallio groups. Mean size of harem groups was 5.0 and bachelor groups 1.8. Haren groups were quite stable, with most of the changes involving immature ♀♀. Considerable shifting occurred within bachelor stallion groups. Social orga- nization closely paralleled that reported for plains and mountain zebras by

KLINGEL (1964, 1965, 1967, 1968, 1969 b, 1969 c) and KLINGEL and KLINGEL (1968 a, 1968 b), for Sable Island ponies by BRUEMMER (1967) and for New Forest ponies by TYLER (1972).

Home Range and Spacing Between Groups

Groups of Pryor Mountain horses occupy home ranges in the sense of BURT (1943) as geographic areas in which most of the time is spent and life activities are carried out. The groups were quite predictable in the areas in which they could be found; each home range included a large feeding area and one or more watering sites. Territoriality, in which the geographic area is defended against other groups, has been reported by KLINGEL (1969 a, 1972) for Grevy's zebra and African asses, and by MOEHLMAN (1974) for some male feral asses in Death Valley, California. SCHLOETH (1958) interprets fecal pile behavior in horses as territorial marking. That the Pryor Mountain horses are not territorial is shown in Fig. 1 by the results of plotting the home ranges over the period of the study of 21 harem groups using the more accessible southern 54.8 % of the Range (7,451.7 ha). If the harems were territorial, one would expect to find a mosaic of areas with relatively well defined boundaries each used more or less exclusively by one group. The opposite situation holds. Only 10 % of the ground area was used exclusively by one harem, and this consisted of fragments on the edges of home ranges. The lowest overlap by other bands was one band overlapping 4; 4 bands overlapped with 5 others, 3 with 7 others, 12 with 8 others and one with 12 others.

Thus, considerable overlap of use of the area by harems occurred. In addition, bachelor groups shared the same areas, and most of the remainder of the harem groups crossed the area to reach water. These results are similar to those reported for New Forest ponies by TYLER (1972). Furthermore, no defence of boundaries or space-related dominance was observed.

Home ranges varied in size depending upon quantity and quality of food resources and closeness to water. Home ranges of the 21 harems plotted varied from 299.8 to 3,200.0 ha with a mean of 1,485.4 ha.

Much of the observing was done from high points from which several groups could be watched at the same time. Even though a number of groups used the same geographic area, minimum spacing between the groups was maintained, primarily through the activities of the dominant stallions. When bands came in contact the dominant stallions would approach each other, posture and threaten, and sometimes engage in a mild fight of pushing and kicking. In fact, maintenance of linear spacing of groups was the most common cause of observed stallion fights (37 out of 84; 44 %). After the encounter, the stallions returned to their respective harems and moved them apart, or maintained a distance of at least 90 m. Individual distance between members within a group was reduced when in close proximity to other groups.

Similar behavior was observed on trails and at water holes. Dominant stallions herded harems off the trail to allow bands coming from the other direction to pass without intermingling. Linear spacing was maintained between groups moving in the same direction. Horses whinnied when approaching water holes, and if a group was already present, the new arrivals would wait until they had left. As many as five harems were observed near a water hole at the same time, each waiting until others had left before moving in to drink. On only one occasion (on an extremely hot day) was a harem observed to move into water while another group was still there. This resulted in considerable tension and threatening, but no fights.

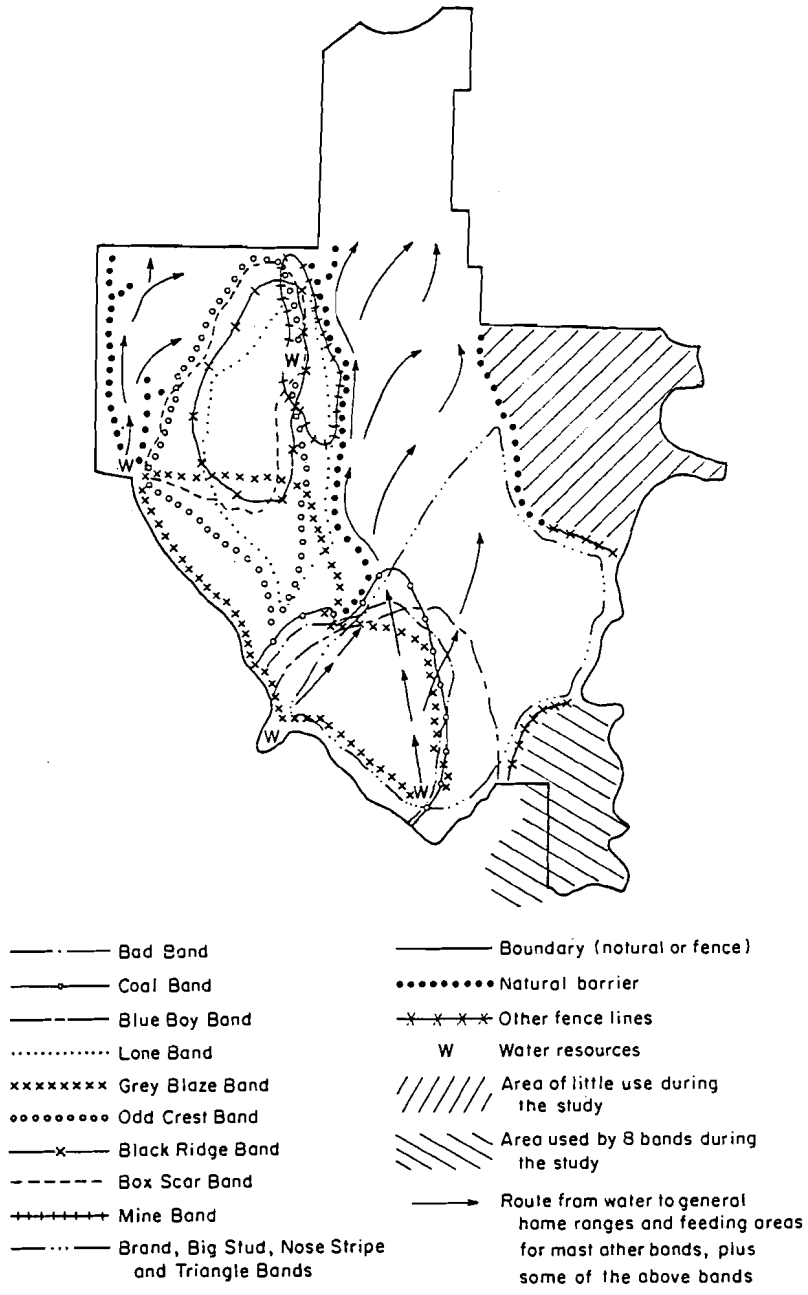
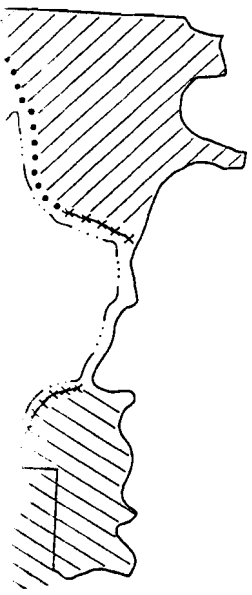


Fig. 1: Map of the Pryor Mountain Wild Horse Range showing home ranges of 21 harem bands. Note large overlap of home ranges

General Activity Patterns

Drinking

No consistent watering schedule was maintained by any group. The horses visited water holes from early morning until after dark. A few groups visited water holes at approximately the same time for several days, then



Boundary (natural or fence)
Natural barrier
Other fence lines
Water resources
Area of little use during the study
Area used by 8 bands during the study
Route from water to general home ranges and feeding areas for most other bands, plus some of the above bands
Range showing home ranges of home ranges

erns

intained by any group. The ntil after dark. A few groups time for several days, then

anged times or, in some cases, even used another water hole. The constant shifts in feeding areas probably explain some of this variability. Although groups also visited water holes at night, watering at this time was much less frequent than in the daytime.

This is in marked contrast with the results of PELLEGRINI (1971) in Nevada where feral horses visited water holes at night. SOLMATIN (1964) has described watering behavior of Onagers *(Equus onager)*, observing that these animals appeared to visit the watering places at about 10 p.m., remaining for one to two hours. They also adhered to regular watering spots and routes to them. Perhaps the amount of human disturbance was an important factor in these instances. JOUBERT (1972) reported that undisturbed mountain zebras watered throughout the day while disturbed ones watered at night.

Generally, a group visited a water hole once a day. However, frequency was affected by weather conditions. Extreme heat caused some groups to drink twice a day and, on one occasion, caused five groups to stay close to a water hole (within 38 m) for more than two hours, which was much longer than usual. Rains that formed temporary water pools in the rocks, or snowfall, which made water available at the higher elevations, eliminated regular travel to water holes, as did cooler weather which reduced need.

Over most of the range, horses stayed within 5 or 6 km of water and generally visited regularly. However, there were shifts to one or two different water sources depending on feeding areas. Groups that usually fed on the higher elevations of Mystery Ridge had the longest distance (8 to 16 km).

This pattern will now be altered because of the construction of a water catchment basin on Mystery Ridge by the BLM. This new water supply will certainly make such long travels for water unnecessary, thus helping to distribute horses more evenly over the Range. A catchment basin in the northeast corner of the Range between the Mystery Ridge cliff and the Bighorn Canyon would have a similar beneficial effect there.

Groups approached water holes on a trail in single file with the dominant stallion or a mare leading. Groups returned to feeding areas in a similar fashion or grazed along the way. When a mare of the group was first to the water, she was often found to be lactating. Occasionally, such mares would even reach the water hole about 100 m ahead of the rest of the group and were often the last to finish drinking. At the Big Coulee water hole, which was a slow seep from a bank, lactating mares were observed to drink only from one of the clearer pools or directly from the seep. As each horse finished drinking, it would wait for the rest of the group to finish when all moved away together. An exception was the Triangle group, in which a mare and foal were observed to remain at the water hole a number of times for as long as 30 min after the rest of the group had left.

The time spent at water holes rarely exceeded 30 min, most groups taking between two and ten minutes to drink and begin leaving. MCKNIGHT (1958) and MOEHLMAN (1974) have reported that burros in the American southwest may remain at water holes for hours. MCKNIGHT also observed feral burros rolling and defecating in the water. Feral horses were observed rolling, but not eliminating in the water.

Feeding

Horses are primarily grazers, feeding with heads close to the ground, taking a few mouthfuls, then moving forward a step or two. Pawing with a front hoof occasionally was used to remove obstacles and facilitate feeding.

Each group grazed as a unit with individual distances of at least several m maintained between members. On rare occasions a group would be spread over a considerable distance while feeding. For example, Bucki, a mare in the Blue Boy harem was observed feeding out of sight and over 100 m from the band. At the approach of other horses, a group would usually stop feeding and gather behind the dominant stallion, as reported in domestic horses by ALTMANN (1951). Night observations revealed similar feeding habits.

Extensive studies of food habits of the horses were not conducted. Nevertheless, some general qualitative statements can be made. The most important and sought-after foods were grasses. However, grasses on the Range were extremely sparse, particularly in the low, semi-desert area, and the horses had to supplement their feed with other types of vegetation. These foods were generally of a coarser nature than the grasses, and in all cases their use was minimal compared to the use of the grasses. In the southeast corner of the Range and on the Tillett ranch in the same area, where water was abundant, horses used marsh grasses, reeds, and numerous forbs. On the rest of the range, which was dry, there were fewer species of forbs available to the horses. The most unusual use of a woody plant was the eating of the roots of winter fat (*Erotia lanata*). The tops of these plants are especially palatable (MCCULLOUGH 1969), but no known nutritional analysis has been done on the roots. Horses obtained roots by pawing the ground to remove the soil from around the tap root, which was either bitten off or pulled out of the ground and eaten. The tap-like roots of two species of milkvetch (*Astragalus kentrophyta* and *Astragalus gilviflorus*) were also eaten. These two plants formed compact, mat-like cushions at ground level but were pawed up and only the roots eaten.

Most grasses on the range were short grasses, which, along with consumption of roots, resulted in a considerable intake of grit. This produced excessive wear on the horses' teeth, as evidenced by jaws of animals found dead.

The new growth of a few woody plants was also observed to be eaten by the wild horses. These included saltbush (*Atriplex* spp.), greasewood (*Sarcobatus vermiculatus*), rabbitbrush (*Chrysothamnus* spp.), big sagebrush (*Artemisia tridentata*), and black sagebrush (*Artemisia nova*), and in rare cases and in minimal amounts, Utah juniper (*Juniperus osteosperma*) and mountain mahogany (*Cercocarpus* spp.).

When food becomes scarce horses may also supplement their diet by eating old feces. On August 14, observations were made of a mature, immature, and yearling ♀ eating old feces from a stallion fecal pile. Similar observations for the winter months were reported to us by LLOYD TILLETT. These observations do not agree with the statement of HAFEZ et al. (1962) that adult horses reject the feces of other horses, but that foals do consume fresh feces of adult horses. They state that this behavior ensures acquisition of the proper intestinal bacterial flora. This would not explain the eating of old feces which we observed, which seemed to be related to a shortage of quality natural forage.

On one occasion in the spring, a lone stallion was observed eating mud of a dark grey color from a nearly dried up water puddle. No similar observations were made at water holes. Horses may obtain minerals in this way. We have observed domestic horses eating mineral soils from a newly ploughed field in Canada, and such behavior is common among wild ungulates (COWAN and BRINK 1949).

Play

Activities such as running about alone or in groups, or chasing one another for no overt reason, were designated as "play". These activities involved much head tossing, sudden stops and starts, and kicking of the hind legs into the air. They were regularly indulged in by foals within a group and even between foals of different groups. The latter was tolerated by the dominant stallions if the foals returned to their groups after play. Foals were observed playing at all times of the day, but primarily in the early evening or when temperatures cooled. Other age classes exhibited similar activity only on rare occasions. This was always restricted to horses within a group and was not tolerated by the dominant stallion, who quickly attempted to end the activity.

HAFEZ et al. (1962) speculated that play functions to increase locomotive skills and to test social dominance of the young ♂♂. The first function of play seems obvious. The second function also seemed apparent, for larger and older foals clearly dominated the smaller ones by mounting behavior and threats. In one case, a foal forced another one to stand by biting and holding on to its mane. Short, mild, and sporadic fights — primarily by pushing — were seen occasionally while foals were playing. Play in New Forest ponies is discussed in detail by TYLER (1972).

Rolling

We observed 81 cases of rolling, 50 (61.7 %) of which were in dust and 31 (38.3 %) in mud and water. 26 of the 31 observations of rolling in mud and water involved mature stallions. A total of 62 (76.5 %) observations of rolling was of ♂♂ and 19 (23.6 %) were of ♀♀. Except for two, all of the ♀♀ were mature. No yearling or foal ♀♀ were observed to roll in dust or water; however, one male yearling and eight male foals were observed to roll. Mature ♂♂ were observed rolling in 47 (73.4 %) cases, as opposed to 17 (26.6 %) for mature ♀♀. Three of the 47 mature stallions observed rolling were stallions from bachelor groups.

6 cases of immature ♂♂ rolling were also recorded. Three observations were of an immature ♂ rolling at the same time with the dominant stallion of a harem group. These all occurred in the Crip group, in which Crip and J.S. rolled together. In the other three cases, the immature ♂♂ rolled alone but were always immediately followed by the dominant stallion of the harem rolling in the same place.

Places used for dusting were on exposed, fine soil which was free of large or sharp rocks. Some locations were found in which the horses had pawed at the ground to loosen the soil to create a dusting place. Pawing and apparent smelling usually preceded rolling. The dusting sites were found scattered throughout the range, but were particularly concentrated around the water holes. A dusting site was not exclusively used by any one horse or group, but was used by any horses who passed by. Sites for rolling in the mud and water were restricted to the watering holes. On occasion, horses would paw and splash water on their undersides after drinking, then occasionally roll in the water. On a few occasions, individuals were so covered with mud that they could be identified only because of the group they were with!

In some cases all or most of the members of a group rolled in succession, in the same dusting site. This was observed six times, and in each case the dominant stallion was the last to roll. If the group was moving across an area

with some members well in front of the stallion, and if a mare or mares rolled, the stallion would also roll in the same pit when he arrived. This may be related to dominance of the stallion over the group, as is also evidenced in the behavior of the stallions after the rolling of immature ♂♂. Three cases appeared to be clear indications of the stallion showing dominance over the immature. The other three incidents involved Crip and J.S. rolling together which may have indicated that Crip, with his abnormal front hoof, was losing his dominance in the group (which in fact, happened). 11 of the cases in which stallions rolled were related to some form of fight, challenge, or threat towards another stallion.

Rest and Sleep

Rest or sleep (Fig. 2) was observed in a number of situations. In some instances shade, offered by vegetation or vertical canyon walls, was sought for a resting place. At other times, high exposed knolls or hillsides were used to take advantage of wind during the heat of the day. Group members rested or slept in a tight group, or stood in small groups of one or two pairs. The common posture for resting was with ears out or forward, head lowered with the neck horizontal to the ground, eyes partially or fully closed, and the weight often taken off one of the hind legs. Foals rested either by lying down with the head up and legs partially folded under the body, or by stretching out with the body flat on the ground. Foals spent much time in these positions when young, but as the summer progressed the time spent lying down decreased. Yearlings spent less time in these positions than foals, but more than mature horses. Mature and immature horses only occasionally rested or slept lying down. These observations agree with those of JOUBERT (1972) on mountain zebras. No observations were made of the entire band lying down. The protective advantage is obvious. This agrees with HAFEZ et al. (1962), who note that such behavior is rare. KLINGEL (1967) also observed that during the rest period there is at least one member of the zebra band standing alert at all times. We saw only one case of the dominant stallion of a harem group resting by lying down. On this occasion, in the Crip group, the whole group except the "number two" stallion, an immature ♂, were found lying down at the same time.

Yawning often occurred at the end of sleep or rest activity. This was performed with the eyes usually closed, ears up, and the mouth held open in a "U" shape with the canine teeth exposed. Stretching followed a yawn, although there were instances when the acts occurred independently. Horses stretch either by extending one hind leg off the ground and to the rear and bending the back up and down, or by extending and raising the neck.

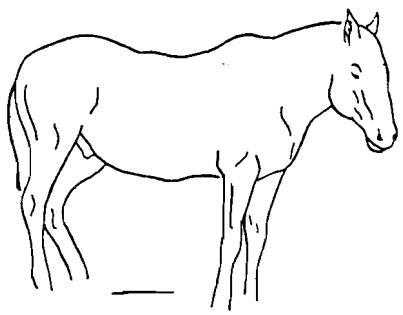


Fig. 2: Posture for rest or sleep

Communication and Social Behavior

Perceptions

Wild horses appeared to have good hearing. They were rarely alarmed by sound alone, but would usually have to see the source before a reaction such as escape behavior was elicited. Hearing was employed to detect and locate intruders and other groups, as well as in communication between individuals and groups.

Olfaction was important to the horses. As with hearing, we found that horses usually would have to see the source of the odor before taking action. On a number of occasions horses were observed using olfaction in a precautionary way. Big Coulee Spring was hidden so that a horse approaching it to drink would have to be within 30 m before it could see other horses at the water hole. Horses periodically tested the air by sniffing as they approached and as they drank. This precaution seemed to be an attempt to prevent one group from approaching too closely to another. Olfaction was also used in association with greeting between horses. They would approach and touch or almost touch nostrils, and smell each other. We also observed stallions periodically sniffing the air in what appeared to be attempts to locate harems which had become separated as they grazed. The most extensive use of olfaction occurred among stallions, particularly by those with harem groups. This occurred over urinations and defecations of mares, at fecal piles and dusting sites, and during encounters and fights.

The keenest sense of the horse, and the one that appeared to be most relied on for detection of intruders, was vision. The eyes of a horse are set at the side of the head, providing a wide field of view. The retina is non-elastic but is arranged on a slope or ramp, the bottom part being nearer to the lens (HAFEZ et al. 1962). With this arrangement, the grass on which the horse is grazing and distant objects are in focus at the same time. With the head up, slight movements of lowering or raising the head are used to focus. HAFEZ et al. also note that eyes of horses are adapted for nocturnal vision. Our nocturnal observations indicated that horses carry on normal activities at night.

Individual Recognition

The wild horses, especially those in the same group, seemed to have no difficulty in recognizing each other as individuals. An example of auditory recognition involved the Triangle harem. Goldylocks and her foal, Gold, would occasionally lag behind at a water hole. When they moved to rejoin the band, Goldylocks would often whinny or neigh. Hearing this, Triangle would stop the movements of the group to wait for the mare and foal, even though they were out of sight and over a quarter of a mile away. Numerous observations were made of mares and foals apparently recognizing each other by sound alone. KILEY (1972), TYLER (1972), and MOEHLMAN (1974) also noted that calls seemed to convey individual identification.

It was obvious that individual recognition occurred by sight. Harem stallions immediately recognized a strange member in the group and, similarly, singled out any of their harem who were temporarily in another group. The frequent smelling of other horses and excretions occurs with such regularity as to suggest individual recognition.

Vocalizations

Numerous observations were made of the louder vocalizations and the situations in which they occurred. Four definite vocalizations were identified: the "snort", the "whinny" or "neigh", the "nicker", and the "squeal". HAFEZ et al. (1962) described the snort as a danger signal, the whinny as a distress call, and the nicker as a sign of relief or close communication and courtship. WARING (1971) used recordings to distinguish seven sounds; squeal, nicker, whinny, groan, blow, snort, and snore. KILEY (1972) described and presented sonograms of six vocalizations: sigh, sigh-nicker, nicker, snort, whinny or neigh, squeal, and roar. She proposes that the vocalizations sigh, sigh-nicker, nicker, whinny, and long whinny, represent a series given in order of increasing excitement, with the snort being derived from the nicker, and the squeal and roar from the whinny.

In our analysis, we did not record the sigh as a separate vocalization, since much of our observing was done at a distance too great to hear this sound. The sigh-nicker is an intergrading vocalization (KILEY 1972) which we recorded as a nicker. Our observations of the nicker agree with those of HAFEZ et al. (1962), WARING (1971), TYLER (1972), and KILEY (1972) that it is given in close communication between horses primarily within the harem group. It is given most commonly in interaction between mothers and foals, and in courtship, and seems to be a close-range cohesion call. WARING (1971) and KILEY (1972) note that it is also directed towards a handler in domestic horses.

The whinny or neigh was used as a distress call by wild horses, but it was used in other situations as well. As a distress call, it was used primarily by mares and younger horses of both sexes. Foals out of sight of their mother or left behind in movements of a band would commonly neigh. A mare who was disturbed about the closeness of another mare to her foal neighed quite loudly, whereupon the stallion immediately moved in and separated the mares. A mare would whinny loudly and frequently for her lost foal.

Stallions used the neigh towards foals who had wandered to another group. In such cases, the foals always returned immediately to their group. The stallions Brand and Rye used neighing to achieve cohesion in their harems while trying to gain stray mares. When two of a group of three bachelor stallions left a water hole, the one remaining neighed. The two waited for him to catch up.

Neighing also was used by stallions in build-ups to fights. They would stand and stare at each other from a distance, occasionally calling back and forth. Mares from different groups once neighed back and forth, which led to a mild fight between the stallions. The neigh was also used occasionally when greeting another horse.

It seems that the whinny is a long-range communication call, which typically functions as a cohesion call, as reported by WARING (1971), KILEY (1972), and TYLER (1972). However, some of the observations of its use suggest it functions in the broader context of indicating location of distant horses which may, in fact, be a prelude to aggressive interactions rather than cohesion.

The use of the snort as a danger signal (HAFEZ et al. 1962; TYLER 1972; KILEY 1972; others) was evident from our study. WARING (1971) referred to this call as a blow, using snort to describe clearing of the nostrils. In our study, a snort was usually issued by the stallion of the group when an intruder was discovered. After the stallion snorted, the group came to the alert and watched the stallion for signals while they, too, studied the intruder. Only rarely did a

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mare snort. If a mare discovered an intruder first, she would come to an alert posture; subsequently, the stallion would come to alert and snort. The call has the physical properties described by MARLER (1959) of carrying well, and being readily localized.

We frequently heard a vocalization, not described by HAFEZ et al. (1962), but which WARING (1971), KILEY (1972), and TYLER (1972) referred to as a squeal. KILEY (1972) described this call as a defensive greeting call, while WARING (1971) reported it in agonistic encounters. TYLER (1972) observed it to be used by mares in relation to stallions and in mare-mare fights. In our observations this call was used exclusively by stallions during fights. It was rarely issued when stallions were smelling noses and was commonly heard again in unison while smelling fecal piles after defecation by one or both. During the kicking and biting sequences of a fight, the squeal was again given at times, though not as consistently as in early stages of the fight. The roar, which KILEY (1972) reported as given by stallions in frustration or rejection by non-receptive mares was not heard in our study. Neither were several of the vocalizations reported by WARING (1971). However, the latter would be expected because of the distance at which we worked, and the gradation of sounds in the horse, which are subtle and difficult to distinguish by ear from a distance.

Alert, Alarm, and Flight Behavior

When a horse detected an intruder, it came to the alert posture (Fig. 3) and watched the intruder. The ears were flicked forward, then back, out, and forward to receive sound cues from other horses or the intruder. It quickly brought its weight evenly to all four feet and slightly raised its tail. While or after assuming this posture, it often gave a whinny to alert other horses. Even if it did not vocalize, its sudden shift to alert posture usually was sufficient to alert other horses.

If the group was disturbed greatly by the intruder, the stallion gave a snort and he and the rest of the group came to the alarm posture. This was similar to the alert posture except that it was a more exaggerated stance, with the head, neck, and tail higher. In alarm (Fig. 4), the stallion took a few quick steps toward the intruder to stare and, possibly, snort again. The group mem-

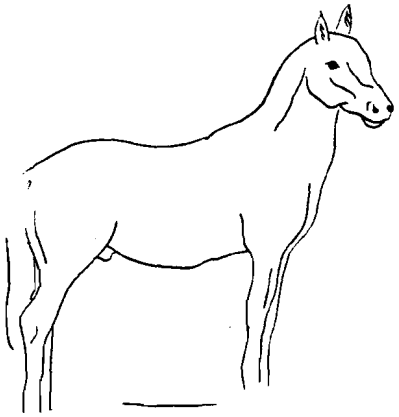

Fig. 3: Alert posture

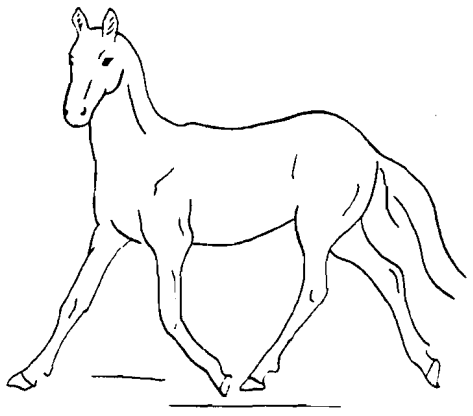

Fig. 4: Alarm and flight

bers bunched and moved to the rear of the stallion so that he was between the group and the intruder. During this interval, the horses often lifted their tails higher and defecated.

Members of the group other than the stallion often became excited about our presence, even whinnying to call the attention of the stallion. If the stallion was not excited by our presence or could not detect us, he remained calm, and the rest of the group would soon calm down as well. If a mare snorted, a rare occurrence, the stallion and other group members came to her side.

If flight behavior was elicited, the stallion turned and moved away from the intruder, and the group moved off with the stallion directing movement from the rear or, in some cases, from the front of the group. Sometimes the stallion moved the group by herding (Fig. 11).

Usually the group moved 50 to 100 m then stopped and watched the intruder again. The degree of alarm depended on the particular group, closeness of the intruder, and degree of surprise.

Aggressive Behavior

Threatening actions by both mares and stallions followed one of two general modes, each consisting of a progression of acts ranging from mild to serious. The first mode involved biting, and the progression from mild threat to serious threat included ears laid back, head swing (Fig. 5), mouth open, attempted bite, and bite. "Ears laid back" involved flattening the ears against the top of the neck with the head usually turned towards the threatened horse. The "head swing" consisted of the ears laid back plus the side swing of the neck and head toward the threatened horse. The "mouth open" consisted of the above described acts with the mouth being opened and the lips tightened in a manner that exposed the incisor teeth and, if present, the canines. The "attempt to bite" involved the above motions and was accompanied by aggressive forward movement, and finally, the infliction of the "bite" itself.

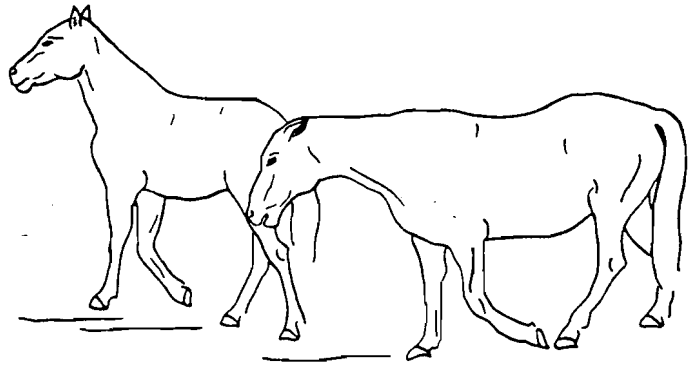

Fig. 5: Aggressive threat: "ears back, head swing" of the biting mode

In the second mode, kicking, the progression was turning the rear to another horse, threatening to kick with one hind leg (Fig. 6), kicking with one hind leg, and kicking with both hind legs (Fig. 12). In the "kick threat", a horse would raise one hind leg off the ground and pull it up under its body in readiness. To accomplish the "kick", the hind leg was struck out to the rear at the other horse. To "kick with both hind legs", it lifted them together and thrust them out to the rear, its weight and balance being taken up on the front legs.

Threat postures were used primarily in maintaining individual distance within the group. Threat was exhibited if another horse came within 1.5 m of one that would not tolerate close association at that moment. Mares also threatened other horses if they came too close to their foals. Threats were used at the water holes to gain drinking space, although for the most part, group members were tolerant of each other in this circumstance. Mares not in heat employed threat postures toward stallions who came too close in attempts to smell and tend them.

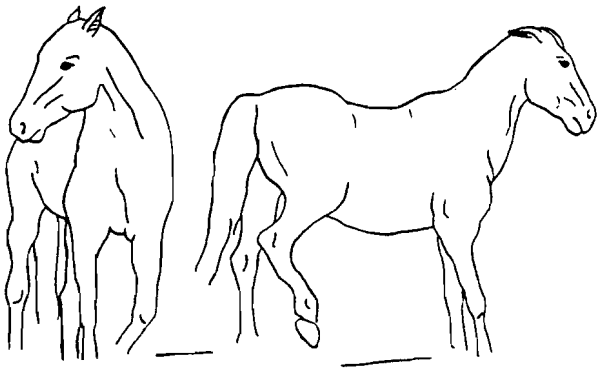

Fig. 6: Aggressive threat: "hind-foot kick" of the kicking mode

As noted below, we were unable to determine a dominance or a rank order expressed through threat displays among the mares of a group. In one situation one mare might threaten another; in a similar situation at another time, the roles would be reversed. Rarely was more than one threatening act used. If a mare threatened another, the threatened mare usually moved away a few steps, ending the encounter.

Threats were commonly employed by mares, perhaps because they were on much the same level of dominance in the group. The use of threats by stallions was considerably less frequent because of their strong dominance, but threats used were more forceful than those of mares. Also, stallions were commonly outside the cluster of the group which resulted in fewer interactions with other group members.

Several threats were used exclusively by stallions: the forefoot kick, herding, and the direct run. A description of "herding" behavior is found in the section under that title. The "forefoot-kick" was commonly used in fights between two stallions and was accomplished by striking forward with a front leg and hoof. The "direct run" was used to move an individual in a group or non-band members away. It involved running directly at a horse with ears laid back and head and neck low, but with no obvious use of the herding posture.

Submissive Behavior

The most common expression of submission was simply moving away. For example, a mare who came too close to a foal was threatened by its mother, and reacted by turning and moving away. Or if a mare was approached by a stallion in courtship and she was not receptive, she threatened the stallion. His response usually was to move off.

The second kind of submissive behavior was not induced by a threat. It involved interactions between the dominant stallions and young ♂♂ in the harem. No ♀ was observed showing this behavior. This act, which we called "teeth-clapping", consisted of the immature ♂ approaching the stallion from

the side or front with his neck and head stretched out, reaching up toward the stallion's head (Fig. 7). The ears of the submissive horse were pointed outwards, the mouth was open with the lips pulled back to expose the incisor teeth, and the lower jaw was moved up and down. TYLER (1972) has referred to this act as "snapping", a term we believe has aggressive overtones. We prefer to use the more descriptive term, "teeth-clapping", for this clearly submissive act.

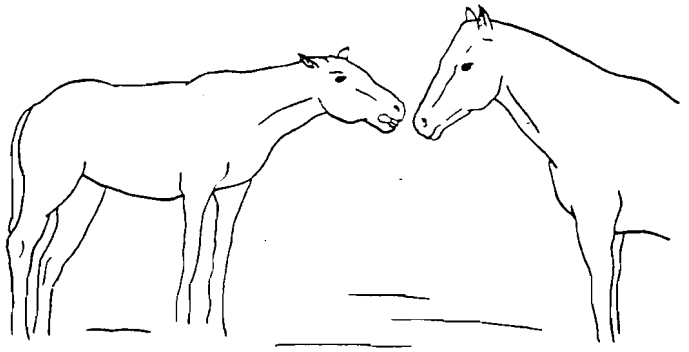

Fig. 7: Teeth-clapping by an immature ♂ towards the dominant stallion of the harem

6 harem bands contained an immature ♂, and we observed 21 instances of teeth-clapping in a variety of situations. Four cases followed fights between dominant stallions of different harems. After the fight, when the stallions returned to their harems the immature ♂ showed this submissive behavior. Two cases occurred in the Crip band on different occasions. J.S., the immature ♂, left the band to fight with another stallion and retrieve a mare and a foal. J.S. apparently was performing the role of a dominant stallion although submissive to Crip, whose mobility was impaired. Upon his return, J.S. teeth-clapped to Crip. 8 observations of teeth-clapping were made after encounters between the dominant stallion and one of the mares. For example, if the stallion were tending a mare and the mare rejected his advances by a threat, the immature ♂ would then approach the dominant stallion in this submissive posture. In another example, in the Sheik harem, Number-Two, the immature, performed teeth-clapping to Sheik after he returned from tending a mare. This was accompanied by an unusual response from Sheik, who reached out and bit onto the skin on the shoulder of Number-Two. They remained fixed in this position with ears laid back for about a minute. Ordinarily, the stallion simply stood and received the submissive posturing of the immature ♂.

Another interesting encounter arose when Sheik retrieved an immature ♀ that had wandered into the Buckskin harem. Following the retrieval, Number-Two approached Sheik in the submissive posture and then a yearling ♂ of the group also exhibited teeth-clapping. Two observations of teeth-clapping were recorded in the Del harem, both given by the immature ♂, Grey Streak. The first occurred after Del threatened Grey Streak, who had been attempting to groom him. This was the only case observed of a threat to an immature inducing teeth-clapping. The second instance occurred when Del rejoined the harem after becoming separated during feeding. He was met with a display of teeth-clapping by Grey Streak.

Two observations of colts giving teeth-clapping were recorded. In one case, Tone Foal of the Del harem walked over to the Buckskin harem and gave

the submissive posture to Short Tail, a yearling ♀. This was the only time the act was observed being given to a ♀. This may have been confusion because of the age of the animal. The other case of repeated occurrence of teeth-clapping by a colt was on the day that Cabin Black, formerly a lone stallion, gained the mare Box and her colt, Little Blaze, from Crip. The stallion spent two days trying to get Box to stand to be mounted. During these intensive encounters, occurring at a frequency of two to four per hour, Little Blaze remained close to his mother, stretching his neck out and teeth-clapping at Cabin Black.

The submissive action by teeth-clapping is believed by ZEEB (1959) to be an expression of ritualized grooming. Mutual grooming, as noted in the section below, can be an appeasement expression or it can be used for mutual social contact. However, in only 5 of the 90 cases of mutual grooming was a dominant stallion observed grooming with an immature ♂. One case followed immediately after a threat by the stallion to the immature ♂. The teeth-clapping and its approach, which is similar to that for grooming, may be a ritualization of a non-aggressive mutual act which allows young ♂♂ to express their subordinate rank, thereby avoiding aggression from clearly stronger animals.

Mutual Grooming

Mutual grooming was common in the daily activities among wild horses. It began with one or both individuals approaching head on. The ears were held forward or out, rather than laid back which indicates threat. The two horses often smelled each others' noses and/or necks before grooming. Mutual grooming began on the side of the neck, withers, and base of the mane, then progressed down the back to the rump and, in some cases, the base of the tail (Fig. 8). While this progression was most common, occasionally grooming was exclusive to the neck or the rump. Horses were also observed to groom along one side, then switch positions and groom along the opposite side. Grooming was accomplished by pulling the lips back slightly and using the incisor teeth. If one stopped grooming, the other also stopped. Only in rare cases was grooming not a mutual effort. The time span for grooming ranged from a few s to 10 min. In 90 observed cases of grooming, 90% lasted less than 3 min, and 10% lasted between 4 and 10 min.

The functional benefit of mutual grooming is the care of the coat and skin in areas difficult for the individual to reach. Self-grooming was accomplished by scratching with a hind hoof or by rubbing against trees and bushes. However, as noted by TYLER (1972), the close contact provided by mutual grooming may also strengthen the bonds between the group mem-

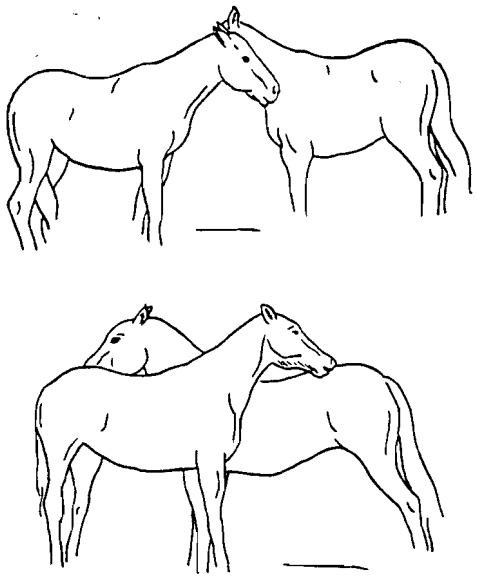

Fig. 8: Mutual grooming of the neck (above) and rump (below)

bers. Appeasement was an obvious reason for grooming in some instances, indicating the relationship of grooming to submissive teeth-clapping behavior or ritualized grooming. Most cases of unilateral grooming were by immature animals following threats by adults.

Mutual grooming occurred only between members of the same group. All combinations of group members were observed grooming, except dominant stallions and foals. In two cases a stallion groomed a mare for short periods during pre-copulatory activity. This was also reported by KLINGEL (1969b) for plains zebras; however, zebra stallions seem to groom more extensively in this situation than do feral horses.

Mutual grooming may also function as a subtle greeting or social contact, although this was not clear since the band members were in close formation almost constantly. The only form of greeting that we were able to identify in horses was naso-nasal contact. ZEEB (1959) notes that specific facial expressions are used by zebras but not horses for greeting and "sexual desire". Like ZEEB, we did not find these expressions in the horses. However, TYLER (1972) reported a greeting expression in New Forest ponies.

Urination and Defecation

Both ♂♂ and ♀♀ assumed stereotyped postures during elimination. Dominant stallions exhibited greater posturing in these acts than did younger ♂♂ or ♀♀. They also commonly smelled their own excrement. ♂♂ and ♀♀ defecated with the tail lifted to some degree, and moved the tail from side to side after defecation. Stallions usually lifted their tails higher when elaborately posturing and defecating on a stallion feces pile or on a mare elimination.

♂♂ urinated with the penis in the sheath and often extended it for a short time after urination. In wild horses, there was particular importance attached to the scent marking function of excretory products. This was most clearly manifested in the behavior of stallions towards eliminations of harem members and that of rival ♂♂. In harem groups, the covering of eliminations of harem members (particularly adult mares) by the dominant stallion, with their own eliminations was an apparent expression of possession or association. In conflicts between rival ♂♂, or within the bachelor groups, order of elimination was strongly linked to dominance.

Table 1: Eliminatory response of harem stallions to eliminations of harem group members

Elimination of harem group member	No. observed	Eliminatory response of harem stallions					
		No elimin.		Defecate		Urinate	
		No.	%	No.	%	No.	%
Defecation by							
Adult ♀	76	46	60.5	4	5.3	26	34.2
Immature both sexes	5	3	60.0	0	0.0	2	40.0
Yearling both sexes	7	7	100.0	0	0.0	0	0.0
Foal both sexes	17	17	100.0	0	0.0	0	0.0
Urination by							
Adult ♀	77	38	49.4	2	2.6	37	48.0
Immature both sexes	6	5	83.3	0	0.0	1	16.7
Yearling both sexes	6	3	50.0	0	0.0	3	50.0
Foal both sexes	12	11	91.7	0	0.0	1	8.3

The relationship of elimination by dominant stallions to that of harem group members, as recorded in the quantitative notes, is given in Table 1. Commonly a stallion detecting the urination or defecation of a harem band member would approach, smell it, step over it, urinate or defecate on it, and then turn and smell again (Fig. 9). Urination, particularly, was performed from an exaggerated stance as compared to simple elimination. The hind legs were extended farther to the rear, the tail lifted higher than normal, and the urine was released in a short but strong spurt.

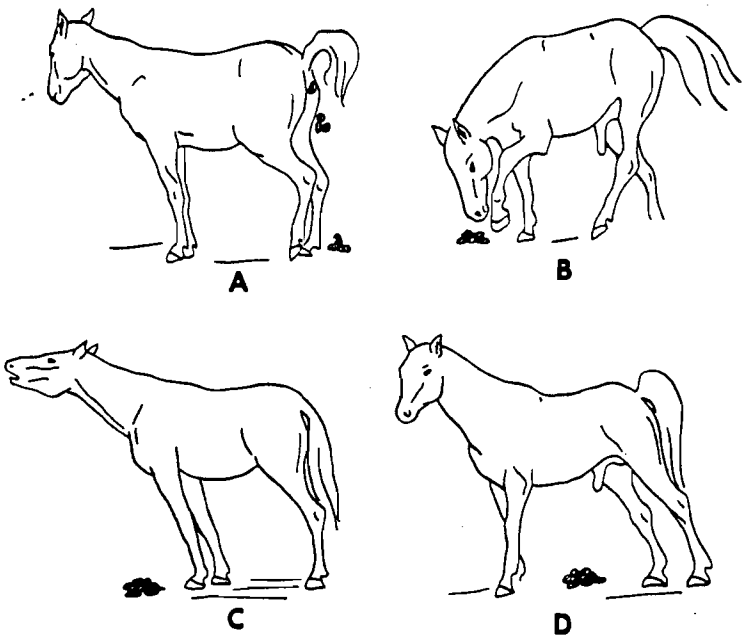

Fig. 9: Behavior of harem stallion in relation to defecation and urination of mares. A. Mare defecating. B. Stallion approaches, smells, and steps over the fecal pile. C. Stallion flehmens following smell. D. Stallion urinates on the mare's feces

Stallions did not always respond to harem members' elimination. From Table 1 it can be seen that stallions responded to 39.5 % of defecations and 50.6 % of urinations of adult mares. Response to eliminations of younger horses was much less frequent. The eliminations of both sexes of younger horses were combined because of small sample size, low sexual connotation to the stallion, and because all stallion responses were urination rather than defecation. This would suggest that in this situation, marking was related to possession or association rather than male dominance as discussed further below.

Failure by a dominant stallion to eliminate on the elimination of harem members sometimes can be explained by his failure to detect them. However, at least three defecations by adult mares were definitely detected by the stallion who smelled and "flehmened", but did not eliminate. Although elimination on harem member elimination occurred throughout the study, it was more frequent during the breeding season, and postures of the stallion were more exaggerated at that time. The sexual connotation of this behavior is further suggested by its being directed mainly towards adult ♀♀ in the harem groups.

KLINGEL (1969 b) also reported that plains zebra stallions sometimes failed to eliminate on eliminations of harem members.

The predominate response of the stallion to harem group elimination was urination (Table 1). Of the 76 follow-ups, 70 (92.1%) were by urination.

Mares were never observed to respond to the excrement of other horses. However, on 5 occasions male foals were observed to apparently smell and then urinate on the urination of a mare, and in two additional cases they smelled the urine of the mare and then flehmened. One of these observations occurred when a mare urinated, followed in succession by the smelling and urinating on it by Divide Legs (a male foal), Grey Streak (an immature ♂), and finally by Del (the dominant stallion). The response of foals and immatures to a mare's excretion was probably an early expression of male sexual behavior.

Dominant stallions eliminated on top of the eliminations of subordinate ♂♂. This order was strictly adhered to in bachelor groups. Usually such adherence to a dominance rank order was true among ♂♂ in harem groups, but exceptions were observed. The clearest example of a dominance order was observed in the Prostitute band, which contained one mare and 6 ♂♂. If the mare or a lower ranking ♂ defecated, the rest or part of the group would follow in order of dominance. Also, in a case where Del chased off the immature, Half Face, Del deliberately approached and urinated on the defecation that Half Face left before he ran. However, in other instances observed in the Del band the immature ♂ defecated on the defecation of a mare without any following action by the dominant stallion. In the Crip band, a mare urination was followed by those of J.S. (an immature), of Crip, and then of Ag (an immature). Other observations clearly set the rank order for these three ♂♂ to be Crip, J.S., then Ag.

Eliminations by stallions not related to elimination of a harem group member are summarized in Table 2. It is apparent that most elimination by stallions, whether urination or defecation, was somehow related to other horses. Of 186 total defecations recorded in the quantitative notes, only 8 (4.3%) occurred without apparent external stimulus, while of 107 urinations, 12 (11.2%) were without external stimulus.

Table 2: Eliminations by stallions in contexts other than response to elimination by harem group members

Situation	Defecation		Urination	
	No.	%	No.	%
No apparent external stimulus	8	4.5	12	32.4
During courtship or tending	5	2.8	7	18.9
On fecal pile in normal movements	69	38.3	2	5.4
On fecal pile during fight	96	53.3	16	43.3
On fecal pile during escape	2	1.1	0	0.0
	180	100.0	37	100.0

While most interactions of stallions with ♀♀ involved urination, those between ♂♂ mostly involved defecation, primarily at fecal piles. Of the total 186 defecations by stallions recorded, 167 (89.8%) occurred at fecal piles. For urinations, 18 out of 107 occurred at fecal piles (16.8%).

Most behavior at fecal piles occurred either among harem stallions or bachelor groups as they passed in normal movements, or during aggressive interactions between stallions. In two cases, piles were used by stallions as they

passed during escape. Numerous other instances of horses of both sexes defecating prior to flight were observed in response to the observer, but these were not included in the tables because the quantitative notes were taken only on horses unaware of the observer. Defecation seemed to be typically associated with alarm, and stallions stopped momentarily at fecal piles to defecate during mild alarm.

Nearly all use of fecal piles was by adult stallions. One observation was made of a yearling ♂ defecating on a pile, and another of a male foal, which was responded to by the harem stallion. Stallions showed typical eliminative behavior when passing by a fecal pile, either alone or with their harems (Fig. 10). They would smell, occasionally paw at the pile with a front hoof, defecate and occasionally urinate. No other stallion need be near to induce the use of the pile.

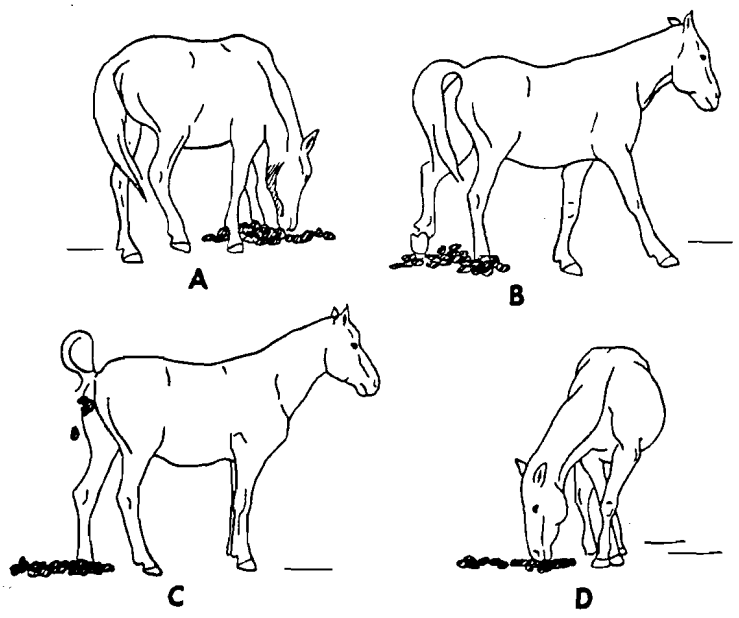

Fig. 10: Behavior of stallion at fecal piles. A. Stallion smells the pile. B. He steps over the pile. C. Defecation. D. Smelling of his own feces by the stallion

Fecal piles were also used in encounters between stallions, ranging from a mild challenge with only a direct stare to more serious fights. If one harem came close to another, the stallions commonly would come out a few m from their harems, stand and stare, smell the fecal pile, defecate, turn again, and lower the head to their own defecation. After this, the two would usually return to their harems and continue their previous activity. In more serious encounters, the two stallions would come together at a pile and proceed through a series of aggressive acts. Usually, before or during the encounter, the two stallions would smell the pile together, defecate in turn or together on it, smell the pile again, and then continue into the fight or separate and end the encounter. Within a bachelor group, this behavior was also observed in mild or "mock" fights. Even in serious fights, which included kicking and biting, often the stallions would stop to perform acts over the fecal piles. The amount of feces dropped by a stallion in any one elimination, or on a fecal pile, ap-

peared to be controlled, as reported by EBHARDT (1954). Stallions were observed to defecate up to three times in succession during a fight.

At the end of the study, a survey was taken of fecal piles in five different areas of the Range. One hundred and 20 fecal piles were examined. Of these, 69 (57.5%) were currently in use and 51 (42.5%) were old piles not in use that year. 54 piles (45%) were on or immediately beside a trail and 66 (55%) were away from trails. The greatest density of fecal piles was found on the trails near the water holes which received the most frequent and consistent use. A number of the piles comprised a series of from two to seven in a row, all touching or within several feet of each other. Piles made up of such series were counted as one. Only one of the piles in a series would be currently in use; the others extended in a row from the most recent pile to the oldest. Some of the recent piles reached a height of over one foot, while in many cases the older piles were so weathered that they had almost disappeared. It was obvious that as one pile became old or had been used for some time the stallions would begin a new one beside it. We estimated that a single pile or one in a series was used for one to three years.

The area covered by the piles ranged from about 3 × 3 for a single pile to 6 feet by 25 feet for a series of connected piles. Of the 120 piles examined, 70 (58.3%) were single piles, 16 (13.3%) two, 17 (14.2%) three, 8 (6.7%) four, 3 (2.5%) five, 5 (4.2%) six, and 1 (.8%) was seven piles in a series.

SCHLOETH (1958) suggested that fecal piles and pawing behavior by horses act as markers for territories, and PELLEGRINI (1971) believed fecal piles had a similar function in feral horses in Nevada. However, he presented no direct observations to support this view. Our evidence, as discussed in the section on home range, is that territoriality does not exist in the Pryor Mountain horses. No specific piles belonged exclusively to any one stallion, nor was any area around the pile defended. However, KLINGEL (1969a) found that Grevy's zebra stallions do maintain territories which are marked by fecal piles; this was also found in the wild ass in Africa (KLINGEL 1972) and some feral ass ♂♂ in Death Valley, California (MOEHLMAN 1974). Plains and mountain zebra stallions were found to defecate on fecal piles (KLINGEL 1967, 1968) but, like horses, were not territorial. According to KLINGEL (1972) the wild ass and Grevy's zebra are not as highly evolved as the other equids. He hypothesizes that territoriality is the older form of social living and that territorial marking is now lost by most *Equus*, but the fecal behavior continues as a "vestigial pattern" inherited from territorial ancestors.

Certainly much more investigation is needed before adequate discussion of the present function of marking behavior by non-territorial *Equus* on ♀ eliminations and stallion fecal piles can be provided. But based upon our observations of feral horses we do not agree with KLINGEL that this behavior is simply vestigial. We propose that fecal deposit behavior (and urine marking as well) has immediate visual and olfactory importance in terms of dominance relationships and associations. We further suggest that chemical information in such deposits persists over time, and serves as a rich source of information on dominance relationships, associations, estrous state, time of passing etc. which can be detected by other horses. In this sense, we view scent marking by horses as analogous to writing in human society, a communication mode by which stimuli can persist over time, and can be perceived long after the initial action was completed.

Flehmen

Flehmen, sometimes referred to as lip-curl or grimace (Fig. 9), is a common behavioral act in ungulates (ESTES 1972). Only the ♂♂ among the wild horses were observed to use this act, which was performed by curling the upper lip upwards and slightly compressing it from the sides and shortening the distance between the nostrils. The mouth was closed but the teeth exposed, and at the same time the head and neck were pointed upwards and generally off to one side of the body, with the ears pointing outwards. Flehmen is induced by olfactory stimuli since the ♂♂ appeared to smell something before exhibiting the flehmen. The 55 situations in which flehmen was observed can be broken down into 7 major categories: 1) smelling a mare's vulva in association with tending or copulation (8); 2) smelling a mare's urine (17); 3) smelling a mare's defecation (4); 4) smelling a fecal pile (7); 5) smelling a fecal pile in relation to a fight (2); 6) smelling his own urine (5); 7) smelling the ground, stimulus not established (12). If a series of flehmens occurred over the same stimulus, it was counted as one. For example, in one case, after smelling a mare's urine, a stallion flehmened 8 times. This was recorded as one.

Harem stallions, bachelor stallions, and a few immature ♂♂ in harems were all observed to flehmen. No yearling ♂♂ were observed to flehmen, but two cases of male foals were recorded. In one, the foal smelled the rear of a mare, not his mother, and flehmened three times. In the other, a foal smelled the urine of his mother and then flehmened.

The use of the flehmen was in evidence throughout the six-month study, but more frequently in the early part of the breeding season. Presumably mares at this time provide more olfactory stimuli to the ♂♂. However, as noted by many workers on ungulate behavior, it is shown in a number of other contexts as well. In at least a third of the cases we observed where the stimulus was identified, it was shown by stallions in relation to themselves or other stallions. Furthermore, TYLER (1972) has observed flehmen in female ponies as well.

Leadership and Herding Behavior

Leadership can be expressed either by the taking of initiative by one animal with the others following, or by the active driving or herding of the group by a stallion.

Leadership by initiative was recorded in 159 cases during movements from place to place, and going to and from water. Of these 106 (66.7 %) were led by the stallion and 53 by a mare. Of the 53 cases in which a mare assumed leadership, 7 were in response to the closeness of other harems, and 9 were due to unnatural disturbances. In these 16 cases, the stallion herded the group, directing its movements. Thus, in only 23.3 % of the observed cases was the mare solely responsible for leadership.

EBHARDT (1954) reported that stallions were the common leaders in Icelandic pony bands. TYLER (1972) found adult mares to be the leaders in New Forest ponies. However, her results may be biased by the few stallions present. With the plains zebra, KLINGEL (1964, 1967) found the oldest zebra mares to be the apparent leaders in group movements, but with the stallion still the dominant animal. KLINGEL (1968) reported that in mountain and Hartmann zebras, the stallion leads to water but the dominant mare leads when leaving water. The same situation was observed occasionally in the wild horses. We were unable to determine a hierarchy among the mares of a group with regard

to position in movements. However, observed leaders were usually older mares or mares with foals or yearlings.

Herding or driving behavior (Fig. 11) consisted of first assuming a threatening posture. The ears were laid back and the neck and head stretched out.

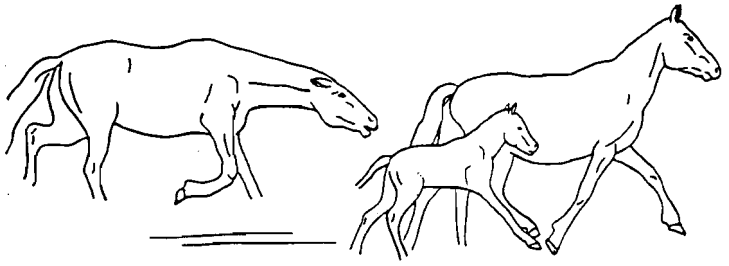

Fig. 11: A harem stallion (left) herding a mare and foal

The strength of this signal was determined by how low the head and neck was, how vigorously the position was assumed, and how fast the stallion moved toward the other horses. If a stallion laid his ears back and stretched his head and neck horizontally but merely walked toward the horses, the group responded with the same speed of action. If the stallion laid his ears back, snapped his head down so that his chin almost touched the ground, and ran towards the horses, they responded rapidly. Often the stallion's head would move from side to side, producing a snake-like motion. The intensity of herding depended on the particular situation that evoked it and the differing temperament of individual stallions. Some stallions exercised close control over harems while others were more lax.

The quantitative observations included 130 instances of herding behavior. In 55 (42 %), the stallion herded his harem away from another group or stallion. Herding by a stallion to indicate direction of movement, to retrieve members, or to reduce spread of the harem, accounted for 39 (30 %) of the instances. The herding of a member of another harem away from the stallion's own harem was observed 15 (12 %) times. 6 (4 %) times a stallion herded in the attempt to gather a mare, usually a stray, into his own harem. Finally, 15 (12 %) observations were made of a stallion herding a mare within a harem in an attempt to copulate.

On rare occasions an immature or secondary ♂ in a harem group exhibited herding behavior, usually simultaneously with the dominant stallion and at a position between the stallion and the rest of the group.

Both initiative leadership and herding behavior in the harem show that the stallions are the principal leaders and they direct most of the movements of the groups. Leadership by stallions, in conjunction with fidelity of mares to the group, results in a high degree of harem stability.

Dominance Hierarchy

A dominance hierarchy was present in the bachelor ♂♂. The largest bachelor group had 8 members. The dominant stallion herded the group as if they were a harem and met with, challenged, and fought with the stallions of other groups. Often this band split into two or three groups with the same stallions dominant in each. Smaller, more stable bachelor groups showed similar hierarchies. Dominance was expressed by threat gestures, herding, mild fights

within the group, and fights with stallions of other bachelor groups or harems. Age was a definite factor in the hierarchies, with younger, smaller stallions being lower while larger, older stallions were dominant.

During the study, three stallions from bachelor groups each gained a mare, and each of the three was the dominant ♂ of his bachelor group before the change. When a bachelor group approached a harem, it was always the dominant ♂ of the bachelor group that met and interacted with the stallion of the harem. The role of the hierarchy in fecal pile behavior has already been discussed. In cases where only one ♂ from the bachelor group defecated on the fecal pile, it was always the dominant ♂.

In bachelor groups, behavior analogous to the marking of mare elimination by the harem stallion was observed occasionally. For example, in the Forest group, when the youngest member urinated, he was followed by the second in rank on the same spot, and lastly by the oldest and dominant ♂, Forest. In the Prostitute band, when the mare defecated or urinated, the ♂♂, in a rank order from lowest to highest, defecated or urinated on the spot. In all cases, the dominant ♂, Blacki, was the final one to defecate. The hierarchy was also clearly expressed in this band by threats always being directed toward stallions lower in rank. The number 1 and 2 stallions, Blacki and Black One respectively, gave evidence of their rank by occasional mild fights which Blacki always won. Often both would herd the rest of the group at the same time. Black One would always be second from the rear, and Blacki at the rear, with a harem stallion.

No consistent dominance hierarchy was observed among individual mares of a harem. In some situations, a mare dominated another in protecting individual space, defending a foal, or establishing position at a watering site or in the resting group. However, the relationship depended upon the circumstances, and reversals were frequent. Further study might reveal that some particular mares do dominate others consistently enough to establish a rank order. Our observations indicated that female hierarchies are poorly developed, and strong dominance of the stallion over the harem overshadows the expression of a female hierarchy.

Dominance hierarchies have been reported for horses by other authors. ZIMEK (1944) and MONTGOMERY (1957) reported them in domestic horses, HJARDT (1954) for Icelandic ponies and TYLER (1972) for New Forest ponies. KLINGEL (1967, 1968) reports that plains, mountain, and Hartmann zebra bands exhibit a dominance hierarchy among the mares that is most clearly expressed by a rank order within the family unit when moving to water. That order was characterized by the most dominant mare in front, followed by her youngest then second youngest offspring, followed by the next most dominant mare, etc. The stallion, although often at the rear of the band, was dominant over all the other band members. It is interesting to note that KLINGEL found no dominance hierarchy in the adults of bachelor groups of zebras.

Stallion Encounters and Fights

Fights between harem stallions, lone bachelors, and bachelor stallions were observed throughout the study. 82 encounters between stallions were recorded. The largest number (37) resulted from nearness of other groups. Typically, these occurred when groups of bachelor stallions came too close to other groups while feeding or moving about the Range. Stallions would approach each other, and an aggressive encounter would follow. Such encounters help to maintain linear spacing between groups as previously discussed. Twelve

fights were the result of attempts by a harem stallion to gain a mare from another harem. Four fights were the result of attempts by stallions to regain mares that had been separated from the harem. In only one case was the mare not recovered as a result of the fight, and that involved a crippled harem stallion who lost mares to two bachelor stallions.

One fight was a unique case. It was a complicated encounter of undetermined cause, which involved 5 harem stallions and two immature ♂♂. All of the harem stallions fought each other in earnest, but the encounter between the two immatures was decidedly milder.

The first part of a stallion fight involved posturing by one or both stallions. Usually one or both would stand and stare at the other in a mild, alert posture, and occasionally paw the ground with a front hoof. In some cases,

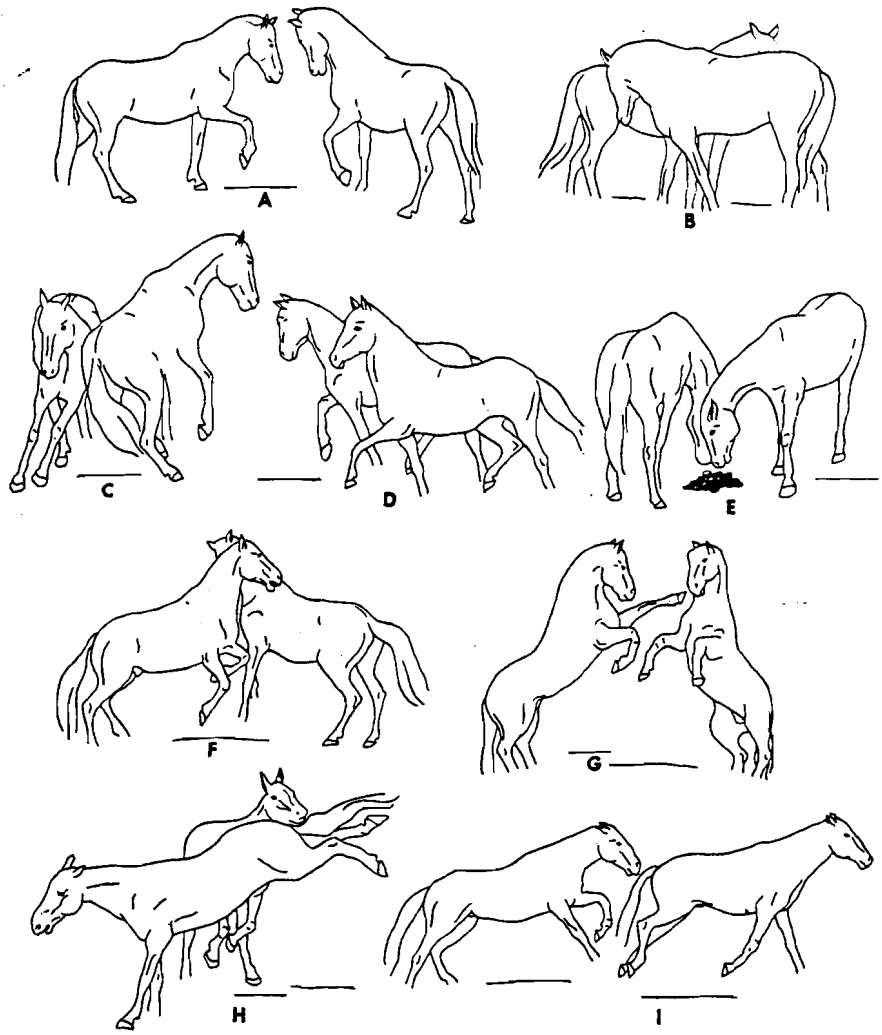

Fig. 12: Typical sequence of acts in an aggressive encounter between dominant stallions. A. Approach and smell noses, B. Smelling flanks. C. Pushing and threatening a hindfoot kick. D. Parallel prance. E. Smelling fecal pile together. F. Shoulder-pushing and biting. G. Rearing and front-foot kicking. H. Hind-foot kicking. I. Defeated stallion chased by the winner (left)

one or both would then walk to a nearby fecal pile and smell, sometimes paw at it, defecate on the pile, and turn and smell it again. Several times during the study the observer became the object of these challenges when close to habituated harems. These challenges were declined.

If the confrontation continued, the next step was an approach to one another by walking, trotting, or prancing with exaggerated movements. The head was held high with ears forward and the neck tightly arched, with occasional head tosses and with the tail lifted to some degree. The front legs were lifted high, often striking forward in a threatening manner. A parallel prance was usually observed in the initial stages as well as during intervals in the rest of the fight.

In all fights except the most serious ones, the two stallions came together and investigated each other. A typical series of acts is illustrated in Fig. 12. First they stood facing one another with their foreheads touching, or almost so, or with their heads side by side but still facing in opposite directions. In this position, and with heads high, necks arched, and ears forward, the stallions appeared to smell each other's nostrils and breath. Often at this point, a squeal would be emitted by one or both stallions. This sound would be heard again at intervals throughout the fight. Often the squeal was accompanied by a front-foot kick usually only striking the ground. Further smelling would then proceed, and this was accomplished by each stallion moving along the side of the other so as to smell the neck, withers, flank, genitals, rump, and occasionally, the tail of the other. In smelling necks, each stallion would turn his head and neck toward the other so that it appeared like two hooks caught together. The ears were held forward while smelling noses and necks, but were usually laid back when smelling the rear of the body. After these investigations were complete some fights involved pushing each other with shoulders, and head swings directed at the opponent's head and neck. Occasionally, a stallion would lay his neck over the other's neck and rear above the other in what appeared to be an attempt to force the opponent to the ground. However, the only time a stallion was observed to fall was when one was pushed over backwards while both were reared on their hind legs and striking with the forefeet.

In the most serious fights, initial posturing was sometimes omitted and the two stallions ran at each other, ears back and mouths open, engaging in immediate and active combat. Kicking and biting were the main actions that characterized the more serious fights. Kicking with the forefeet was used in a face-to-face confrontation, using one leg while standing or both while reared on the hind legs. Intense forefoot kicking exchanges usually were accompanied by biting. Scars on the sides, rumps, and necks of stallions attest to the effective delivery of bites.

Kicks with one or both hindfeet were used, although they were always in short and intermittent parts of the fight. Because hind-foot kicks are extremely powerful, the stallions made great efforts to avoid receiving such a kick, which gave many fights the appearance of consisting of active and passive phases. Broken jaws on skeletons recovered from the Range (3 out of 35) might well indicate the fate of a horse receiving a well-placed kick.

Behavior by both opponents at the same fecal pile often occurred during interludes between active fighting. The procedure was to smell, defecate, turn and smell again, either in unison or in turn. Great interest was shown in smelling the feces of the opponent. In serious fights we found no relationship between which stallion defecated first and which initiated the fight or won. However, in "mock fights" it was always the dominant stallion who defecated

last. This would be followed by resumption of active fighting, or an end to the confrontation, or to parallel prancing, again followed by a defecation sequence at the same or a nearby fecal pile.

Some encounters consisted of, or, in the case of fights, were terminated by, a chase. The stallions layed their ears back and, while running, the pursuer would attempt to bite the rump and tail of the escaping horse. In fights between harem stallions, it was seldom that a clear winner could be determined, except in the cases where one chased the other. Most fights ended in an apparent draw with both stallions simultaneously separating and returning to their respective groups.

The number of acts used in a fight, the order of their occurrence, the intensity of their use, and the general seriousness of a fight were all variable. Examples of some fights are illustrated in Table 3 where the numbers signify the acts used and the order of their occurrence. For example, a mild encounter may consist only of a simple stand-stare by one stallion, and a response of facing or moving away by the other. In a more serious encounter, the stand-stare, walk-together, smell noses, squeal, fore-foot kick, smell-defecate-smell, and head-swing may all be used. In a serious fight, the preliminary challenge and display may be omitted, and the two stallions may come together and begin biting, fore-foot kicking, and hind-foot kicking in a violent and determined manner.

The intensity of the acts was a measure of the seriousness of a fight which could be judged, for example, by how determined and aggressive the bites

Table 3: Sequence of acts in encounters and fights between dominant stallions

Behavior Acts	Behavioral Acts Used and Order of Progression (or first time used in the encounter)																							
	Mild Encounter								Serious Encounter								Serious Fight							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Stand-stare	1	1		1					1		1	2		1			1	1		1		1		
SDS* Piles Apart	2			4		4			3			1				1						2		
Whinny				2	2	1								2										3
Parallel-prance			2	3	3	3		4		2		3			1									2
Walk together		2	1		1		1		2		2		1						1	2				
Trot together								1		1							2				1			
Prance together						2								3				2						
Run together																2							1	1
Smell fecal Pile							2		4	9														
Smell Noses									5	3	3	4	2	4	2		3	3	3		2			
Smell Neck									6	4	4	5	4	6							6			
Smell Withers									7	5			5	7										
Smell Flank									8	6			6	8										
Smell Genitals										7			7											
Smell Rump									9	8			8	9										
Squeal							3	3			5		3	5	3		4	4	4		3			
Front-foot kick, One leg									10		6			10	4			5	5		4			
Shoulder-push									11		8										8			
Head-swing									12	10	7										9			
SDS* Same Pile			3				4	2									7		2	3	5			6
SDS* Different Piles					4							8		14					8	4				
Front-foot kick, Both Legs												6		11		3	6	6	6				2	4
Bite																4	5	7					3	5
Hind-foot-kick, One Leg														12	5									
Hind-foot-kick, Both Legs													9	13	6	5			7	5	10		4	
Chase and Bite																6						4		
Chase With Mouth Open																						3	5	
One Turn, Face Away		3			5							7		15										
Mutual Separation	3		4	5		5	5	5	13	11	9		10		7		8	8	9	6	11			7

* Smell-defecate-smell on a fecal pile.

...e, how exaggerated the postures were, and how much of the fight consisted of biting and kicking as compared to displays. "Mock fights" between immature ♂♂ or bachelor stallions, for instance, lacked the exaggeration in posturing, and biting and kicking were executed with much less intensity, seeming to be more symbolic gestures (intention movements) than attempts to deliver injury.

The ear, head, and tail positions changed continually during fights. In the challenge or display stage the ears were forward, head high, neck arched, mouth closed, and tail up. In the active stage, acts such as kicking, biting at the neck and throat, and pushing and head swings were accompanied by the ears being laid back, mouth opened, and head stretched out toward the opponent. In the passive stage, the ears were held out or forward, mouth closed, and the neck or body swung away from the opponent.

While stallions were engaged in a fight, the mares, as a rule, seemed quite uninterested in the activity. They either continued feeding or grouped up and stood waiting for the stallion to return.

Smelling of the genitals was observed in mature stallion encounters, but it was more common between immature ♂♂. Both mature and immature stallions were observed licking another's penis. In all cases of smelling or licking the genitals, it was done by the lower in rank of the two, or both together.

In many respects, horses and zebras fight in a similar manner. However, according to KLINGEL and KLINGEL (1968 a) plains zebra fights involve a considerable amount of biting directed toward the legs, which they defend by dropping to their knees or haunches. This was observed only rarely in immature wild horses, while in serious fights bites were directed to the head, neck, and body. It also appeared that horses engaged in more forefoot kicking, especially while reared on their hind legs, than do zebras.

Reproductive Behavior

Breeding

Post-partum estrous and a constricted breeding season were shown by the horses (FEIST and MCCULLOUGH 1975). ♀♀ usually became sexually mature at three years, but two cases of two year olds successfully breeding were observed.

Stallions tended mares from May 1 to July 12, and often tried to copulate. However, only 8 successful copulations were observed in the study. All but one were performed by dominant stallions with mares of the harem.

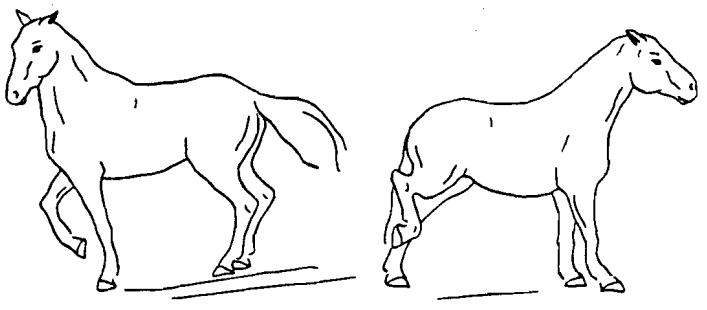

Fig. 13: Unreceptive mare (right) rejects sexual approach of stallion who then moves away. Mare lays back ears and kicks with hind-foot

During the breeding season heightened sexual activity by the stallion included more direct and frequent smelling of a mare's vulva, and increased displays over excrement of mares. As mares approached estrous, the stallion pressed them more persistently. Unreceptive mares rejected these advances by laying back their ears and moving away, or threatening, or delivering hind-foot kicks with one or both legs (Fig. 13).

Receptive mares indicated readiness for mounting by standing still and raising the tail (Fig. 14). This was followed by mounting with erect penis, intromission, and pelvic thrusts. Following ejaculation, the stallion dismounted and stood for a moment. He then smelled the ground beneath himself, flehmened, and urinated. This sequence often was repeated. A second copulation with the same mare sometimes occurred a few minutes later. Typically one or more mountings without erection of the penis preceded actual copulation. Nursing of a foal is commonly observed following mountings.

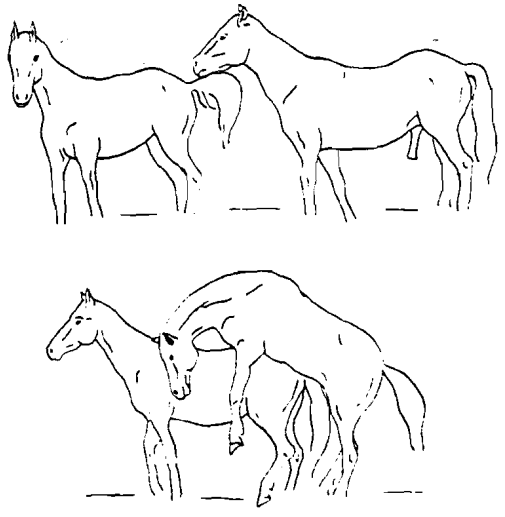

Fig. 14: Copulatory behavior. Above The mare (left) indicates receptivity by standing and raising the tail. The stallion approaches and smells. Below Mounting and intromission

On one occasion, another mare kicked at the copulating pair. ZEEB (1959) observed similar behavior in horses where older mares interfered with copulation of younger mares. KLINGEL (1968b) reported that in zebras, band members move into close contact with a mating pair in the harem.

ZEEB (1959) and TYLER (1972) reported mares in heat presenting themselves to a stallion. This situation seems characteristic of groups with unbalanced sex ratios with few stallions. It was observed once in the Sheik band, which contained the most mares. A mare in heat approached the stallion, and backed into him with her tail lifted. He drove her away with a strong threat. A half-hour later she returned and again presented. Copulation followed.

The lone instance of a copulation not involving the harem stallion involved an immature mare of the Sheik harem. She left the Sheik band to accept copulation with the dominant stallion from another harem group. Sheik stood passively watching through most of the sequence of events. During copulation he approached and drove the ♀ back to his harem, and then moved the harem away. KLINGEL (1969) reported that zebra mares approaching sexual maturity are abducted by rival stallions. This situation is not so clear in the horses, and young mares may voluntarily leave a harem band.

Many copulations and attempts to copulate occurred in situations of disturbance, such as an encounter between the harem stallion and another stallion, or our presence. This was true for 70 % of the observed breeding activity. It seems possible that some form of threat or stress to a stallion may influence

attempts to copulate. Such behavior would seem to have selective advantage since the stallion would attempt to maximize reproductive success before engaging in activities which involve risk of mortality, injury, and displacement by rival ♂♂.

Mounting is sometimes observed outside the normal breeding context. On four occasions male foals mounted other horses. One involved a male foal mounting a female foal. The others involved mature mares, in one case, the mother. A female yearling was observed mounting a mare. An immature ♂ attempted to mount a female yearling from another harem. The dominant stallion of that harem disrupted the encounter.

Twice ♂♂ were observed to establish or reinforce their dominance over subordinate ♂♂ by mounting them. One harem stallion, following rejection by a ♀, attempted mounting of an immature stallion in the harem. The penis was not extended. The other case involved a bachelor stallion mounting an immature ♂ who had momentarily moved away from the harem. The bachelor stallion mounted four times with an extended, but not erect, penis. This encounter also involved playful fighting, with nipping at the knees. This mode of fighting is common in zebras, but virtually absent in fighting in horses. Dominance apparently can be expressed in stallions by sexual patterns; and as previously observed, dominant stallions in bachelor stallion groups frequently treat the group as if it were a harem.

Extraneous Penis Extensions

We recorded 59 observations of ♂♂ with an extended penis in situations not connected with acts of copulation or urination. Of these, 31 included flexing of the penis after extension. The observations, which occurred at all times of the day, averaged 2 min in duration. Thirty-six stallions of harem groups, 6 immatures or secondary ♂♂ from harem groups, and 17 bachelor stallions (lone or in bands) were involved. No observations of male foals or yearlings were recorded except when all six ♂♂ of the Prostitute band (which included a yearling) were observed standing in a loose group, each with his penis extended.

The 59 cases were classified with respect to possible cause. Twelve were related to encounters with another stallion and occurred during the stand-stare, a common posture in the early stages of a fight. Only 10 cases were believed to have been caused by direct arousal from a mare or from smelling a mare's excretions. In the majority of the observations (37) we could not detect a clear external stimulus which induced behavior. In these cases, the ♂♂ were standing alone or a short distance from the harem, either quietly feeding or in a rest posture.

In some instances, extension might have been due to relaxation. However, in 31 of the 59 cases the penis was also rigid and flexed after extension, and 10 were accompanied by pelvic thrusts similar to those used in copulation. None of these extraneous penis extensions resulted in the obvious emission of urine or seminal fluid.

Mare-foal Relations

Foals were born from about mid-April to late June, with most births occurring in May and early June. No actual deliveries were observed, and it is our belief that mares usually seclude themselves from the harem prior to birth as reported by IMANISHI (1953), PELLEGRINI (1971), and TYLER (1972). We found the remains of two extremely small foals which may have been still-

born or died shortly after birth. Both were in sheltered clumps of juniper. However, the possibility of their being dragged there by scavengers cannot be ruled out.

The bond between mother and offspring was most apparent in the close association of the two. The foal remains at the mother's side in the band during all movements, and will get up from rest or sleep whenever there is movement. The ♀ is strongly protective of the foal, and issues a low whinnying if the foal becomes separated. She will threaten other horses which approach too closely to the foal. She positions herself between the foal and the intruder. Dominant stallions are also protective of the foal and will herd it with the band, and bring it back to the band if it becomes separated (FEIST and MCCULLOUGH 1975).

Dominant stallions also will separate band members if the mother is unable to maintain the distance of other horses from the foal. The mother gives a whinny in these cases and the stallion moves the other horse away. Such protectiveness is important since lost or orphaned foals are attacked by other horses.

Nursing

Nursing (Fig. 15) was usually initiated by the foal walking directly toward the mare, often crossing under her neck, and then moving along her side to the udder. Mares rejected these approaches only in 6.2 % of the cases. If she accepted, the mare would raise her head, stop eating, and stand still. As a foal began nursing, most mares would take a short step forward with their front legs, leaving the back ones stationary to facilitate nursing. At the end of nursing, foals began feeding on vegetation, stood or walked alongside the mare, or in most cases lay down a few yards away from the mare. If a yearling was nursing, it would commonly return to eating vegetation.

There were no observed cases of a foal or yearling nursing from a mare other than its own mother.

A total of 244 observations of nursing was recorded during the study (FEIST and MCCULLOUGH 1975). Of these, 222 were by foals, 21 were by yearlings, and one by an immature. For the six individual yearlings which nursed, it was not clear if the mare failed to produce a foal, or if the foal died shortly after birth. One ♀ nursed both a foal and yearling. The one case of an immature nursing involved a mare who dropped a foal which died soon after birth.

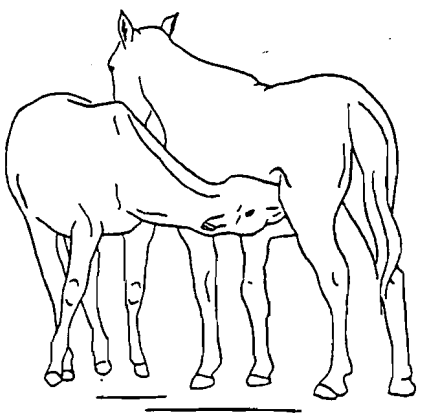

Fig. 15: Typical nursing posture, in this case by a yearling. The mare stands with hind-legs slightly extended

Nursing lasted from a few s to 2 min; the median time was about one and a half min. Foals nursed 1.85 times per h, while yearlings nursed (.97) times per h, or about one-half as often as foals. Length of nursing bouts was similar for foals and yearlings.

Termination of nursing was voluntary in 75.7 % of the observa-

tions, and involuntary in 24.3 % when the mare moved or there was interference by another horse.

The side of the mare from which foals or yearlings nursed was recorded in 230 cases; 110 were on the right side, 116 were on the left side and four nursings were from behind. The nursings from behind were all by the foal of a mare who was suckling both her yearling and foal. The yearling displaced the foal at nursing with no interference from the mare, and in these cases the foal usually moved to the rear position.

General Discussion

An impressive aspect of the social behavior of horses is the conservatism of social organization. The domestic horse has a long history of human-controlled artificial selection for characters having little or no bearing on survival in the wild, and for management in captivity which has not allowed expression of normal social structure. Despite this period of manipulation by man, once horses manage to escape and live in a wild or semi-wild state, the typical wild social organization emerges. This social organization is typical of what is known of the well-studied plains zebra, and apparently of the less-well-known remaining populations of *Equus caballus*. Reemergence of the pattern among feral horses is reported by IMANISHI (1953) in Japan, BRUEMMER (1967) on Sable Island off of Nova Scotia, TYLER (1972) in England, PELLEGRINI (1971) Nevada and FEIST and MCCULLOUGH (1975) in Wyoming-Montana. Although details are not available to us, we understand that similar results are being obtained on other feral populations. Certainly these feral populations represent a wide variation in genetic make-up, past history of handling in captivity, and length of time in the wild or semi-wild state. The constancy of social organization is remarkable, and deserving of closer study.

One of the most interesting differences between the results of our work and that of TYLER (1972) is the difference in behavior of ♀♀. In TYLER's population, ♂♂ were removed by man, and a population of mostly ♀♀ resulted. She observed many kinds of behavior, including vocalizations, being shown by ♀♀ which we observed only in ♂♂. In our population, dominant stallions are extremely dominant, and apparently they suppressed a number of behavior expressions in ♀♀. Nearly all alert-alarm behavior and aggressive behavior is expressed by ♂♂. Dominant ♂♂ quickly assume control if a ♀ first detects danger, and they intervene in mare-mare antagonisms and even play within the harem. They play a major role in keeping the group together. The dominant stallions are so successful in their control of the harem group that a remarkable homeostasis is achieved. We believe that this accounts for the very poorly developed social hierarchy among harem ♀♀ and the reduction of mare-mare aggression.

Evidence seems to be accumulating to suggest that in year-long harem groups dominant ♂♂ do not breed with their female offspring. Because harem groups persist for years, in most cases juvenile ♀♀ would be copulating with their own fathers. Why are rival zebra stallions able to abduct juvenile ♀♀, but not adult ♀♀? Why would a dominant stallion horse passively allow copulation of a young ♀ in his harem with a rival ♂? Why do vicuña dominant ♂♂ actively drive juvenile ♀♀ from the family group (FRANKLIN 1974)?

Surely the social system would collapse if rival ♂♂ were equally successful at abducting adult ♀♀. This would imply that fundamental to this phenomenon is the behavior of the dominant ♂. In vicuna, the dominant ♂ actively excludes juvenile ♀♀, while in horses it is more passive. One stallion allowed

copulation by a rival, and juvenile ♀♀ were responsible for most of the harem changes observed. The fact that rival zebras select juvenile ♀♀ to abduct suggests that such attempts are more successful than attempts at adult ♀♀, and ultimately that behavior of the dominant stallion must differ in the two cases.

For a polygynous ♂ to give up a potential mate implies that there is a substantial cost of such matings which either outweighs the benefits in cases of passive or active exclusion of juvenile ♀♀, or comes close enough in the case of abduction that the effort required of the dominant ♂ to retain the ♀ is not warranted.

The cost involved would seem to be the potentially deleterious effects of inbreeding. Clearly this question of fundamental evolutionary significance should be explored more completely.

Summary

A behavioral study of a population of feral horses in the western United States was conducted between 1 May and 1 November, 1970. Horses were organized into stable harem groups with a dominant stallion, and less-stable bachelor male groups. Home ranges of groups overlapped extensively, and territoriality was not present. Linear spacing between groups was maintained by interactions between dominant stallions. Daily activities involved mainly grazing, resting and moving to water during dry periods. Grasses were the major food, forbs and even roots were consumed on this poor range. During dry periods, water sites were visited once a day at any time of day, or less commonly, at night.

Stereotyped postures and modes of communication are described. Detailed quantitative notes were recorded at one min intervals on horses unaware of the observer. In addition, six groups were habituated to enable close-range observation. Harem group stability was maintained by strong dominance of the harem stallion and fidelity of group members. Elimination of mares was often marked by harem stallions, mainly with urine. Both mares and stallions rejected non-group horses. Most stallion fights and herding attempts involved keeping harems separated, with relatively few involving attempts at gaining additional mares. ♀♀ in harem groups showed poorly developed hierarchies. Immature ♂♂ were tolerated by the dominant ♂ because of their strongly submissive behavior. Leadership, as expressed by both herding and initiating movement, was most commonly shown by the dominant stallion.

Linear dominance hierarchies were present in bachelor male groups. The dominant ♂ behaved towards the group as if it were a harem. ♂-♂ interactions, both between harem stallions and between bachelor stallions involved prominant use of feces. Bachelor group hierarchies were clearly expressed at fecal piles where the lowest ranking ♂ defecated first, followed in order by the others, with the dominant animal last. All ♂♂ used the same fecal piles which were distributed widely over the range, but with concentrations along trails and by water holes.

Foaling was concentrated in May and early June with mares showing a post-partum estrous. All breeding was done by harem stallions. Mares were strongly protective of their foals, and dominant stallions showed protective behavior towards the foals of the harem. Nursing was initiated by the offspring and was seldom rejected. Yearlings were commonly nursed by mares if they failed to breed or lost the new foal early. One immature (2—3 years) nursed from a mare which had lost her new foal. Foals nursed 1.85 times/h

...d yearlings 0.97 times/h. Nursing bouts averaged about 1.5 min in length and did not differ between foals and yearlings. Termination was voluntary 76% of the time.

Equids show pronounced conservatism in social structure, with feral ponies and horses displaying organization similar to most zebras. Strong dominance of harem stallions apparently suppressed much behavior of ♀♀.

Zusammenfassung

Von Mai bis November 1970 wurden verschiedene Wildpferd-Gruppen in ... westlichen Vereinigten Staaten untersucht. Die Pferde lebten das ganze Jahr in stabilen Harems mit einem dominanten Hengst oder in weniger stabilen Gruppen aus jungen ♂♂. Weidegebiete überschnitten sich sehr stark, Territorien waren nicht feststellbar. Der Abstand zwischen Gruppen wurde vom dominanten Hengst gewahrt. Der Tageslauf umfaßte regelmäßig Grasen, Ruhen und bei Trockenheit Wanderungen zur Wasserstelle.

Wiederkehrende Verhaltens- und Verständigungsweisen werden beschrieben. Unbemerkt von den Pferden wurde das Verhalten in Minutenabständen protokolliert. Zusätzlich wurden sechs Gruppen daran gewöhnt, aus nächster Nähe beobachtet zu werden. Die Konstanz der Haremsgruppen wurde durch ...rke Dominanz des Hengstes und die Treue der Gruppenmitglieder gewahrt. ... Exkremente der Stuten wurden oft von den Haremshengs... mit Urin ...arkiert.

Sowohl Stuten als auch Hengste wiesen gruppenfremde Pferde ab. Kämpfe zwischen Hengsten und Zusammentreiben der Stuten dienten meist dazu, die Harems getrennt zu halten; selten ging es darum, zusätzliche Stuten zu bekommen.

Harems-♀♀ zeigten nur wenig entwickelte Rangordnungen. Halberwachsene Hengste wurden von den dominanten ♂♂ wegen ihrer starken Unterwürfigkeit geduldet. Führer, erkennbar am Hüteverhalten wie am Initiieren von Aktivitäten waren meist die dominanten Hengste. In den Junggesellen-...ppen bestand eine lineare Rangordnung. Das dominante ♂ behandelte die ...ppe wie seinen Harem. Bei Interaktionen zwischen Harems-Hengsten wie zwischen Junghengsten spielte Koten eine große Rolle. Die Rangordnung in Junghengst-Gruppen war beim Koten erkennbar: der Rangniedrigste kotete zuerst, die jeweils Ranghöheren folgten. Alle ♂♂ benutzten dieselben Kotplätze, die weitverstreut an Pfaden und Wasserstellen lagen.

Im Mai und Anfang Juni fohlten die Stuten, die sofort wieder rossig und dann ausschließlich vom Haremshengst gedeckt wurden. Die Stuten verteidigten ihre Fohlen sehr stark, und auch die dominanten Hengste zeigten Verteidigungsverhalten. Die Fohlen saugten von sich aus und wurden selten abgewiesen. Einjährige wurden gewöhnlich von Stuten gesäugt, die selbst keine oder ihre neugeborenen Fohlen verloren hatten. Fohlen tranken 1,85mal pro h, ein...hrige 0,97mal pro h, und zwar durchschnittlich 1,5 min lang. In 76% aller ...lle hörten die Tiere freiwillig zu trinken auf.

Pferde haben eine sehr ursprüngliche Sozialstruktur, die bei wilden Ponies und Pferden der bei den meisten Zebras ähnlich ist.

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This paper is dedicated to the late John P. CLARK in warm remembrance of the experiences the three of us shared.

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Effects of neutering on bodyweight, metabolic rate and glucose tolerance of domestic cats

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Abstract

Few controlled studies have been made of the possible mechanisms and physiological consequences of weight gain after cats have been neutered. In this study, six male and six female cats were gonadectomised and compared with five entire male and six entire female cats, before they were neutered and one and three months later. The neutered males gained significantly more weight (mean [SEM] per cent) than the entire males (30.2 [5·2] v 11·8 [2·3]) and the entire females gained 40·0 (7·3) v 16·1 (3·3) per cent, ($P<0·05$). The castrated males gained more weight as fat than the sexually intact males (22·0 [3·3] v 8·8 [4S] per cent, $P<0·05$). There was a significant increase ($P<0·05$) in daily food intake after neutering. Spayed females underwent a significant decrease in fasting metabolic rate (83·7 [5·5] v 67·2 [23] kcal/kg bodyweight$^{0·'75}$/day $P<0·05$). Gonadectomy had minimal effects on serum thyroid hormone concentrations, the resting or fasting metabolic rates in males, or on indices of glucose tolerance.

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Optimal Control of Wild Horse Populations with Nonlethal Methods

Robert Fonner and Alok K. Bohara

ABSTRACT. *Effective management of wild horse populations has proven difficult for land managers. This paper develops and evaluates dynamic optimization models of wild horse management for three nonlethal population control methods: removals, fertility control, and a hybrid method combining removals and fertility control. Two state variables are included in the models to account for horses on the range and horses in off-range holding facilities. The optimization problems are solved and dynamic simulations are generated for each method. The results indicate that a strategic approach to nonlethal wild horse population control has the potential to significantly improve management efficiency.* (JEL Q28, Q57)

I. INTRODUCTION

Wildlife populations provide a variety of amenities to society, ranging from food and recreation to ecosystem regulation and existence values. Wildlife populations can also impose costs on society through interference with human activities, degradation of habitat, and spreading of disease. Species that both provide a valuable resource and impose societal costs are classified in the literature as multi-use species (e.g., Horan and Bulte 2004; Zivin, Hueth, and Zilberman 2000). Some multi-use species require active management to keep populations from imposing net societal costs. Striking a balance between the costs and benefits of multi-use species can involve active population management. Direct population control is commonly carried out through increased regulated hunting or trapping (Rondeau 2001). However, when multi-use species are endeared to the public, lethal population control methods may not be palatable from a societal perspective. In these cases, wildlife can be captured then relocated, or captured then held in captivity. A third alternative, fertility control, is an emerging nonlethal population control method that can be implemented without displacing animals from their range.

This study evaluates alternative nonlethal strategies for controlling wild horse populations in the American West—a multi-use species for which traditional population control methods are not a politically feasible option. Dynamic economic models of optimal wild horse management are developed for two nonlethal management methods: horse removals and fertility control. A third "hybrid" model is also developed to investigate the effectiveness of using removals and fertility control in tandem. The models account for how management decisions and horse biology influence the dynamics of horse populations on the range, and the population of horses in holding facilities. The objective functions incorporate the costs of on-range management, the costs of off-range holding, and the net benefits associated with wild horse populations. Model parameters are calibrated using published studies on wild horse population biology and Bureau of Land Management (BLM) expenditure data. The value of ongoing status quo management is also estimated, and policy simulations are generated for horse-removal, fertility-control, and hybrid management scenarios. Additional scenarios are developed to assess the sensitivity of model results to shorter time horizons and a cost minimization specification. The simulation results are then examined to glean insights for effectively managing wild horse populations.

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This study fits within a growing body of literature examining the dynamics of multi-use species management. A key characteristic of these analyses is that the shadow price of multi-use species can be either positive or negative, which can lead to multiple potentially optimal equilibria (Rondeau 2001; Zivin, Hueth, and Zilberman 2000). Prior studies have investigated the dynamics of multi-use species management in the context of controlling beaver, deer, and wild pig populations (Huffaker, Bhat, and Lenhart 1992; Rondeau and Conrad 2003; Zivin, Hueth, and Zilberman 2000); wolf reintroductions (Rondeau 2001); and invasive species management with biological controls (Fenichel, Horan, and Bence 2010). This article contributes to the dynamic multispecies management literature an analysis of nonlethal population control using removals with off-site holding, and fertility-control methods.

II. BACKGROUND

Free-roaming horses are an enduring icon of the American West, but an effective and sustainable population management plan has eluded land managers for decades. Western wild horse populations are descendants of the domestic animals that were introduced by Spanish explorers in the early 1500s. Once introduced, the horse populations grew quickly, and by the year 1800 wild horses roamed in large herds from Texas to California (Dobie 1952).

Rapid settlement of the western United States and the subsequent development of western lands led to conflicts with wild horse populations in the 1800s and 1900s. During this time, wild horses were sought out and destroyed or captured for commercial slaughter to prevent their interference with grazing and agricultural activities (Phillips 2012). By 1971, the total U.S. wild horse population was reduced to approximately 9,500 a (Pitt 1984). Some wild horse advocates viewed wild horse management practices as inhumane, and by the mid-twentieth century these advocates had organized to lobby Congress for wild horse protections. The campaign garnered public interest and media coverage; and in 1959 Congress passed legislation to prohibit the use of aircraft or motorized vehicles to hunt horses and burros on public lands (Pitt 1984).

In 1971 Congress passed the Wild and Free-Roaming Horses and Burros Act, legislation that continues to guide the BLM's management of wild horse populations. The act banned private horse gathers and tasked the BLM with "protection, management and control of wild free-roaming horses and burros on public lands" (National Research Council [NRC] 2013). The bill's language guides the BLM to manage horses and burros at "the minimal feasible level" to "achieve and maintain a thriving natural ecological balance on the public lands" (NRC 2013). These directives must be balanced with the BLM's mandate to manage public lands for multiple uses. Without active control, horse populations can become a stress on grazing land (Pimentel, Zuniga, and Morrison 2005). To prevent rangeland degradation, the BLM actively gathers wild horses and removes them from the range with the goal of keeping populations within predetermined appropriate management level (AML) bounds. Adoptive homes are sought for removed horses, and unadopted animals are sent to long-term holding facilities. Current BLM policy does not support selling unwanted horses for slaughter.

In recent years, unwanted horses have been accumulating at holding facilities. During the 2015 fiscal year, 66% of the Wild Horse and Burro Program's (WHBP) $77.2 million budget was dedicated to maintaining captive horses (BLM 2016b). In 2000, by comparison, holding accounted for 46% the $19.8 million total program budget (Government Accountability Office 2008). Furthermore, Garrott and Oli (2013) estimated the total net present value (NPV) cost of caring for the horses currently in holding, if no more were added, at nearly $350 million. Under the existing management program, these authors estimate that the total 2013–2030 costs of maintaining unadopted horses in captivity would total $1.1 billion.

Facing an uncertain future, the BLM requested that the NRC scientifically evaluate the challenges facing the WHBP (NRC 2013). In their report, the council offered guidance for developing dynamic models that could assist the BLM with identifying cost-effective management options. Specifically, a need was

expressed for a model that evaluates the dynamics of on-range and off-range populations, and the costs and consequences of existing and alternative management regimes (NRC 2013).

The findings of the NRC report have not yet led to appreciable shifts in the BLM's wild horse management strategy. Meanwhile, horse populations remain above the AML in most areas, and over 45,000 horses are held in off-range facilities (BLM 2015c). The unsustainable nature of this situation recently garnered coverage by several major media outlets, heightening awareness of wild horse management issues (*The Economist* 2016; Phillips 2016). In September 2016, the citizen-led Wild Horse and Burro Advisory Board recommended euthanizing horses in holding after offering them for adoption. However, following public outcry from animal advocates, the BLM stated that it does not intend to change its policy against wild horse euthanization (Chokshi 2016). Here, we evaluate management alternatives using nonlethal strategies to inform future management actions under current BLM policy.

III. MODELS OF OPTIMAL WILD HORSE POPULATION CONTROL

This section presents dynamic models of optimal wild horse population control for two management methods: removing horses from the range and treating horses with a fertility-control vaccine. A hybrid management regime combining removals with fertility control is then developed. The optimal management policy under a given set of assumptions is to choose a sequence of annual population control efforts that maximizes the net benefits associated with wild horse populations over time.

Both removal and fertility-control methods require that horses be gathered on the range. With removal, gathered horses are transported to short-term facilities where adoptive homes are sought for the animals. Horses that go unadopted are shipped to long-term holding facilities. One of the most promising and practical fertility-control methods for wild horse populations is the long-lasting formulation of porcine zona pellucida vaccine (PZP-22), an imunocontraceptive that is administered through injection after horses are gathered. Treated horses are released back to the range, where the contraceptive remains about 85% effective after 22 months (NRC 2013).

The annual cycle of horse management in the optimization models coincides with the fiscal year. Management actions begin October 1 and continue through the end of February. Wild horse foaling (i.e., birthing) occurs from March 1 to June 30, and the BLM prohibits helicopter gathers during that period (BLM 2010). Net population benefits are assumed to accrue from July 1 through September 30.

Optimal Horse Removal

The NPV of a population of wild horses managed in perpetuity with removal methods is maximized where the chosen sequence of removals $\{Y_0,\ldots,Y_\infty\}$ solves the problem in [1].

$$\max_{\{Y_t\}} \sum_{t=0}^{\infty} \rho^t [B(X_t) - C^{\mathrm{g}}(Y_t) - C^{\mathrm{I}}(I_{t-1},X_{t-1},Y_t) - C^{\mathrm{TR}}(X_{t-1},Y_t) - C^{\mathrm{MISC}}(X_{t-1},Y_t) - CADPT\gamma_t - SC_t], \quad [1]$$

subject to

$$X_t - X_{t-1} = F(X_{t-1}) - Y_t\left[1 + \frac{F(X_{t-1})}{X_{t-1}}\right],$$

$$I_t - I_{t-1} = -I_{t-1}m_{\mathrm{I}} + Y_t\left[1 + \frac{F(X_{t-1})}{X_{t-1}}\right] - \gamma_t,$$

$$Y_t \le \min\{Y^{\max}, \delta X_{t-1}\} \forall t,$$

$$X_{-1} \text{ is given,}$$

$$I_{-1} \text{ is given,}$$

$$X_t^{\min} \ge AML \forall t,$$

$$SC_t = \begin{cases} 0 \text{ if } Y_t = 0 \\ SC \text{ if } Y_t > 0 \end{cases}.$$

Initial population X_{-1} is the population size when the first management cycle begins. The choice variable Y_t is the number of horses gathered during the first five months of period

t. In the absence of management, the horse population grows by $F(X_{t-1})$ during period t foaling. Under optimal removal management, the population transitions to size $(X_{t-1} - Y_t)$ after removals are implemented and then grows to size X_t before benefits are realized in the latter part of period t. Many of the female horses removed from the range are pregnant and give birth off-range rather than on-range. Thus the effective impact of removals on horse population growth is $Y_t[1 + F(X_{t-1})/X_{t-1}]$, where $F(X_{t-1})/X_{t-1}$ approximates the per capita growth increment for horses in a population of size X_{t-1}. This also implies that removing Y_t horses from the range results in $Y_t[1 + F(X_{t-1})/X_{t-1}]$ captive horse births during period t. Furthermore, the total cost of gathering horses, $C^{g}(Y_t)$, depends on the number of horses gathered. Initiating management actions at the beginning of a management period is associated with setup costs $SC_t = SC$.

The inventory of horses in long-term holding at the beginning of period t equals I_{t-1}. These horses are held in long-term facilities until the end of the period when a discrete mortality event occurs at rate m_{I}. The horse inventory in short-term holding during period t equals effective removals in that period, $Y_t[1 + F(X_{t-1})/X_{t-1}]$. Adoptions occur at the end of the period, and the remaining horses are shipped to long-term holding. The costs of off-range holding during time t are given by $C^{\mathrm{I}}(I_{t-1}, X_{t-1}, Y_t)$. The costs of shipping horses from short-term to long-term holding are $C^{\mathrm{TR}}(X_{t-1}, Y_t)$.

The parameter γ_t represents the horses adopted in period t, and γ is the annual state adoption demand. The first γ removed and captive-born horses are adopted and the rest go to long-term holding facilities. Thus, the number of horses adopted in period t equals $Y_t[1 + F(X_{t-1})/X_{t-1}]$ if effective removals (i.e., removed plus captive-born horses) are less than or equal to γ, and equals γ otherwise. The costs associated with administering an adoption are $CADPT$, and total adoption costs in period t equal $\gamma_t CADPT$. When removals plus captive births exceed adoption demand, adoption costs are a fixed cost of management. The miscellaneous costs related to compliance inspections, horse preparation, and additional holding are given by $C^{\mathrm{MISC}}(X_{t-1}, Y_t)$.

Wild horses are associated with economic values beyond the costs of population management. Wild horses provide recreation benefits for wildlife viewers and nonuse benefits for individuals who value knowing that wild horses roam free on the western range. Horse populations can also impose economic costs through displacement of wildlife species and domestic livestock, and through degradation of rangelands. The net economic benefits provided by a wild horse population of size X_t are given by the function $B(X_t)$. It is assumed that $B(X_t)$ is a strictly concave, single-peaked function that reaches its maximum where $X = \bar{X}$. Thus, the marginal benefits function $B_x > 0$ for $X \in [0, \bar{X})$ and $B_x < 0$ for $X \in (\bar{X}, \infty]$.

Several constraints are placed on the optimal horse management solution. During a given period, the horse population is smallest after removals occur but before foaling begins. The models specify that this minimum population level, $X_t^{\min}$, must be above the low-range AML set by the BLM. Wild horse managers may be constrained by the number and proportion of animals that can be gathered in a given year (NRC 2013). Constraint parameter δ is the maximum proportion of the population that can be gathered in a single period. Gathers are also constrained in the model by the maximum total number of horses that can be gathered in a single period, $Y^{\max}$. Finally, the discount factor ρ reflects time preference and is bound between 0 and 1 (inclusive).

Optimal Fertility Control

The optimal fertility-control program is a sequence of fertility treatment choices that maximizes the following problem:

$$\max_{\{Y_t\}} \sum_{t=0,2,4,\ldots}^{\infty} \rho^t \left[B(X_t) + \rho B(X_{t+1}) - C^{\mathrm{g}}(Y_t) - C^{\mathrm{fc}}(Y_t, HMA) - C^{\mathrm{I}}(I_{t-1}) - SC_t \right], \quad [2]$$

subject to

$$X_{t+2} - X_t = F_{t+1}(X_t, Y_t) + F_{t+2}(X_{t+1}, Y_t),$$
$$I_{t+1} - I_{t-1} = I_t(1 - m_{\mathrm{I}}) - I_{t-1},$$
$$Y_t \le \min\{Y^{\max}, \delta \tilde{X}_t\},$$
$$X_{-1} \text{ is given},$$
$$I_{-1} \text{ is given},$$
$$\tilde{X}_t \ge AML \forall t,$$
$$SC_t = \begin{cases} 0 \text{ if } Y_t = 0 \\ SC \text{ if } Y_t > 0 \end{cases}.$$

As in the removals model, the horse population is of size X_{-1} when fertility-control management commences, and Y_t horses are gathered during the first five months of period t. All gathered horses are released back to the range after the mares (i.e., female horses) are treated with contraceptives. Gathers are assumed to occur once every two periods to coincide with the approximate effectiveness duration of PZP-22.

Fertility-control treatments prevent conception but do not interfere with the development of horses in utero (Kirkpatrick and Turner 2003). Thus, fertility treatments applied in period t begin to impact population growth in period $t+1$. Because of this delayed impact, the population grows to size $X_0 = F(X_{-1}) + X_{-1}$ by the beginning of the second period regardless of the initial level of fertility-control effort. The fertility-control formulation considered in this model is effective for two periods, so the treatments applied in period t impact population growth in periods $t+1$ and $t+2$. Fertility-control treatments directly impact the population growth rate. The population growth increments impacted by fertility control undertaken in period t are given by $F_{t+1}(X_t, Y_t)$ and $F_{t+2}(X_{t+1}, Y_t)$. There are no horse removals in the fertility-control optimization model. Thus, the initial horse inventory decays according to mortality rate m_{I} until there are no horses left in holding. The costs of horse holding are determined in the same manner as in the removals model, except that gathers and population size have no impact on holding costs and no are horses held in short-term holding.

Costs specific to fertility-control management (C^{fc}) are assumed to be a function of the number of horses gathered for treatment (Y_t) and the number of BLM herd management areas (*HMA*) where fertility control is undertaken. Gather constraints under fertility-control management mirror the constraints under removals, with one notable exception. The delayed effect of fertility control necessitates a more restrictive gather constraint to facilitate dynamic programming of the solution. The constraint $\tilde{X}_{t-1}$ is the lower bound of the previous period population.

Hybrid Management: Combining Removals and Fertility Control

Horse-removal and fertility-control methods have thus far been considered independently. Each of these approaches has advantages that could be leveraged in a population management regime utilizing both removals and fertility control. The following section discusses the strengths and weaknesses of removals and fertility control and proposes a hybrid management regime that combines both methods.

Compared to removals, fertility control is a more benign method of population management that better aligns with the BLM's mandate to manage horses at the "minimum feasible level." Fertility control may also represent a low-cost population control alternative as it does not require off-range management of horses. However, reducing or stabilizing horse populations with fertility control alone requires that a large share of the population be frequently gathered and treated (NRC 2013).

Removal management directly decreases the base from which a population grows and prevents removed pregnant females from giving birth on the range. Furthermore, removals have an immediate impact on the population compared to the delayed effect of fertility control. These characteristics make removal management a more effective means of reducing horse populations in terms of population reduction per unit of gathering effort. Management with removals also has drawbacks. It requires costly long-term management of off-range horses and is a more intensive management alternative compared to fertility control.

Because removals and fertility each have respective strengths and weaknesses, a management alternative that allows for both removals and fertility control may be preferred to management under a single method. Specifically, a "hybrid" management program could allow for population reductions while reducing the number of horse removals required to maintain populations at a desirable level. Hybrid management may also reduce the intensity of population management subject to maintaining ecological balance on the range.

The NRC's report describes a possible hybrid management scenario (NRC 2013). Following this description, the hybrid scenario is specified to involve several years of intensive removals to reduce the population, followed by a management regime that incorporates fertility control. Specifically, hybrid management involves z consecutive years of removals at the $Y_t = \min\{Y^{\max}, \delta\tilde{X}_t\}$ level until the population is within the AML range. Following the initial reduction phase, horses are managed on a two-year gather cycle with a fixed number of removals and optimally chosen fertility-control effort. The hybrid management model is presented in the Appendix. The number of horses removed at the beginning of each management cycle, q, is the minimum removal effort that maintains a population below carrying capacity in the long term under optimally chosen fertility control.

IV. MODEL CALIBRATION

The objective of this article is to inform wild horse management through development and evaluation of dynamic bioeconomic models. Because the form of the defined optimization problems makes derivation of analytical solutions impractical,[1] we proceed by exploring the problems empirically and develop policy insights through dynamic simulations. This section specifies functional forms, parameters, and assumptions for the optimal horse management models.

[1] These characteristics include having objective functions that are nonmonotone in X, nonlinear transition functions for X and I, and multiple state variables.

Separate models were developed for Nevada and Oregon based on data availability. Thus, all of the herds within in a state were modeled as a single meta-population. This treatment is appropriate given the significant migration that can occur between herds (e.g., see BLM 2015b), but it also imposes no migration between states and equal density dependence (i.e., X/K) across herds.

The biological parameters specified for the models are based primarily on studies of the Beatys Butte herd in eastern Oregon. Due to data limitations, the models do not incorporate burro-specific cost or population biology parameters. Monetary costs and benefits in the models are inflation-adjusted to 2015 dollars. The discount factor (ρ) reflects a 4% discount rate in the base model. The functional forms and associated parameter values specified for the models are summarized in Table 1.

Wild Horse Population Biology

Eberhardt and Breiwick (2012) examined growth data from four wild horse populations, including one in the western United States. They found that the populations grew according to a theta logistic growth function, with the parameter controlling the inflection point equal to two, as shown in equation [3]. The carrying capacity (K) is the maximum population that can be sustained by an environment given available resources, and the intrinsic rate of growth (r) is equal to $b - m$, the birth rate minus the mortality rate.

$$F(X_{t-1}) = X_{t-1} r\left[1 - \left(\frac{X_{t-1}}{K}\right)^2\right]. \quad [3]$$

The theta logistic growth function exhibits properties that add intuition to the equation. The term $X_{t-1}r$ represents the intrinsic increment of growth of the population, and $X_{t-1}r(X_{t-1}/K)^2$ captures the density-dependent population effects. As carrying capacity K goes to infinity, the population is unconstrained by density effects, and population growth is equal to the intrinsic increment of growth. As the population approaches the carrying capacity, growth goes to zero.

TABLE 1
Model Calibration

Description	Functional Form	Parameter Values	References
Discount factor	$\rho = \frac{1}{1+\delta}$	$\delta = 0.04$	
Initial population	X_{-1}	$X_{-1}^{NV} = 41{,}161; X_{-1}^{OR} = 3{,}841$	BLM 2016a
Initial holding inventory	I_{-1}	$I_{-1}^{NV} = 24{,}335; I_{-1}^{OR} = 2{,}707$	BLM 2015c
Appropriate management level ranges	$AML_{low}; AML_{high}$	NV: $AML_{low} = 7{,}597, AML_{high} = 12{,}811$; OR: $AML_{low} = 1{,}374, AML_{high} = 2{,}715$	BLM 2016a
Annual adoption demand	γ	$\gamma^{NV} = 75; \gamma^{OR} = 196$	BLM 2015a
Population growth function	$F(X_{t-1}) = X_{t-1} r\left[1 - \left(\frac{X_{t-1}}{K}\right)^2\right]$	$r = 0.28; K^{NV} = 61{,}595; K^{OR} = 13{,}054$	Eberhardt and Breiwick 2012
Population transition function with removals	$X_t - X_{t-1} = F(X_{t-1}) - Y_t\left(1 + \frac{F(X_{t-1})}{X_{t-1}}\right)$		
Population transition with biannual fertility control	$X_{t+2} - X_t = F_{t+1}(X_t, Y_t) + F_{t+2}(X_{t+1}, Y_t); r_{t+1}(X_t, Y_t) = r_0 - \frac{\pi_1 \theta Y_t}{X_t}; r_{t+2}(X_{t+1}, Y_t) = r_0 - \frac{\pi_2 \theta Y_t}{X_{t+1}}$	$r_0 = 0.28; \theta = .354; \pi_1 = 0.94; \pi_2 = 0.82$	Eberhardt, Majorowicz, and Wilcox 1982; Eberhardt and Breiwick 2012; Bartholow 2007
Holding inventory transition function	$I_t - I_{t-1} = -I_{t-1} m_I + Y_t\left[+\frac{F(X_{t-1})}{X_{t-1}}\right] - \gamma$	$m_I = 0.0952$	Garrot and Oli 2013
Net population benefits	$B(X_t) = aX_t \ln\left(\frac{b}{X_t}\right)$	NV: $a = 25{,}85.5, b = 34{,}823.9$; OR: $a = 25{,}85.5, b = 7{,}380.1$	Bastian et al. 1999; Rondeau and Conrad 2003

(table continued on following page)

TABLE 1
Model Calibration *(continued)*

Description	Functional Form	Parameter Values	References
Gather costs	$C^{g}_{NV}(Y_t) = \beta_1 Y_t + \beta_2 Y_t^2$; $C^{g}_{OR}(Y_t) = \beta_1 Y_t$	NV: $\beta_1 = \$343.5, \beta_2 = \$0.07$; OR: $\beta_1 = \$489.5$	BLM 2015a
Holding costs	$C^{I}(I_t, X_{t-1}, Y_t) = I_{t-1} CLT + Y_t CST_g + Y_t \frac{F(X_{t-1})}{X_{t-1}} CST_{cb}$	$CLT = 1.52 \frac{\$}{day} * 365$ days; $CST_g = 5.37 \frac{\$}{day} * 290$ days; $CST_{cb} = 5.37 \frac{\$}{day} * 150$ days	BLM 2016b
Transport costs	$C^{TR}(X_{t-1}, Y_t) = CTR\left[S_I^{ST} Y_t \left(1 + \frac{F(X_{t-1})}{X_{t-1}}\right) - \gamma\right]$	$CTR = \$123.4; S_I^{ST} = 0.968$	Arneson, Beutler, and Hurst 2002; Garrot and Oli 2013
Miscellaneous costs	$C^{MISC}(X_{t-1}, Y_t) = CMISC\left[Y_t \left(1 + \frac{F(X_{t-1})}{X_{t-1}}\right) - \gamma\right]$	$CMISC = \$210$ if adopted; $CMISC = \$726$ if unadopted	BLM 2014
Gather startup costs	SC	NV: $SC = \$45,447.4$; OR: $SC = \$154,842.3$	BLM 2015a
Adoption costs	$\gamma * CADPT$	$CADPT = \$2,582$	Zeigler 2012; BLM 2014
Biannual fertility-control costs	$C^{fc}(Y_t) = HMA * M + \rho HMA * M + CFC * 0.5 Y_t$	$HMA^{NV} = 84; HMA^{OR} = 18$; $M = \$6,997; CFC = \$299$	Bartholow 2007

For the Beatys Butte herd in eastern Oregon, Eberhardt and Breiwick (2012) estimated an intrinsic growth rate of 0.28 and a carrying capacity of 1,202 horses. The models developed in this study assume that in the absence of management, state horse populations grow according to equation [3] with $r = 0.28$. The states are also assumed to have the same carrying capacity to high-bound AML ratio as the Beatys Butte herd.

The models specify the initial population size based on the BLM's population estimates from March 1, 2016. Because the models require initial population estimates from the beginning of the fiscal year, the BLM population estimates are assumed to grow under model assumptions without management. In the optimization models, $X_{-1} = F(X_{\mathrm{BLM}}) + X_{\mathrm{BLM}}$, where X_{BLM} is the 2016 BLM estimate and X_{-1} is the population at the start of management.

Wild Horse Gather Costs

The annual cost of conducting horse gathers depends on the number of horses gathered. State-level gather expenditure data were obtained from the BLM for fiscal years 2009 through 2015 to parameterize this relationship. The gather expenditure data were escalated to the 2015 fiscal year and then merged with gather statistics from the WHBP website (BLM 2016a). Linear models regressing gather expenditures on the number of horses gathered were estimated for two candidate specifications: a linear specification to represent constant marginal gather costs, and a quadratic specification to represent increasing marginal costs. The quadratic specification provides a superior model fit for the Nevada data, and the linear specification provides a better fit for the Oregon data, as determined by the adjusted R^2 values of the regressions. The final Oregon and Nevada fitted regression lines explain 75% and 87% of the variation in annual gather costs, respectively, according to the final model R^2 values. The model constants are estimates of gather setup costs (SC), and the model coefficients are marginal gather cost estimates. Setting up gather operations is estimated to cost approximately $45,000 Nevada and $155,000 in Oregon. The marginal cost of gathers in Oregon is constant at $490 per horse. In Nevada, marginal gather costs are increasing. The cost of gathering the first horse is $345, and the marginal cost of gathering the 5,000th horse is $1,078. Other potential determinants of state-level gather costs were investigated by sequentially including additional covariates in the regressions described above. However, none of the candidate covariates improved model fit, according to the adjusted R^2, suggesting that gather costs are best predicted by the number of horses gathered without additional covariates.

The gather constraint parameters are specified as follows. The maximum number of horses that can be gathered in a given period ($Y^{\max}$) is set just under the maximum number gathered in a single fiscal year in Oregon ($Y^{\max} = 1{,}250$) from 2009 to 2015. In Nevada, where the current population is a greater share of the AML, the constraint is set to 140% of the maximum number gathered in a single fiscal year in Nevada from 2009 to 2015 ($Y^{\max} = 8{,}000$). Further, no more than half of the pregather population can be removed in a single period in either state ($\delta = 0.5$).

Off-range Horse Inventory

Horses removed from the range are transferred to short-term holding facilities where adoptive homes are sought for them. Horses that go unadopted are transported to long-term holding pastures to live out the rest of their lives. Thus, the inventory of horses in holding over time depends on the number of horses removed from the range, the number of captive-born horses, off-range horse mortality (assuming no reproduction), and the initial inventory of horses.

The models assume zero mortality for removed and captive born horses during the period they were gathered. Mortality for horses in long-term holding occurs as a discrete event at the end of each period. The rate of annual mortality for horses in holding (m_1) was specified as follows. First, inventory accrual was simulated over a 35-year period by looping over 1996–2015 removals and adoptions for Nevada and applying the gender-age-specific survival rates from Garrott and Oli (2013).

The simulations assumed that half of the horses removed were mares and that the age of removed horses is a Poisson process with $\lambda = 2$. The simulations yielded age distributions of horses in holding by gender. The mortality rate for horses in holding, $m_I = 0.095$, was then calculated as the overall annual mortality rate in the 36th year, given the age distributions derived in the simulations and the gender-age-specific survival rates. The initial holding inventories for Nevada and Oregon were approximated as the product of the total number of horses in holding as of September 2015 times the state's share of the total horse population, where $I_{-1}^{NV} = 24{,}335$ and $I_{-1}^{OR} = 2{,}707$.

Off-range Management Costs

Horses removed from the range are transported to short-term holding corrals where they remain until they are adopted or transported to long-term pastures. The models assume that adoptions occur as a discrete event just before the end of each period, and that all unadopted horses are transported to long-term holding facilities before the next period begins. The models also assume that future annual adoption demand remains constant at the 2010–2014 average and that long-term holding capacity is not limiting.

Gathers occur between October and February, and captive-born horses are foaled during the spring of the same period in which their mothers are gathered. To reflect this timing the models assume that I_{t-1} horses are held in long-term holding for the entirety of period t, removed horses are held in short-term holding for 290 days, and captive-born horses are held for 150 days of period t.

Off-range management costs were parameterized with WHBP adoption, horse holding, and expenditure data from the BLM. Per-horse adoption expenditures averaged $2,707 annually for fiscal years 2011–2015, and adopters of wild horses paid a $125 fee (BLM 2016b). Annual adoption demand in Nevada and Oregon averaged 75 and 196 horses, respectively, from fiscal years 2010–2014 (i.e., $\gamma^{NV} = 75$; $\gamma^{OR} = 196$). During fiscal years 2011–2015, long-term holding expenditures averaged $1.52 per day and short-term holding expenditures averaged $5.37 per day.[2] The cost of shipping unadopted horses to long-term facilities is $3.29/mi/40 horses (Arneson, Beutler, and Hurst 2002). The typical distance from short-term facilities to long-term facilities is approximately 1,500 miles, roughly the distance from short-term holding facilities in Reno, Nevada, or Burns, Oregon, to long-term holding pastures the midwestern United States. Horses removed from the range are associated with miscellaneous costs related to compliance inspections, horse preparation, and additional holding. Total miscellaneous costs are $210 for adopted horses and $726 for unadopted horses (BLM 2014).

Fertility-Control Effectiveness and Duration

The relationship between horse population growth and fertility-control effort is specified next. Recall that Eberhardt and Breiwick (2012) estimated the intrinsic growth rate for the Beatys Butte herd at $r = 0.28$ without managment. Another study of the Beatys Butte herd (Eberhardt, Majorowicz, and Wilcox 1982) estimated the average annual survival rate at 0.926. Thus, the intrinsic rate of growth at Beatys butte equals 0.28 with no fertility control, and equals –0.074 when every female in the herd receives effective contraception. In reality, PZP-22 is not 100% effective at preventing horse pregnancy. Bartholow (2007) reports that PZP-22 was 94% effective in the first year after treatment and 82% effective in the second year for Nevada's Clan Alpine herd.

To maintain tractability, the optimal fertility-control model assumes an equal sex ratio in horse populations, homogeneity across gender-specific gather production, and no gender selectivity in gathers. These assumptions yield the simplifying condition that half of the horses gathered are treated with fertil-

[2] For each fiscal year from 2011 to 2015, the number of horses in holding (short and long) is calculated as the average of monthly holding inventories reported by the BLM. The per day holding cost is then calculated as the average number of horses in holding divided by the total (short and long) holding expenditures reported by the BLM for that fiscal year.

ity-control agents and the other half are returned to the range.

Contraceptive applications occur on a biannual cycle in the fertility-control model so that two foaling seasons occur between management actions. In the first mating period after fertility-control application, fertility-control effectiveness is $\pi_1 = 0.94$ and the intrinsic rate of growth is r_{t+1}. In the second mating period after management, fertility-control effectiveness is $\pi_2 = 0.82$ and the intrinsic rate of growth is r_{t+2}. A linear functional form is specified between the growth rate and the proportion of females treated with contraceptive. Combined with the previously defined parameters, a linear functional form yields equations [4] and [5].

$$r_{t+1}(X_t,Y_t) = r_0 - \pi_1 0.354\frac{Y_t}{X_t}, \quad [4]$$

$$r_{t+2}(X_{t+1},Y_t) = r_0 - \pi_2 0.354\frac{Y_t}{X_{t+1}}. \quad [5]$$

The transition of the horse stock over time is then given by the theta logistic population growth function in equation [3], where the intrinsic rate of growth is $r_{t+1}(X_t,Y_t)$ in the second foaling period after fertility-control management and $r_{t+2}(X_{t+1},Y_t)$ in the third foaling period after management.

Fertility-Control Costs

The costs of fertility-control management are parameterized as follows. The combined cost of obtaining contraceptives and administering them on the range is approximately $300 per application (Bartholow 2007). Fertility-control management requires additional population monitoring compared to management with removals. The cost of fertility-control monitoring (M) is approximately $7,000 per HMA per year (Bartholow 2007). There are 18 distinct HMAs on BLM lands in Oregon and 84 in Nevada. Because management is considered at the state level, the models assume all HMAs on BLM lands are monitored for the two years after a fertility control cycle is initiated. The second year of fertility monitoring is discounted, so that if fertility control is initiated in fiscal year t, then monitoring costs per HMA for that management cycle equal $M_t = M + \rho M$. If fertility control is not initiated, then no monitoring costs are incurred during that management cycle.

Horse Population Net Benefits

Wild horse populations are associated with significant societal benefits. On the other hand, large horse populations can impose costs on stakeholders through displacement of alternative land uses and degradation to range lands. Pimentel, Zuniga, and Morrison (2005) estimated that U.S. wild horse and burro populations cause $5 million in forage losses annually. Bastian et al. (1999) found that the marginal opportunity costs of additional horses on the range in excess of target population levels are well over $4,060 in in 2015 dollars. Wild horses also provide use and nonuse benefits to wild horse supporters. However, these benefits are challenging to measure because their value is not revealed through market transactions.

The function describing horse population net benefits, $B(X)$, represents the aggregation of all costs and benefits attributable to that population. The shape of $B(X)$ depends on the shape of the marginal benefits and marginal costs functions. Bastian et al. (1999) found that the marginal costs of adding horses to a population were increasing through the population size associated with the $4,060 figure mentioned above. Intuitively, adding horses to a small population under BLM management (i.e., management subject to an overall grazing limit) would displace less valuable wildlife and livestock resources compared to when the horse population is already large. Conversely, attempts in the economic literature to measure benefit functions for wildlife populations and habitats support decreasing marginal benefits, (e.g., Layton, Brown, and Plummer 1999; Rollins and Lyke 1998), particularly when the wildlife population is above its minimum viable population (e.g., Bandara and Tisdell 2005; Ojea and Loureiro 2009). Decreasing marginal costs and increasing marginal benefits imply that the total net benefits function for horse populations, $B(X)$, is a single-peaked function. This analysis fol-

lows Rondeau and Conrad (2003) in assuming that total net benefits can be represented by a Gompertz function where

$$B(X) = \begin{cases} 0 \text{ if } X = 0 \\ aX\ln\left(\frac{b}{X}\right) \text{ if } X > 0 \end{cases}. \quad [6]$$

The Gompertz function is a single-peaked function that satisfies the characteristics of net horse benefits specified previously (i.e., that $B_X > 0$ for $X \in [0,\bar{X})$ and $B_X < 0$ for $X \in (\bar{X},\infty]$). The high-bound AML is assumed to be the population level $\bar{X}$ that corresponds with the maximum of $B(X)$. The marginal opportunity cost of an additional wild horse at high population levels is approximately \$4,060 (Bastian et al. 1999), and the models assume that this is the marginal net benefit at carrying capacity. The resulting system of equations (i.e., $B_X(\bar{X}) = 0$ and $B_X(K) = -4{,}060$) was then solved for parameters a and b. In the Nevada model, parameters a and b are equal to 2,586 and 34,824, respectively. The resulting function implies that a state population of 10 horses in Nevada produces \$211,000 in annual net benefits, while a population at the high-range AML produces \$33.1 million in annual net benefits. At carrying capacity, the Nevada horse population yields net benefits of −\$90.8 million annually. In the Oregon model, parameter a equals 2,586, and parameter b equals 7,380.

V. RESULTS

This section presents simulations of the specified optimal management models and compares them to current management practices. A 50-year time horizon is used to compare the base scenarios, and sensitivity scenarios with shorter horizons are also explored. A long-term time horizon is appropriate for identifying optimal management strategies because horse removals conducted in the current period are associated with holding costs that extend decades into the future.

Solving the Dynamic Optimization Problem

The solution to the removals problem in [1] is identified by solving the associated Bellman equation in [7], subject to the previously defined constraints and the terminal condition $V(T+1,X_t) = 0$, where T is the finite time horizon.

$$\begin{aligned} V(t,X_{t-1},I_{t-1}) = \max_{\{Y_t\}} \{ & \rho^t [B(X_t) - C^{\mathrm{g}}(Y_t) \\ & - C^{\mathrm{I}}(I_{t-1},X_{t-1},Y_t) - C^{\mathrm{TR}}(X_{t-1},Y_t) \\ & - C^{\mathrm{MISC}}(X_{t-1},Y_t) - CADPT\gamma_t - SC_t] \} \\ & + \rho^{t+1} V(t+1,X_t,I_t). \end{aligned} \quad [7]$$

The function $V(t,X,I)$ is the maximum achievable NPV benefits starting at time t with population level X and given action Y. All subsequent actions are assumed to be taken optimally, given the action taken in the current period. Bellman equations [8] and [9] are the equivalent for optimal fertility-control and hybrid population management, respectively.

$$\begin{aligned} V(t,X_t) = \max_{\{Y_t\}} \{ & B(X_t) + \rho B(X_{t+1}) - \mathrm{C}^{\mathrm{g}}(Y_t) \\ & - C^{\mathrm{fc}}(Y_t,HMA) - C^{\mathrm{I}}(I_{t-1}) - SC_t \} \\ & + \rho^{t+1} V(t+1,X_{t+1},I_t), \end{aligned} \quad [8]$$

$$\begin{aligned} V(t,X_t) = \max_{\{Y_t\}} \{ & \rho^t [B(X_t) + \rho B(X_{t+1}) - C^{\mathrm{g}}(Y_t+q) \\ & - C^{\mathrm{fc}}(Y_t) - C^{\mathrm{I}}(I_{t-1},\tilde{X}_t,q) - C^{\mathrm{TR}}(\tilde{X}_t,q) \\ & - C^{\mathrm{MISC}}(\tilde{X}_t,q) - 2CADPT\gamma - SC] \} \\ & + \rho^{t+1} V(t+1,X_{t+1},I_t). \end{aligned} \quad [9]$$

The Bellman equations were programmed in MATLAB (MathWorks 2015) and solved with a backward induction algorithm. The terminal period (T) was set to 65 years, so that realization of the terminal condition is not taken into account in the optimal management simulations. Robustness checks with longer-horizon models suggest that the solutions are stationary. The problems were discretized to limit their dimensionality and facilitate their solution via dynamic programming.[3]

The Nevada and Oregon scenarios were simulated with a 50-year planning horizon for

[3] Discretization involved grouping the horses into units and then rounding the state variables to the nearest unit before each value calculation. For removal and hybrid management, the Nevada and Oregon models grouped horses into 1,000-horse and 150-horse units, respectively. The fertility-control models grouped horses into 10-horse units.

both the horse-removal and fertility-control models. The steady-state solutions to the optimal removal and optimal fertility-control models are summarized for Nevada and Oregon in Table 2. The population and holding inventory paths for the optimal solutions and the status quo scenario are presented for Nevada and Oregon in Figure 1 and Figure 2, respectively.

Status Quo Management Scenario

Status quo horse management practices were simulated under model assumptions to provide a baseline for comparison. BLM horse population estimates from 2009 to 2014 and associated management actions from that period were evaluated to characterize current state-level management in Nevada and Oregon. Notably, the intensity of removal efforts varied substantially from year to year in both states. In Nevada, annual gathers ranged from 1.5% to 30.5% of the estimated prefoal population.[4] Annual Oregon gathers ranged from 0.0% to 54.0% of the prefoal population. The status quo management scenarios assume that future gather, removal, and fertility-control efforts reflect a state's recent management practices. Specifically, the scenarios follow a six-year management cycle based on the proportion of horses managed in the six-year period during fiscal years 2009–2014, subject to state gathers not exceeding $Y^{\max}$ or δX_{t-1}.

The periodic nature of removals in the status quo scenarios gives rise to oscillations in horse populations and holding inventories, as displayed in Figures 1 and 2. In terms of long-term trends, populations and inventories increase in the earlier periods before leveling off in the later periods. The cumulative NPV of status quo horse management over a 50-year time horizon is –$1.52 billion in Nevada and –$255 million in Oregon. The cumulative NPV program benefits over time for each scenario are presented in Figure 3, and the cumulative NPV management costs over time are presented in Figure 4.

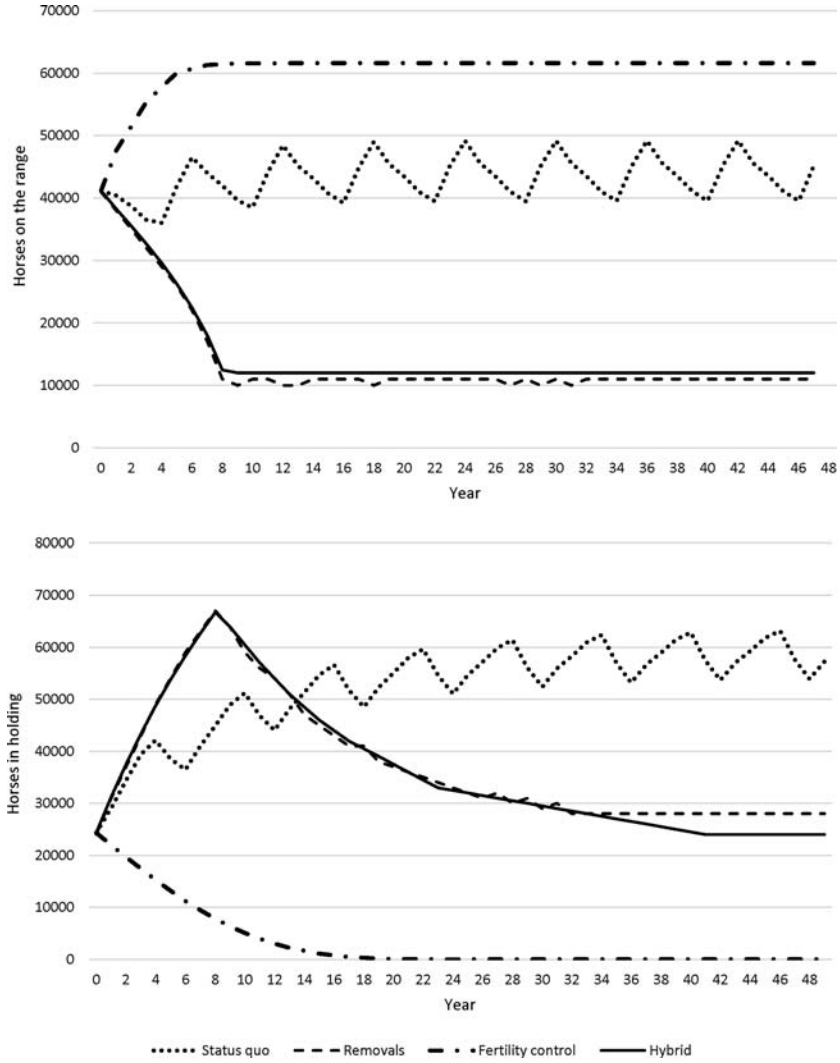
[4] The status quo gather proportions were constructed with the March 1 BLM population estimate in the denominator and the horses gathered over the subsequent 12 months in the numerator.

TABLE 2
Optimal Steady-State Management Programs and Cumulative Optimal Program Benefits

Scenario	Pregather Population	Removals	Fertility Treatments	Total Gathers	Horses in Holding	Percent Gathered	Cumulative Net Benefits[a]	Change from Status Quo[a]
Nevada								
Removals	11,000	2,000	0	2,000	28,000	18	(230.1)	1,290.5
Fertility control	61,600	0	0	0	0	0	(2,005.5)	(484.9)
Hybrid	12,000	3,750	1,000	5,750	24,000	48	(265.1)	1,255.5
Oregon								
Removals	2,250	450	0	450	4,050	20	34.7	290.1
Fertility control	13,050	0	0	0	0	0	(371.6)	(116.2)
Hybrid	2,250	677	150	977	2,100	43	44.3	299.8

[a] Millions of dollars. Parentheses indicate negative values.

FIGURE 1
Population and Holding Inventory Paths for Nevada Simulations

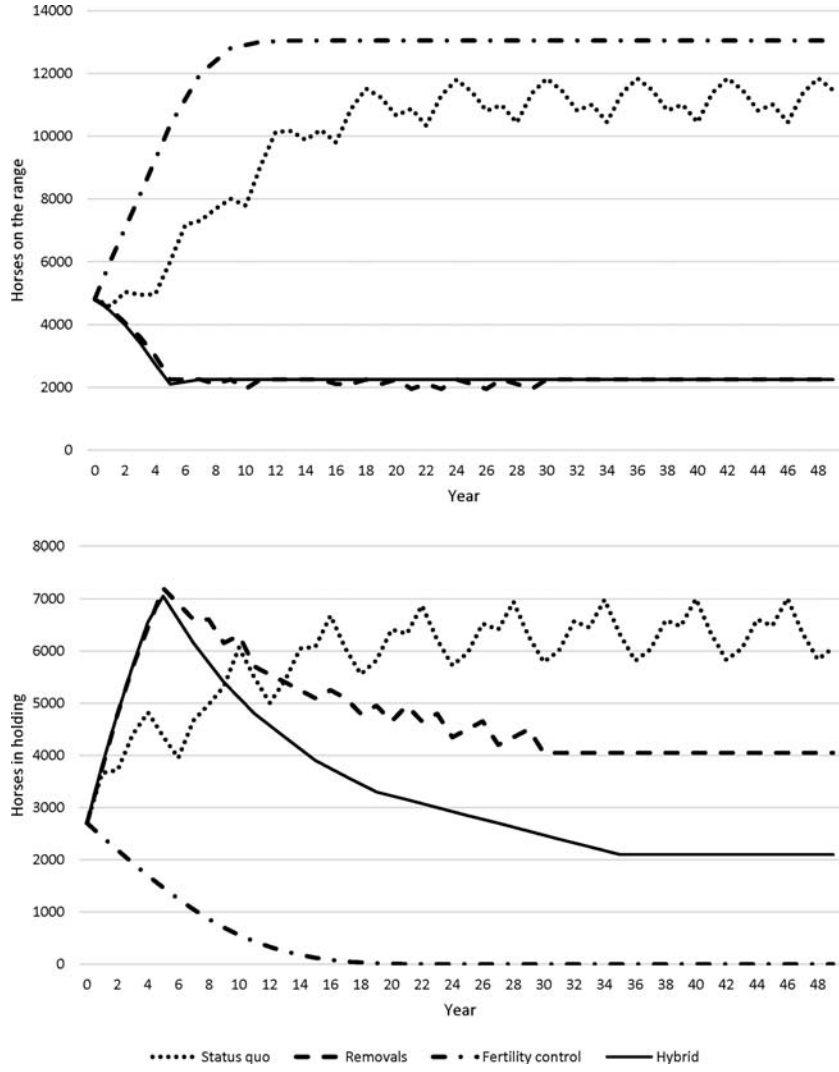


Management Simulations

Horse Removal

The Nevada and Oregon optimal removal scenarios involve an initial reduction of the population to a steady-state equilibrium. In Oregon, the optimal program involves five years of intensive removal effort at or near $Y^{\max}$. At steady-state equilibrium, 450 horses are removed from the range each year to maintain a population of 2,250 horses and a holding inventory of 4,050 horses. The 50-year cumulative NPV of the Oregon optimal removals program is –$34.7 million, a savings of $290.1 million compared to the status quo scenario. Similarly, the optimal removal program in Nevada involves intensive removals over the first eight years of management. Following the initial population reduction, a steady-state management regime commences where 2,000 horses are removed annually to maintain the population at 11,000 and the holding inventory at 28,000. The 50-year cumulative NPV of optimal removals in Nevada

FIGURE 2
Population and Holding Inventory Paths for Oregon Simulations



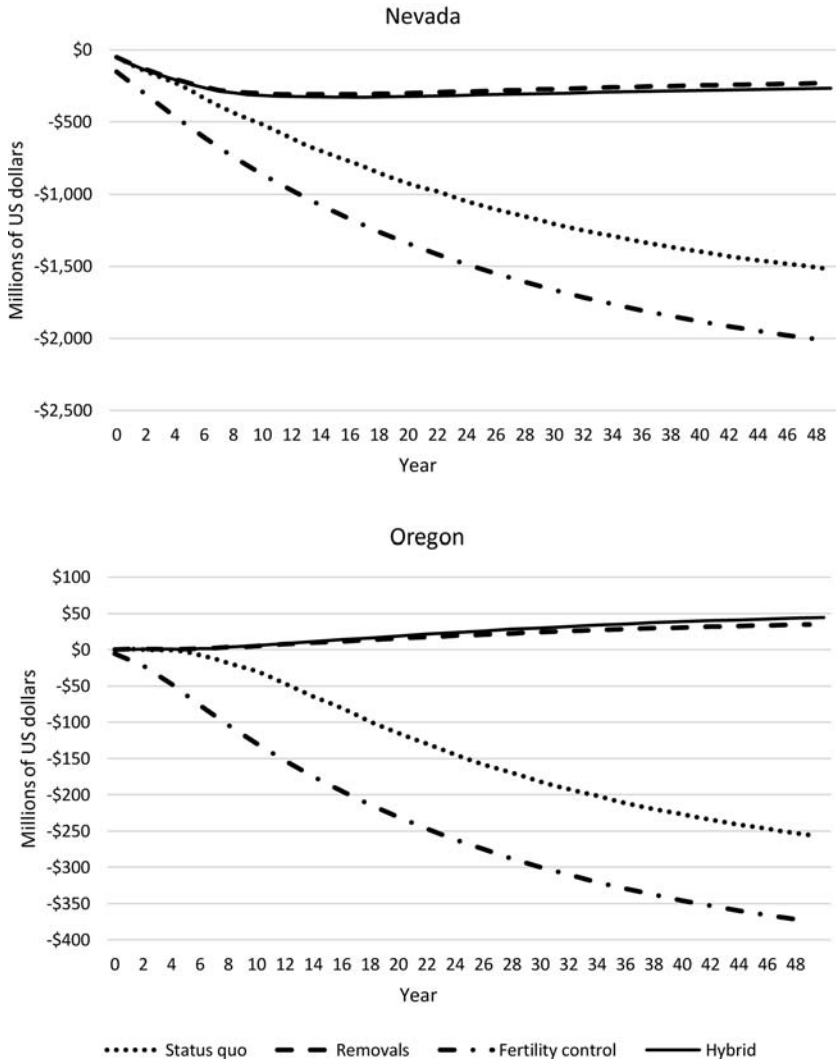
is –$230.1 million, a savings of $1.3 billion over that period compared to the status quo scenario.

As a check to the dynamic programming solutions, optimal steady-state values for removals, population size, and holding inventory were derived numerically using the discrete-time maximum principle (Canon, Cullum, and Polak 1970). While minor differences in the solutions occur due to rounding errors, the numerically derived steady-state values verify the dynamic programming solutions.[5]

The optimal management scenario results yield insights into the trade-offs involved in maximizing population benefits over time. The absolute gather constraint is binding while the populations are reduced in the initial

[5] The steady-state values derived using the maximum principle are as follows: $X_{SS}^{NV} = 10{,}141; I_{SS}^{NV} = 28{,}242; Y_{SS}^{NV} = 2{,}171; X_{SS}^{OR} = 2{,}175; I_{SS}^{OR} = 4{,}163; Y_{SS}^{OR} = 466$.

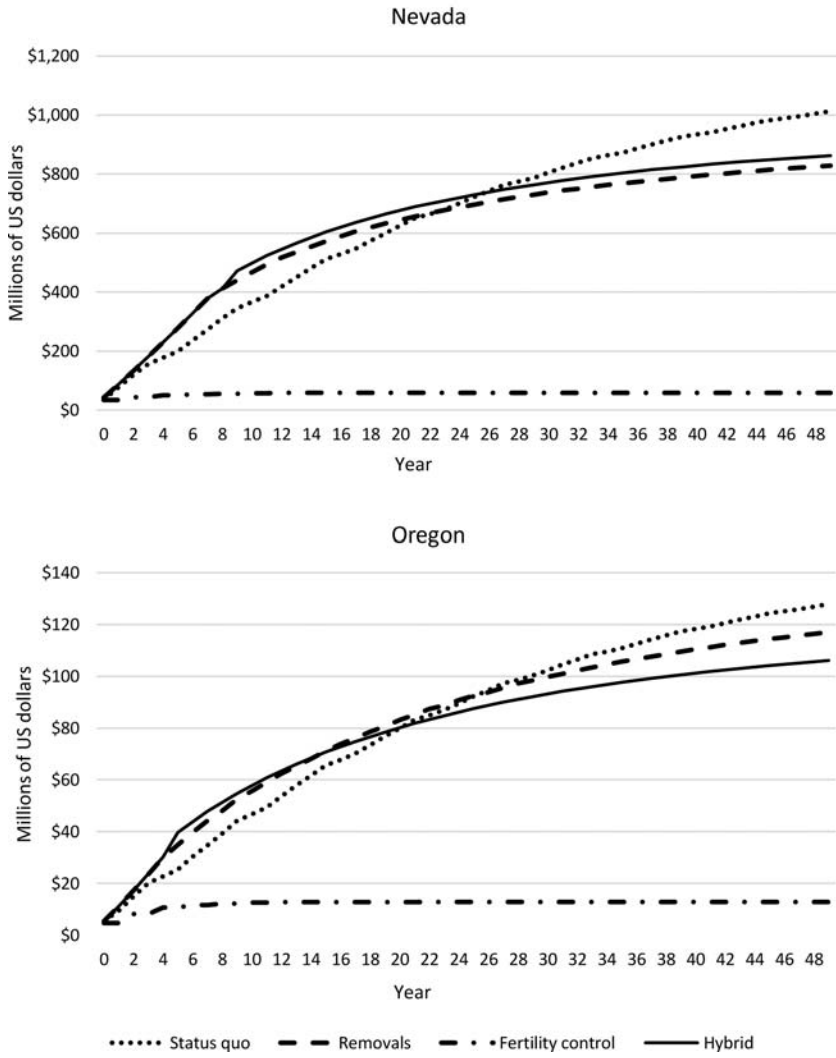
FIGURE 3
Cumulative Net Present Value Net Benefits of Management Programs



periods (i.e., $Y_t = Y^{\max}$). Whereas the removals optimization problem does not meet the sufficient conditions for bang-bang control defined by Spence and Starrett (1975),[6] the removal simulations suggest the optimal management path is indeed to approach a steady state as rapidly as possible. At the optimal steady state, neither the Oregon nor the Nevada optimal removals program is bound by the gather constraints imposed on the model (i.e., $Y^{SS} < \min\{Y^{\max}, \delta X^{SS}\}$). Thus, while the gather constraints regulate the speed with which the population is reduced to steady state, they do not constrain management in optimal steady-state equilibrium.

[6] The Nevada removal model specifies nonlinear gather costs in Y. Both state removal models specify setup costs and stock-dependent variable costs that violate the sufficient conditions of quasi-concavity and separability, respectively.

FIGURE 4
Cumulative Net Present Value of Wild Horse Management Costs



The management costs incurred over a single fiscal year in the optimal removals steady state are higher for the larger Nevada population ($22.4 million) than they are for the smaller Oregon population ($4.3 million). Likewise, in steady state, the larger Nevada population yields higher annual net benefits ($32.8 million) than the Oregon population ($6.9 million). The annual value of net program benefits from optimal removals in steady state is $10.3 million in Nevada and $2.6 million in Oregon.

Horse Fertility Control

The NRC report suggested that fertility control alone cannot address the challenges associated with managing western wild horses. The optimal fertility-control scenarios support this sentiment. The optimal fertility-control management program is to undertake fertility control in early management cycles and then to undertake no management once the population grows toward its carrying capacity. In Oregon, the optimal fertility-control

TABLE 3
Optimal Two-Year Management Cycles at the Steady State (Millions of U.S. Dollars)

Scenario	Net Population Benefits	Management Costs[a]	Net Benefits[a]
Nevada			
Removals	64.3	44.0	20.3
Hybrid	64.1	46.1	18.0
Difference	− 0.4%	4.8%	− 11.3%
Oregon			
Removals	13.6	8.4	5.1
Hybrid	13.2	6.4	6.7
Difference	− 2.7%	− 23.8%	31.4%

[a] Includes costs of off-range holding. For discounting purposes, $t = 0$ in the first year of the optimal management cycle, and $t = 1$ in the second year.

program is to treat around 600 females for the first three cycles and then cease fertility control in all subsequent periods. The optimal fertility-control program in Nevada is to treat 3,940 females in the first period and then undertake no further management actions. Without management, the horse populations grow to their carrying capacity (K) and remain at that level for the duration of the management horizon. The inventory of horses in holding declines to zero deterministically in all of the fertility-control scenarios. Intuitively, it is impossible to reduce the population with fertility control alone given the model gather constraints. Thus, after slowing growth in the initial period(s), it is optimal to just let the populations grow to their carrying capacity instead of applying costly fertility control. Because horse populations are not effectively managed in the fertility-control scenarios, subsequent discussion focuses on the removal and hybrid methods.

Hybrid Management

Fertility-control alone is not a viable population management strategy. The hybrid management scenarios explore the efficacy of fertility control when applied in tandem with horse removals. The hybrid scenarios begin with intensive removals over successive years until the state population is brought within the AML range. Next, a program of fixed removals with optimally chosen fertility-control effort ensues on a two-year cycle. Fixed removals are undertaken at the lowest possible level subject to maintaining a stable population below carrying capacity in the long run. When implemented in concert with removals, fertility control helps stabilize the horse population by decreasing the intrinsic rate of growth. The Nevada hybrid scenario involves nine years of intensive removals followed by a steady-state gather program that stabilizes the population at 12,000. The steady-state program involves gathering 5,750 horses biannually, of which 3,750 are removed and 1,000 are treated with fertility-control agents. Adoptive homes are found for 150 of the removed horses and 3,600 go to long-term holding facilities. A steady-state inventory of 28,000 horses is maintained in holding facilities. The hybrid program in Oregon involves six years of removals followed by a biannual steady-state management program that maintains a pregather population of 2,250 horses. The program involves gathering 977 horses every other year, including 677 removals and 150 horses treated with fertility control. In Oregon, 392 of the removed horses are adopted and 285 are sent to long-term holding facilities. A holding inventory of 2,100 horses is maintained in the steady state.

The NPV costs and benefits of the optimal removal and hybrid management programs over a two-year cycle are presented in Table 3. The steady-state NPV management costs of a two-year hybrid management cycle in Nevada are $46.1 million, an increase of 4.8% compared to the NPV costs removals over the same time period. In Oregon, the NPV of steady-state hybrid management over a cycle is $6.4 million, which represents a 23% re-

duction compared to removals. In terms of program net benefits, a cycle of steady-state hybrid management yields NPV $18.0 million in Nevada and $6.7 million in Oregon. Compared to optimal removals in the steady state, hybrid management decreases two-year net program benefits by 11.3% in Nevada and increases net program benefits by 31.4% in Oregon. The superior performance of the Oregon hybrid scenario is related to Oregon's higher adoption demand, which facilitates a lower steady-state holding inventory.

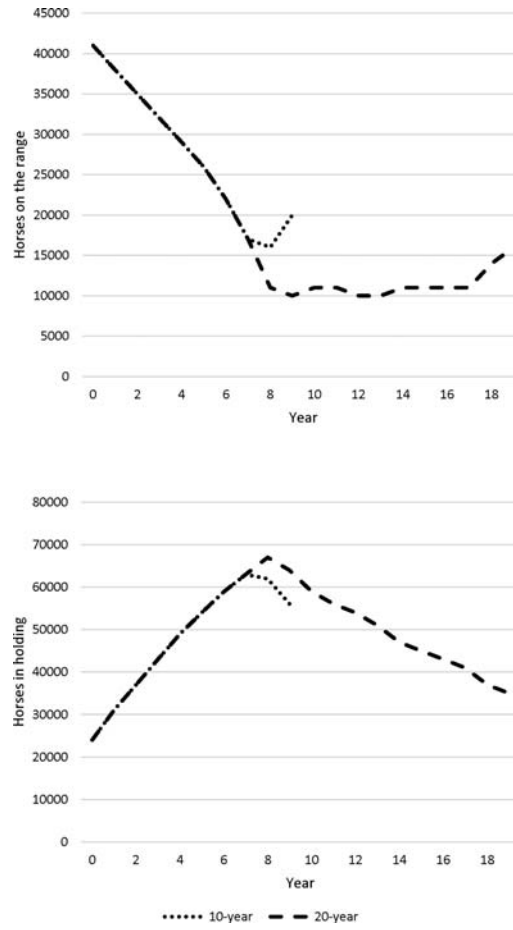
Time-Horizon Sensitivities

A long-term time horizon is appropriate for capturing the economic impact of wild horse management decisions because removals in the current period result in holding costs for the life span of removed horses. In practice, however, land managers may base their management decisions on shorter time horizons, due to budgetary constraints, management uncertainties, or other factors. Additionally, the models assume that specified parameters remain constant over time, and setting a shorter time horizon makes this assumption more likely to hold. The Nevada removals models were solved under 10-year and 20-year time horizons (i.e., $T = 10$ and $T = 20$) to explore how shorter time horizons influence optimal management. The optimal population and holding inventory paths from these simulations are shown in Figure 5. The paths suggest that even when considered under a shorter time horizon, it is optimal to reduce the population rapidly in the early periods to avoid incurring costs (i.e., negative net population benefits) associated with a larger population. The population uptick at the end of the shortened time horizons occurs to satisfy the terminal condition.

Removals under Cost Minimization

Allowing horse populations to become self-limited can reduce population growth rates, and potentially reduce the number of horses removed and sent to holding compared to current management. On the other hand, larger populations grow from a bigger base and thus produce more horses annually for a given growth rate. If managers determine

FIGURE 5
Optimal Paths for Removal Management in Nevada under Alternate Time Horizon



horse populations should not exceed some maximum level, the question arises as to whether it is cost-effective to maintain self-limited populations just below the threshold, or if it is less costly to maintain smaller, but faster growing populations. To explore this question, the next section examines optimal horse removals under a cost minimization objective, where the net population benefits term is dropped from the objective function. Dropping $B(X)$ from [1] and imposing a maximum population constraint frames management decisions in terms of cost-effectiveness, where management costs are minimized subject to maintaining the horse population under some ceiling.

The cost-effectiveness scenarios were run for optimal removals management in Nevada. If the maximum population constraint is set above 50,000, or roughly 80% of carrying capacity, then it is cost-effective to actively manage a large population at the constraint. If the maximum population is set below this level, then it is cost-effective to manage a population of 11,000 horses, the optimal steady-state solution to the model in equation [1].

VI. DISCUSSION

Dynamic, benefit maximizing models of wild horse management were developed for three nonlethal population control methods using published research on wild horse population biology and expenditure data from the BLM. The optimal model solutions were compared to each other and to a scenario that represents status quo management. Finally, optimal removal models were simulated with shorter time horizons, and with a cost-minimization objective to assess the cost-effectiveness of maintaining populations under a specified level.

The model simulation results provide evidence of how to undertake state-level horse management to maximize benefits for stakeholders. Importantly, the results indicate that societally efficient management programs involve an initial reduction of state horse populations from their 2015 levels. This result is robust to the time-horizon specifications, and to the cost minimization specifications where the maximum population is set less than 80% of carrying capacity. The upshot here is that even in the face of costly removals and holding, efficient management involves reducing populations from current levels and maintaining them within their AML ranges. Moreover, the removal and hybrid management simulations suggest that with current technology nonlethal wild horse management requires maintaining off-range populations comparable to, or greater than, the on-range populations.

A second takeaway from the results is that, as defined, fertility control alone is not an effective method for managing horse populations. The reason is that nearly all of the mares must be effectively treated to reduce horse populations using fertility control only. Further, the gather constraints imposed by the model make reducing or stabilizing horse populations with fertility impossible. If all mares in the population could somehow be effectively treated in each period, fertility control has the potential to slowly reduce the population at the mortality rate. However, it is unlikely all mares could be treated, and even if they could, fertility control would not produce a significant population reduction over the short term.

While fertility control alone is not an effective population management strategy, the hybrid management results indicate that fertility control can reduce the total number of horses removed from the range while maintaining comparable management costs and net program benefits. At the optimal steady state, for example, there are 6% fewer horses removed over each two-year hybrid management cycle compared to optimal removals in Nevada, and 25% fewer in Oregon.

When framed in terms of cost minimization, the models yield the cost-effective solution for keeping populations under some specified maximum level. For removals in Nevada, it is cost-effective to reduce populations and maintain them at the base model steady state unless the population constraint is set above 50,000, or roughly 80% of carrying capacity. Thus, maintaining small populations that are not resource limited is less costly than managing large, resource-constrained populations unless the large populations are near the carrying capacity. Further, managing medium-sized populations is costly because many horses need to be removed to stabilize fast-growing populations that are not resource limited.

As shown in Figure 4, cumulative NPV management costs are higher in the optimal scenarios than in status quo management until at least the twentieth management year across scenarios. Thus, realizing the gains associated with optimal management requires enduring higher initial management costs in return for greater population benefits and lower management costs in the future. Furthermore, while the optimal scenarios involve intensive management in the initial periods, the status quo scenario is the most intensive manage-

ment method across the 50-year management horizon. In the Nevada status quo scenario, over 280,000 horses are taken to long-term holding facilities across the management horizon, compared to around 175,000 with optimal removals and 170,000 with hybrid management.

Overall, the simulation results show that significant economic gains can be realized with a more strategic approach to wild horse population management. Relative to the status quo scenario, the results indicate that implementing optimal removals or hybrid management would result in 50-year NPV program savings of $1.3 billion in Nevada. Undertaking optimal hybrid management would save $300 million compared to the status quo in Oregon. Realizing these savings, however, is a matter of incurring short-term costs to realize future benefits.

Separate models were developed for Nevada and Oregon, and comparison of the results across states yields additional management insights. Oregon contains almost 3 million HMA acres compared to 14 million in Nevada. Likewise, the Oregon models specify a smaller carrying capacity, AML, and initial population to carrying capacity ratio compared to the Nevada models. Adoption demand is greater in Oregon than in Nevada despite Oregon having a much smaller horse population. This allows Oregon to send a smaller proportion of removed horses to long-term holding. These state-specific management differences reflect the difficulties inherent in managing the large Nevada population of horses. Though the larger Nevada population yields nearly five times the population benefits at the optimal steady state, Oregon yields greater 50-year program benefits due to the high costs of managing the Nevada population.

Implementing the optimal management programs identified in this study would require allocating additional resources to the WHBP to fund intensive removals in the early periods and associated increases in the horse-holding inventory. The BLM currently has capacity for around 32,000 horses in long-term facilities. Implementing the optimal management programs would require temporarily increasing that capacity to around 70,000 to handle the Nevada population alone (Nevada's horse population represents roughly half of the overall wild horse population in the west.) Securing the resources to implement these programs would surely be challenging. However, the model results suggest that the investment would yield benefits that outweigh the costs.

While the models developed in this article represent a step toward understanding the economic consequences of alternative wild horse management regimes, they also have some notable limitations. First, the models do not capture potentially significant heterogeneity in biological and economic parameters across the state. More granular models may increase overall precision and provide guidance regarding how to best allocate management effort among state HMAs. Second, the models implicitly assume that specified economic and biological relationships remain constant across time. Aside from uncertain shifts in preferences or management costs, horse habitats are likely to change in the future due to climate change. In the Great Basin, where most of Oregon's and Nevada's wild horses reside, climate change is expected to increase overall precipitation, but also increase interannual precipitation variability. In summer, stream flows are expected to decline (Chambers 2008), which could have limiting impacts for wild horse populations. Beschta et al. (2013) suggest that reducing wild horse grazing is a prudent policy for mitigating the expected effects of climate change on western public range lands. Future research efforts could incorporate emerging climate science and related ecosystem impacts into horse management models. Despite its limitations, this article presents significant evidence that current wild horse management is inefficient, and that significant efficiency gains are possible if management actions are undertaken strategically.

APPENDIX: HYBRID POPULATION CONTROL

$$\max_{\{Y_t\}} \sum_{t=z+1,z+3,z+5,\ldots}^{\infty} \rho^t \big[B(X_t)+\rho B(X_{t+1}) - C^{g}(Y_t+q) - C^{fc}(Y_t,HMA) - C^{I}(I_{t-1},X_t,q) - C^{TR}(X_t,q) - C^{MISC}(X_t,q) - 2CADPT\gamma - SC\big], \quad [A1]$$

subject to

$$X_{t+2} - X_t = F_{t+1}(X_t, Y_t) + F_{t+2}(X_{t+1}, Y_t) - q\left[1 + \frac{X_{t+1}}{F_{t+2}(X_{t+1}, Y_t)}\right],$$

$$I_{t+1} - I_{t-1} = (1 - m_I)\left\{I_{t-1}(1 - m_I) + q\left[1 + \frac{\tilde{X}_{t-1}}{F(\tilde{X}_{t-1})}\right] - 2\gamma\right\} - I_{t-1},$$

$$Y_t \leq \min\{Y^{\max}, \delta\tilde{X}_t\},$$

$X_z; I_z$ = states when fertility control begins,

$$\tilde{X}_t^{\min} \geq AML \forall t,$$

where

$$\tilde{X}_{t-1} = \frac{X_t}{(1 + r_0)} + q,$$

$$\tilde{X}_t^{\min} = \frac{X_t}{(1 + r_0)},$$

$$X_{z+1} = X_z + F(X_z) + - q\left[1 + \frac{X_z}{F(X_z)}\right],$$

z = initial reduction periods.

Acknowledgments

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Effects of Ovariectomy and Season on Plasma Luteinizing Hormone in Mares

M. C. GARCIA, O. J. GINTHER

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Six pony mares were ovariectomized (OVX) on day 16 of diestrus during June and July, 1972, to study short term changes in plasma luteinizing hormone (LH) concentrations. Plasma LH was higher ($P < .05$) 3 days after OVX (1.76 ng/ml) than the day after OVX (1.01 ng/ml), and a gradual increase occurred over the first 2 weeks. Elevated plasma LH concentrations similar to mid-estrus levels were present from the 2nd to 11th week post-OVX.

In another experiment, the same 6 OVX mares were bled once a month from February, 1973, to January, 1974, to study long-term changes in plasma LH in relation to the anestrous (November-April) and breeding seasons (May-October). Mean monthly plasma LH concentrations in OVX mares during all months of the anestrous season were lower ($P < .05$) than mean monthly plasma LH during the months of June-September of the breeding season. During the transition from anestrous to breeding seasons (April, May) and breeding to anestrous seasons (October, November), mean plasma LH concentrations were not significantly different between April and May (1.39 us 1.84 ng/ml) and between October and November (3.02 vs 2.56 ng/ml), but April and May levels were lower ($P < .05$) than October and November levels. Mean monthly plasma LH levels in OVX mares during the anestrous season were similar to those in intact mares during the anestrous season and at mid-diestrus during the breeding season. Mean monthly plasma LH levels in OVX mares during the breeding season were similar to plasma LH levels at mid-estrus in intact mares.

These data indicate the presence of inhibitory (most likely progesterone) and stimulatory (most likely estrogen) influences from the ovary which modulate the endogenous LH pattern in mares during the breeding season. (*Endocrinology***98**: 958, 1976)

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CONSERVATION

A Critical Crossroad for BLM's Wild Horse Program

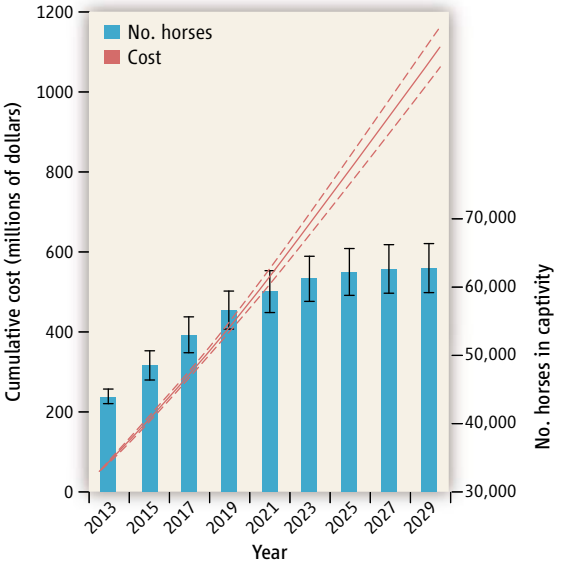
Captive wild horses will cost the United States over $1 billion by 2030 unless management approaches change

Robert A. Garrott,[1]* and Madan K. Oli[2]

Although horses evolved in North America, they went extinct 10 to 12 thousand years before the present. Spanish conquistadors returned domestic horses to the continent in the mid-1500s, and since then, domestic horses have escaped captivity and also were purposefully released onto western rangelands where they thrive today. Most free-roaming horse populations are managed primarily by the Bureau of Land Management (BLM) of the U.S. Department of the Interior (*1*).

In 1971, the Wild Free-Roaming Horses and Burros Act (*2*) was passed, which establishes public ownership of free-roaming horses and instructs federal agencies to "protect and manage" horses as "an integral part of the natural system of the public lands." The act mandates that BLM and other federal agencies monitor horse numbers, determine appropriate population levels, and remove excess horses from public lands "to preserve and maintain a thriving natural ecological balance." However, at present, tens of thousands of horses are not living the life of "wild mustangs" as Congress intended in passing the Wild and Free-Roaming Horses and Burros Act; instead, they have reverted back to the status of captive, domestic livestock, and the cost of the captive horse program is increasingly unsustainable.

The BLM currently reports ~33,000 free-roaming horses in the western United States. The WHB Program has 179 designated Herd Management Areas, with population objectives for each, and an aggregate maximum goal of 23,622 horses (*3*). Since its inception, the WHB Program has been embroiled in controversy over appropriate population goals, management activities, and the accuracy of its estimates of population size and growth rates (*4*–*7*). The BLM monitors numbers using aerial surveys and captures and removes horses from rangelands when horse numbers exceed target population size. As of 2012, BLM has removed >195,000 horses from public lands under this program (*3*). The act requires BLM to place removed horses into "private maintenance and care for which … an adoption demand exists," with unadoptable, old, and excess animals, beyond the adoption demand, either sold or "destroyed in the most humane manner possible" (*2*). The number of horses BLM removes from public lands routinely exceeds adoption demand (*8*). However, because of pressure from horse advocates, administrative directives and congressional appropriation bills prohibit killing healthy horses (*9*); therefore, BLM is left with large numbers of captive horses that must be maintained indefinitely.



Projections of U.S. captive wild horses and costs (with 95% confidence intervals). (see SM for more details).

These policies have led to a complex and costly system for maintaining captive horses. Horses removed from public lands are processed in short-term holding facilities similar to cattle feed lots and held for several months or more. Many mature females are pregnant when captured and foal in the facilities, which adds to the captive population. Most adult males are castrated. Horses can be adopted from the short-term holding facilities, and some are removed through deaths, sales, or release back onto public lands. Excess animals are eventually transferred to private pastures, primarily in the Midwest, that are contracted as long-term holding facilities. Although small numbers are sold, horses typically remain in these facilities until they die of natural causes. Currently, the ~45,000 horses in captivity exceeded the estimated 33,000 free-ranging horses on public rangelands (*3*).

In a recently released National Research Council (NRC) report (*7*), a committee of 14 scientists concluded that, left unmanaged, horse numbers on public lands would triple every 6 to 8 years until food and water became limited. Under these conditions, horses would be in poor health, reproduction would be suppressed, and deaths from starvation and dehydration would become common, with mass mortality events possible during periods of drought. High horse populations and limited resources would also degrade rangelands, which would affect all native species and public uses of these lands. Thus, although the NRC committee concluded that BLM must actively manage wild horse populations, it asserts that continuing "business as usual" will impede effective management of wild horses on public lands and recommends management changes to put the WHB Program on a more sustainable course (*7*).

Although the NRC report focused on scientific issues, a major impetus for changing the WHB Program is the escalating costs of maintaining increasing numbers of horses in captivity. The WHB Program budget grew from $19.8 million in 2000 (*10*) to $74.9 million in 2012 (*3*). More than 60% of the 2012 budget was used to maintain captive horses, 11% expended to capture and remove animals from public lands, and 10% for adoption programs (*3*). To inform the policy debate, we extend the implications of the NRC report to provide a projection of the captive horse population and associated costs if current policies and management approaches continue.

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The Consequences of Business as Usual

We used records of the sex and age of 165,459 horses removed from public lands and placed in captive facilities between 1990 and 2011 to develop demographic projections and associated costs (see the chart) (Fig. 1) [see supplementary materials (SM) for details]. We made two basic assumptions. First, we projected the life span of 33,946 unadoptable horses currently in long-term holding facilities. Under this scenario, BLM will accrue 366,846 horse-years of maintenance costs over a 30-year period before all animals currently in captivity die. Given that $45.9 million was budgeted to maintain 42,835 horses in captivity during 2011 (*10*), annual per-horse maintenance costs would be ~$1074. Thus, the 30-year cost of maintaining horses currently in long-term holding facilities would be ~$394 million ($449 million with 2% annual inflation).

Second, we assumed the current management program would continue, with a mean of 9412 horses in short-term holding facilities and 3803 horses transferred annually to long-term holding facilities. Expenses would be ~$1.1 billion between 2013 and 2030, and annual costs thereafter would be $67 million. Although some citizens may support such expenditures, these projections reinforce assertions (*7*, *8*) that BLM's current approach to managing horse numbers is not sustainable.

A Way Forward

Over 30 years of research on contraceptive agents for feral horses have resulted in the development of effective vaccines that prevent pregnancy in both captive and free-ranging mares for 1 to 3 years (*7*). Experimental application of these vaccines has contributed to the control of a small horse population on an Atlantic Coast barrier island, and limited experiments on western horse herds have also produced encouraging results. The Environmental Protection Agency recently sanctioned two contraceptive vaccines, which enables their routine use for horse population management on western rangelands (*7*). The average life-expectancy of horses entering long-term holding facilities is about 15 years (see SM). Therefore, every horse that does not enter long-term holding facilities would save, because of contraceptive application, $16,110 in maintenance cost (or savings of $1 million for every 62 horses), not considering the modest cost of contraceptive application.

Uncertainties exist when attempting to deliver vaccines to large numbers of horses. Although concerns have been raised regarding behavioral, physiological, and demographic consequences of contraceptive vaccines, none of these concerns preclude their use for managing rapidly increasing populations (*7*). The level of reduction in population growth that can be realistically attained for western horse herds through application of contraceptive vaccines remains unclear. Large-scale management experiments, perhaps within an adaptive management framework, would help address some of this uncertainty (*11*).

We estimate that the typical 15 to 20% annual population increase of western horse herds (*7*) could be halved by means of contraceptive vaccines. If a gather-and-removal effort reduced the western horse population to the BLM's goal of 23,622 horses and aggressive contraception treatment was initiated, then BLM need only remove 2000 to 3000 horses annually to maintain its goal. The number of horses annually removed would then more closely match adoption demand and that would minimize or eliminate the need for long-term maintenance of unadopted animals.

Although broad-scale use of contraceptives can help reduce the number of horses that BLM must remove to control populations, it is not a panacea. Current contraceptive vaccines are most effective when hand-injected, and remote delivery of vaccines via dart is impractical for most free-ranging horse populations (*7*). Accordingly, vaccine delivery will require continuing, and perhaps increasing, the frequency of horse captures. It may also take a decade of experiment and refinement of treatment regimens and contraceptive methods to scale-up management to include the entire population of horses on western rangelands. Furthermore, most populations already exceed management objectives, and population modeling efforts indicate that current contraceptives can only reduce, not stabilize or reverse, population growth. Thus, even with the changes suggested by the NRC report, BLM must continue removing horses from public lands for the foreseeable future.

It is up to society to decide how they want their government to manage wild horses, how many wild horses should be accommodated on public lands, what should be done with excess horses, and how much they are willing to spend. The debate has gone on for many decades; however, we are at a critical juncture. BLM recently announced that because of funding constraints and lack of additional capacity for maintaining captive animals, it will substantially reduce scheduled removals of horses from public lands this year. This decision has been made despite worsening drought conditions that are causing severe shortages of forage and water for horses, wildlife, and livestock (*12*). Terminating or reducing active management will lead to a rapid increase in the number of horses, further exacerbating resource limitations.

The current situation in Australia provides a sobering view of what might be in store for western rangelands in the United States if the current management dilemma is not resolved. The wild horse population in Australia is estimated to exceed 400,000 animals (*13*), and severe drought conditions in central Australia have reportedly forced government agencies to propose shooting 10,000 horses from helicopters to reduce animal suffering and environmental degradation (*14*). One can only hope that there is the political will to change current policy and place BLM's WHB Program on a more sustainable trajectory.

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Supplementary Materials

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LIMITATIONS OF MALE-ORIENTED CONTRACEPTION FOR CONTROLLING FERAL HORSE POPULATIONS

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Abstract: We developed a computer program to simulate key features of feral horse (*Equus caballus*) population dynamics and breeding cycles because of the continuing controversy over male- versus female-oriented contraceptive approaches to population management. We used the program to investigate the potential efficacy of male-oriented contraception as a population management tool, and to explore the impacts of such programs on the normal seasonal foaling pattern. The treatment strategies we tested included sterilizing only dominant harem stallions and sterilizing males without regard to their social status. Sterilizing only dominant harem stallions resulted in relatively modest reductions in population growth. Substantial reproduction may occur even when 100% of the dominant harem stallions are sterilized if other males perform as little as 10% of the breeding. The long breeding season allows mares to cycle 6–10 times if not successfully bred and provides many opportunities for mares to breed with males outside the harem. Adequate suppression of population growth may be attained only if a large proportion of all males in the population, regardless of their social status, could be sterilized. Both treatment strategies resulted in the disruption of the normal seasonal foaling pattern. The foaling season became less synchronized and more skewed toward summer and autumn as the proportion of sterile males in the population increased.

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During the last 15 years there has been considerable interest in controlling feral horse populations in the western United States by inhibiting reproduction with contraceptive devices. From the outset of contraceptive research on feral horses, there has been controversy over whether male- or female-oriented contraceptive approaches were most practical for population management (Natl. Res. Counc. 1982, Turner and Kirkpatrick 1986*a*). Two studies in which dominant harem stallions were sterilized (Turner and Kirkpatrick 1982, 1986*a*; T. C. Eagle et al. unpubl. data) suggested a decrease in foal production, indicating that male-oriented contraception may have promise as a management tool.

We became concerned that applying male-oriented contraception may cause some undesirable changes in the seasonal reproductive patterns of feral horses. Horses have a 340–350 day gestation period and undergo a post-foaling heat approximately 5–15 days after parturition (Ginther 1979). These characteristics essentially lock mares into a relatively fixed yearly reproductive cycle, dictating that if a mare conceives during the post-foal heat she will produce consecutive foals at essentially the same time each year. Horses are polyestrous (Ginther 1979); therefore, if a portion of the males in a population are rendered infertile by some contraceptive technique, the mares bred by these males will cycle and become receptive again. If in-

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fertile males continue to breed the mare, she will continue cycling until late summer or autumn when ovarian activity ceases (Turner and Kirkpatrick 1986*b*, Plotka et al. 1989). We hypothesized that if mares cycle throughout the breeding season, there are enough opportunities to breed and conceive even if the probability of mating with a fertile male is small. Each time a mare cycles, she drifts further from the spring breeding season, and the foal will be born later in the season if she is successfully bred. Once out of the normal seasonal breeding and foaling cycle, the yearly cycle of gestation and post-foal heats could make it difficult for the mare to regain seasonal synchrony.

We constructed a computer program that incorporated key features of feral horse population dynamics and breeding cycles. The program then was used to perform simulations to investigate the potential efficacy of male-oriented contraception as a population management tool, and to explore the impacts of such programs on the normal seasonal foaling pattern.

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METHODS

Defining the Foaling Season

We obtained information on the normal foaling season of feral horses from records of horses removed from BLM lands in Nevada between 1979 and 1987. Of the 33,521 horses captured during this time, 5,993 animals were judged to be foals (Garrott 1991*a*). The age of 4,678 of the foals was estimated to the nearest month based on the eruption patterns of incisors (Am. Assoc. Equine Practitioners 1981). The birth month of these foals was estimated by back dating to develop a histogram of the seasonal foaling pattern. Most foals are born between April and July; however, foaling may occur throughout the year (Fig. 1). Because the criteria used to estimate the ages of foals was not precise and aging errors undoubtedly occurred, the histogram derived from these data must be considered an approximation of the true foaling distribution. However, because our derived foaling distribution (Fig. 1) approximates observations from field studies (Feist and McCullough 1975, Boyd 1979, Keiper 1979, Berger 1986, Kirkpatrick and Turner 1986), we assume it adequately represents the seasonal foaling pattern. Because mares enter estrus soon after foaling (Ginther 1979), we also assume these data represent the normal seasonal breeding pattern. We fit a cosine curve to the foaling data (Fig. 1), emulating the asymmetry in order to create a frequency distribution that could be used in the model to randomly assign each mare a foaling date. The limited reproduction occurring between November and February (3%) was ignored.

Development of the Simulation Program

The simulation model was comprised of 2 principal components, a population model and an annual breeding cycle model for individual mares. The population model, constructed only for females, utilized age-specific fecundity and survival schedules to simulate population growth. Mares were assigned to 1 of 26 age classes ranging from zero (foal) to 25 years old. The number of females in each age class and age-specific fecundity and survival schedules were user-defined. A series of feral horse survivorship and fecundity schedules were formulated by Garrott et al. (1991*b*) using models presented by Eberhardt (1985). We used the F2 fecundity schedule, which was derived from reproductive data compiled for 1,144 mares captured immediately after the foaling season in Nevada (Garrott et al. 1991*a*). The S1 survivorship schedule (Garrott et al. 1991*b*) was used because it represents the only age-specific survival data available for a feral horse population, and was developed from 11 years of data collected on animals in the Pryor Mountain herd in Montana (Garrott and Taylor 1990). The survivorship schedule was converted according to Caughley (1977) to obtain the annual age-specific survival rates required for the population model.

We determined the stable age distribution for these 2 schedules by projecting an arbitrary initial population forward 10 years using a Leslie matrix type model (Leslie 1945). The stable age distribution was used to calculate the number of mares in each age class for an initial population of 1,000 animals. No density-dependent

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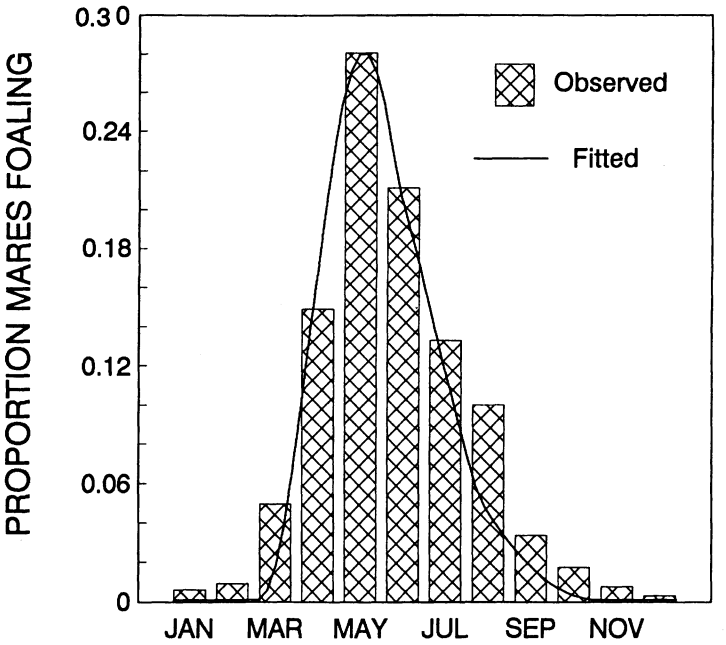

Fig. 1. Annual feral horse foaling pattern and frequency distribution fitted to data obtained from 4,678 foals, in Nevada 1979–87. Age of foals estimated to the nearest month using incisor eruption patterns; date of birth determined by back dating.

mechanisms were incorporated into the model, creating a population growing geometrically at a rate of approximately 21% annually. Many feral horse herds throughout the western United States have grown at annual rates of 15–27% (Garrott et al. 1991*b*); thus, the demographic characteristics in this model reflect typical feral horse populations.

The social structure of feral horse populations has an important influence on breeding and, hence, the efficacy of any contraception program. The primary social unit is the harem group, which is composed of mares and their young from the previous 1–2 reproductive seasons, a dominant stallion, and an occasional subdominant male. All females are normally associated with a harem, with bachelor males forming loose aggregations (Feist and McCullough 1975, Miller 1979, Berger 1986). Although harem stallions attempt to maintain exclusive access to mares for breeding, some mares are bred by other males (Nelson 1978, Miller 1979, Bowling and Touchberry 1990). This social structure suggests 2 treatment strategies that could be used in a male-oriented contraception program: sterilization of only dominant harem stallions (Turner and Kirkpatrick 1982, 1986*a*; T. C. Eagle et al. unpubl. data) or sterilization of a proportion of all males regardless of their social rank. The degree of population control that can be attained by sterilizing males will be dependent upon which of these 2 strategies is employed, the proportion of males treated, the probability a mare will breed with her harem stallion, and the probability of mating with a treated male if the mare breeds outside the harem.

We constructed the model to allow the user to define whether sterilization would be applied to only harem stallions or to all males, the proportion of males that were treated, and the probability that a mare will breed with her harem stallion. We assumed that treatments were applied randomly and that all males in the population had equal access to mares breeding outside of the harem structure. Therefore, in simulations where a proportion of all males were sterilized, the probability of mating with a sterile male, if a mare bred outside the harem, was equal to the proportion of males sterilized. For simulations where only dominant harem stallions were sterilized, this probability was not only dependent on the proportion of harem stallions sterilized, but also on the proportion of males in the population that were dominant stallions. We assumed a population sex ratio of 82 males/100 females (Garrott 1991*c*) and that an average harem contained 4 mares (Berger 1986).

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A population of 1,000 mares, therefore, would contain 820 males, 250 of which were dominant stallions. Hence, 0.30 of the males (250/820) in the population would be dominant stallions, and the probability of mares mating with a sterilized male outside the harem can be calculated as 0.30 times the proportion of dominant stallions treated.

The breeding cycle component of the program traced individual mares through the annual reproductive cycle. When the initial population was read into the program, each mare was stochastically assigned a foaling date using the frequency distribution fitted to the Nevada foaling data (Fig. 1). At the start of the annual loop, the program stochastically determined if each mare survived to the beginning of the breeding season. If a mare survived, her age was incremented by 1 year, and a second random number was drawn to determine if she would attempt to breed. The survival and breeding determinations were made based on the mare's age and the age-specific survival and fecundity schedules. For mares attempting to breed, we used the user-defined probabilities previously discussed to determine stochastically if she bred with the dominant harem stallion or outside the harem, and whether her mate was sterile or fertile. If she mated with a sterile male, her foaling date was incremented by 21 days, the average estrous cycle (Ginther 1979), and additional random numbers were drawn to determine if her second mating was within or outside the harem and if her mate was sterile or fertile. This sequence continued until the mare was bred by a fertile male or cycled beyond October, at which time she was assumed to cease cycling and remained barren. If the mare was successfully bred, she was assumed to foal 1 year later. The sex of each foal produced was assigned stochastically, and all female foals were added to the mare population. A new foaling date was drawn from the foaling distribution for all mares that remained barren for a year. This feature of the program allowed any mare that had her annual reproductive cycle altered due to repeated mating with infertile males to reestablish a normal seasonal reproductive pattern.

We conducted each simulation for 10 years and performed 50 repetitions for each treatment. Demographic output included the number of mares in the population at the beginning of the year, the number of mares that died, the number of foals born, and the number of mares in the population at the end of the year. The growth rate of the population was determined using log-linear regression techniques (Eberhardt 1987). Reproductive output included the number of foals born in each month, the number of estrous cycles required for each mare to conceive, and the number of mares that failed to conceive because all matings were with infertile males.

We simulated management programs where only dominant harem stallions were sterilized and programs where males were sterilized regardless of their social rank. For each of these treatment strategies, 3 probabilities of mating with the harem stallion were simulated: 0.70, 0.80, and 0.90; and for each of these probabilities, 12 levels of sterilization were simulated: 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 0.95, and 1.00. Since the model was stochastic, each simulation resulted in unique results; therefore, the results are presented as means of 50 simulations for each of the 72 combinations of treatment strategy, probability of mating with the harem stallion, and the proportion of males sterilized.

RESULTS

Effects of Sterile Males on Population Growth

Results of the simulations indicate that the effectiveness of male-oriented contraception techniques to suppress population growth increased with increasingly severe treatment levels and as the probability of mating with the harem stallion increased (Fig. 2). These trends were evident whether sterilizations were limited to only harem stallions or were applied to a sample of all males regardless of their social rank. The general effectiveness of the 2 treatment strategies, however, was dissimilar. Sterilizing only dominant harem stallions resulted in relatively modest reductions in population growth, while sterilizing a sample of all males in the population severely depressed population growth at the higher treatment levels.

Effects of Sterile Males on the Foaling Season

Our simulations indicated that the normal seasonal foaling pattern of feral horses would be disrupted by introducing substantial numbers of infertile males into the population. As the probability of mating with an infertile male increased, the foaling distribution became more

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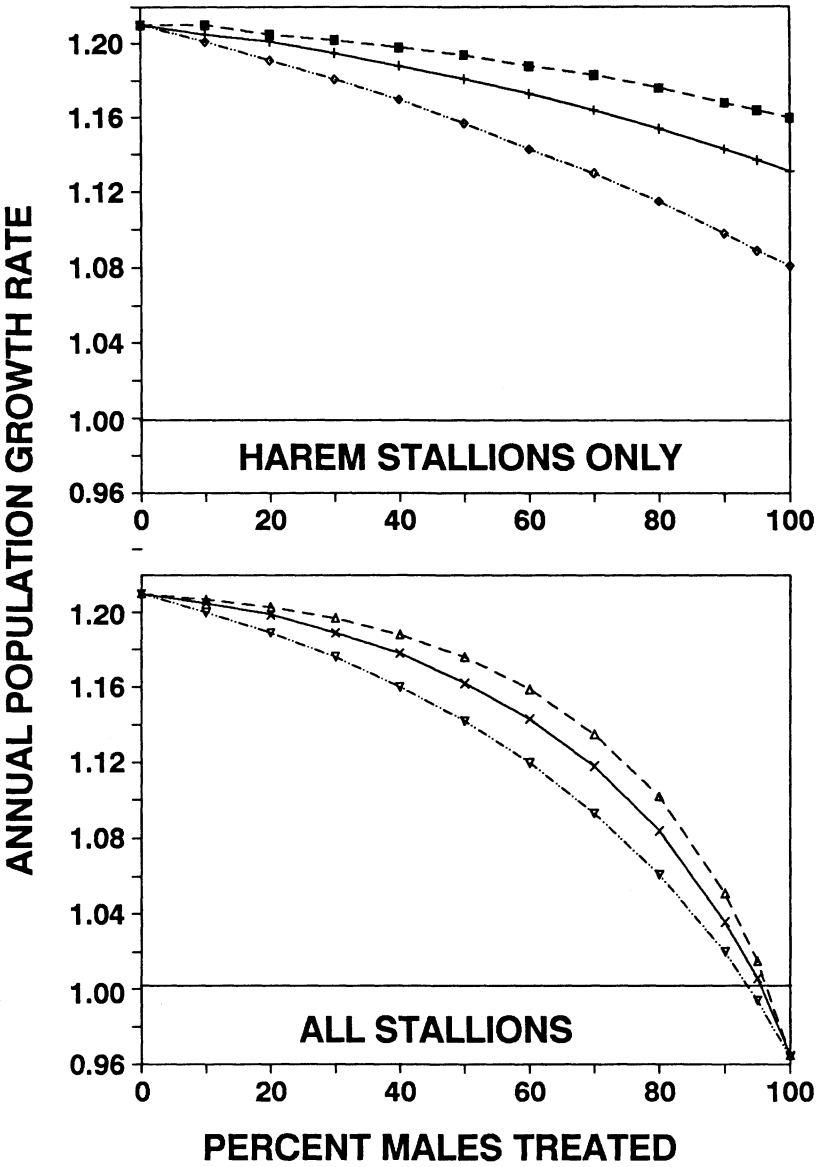

Fig. 2. Comparison of changes in the annual growth rate of a simulated feral horse population subjected to sterilization of dominant harem stallions, and sterilization of males without regard to their social status. Simulations performed assumed the probability of a mare mating with her harem stallion was 0.70 (dashed line), 0.80 (solid line), and 0.90 (dash-dotted line).

flattened and shifted increasingly toward late summer and autumn months (Fig. 3). This disruption was not gradual and occurred during the first foaling season after sterilized males participated in the breeding season. Once the shift in the foaling distribution occurred, it remained relatively unchanged throughout the 10-year simulation (Fig. 3).

Although the shift in foaling seasons was evident for both treatment strategies (sterilizing only dominant harem stallions and sterilizing males without regard to their social status), the magnitude of the shifts differed. In untreated populations, the seasonal foaling distribution used in the model dictated that approximately 8% of the foals were born late in the reproductive season, between August and October. Sterilizing males resulted in a nearly linear increase in the proportion of late-born foals in the simulations where only dominant harem stallions

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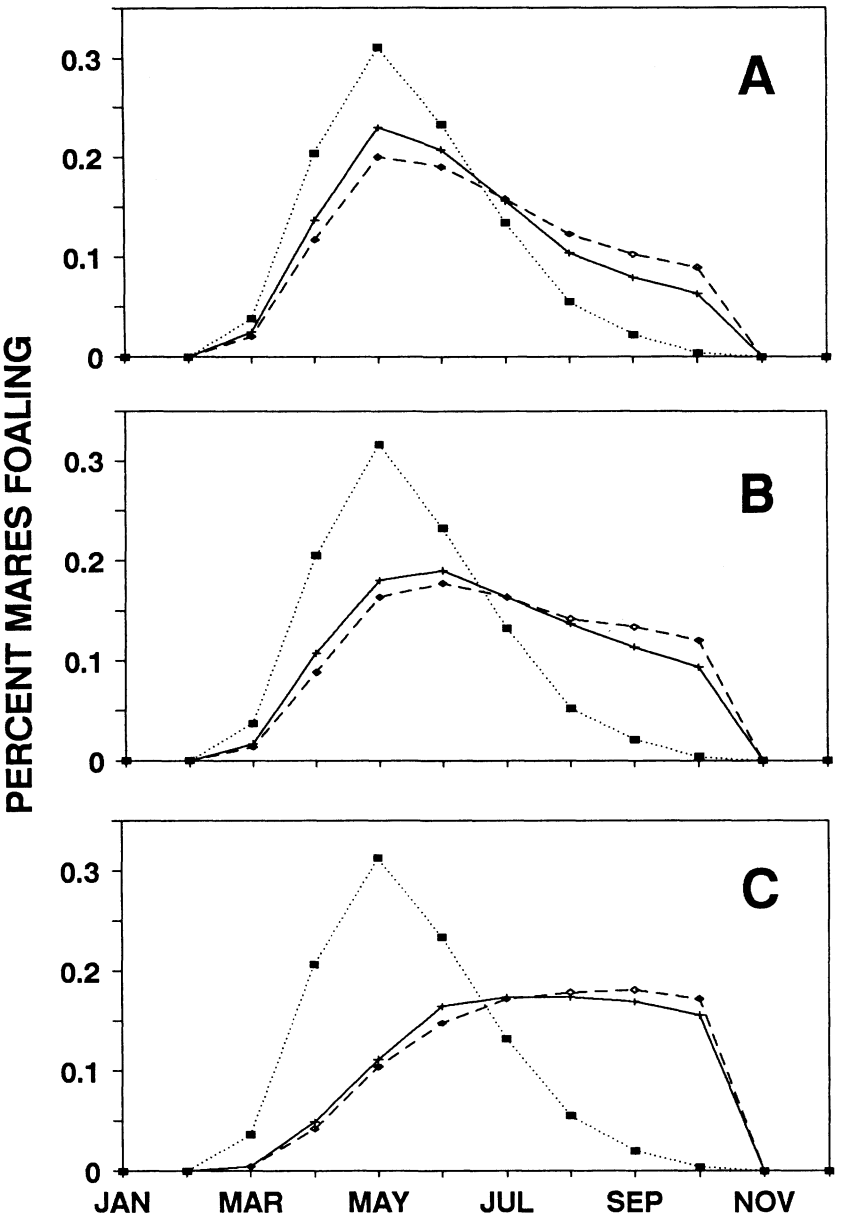

Fig. 3. Temporal changes in the seasonal foaling pattern of a simulated feral horse population when (A) 60%, (B) 80%, and (C) 100% of the dominant harem stallions were sterilized. Dotted lines represent normal foaling distribution at year 0, solid lines are distributions at year 2, and dashed lines are distributions at year 10. Simulations assumed that mares had a 90% probability of mating with their harem stallion during each period of receptivity.

were sterilized. When ≥80% of the stallions were sterilized, 40–50% of the foals were born during the August–October period (Fig. 4). The magnitude of the shift in the foaling season was not as pronounced for simulations where a sample of all males was sterilized. Under this treatment regime, the relationship between the proportion of males sterilized and the proportion of late-born foals was curvilinear with the most severe treatments resulting in 20–40% of the foals being born during the August–October period (Fig. 4).

DISCUSSION

The effectiveness of any male-oriented contraceptive program depends on each female

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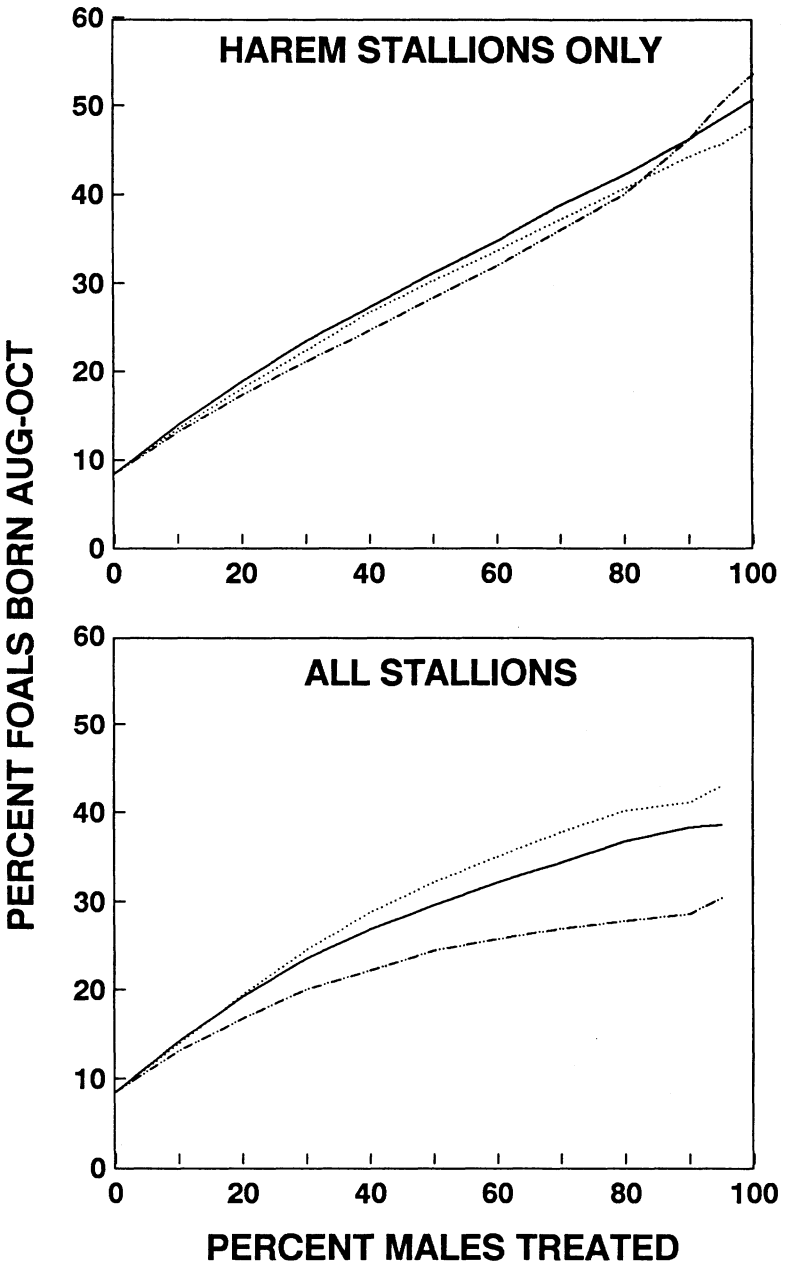

Fig. 4. Comparison of changes in the proportion of late-born (Aug–Oct) foals in a simulated feral horse population subjected to sterilization of dominant harem stallions, and sterilization of males without regard to social status. Simulations were performed assuming the probability of a mare mating with her harem stallion was 0.70 (dotted line), 0.80 (solid line), and 0.90 (dash-dotted line).

mating with an infertile male during all periods of receptivity throughout the March–October interval of cycling. The efficacy of sterilizing only dominant harem stallions, therefore, depends heavily on the ability of the stallions to maintain exclusive breeding access to their mares. Berger (1986) found that dominant harem stallions in the Granite Mountain herd in western Nevada performed nearly all copulations with their mares; hence, in some populations, such regimented breeding may exist. However, several other studies have provided

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evidence that not all mares in a harem are bred exclusively by the dominant stallion (Nelson 1978, Miller 1979, Bowling and Touchberry 1990).

Our simulation results indicate that significant reproduction may occur even when 100% of the dominant harem stallions are sterilized, if other males perform as little as 10% of the breeding. The long breeding season allows mares to cycle 6–10 times if not successfully bred and provides many opportunities for them to breed with males outside the harem. Our results indicate that a male-oriented contraceptive program will effectively suppress population growth only when a large proportion of all males are sterilized. A potential consequence of introducing substantial numbers of infertile males into the population, however, is disrupted normal seasonal foaling. This shift in the foaling season is caused by the repeated cycling of mares before mating with a fertile male. As the probability of mating with an infertile male increased, fewer mares conceived during their first estrous cycle and increasingly greater numbers of mares did not mate with a fertile male until cycling several times. Because each estrous cycle required 21 days to complete, every time a mare came into estrus and failed to mate with a fertile male, her potential conception date was delayed by 3 weeks, and her foal would be born 3 weeks later in the following summer. Such events were more common when only dominant harem stallions were sterilized because large numbers of fertile males were available for breeding (Fig. 4).

RESEARCH AND MANAGEMENT IMPLICATIONS

Male-oriented contraception is attractive because there is a perception that sterilizing dominant harem stallions, which represent a small proportion of any population, will result in inhibiting reproduction in a large proportion of the mares in the population. Such a concept seems to be more practical and economical than female-oriented contraception where a larger proportion of the population must be treated (Garrott 1991*b*). Some animal welfare groups also appear to favor male-oriented contraception because they believe the techniques may be less intrusive and require treating fewer animals, and therefore, more humane than female-oriented contraception. Although several studies have demonstrated that sterilizing dominant harem stallions can reduce foaling rates of individual harems (Turner and Kirkpatrick 1982, 1986*a*; T. C. Eagle et al. unpubl. data), results of our simulations suggest that such a treatment strategy would not be an effective population management tool. Adequate suppression of population growth may be attained only if a large proportion of all males in the population, regardless of their social status, could be sterilized.

The probable disruption of the seasonal foaling pattern is a potential obstacle to the application of male-oriented contraceptive techniques as a population management tool. Seasonal reproduction has evolved in most temperate animals presumably as a response to the predictable pattern of food abundance and scarcity caused by seasonal climatic changes. Shifting the foaling season toward the summer or autumn months would result in large numbers of relatively young foals entering the winter without adequate forage for themselves and the lactating dams, or adequate body reserves to endure long periods of nutritional stress. The consequences of such conditions would undoubtedly be higher mortality of foals during the winter. Although this would have the effect of reducing population growth, some would question the appropriateness of such a mechanism.

Our simulation program had many limitations and was extremely generalized. Its major weakness was the rigid and relatively simplistic way we modeled behavioral and social mechanisms. These mechanisms undoubtedly influence mating and reproduction in the real world and are much more complex and flexible than the mechanisms we built into the model. Despite these limitations, we think the general conclusions that can be drawn from this exercise are valid and, in many ways, intuitive when one considers some of the fundamental aspects of feral horse reproduction. Our results also may apply to other polyestrous large mammals that restrict their reproduction to specific seasons.

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Review of Castration Complications: Strategies for Treatment in the Field

Liberty M. Getman, DVM, Diplomate ACVS

Castration is a commonly performed procedure, but it has a relatively high complication rate. Complications encountered can include post-operative swelling and edema, infection, hemorrhage, eventration, peritonitis, penile damage, and hydrocele. Prompt recognition of a complication and the institution of appropriate therapy are essential for the treatment of these complications. This report reviews treatments that can be performed in the field and indicates the cases that should be referred for more aggressive treatment. Author's address: Department of Clinical Studies, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, Pennsylvania 19348; e-mail: libertyg@vet.upenn.edu. © 2009 AAEP.

1. Introduction

Castration is the most common surgical procedure performed by equine veterinarians in the field. Although the surgery is technically easy to perform, the complication rate associated with it is relatively high with 20–38% of horses experiencing a complication.[1,2] Additionally, equine castration complications are the most common cause of malpractice claims against veterinarians in North America.[3] Pre-operative identification of risk factors for the development of complications can ensure that proper measures are taken to reduce these risks. This can help eliminate some complications, but even with appropriate pre-operative evaluation and proper surgical technique, complications can still occur. Therefore, prompt recognition of complications and quick application of appropriate therapy are essential for the treatment of post-operative complications. This may include treatment that can be performed in the field or in some cases, treatment that is best performed at a referral center.

2. Post-Operative Swelling and Edema

Post-operative swelling and edema of the prepuce and scrotal area are the most common "complications" of equine castration, although some degree of swelling develops in almost every horse after castration. This generally peaks 3–4 days after surgery and resolves completely in 10–12 days. Swelling beyond this time or swelling associated with clinical signs such as a stiff gait or reluctance to move or urinate is abnormal (Fig. 1). This is often caused by failure to remove enough scrotal skin, failure to stretch the skin and SC tissue after castration, or inadequate exercise after castration, any of which allows the scrotal incision(s) to close prematurely and excess fluid to accumulate. Older horses are also reported to be more prone to developing excessive edema after castration than younger horses.[4]

Swelling beyond the initial post-operative period is also suggestive of an infectious process, which should be treated appropriately (see below) if suspected. Horses with swelling that is not suspected to be infectious in origin can be treated at the farm

NOTES

Fig. 1. Horse with excessive post-operative swelling and edema after castration.

by establishing drainage and administering non-steroidal anti-inflammatory drugs (NSAIDs). The horse is sedated, the area is aseptically prepared, and the incision is manually opened and stretched using sterile gloves. It is imperative that the owner follow this treatment up by instituting daily (or twice daily) exercise to prevent premature closure of the incision. If left untreated, excessive swelling can lead to the development of paraphimosis, surgical site infection, and dysuria.

3. Infection

Infection is the second most common complication after equine castration; 3–20% of horses are reported to experience this complication.[1,2] Clinical signs can occur anywhere from days to years after surgery, and they include fever, scrotal/preputial swelling, lameness, and incisional discharge. Use of ligatures around the spermatic cord has been associated with a higher risk of post-operative infection.[2,4] Different forms of post-castration infection exist. Champignon, or infection caused by *Streptococcus sp.*, is characterized by the presence of a purulent discharge from the spermatic cord stump surrounded by a mound of granulation tissue. This form was more common historically when non-sterile materials were used as ligatures. Scirrhous cord refers to chronic infection of the spermatic cord stump, typically with a *Staphylococcus sp.* In these cases, the scrotal incision heals, but the infected stump continues to enlarge and abscess; eventually, a draining tract develops. This may take months to years to become evident. The abscessed stump is palpable externally as a firm mound of tissue in the inguinal area (Fig. 2). In some cases, the abscesses can involve the entire spermatic cord stump through the inguinal ring and into the abdomen; in these horses, the abscessed stump can be palpated per rectum as a firm mass.

Any horse with a fever and scrotal/preputial swelling after castration should be treated as a post-castration infection until proven otherwise. Treatment in the field can be successful, especially if it is instituted early. This should consist of opening and stretching the incision using the aseptic technique as described above, lavage of the incision with sterile isotonic fluids (with or without dilute antiseptics), and administration of broad-spectrum antimicrobials. This treatment protocol has been found to be more effective than doing any of the above components alone.[1] The incision may need to be reopened periodically, and exercise should be instituted to prevent premature closure of the wound. NSAIDs should be used for analgesic and anti-inflammatory purposes. It is also advisable to obtain a deep sample from the incision for bacterial culture and sensitivity testing before the administration of antimicrobials to direct future therapy. This can be done after an aseptic preparation of the area around the incision by inserting a sterile cotton-tipped applicator deep into the incision while wearing sterile gloves and then placing it into culture media. Empirical broad-spectrum antimicrobial therapy can then be initiated pending these results. The most common isolate from post-cas-

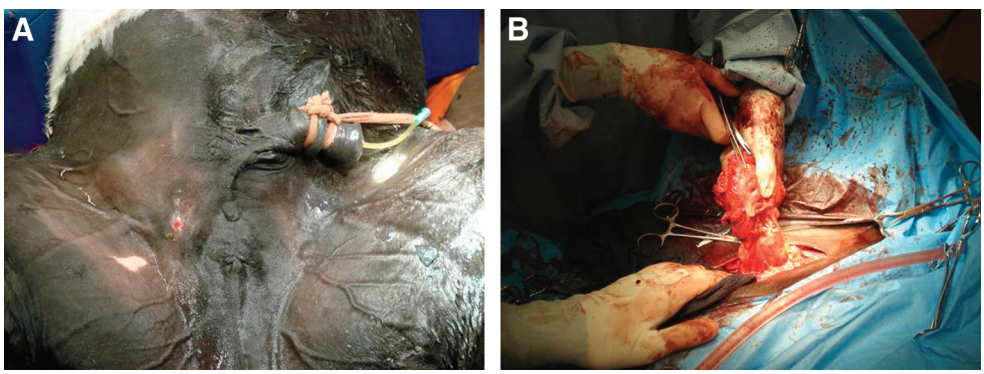

Fig. 2. (A) A pony with a chronic infection of the spermatic cord after castration ("scirrhous cord"). Note the swelling that is represented by a firm mass and the draining tract. (B) Infected spermatic cord stump resected at surgery.

tration infections is *Streptococcus zooepidemicus*, but other bacteria including *Staphylococcus sp.,* Enterobacteriaceae, and *Pseudomonas* are also isolated frequently. In our hospital, oral antimicrobials such as trimethoprim sulfamethoxazole, chloramphenicol, or doxycycline are often used initially with good results; however, if the horse is showing signs of systemic illness, it may be advisable to use broad-spectrum IV antimicrobials such as potassium penicillin or a cephalosporin in combination with an aminoglycoside for initial therapy.

Most infections will resolve with this therapy at the farm if treated early. For those that do not resolve or in cases where signs do not occur until months after the castration, then referral to a surgical center is warranted, because these cases will require surgical resection of the infected tissue for complete resolution. Other cases that warrant referral are those with systemic signs of sepsis or endotoxemia and those where infection with a *Clostridial sp.* is suspected. Although rare, tetanus and botulism can occur in unvaccinated horses, and wound infections with other *Clostridial sp*. can cause necrotizing cellulitis, myositis, and systemic endotoxemia. Infections with any *Clostridial sp.* can result in serious illness and death, and if suspected, these horses should be referred as soon as possible.

4. Hemorrhage

Some bleeding is usual for the first ~5 min after the emasculator is removed (or the horse stands up from anesthesia); however, hemorrhage in the form of a fast drip or a stream beyond 15 min is excessive and should be addressed.[4,5] The most common source of post-castration hemorrhage is the testicular artery; less commonly, the hemorrhage is from a scrotal vessel (branches of the external pudendal vessels) or a vessel within the cremaster muscle. Hemorrhage typically occurs because of improper application or function of the emasculators, including non-perpendicular placement of the emasculators on the spermatic cord, inclusion of scrotal skin within the emasculators, tension of the spermatic cord during emasculation, premature release of the emasculators, and use of emasculators that are too sharp.

Initial treatment can (and should) be performed in the field and is often successful in identifying and eliminating the source of hemorrhage. In the acute situation, the horse should be sedated if it is systemically stable, and the spermatic cord stump should be identified and inspected for the source of hemorrhage. This may require injection of local anesthetic into the stump. If the stump is found to be the source of the hemorrhage, then it should be emasculated again, and a transfixation ligature with large, absorbable suture material[a] should be placed proximal to the emasculator while it is in place for additional hemostasis after its removal. If the stump is retracted too far within the scrotal incision to be emasculated, then it can be grasped with crushing forceps, which are then left in place for 24–48 h. If the source of hemorrhage is determined to be a scrotal vessel, then the vessel should be clamped and ligated routinely. For hemorrhage originating from the cremaster muscle, emasculation and placement of a transfixation ligature should be performed. If the source of the hemorrhage cannot be identified, then the scrotal incision should be packed with sterile gauze packing and sutured closed. Adjunctive therapy with substances that decrease fibrinolysis, such as aminocaproic acid (100 mg/kg, IV), can also be used. Although packing the incision with gauze may provide adequate hemostasis in some cases, referral should be considered if the hemorrhage does not resolve quickly.

Referral should be considered in cases where the source of hemorrhage cannot be identified, substantial blood loss has already occurred before the examination, the horse is in hypovolemic shock, or intra-abdominal hemorrhage is suspected. Horses in hypovolemic shock will show clinical signs such as tachycardia, tachypnea, pale mucous membranes, dull mentation, weak pulses, cold extremities, and generalized weakness. It is important to remember that the PCV and total protein values in these horses are often normal initially, and decreases will not be evident for 6 h (total protein) or 12–24 h (PCV). If possible, these horses should be stabilized with IV fluid therapy before transport. Horses with intra-abdominal hemorrhage will show signs of hypovolemic shock, but they may also show signs of colic. The diagnosis can be quickly made by seeing swirling, hyperechoic abdominal fluid on ultrasonographic evaluation. These horses often require general anesthesia to identify and eliminate the source of hemorrhage. All horses with post-castration hemorrhage, regardless of if the source is quickly identified, are at an increased risk for the development of an infection at the surgical site because of the presence of the blood clot (and potentially suture material or gauze packing). Therefore, they should be treated with a course of broad-spectrum antimicrobial therapy.

5. Eventration

Eventration after castration is rare, occurring in only 0.2–2.6% of cases, but it is the complication that is most likely to be fatal. Therefore, referral is always recommended in cases where the intestine is involved.[2,4–7] Horses with omental eventration do not normally require referral. The omentum can be removed by ligation and transection or emasculation, and generally, there are no complications associated with its protrusion. However, it is advisable in these cases to perform a rectal exam to evaluate the size of the internal inguinal rings and ensure that there is no intestinal protrusion through the rings.

Intestinal eventration usually occurs within 4 h of castration, but there are reports of it occurring as

Fig. 3. (A) A yearling that developed small intestinal eventration 4 h after a routine closed castration. Because of the large amount of intestine eventrated, it was not possible to replace it into the scrotum. (B) Therefore, a hand towel was sutured to the skin to form a sling. (C) This was covered with a sheet for additional support during transport to the referral center.

late as 12 days after surgery.[4–7] Risk factors include breed (Standardbreds and Draft Horses are over-represented breeds, and anecdotally, Saddlebreds and Tennessee Walking Horses are also at increased risk), pre-existing inguinal hernias, presence of an inguinal hernia as a foal, and an internal inguinal ring that 2 or more fingers can fit into on rectal palpation. In these cases, it is recommended that a closed or modified open castration with a ligature placed around the cord be performed. A closed castration without a ligature will not decrease the risk of eventration, but the addition of a ligature has been shown to decrease the risk.

After intestinal eventration occurs, initial therapy is aimed at keeping the bowel safe from damage and further contamination and preparing the horse for transport to a referral center. The protruding portion of bowel should be cleaned of all gross contamination with sterile saline and then replaced into the scrotum, which is sutured closed or closed with several towel clamps. If this is not possible because of the amount of bowel that is prolapsed, then a moist towel or drape should be made into a sling and used to support the bowel during transport (Fig. 3). The horse should be given sedation (if systemically stable), broad-spectrum IV antimicrobials, and flunixin meglumine for analgesic and anti-endotoxic therapy. Survival rates after surgical treatment of intestinal eventration range from 36% to 87%, and the lowest survival rates are associated with an inguinal-only surgical approach, increased length of prolapsed bowel, and the need to perform a resection and anastomosis.[6,7]

6. Peritonitis

Septic peritonitis rarely occurs after castration; however, the vaginal and peritoneal cavities do communicate, and therefore, the potential for infection to spread from the spermatic cord to the abdomen does exist. Clinical signs of septic peritonitis include fever, depression, tachycardia, dehydration, colic, diarrhea, and anorexia. Abdominocentesis should be performed in horses displaying these signs, and the fluid should be submitted for cytological evaluation, bacterial culture, and sensitivity testing. However, it is important to note that nonseptic peritonitis occurs frequently in horses after castration and is not clinically significant, but it will cause the cell count in the abdominal fluid to be elevated; 10,000 cells/μl is common, and counts can reach as high as 100,000 cells/μl for up to 5 days after surgery without causing any clinical signs.[4,5] This is thought to be caused by the inflammation from the presence of blood in the abdominal cavity. Therefore, in horses with clinical signs of peritonitis, it may be more useful to evaluate the fluid cytologically for the presence of bacteria or degenerative neutrophils to make a definitive diagnosis rather than relying on absolute cell counts.

Horses with clinical signs of septic peritonitis should be referred for treatment if possible. This should include broad-spectrum IV antimicrobials (pending the results of culture and sensitivity testing of abdominal fluid), NSAIDs, and anti-endotoxic treatments such as polymixin B and hyperimmune plasma. In severe cases, the placement of an indwelling abdominal drain may be warranted to perform lavage 3–4 times daily. Alternatively, an

exploratory celiotomy can be performed for a more thorough lavage, and a drain can be placed at the time of surgery.

7. Penile Damage

Iatrogenic penile damage has been reported after castration and usually occurs when inexperienced veterinarians mistake the penile shaft for a testicle. This can obviously be avoided by having a sound knowledge of the anatomy of the area. If the penis is partially dissected from the surrounding soft tissue while searching for an inguinal or small testicle, then the resultant inflammation and trauma can lead to soft tissue swelling and paraphimosis. In these cases, cold hydrotherapy of the penis and scrotum to reduce the swelling is indicated, and the penis should be manually replaced into the prepuce and then held in place with a sling or purse string suture until the swelling subsides enough for the penis to retract normally. If the penis is inadvertently transected during the procedure, then the horse should be referred for immediate surgical repair or partial phallectomy.

8. Hydrocele

A hydrocele is an accumulation of sterile serous fluid within the vaginal cavity that usually occurs months to years after castration. It occurs more commonly after open castrations, and mules are predisposed to developing the condition. Clinically, these horses have a flocculent, non-painful scrotal swelling. Hydroceles generally do not require any treatment unless the owner requests that it be removed for cosmetic purposes or in rare cases, where the swelling is severe enough to cause lameness. Surgical removal under general anesthesia is the treatment of choice, because the swelling will recur after simple drainage alone.

9. Conclusions

Although castration complications are relatively common, most of these can be resolved quickly with prompt treatment at the farm. However, complications with acute, life-threatening potential, such as hemorrhage, eventration, and infection or peritonitis with systemic signs of endotoxemia, are true emergencies, and these horses should be considered candidates for referral. Horses with suspected penile damage should also be referred promptly. Other complications may require referral at a later stage if the horses do not respond to the initial therapy attempted at the farm. These include cases with significant or chronic infections and those with peritonitis. Most cases of post-operative swelling and edema can be managed at the farm.

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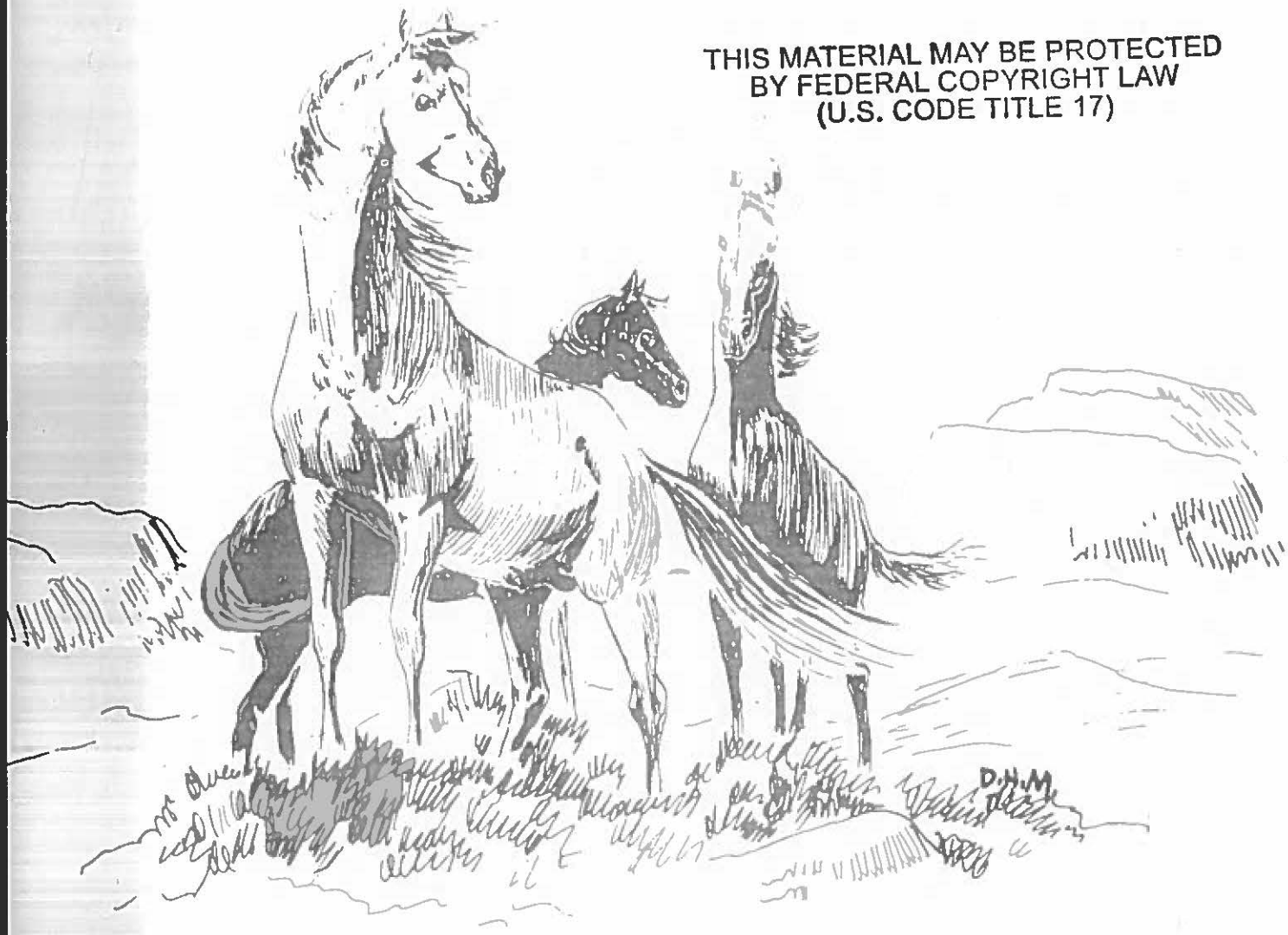

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THE WILD HORSE POPULATION OF STONE CABIN VALLEY, NEVADA: A PRELIMINARY REPORT

Nancy F. Green and Howard D. Green*

Introduction

In Summer 1975 and Spring and Summer 1976 we studied the wild horse population of Stone Cabin Valley, Nye County, Nevada. Stone Cabin Valley covers over 380,000 acres and, at the beginning of the study, supported one of the largest wild horse populations in Nevada. The primary objectives of our study were: 1) to determine the number of wild horses in the Valley and gather data on sex and age composition and recruitment; 2) to evaluate habitat utilization in relation to forage quantity and quality, including determination of home range size, movement patterns, and food habits, and 3) to study social organization and behavior. This report is a preliminary analysis of our data.

Acknowledgements: Support was provided by the American Horse Protection Association for Summer 1975; research in Summer 1976 was funded by Wild Horse Organized Assistance. We also would like to thank the personnel of the Tonopah Area Office of the Bureau of Land Management for their help and cooperation.

Methods

Data was collected June 15-September 25, 1975 and March 10-May 25, 1976. At monthly intervals in June, July, and August 1976 we returned to the Valley and spent 3 days collecting census and distribution data, and clipped forage samples for nutrient analysis.

A 110 mile census route on established roads was used during Summer 1975 to determine the number and distribution of wild horses in the Valley. Stops were made at regular intervals and, using 20 power spotting scopes, the surrounding area was scanned and all horses counted. The sex and age of individuals was recorded when possible. The distribution of horses was plotted on grid maps of the Valley, and the number of horses in each vegetation type was determined.

Data on sex and age ratios were gathered by making detailed descriptions of as many bands as possible. This was a difficult process as it usually was not possible to get within 300 meters of a band without causing flight. Distinguishing between immature (2-3 year old) and adult horses was especially difficult; therefore the immature age class data are included in the adult category in this report.

Detailed descriptions were made of the color and markings of each horse in a band. Each band was assigned a number and a band list index was developed to provide a rapid means of determining which bands had been described. Home ranges of several bands were determined by recording the date time, and location of the bands each time they were observed.

Samples of fresh fecal material were collected in various vegetation types during the study. Food habits data are being analyzed by the Composition Analysis Laboratory at Colorado State University, and results will be presented in a separate report (H. Green, in prep).

The "instantaneous" and "all-occurrences" methods (Altmann 1974) were used to collect over 500 hours of detailed notes on wild horse behavior.

Results and Discussion

Population Size

Census data for Summer 1975 range from a low of 538 horses seen on July 4, 1975 to a high of 786 counted on July 17, 1975. The average number of horses seen was 703. It is likely that we missed seeing horses that were lying down as they were very difficult to detect at distances greater than 500 meters, expecially if they were bedded in vegetation more than one meter high. Not all areas were equally visible from the census route due to the rolling topography in portions of the Valley. Thus our data are a minimum estimate of the horses present in the Valley.

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Foaling

Most foals were born between mid-March and mid-June. In 1975 we found 12 foals per 100 adults. About 50% of the mares did not have yearlings or foals with them. Almost without exception, mares with young had either a yearling or a foal, but not both. We do not know if this is because they did not breed the previous Spring (an unlikely alternative), aborted during pregnancy, or lost foals at or soon after birth to various mortality factors. Mares with foals or nursing yearlings were thin even at the end of Summer when the other horses had gained weight. It would be difficult for these mares to successfully overwinter since Winter and early Spring forage is in short supply. Mares unable to meet the energy demands of pregnancy probably abort or give birth to weak foals susceptible to mortality factors.

In Spring and Summer of 1976 we found 10 yearlings per 100 adults, indicating that some mortality among foals does occur during Winter.

The foal crop in Spring 1976 was considerably higher than that in 1975; a Bureau of Land Management (BLM) aerial survey conducted in September 1976 found 18 foals per 100 adults (M. Hedrick, pers. com.). Our data indicates that given relatively high moisture and subsequent improvement in forage, mares may raise foals in successive years. That they do not successfully foal each year is most likely due to variations in weather and forage conditions. Similar findings have been reported for the Plains zebra (Klingel 1969a).

Sex and Age Ratios

Detailed descriptions of 61 bands (321 horses) were obtained by repeated observations of groups. Band size averaged 5.3 horses and ranged from 1 to 15. Of the 248 adults, 53% were females and 47% were males. The number and percent of horses in each category is given in Table 1.

Table 1. Number and percent of horses in each age category for 61 bands. Summer 1975.

	Adult Males	Adult Females	Yearlings	Foals	Total
Number	117	131	44	29	321
% of total	36.4	40.8	13.7	9.0	

In Summer and Fall 1975, BLM trapped and removed 209 horses; 54% were females and 47% were males, essentially identical to our findings.

Mortality

Incomplete skeletal remains of seven adult horses, 3 males and 4 females, were found in the Summer of 1975. Most of the skeletons obviously had been lying exposed for several years and an exact determination of the cause of death was not possible. The mandible of one male skull was cracked and it is possible that the horse had difficulty feeding and may have starved. A cracked mandible would most likely result from receiving a rear kick from another horse during a fight.

Mortality among immature and adult horses is most likely the result of poor nutrition, disease, old age, or injuries sustained during fighting. We observed numerous aggressive interactions between stallions, but most were highly ritualized and did not involve injury to either participant. However, some fights between stallions included biting and kicking, especially during the Spring of 1976 when mares were in breeding condition. It certainly is possible that blows sustained during such fights could result in broken bones or internal injuries sufficiently damaging to cause death.

In March of 1976 most of the horses, especially mares with nursing young, were very thin. Forage availability was limited in both quantity and quality during Winter. Pregnant mares and those nursing young have high energy requirements and failure to meet these energy demands undoubtedly is a source of mortality. A dead mare was found at a water hole on March 12, 1976. She was extremely thin, her ribs and hips protruded. In April we found a mare lying near a water hole, she was unable to stand. There was a small amount of dried blood on her hind legs. She was very thin and appeared to be in shock; she made no attempt to move when approached and did not respond to the approach of her foal. The foal, a filly, was judged to be about two weeks old. It was not possible to determine the cause of the mare's condition. She may have had trouble giving birth and finally became paralyzed, she may have been kicked and paralyzed by fighting males, or perhaps she was undernourished prior to having her foal and nursing was simply too great an energy drain on her. It was our judgment that she could not be saved. BLM personnel concurred in this judgment and the mare was shot. We took care of the foal for several days until she could be transported to the BLM corrals at Battle Mountain. Without our intervention the foal certainly would have died.

Four old solitary horses were observed and were obviously in poor health. Their movements were lethargic and they seemed unable to raise their heads. It is possible that these animals were unable to obtain sufficient forage

due to tooth wear. Most of the forage is covered with grit and is highly abrasive, and tooth wear in old horses may lead to starvation.

Most mortality seemed to occur among foals. We found carcasses of four foals. Three had sanded hooves, and one had no exterior signs that indicated cause of death. Several lame foals were observed who probably had sanded hooves. Sanding is a condition in which the sole of the foot cracks and sand and rocks collect in the hoof, causing lameness. Lame foals have difficulty staying with their bands and often lag behind by as much as 300 yards. On July 4, 1975 we found a filly lying by a water hole. There were no other horses in sight for at least three miles; she apparently was abandoned by her band. She had three sanded hooves and could barely stand or walk. We suspect that many sanded foals do not recover and, unable to keep up with their bands, are abandoned to die.

Another source of foal mortality involves problems during or shortly after birth. In April 1976 we found an abandoned colt which was not more than 48 hours old. He was extremely lethargic and constipated. Intestinal blockages are not uncommon among newborn domestic foals and can result in death if not treated. We immediately gave the colt water and transported him to our camp. We were able to correct the constipation problem and the colt eventually was adopted by people from the East Coast. It is difficult to determine how often intestinal blockages or similar problems occur, but they are clearly a mortality factor among foals.

A total estimate of foal mortality is difficult to derive since most carcasses are scavenged quickly by coyotes and birds. Also, we were limited to finding carcasses visible from the road or near our vegetation transects.

In summary we found the following sources of foal mortality: death of the mother when the foal was young and dependent on her milk, leading to subsequent death of the foal; lameness; and intestinal blockage or other internal problems during or soon after birth. All of these are natural mortality factors. High mortality among the newborn of most species is a common phenomenon (Caughley 1966).

Social Organization and Behavior

The social organization of wild horses is very similar to that of the closely related Plains zebra (Klingel 1969b). The basic unit of social organization is the harem band, usually composed of one adult male and one or more adult females with their offspring. Of the 53 harem bands described in detail in 1975, 24 (45%) contained more than one adult male (note that we have combined the immature and adult age classes so the actual percent of bands with more than one adult male is lower than what is indicated by our data). In the Spring of 1976 we obtained detailed descriptions of 67 harem bands, 36 (54%) of which contained more than one adult male. We found that in harem bands with more than one adult male, one stallion was consistently the harem master.

Several bachelor (all-male) bands were observed in 1975, ranging in size from one to four horses. Bachelor bands were composed of adults or adults and immatures, but never contained yearlings or foals. A few adult males were solitary.

The harem bands tended to be stable in composition in terms of adult members. We were able to re-locate many of the bands we saw in 1975 in the Spring of 1976 and most of them had not changed in number. Changes in band composition were usually the result of immature horses leaving their original band. We documented such changes for two distinctly marked young mares. Also, in late Summer 1975 we found that yearling females sometimes approached and fed with other harem bands, usually close to the other harem stallion; they returned to their own band after five to thirty minutes. Harem stallions did not attempt to prevent this type of movement by yearling females in the Summer, but did attempt to prevent it during the Spring breeding period by herding females of all ages back toward the harem if they strayed. Feist (1971) found that immature females accounted for most of the changes in band composition he observed in the Pryor Mountain wild horse population, and concluded that stallions may not attempt to breed or hold their own offspring in the band. Most shifts of immature females, observed by Feist, occurred during the breeding period.

Klingel (1969a) reported that shifts between bands of zebras were usually the result of immature females being abducted by other harem masters or bachelors during the breeding period. He found that stallions tried to prevent such abductions but usually failed, especially if several stallions simultaneously attempted to abduct a young female. Thus, the change by immature females from their original harem band to some other band seems to be a common pattern in equine species. One effect of this behavior is that inbreeding is probably not a problem, as has been suggested in some of the wild horse literature.

Immature males were present in some of the bachelor bands. No instances of immature males being chased from their original band by the harem master were observed. We have no data as to how or why young males leave their original harem. Their presence was generally tolerated by the harem stallion, who rarely interacted with young males. Foal, yearling, and occasionally immature males showed a submissive gesture called teeth-clapping when they were close to stallions. Teeth-clapping probably serves to acknowledge the dominance of the stallion and may prevent aggression of stallions toward young males in the band.

Changes in harem band composition can occur for several other reasons. If the harem stallion dies the mares and other harem members may be adopted by another stallion. We documented this for one band: a stallion who already had a mare and 2-year-old son adopted two mares and their foals. He was aggressive toward the 2-year-old male from the new group, who eventually left. Band composition also changed as a result of a single harem being split by two stallions (two observations). Bands also may change if the harem stallion is defeated and driven away by another harem master or a solitary male. The new stallion takes over the harem.

Wild horses spend a vast majority of their time (about 90%) either feeding or resting. Large aggregations of bands feeding in close proximity were seen during Summer 1975. The density of horses during these feeding aggregations was as high as 90 horses per square mile. There was little inter-band aggression during Summer months. In the 1976 Spring breeding period, however, we saw many more aggressive interactions between harem leaders, and inter-band spacing was much greater than it had been the previous summer.

A detailed analysis of the behavior of wild horses, with emphasis on stallions, will be published separately (N. Green, in prep.).

Home Range and Habitat Utilization

The home range of one band often overlaps the home ranges of several other bands. We saw no evidence of one band defending any particular area or excluding other bands from their home range, nor did we see any evidence that any one band had exclusive use of an area, i.e. we observed no form of territoriality. Figure 1 shows the home ranges of five bands in Spring and Summer 1976. The size of the home ranges is quite variable: that of Band 218 was only 4.2 square miles, while Band 96 had a home range of 30.2 square miles. It should be kept in mind that these are Spring and Summer home ranges and do not include possible seasonal movements. If any of these bands migrate seasonally their home ranges would be considerably larger.

Most bands had a smaller area within their home range in which they spent most of their time; this is referred to as the maximum activity area. In the Southern part of the Valley (below Highway 6) each band's maximum activity area was located within three miles of a water source. Movements outside of the maximum activity areas were instigated for reasons which are not completely understood. The southern-most extensions of the home ranges of bands 86, 96 and 213 (Fig. 1) were the result of these bands moving out of their maximum activity areas in response to heavy rain or snowfall. On three occasions in Spring 1976 following several hours of rain or snow many of the bands which normally occupied the area around the South Central Water Hole, Haw's Canyon, and Haw's Trap moved to an area East of the Pond and Reed's Ranch (Figure 2). The area normally is grazed very little by horses even though forage is relatively abundant. Water is available at Reed's Ranch and the Pond on a seasonal basis, being turned off in Summer and Fall. It may be that this area is little used by horses, despite the good forage, because they have no way of determining when water will be available there. However, following heavy rain or snow large pools of water accumulate in shallow depressions. Many bands (as many as 150 horses) move South and feed in the area until the standing water dries up, at which time they return to their maximum activity areas.

In the North end of Stone Cabin Valley (North of Highway 6) the distribution patterns of horses are different. Several water sources are available all year (Fig. 2). However, most are located in areas of very poor forage (in some cases this seems to be due to over-grazing by cattle around waterholes), and the area of maximum concentration for most bands is located several miles from the water sources in an area of better forage. These bands travel up to 7 miles each day to water.

These data suggest that at least two environmental factors, water and forage availability, influence the distribution of horses.

In the southern part of the study area most horses stay in an area where both food and water are available (Figure 2) and move South to take advantage of better food sources when water becomes available in those areas. In the North end of the Valley where there are no locations with both ample forage and water, the maximum concentration of horses is found in areas of good forage and the horses travel longer distances to water.

Within the Valley there are three broad habitat areas: the valley floor, the foothills and mountains, and the mountain meadows. The valley floor, which covers a majority of the study site, is an area of relatively flat topography vegetated primarily by low brush species such as *Atriplex canescens* (four-wing saltbush), *A. confertifolia* (shadscale), *Tetradymia glabrata* (horse brush) and perennial grasses such as *Hilaria jamesii* (galleta grass) and *Oryzopsis hymenoides* (Indian rice grass). Surrounding the valley floor on three sides are foothills primarily vegetated by *Artemesia tridentata* (big sage) and *A. nova* (black sage); the foothills grade into the mountains where Pinyon and Juniper trees are dominant. At several locations in the mountain ranges there are meadows primarily vegetated by sedges and annual and perennial grasses.

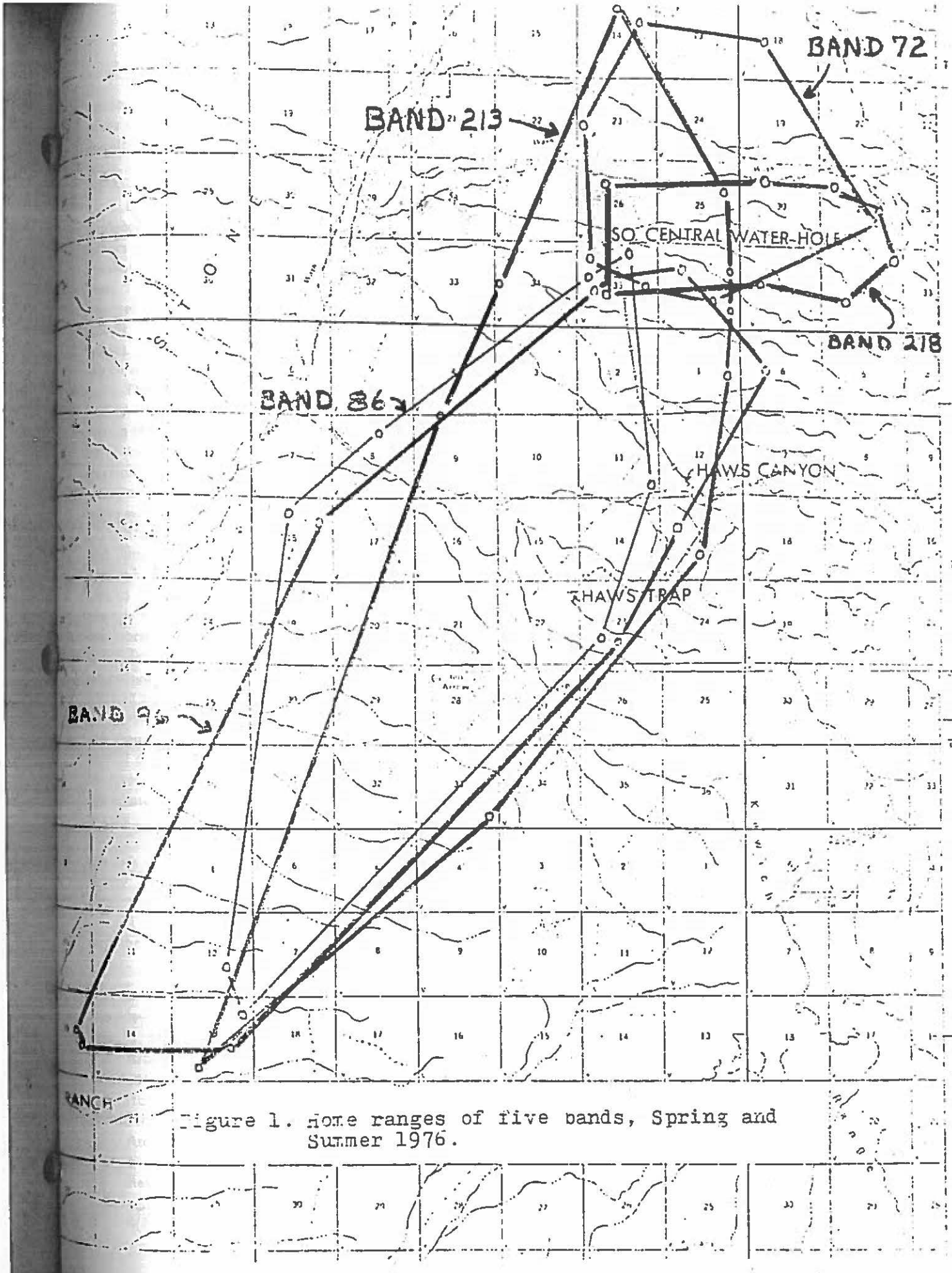
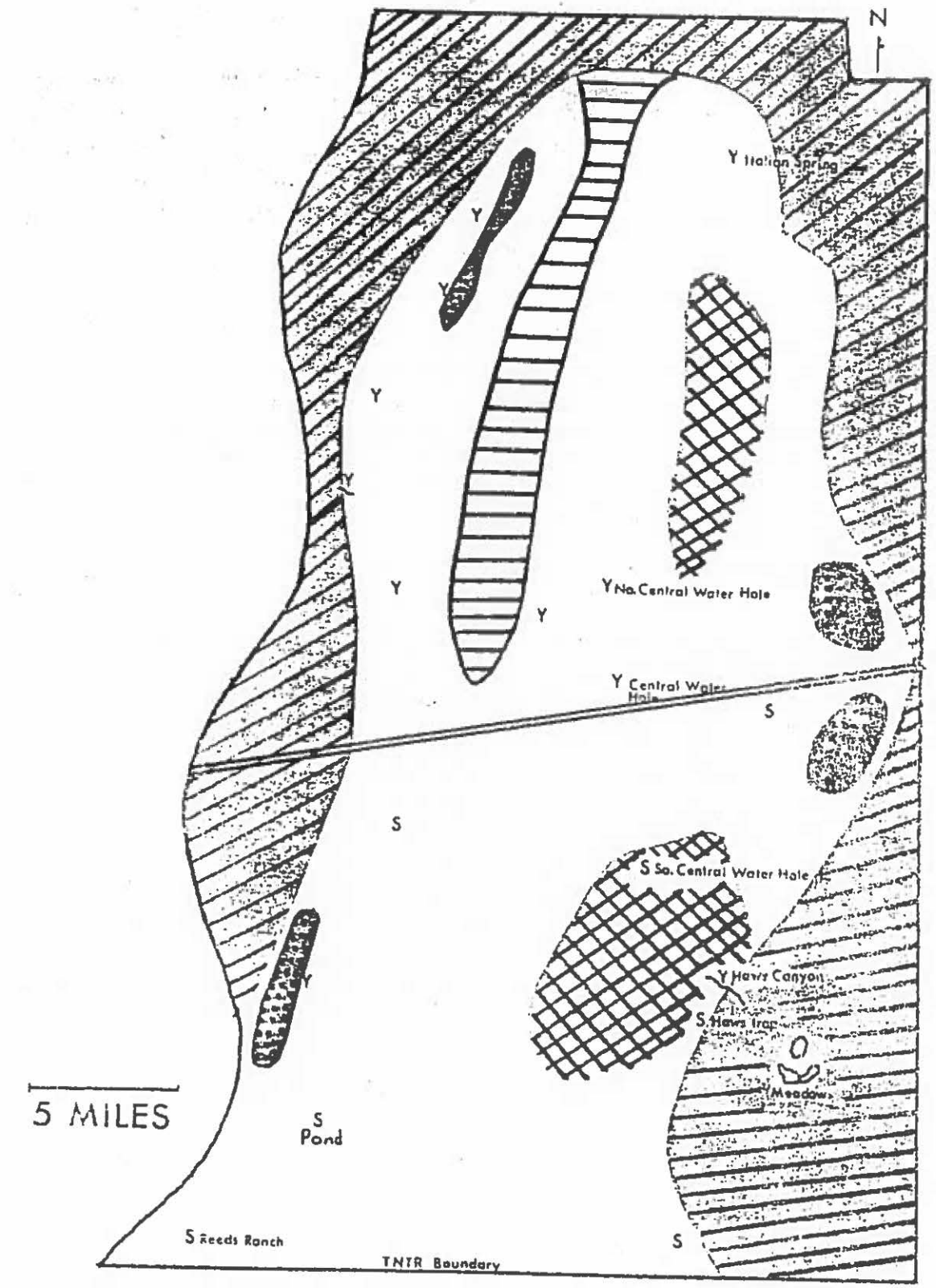


Figure 1. Home ranges of five bands, Spring and Summer 1976.

Figure 2. Habitat utilization by wild horses in Stone Cabin Valley. Summer 1975, Spring and Summer 1976.



Most of the horses inhabit the valley floor. Meadows have small populations of horses in Summer. The rest of the mountain areas are lightly populated by dispersed bands of horses. The bands occupying each of these broad habitat areas showed a remarkable fidelity to their area during the period of the study. Valley horses were not observed in meadow areas, and valley bands did not move into the mountain areas further than the first low foothills. Correspondingly, bands which occupied the meadow areas were not observed in the valley floor.

SEASONAL MOVEMENTS

Due to the short time span of this study few direct observations of seasonal movements were obtained. Indirect evidence of seasonal movements suggests that some North-South migration does take place. Ranchers in the Valley and others who have observed the horse population levels on a year-round basis agree that there are many fewer horses present in Winter than in Summer. On our first census in the Spring of 1976 (March 29) relatively few horses, 221, were seen. This low population level was still evident on the second census made on April 8, 1976 when only 204 horses were counted. As the season progressed more horses were counted on each census: April 19 - 334 horses; April 28 - 527 horses; May 8 - 446 horses; May 18 - 570 horses; June 18 - 315 horses; July 18 - 317 horses; and August 18 - 395 horses. (The decrease in the census numbers in June, July and August probably was due to the trapping operation which removed 205 horses from the Valley between May 20 and June 29, 1976.)

These census data indicate that at least some of the bands spend the Winter in places other than Stone Cabin Valley. Some of the bands with which we were familiar in the Summer of 1975 were not observed in the Valley until late in the Spring of 1976, after which they were observed frequently. Their sudden reappearance in the Valley indicates that they probably had returned to the Valley from some other location. Other bands which we had described the previous Summer were present in the Valley early in the Spring of 1976 and remained there at least through August, when our study terminated. These data indicate that some of the bands stay in the Valley year-round, while others make seasonal movements to other areas.

Stone Cabin Valley is bordered on the North, East and West by moderately high mountains vegetated by Pinyon and Juniper trees. The mountains appear to act as ecological barriers to horse movements. To the South the study area is bounded by the Tonopah Nuclear Test Range (TNTR). There are no fences or other barriers and the horses are free to move on and off the TNTR. Individuals who are allowed on the Test Range report that the horse population there is high during Winter. We made one trip South of the Test Range boundary in the Summer of 1975. There were few horses south of the Stone Cabin Valley-Test Range boundary. These data suggest that some of the horses which spend late Spring and Summer in the Valley may spend the Winter on the Test Range to the South. This is further supported by a series of observations made of Band 500 during Spring 1976. Band 500 spent the entire Summer of 1975 in a mountain meadow area in the Kawich Mountain Range, east of the Valley. During the entire Summer of 1975 we never observed these horses outside of the immediate meadow area. On April 29, 1976 we observed Band 500 in the main Valley area, 6 miles southwest of the meadow and 7.5 miles north of the Test Range border. For the next several days we observed the band slowly move further northeast, closer to the meadow. On May 10 the band was observed in the meadow, where they remained for the Summer. The meadow area was covered with snow for much of the Winter, forcing horses out until Spring. From our observations of the direction from which Band 500 returned to the meadow, it is likely that they were coming from the Test Range.

Seasonal movements are probably a response to both water and forage availability. It is our opinion that due to the lack of water and forage on the Test Range, many horses spend the Summer North of the TNTR, in Stone Cabin Valley. In winter, snow is sometimes available to eat and snowmelt water is available in natural catchments, thus eliminating lack of water as a hinderance to southern movement.

A detailed analysis of the distribution of horses in relation to the quantity and nutrient quality of forage will be published separately (H. Green, in prep.)

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Nd:YAG Laser Photoablation of Endometrial Cysts: A Review of 55 Cases (2000–2001)

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Videoendoscopic assisted laser photoablation of endometrial cysts is an effective method of cyst reduction. The group of mares in this review had been barren for at least one breeding season. Following laser treatment a high percentage of these mares conceived. Authors' address: Equine Services Surgical Hospital, P.O. Box 464, Simpsonville, KY 40067. © 2002 AAEP.

1. Introduction

Endometrial cysts have been previously described as originating from uterine glands or from lymphatic lacunae.[1–3] Glandular cysts have been described as being <10 mm in diameter, with a significance that is largely unknown.[4] Lymphatic cysts, however, are lymphatic lacunae that can be significantly larger than 10 mm in diameter. Ragon reported that these cysts could be found in the endometrium or myometrium. The cysts may appear as unilocular or multilocular, be round or elongated, and their presence is associated with both normal and chronically inflamed endometria.[5]

Ragon also stated the prevalence of glandular cysts is unknown, but regardless of the population of mares studied (fertile or subfertile), the incidence of lymphatic cysts increases with age.[5] In one study, 73.1% of mares over 14 yr of age had endometrial cysts.[6] In a study of mares acquired from a slaughter-house market, the mean age of mares with endometrial cysts was reported to be 13.4 yr of age.[7]

Uterine cysts are most commonly diagnosed by ultrasonography. Prior to the advent of trans-rectal ultrasound, postmortem examination was the most common method of diagnosis.[8] Recently hysteroscopy has become more common in equine practice. Hysteroscopy allows for a thorough visual inspection of the uterus and potential pathology, which other diagnostic procedures cannot provide. Hysteroscopy has been found to be a useful diagnostic technique, and has been used on a routine basis at Equine Services since 1977.[a]

The effect of uterine cysts on fertility has been debated. McKinnon et al suggested that the relationship between infertility and uterine cysts was axiomatic.[4] These authors also stated that later in pregnancy, contact between the cyst wall and yolk sac or allantois may prevent absorption of nutrients. Ragon noted in her review that the presence of uterine cysts has been associated with decreased conception rates, increased early embryonic death, and decreased foaling rates.[5] It has also been shown that endometrial cysts are closely associated with increasing age and biopsy scores.[7] This makes the specific effect of uterine cysts on infertility difficult to predict. The purpose of this study was to determine the conception rate in a group of previously barren mares, after photoablation of endometrial lymphatic cysts with Nd:YAG laser.

NOTES

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2. Materials and Methods

Records of fifty-five barren mares presented for photoablation of endometrial cysts during 2000 and 2001 were reviewed. Of the cases seen, three mares were evaluated twice. The mares were placed in stocks and sedated to effect with a combination of detomadine[b] (0.01–0.02 mg/kg, i.v.) or xylazine[c] (0.3–0.4 mg/kg, i.v.) and butorphanol[d] (0.01–0.02 mg/kg, i.v.). The rectum was emptied of manure, and the tail was wrapped and secured out of the working area. The perineal region was then thoroughly cleansed for aseptic procedure. The operator is gloved with a sterile sleeve. A one-meter videoendoscope[e] is cold sterilized[f] then rinsed and flushed with sterile saline and guided through the cervix into the uterine body. A sterile artificial insemination pipette is then placed alongside the videoendoscope into the uterine body. A sterilized hand-held bulb-type air pump is attached to the pipette and used to insufflate the uterus with room air. The cervix must be tightly closed during the procedure, or the uterus will not insufflate.

Once the uterus is insufflated and the endometrium in the uterine body can be clearly visualized, the endoscope is passed into both of the uterine horns. It is helpful to make a thorough inspection of the uterus and locate the cysts prior to photoablation. Other important structures that should be clearly visible include the corpus-cornual junction, both utero-tubal papillae, and the endoscopic appearance of sub-endometrial blood vessels. The endometrium should also be evaluated for the appearance of scarring or atrophy. Although this is a subjective assessment, we believe experience will allow the operator to provide a more accurate prognosis of fertility in the mare. Bracher et al. and Inoue et al. have clearly shown the usefulness of hysteroscopy for the evaluation and prediction of the reproductive ability of mares.[9,10]

After all cysts have been located, the laser fiber is passed through the biopsy port of the endoscope until 1–2 cm of the fiber is visible. An Nd:YAG laser system with a 4-m long, 800-μm diameter transmitting fiber is used.[g] The beam is passed through a fiber, with a 1-mm orb tip. Coaxial cooling is not used with this contact fiber. The laser output is set at 15 W. It is helpful to start at the tip of a horn and work in a posterior direction. The tip of the laser fiber is guided to a cyst and placed in contact with the central dome of the cyst and energy is applied. The cyst is photoablated until all lymphatic fluid has leaked out and the cyst is reduced in size. Once fluid empties, the remaining cystic tissue will collapse around the fiber and reduce back to the level of the endometrium as energy is applied. Energy may have to be applied to several points on a large cyst in order to accomplish this. If numerous lymphatic cysts are present, the uterus may have to be emptied of air and smoke, then re-insufflated in order to clearly visualize all cystic structures. When all cysts have been satisfactorily ablated, all air is emptied from the uterus. If uterine biopsy is to be done, it should be done following cyst reduction.

The perineal region can then be cleansed again and a Foley catheter is passed in order to lavage the uterus. The uterus is lavaged with 1 L of 7% hydrogen peroxide in sterile saline, followed by a 2% povidone iodine solution in sterile saline. It is felt that the hydrogen peroxide lavage helps to effectively remove debris resulting from the procedure. The follow-up povidone iodine solution is used as an anti-microbial lavage, to aid in prevention of possible infection secondary to the procedure. The recommended take-home instructions include lavage of the uterus twice more before breeding the mare.

3. Results

The 55 mares in this case review had been barren for at least one season. A total of 269 cysts were photoablated. Many cysts were multiloculated structures that were counted as one cyst. The mean age of this group of mares was 18.8 yr, with a median of 18 yr of age. The range was from 13, to a group of four mares, all 24 yr of age. The cysts were divided as to their location within the uterus. They were divided into four groups; those in the left horn, right horn, at the corpus-cornual junction and in the uterine body. In this group of mares, 21.6% of cysts were located in the left horn, 28.2% of cysts were in the right horn, 16.4% of cysts were at the corpus-cornual junction, and 33.8% of cysts were in the uterine body.

Follow-up of the 29 mares treated in the year 2000 was attempted. Of these mares, 25 were located for further information. Of the four lost to follow-up, one had died, and we were unable to contact the owners of the other three. Of the 25 available, 18 had conceived in either 2000 or 2001. These mares were bred an average of 2.3 cycles per conception. Mares that conceived had an average of six cysts. The group of mares that did not conceive had an average of five cysts.

Follow-up data on the mares treated during 2001 was obtained for 14 mares. Of this group, eight are known to have conceived. They were bred an average of 1.4 cycles per conception. Of the six that did not conceive, one was retired from breeding due to age, and another was diagnosed with a granulosa cell tumor. Mares that conceived had an average of 7 cysts, whereas those that did not had an average of 4 cysts.

4. Discussion

As has been previously stated lymphatic cysts have been associated with infertility in the mare. Tannus and Thun found that pregnancy rates at 14 d and 40 d were significantly lower in mares with cysts compared to mares without cysts.[6] Several different methods describing removal of endometrial cysts were previously reviewed. These include me-

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chanical curettage, hypertonic saline infusions, aspiration, rupture with endoscopic biopsy instruments, electrocoagulation, and photoablation with laser.[4]

Blikslager et al. previously described a technique for photoablation of uterine cysts with Nd:YAG laser on a small group of mares, both *in vivo* and *in vitro*.[11] They used a coaxial cooled fiber, at much higher power than is described in this paper. *In vivo*, they performed the procedure in two stages. Initially they were placing the probe 1 cm from the cyst surface, blanching the entire cyst at 50 W. This was followed by membrane puncture at 100 W. We have not found this level of power to be necessary in order to adequately photoablate uterine cysts. Their *in vitro* study also described creating Grade 4 lesions by using high-energy densities. This is obviously undesirable in clinical cases.

Nd:YAG laser has been used for treatment of endometrial cysts at Equine Services since 1986.[a] We have found Nd:YAG laser to be an effective, affordable treatment for ablation of intrauterine cysts. Continued follow-up of mares, including the live foal rate of those that conceived following this procedure, would provide further valuable information regarding the usefulness of this procedure.

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[a]Personal communication, Dr. Scott Bennett, Equine Services, Simpsonville, KY, 2002.
[b]Dormosedan®, Orion Corp., Epsoo, Finland.
[c]Xyla-Ject®, Phoenix Pharmaceuticals, St. Joseph, MO 64503.
[d]Torbugesic®, Ft. Dodge Animal Health, Ft. Dodge, IA 50501.
[e]Pentax Precision Instrument Corp., Orangeburg, NY 10962.
[f]Cidex plus® Johnson and Johnson Co., Irvine, CA 92618.
[g]Surgical Laser Technologies Inc., Oaks, PA 19456.

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A dynamic simulation model for evaluating effects of removal and contraception on genetic variation and demography of Pryor Mountain wild horses

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Abstract

I used an individual-based model to simulate population dynamics and changes in genetic variation of horses in the Pryor Mountain Wild Horse Range under a range of management strategies. Management strategies included population objectives of 90 to 180 horses and removal and/or contraception of animals of random, young, or old ages. Populations controlled only by removals fluctuated widely in size and generally exceeded objectives by >20%. Populations controlled by contraceptives remained within 3% of objective. Over a 200-year period, populations were very likely to lose alleles with an initial frequency of <3%, which included nearly 30% of alleles in the population. Allelic diversity was lost faster than population-level heterozygosity. Removal or contraceptive treatment of older animals hastened the loss of genetic variation compared to treatment of random-aged or young animals. Rate of loss of genetic variation was directly related to generation interval, and differences in loss of genetic variation were greater between treatments of old and young animals than between populations that differed in size by a factor of 2. These results apply to management of many long-lived species in harvested populations and they illustrate the importance of actions that modify age structure and generation interval. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Contraception; Genetic variation; Infertility; Population control; Simulation model

1. Introduction

About 150 horses inhabited the Pryor Mountain Wild Horse Range (PMWHR) in 1998, close to the estimated range carrying capacity (BLM, 1997). The small size of this population concerned those mandated to sustain a viable and healthy population, because genetic diversity rapidly disappears in small populations (Wright, 1931). The Pryor horse population has the reproductive potential to double in size every 4–5 years and attain a size that poses risks to habitats and to populations of mountain sheep and deer that also inhabit the PMWHR (Garrott and Taylor, 1990). Management of the Pryor horse population is illustrative of the general predicament faced by agencies with a mandate to control an isolated population characterized by high reproductive potential, long life span, and limited area of available habitat. In these situations, management plans must balance measures to minimize risk of long-term habitat degradation while maximizing long-term retention of genetic variation and the likelihood of population persistence. There are thus conflicting needs to minimize population size to control habitat degradation while maximizing population size to conserve genetic resources.

Feral horses in the United States are protected by the Wild Free-roaming Horse and Burro Act of 1971 and management of most feral horse populations falls under the jurisdiction of the Bureau of Land Management (BLM, 1997). Wild horse populations in the western United States have exhibited growth rates that frequently exceed 15% per year (Garrott et al., 1991a) and they can rapidly achieve population sizes that raise concerns about long-term habitat damage. A total of about 35,000 horses inhabit ranges on public lands in the western United States, but only 25% of the 186 wild

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horse populations under active management have a population objective of greater than 150 horses (BLM, 1997). The small size of these populations raised concerns about long-term maintenance of genetic variation and emphasized the need to identify methods to control population size while retaining genetic variation.

Wild horse populations have been controlled primarily through periodic gathers and removals. Programs were developed to place wild horses in private ownership, but this has proven to be exceedingly expensive (> $16 million in 1995; BLM, 1997) and it has become impossible to place all horses acquired by removal programs. As a result, there is great interest in the potential use of contraceptives to reduce fertility of horses and other overabundant species (Sturtevant, 1970; Hoffman and Wright, 1990; Garrott et al., 1991b; Holden, 1992; Eagle et al., 1992; Fayrer-Hosken et al., 1997). Use of contraceptives could provide opportunities for a variety of new management scenarios, including permanent infertility, delayed reproduction, or permitting mares to reproduce once and then preventing further breeding. Simulations of horses population dynamics have shown that a relatively high level of infertility would likely be needed to maintain a low growth rate in horse populations (Garrott, 1991), but no analyses have specifically considered the consequences of alternative management actions on long-term persistence of genetic variation in horses or similar mammals.

Management strategies to control growth rates of abundant populations by removal or contraception must focus on three decisions: How many animals should there be? What sex and age of animals are to be removed or made infertile? How often are animals removed or treated? The model described below was developed to address these questions by simulating removals only, contraceptives only, or a combination of removal and contraception. The model was specifically applied to the Pryor wild horse population to evaluate a set of realistic management alternatives. Results from the simulations lend insight to the consequences of management actions used or considered for control of overabundant species including bison, deer, elk, elephants, mountain goats, and some carnivores (Hoffman and Wright, 1990; Bradley, 1994; Fayrer-Hosken et al., 1997; Kirkpatrick et al., 1997). To preserve genetic variation, guidelines for managers almost always emphasize actions that increase overall population size or reduce reproductive variance. I offer analyses that clearly demonstrate the importance of age-related management actions and show that actions that increase (or decrease) generation time of long-lived species can have an effect equal to doubling population size. These results are important because it is frequently much easier to modify age structure than increase population size.

2. Method

I developed an individual-based population model to simulate the dynamics of the PMWHR horse population. The model operated on a yearly time step and it simulated the processes of breeding, recruitment, removal, contraception, natural mortality, and aging. Each individual in the population was explicitly represented and the sex, age, genetic constitution, number of mates, and reproductive status of all animals was followed from birth to death. Model outputs included sex and age of each individual in the population, number of mates (by age) of males and females, and the frequency of all alleles.

2.1. Model processes and parameter estimates

Wild horses on the PMWHR have high rates of survival and reproduction (Garrott and Taylor, 1990; Singer et al., 1998), consistent with observations of wild horse populations throughout the western United States (Berger, 1986; Garrott et al., 1991a). Estimates of fecundity and survival were derived from observations of the Pryor population, emphasizing data subsequent to management changes that occurred about 1990 (Garrott and Taylor, 1990; Singer et al., 1998). Horses in the PMWHR have been intensively studied since the 1980s and about 95% of all horses are individually known and repeatedly observed each year. Age-specific survival rates are thus known with a high degree of confidence up to about 15 years of age. Relatively few horses have lived longer than 15 years and these older horses make up a small proportion of the population. No density dependent regulation of survival or reproduction was included in the model because the horse population has been maintained at a size well below estimates of range carrying capacity and evidence for density dependence is lacking. Recent analyses found a significant correlation between growth rate and population size, but neither recruitment nor survival varied significantly with population size ($P > 0.10$; Singer et al., 1998).

Breeding was simulated by comparing a uniform random deviate to the probability of recruitment for each mare, and then selecting a mate. Age-specific breeding success of wild stallions was used to simulate paternity (Berger, 1986, p. 200). It is difficult to determine mating success in the field and tenuous to extrapolate results to another site. However, sensitivity analysis showed that model results were insensitive to age-specific breeding success, but were more sensitive to the probability that a male bred during its lifetime. Estimates of breeding success (Berger, 1986; Singer et al., 1998) show that that virtually all prime-aged stallions breed. The likelihood that a particular stallion mated with a female was determined by the number of breeding-age males in the

population and the age-specific probability of breeding. Every male in the population was assigned the appropriate age-specific probability of breeding (Table 1) and the cumulative breeding probabilities were placed in a vector with a length equal to the total number of breeding-age stallions. For each female that bred, a uniform random deviate between 0 and the sum of breeding probabilities was generated and compared to values in the vector. The male that corresponded to the value of the deviate was chosen as the mate for that female. All matings were independent. Factors that determined harem formation, size, and retention were too poorly known to include in the model, but harem interchange rates in the Pryor horse population are so high (Singer et al., 1998) that the age-specific algorithm was probably sufficient for this situation. If only harem-holding stallions mated, simulation results reported here will have overestimated retention of genetic variation.

After the parents of an offspring where determined, Mendelian inheritance was simulated by randomly selecting one allele from each parent at 10 independent loci. At the beginning of each set of simulations, individuals were assigned alleles in the frequency in which they occurred in the Pryor population (G. Cothran, pers. comm.). Each simulation began with a total of 41 alleles distributed over 10 loci; two to 13 different alleles occurred at each locus with an initial frequency of 0.0035 to 0.844.

2.2. Population objective, removal and contraception treatments

The current management plan for the PMWHR includes a population objective of 95 horses, excluding foals (BLM, 1992). A population objective, or appropriate management level (AML), is the optimum number of horses that remain after a removal event in order to maintain a "thriving ecological balance". Foals are usually excluded in determining the AML, and in this paper foals were always excluded from counts used to determine compliance with a population objective or to categorize a population size. Simulations included an AML of 90 horses, and increases in AML to 120, 150, and 180 horses. These objectives might be accommodated by supplemental feeding, acquisition of additional habitat, or other strategies. No specific means to attain a particular objective is implied.

Removals and/or contraceptive treatments of wild horses were simulated by applying rules based on current population size, post-treatment population objective, sex and age of animals to be treated, the minimum number of horses in each sex/age class that were to be unaffected by the treatment, and for removals, the length of time since a previous removal. To determine the annual control treatment, the population was first compared to size thresholds used to categorize the population as small, normal, or large. If the population size was less than or equal to the lower threshold, it was categorized as small. If the population size was greater than the lower threshold and less than or equal to the higher threshold, it was categorized as normal. If greater than the upper threshold it was considered large.

Population size could alter both the timing and magnitude of treatment. It is expensive to gather and remove wild horses and managers seek to minimize the frequency of these events (Boyles, 1986; BLM, 1997). The model therefore simulated a removal event only when the population size had grown to a sufficient size. Removals or contraceptive treatments always occurred when the population size was categorized as large, regardless of how long it had been since a previous treatment. Removals also occurred when the population size was categorized as normal and a threshold number of years had passed since the previous removal. This time threshold was set at 3 years for removal-only treatments and 5 years for removal-and-contraceptive treatments. For removal-only treatments, the threshold between a small and normal population size was set at 150% of the population objective and the threshold between a normal and large population size was set at 200% of the population objective. A removal never occurred when the population size was categorized as small.

Several variables determined which animals were removed from the population. The first variable was the

Table 1
Demographic parameters used to simulate wild horse dynamics

Males			Females		
Age	Survival	Probability of mating	Age	Survival	Probability of recruiting
1–4	0.98	0.00	1–2	0.98	0.00
5–6	0.98	0.10	3–4	0.98	0.50
7–8	0.98	0.26	5–16	0.98	0.60
9–16	0.98	0.64	17–22	0.75	0.47
17–19	0.75	0.50	23–24	0.50	0.47
20–22	0.62	0.25	25	0.00	0.00
23–24	0.50	0.25			
25	0.00	0.00			

population objective, which was the number of horses (excluding foals) remaining after a removal was completed. Removal treatments were categorized as young, old, or random for the age of horses emphasized in the treatment. Random animal treatments selected horses randomly with regard to sex or age until the population objective was reached. For old animal treatments, the oldest horses in the population were selected first, leaving a few males and females in each multi-year age class. Similarly, young treatments first removed the youngest animals, again leaving a few males and females in each multi-year age class. Males and females were removed to leave a slightly female-biased sex ratio, consistent with observations of other wild horse populations and prior BLM removals (Berger, 1986, p. 91).

The proportion of mares treated with contraceptives varied with population size. For each contraception-only treatment, the level of contraception was initially calibrated to achieve a stable population size. However, demographic stochasticity often resulted in populations that either grew or declined. In these situations, the rate of application of contraceptives was increased or reduced until the population declined or increased to a size that fell within the normal range. For populations regulated by contraceptives, populations were categorized as normal when they were 85 to 110% of the population objective. Contraceptive treatments were administered every year and treated mares remained infertile for one year. As with removals, females were selected for contraceptive treatments based on age, using rules that selected breeding-age females randomly, or that resulted in treatment of the oldest or youngest mares first.

Simulations that used both infertility and removal employed a constant rate of application of contraceptives and a threshold population size for determining the frequency of harvest. The rate of contraception was initially calibrated to result in removal of about 50 horses every 5 years.

2.3. Genetic analyses

Observed heterozygosity [H_o; Eq. (1)], expected heterozygosity [H_e; Eq. (2)], and the inbreeding coefficient [F_{is}; Eq. (3)] were calculated following Hartl and Clark (1997), where N is the number of loci.

$$H_o = \frac{\sum_{n=1}^{N} \frac{\text{heterozygotes at locus}_n}{\text{number of individuals}}}{N} \quad (1)$$

$$H_e = \frac{\sum_{n=1}^{N} \left(1 - \sum_{k=1}^{\infty} (\text{freq. of allele k})^2\right)}{N} \quad (2)$$

$$F_{is} = 1 - \frac{H_o}{H_e} \quad (3)$$

In the absence of agency-mandated objectives for conserving genetic diversity, changes in genetic variation were evaluated relative to a goal to achieve a 90% probability of conserving 90% of selectively neutral genetic variation for 200 years. This is a slightly less ambitious objective than suggested by Soule et al. (1986). The frequency of each allele at each locus was recorded and used to evaluate rate of loss of alleles.

2.4. Initial conditions, simulation procedures, and evaluation of results

All simulations started with an initial population of 147 horses that matched the sex and age structure of the PMWHR horse population in fall 1998. The initial population consisted of 65 males aged 1 to 22 years and 82 females aged 1 to 17 years. Treatments were evaluated from results of 500 simulations that each lasted 200 years.

A variety of output files were written to permit evaluation of the causes of differences between treatments. Each year, the age structure of the population, the age and sex of individuals removed, and the age of females treated with contraceptives was recorded. Summary files included data on age-specific fecundity and lifetime reproductive success of males and females. These outputs were used to construct life tables of simulated populations, from which standard demographic statistics such as growth rate and generation time were calculated (Carey, 1993). Annual growth rates for removal treatments accommodated removals by adding the number of horses removed back to the population, such that

$$\lambda_t = \frac{N_t}{N_{t-1} + R_{t-1}} \quad (4)$$

where λ_t is the apparent annual growth rate at year t, N is the total number of animals in the given year, and R_{t-1} is the number of animals removed from the population between year $t-1$ and t. The initial age structure led to instability for the first 10–25 years in simulations of populations regulated by contraceptives, and analyses of rates of contraception and average population processes excluded data from the first 30 years of each simulation.

3. Results

3.1. Treatment levels and demographic responses

Removal and contraceptive treatments resulted in population dynamics that were fundamentally different. Populations controlled by removals increased rapidly and declined precipitously when a removal occurred. In

contrast, populations controlled by contraceptives remained relatively stable. As a result of these dynamics, the average size of populations controlled by removals was 18–27% greater than the AML, whereas populations controlled by contraception remained within 3% of the AML (Fig. 1; dark bars referring to horses 1 year and older). Populations controlled by a combination of contraception and removal were on average 18–5% greater than the AML for population objectives of 90–180, respectively. The coefficient of variation of population size for populations controlled by removal averaged 17%, compared to 8% for populations controlled by contraceptives alone and 11% for populations controlled by contraceptives and removals.

Average annual growth rate (λ) of populations controlled by removals was 1.15 when young horses were removed, 1.16 when old horses were removed, and 1.17 for removal of random-aged horses. Population objective had no influence on average growth rate and the maximum difference in growth rate within a removal treatment (old, young, or random) was 0.004. For populations controlled by contraception and removal of younger horses, λ varied with population objective and ranged from 1.05 to 1.09 for simulated populations with objectives from 180 to 90, respectively. For these treatments, differences in λ were driven by differences in the application rate of contraceptives (Table 2), which were adjusted to achieve a removal of about 50 horses every 5 years.

To achieve a stable population with contraceptive-only treatments, an average of 78, 73, or 74% of all mares 2 years or older were rendered infertile for treatments of young, old, or random-aged mares (Table 2). Depending on population objective, this resulted in

Table 2
Percent and number of horses 1 year or older affected by simulated contraception treatments of the Pryor wild horse population, and the average number of foals in the population

	Population objective	Percent treated	Number treated (mean±1 S.D.)	Average number of foals
Contraception-only treatments				
Young age	90	78	32±7.6	5.7
	120	78	44±8.9	7.7
	150	78	54±10.1	9.6
	180	78	65±11.2	11.5
Old age	90	73	31±6.4	5.8
	120	74	43±8.8	7.9
	150	73	52±8.5	9.7
	180	74	62±9.4	11.6
Random age	90	74	32±8.1	5.9
	120	74	43±8.8	7.9
	150	74	54±9.9	10.0
	180	74	64±10.9	12.0
Contraception and removal treatments				
Young age	90	50	25±4.7	17.2
	120	58	37±5.2	18.3
	150	61	46±6.0	20.1
	180	64	57±6.6	21.5

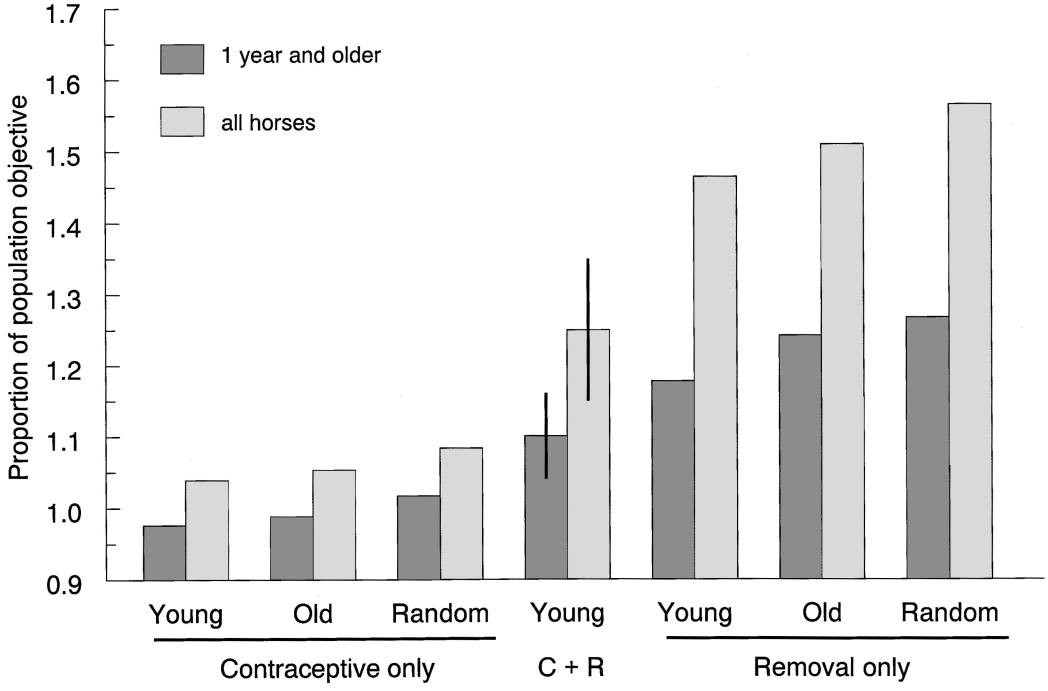

Fig. 1. Average size of populations with (dark bars) and without foals (light bars) as a proportion of population objective; C + R = control using both contraceptives and removals. Population size as a proportion of the population objective varied with the size of the population objective for only C + R treatments; vertical lines show the largest values (for an objective of 90) and smallest value (for an objective of 180).

treatment of an average of 32 to 65 mares per year. For treatments that combined both contraceptives and periodic removals, an average of 50, 58, 61 or 64% of breeding-age mares were rendered infertile each year for population objectives of 90, 120, 150, and 180 horses, respectively. These levels of infertility required treating, on average, 25 to 57 mares each year. When simulated populations were controlled by a combination of removals and contraception, more than 90% of all removals took place at a 5 or 6 year interval regardless of population objective. About 55 horses (range of means = 53–58) were culled from the population each time a removal occurred (Table 3).

For removal-only treatments, more than 90% of removals occurred at three year intervals regardless of population objective or the age of horses removed. Each time a removal occurred, an average about 35% of all horses were removed (range of means = 33 to 37%; Table 3). The number of animals removed was directly related to growth rates; the lowest rate of removal was for treatments of younger horses and the greatest was for random removals (Table 3). Removal of a random selection of horses required harvesting about 20% more horses than when younger horses were removed.

Removals and infertility treatments resulted in populations that had drastically different age structures, but differences in age structure were due entirely to the removal or contraceptive treatment and not to population objective. Within a removal or contraceptive treatment, the proportion of the population in a particular age class remained constant across all population objectives simulated. All simulated removal treatments resulted in age structures characteristic of growing populations, while contraception resulted in the flat age structure characteristic of a stable population (Fig. 2). The average number of foals in the population at the time of a removal varied from 20 to 67 depending on AML and treatment (Table 3). Because foals were not counted towards the AML, populations with a large number of foals in the year of a removal far exceeded the AML in the subsequent year. This occurred because survival rates were high for both foals and adults, and thus the number of foals was far in excess of the number of animals dying from natural causes.

Contraception of older mares or removal of older horses resulted in much shorter generation times than treatments that removed younger females or that delayed reproduction. Average generation time of mares varied by more than a factor of 3, from an average of 4.7 years (contraception of older mares) to a maximum of 16.8 years (contraception of younger mares; Fig. 3). Differences in generation time for males varied far less than that of females, from a minimum of 10.4 years to a maximum of 12.6 years (Fig. 3). Generation time for males was the same for all treatments except for removals of random or old horses; these treatments resulted in shorter generation intervals. For both sexes, differences related to population objectives were small compared to those between treatments that affected different-aged segments of the population.

Table 3
Simulated removal treatments for the Pryor horse population[a]

Age treated	Population objective	Number removed per event (mean±S.D.)	Number removed per year	Proportion removed per event	Harvest interval (years)	Foals at time of harvest
Contraception and removal treatments						
Young age	90	58±10.7	12	0.35	5	20.1
	120	54±9.7	11	0.28	5	20.8
	150	54±10.0	11	0.24	5	22.5
	180	53±9.8	11	0.21	5	23.8
Removal-only treatments						
Young age	90	61±8.3	20	0.34	3	30.2
	120	81±9.7	27	0.33	3	40.2
	150	100±11.1	33	0.33	3	49.9
	180	119±13.1	40	0.33	3	59.8
Old age	90	65±8.2	22	0.35	3	31.9
	120	87±9.9	29	0.35	3	42.6
	150	108±11.1	36	0.35	3	52.8
	180	129±12.4	43	0.35	3	63.3
Random age	90	72±7.7	24	0.37	3	33.7
	120	96±9.0	32	0.37	3	44.9
	150	120±10.3	40	0.37	3	55.9
	180	144±11.4	48	0.37	3	67.1

[a] Mean percent and number of horses 1 year and older removed, and the average number of foals at the time of a removal.

3.2. Effects of treatments on heterozygosity

Initial H_o averaged 0.433 (range of means = 0.432–0.434; range of individual simulations = 0.388–0.479). After 200 years, mean H_o varied from a low of 0.301 (70% of initial H_o), for control by removal of old animals with a population objective of 90, to a high of 0.413 (95% of initial H_o) for populations controlled by contraception and removal of younger mares with a population objective of 180 (Fig. 4). The method of control and age of animal treated or removed had a greater effect on retention or loss of H_o than did population size over the ranges simulated. Treatments that affected older individuals uniformly resulted in more rapid declines in H_o than treatments of young animals. More than 10% of all simulated combinations of population objective and control strategy failed to retain 90% of initial H_o. Using H_o as a measure of genetic diversity, these simulated control strategies thus failed to meet the objective to achieve a 90% probability of retaining 90% of neutrally selective diversity for 200 years.

Inbreeding coefficients (F_{is}) after 200 years of simulation averaged 0.092 (range of means = 0.086–0.096) across all treatments and population objectives. F_{is} tended to be lower for small population sizes, a trend especially pronounced for populations with objectives of 90 and 120 horses. This counterintuitive trend probably resulted from the greater loss of rare alleles in smaller populations, which also reduced H_e in these scenarios (see below).

The use of allele frequencies from the Pryor population resulted in populations in which 34% of all alleles had an initial frequency of 0.05 or less and in which 12% of all alleles occurred at a frequency of 0.02 or less. With an AML of 90, all simulated control strategies resulted in an average loss of more than 25% of alleles (Fig. 5). Almost half (48%) of all alleles failed to persist

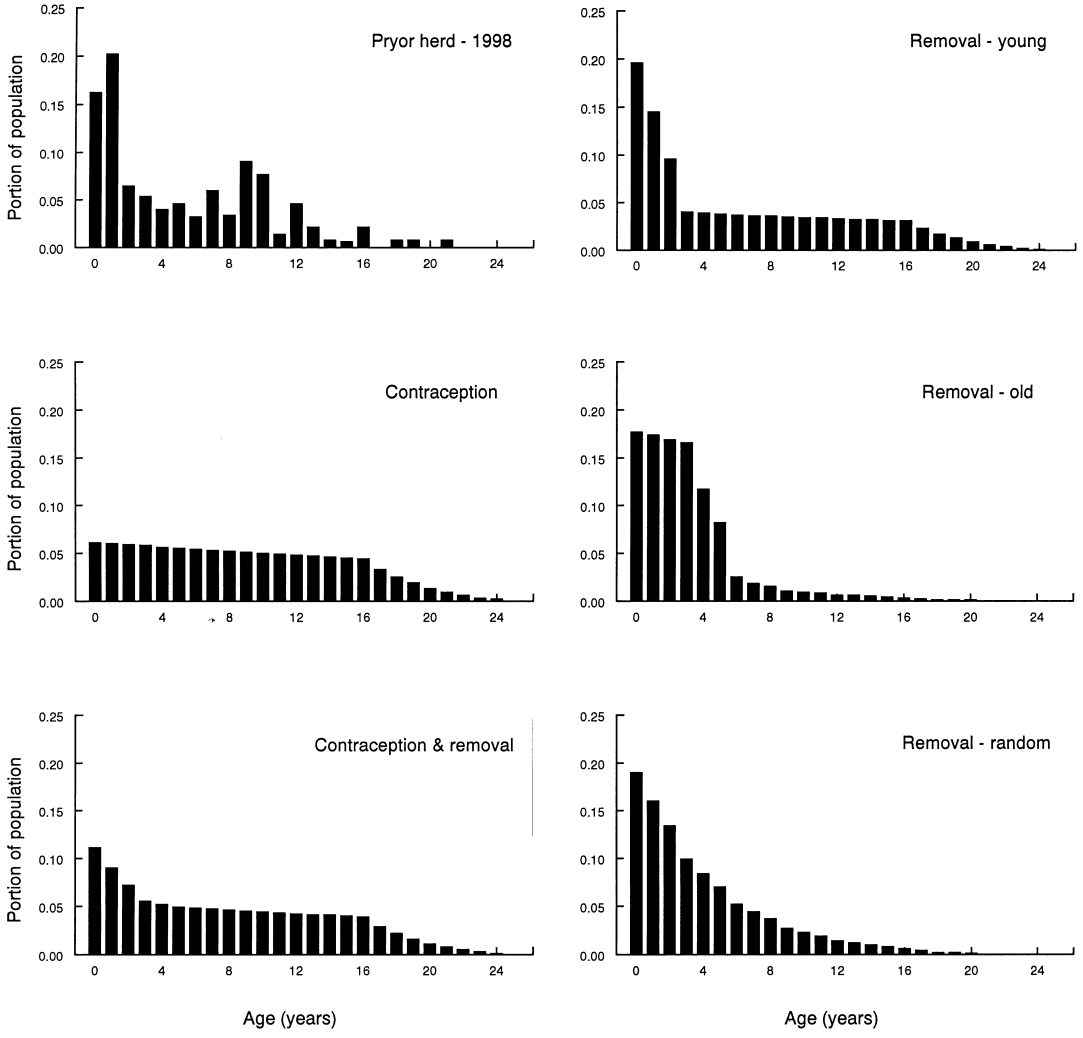

Fig. 2. Age structure of the initial population and simulated populations controlled by contraception, removal, or a combination of both. Age structure of the population was the same regardless of population objective or ages of mares rendered infertile.

for 200 years when old animals were removed from herds with a population objective of 90. Among treatments simulated, contraception of young animals or a combination of contraception and removal of young animals resulted in the greatest persistence of alleles, and an average of 83% of alleles persisted for 200 years under these treatments. These treatments had a 90% chance of retaining 76% of the initial alleles for 200 years. The lower 10th percentile of observations for other treatments included retention of as few as 47% of the initial alleles.

Rates of loss of alleles were highly and non-linearly related to allele frequency. Alleles with an initial frequency of less than about 0.025 tended to be lost very rapid during the first 50 years of simulations. The absolute rate of loss of alleles with initial frequencies greater than about 0.025 tended remain constant throughout the 200 year simulations (Fig. 6).

4. Discussion

4.1. Population control

Simulation results clearly identified contrasting effects of population control by contraception or removals. Compared to removals, infertility treatments reduced variance in population size and resulted in lower standing populations. Because the average sizes of populations managed by infertility were about 25% smaller than those managed by removals, objectives for populations controlled by infertility could be increased to reflect these differences in average size, further enhancing the retention of genetic variation.

Existing chemical contraceptive treatments are effective for a limited period, and they require repeated applications to maintain infertility. Short-duration contraceptives have been used to successfully control growth of horses on Cumberland Island, Georgia and Assateague Island, VA (Kirkpatrick et al., 1997) and they may be suitable for other populations where individuals are easily approached, such as the Pryor herd. A very intensive, and probably very expensive, effort would be required to use short-duration contraceptives as the primary means to control a free-ranging horse population. These treatments are not likely to be successfully applied to widely dispersed populations, but the advantages of contraceptives over removals has stimulated active and promising research that will likely lead to longer-acting contraceptives that can be applied via a "biobullet" or other remote means (Muller et al., 1997). Infertility treatments remain a high priority because they can be far less expensive than removal and adoption programs and they generally receive widespread public support (Garrott et al., 1992; BLM, 1997).

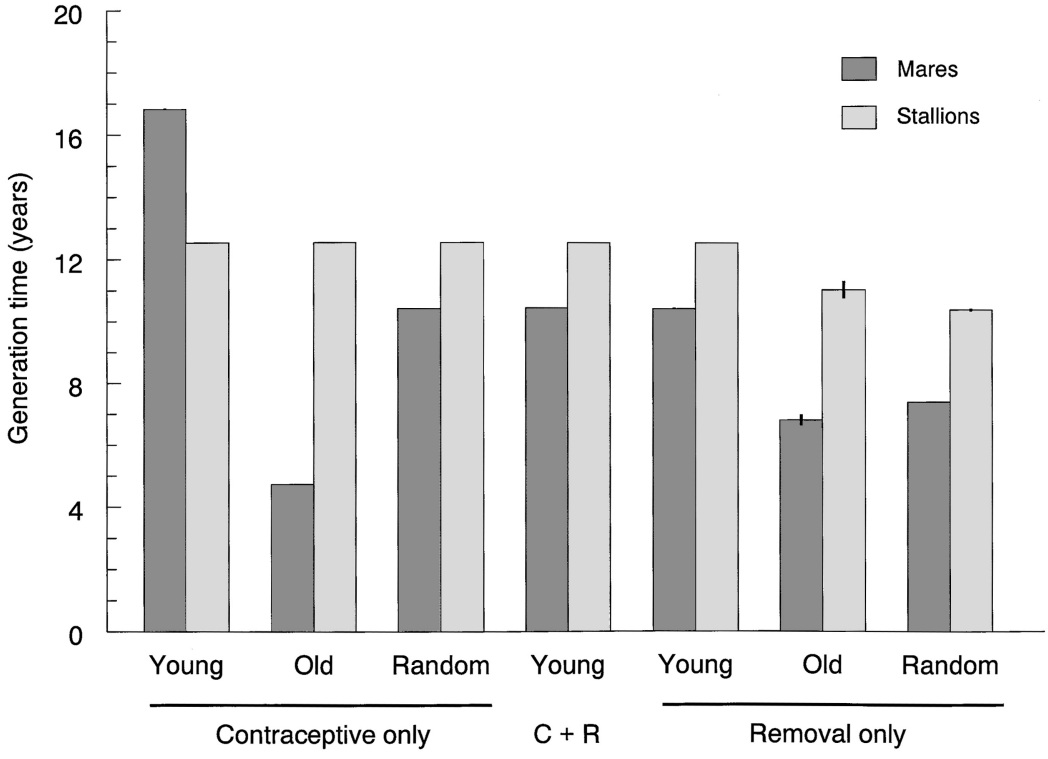

Fig. 3. Generation time of mares (dark bars) and stallions (light bars) in simulated populations controlled by contraceptives only, contraceptives and removal (C + R), or removal only of young, old, or random-age horses. Vertical lines are±1 S.D. of average from populations with management objectives of 90 to 180 horses.

Regardless of the control methods used, intensive management will be necessary to restrict the size of populations with high growth potentials. Contraceptives could eliminate or reduce the need to remove and place wild horses, but a complete reliance on contraceptives can lead to other problems. Contraceptive-only treatments provide no mechanism for controlling the sex ratio of a population, which may become highly skewed, and they provide a rather weak means for controlling populations which have exceeded a size objective. The most flexible and realistic program is thus likely to combine the advantages of infertility and removals. Contraceptives can be used to slow growth rates, while removals provide a means to modify sex ratio or rapidly bring a population into compliance with a size objective.

4.2. Genetic considerations — alternatives to increasing population size?

Simulation results emphasized the importance of age structure and demography on processes that govern the rate of loss of genetic variation. Recommendations for preserving genetic variation have focused on increasing population size, and few papers even consider other strategies that may dramatically affect long-term persistence of genetic diversity. This oversight is not due to misunderstanding among geneticists (Hedrick and Miller, 1992, Fig. 5; Lande, 1995, Fig. 14.4), but rather a lack of appreciation among wildlife mangers and biologists of the important roles of generation time and effective population size in maintaining genetic variation (but see Ryman et al., 1981). Age-specific treat-

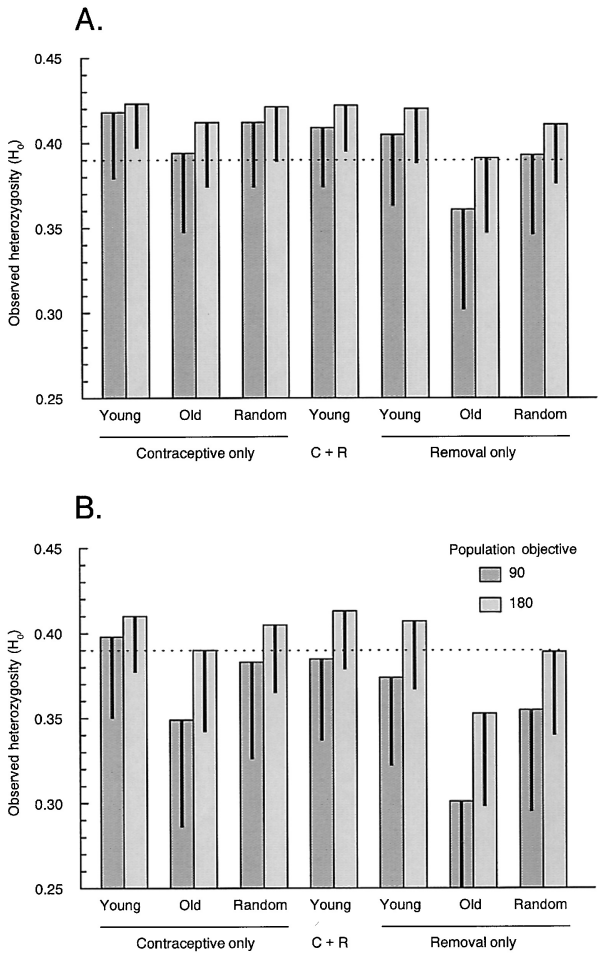

Fig. 4. Observed heterozygosity after 100 years (A) and 200 years (B) in simulated populations controlled by contraceptives only, contraceptives and removal (C + R), or removal only of young, old, or a selection of random-age individuals, for population objectives of 90 (dark bars) or 180 (light bars). Vertical lines descend to the 10th percentile of observations; the horizontal line is at 90% of initial heterozygosity. Initial heterozygosity was 0.433.

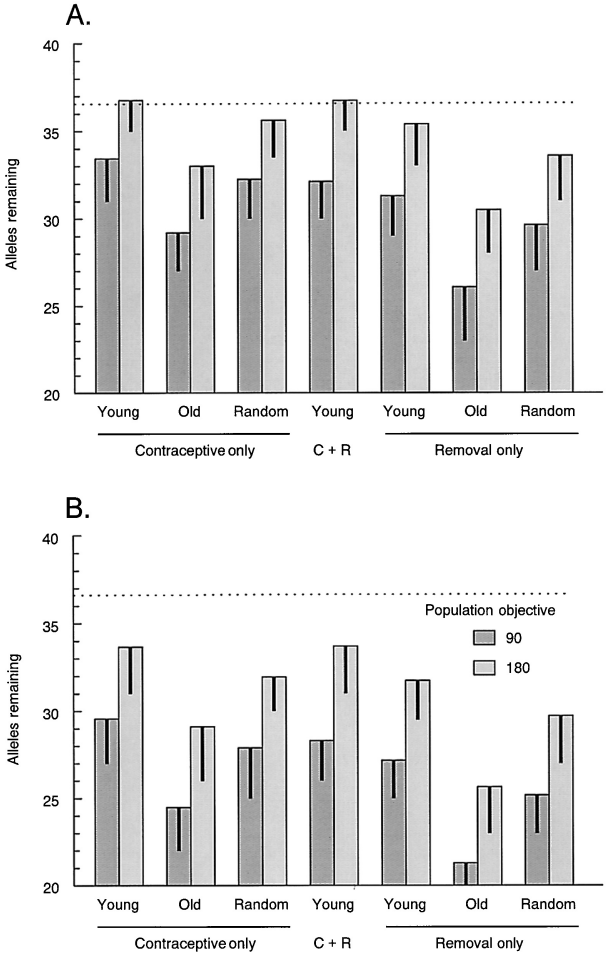

Fig. 5. Number of alleles persisting in simulated populations controlled by contraceptives only, contraceptives and removal (C + R), or removal only of young, old, or a selection of random-age individuals, after 100 years (A) and 200 years (B), for population objectives of 90 (dark bars) or 180 (light bars) horses. Vertical lines descend to the 10th percentile of observations; the horizontal line illustrates 90% of the number of alleleles that were present at the start of simulations.

ments may offer viable alternatives to increases in population size for long-lived species subject to management control. Dramatic differences in the rate of loss of genetic variation between treatments that removed young or old animals are especially pertinent to evaluating management plans because many large mammals of conservation interest are still harvested, either for sport alone or as part of a community-based conservation plan. Both types of harvest usually emphasize removal of older animals from the populations. Consistent harvest of older individuals can have a large effect on retention of genetic variation even when population size exceeds those simulated here by an order of magnitude (Ryman et al., 1981).

The use of multiple measures of genetic variation revealed that under these situations allelic diversity was a much more sensitive indicator of changes in genetic variation than was H_o. Both measures indicated a high probability ($>10\%$) of losing more than 10% of initial variation, even using the best management option simulated. Additional simulations of the contraceptive-and-removal strategy revealed that a population objective of about 500 was necessary to achieve a 90% probability of retaining 90% of alleles, and an objective of 250 was necessary to achieve a 90% probability of retaining 90% of initial observed heterozygosity. It is impossible to implement management plans that achieve levels of contraception or removals as precise as those simulated, thus rates of loss of genetic variation observed under field conditions will vary from those simulated. For treatments of young animals, observed rates of loss of variation will be greater than simulated, while the opposite will likely result from treatments of older animals.

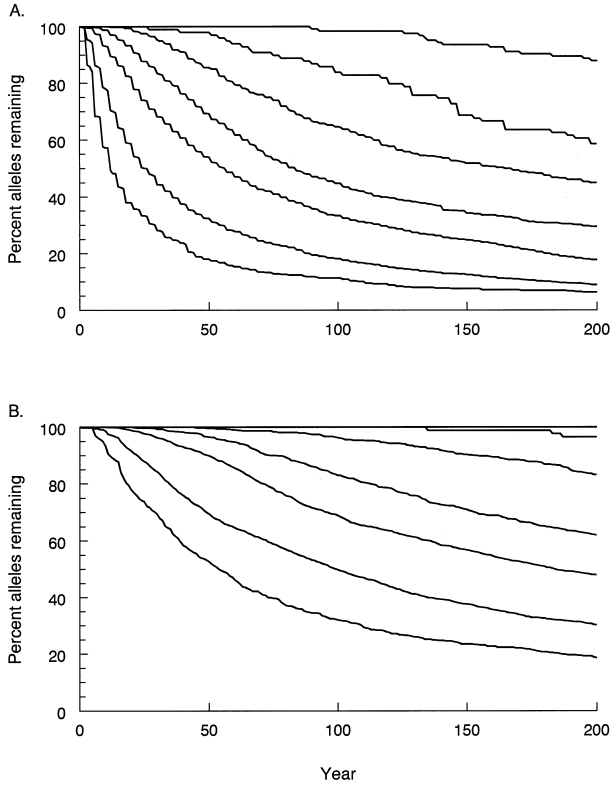

Fig. 6. Persistence of alleles with different initial frequencies over 200 years of simulation. A. Populations controlled by removal of random-aged horses with a population objective of 90. B. Population controlled by contraceptives and removal with a population objective of 150. From the bottom of the plots, lines pertain to alleles that occurred with initial frequencies of 0.005, 0.01, 0.02, 0.05, 0.10, 0.15, and 0.20.

Projected losses of genetic variation were strongly influenced by the frequency distribution of alleles used to initialize simulations. The Pryor population exhibited an unusually high level of heterozygosity (Wooten and Smith, 1985) and it contained many rare alleles. If this population had been isolated for an extended period, many of the rarer alleles would already have been lost and the effects of population size or age of treated animals would have been less. This leads to a rather surprising conclusion: as existing genetic variation declines, fewer individuals are needed to retain a high proportion of the existing variation. This can occur because rare alleles will have already been lost from the population, and those alleles that occur at a relative high frequency are lost slowly (Fig. 6). In natural populations the level of genetic variation will reflect processes that increase and decrease natural variation. Mutations increase variation, but they occur at a slow rate and new variation must be maintained to contribute to long-term heterozygosity. On the other hand, genetic drift results in loss of variation.

In this model, changes in genetic variation resulted only from genetic drift. While the inclusion of mutations would have resulted in lower rates of loss of variation, genetic drift is by far the dominant process leading to changes in gene frequencies in small populations (Nunney and Campbell, 1993; Lynch, 1996). This occurs for two main reasons. First, sampling errors are exacerbated in small populations, and second, the proportion of genetic variation that is selectively neutral increases as population size declines. Selection tends to be far less important in small populations because a much larger selection coefficient (selective advantage) is necessary for selection to act in a deterministic manner as population size declines. For quantitative characters, this situation arises from the distribution of selective forces over a large number of loci, thus selection on any one locus is small enough to be overwhelmed by random genetic drift (Lynch, 1996). A consequence of the dominance of random drift is that an allele with strongly negative effects can become fixed in the population. In large populations, a small selective disadvantage may be sufficient to reduce the frequency of a deleterious allele, whereas this is unlikely to occur in small populations.

Several factors not included in the simulation analysis could result in more rapid loss of genetic variation than these results suggest. Periodic catastrophes could lead to temporary reductions in population size. The Pryor population suffered high winter loss in 1977–1978 (Garrott and Taylor, 1990), and catastrophes are thought to be an important factor in other large mammal populations (Young, 1994, 1999; Ebb and Boyce, 1999). Other factors that may be important and that require further investigation include density dependence, compensatory reproduction, and the role of environmental stochasticity. All of these factors could increase the rate of loss of genetic variation. The results of these simulations should thus be interpreted conservatively if they are used to guide management decisions.

These simulations illustrated several important genetic and demographic concepts. For managed populations, actions that delay the age of reproduction and increase generation interval will reduce the loss of genetic variation. Allelic diversity can be a much more sensitive index to loss of genetic variation than population-level heterozygosity, especially when there are many alleles at a locus. Compared to periodic removals, use of contraceptives under simulated conditions resulted in smaller populations and a much less variation in population size. Management plans that use contraceptives to control the rate of growth and that employ occasional removals to control sex ratio and reduce population size could emphasize the advantages of both approaches, while avoiding the most undesirable characteristics. Overall, these results support use of management strategies that rely less on removal and more on infertility treatments to reduce long-term loss of genetic variation.

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Effects of sterilization on movements of feral cats at a wildland–urban interface

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Journal of Mammalogy, 91(2):482–489, 2010

Effects of sterilization on movements of feral cats at a wildland–urban interface

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Trap–neuter–release (TNR) programs, in which feral cats are sterilized and fed in unconfined colonies, have been advocated as a humane and effective way to reduce the impacts of feral cats on native wildlife. Little is known, however, about the effects of sterilization on feral cat movements and space use, particularly where colonies are located near natural areas. We determined home-range area and overlap and characterized the long-range movements of 14 sterilized and 13 intact radiocollared cats on Catalina Island, California, from 2002 to 2004. Male home ranges were significantly larger than those of females, but no significant differences were revealed in home-range areas or overlap between sterilized and intact cats. Cats regularly moved between natural habitats in the interior of the island and human-populated areas regardless of sex or treatment status, although most (68%; 17/25) of the cats that moved long distances were female. Island-wide, the cat population was estimated to be 600–750 cats, with >70% associated with developed areas, including existing TNR colonies. The influx of subsidized cats to natural habitats, combined with their high vagility and low trappability, makes TNR an unlikely solution for controlling feral cats on a large, rugged island like Catalina and, more generally, in other locations where human populations abut ecologically sensitive areas. DOI: 10.1644/09-MAMM-A-111.1.

Key words: California Channel Islands, Catalina Island, feral cats, home range, invasive predators, island conservation, trap–neuter–release

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Domestic cats (*Felis catus*) have been introduced to islands and continental areas worldwide where they have been implicated in the extinction of native species (Dickman 1996; Nogales et al. 2004). Free-roaming cats harm native wildlife in several ways. First, cats are opportunistic predators, with large effects on prey that are naïve to cat predation (Courchamp and Sugihara 1999). Second, free-roaming cats can compete with native predators for prey. Human-subsidized cats can reach densities exceeding several hundred cats/km^2 (Liberg et al. 2000), and individuals can spill over into less densely populated wildland areas where they reduce prey for native predators (George 1974). Finally, free-roaming cats often carry diseases and parasites that can spread to wildlife (Jessup et al. 1993; Riley et al. 2004).

The introduction of feral cats has been particularly devastating for island species (Nogales et al. 2004). Although eradication and control of invasive predators has been successful on uninhabited islands, such efforts are more complicated on islands inhabited by humans and their pets and by nontarget wildlife (Levy and Crawford 2004). A nonlethal approach to controlling cat populations, known as trap–neuter–release (TNR), involves trapping and sterilizing cats and returning them to unconfined ''colonies,'' where they are fed daily by volunteer caretakers. Longcore et al. (2009), however, point out that the TNR phenomenon is received by the public as an issue of animal welfare, not one of environmental impact. As a result, although TNR is considered only an interim solution to cat overpopulation (Slater 2002), it has been widely implemented to control feral cat populations in urban, rural, and wildland areas, often with detrimental effects on native wildlife (Jessup 2005). In reality, most TNR programs are volunteer-based, understaffed, and cannot sterilize all cats in a colony or monitor colony health or population changes over time (Jessup 2004). Even in intensively managed colonies with a high rate of sterilization (75–80%), the estimated time to extinction of a TNR colony may be more than a decade (Nutter 2005). Finally, TNR research fails to address critical issues such as spillover predation by cats in adjacent wildland areas, zoonotic and wildlife disease risks, and the health and welfare of feral cats (Castillo and Clarke 2003). Thus, many of the actual

consequences of TNR remain poorly documented, making it difficult to assess the true efficacy of such programs.

The feral cat population on Catalina Island provides an ideal opportunity to assess the impact of TNR on cat populations living at the wildland–urban interface. Cats likely were first introduced to the Channel Islands off California, during the 19th century (Phillips and Schmidt 1997), and feral populations are currently resident on 3 of the 8 Channel Islands: San Nicolas, San Clemente, and Santa Catalina (hereafter, Catalina). Catalina has a human population of approximately 4,000 permanent residents and a tourism-based economy with effectively no regulation of exotic or domestic animals brought to the island (Guttilla 2007). Human activity is restricted primarily to the periphery of the island, with the interior of Catalina consisting of rugged, relatively intact native habitat. At the same time, for >20 years, the Catalina Island Humane Society has practiced TNR at designated cat colonies in Avalon and Two Harbors, the 2 largest human settlements on the island. Proponents of TNR have argued that colony-fed sterilized cats pose little threat to native species in the adjacent wildland interior and that because sterilized cats roam less than reproductively intact cats but retain their territorial behavior (Hawkins et al. 2004; Zaunbrecher and Smith 1993), they should prevent immigration of new cats (i.e., the vacuum effect—Mahlow and Slater 1996; Neville and Remfry 1984), thereby eventually leading to population decline due to attrition.

To explore the effects of sterilization on the persistence of feral cat populations we examined the home-range behavior and movements of sterilized and intact radiocollared feral cats living in the interior of Catalina. We predicted that home ranges of sterilized cats would be smaller than those of intact cats because the former would not wander as far in search of mates. In addition, we predicted that sterilized cats would be more sedentary and less territorial and would therefore exhibit greater overlap in their home ranges than intact cats (Castillo and Clarke 2003; Lee et al. 2002). We also examined the extent of movement of cats between the interior and developed areas, including TNR colonies, to determine whether these areas are a source of cats for other parts of the island. Last, we used our livetrapping efforts to estimate the population size of feral cats on Catalina Island. Although our results apply most directly to the management of cats on Catalina, they should improve our understanding of the consequences of maintaining large, unrestricted populations of human-subsidized cats adjacent to ecologically sensitive continental areas.

Methods

Study site.—Catalina is the 3rd largest (194 km^2) of the California Channel Islands, which are located approximately 35 km off the coast of Southern California (Fig. 1). The island is 13 km wide and 22 km long, with rugged terrain ranging in elevation from sea level to 631 m. It has a Mediterranean climate characterized by hot, dry summers and cool, wet winters. Annual temperatures range from 9°C to 24°C, with a mean (± 1 *SD*) annual precipitation of 300 (± 146) mm (Catalina Island Conservancy, www.catalinaconservancy.org). The predominant types of vegetation are coastal sage scrub, island chaparral, and grassland (Knapp 2005). The wet season (November–April) generally corresponds to the cat breeding season, with little reproduction by feral cats reported during the drier part of the year (May–October).

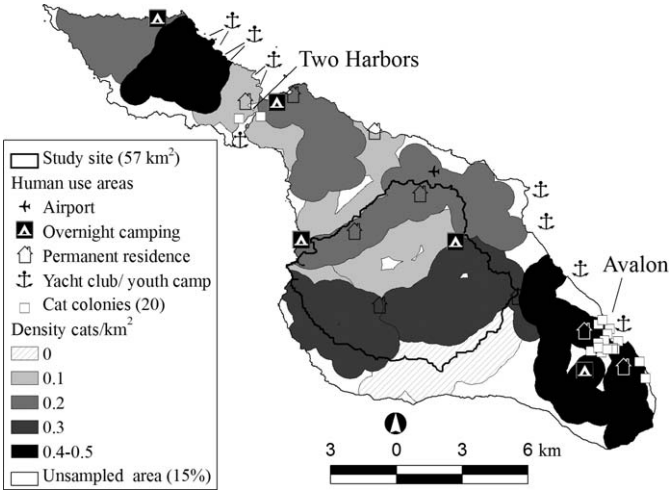

Fig. 1.—Feral cat densities estimated from 2004 island-wide stratified trap efforts on Catalina Island. Filled areas represent bands of different cat densities, on the basis of the number of captures in different trap lines (see text for procedure used to estimate area sampled for determination of densities). Human-inhabited areas and known TNR (trap–neuter–release) colonies are shown because of the higher cat densities observed near those areas. The dark line represents the boundary of targeted trapping efforts, which was approximately 57 km^2 or 30% of the island.

Animal capture and handling.—To capture cats for radiotracking we conducted targeted trapping in the Middle Canyon and Cottonwood Canyon watersheds (57 km^2) on the eastern half of Catalina (Fig. 1). Targeted trapping consisted of setting traps along roads, trails, canyon bottoms, and ridge tops where we detected fresh cat sign; trapping efforts were not standardized with respect to duration or trap placement. In 2004, these efforts were expanded with island-wide transects (250–350 m) placed systematically along the interior road system. We trapped cats with wire-mesh live traps (Model 106; Tomahawk Live Trap Co., Tomahawk, Wisconsin) that were custom-manufactured with smaller mesh to reduce tooth injuries. Traps were covered with industrial shade cloth and equipped with bite bars consisting of nontoxic plastic hose affixed to the back of traps with wire (Guttilla 2007). Traps were lined with grass and baited with wet and dry commercial cat food. We anesthetized cats in traps with an intramuscular injection of ketamine HCl (10 mg/kg) and acepromazine (0.1 mg/kg). Cats were weighed and tested for exposure to feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). Tests for FIV and FeLV antibodies were run in-house using a commercially available enzyme-linked immunosorbent assay test kit (Snap Combo kit; IDEXX Veterinary Laboratory, West Sacramento, California). FeLV- and FIV-

positive cats were euthanized to prevent infected cats from suffering disease symptoms, to reduce opportunities for disease transmission to other cats and wildlife, and to minimize the loss of experimentally sterilized cats.

We marked adult cats with passive integrated transponder tags (AVID, Inc., Norco, California; Biomark, Inc., Boise, Idaho), which were implanted subdermally between the shoulder blades. A subset of 27 of the adult cats captured was equipped with 45-g radiocollars (Communication Specialists, Orange, California). Fourteen (7 male, 7 female) of the radiocollared cats were sterilized using standard spaying and neutering procedures (Guttilla 2007). The remaining 13 (5 male, 8 female) were left intact as controls. Cats were selected for sterilization opportunistically, as determined by the availability of a veterinarian (on contract with the Institute for Wildlife Studies, Arcata, California) on-island and the timing of cat captures (Guttilla 2007). All radiocollared cats were negative for both FIV and FeLV and weighed at least 1.5 kg. To identify cats as having been sterilized, approximately 1 cm of the distal portion of left ear was cut (tipped). Ear-tipping is common practice in TNR programs (Scott et al. 2002), and TNR colony cats on Catalina have their right ear tipped (Guttilla 2007). All procedures were consistent with guidelines set by the American Society of Mammalogists (Gannon et al. 2007) and were approved by the Institutional Animal Care and Use Committee at California State University Fullerton.

Radiotelemetry.—Before radiotracking cats we placed radiocollars at known locations to determine accuracy of directional bearings in estimating cat locations, giving an estimated bearing error of 12° (White and Garrott 1990). We radiotracked cats from November 2002 to July 2004 using an R-1000 radio receiver and hand-held 3-element Yagi antenna (Communication Specialists, Orange, California). Most locations (83%; $n = 1{,}614$) were recorded between sunset and sunrise when cats are most active (Barratt 1997). We attempted to obtain 3 locations per cat per week. For each fix a single observer moving on foot or by vehicle triangulated the location of the cat. Mean ($\pm$ 1 *SD*) time between the 1st and last bearings for a fix was 21 ($\pm$ 16) min ($n = 2{,}004$ locations). We tracked individual cats for 1–4 wet/dry seasons (mean $\pm$ 1 *SD*: 2 $\pm$ 1 seasons); tracking continued until either collar failure or death of the collared cats.

Home-range analyses.—We plotted telemetry fixes in location of a signal (LOAS 2.07; Ecological Software Solutions, Urnäsch, Switzerland) using the maximum likelihood estimator (MLE) algorithm, with best biangulation as the default estimator when MLE failed. LOAS converts radiolocation estimates into a geographic information systems format so that home-range analyses can be performed. Home-range boundaries were determined in ARCVIEW 3.2a, using the spatial analyst (Environmental Systems Research Institute Inc., Redlands, California) and animal movement (Hooge and Eichenlaub USGS, Anchorage, Alaska) extensions. Home-range boundaries were calculated using minimum convex polygon (MCP) and fixed kernel procedures (KHR, optimized by least-squares cross validation—Worton 1989); 2 procedures were used to better account for differences in the precision and assumptions associated with each estimator (Harris et al. 1990). Home-range area was calculated from 95% of the radio fixes for each animal (95% MCP, 95% KHR), assuming that the remaining 5% of fixes, as determined by the software, were outliers that represented excursions. Core home-range, defined as the area used disproportionately more than other areas (Samuel et al. 1985), was estimated using 50% KHR.

All statistical analyses were performed in SAS v. 9.1 (SAS Institute Inc., Cary, North Carolina). Home-range data were analyzed by season (breeding, nonbreeding), with 18–50 fixes (28 $\pm$ 6; mean $\pm$ 1 *SD*) per cat per season tracked. We used Pearson correlations (r) to determine if a relationship existed between the number of fixes and home-range area that would indicate that we had sufficient numbers of fixes to estimate home-range areas. Initial inspection of these data also indicated that they did not meet the assumptions of parametric tests, and thus estimates of home-range area were natural log-transformed before analysis. We used a 2-way analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) tests to determine the effects of sex and reproductive status (treatment = intact versus sterilized) on the areas of the home ranges occupied by our study animals during the breeding and nonbreeding seasons. We used t-tests to compare home-range areas of cats, pooling across sexes and treatments, and Pearson correlations to determine the relationship between home-range area and body size.

Home-range overlap was calculated for all pairs of overlapping cats during a given season and year, using both 50% and 95% KHR estimates. The area of overlap for a given pair of cats was calculated as the percentage of each cat's home range during a given season and year. Percentage overlap data were normalized by arcsine-square root transformation. We used 3-way ANOVAs to determine the effects of season, reproductive status (sterilized versus intact), and sex on percentage overlap followed by Tukey HSD test for pairwise comparisons subsequent to significant ANOVA outcomes.

Long-distance movements.—We recorded long-distance movements of radiocollared cats from their home ranges in the island interior to areas associated with human activities. These were calculated as the linear distance between the last observation within a cat's home range and the 1st observation in a new area, and were equivalent to $\geq$2 diameters of a given cat's home range. Long-distance movements also were recorded for uncollared TNR colony cats trapped in the interior of the island; these animals were identified visually on the basis of their right-tipped ears. These cats came from TNR colonies located in Avalon and Two Harbors. We estimated the linear distance from trap locations to both Avalon and Two Harbors and recorded the shorter of the 2 distances. All estimates of movements and distance traveled were conservative because they did not take into account the route traveled or the topography of the island.

Table 1.—Estimates of the numbers of free-roaming feral cats in the interior of Catalina Island, California, between 2002 and 2004 on the basis of trapping, direct observations, and spotlight counts. Spotlight surveys were conducted in November 2003 and January–September 2004 (Stapp and Guttilla 2006). The estimated population size assumes that only 50% of the interior population was trapped in a given year.

Method	Year		
	2002	2003	2004
Targeted and systematic trapping	50	70	75
Known to be alive (visuals or radiocollars) but not trapped	53	66	9
Monthly spotlight surveys		5	22
Total known individuals	103	141	106
Estimated population size	128	176	144

Population size and density.—We estimated population density of feral cats from targeted and systematic trapping efforts as the number of individual cats captured divided by the effective trapping area. The effective trapping area was calculated by assuming that the area covered by each trap was equivalent to the mean 95% MCP home-range area (1.56 km^2), resulting in a 705-m radius buffer around each location; areas then were summed for each trapping transect. The total area of coverage from targeted efforts ranged from 39 to 77 km^2, whereas systematic, island-wide efforts in 2004 had an effective area of 165 km^2 (85% of the island). Trap success was adjusted to exclude disturbed traps or those that captured nontarget species. To estimate the size of feral cat population in the interior of the island we combined capture records from targeted and systematic trapping with information on radiocollared cats known to be alive (Stapp and Guttilla 2006). We also surveyed TNR colony caretakers in Avalon and Two Harbors and interior rural residents to estimate the number of colony and feral cats that were dependent on human foods. These two values were summed to estimate the total number of free-roaming cats on Catalina.

Results

We trapped 142 cats between May 2002 and October 2004. Forty-one (28.9%) of 134 cats tested positive for FeLV and/or FIV (30/73 male, 11/61 female) and were euthanized (FeLV prevalence = 15.7% [13 male, 8 female]; FIV prevalence = 17.9% [20 male, 4 female]). These included 3 (17%) of 18 previously sterilized TNR colony cats that were caught in the interior of the island. Of the remaining 101 cats, 35 sterilized (15 previously sterilized; 20 sterilized in our study) and 66 intact animals were released at the point of capture. Fourteen of the sterilized individuals (7 male, 7 female) and 13 of the intact animals (5 male, 8 female) were the radiocollared animals used to assess home-range size and overlap.

Trap success for both targeted and island-wide trapping (9,242 trap-nights) was low (combined mean $\pm$ 1 *SD* = 2.7% $\pm$ 0.4%; $n = 3$ years). Cat densities from targeted trapping (0.8 $\pm$ 0.4 cats/km^2) were higher than those for island-wide efforts (0.3 $\pm$ 0.1 cats/km^2). The highest densities during island-wide trapping were near areas of human settlement (Fig. 1). The west end of the island, which supports several camps and yacht clubs, also had high cat densities (0.5/km^2), although estimated densities near Two Harbors (0.1–0.2 cats/km^2), the location of several TNR colonies, were surprisingly low.

Feral cats were difficult to recapture; nearly 50% of cats known to be alive in 2002 and 2003 evaded traps (Table 1). Under the simple model that only 50% of the population was trappable in a given year, estimates from all sources suggested that the feral population in the interior ranged from 128 to 176 cats across years. Similarly, if we extrapolate the range of feral cat densities generated from trapping (0.3–0.8 km^{-2}) to the entire island (194 km^2), estimates of the interior feral cat population size ranged from 58 (0.3 km^{-2}) to 155 (0.8 km^{-2}) cats. Assuming that the numbers of TNR colony (471) and interior feral cats (85) reported by residents were stable over time, we suggest that approximately 614–732 free-roaming cats were on the island during our study.

We recorded 2,004 fixes for the 27 animals monitored via radiotelemetry between November 2002 and July 2004. Four cats were tracked during all 4 seasons, 9 cats were tracked for 3 consecutive seasons, 4 cats were tracked for 2 consecutive seasons, and the remaining 10 cats were tracked for 1 season. To account for differences in the number of seasons during which individuals were monitored, mean seasonal home-range sizes were calculated for the 13 cats that were tracked for 2 dry or 2 wet seasons. Mean ($\pm$ 1 *SD*) number of fixes per season per cat was 29 $\pm$ 6, with no significant correlation between the number of fixes and home-range area ($n = 61$; 95% MCP: $r = -0.005$, $P = 0.975$; 95% KHR: $r = -0.062$, $P = 0.682$).

For all 3 home-range estimators used (95% MCP, 95% KHR, 50% KHR), sex was the only factor that significantly affected home-range area, and only during the dry, nonbreeding season (dry season: overall model, $d.f. = 3, 16$, $P \leq 0.027$, sex effect $P \leq 0.004$, treatment, sex $\times$ treatment effects $P \geq 0.299$; wet season: overall model $d.f. = 3, 20$, $P \geq 0.089$). Combining treatments, mean home-range area for males was 2–4 times larger than that for females, depending upon the estimator (Table 2; *t*-tests, dry season, $P \leq 0.002$; wet season, $P < 0.064$). Although seasonal home ranges of intact and sterilized females were similar, home ranges of intact males tended to be larger (although not statistically different; $P \geq 0.173$ for the 3 estimators) than those of sterilized males during both seasons (Table 2). The large variation in 95% KHR estimates of home ranges for intact males was largely the result of 1 individual that had a home range in both the breeding (13.6 km^2) and nonbreeding (18.7 km^2) seasons that was 2–4 times larger than the average for intact males. Home-range area was not correlated with body mass for males ($n = 12$, $P \geq 0.550$ for the 3 estimators), and correlations between home-range area and mass for females were marginally nonsignificant for 50% KHR ($n = 15$, $r = -0.49$, $P = 0.065$) and 95% KHR ($r = -0.45$, $P = 0.089$).

TABLE 2.—Home-range area estimates (km^2; mean ± 1 *SE*) of sterilized and intact feral cats radiotracked during dry (nonbreeding) and wet (breeding) seasons on Catalina Island between November 2002 and July 2004.

	Dry (nonbreeding) season			Wet (breeding) season		
Treatment group	*n*	95% MCP	95% KHR	*n*	95% MCP	95% KHR
Female intact	6	0.8 ± 0.2	1.5 ± 0.3	7	1.7 ± 0.6	1.9 ± 0.4
Female sterilized	6	0.9 ± 0.2	1.7 ± 0.4	6	1.3 ± 0.5	3.1 ± 1.4
Male intact	4	2.8 ± 1.0	8.2 ± 3.9	5	3.7 ± 1.2	7.4 ± 2.1
Male sterilized	4	2.1 ± 0.3	3.8 ± 0.3	6	2.2 ± 0.6	4.1 ± 1.1
All males	8	2.5 ± 0.5	6.0 ± 2.0	11	2.9 ± 0.6	5.6 ± 1.2
All females	12	0.9 ± 0.1	1.6 ± 0.3	13	1.5 ± 0.4	2.5 ± 0.7
All cats	20	1.5 ± 0.2	3.4 ± 0.9	24	2.1 ± 0.4	3.9 ± 0.7

We observed no difference in home-range overlap between seasons, sex, or treatment groups for 50% KHR ($n = 58$ pairs; overall $F_{7,50} = 1.39$, $P = 0.231$). For 95% KHR, sex was the only factor that was significantly associated with differences in overlap ($n = 248$ pairs; overall $F_{9,238} = 2.00$, $P = 0.040$; sex effect: $F_{2,238} = 4.88$, $P = 0.008$). Males overlapped significantly more with other males (mean ± 1 *SE*: 23.5% ± 2.7%) and with females (20.6% ± 2.0%) than females overlapped with each other (10.3% ± 1.5%; Tukey HSD test, $P < 0.05$). Neither season ($F_{1,238} = 0.06$, $P = 0.811$), reproductive status ($F_{2,238} = 1.47$, $P = 0.232$), nor the interaction of sex and reproductive status ($F_{4,238} = 1.09$, $P = 0.363$; Fig. 2) significantly influenced the degree of home-range overlap.

Eighteen previously sterilized cats (13% of captures, identified by tipped right ears) were trapped in the island interior (Fig. 3a) ≥10 km from Two Harbors and Avalon. Most of these were females (12, versus 6 males). Long-distance movements from the island interior into peripheral, human-populated areas were recorded for 7 (26%) of the 27 radiocollared cats, including animals of both sexes (2 males, 5

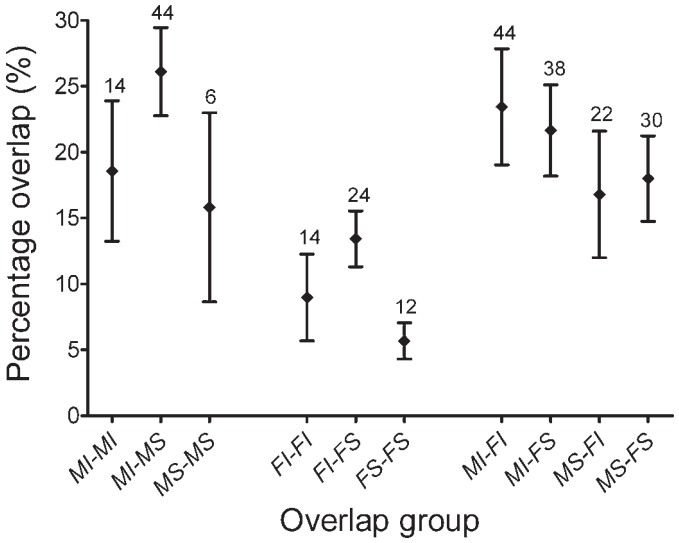

FIG. 2.—Mean percent home-range overlap (± *SE*) of intact and sterilized feral cats, grouped by sex, estimated by fixed kernel (95% KHR). Sample sizes of overlapping groups are listed above error bars. MI = intact male; MS = sterilized male; FI = intact female; FS = sterilized female.

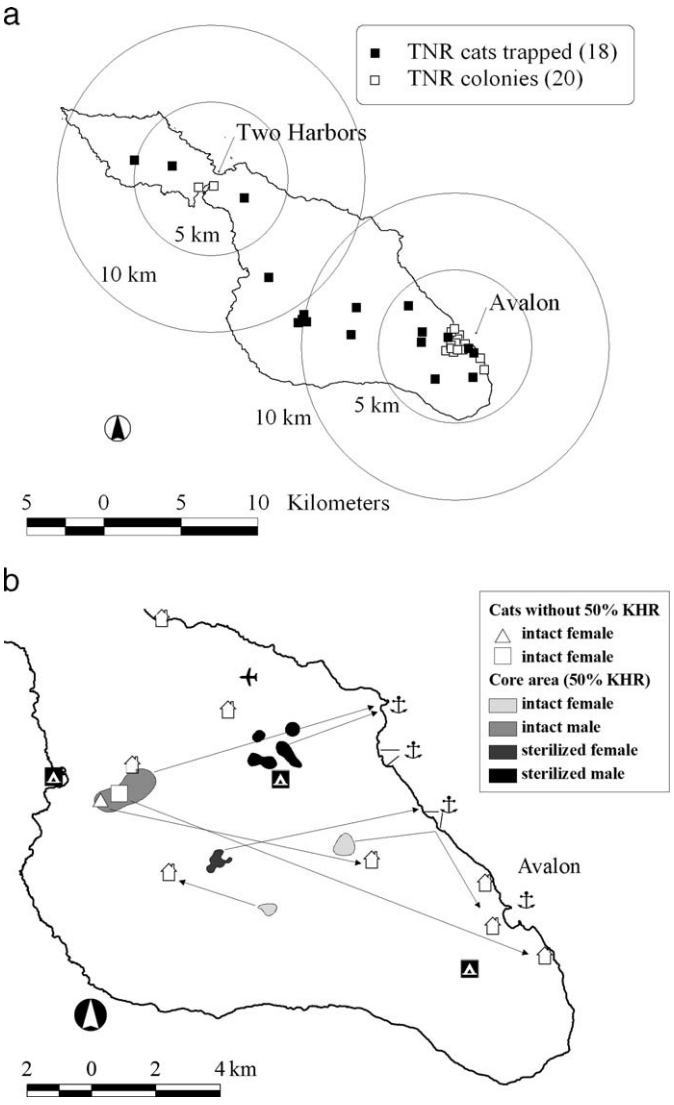

FIG. 3.—a) Trap locations of sterilized colony cats (TNR cats; $n = 18$) captured in the interior of Catalina Island in 2003–2004. Circles denote distances of 5 and 10 km from TNR colonies in Avalon and Two Harbors. b) Long-distance movements of 7 radiocollared cats from the wildland interior into human areas. Polygons are 50% KHR core home-range areas of cats before movements in the direction of the arrows. The open triangle and square denote trap locations of 2 intact females for which core areas could not be calculated before movements. See Fig. 1 for description of other symbols of human use areas.

females) and both intact and sterilized cats (5 intact, 2 sterilized). The mean (± *SD*) distance moved by radiocollared cats was 8 (± 4) km (Fig. 3b), although movements exceeding 14 km were recorded. On average, females (9 ± 4 km) tended to move farther than males (6 ± 3 km), although sample sizes were too small for meaningful statistical analysis.

Discussion

Home ranges of male feral cats were significantly larger than those of females, a finding that has been reported elsewhere (Liberg et al. 2000). However, contrary to our predictions, we found no significant differences in home-range areas of sterilized and reproductively intact cats of either sex. We caution that although we tracked many cats across multiple seasons, sample sizes, especially for males, were relatively low. We expected males to vary more in ranging behavior, suggesting that very large numbers of individuals might be needed to estimate range size with any precision. Our results, however, provide little evidence that sterilization markedly reduces ranging behavior for either sex, at least for cats sterilized as adults.

Previous studies (e.g., Fettman et al. 1997) have revealed that sterilization does not reduce feeding activity and may actually increase longevity (Levy and Crawford 2004). Together with these findings, our results suggest that sterilization likely would not reduce the impact of feral cats on native prey, which, on Catalina, includes a variety of small mammal, bird, amphibian, reptile, and invertebrate species (Guttilla 2007). In addition, the removal of diseased cats before sterilization—a common practice in some TNR programs (Hughes and Slater 2002)—may result in a healthier population than one where sterilization is not conducted. Although this may benefit free-roaming pet cats that interact with feral ones (and ultimately, wildlife), returning healthy cats, even if sterilized, to the environment has no clear ecological benefits.

Our results also revealed that, contrary to our predictions, sterilization had no effect on the degree of home-range overlap among individuals. Because uncollared cats were known to be present in our study area, our measurements of home-range overlap might have underestimated the actual overlap among feral cats. Extensive overlap among male cats has been reported for other islands (Harper 2004; Konecny 1987), although the lower frequency of male–male overlap observed for core areas relative to 95% use areas suggests that core areas may be defended. Sterilized and intact males behaved similarly, however, suggesting that territorial behavior was not affected by being neutered (see also Nutter 2005). Because home ranges of sterilized cats did not overlap more than those of intact cats, sterilization alone would not necessarily lead to an increase in cat densities.

On the surface our finding that sterilization did not significantly change the spatial distribution of feral cats appears to support the argument that sterilization prevents immigration of new animals (i.e., the vacuum effect) and therefore may lead to population decline via attrition. This argument, however, assumes that no consistent source of new, unsterilized cats exists. We found evidence of considerable long-distance movement of both sterilized and intact cats between the wildland interior and developed areas; most of the cats moving long distances were females. The presence of TNR colony cats in the island interior was either the result of cats dispersing from unnaturally high densities near feeding areas or from the deliberate, illegal release of unwanted colony cats into the interior. The number of radiocollared cats observed moving from wildland to populated areas, however, suggests that long-distance movements may be common and that these populations are not distinct.

Our study also provides the 1st estimates of population size for feral cats on Catalina (600–750 cats). These data suggest that the numbers of cats dependent on humans may be 3–4 times larger than the feral population in the island interior. The low trappability of cats in our study population suggests that this estimate is likely conservative. The higher cat densities observed near some developed areas may be spillover from overcrowded conditions associated with TNR colonies and unsterilized pets, which may affect native prey populations in areas immediately adjacent to colonies. For example, low trap success near TNR colonies at Two Harbors, where some 175 stray and colony cats were present but not caught (Guttilla 2007), corroborates previous studies (e.g., Nutter et al. 2004) suggesting that cats become trap-wary, making them difficult to capture and complicating sterilization and control efforts.

From a management perspective our study revealed that hundreds of stray and feral cats live on Catalina. Collectively, our findings suggest that an island-wide TNR program would probably fail to alleviate threats of feral cats to wildlife and would run counter to efforts to protect vulnerable species and restore native ecosystems. TNR also does not address public health risks and nuisance issues in populated areas of Catalina, and sterilized cats are still susceptible to diseases and parasites that can be transmitted to wildlife, humans, and pets (Lee et al. 2002). For these reasons and out of consideration of the welfare of the cats themselves, many animal welfare organizations, including the American Society for the Prevention of Cruelty to Animals, People for the Ethical Treatment of Animals, American Veterinary Medical Association, and Humane Society of the United States currently oppose TNR programs in and adjacent to ecologically sensitive areas where wildlife may be at risk (Guttilla 2007).

We believe that efforts to minimize the effects of feral cats on the interior of Catalina depend upon effective management and control of high-density cat populations in Avalon, Two Harbors, and other coastal areas. Until resources are available to implement more proactive control measures in these areas, cats trapped in the island interior should be removed and delivered to a shelter; if they are deemed adoptable, cats should be sterilized and added to the adoption pool on the mainland. If they are not adoptable or if there are insufficient resources to support relocation, they should be euthanized. Because TNR colonies in human-populated areas of the island

are a likely source of feral cats to maintain an interior cat population, steps should be taken to reduce and eliminate these colonies and adopt and enforce stricter policies for responsible pet ownership and regulation of the importation of exotic animals to the island.

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Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline

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Most of the white-tailed eagle (*Haliaeetus albicilla*) populations in Europe experienced dramatic declines during the twentieth century. However, owing to intense conservation actions and the ban of DDT and other persistent pollutants, populations are currently recovering. We show that despite passing through demographic bottlenecks, white-tailed eagle populations have retained significant levels of genetic diversity. Both genetic and ringing data indicate that migration between populations has not been a major factor for the maintenance of genetic variability. We argue that the long generation time of eagles has acted as an intrinsic buffer against loss of genetic diversity, leading to a shorter effective time of the experienced bottleneck. Notably, conservation actions taken in several small sub-populations have ensured the preservation of a larger proportion of the total genetic diversity than if conservation had focused on the population stronghold in Norway. For conservation programmes targeting other endangered, long-lived species, our results highlight the possibility for local retention of high genetic diversity in isolated remnant populations.

Keywords: bottleneck; conservation genetics; generation time; microsatellites; mitochondrial DNA; single large or several small

1. INTRODUCTION

The white-tailed eagle (*Haliaeetus albicilla*) is used as a major flagship and umbrella species for conservation work throughout large parts of Europe. The species was once widely distributed across the continent (Helander & Stjernberg 2003), but direct persecution and low reproductive success as a result of environmental pollutants led to dramatic declines during the twentieth century (Bijleveld 1974; Helander *et al.* 2002). By the 1970s, many local populations in Europe had gone extinct and most surviving sub-populations barely reached one or a few tens of breeding pairs (figure 1). However, a stronghold remained along the Norwegian Atlantic coast with at least 700–800 breeding pairs (Folkestad 2003), corresponding to more than 70% of the northern and central European population (Helander *et al.* 2003). The population along the Norwegian coast utilized relatively uncontaminated food resources from the northern Atlantic and was thus largely saved from the detrimental effects of harmful pollutants (Helander *et al.* 1982). On the other hand, around the heavily polluted Baltic Sea, white-tailed eagles suffered strongly reduced breeding success (Helander *et al.* 1982).

With the ban in the 1970s of DDT, PCB and other detrimental chemicals, European white-tailed eagle populations soon started to recover, a process that is still ongoing (figure 1). For instance, the number of territorial pairs in southern and central Sweden increased from about 40 in 1974 to 250 in 2000 (Helander 2003*a*), and in Estonia from 10–15 between 1965 and 1975 to 116 in 2002 (Volke & Randla 2003). This population expansion appears to have been based mainly on local recruitment, since almost no inter-population exchange of breeders has been recorded from ringing data. In particular, the large Norwegian population does not seem to have acted as a major founding source for recovering populations; none of the more than 3000 eagles colour-ringed in Norway has so far been found breeding outside Norway (Folkestad unpublished data). Further, among more than 150 ringed breeding birds in Sweden, all but a few individuals from the nearby Finnish coast were offspring of resident pairs (Helander 2003*b*). Similarly, most of the few recorded emigrants from the German eagle population settled to breed nearby in Poland (Köppen 2003). Given this apparent trend of local recruitment, current populations might have been depleted of genetic diversity by the recent demographic declines.

Loss of genetic diversity can reduce both short-term viability (e.g. Saccheri *et al.* 1998) and long-term adaptability (Frankham 2005). To assess the genetic impact of population declines during the twentieth century, we therefore studied the genetic variability at mitochondrial DNA (mtDNA) control region sequences and autosomal microsatellite markers in north and central European white-tailed eagle populations.

2. MATERIAL AND METHODS

Several populations from northern and central Europe were sampled, yielding a total number of 218 presumably unrelated individuals: Estonia (EST, $n=13$ samples), Germany (GER, $n=18$), Swedish Lapland (LAP, $n=24$), southern and central Sweden (SWE, $n=117$), Kola peninsula, Russia (KOLA, $n=10$) and Norway (NOR, $n=36$). Most of the samples consisted of blood, but nine samples were moulted feathers that had been collected in the field.

Polymerase chain reaction (PCR) of 26 autosomal microsatellite loci was performed (details are given in Hailer *et al.* (2005) and the electronic supplementary material). In order to directly compare

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the amount of genetic variation (expected and observed heterozygosity, H_E and H_O, and the mean number of alleles per locus, N_A) between population samples, we standardized for sample size by employing a bootstrapping procedure (see electronic supplementary material). We tested for deviation from Hardy–Weinberg equilibrium (HWE) and genotypic differentiation using GENEPOP 3.4 (Raymond & Rousset 1995). GENETIX 4.05.2 (Belkhir *et al.* 2004) was used to calculate F_{ST} and its 95% confidence interval. Assignment tests were carried out using GENECLASS 2.0.d (Piry *et al.* 2004) and demographic simulations were performed in BOTTLESIM (Kuo & Janzen 2004). For the latter, population size was held constant at 300 actively reproducing pairs for 10 years, followed by a bottleneck of 30 reproducing pairs for 40 years (see electronic supplementary material for details). The program BOTTLENECK (Cornuet & Luikart 1996) was used to detect possible traces of population bottlenecks on the allele frequency spectrum, applying the recommended settings and tests for microsatellites (two-phased model: 90% one-step mutations and 10% multi-step changes; evaluation with standardized differences and Wilcoxon tests).

A part of the mtDNA control region was amplified and sequenced using the primers *HalHVR1F* (5′-CCCCCCCTATG-TATTATTGT-3′) and *HalHVR1R* (5′-TCTCAGTGAAGAGC-GAGAGA-3′) developed for *H. albicilla*, yielding a total of 500 bp for analysis. Similar to the microsatellite analyses, we used a bootstrapping procedure to correct diversity estimates for sample size (see the electronic supplementary material). DnaSP 4.10 (Rozas *et al.* 2003) was used to determine Tajima's *D* (Tajima 1989) and G_{ST}, an estimator of genetic differentiation among populations, using haplotype identity as the underlying statistic. Sequences obtained have been placed in the EMBL database (accession numbers AM156933–AM156934 and AM156936–AM156943).

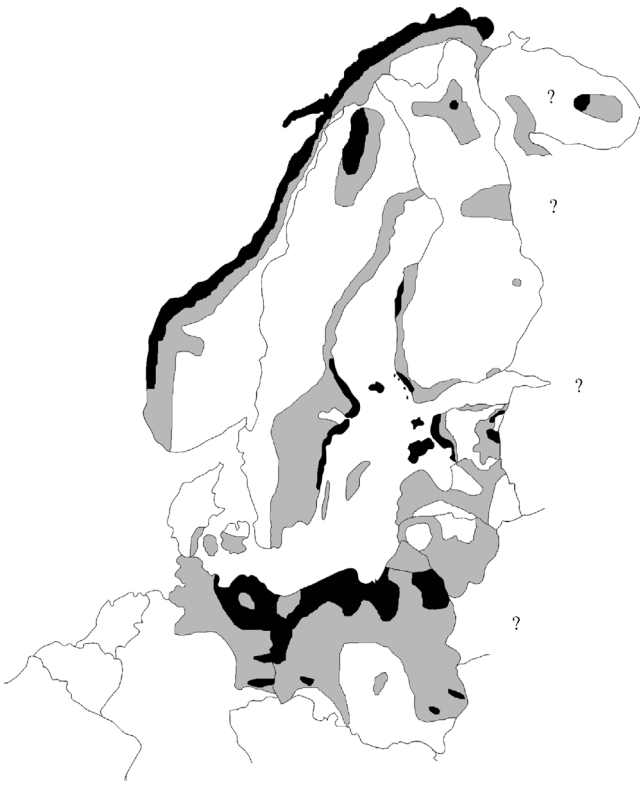

Figure 1. Distribution of the white-tailed eagle in north–central Europe. Black shading indicates regions where the species survived the mid-twentieth century population crash, light grey shading refers to regions re-colonized in the phase of population recovery since 1980.

3. RESULTS

A total of 139 alleles were observed across the 26 microsatellite markers. Significant heterozygote deficit was detected in the SWE, LAP and NOR populations. However, the signal disappeared when the locus *Hal 10* was excluded; we take this as an indication that this locus exhibits a null allele with restricted geographical distribution and that the populations otherwise do not deviate significantly from HWE.

Observed and expected heterozygosities estimated from data corrected for inter-population variation in sample size were in the range of 0.41–0.51 for all populations (table 1). Similarly, the mean number of alleles per locus did not vary much among populations, being in the range of 2.9–3.5. Importantly, there was no evidence for the large Norwegian population to show higher levels of genetic diversity ($H_E=0.48$, $H_O=0.49$, $N_A=3.1$), as compared to the other populations that had experienced a significant demographic bottleneck. Further, no population showed significant allele frequency shifts ($p>0.05$) which are expected after strong demographic bottlenecks.

One possible explanation for the homogeneous levels of genetic diversity among the populations could be that gene flow had passed unnoticed in the field surveys. Consistent with this idea, overall genetic differentiation was low ($F_{ST}=0.036$). However, F_{ST} was significantly different from zero (95% confidence intervals 0.029–0.044) and the test of genotypic differentiation found that all populations were significantly differentiated from each other ($p<0.01$ in all pairwise comparisons). Furthermore, an assignment test also indicated that populations were distinct units: despite the low overall F_{ST} value, about 80% of the analysed nestlings assigned to their natal population. Thirty-seven alleles found in other European populations were not observed in Norway, while only three of the Norwegian alleles were not found in the rest of the Europe.

Ten different mtDNA haplotypes were observed. Tajima's *D* was 1.522, but differed non-significantly from zero ($p>0.05$), compatible with neutral evolution of the DNA sequences. Haplotype frequencies differed significantly among populations, reflected by an overall G_{ST} of 0.228. The stronger population differentiation in mtDNA than nuclear markers probably reflects differences in effective population size between the marker types. Higher dispersal rates in males than in females are uncommon in birds (Greenwood 1980), and not supported by empirical data in white-tailed eagles (Helander 2003*b*).

The corrected number of haplotypes per population varied between 1.3 (Norway) and 7 (Kola peninsula; table 2), again indicating that bottlenecked populations do not contain lower levels of genetic diversity as compared to the Norwegian population. Further, our results confirm that the European populations could not have been derived largely from the coastal Norwegian population: only two mtDNA haplotypes were observed in Norway, one haplotype being very frequent (97%).

4. DISCUSSION

In summary, our data suggest that effective dispersal between populations has not been common during the past decades. We conclude that sustained genetic

Table 1. Genetic diversity at microsatellite loci. Number of samples analysed, unbiased expected (H_E) and observed (H_O) heterozygosity, mean number of alleles per locus (N_A) and their inter-locus standard deviations. For comparison between populations, standardized diversity values are also given.

population	sample size	H_E (s.d.)	corrected H_E	H_O (s.d.)	corrected H_O	N_A (s.d.)	corrected N_A
Norway	36	0.49 (0.05)	0.48	0.49 (0.02)	0.49	3.9 (2.3)	3.1
Estonia	13	0.49 (0.05)	0.48	0.50 (0.03)	0.50	3.5 (1.7)	3.1
Germany	18	0.43 (0.05)	0.42	0.42 (0.02)	0.41	3.4 (1.7)	2.9
Kola peninsula[a]	10	0.52 (0.05)	—	0.51 (0.03)	—	3.8 (2.0)	—
Lapland	24	0.51 (0.05)	0.49	0.49 (0.02)	0.49	4.2 (2.3)	3.4
mid and southern Sweden	117	0.51 (0.05)	0.51	0.50 (0.01)	0.51	4.8 (2.2)	3.5

[a]diversity statistics of the remaining populations were standardized to sample size of $n=10$, corresponding to the number for Kola peninsula.

Table 2. Genetic diversity at mitochondrial control region sequences. The number of analysed eagle territories (maternal lineages) is denoted by n, and N denotes the number of unique haplotypes found in each population. To correct for unequal sample sizes among populations, the mean number of alleles encountered in 100 bootstrap samples of 10 individuals each, and the standard deviation of these values, is presented.

population	n	N	corrected N (s.d.)
Norway	33	2	1.3 (0.5)
Estonia	12	5	4.0 (0.7)
Germany	18	3	2.6 (0.5)
Kola peninsula[a]	10	7	—
Lapland	22	6	3.7 (1.0)
mid and southern Sweden	44	4	2.9 (0.6)

[a]diversity statistics of the remaining populations were standardized to sample size of $n=10$, corresponding to the number for Kola peninsula.

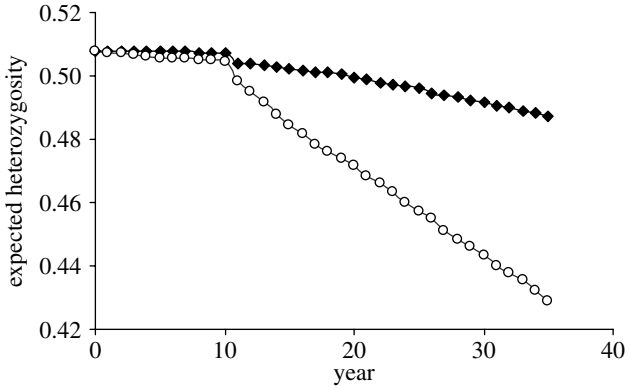

Figure 2. Loss of genetic diversity (as measured by expected heterozygosity) in a bottleneck of 30 reproducing pairs and overlapping generations. Filled diamonds and open circles consider an individual lifespan of 17 years and 1 year, respectively. The simulation was started at a population size corresponding to 300 reproducing pairs (years 0–10).

diversity in extant north–central European white-tailed eagle populations has a predominantly local origin, although some immigration may have contributed to local gene pools. We suggest that the long generation time of white-tailed eagles (average life span is around 17 years; Helander 2003*b*; Struwe-Juhl 2003) has minimized genetic drift and acted as an intrinsic buffer against rapid loss of genetic diversity. When a randomly mating population passes through a bottleneck, reduction of genetic diversity depends on population size and time according to $H_t=H_0(1-1/(2N_e))^t$; H_0 and H_t being the past and present heterozygosity, respectively, N_e the effective population size and t the time measured in number of generations. A bottleneck lasting about 20–30 years is equivalent to about two white-tailed eagle generations. Simulation of a bottleneck comprising 30 reproductive pairs (which corresponds to the situation in several European populations during the 1970s; see §1) shows that only about 4% of the original heterozygosity is expected to be lost in an eagle population during that time (figure 2). By contrast, for an organism with a generation time of 1 year, the corresponding loss of genetic diversity is considerably higher (16%; figure 2). Our results are compatible with the relatively high levels of genetic diversity seen in remnant and bottlenecked populations of other long-lived species, e.g. black rhinoceros (Swart *et al.* 1994) and greater one-horned rhinoceros (Dinerstein & McCracken 1990).

Starting in the late 1960s, several countries in Europe developed conservation programmes to protect eagles (Helander 1990). Nest site protection enabled fertile pairs to raise fledging young. Carcasses free of contaminants were laid out in winter and, as a result, juvenile survival was increased to 80% (Helander 2003*b*). Our results, therefore, indicate that these conservation efforts have led to the preservation of ample genetic diversity in local eagle populations. Moreover, conservation of eagles at local scales has resulted in the preservation of more total genetic diversity than if all conservation efforts had been centred on the coastal Norwegian population.

There has been an intense debate among conservation biologists about reserve design (the SLOSS debate—single large or several small; reviewed in Kingsland 2002). This debate was directly linked to the relative importance of local conservation versus a focus on population strongholds. Our results illustrate the high conservation value of local populations for species with a long generation time. A number of other species of conservation concern also have long generation times (e.g. raptors, turtles, large mammals and orchids) and for many of these significant genetic diversity may still exist in local populations. For the white-tailed eagle, protection of the local populations did not arrive too late.

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Monitoring distances travelled by horses using GPS tracking collars

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Objective The aims of this work were to (1) develop a low-cost equine movement tracking collar based on readily available components, (2) conduct preliminary studies assessing the effects of both paddock size and internal fence design on the movements of domestic horses, with and without foals at foot, and (3) describe distances moved by mares and their foals. Additional monitoring of free-ranging feral horses was conducted to allow preliminary comparisons with the movement of confined domestic horses.

Procedures A lightweight global positioning system (GPS) data logger modified from a personal/vehicle tracker and mounted on a collar was used to monitor the movement of domestic horses in a range of paddock sizes and internal fence designs for 6.5-day periods.

Results In the paddocks used (0.8–16 ha), groups of domestic horses exhibited a logarithmic response in mean daily distance travelled as a function of increasing paddock size, tending asymptotically towards approximately 7.5 km/day. The distance moved by newborn foals was similar to their dams, with total distance travelled also dependent on paddock size. Without altering available paddock area, paddock design, with the exception of a spiral design, did not significantly affect mean daily distance travelled. Feral horses (17.9 km/day) travelled substantially greater mean daily distances than domestic horses (7.2 km/day in 16-ha paddock), even when allowing for larger paddock size.

Conclusions Horses kept in stables or small yards and paddocks are quite sedentary in comparison with their feral relatives. For a given paddock area, most designs did not significantly affect mean daily distance travelled.

Keywords behaviour; feral horses; foals; global positioning system (GPS); horses; paddock design

Abbreviation CI, confidence interval; GPS, global positioning system

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Extent of movement and distances travelled by domestic horses may affect their general health and hoof quality. It is believed that domestic horses have become more sedentary since the industrial revolution, with many now maintained in small paddocks and stables because the amount of land available for horse maintenance has decreased. The Thoroughbred racehorse, for example, is an athletic horse that is typically boxed in a stable for 23 hours/day and moved to the training track for a short gallop once daily.[1] The psychological, physiological and musculoskeletal consequences of this relatively sedentary lifestyle are not fully understood, but the low level of mobility from an early age may adversely affect the shape, structure and function of the foot. Domestication has also necessitated an altered diet,[1] and horses are routinely fed high-energy food twice daily. Confinement and altered feeding patterns parallel the modern human lifestyle that is thought to be a major contributor to current high prevalences of obesity, cardiovascular disease, arthritis and diabetes. For example, the prevalence of osteochondritis dissecans was 26% in 1000 yearling Thoroughbred horses surveyed radiographically, and diet and environment were cited as contributing factors.[2] Another radiographic study of 315 yearling Standardbred horses revealed bone and joint lesions in 58% of horses, 38% of which required surgical correction.[3] Hyperinsulinaemia has recently been associated with laminitis,[4] and both a diet high in non-structural carbohydrates and low levels of activity are associated with hyperinsulinaemia.[5]

It has been hypothesised that horses move more in large paddocks and that paddock design may also affect the distance moved; for example, it has been suggested that a 'racetrack' design is a useful strategy for increasing the distance moved by horses.[6] The effectiveness of this design has not been investigated. Understanding the relationships between both paddock size and internal fence design and distances moved would allow development of practical management strategies to increase horse movement.

The velocity of horse movement varies considerably, making locomotion studies in the field challenging. Global positioning systems (GPS) are now sufficiently lightweight and accurate to measure speed variation and to distinguish small movements of fast-moving animals, so accurate determination of horse movement profiles has become feasible. GPS have been used to measure locomotion variables in horses during cross-country riding[7] and in foals at pasture.[8]

Little is known about the movement patterns of domestic horses or the effects of the various forms of yard and paddock confinement on distance travelled. Also, to the authors' knowledge, there have been no reports of the distances travelled by free-ranging, non-domesticated horses. In studies of domestic cattle, those in a rangeland environment were observed to walk up to 27 km between visits to water,[9] whereas cattle in a more intensive grazing situation walked up to 5.5 km during a 24-h period that included grazing, watering and socialising.[10] Free-ranging cattle in Africa walk about 5000 km annually (an average of 14 km/day).[11] Equidae are generally more mobile than cattle and capable of covering greater distances between feeding and watering.[12] In one study, female zebra walked approximately 15 km over a 12-h period between water and their feeding region, remaining for 3 to 4 days before walking back to water.[13]

The aims of this work were to (1) develop a low-cost equine movement tracking collar based on readily available components, (2) conduct

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preliminary studies of the effects of both paddock size and internal fence design on the movements of domestic horses and (3) describe distances moved by mares and their foals. Additional monitoring of free-ranging feral horses was conducted to allow preliminary comparisons with the movement of confined domestic horses.

Materials and methods

GPS data logger

The University of Queensland Equine GPS data logger was constructed from a Wintec G-Rays GPS unit (G-Rays 2 WBT-201 data logger, Wintec Wireless Electronics, Taiwan). The store-on-board GPS unit was modified by the addition of a circuit board to control the power input from an additional three AA-battery pack. The total weight of the GPS, battery and collar for domestic horse tracking was 450 g. With the addition of a very high frequency location beacon for feral horse tracking, the collars weighed 700 g. The GPS unit was mounted on a neck collar with a full sky view, counterweighted by the battery pack, which hung under the horse's neck. The 130,000 position storage capacity of the unit and the life of a single battery power source (6.5 days) allowed data logging at the rate of a position every 5 s.

Data defining both the horizontal and vertical positions were downloaded via Google Earth Plus (Google Earth Plus-google.com). The Wintec software was used to perform distance calculations from the raw GPS positional data. The pattern of travel was superimposed on Google Earth satellite photographs and checked for plausibility and data error.

Horse movement assessments

Four preliminary studies were conducted to explore the effects of paddock design and size on distance travelled. In each study, the average daily distance moved for each horse was calculated, based on the total distance moved for the 6-day observation period. Descriptive statistics were calculated using Microsoft Excel and other statistical analyses were performed using Stata (version 10, StataCorp, College Station, TX, USA).

The project was approved by the University of Queensland Animal Ethics Committee.

Effect of paddock size. Data loggers were fitted to each of four mature-age (mean ± SD, 6.75 ± 2.2 years), pregnant (approximately 310 days of gestation) domestic Quarterhorse × Australian Stockhorse mares due to foal within 2 weeks of each other. These horses were grazed together for three consecutive 6-day periods in each of three different paddocks, sized in sequential order 0.8, 4 and 16 ha. All paddocks were on the same farm and the study mares were familiar with all paddocks. Mares were placed in each paddock at the same time. They were not hand-fed and all paddocks had sufficient pasture mass to ensure adequate feed intake over the confinement intervals.

A further seven domestic Quarterhorses (3 geldings, 4 mares; age 3–4 years) were each simultaneously tracked for 6 days on a commercial horse-training property. These horses were housed in familiar yards measuring 6 m^2 and were hand-fed twice daily throughout the study.

The relationship between paddock size and average distance moved for the group (calculated as the average of each horse's average distance moved daily) was described as a function of the natural logarithm of paddock size using data from the groups of domestic horses.

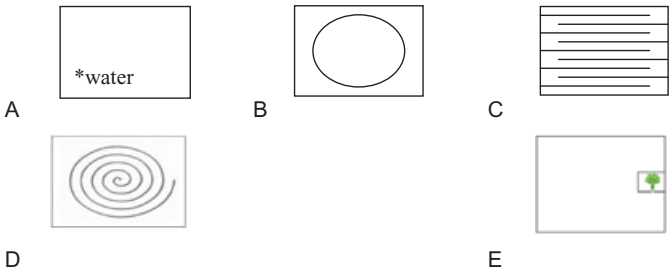

Figure 1. Internal fence designs compared in a study assessing the effects on distance travelled by horses: (A) open, B) racetrack, (C) maze, (D) spiral, (E) open and no tree. *Location of the waterpoint.

Effect of internal fence design. A 5-period cross-over design was used to assess the effect of various internal fence designs on distance travelled. Five horses (4 mares, 1 gelding; age 3–9 years) were each sequentially grazed separately in the five designs. The same rectangular paddock (100 × 50 m) was used throughout the study. There was one tree in the paddock, which provided the only area of shade for the horses, and a stationary trough was the only water point. The internal fence design of the paddock was varied using electric fencing. All horses were accustomed to electric fencing. The five internal fence designs are shown in Figure 1.

Open: The paddock perimeter was fenced with electric fencing (Figure 1A).
Racetrack: The open design with an additional internal oval ring of fencing to create an 8-m wide racetrack around the perimeter of the paddock (Figure 1B). Horses were confined to the racetrack strip and excluded from the centre of the paddock.
Maze: The open paddock was divided into 10 strips, 10 × 50 m long, with gaps at alternate ends to make all strips accessible (Figure 1C). Horses were required to move through each of the 10 strips to travel from one end of the paddock to the other, effectively travelling at least 500 m when moving from one end of the 100-m paddock to the other.
Spiral: The internal fencing had a spiral configuration so that the horses had to travel in circles of diminishing radius to move into and away from the centre of the paddock (Figure 1D).
Open and no tree: Because the horses spent a disproportionate amount of time standing under the shade tree in the open paddock, the open design was used with the tree fenced off to prevent this (Figure 1E).

The maze and spiral designs were not expected to have practical applications in horse management, but were included in the study to determine the effect of complex designs on movement. Each of the five horses were assessed separately in each paddock design, but always in the company of a subordinate yearling gelding to provide company and minimise the likelihood of separation anxiety, which may have affected the movements of the dominant horse. Each horse spent 6 consecutive days in each design, with no delay or 'washout period' between designs. The first horse was rotated through the five designs in an ad hoc order, then each of the remaining four horses was

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allocated to first receive one of the designs using a random draw, followed by each other design in the same order as the first horse. The paddock was spelled for 2 weeks between each horse and pasture cover was maintained to ensure grazing without supplementary feeding during each experiment. Mean distance travelled daily was calculated for each horse for each design and log-transformed. Mean distances travelled were compared between designs using linear regression with horse fitted as a fixed effect.

Mare and foal comparison. Three days after each mare's foaling, the same four mares used in study 1 (paddock size) were grazed with their foals in the 4-ha paddock for a 6-day period. To avoid interference with development of the mare–foal bond, GPS collars were applied to each mare and foal when the foal was 3 days of age. Each mare and foal was again monitored simultaneously when the foals were aged 3 to 5 weeks while in a different paddock (5 ha) on the same farm to determine if there was a difference in activity between newborn foals (a period when they are often stabled) and more mature foals. Effects of class (mare or foal) and foal age (3–9 days vs 3–5 weeks) on average distances moved daily were analysed using linear regression with pair (mare–foal combination) fitted as a fixed effect. Effects of pregnancy/lactation were analysed using linear regression with data only from mares with mare fitted as a fixed effect. Correlation coefficients were estimated for the association between the distances moved daily by mares and foals; separate estimates were made for 6-day periods commencing when foals were aged 3 days and then at 3 to 5 weeks. The 95% confidence interval (CI) for the correlation coefficients was calculated using Fisher's transformation.

Feral horse monitoring. Three mature feral horses from different family bands (1 mare, 2 stallions) and living wild in a 4000-ha paddock were tracked for 6 consecutive days. In order to apply the collars, horses were darted with 0.7 mL etorphine hydrochloride (Imobilon, Novartis SA Pty Ltd) when they approached water. When the horse went into lateral recumbency, the collar was placed and the immobilising agent reversed using diprenorphine hydrochloride (Revivon, Novartis SA Pty Ltd, IV). Horses returned to their family band within 15 min of darting and appeared both unaware of the initial human contact and unaffected by their collars. After a minimum of 6 days, the same feral horses were relocated using the very high frequency beacon on their collar and again immobilised by darting, the collars were removed, the immobilising agent reversed and the horses released.

Results

GPS collar performance

The data logger device logged positional data at 5-s intervals for 6.5 days. The unit ran continuously and did not record either the usual gaps in data or the error often associated with reconfiguring position in relation to satellites; it was 100% reliable over 384 data logging days.

Horse movement assessments

Paddock size study. Average distances moved for the groups were greater in larger paddocks (Figure 2A). The average for the group was: 4.7 km/day (range 4.2–5.2 km/day) for the 0.4-ha paddock; 6.1 km/day (range 5.8–6.5 km/day) for the 4-ha paddock; 7.2 km/day (range 6.7–7.6 km/day) for the 16-ha paddock. The average for the feral horses in the 4000-ha paddock was 17.9 km/day (range 12.52–5.9 km/day). The average for horses kept in the 6 × 6 m yard was 1.1 km/day (range 0.2–1.9 km/day).

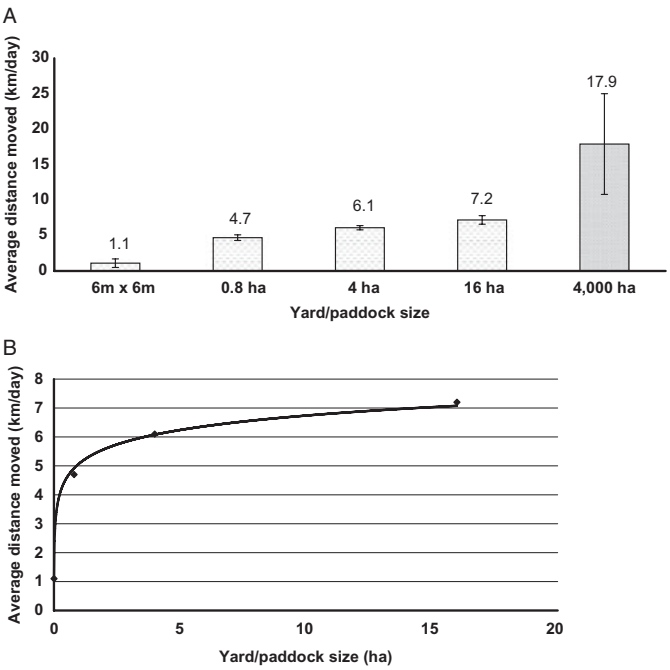

Figure 2. (A) Average distances (km/day) moved by groups of horses (± SD of each horse's distance) over 6 days in various sized yards and paddocks. Light shade indicates domestic horses; dark shade indicates feral horses. (B) Scattergraph of average distances moved by groups of domestic horses against yard/paddock area and logathithmic line of best fit of average distance moved by group as a function of yard/paddock area.

The average distances moved by the groups of domestic horses could be described as a logarithmic function of paddock area: Average distance moved/day = 0.72 (SE 0.029)**ln*(paddock size in ha) + 5.08 (SE 0.094)($R^2 = 0.997$) (Figure 2B).

Internal fence design study. Mean distances travelled daily by the five horses for each of the internal fence designs are summarised in Table 1. The average of the distances travelled by each horse was greatest for the open paddock design and smallest for the spiral design (ratio of geometric means 0.72, 95% CI 0.55–0.96, P = 0.03). There were no significant differences (P > 0.35) in the distances travelled by horses between the 'open' paddock and either the 'maze' or 'open and no tree' paddock designs. There was also no significant difference between the 'open' and 'racetrack' paddock design (ratio of geometric means 0.94, 95% CI 0.71–1.24, P = 0.63).

Mare and foal comparison study. Prior to foaling, the average distance travelled for the group of mares (derived from study 1) was 6.1 km/day (range between mares 5.8–6.5 km/day). During the 6-day periods from 3 to 9 days and 3 to 5 weeks after foaling, the average distances moved by mares were 7.2 km/day (range 6.9–7.4 km/day) and 7.7 km/day (range 5.9–10.7 km/day), respectively (Figure 3). Daily distances moved by foals were 7.3 km/day (range 4.5–9.5 km/day) and 7.3 km/day (range 4.5–9.3 km), respectively. After account-

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Table 1. Distances moved by five horses in paddocks with various internal fence designs

Internal fence design	Distance moved (km/day)			Ratio of geometric means (95% CI)	P value
	Range between horses[1]	Arithmetic mean[2]	Geometric mean[2]		
Open	2.8–5.3	4.1	4.0	Reference group	
Racetrack	2.7–6.0	3.9	3.7	0.94 (0.71–1.24)	0.63
Maze	2.5–6.8	3.8	3.5	0.88 (0.67–1.17)	0.36
Spiral	2.1–5.8	3.1	2.9	0.72 (0.55–0.96)	0.03
Open and no tree	2.8–4.9	3.9	3.8	0.96 (0.73–1.28)	0.78

[1]Average of distances moved by horse daily on 6 consecutive days. [2]Means of daily average for each horse. CI, confidence interval.

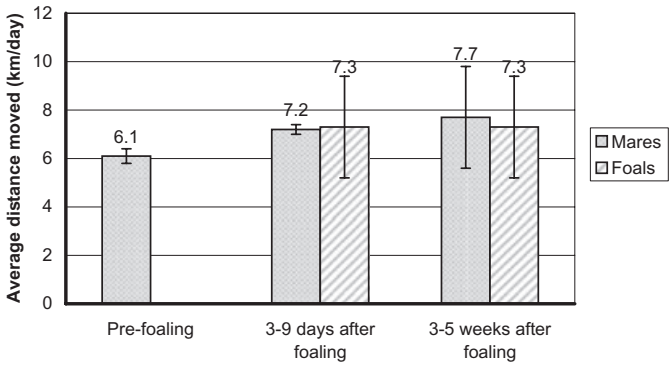

Figure 3. Mean (±SD) distances walked by mares before foaling, and by mares and their foals 3 to 9 days and 3 to 5 weeks after foaling.

ing for mare–foal pair and class (mare or foal), the difference in mean distance walked between 3 to 9 days and 3 to 5 weeks after foaling was not statistically significantly diffferent (difference between means 0.2; 95% CI −1.8–2.2; P = 0.83). The observed correlation coefficient suggested that distances moved were quite closely correlated between mares and their foals during the first tracking period (3–9 days after foaling), but the estimate was imprecise (r = 0.81; 95% CI −0.68–1.00; P = 0.19). During the second study period (3–5 weeks after foaling), the correlation coefficient was 0.47 (95% CI −0.90–0.99; P = 0.53).

Feral horse monitoring. The average distance travelled over 6 days by the three feral horses in the 4000-ha paddock was 17.9 km/day (range between horses 12.5–25.9 km/day) (Figure 2A).

Discussion

GPS collar performance

The weight of the GPS collar with the location beacon attached was approximately 0.15% of total body mass, which was substantially less than in a previous report monitoring zebra in which a collar weight of 0.4% of body mass was found to interfere less with the rate of travel during grazing than a heavier collar weighing 0.6% of body mass.[13] The effects of the collars used in the current study on distance moved could not be assessed but were assumed to be minimal. The collars did not appear to disturb the movement of the horses as neither skin abrasions nor damage to the collars was evident after horses had worn them for 6.5 days.

Horse movement assessments

Paddock size study. Results from this study should be interpreted with caution. Because the horses were run together in each paddock and because they tended to move together, the group, rather than the individual horse, should be viewed as the unit of analysis and the results reflect effects of paddock size on average distance travelled for groups, not for individual horses. (The broodmares used in the study were normally housed together and their owner did not allow the mares to be separated, fearing that this may stress the mares in this important late stage of pregnancy.) In addition, under this study design, confounding may have occurred because of differences between paddocks, other than size, and horse-level factors related to the use of different horses in yards and paddocks. External validity of this study may be low because the relationship between paddock size and distance moved may depend on feed and water availability, terrain and horse characteristics. Distance travelled by heavily pregnant mares may vary from other classes of horses. Finally, groups of horses moved through the different sized paddocks in a non-random order. If carryover effects occur, they may have confounded the results.

Despite these limitations, the substantially lower average distances moved by the study horses in small enclosures as compared with the groups in open paddocks quantifies the relative inactivity of these horses outside of scheduled training sessions. Many athletic performance horses, such as Thoroughbred racehorses, Standardbred trotters and pacers, elite dressage horses, eventers and even long-distance endurance horses, spend the majority of time inside small box stalls or yards. Arena-trained horses, such as working Quarterhorses and dressage horses, may only cover between 1 and 2.4 km in a hard 30- to 40-min training session,[14] so total distance travelled daily during both training and confinement is still substantially lower than the daily movement of the group of unworked horses in the 4-ha paddock in the present study. Similarly, many racing Thoroughbred horses cover only 2 to 3 km in each training session and progress to their first start only 68 days following the start of training preparation.[15] If movement in the box or yard is 1.1 km/day, as observed in the current study, the total distance moved would be only 3 to 4 km/day, which is less than

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the average daily distance moved by the group of unworked horses in the 0.8-ha study paddock, is approximately 75% of that travelled by the group of unworked horses in the 16-ha study paddock and is approximately 25% of the average of the daily distances covered by the feral horses in this study. The ideal exercise regimen for racehorses has not been defined, although a total distance of between 5 and 9 km/day over a 10-week period has been proposed.[16] It is possible that athletic horses should move over greater distances daily for optimal health.

Distances moved by horses for the purpose of fitness conditioning may have different effects from the same daily distance travelled during sedentary activity, because of the differences in speed between these circumstances. Although the measured values of distance travelled serve to indicate relative movement activity, further processing of GPS positional logs into descriptors of speed, as well as the amount of movement, will add to the interpretative power of the recorded data. The low-cost, off-the-shelf GPS technology used in the present study potentially could be used for that purpose.

These results suggest that the distances travelled daily by groups of domestic horses may reach a maximum of approximately 7.5 km for paddock sizes exceeding the maximum used in this study (i.e. 16 ha). If confirmed with replicated studies in a range of environments, this would indicate that horses in paddock sizes exceeding 16 ha can be considered to be 'unconfined' for the purposes of gauging the effect on daily travel behaviour. However, the minimum paddock size that constitutes 'unconfined' is likely to depend on available feed, water resources and the number of horses in the group.

Internal fence design study. A 'trail' paddock design based on observations of feral horses, and simulated in the present study by the racetrack design (Figure 1B), has been proposed to promote natural movement of horses.[6] Interestingly, in the present study the average mean daily distance travelled was highest for the open paddock design. The study horses appeared to move less at night when exposed to complex internal fencing, possibly because of the greater risk of contacting the fences. Although the results of our study did not demonstrate that distance moved differed between the open paddock and racetrack designs, the racetrack design offers management benefits. The large central area of the paddock can be used for other activities, such as pasture rehabilitation, hay making or as an arena.

Mare and foal comparison study. The study results demonstrate that domestic newborn foals are as active as their mothers and capable of moving 5 to 10 km/day when aged 3 to 9 days. Early mobility is essential for feral horses because the mare and newborn foal must move with the family band soon after parturition; foals that do not stand and walk promptly may perish. However, conventional management practice for domesticated horses is to leave foals in box stalls or small yards until the feet and legs are 'hardened' enough to be able to withstand the rigours of outdoor mobility. In this situation, based on findings from the paddock size study reported here, foals would be unlikely to move more than 1 km/day in the first few weeks of life (compared with 7.3 km) and, because of the confined area, would be unlikely to reach speeds faster than walking. The musculoskeletal and neuromotor systems of the foal, as with most biological tissues, require mechanical stimulation to optimise growth and development. Although the effects of exercise in early foalhood are unknown, lack of early stimulation and tissue adaptation may compromise optimum development. Given adequate space, foals spend a substantial amount of their locomotion time at the trot or canter and high workloads during early development might be important in conditioning the musculoskeletal system. Researchers recently concluded that a 30% increase in foal workload did not adversely affect the musculoskeletal health of performance horses[17] and in a subsequent study there were no adverse effects of foal workload on the racing careers of these horses.[18] In a cohort study of 401 foals, the authors concluded that foals should be allowed to exercise regularly as a preventative measure for developmental orthopaedic disease and osteochondrosis, and that limiting paddock size may also help prevent cartilage damage.[19] However, it is not known whether the increased risk of cartilage damage in foals grazed in larger paddocks was because of increased exercise or because of access to a larger selection of pasture. This highlights the need to objectively measure exercise levels in horses in various environments.

Feral horse monitoring. Distances travelled by the feral horses were far greater than those observed for domestic horses and indicate that there are important behavioural differences between unworked domestic horses and feral horses, which is not surprising given the difference in resource availability (feed and water) between domestic and feral horses.

Conclusions

A lightweight GPS data logger, modified from a personal/vehicle tracker and mounted on a collar, was a practical and reliable method for measuring horse movement over extended periods. Horses kept in small paddocks are quite sedentary compared with their feral relatives and stabled horses and horses kept in small yards perform very little physical activity. Newborn domestic foals move as much as their mothers. Horse movement is limited to an extent by paddock size. Several popular paddock designs had no significant effect on distance travelled by unworked domestic horses.

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OBITUARY

John Allan Macfarlane

1945–2009

At the end of 1968 Mac was called in by Prof. Marsh Edwards for a viva in veterinary medicine. Marsh said, "Well, Mac, do you know why you are here?'

'To see if I pass or fail!'

'No, no Mac, to see if you get a credit or a pass,' Marsh pointed out.

On hearing this, Mac stood up and muttered, ' Credit!! Never had one in my life, don't intend to start now!', and walked out.

In many ways, this typified John Allan Macfarlane – 'do the job, do it well and walk away when it's finished'.

John Mac died in a Brisbane hospital in May 2009 after a battle with leukemia. He was optimistic to the last, hoping for a cure.

He was born in Sydney in May 1945 and educated at Tudor House, Bowral, then The King's School, from where he gained entry to the University of Sydney to study veterinary science.

After graduation John headed to Narrabri, working for the Rural Land's Protection Board (RLPB), and there he developed a love for sheep and the sheep industry to which he devoted most of his life. In December 1986 he began work as the District Veterinarian in the Armidale RLPB and he was there till he retired in July 2007.

The control and eradication of virulent footrot, and management and advice to sheep farmers on controlling anthelmintic resistant intestinal parasites took up much of John's time, but somehow he gained a Masters Degree in Rural Science and in Natural Resources, and passed his examination in Sheep Medicine for the Australian College of Veterinary Scientists.

It was following the discovery of ovine Johne's disease (OJD) in the Central Tablelands in the 1980s, that John's veterinary prowess came to the fore. The Regional Veterinary Laboratory in Armidale where many sheep in poor condition were being autopsied was not finding OJD as the cause. It appeared that the prevalence of OJD varied around the State. That RVL was closed in 1997, but John Mac continued his surveillance of the disease. Over 400 thin sheep presented for slaughter at the local pet food abattoir were autopsied, till the RVLs at Orange and Camden asked him to stop sending samples. All samples from those sheep proved to be negative. The RLPB chose 100 flocks at random for footrot surveillance and the rangers were permitted to collect faecal samples from 100 older ewes for pooled faecal culture. These all proved negative too.

At the urging of some sheep veterinarians, leaders of the industry in New England and senior veterinary personnel in the NSW Department of Agriculture, John oversaw the formation of the NE OJD Coordinating Committee, which sought to warn sheep farmers in NE and elsewhere of the dangers of bringing OJD into the district. Through an education program, John and the Board highlighted the epidemiology of OJD so that farmers moved from an 'awareness' to an 'understanding' of this insidious disease. It is testament to his work that there are still no known infected flocks in New England.

John was not an 'upfront' person. He did not like 'leading the charge', but nevertheless he was a good leader of a team, always ready to backup a spokesman with technical details. He was adept at delegating authority to others. Whether it be at a Senate Inquiry into OJD or deliberations with the Minister for Agriculture (or his senior staff), John would provide the information and let others do the talking. It was this self-effacing feature that was one of the most appealing parts of his character.

When in 2004 the Minister for Agriculture gazetted New England and other large parts of NSW as an OJD Exclusion Area, John did not slow down, but kept up the pressure through the RLPB system to maintain vigilance lest OJD 'creep in'. The sheep industry in NE and NSW should salute him.

The veterinary profession has lost one of its great members, a good friend and quiet achiever who loved his wife and family, sailing and a good red wine. To Sally and their children Angus, Sam, Katherine and Jane, we extend our love and deepest sympathy.

D Moen

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Distances travelled by feral horses in 'outback' Australia

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Keywords: horse; feral horses; GPS; distance; water frequency

Summary

Reasons for performing study: **The distance travelled by Australian feral horses in an unrestricted environment has not previously been determined. It is important to investigate horse movement in wilderness environments to establish baseline data against which the movement of domestically managed horses and wild equids can be compared.**

Objectives: **To determine the travel dynamics of 2 groups of feral horses in unrestricted but different wilderness environments.**

Methods: **Twelve feral horses living in 2 wilderness environments (2000 vs. 20,000 km^2) in outback Australia were tracked for 6.5 consecutive days using custom designed, collar mounted global positioning systems (GPS). Collars were attached after darting and immobilising the horses. The collars were recovered after a minimum of 6.5 days by re-darting the horses. Average daily distance travelled was calculated. Range use and watering patterns of horses were analysed by viewing GPS tracks overlaid on satellite photographs of the study area.**

Results: **Average distance travelled was 15.9 ± 1.9 km/day (range 8.1–28.3 km/day). Horses were recorded up to 55 km from their watering points and some horses walked for 12 h to water from feeding grounds. Mean watering frequency was 2.67 days (range 1–4 days). Central Australian horses watered less frequently and showed a different range use compared to horses from central Queensland. Central Australian horses walked for long distances in direct lines to patchy food sources whereas central Queensland horses were able to graze close to water sources and moved in a more or less circular pattern around the central water source.**

Conclusions: **The distances travelled by feral horses were far greater than those previously observed for managed domestic horses and other species of equid. Feral horses are able to travel long distances and withstand long periods without water, allowing them to survive in semi-arid conditions.**

Introduction

Movement and the distances travelled by domestic horses (*Equus caballus*) may affect general health. Domestic horses have become more sedentary since the industrial revolution, with many now kept in small paddocks and stables as land available for horse maintenance has declined. The athletic Thoroughbred racehorse, for example is typically boxed in a stable for 23 h/day and moved to the training track for a short gallop once daily (Richards *et al*. 2006). Hampson *et al*. (2010) found that horses kept in small yards travelled only 1.1 km/day and those housed in a 16 hectare paddock travelled a mean of 7.2 km/day. The psychological, physiological and musculoskeletal consequences of this relatively sedentary lifestyle are not fully understood, but the low level of mobility from an early age may adversely affect musculoskeletal health. Domestication of the horse has not only affected mobility but has been associated with an altered diet. Horses in training are routinely fed high energy diets twice daily and may have an abundance of pasture and water (Southwood *et al*. 1993). Confinement and altered feeding patterns parallel the modern human lifestyle that is thought to be responsible for endemic levels of obesity, cardiovascular disease, arthritis and diabetes (Johnson *et al*. 2004; Perkins *et al*. 2005; Richards *et al*. 2006; Lepeule *et al*. 2009).

Global positioning system (GPS) technology has been shown to be able to accurately measure horse movement, including distance travelled, range use and watering patterns of wild/feral equids. This method was used previously by the current research team in a pilot study to monitor 3 feral horses in Australia and found that the mean daily distance travelled was 17.9 km (Hampson *et al*. 2010). This was significantly further than wild asses (*E. hemionus*) (8.3 km/day; Kaczensky *et al*. 2008) and wild Przewalski horses (*E. przewalskii*) (3.5 km/day; Kaczensky *et al*. 2008) in Mongolia. However, female zebras (*E. burchelli*) were documented to walk approximately 15 km over a 12 h period between water and their feeding region, where they remained for 3–4 days before walking back to water (Brooks *et al*. 2007).

A total published sample size of 3 individual free roaming horses is not representative of the population and does not allow robust comparison of movement patterns to other equids. Since there appears to be substantial variation within and between equid species in locomotory habits, the aim of this further work was to monitor free-ranging feral horses in order to compare feral horse movement with the movement ecology of other equid species and managed domestic horses.

Materials and methods

Twelve feral horses were tracked using GPS data loggers to determine movement and watering patterns.

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GPS data logger

The University of Queensland Equine GPS data logger was constructed from a Wintec G-Rays GPS unit (G-Rays 2 WBT-201data logger)[1]. The store-on-board GPS unit was modified by the addition of a circuit board which controlled the power input from an additional 3 AA battery pack. The total weight of the GPS, battery, collar and a very high frequency (VHF) location beacon was 700 g. The GPS unit was mounted on a neck collar with a full sky view, counterweighted by the battery pack and VHF beacon, which hung under the horse's neck. The 130,000 position storage capacity of the unit and the life of a single battery power source (6.5 days) allowed data logging at the rate of one position every 5 s (Hampson *et al*. 2010).

Data defining both horizontal and vertical positions were downloaded via Google Earth Plus[2]. The Wintec software was used to perform distance calculations from the raw GPS positional data. The pattern of travel was also superimposed on Google Earth satellite photographs of the study area and checked for plausibility and data error. In a previous study (Hampson *et al*. 2010) the same GPS unit was determined to be sufficiently accurate to measure domestic horse movement in small paddocks and feral horse movements in large paddocks with no 'outlier' data points.

Subjects

Twelve mature feral horses, from different family bands (8 mares, 4 stallions), living in 2 different wilderness environments, were tracked for 6.5 consecutive days. Four mares and 4 stallions were tracked at one location, which was semi-arid country on a large cattle property (Babbiloora) in central Queensland (latitude –25.18, longitude 147.49). The area receives a mean annual rainfall of 528.5 mm, and 157 mm had fallen in the 6 months prior to data collection (Bureau of Meteorology, Australia). The area had a large body of feed in all areas of the paddock accessed by the horses. Four mares were tracked at the second location, which was a semi-arid environment near Kings Canyon in central Australia (latitude –24.50, longitude 132.10). This 20,000 km^2 area consists of a valley system bordered by high prominent escarpments and is surrounded by vegetated and nonvegetated sand dunes measuring 15 m in height. Mean annual rainfall is 335 mm and rainfall was 331 mm in the 6 months prior to data collection (Bureau of Meteorology, Australia). There was high competition for feed resources in this area between feral cattle, horses and camels. Vegetation was scarce within 15 km of the single permanent water hole. All other semi-permanent waterholes in the area were dry at the time of the study as confirmed by the local indigenous people and stockmen. Ground and aerial searches from helicopter also confirmed that all animal tracks converged at this single waterhole. Feral horses have inhabited both areas for in excess of 140 years (Berman 1991) and experience very little pressure from human intervention. The breed origins of the horses were uniform with influence from early Thoroughbred, Arab and station hack horses.

GPS collar placement

In order to apply the collars, horses were darted i.m. with 0.7 ml etorphine hydrochloride (Imobilon)[3] when they approached water. When the horse became laterally recumbent, the collar was attached to the upper neck and the immobilising agent reversed using 1.4 ml diprenorphine hydrochloride (Revivon)[3] injected i.v. Horses returned to their usual band within 15 min of darting and appeared unaffected by the neck collar and the initial human contact. After a minimum of 6.5 days, the same feral horses were relocated using the VHF beacon on their collar and again immobilised by darting. The collars were removed, the immobilising agent reversed and the horses released.

Watering frequency

Both study locations had only one watering point available to the horses at the time of the study. Watering frequency was determined by the frequency that horses approximated the position of the water point on the Google Earth satellite photograph of the study area.

Statistical analysis

Distances travelled were compared between groups depending on sex and location of the horses using a nonparametric Wilcoxon *t* test. Statistical significance was set at 0.05. All values are presented as mean ± s.e.

The project was approved by the University of Queensland Animal Ethics Committee (AEC-PCA) monitoring compliance with the Animal Welfare Act (2001) and The Code of Practice for the care and use of animals for scientific purposes (approval number SVS/393/07/AHF).

Results

GPS analysis of travel distance

Mean daily distances travelled by all horses over the 6.5 day period of GPS tracking are presented in Figure 1. There was a large range of mean daily distances (8.1–28.3 km) covered by the horses. The mean daily distance travelled by all horses combined was 15.9 ± 1.9 km. During the period of tracking, stallions (18.2 ± 2.91 km/day) tended to travelled further on average than mares (14.8 ± 2.5 km/day) but this difference was not significant due to the small sample size. Mean daily distance travelled was 16.8 ± 1.71 km by central Queensland horses and 14.7 ± 4.17 km for central Australian horses. The mean daily distance travelled, regardless of gender was not significantly affected by location. However, central Queensland horses tended to travel a similar distance each day and

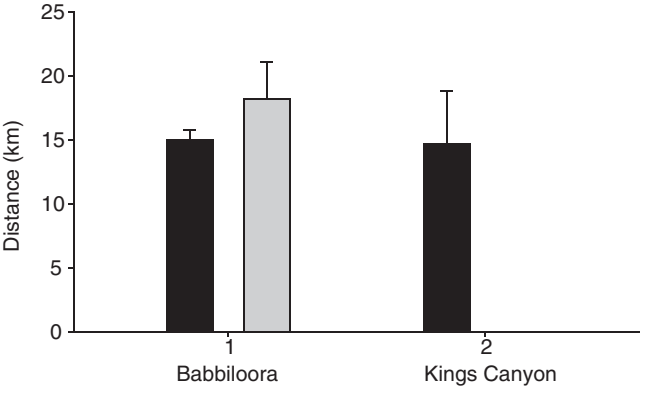

Fig 1: Mean ± s.e. daily distance (km) travelled by female (■) and male (□) feral horses in 2 inland Australian locations, Babbiloora (central Queensland) and Kings Canyon (central Australia). Distances travelled were measured by GPS tracking collar. Stallions did not travel significantly further than mares.

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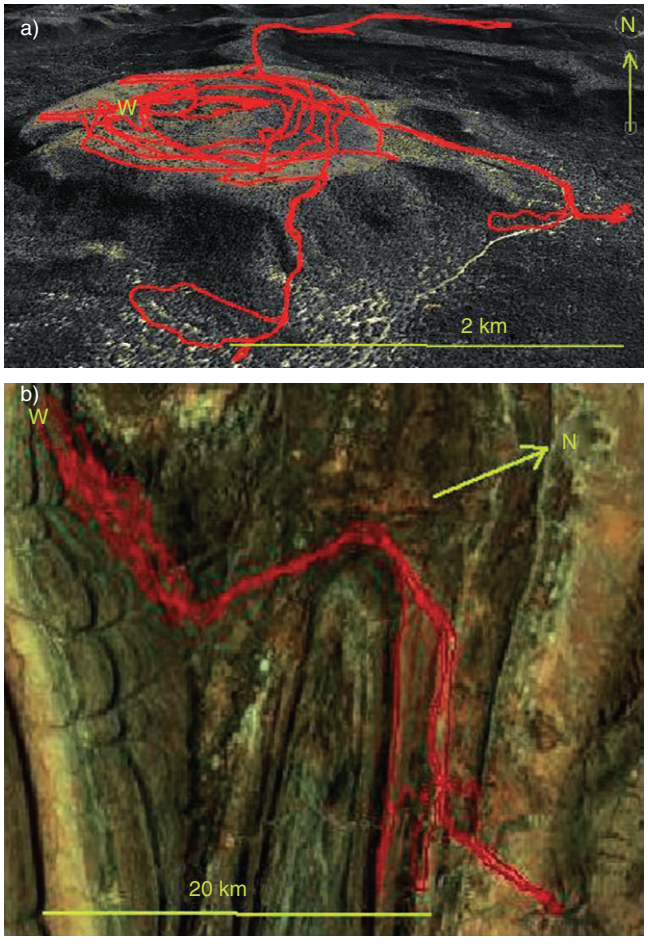

Fig 2: Comparison of typical ranges of horses in the 2 different environments. a) Shows the GPS path of a band stallion in prime grazing country. Each daily travel distance is consistent and within 3.5 km of a central waterhole (W). b) Shows the GPS path of a desert mare with a young foal at foot. Water (W) is at one end of the path and the preferred feeding ground is a distance of 22.5 km from water. True North is shown by 'N'. The end of the path is around 40 km in a straight line from the marked water.

never more than approximately 8 km from water. Central Australian horses travelled significantly greater single day distances when travelling to and from water and were frequently recorded at distances of 15–55 km from water. Long distance travelling days (mini-migrations), to and from water, were followed by short travelling days within good feeding locations (Fig 2).

Watering frequency

The mean watering frequency of central Queensland horses was 2.1 ± 0.9 days (range 1–3 days). Central Australian horses watered less frequently and this difference was significant ($P<0.05$). Their mean watering frequency was 3.2 ± 0.8 days (range 2–4 days).

Discussion

There is a large variability of range use ecology within the Equid family. There have been several previous studies reporting the home range size of feral horses determined by repeated sightings during ground based observations. The accuracy of observation vs. GPS data logging has not been established but observation has previously been the gold standard for animal ecologists (Linklater *et al*. 2000). Since the introduction of accurate and reliable GPS, animal ecologists are now able to monitor animals regularly for long periods without risk of interference. Linklater *et al*. (2000) reported a home range size for Kaimanawa feral horses of 0.96–17.7 km^2 for 48 horse bands (horse groups). Kaimanawa horses have a similar breed origin to the Australian feral horses (Halkett 1996). Linklater *et al*. (2000) found that all band home ranges overlapped with others and were located within an area of approximately 7 × 4 km. Similar spatial organisation of feral horses was reported by Klingel (1975), Salter and Hudson (1979) and Feist and McCouough (1975) in feral horse populations confined in small ranges. However, the study of arid and semi-arid habitat horses in Nevada (Berger 1986), western Alberta (Salter and Hudson 1982) and Wyoming (Miller 1983), have reported larger home ranges. These studies relate the increased size of the home ranges to resource demand. Kaczensky *et al*. (2008) were the first to report equid movement and home range size using GPS technology. Over a 4 year period in Mongolia, wild Przewalski horses had ranges of 152–826 km^2, significantly larger than reported for feral horses elsewhere. Over this 4 year period Przewalski horses travelled approximately 60 km from one end of their range to the other but the mean daily distance was 3.5 ± 0.9 km. On average, Przewalski horses ventured only 9 ± 2.9 km from the nearest water. The same study reported home ranges of 4449–6835 km^2 with a mean daily distance of 8.3 ± 0.7 km for Asiatic wild asses. Asses ventured a mean of 13.5 ± 0.9 km away from water. Other *Equidae* studied include the plains zebras which were recorded using GPS trackers walking up to 15 km away from water to feed and returning to water at 3–4 day intervals (Brooks *et al*. 2007). The current study used GPS technology to determine feral horse movement and watering patterns. We found that central Australian and central Queensland feral horses travelled larger mean daily distances than other equids reported previously. Central Australian horses, in particular, travelled up to 55 km from water and had large interwatering intervals.

The current study found that even though the average daily distance did not vary significantly between the 2 populations from contrasting habitats, the day by day routine of the horses was quite different and appeared to be dependent on resource location. In central Queensland, there was little requirement for horses to travel large distances between food and water. Pasture was widespread and pasture quantity close to water was similar to that at a distance away from water. However, in central Australia, resource distribution was scarce and patchy. Feed tended to grow solely along gully floors and low lying areas where water may have pooled and soaked the ground for several days following infrequent rain. Berman (1991) studied the ecology of feral horses in the same study area in the mid 1980s and measured the herb cover at distance intervals from the same permanent water source used by horses in the current study. Herb cover increased relative to the distance away from water (Fig 3), indicating the large competition for food resources close to water. Berman (1991) found evidence of horses 65 km away from the only available water source.

The current study located horses 55 km from Berman's (1991) water point. In consideration of the long walks the horses must make to access water from distant feed grounds it is not surprising that watering frequencies were as low as every fourth day. Scheibe *et al*. (1998) found that Przewalski horses drank less frequently than domestic horses and drank a higher volume of water relative to body size. They suggested that drinking frequency was not as

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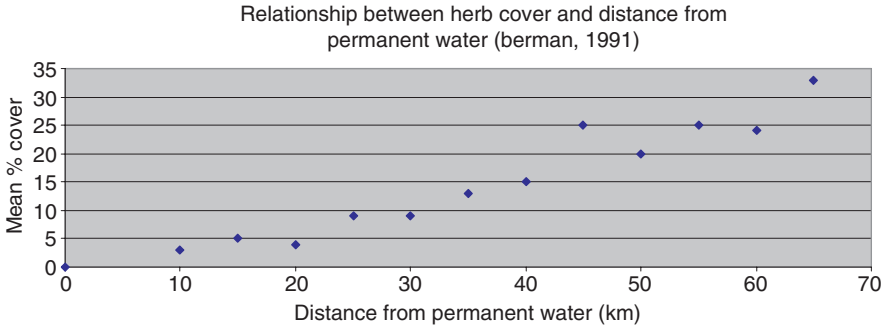

Fig 3: The relationship between herb cover and distance from permanent water in the desert horse habitat recorded by Berman (1991). Horses had to travel long distances from water to access feeding grounds. Feed within a range of 20 km from water was minimal. A similar situation existed in this location during the present study. This graph was drawn from data from Berman (1991) with permission from the author.

important to wild horses as the amount of water consumed. Horses that drank less frequently also ingested larger volumes of water. These authors, as well as Bouman and Bouman (1994) and Sneddon *et al*. (1991) all suggested that desert-dwelling horses are genetically adapted to hot and dry climates as part of the evolutionary strategy of the species. Other equid species have apparently also adapted to arid conditions. Maloiy (1970) subjected Somali donkeys (*E. asinus*) to heat stress and dehydration. Donkeys survived water loss equivalent to 30% of their original bodyweight in temperatures up to 40°C and survived up to 12 days without water. Donkeys were able to drink enough water (24–30 l) in 2–5 min to restore the deficit. Maloiy (1970) concluded that these observations represented a thermal and metabolic adaptation of desert mammals to heat and aridity. This adaptation did not totally eliminate the need to drink water, but permitted a considerable degree of dehydration. Donkeys are capable of living and reproducing in arid habitats similarly to camels in the Middle East, northern and eastern Africa and central Asia.

Central Australian horses may also be genetically adapted to arid conditions. The population of feral horses in the semi-arid areas of central Australia can be reduced by up to 75% in times of severe drought (Berman 1991). The ability to travel up to 65 km from water to access feed (Berman 1991) and survive for at least 4 days without water (present study) may represent a physiological adaptation of the original domestic horse stock that were the founders of the feral horses over 140 years ago. The strong pressure of the frequent severe droughts in central Australia for natural selection may have driven a genetic change. In contrast, domestic horses travel very small distances compared to their feral relatives (Hampson *et al*. 2010), generally have free access to water and have been documented to drink several times daily (Scheibe *et al*. 1998). Scheibe *et al*. (1998) suggested that the high water consumption of domestic horses may be a learned behaviour acquired under conditions of abundance.

The current study determined the range of 12 adult feral horses in 2 different wilderness environments over a 6.5 day period. Mean distance travelled was 15.9 ± 1.9 km/day (range 8.1–28.3 km/day). Horses in the central Queensland habitat, with plentiful pasture, tended to remain close to water (maximum 8 km from water). Central Australan horses were recorded up to 55 km from water and some horses walked for 12 h to reach water. Feed was scarce within a range of 15 km from the water point in the central Australian habitat due to competitive grazing by large numbers of feral horses, feral cattle and feral camels. These horses watered less frequently (3.2 ± 0.8 days; range 2–4 days) than central Queensland horses. Central Australian feral horses travelled longer mean daily distances and recorded longer watering intervals than other wild equids (Rubenstein 1989; Berman 1991; Scheibe *et al*. 1998; Adriansen and Nielson 2005; Brooks *et al*. 2007; Kaczensky *et al*. 2008). The unique ecology of the central Australian horse, as shown by this study, warrants further study.

The ability to survive the extreme environment of central Australia may be within the phenotype of all horses and simply a testament to the versatility of *E. caballus* as a species. On the other hand the central Australian population, subjected to the extreme selection pressure of the harsh environment, may have already inherited traits that set them apart from their domestically managed cousins.

Conflicts of interest

The authors have declared no potential conflicts.

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Review

Is Wildlife Fertility Control Always Humane?

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Simple Summary: There are various fertility control methods (modalities) currently available that aim to reduce the abundance of problematic free-ranging mammalian wildlife. Here, we propose that dissimilarities in the mechanism of action indicate these methods produce great variation in animal welfare outcomes. We present a framework to assist managers in minimising animal welfare risks.

Abstract: Investigation of fertility control techniques to reduce reproductive rates in wildlife populations has been the source of much research. Techniques targeting wildlife fertility have been diverse. Most research into fertility control methods has focused upon efficacy, with few studies rigorously assessing animal welfare beyond opportunistic anecdote. However, fertility control techniques represent several very different mechanisms of action (modalities), each with their own different animal welfare risks. We provide a review of the mechanisms of action for fertility control methods, and consider the role of manipulation of reproductive hormones ("endocrine suppression") for the long-term ability of animals to behave normally. We consider the potential welfare costs of animal manipulation techniques that are required to administer fertility treatments, including capture, restraint, surgery and drug delivery, and the requirement for repeated administration within the lifetime of an animal. We challenge the assumption that fertility control modalities generate similar and desirable animal welfare outcomes, and we argue that knowledge of reproductive physiology and behaviour should be more adeptly applied to wild animal management decisions. We encourage wildlife managers to carefully assess long-term behavioural risks, associated animal handling techniques, and the importance of positive welfare states when selecting fertility control methods as a means of population control.

Keywords: behaviour; capture; endocrinology; fertility; immunocontraception; physiology; reproduction; sterilization; welfare; wildlife

1. Introduction

Animal welfare has been steadily gaining importance and society now recognises it has an obligation to consider the welfare of all animals, including free-ranging species that are perceived to be overabundant or pests. As community and ethical opposition to the killing of animals has risen, especially for charismatic species (e.g., feral horses (*Equus caballus*); [1]), more focus has been devoted to non-lethal methods, particularly fertility control [2]. Fertility control methods have often been grouped together and described using terms such as "humane" [3–5], or "benign" [6,7]. These are not universal opinions though, as some authors have urged consideration of potential animal welfare impacts [8,9], including recognition that the welfare consequences of different methods may vary considerably [10].

Despite the importance of animal welfare, there are other aspects of fertility control methods that cannot be ignored. Efficacy will always need to be considered because if a method is chosen that has a reduced welfare impact on the individual, but does not achieve its animal management goals, then arguably doing nothing at all would have resulted in better animal welfare outcomes [11]. The overall welfare impacts of fertility control techniques should also be considered alongside the impacts of other control tools that may be considered as an alternative [12]. In any context, once an "optimal" decision has been made, it should not be blindly extrapolated to all species for all circumstances. With this in mind, there are large gaps in the published literature as most research has focused on the outcomes of fertility control programs in terms of reproductive output (its efficacy). Much less research has examined how each method induces decreased reproductive output, and at what cost to the animal. This evolution of research into wildlife fertility control methods appears to be tracking the same course that investigations into the use of lethal toxins for wildlife control followed, where efficacy studies preceded assessments of welfare impacts [13].

Aims of This Review

Once it has been decided that a fertility control program will be employed, there is considerable uncertainty when choosing a specific fertility control product or technique. Several authors have attempted to address the broad side-effects associated with fertility control of wildlife, including animal welfare impacts [9,14,15], but the relative risks of each fertility control method has so far escaped critical review. However, the American Zoo and Aquarium (AZA) Contraception Advisory Group has provided several articles examining the relative risks of fertility control modalities relevant to collection animals (e.g., [16]). Equivalent critical reviews have been performed for the manipulation of reproductive hormones in human medicine [17], highlighting that divergent properties of fertility control methods are related to dissimilarities in mechanisms of action, and ultimately produce variable clinical consequences.

This review attempts to demonstrate the differences between broad categories of fertility control techniques, with a view to facilitating future selection of practices. Fertility control has been applied to non-mammalian wildlife (such as birds; [18]) but this review will restrict its examination to mammalian

wildlife. The range of fertility control approaches used to manage mammalian wildlife is discussed, with a focus on mechanisms of action, the importance of maintaining natural behaviour and the need to assess all stages of animal manipulation involved in administering required treatments. We suggest principles to advance the scientific rigour behind the selection of fertility control techniques toward a "best practice" model to maximise animal welfare outcomes.

The central aim of this study is to specifically provide a framework for assessing animal welfare risks for various fertility control methods for mammalian wildlife. It cannot be disputed that efficacy and financial costs are important considerations when management decisions are being made, however, these issues have been thoroughly addressed by other reviews [2] and so will not be discussed in detail in this study. The aims of this study are not to compare fertility control with lethal methods, and not to assess side-effects of fertility control methods as a whole. This review explicitly examines animal welfare risks specific to each class of fertility control method and presents a framework for selecting fertility control methods that minimise animal welfare risks.

2. Fertility Control Methods Currently Used

A wide diversity of methods has been applied to wildlife fertility control over several decades. Table 1 lists the most commonly applied fertility control approaches in free-ranging mammals. Fertility control methods vary greatly in the mechanism of action of the treatment applied, as well as the administration method used to deliver them to animals (e.g., surgery *versus* remote injection or "darting"). Table 1 also illustrates that within individual species (e.g., white-tailed deer (*Odocoileus virginianus*)), multiple fertility control methods, representing several mechanisms of action, have been employed. These methods, and many more, have previously been reviewed in detail [2,15]. Several approaches to classifying the various fertility control methods have been suggested (e.g., physical *versus* chemical), but given the inextricable association between any method's mechanism of action and its effect, we argue that classification according to mechanism of action is the most logical approach.

2.1. Mechanisms of Action

Owing to the diversity of fertility control techniques currently available (Table 1; [2]), an appreciation of welfare impacts is not possible unless focus is first placed upon mechanisms of action. "Mechanistic" approaches have been central to rationalising and standardising the use of other wildlife management techniques. For example, rationalisation of a vast array of trade and colloquial names has been possible for toxins [13] and kill-traps [19] that are subjected to welfare assessment. All effects are produced by a mechanism of action, and dissimilarities in these processes in fertility control need to be understood to avoid misunderstanding [20]. For this reason we recommend the use of the term fertility control modalities to highlight the importance of considering how a method works and the long term welfare outcome at the individual animal level, rather than simply considering efficacy at a population level.

Table 1. Wildlife fertility control modalities that have been trialled, categorised according to their mechanism of action.

Class	Technique	Route	Description	Duration of Effect	Examples of Products	Sex Sterilized	Example Application	
							Species	Reference
Endocrine suppression	Castration (gonadectomy)	Surgical	Removal of testicles	Permanent	N/A	Male	Eastern grey kangaroos (*Macopus giganteus*)	[21]
	Ovariectomy/ovariohysterectomy (gonadectomy)	Surgical	Removal of ovaries +/− uterus	Permanent	N/A	Female	Brushtail possums (*Trichosurus vulpecula*)	[22]
	GnRH agonism	Implant	Binds to GnRH receptors	1–2 years	Deslorelin® Suplorelin®	Female Male	Tammar wallabies (*Macropud eugenii*)	[23,24]
	GnRH immunocontraception	Injection	Induced immunity to GnRH	>3 years	GonaCon®	Female Male	Wild boar (*Sus scrofa*)	[25]
	Synthetic testosterone	Injection	Suppressed spermatogenesis	Temporary	N/A	Male	Wild horses (*Equus caballus*)	[26]
	Synthetic progestins	Implant	Prevention of ovulation	3–5 years	Norplant®	Female	Koalas (*Phascolarctos cinereus*)	[27]
	Synthetic prostaglandin	Implant/biobullet	Pregnancy termination	Temporary	N/A	Female	White-tailed deer (*Odocoileus virginianus*)	[28]
	Synthetic oestrogen	Oral bait	Prevention of implantation	Temporary	N/A	Female	White-tailed deer (*O. virginianus*)	[29]
	Synthetic oestrogen	Implant	Prevention of implantation	Temporary	N/A	Female	White-tailed deer (*O. virginianus*)	[30]
Physical non-endocrine	Vasectomy	Surgical	Ligation of vas deferens	Permanent	N/A	Male	Eastern grey kangaroos (*M. giganteus*)	[21]
	Epididymectomy	Surgical	Removal of epididymis	Permanent	N/A	Male	Feral goats (*Capra hircus*)	[31]
	Tubal ligation	Surgical	Ligation of oviducts	Permanent	N/A	Female	White-tailed deer (*O. virginianus*)	[32]
	Oviduct "webbing"	Surgical	Removal of oviducts	Permanent	N/A	Female	Feral cattle (*Bos taurus*)	[33]
	Intra-testicular injection	Injection	Sclerosis of testicles	Permanent	Neutersol®	Male	American black bears (*Ursus americanus*)	[34]
	Intra-uterine devices	Implant	Endometrium irritation	Temporary	N/A	Female	Wild horses (*E. caballus*)	[35]
Chemical non-endocrine	Zona pellucida immunocontraception	Injection	Induced immunity to ovum proteins	>3 years	ZonaStat-H® SpayVac®	Female	Tule elk (*Cervus elaphus nannodes*)	[36]

2.2. Broad Classes of Fertility Control Modalities

In order to clarify the modalities for each fertility control agent, the following broad classes should be adopted: endocrine suppression methods, physical non-endocrine methods, and chemical non-endocrine methods (Table 1).

"Endocrine suppression" [37,38] (or endocrine disruption) methods alter the actions of naturally occurring reproductive hormones. Endocrine processes may be targeted centrally, depressing all reproductive hormonal activity (e.g., gonadotrophin-releasing-hormone (GnRH) agonists) or peripherally (e.g., progestin antagonists). Such is the complex nature of reproductive endocrine feedback processes that all supplementation, agonism, antagonism, or removal of endocrine hormones will ultimately lead to the suppression of natural reproductive hormone effects. Hence, we refer to all processes that affect reproductive hormones as inducing hormonal incompetence [39]. There are four main modalities in this category. Firstly, disruption can be achieved through the surgical removal of gonads (gonadectomy). Secondly, disruption can be achieved through the supply of exogenous (synthetic or externally derived) versions of naturally occurring reproductive hormones (*i.e.*, testosterone, oestrogen, progestins; Table 1) that interfere with normal feedback control and secretion of endogenous hormones. This method is commonly utilised in human contraception [17,40,41]. Thirdly, endocrine disruption can be achieved by agonism or antagonism of naturally occurring (endogenous) reproductive hormones (e.g., GnRH), via the administration of chemicals that actively compete with hormone binding sites. Finally, immunocontraception can be used to induce an autoimmune response through vaccination against naturally occurring reproductive hormones (e.g., GnRH).

Physical non-endocrine methods rely on physical (rather than chemical) means to disrupt the normal functioning of the reproductive system, and do not alter naturally occurring reproductive hormone levels. These methods encompass surgical techniques that *occlude* reproductive passages (but do not remove endocrine reproductive organs) or the use of implants that physically irritate the female reproductive tract (e.g., non-hormonal intrauterine devices; [35]). Surgical techniques that occlude reproductive passages but leave endocrine reproductive function intact include modalities that ligate (oviduct ligation, vasectomy) or remove (oviduct removal, epididymectomy) reproductive passages, but not gonads (Table 1). It is important to note that traditional surgical techniques used for fertility control in companion animals remove gonads ("gonadectomy"), including castration and ovariectomy/ovariohysterectomy (Table 1), and render reproductive hormone patterns nonfunctional [42,43]. We therefore classify these methods as inducing endocrine suppression despite their application through physical means (surgery).

Chemical non-endocrine methods rely on inducing an autoimmune response through vaccination against naturally occurring ovum proteins, most commonly the zona pellucida (ZP) [44], to prevent fertilisation of ova. This is achieved in the same way that a vaccine is used to mount an immune response against a pathogenic microbe (e.g., virus or bacterium) except that the immune response is being directed towards the animal's own ovum proteins. Once the animal's immune system disrupts the capacity for an ovum to be fertilised, affected females are rendered infertile irrespective of how many times they are mated by a male [44]. Similarities in the effects of physical and chemical non-endocrine modalities have been demonstrated by the use of physical occlusion surgeries to model the effect of ovum protein immunocontraception in epidemiological studies [45].

This first principles approach of classifying fertility control methods according to modalities should enable more meaningful comparisons and improved assessments of welfare risks at an individual animal level. This approach should also help to clarify some misconceptions arising from the oversimplification that has been used in the past to describe classes of methods (e.g., [46]). We therefore advocate a focus on modalities, rather than colloquialisms or trade names [47], when considering the implications for individual animals for any chosen treatment.

2.3. Best Practice Approaches

For some time, many contentious lethal (e.g., shooting, toxins) and non-lethal (e.g., marking) wildlife management methods have been closely scrutinised, with strong focus placed upon animal welfare impacts, rather than efficacy in isolation [19,48]. Quantitative assessment methods have allowed ranking of humaneness for many lethal wildlife control methods [12], in combination with the development of "best practice" approaches (e.g., [49]). In contrast, animal welfare assessment of fertility control methods has not been evaluated with the same level of scientific rigour and much uncertainty remains regarding animal welfare impacts [50]. As identified in reviews [9], most research into welfare risks has used qualitative, anecdotal evidence, and/or presented opportunistic, qualitative data lacking appropriate controls, sampling methodology or time scales. For these reasons, reviews have identified the assessment of welfare risks for fertility control as a research priority [9]. In the absence of quantitative humaneness assessment templates for fertility control modalities, "best practice" standards have not been developed and methods are still largely chosen on an *ad hoc* basis, and there remains much variability in how modalities are chosen and applied.

3. Animal Welfare Risks

It is proposed that any animal treatment can have short-term adverse effects on welfare (for example those caused by an injection) followed by long-term effects, which may be very different [51]. Both should be assessed. At the same time, modern approaches to animal welfare propose that avoiding negative welfare states (harms) should be considered in addition to the provision of opportunities for pleasurable experiences (such as social play, courtship or parenting [52,53]). This approach dictates that animals need positive experiences, in addition to minimised suffering [52,54]. Below we present a list of all of the animal welfare risks associated with fertility control, from administration (short term) to the response to treatment (long term) and include those imposing negative, and restricting positive welfare outcomes, respectively.

3.1. Short-Term (Administration) Negative Animal Welfare State Risks

3.1.1. Animal Capture, Restraint, Anaesthesia, Marking and Surgery

Most stages of wildlife management techniques that involve animal manipulation are likely to impose welfare costs. This is recognised for lethal wildlife control techniques, where animal welfare assessment frameworks require consideration of stages of animal manipulation prior to the killing method [12]. Some wildlife fertility control programs require animals to be captured and handled [55] or undergo anaesthesia and surgery [21] while other techniques allow animals to be darted (remotely treated)

in situ [56]. Classes of fertility control methods that currently require animal capture are shown in Table 2. These restraint procedures, required to administer a fertility treatment, may impose animal welfare costs that are in addition to the physiological and behavioural effects of the fertility control procedure or agent.

Table 2. Animal welfare risks from administration techniques used to implement common currently used fertility control modalities in mammalian wildlife. All potential stages of animal manipulation are shown to demonstrate techniques that require multiple stages of animal manipulation and those that do not.

Mechanism	Technique	Effect of Administration					
		Capture	Anaesthesia	Surgery	Remote Administration	Multiple Doses *	Frequency of Treatment
Chemical non-endocrine	ZP immunocontraception	−	−	−	+	±	3 years
Physical non-endocrine	Vasectomy	+	+	+	−	−	Permanent
	Tubal ligation	+	+	+	−	−	Permanent
Endocrine suppression	GnRH immunocontraception	−	−	−	+	−	3 years
	GnRH agonism	±	−	−	±	−	1–2 years
	Synthetic progestins	±	−	−	±		3–5 years
	Castration	+	+	+	−	−	Permanent
	Ovariohysterectomy	+	+	+	−	−	Permanent

* 2 doses are often required to ensure the efficacy of a single treatment; ± Can be administered via remote delivery or via capture, restraint and implantation.

In contrast, other fertility control programs have recognised the potential animal welfare costs of administration procedures and have chosen methods that allow for remote delivery of a single dose of an agent [57], effectively minimising stress other than that associated with potential darting injuries [58] or injection site reactions [59]. With the use of many modalities, for example, following remote drug delivery (darting) and surgical procedures, there is a requirement for visual marking of treated animals [60,61]. Marking in itself has been demonstrated to impose animal welfare impacts in many wildlife species [48].

3.1.2. Repeated Treatments

There are variations in the durations of action for different fertility control approaches. Some methods (e.g., surgical intervention) produce permanent infertility while some exogenous endocrine methods may affect fertility for less than one year (e.g., exogenous prostaglandins used to terminate pregnancy; [28]). Some chemical fertility control methods will require repeated administration every 1−2 years (e.g., GnRH agonists; [24]) or require booster administration 1−4 weeks after initial treatment (e.g., older ZP vaccines; [60]). All repeat treatments will impose additional animal welfare costs, as animals will need to be captured/injected several times. Some programs addressed the requirement for booster doses for older ZP vaccines by maintaining wild animals in captivity between injections, a process that can be highly stressful for free-ranging species [62]. For long-lived species (>5 years), multiple rounds of administration may be required to maintain infertility in treated animals [63]. An additional consideration is the aversion behaviour of wild animals after previous exposure to a stressful method.

For example, studies requiring animals to be re-darted [60] or re-trapped [64] have observed greater difficulty in re-administration of a treatment.

3.1.3. Injection or Implantation Site Local Inflammatory Reactions

Many remotely delivered immunocontraceptive modalities, being adjuvanted vaccines that rely on stimulating the immune system, have been shown to induce granulomatous inflammation at the site of injection [59,65]. Some studies have suggested that the welfare impact of such reactions is likely to be similar to the use of any adjuvanted vaccine. Likewise, many forms of chemical implants have demonstrated similar local inflammation effects associated with their administration [66]. The incidence of such adverse reactions, and their impact on welfare in free ranging species may be unknown, given the limited monitoring of many animals post-administration.

3.2. Long-Term (Treatment) Negative Welfare States Risks

3.2.1. Physiological Changes

Significant or prolonged physiological changes may be considered an animal welfare concern as they may alter an animal's ability to perform natural behaviour [67] and/or prevent adaptation to environmental challenges. Physiological responses may affect the animal directly, in terms of increased metabolic rate or redistribution of body reserves, or indirectly, in terms of loss of fitness, such as loss of appetite or decreased immunity [68]. All animals have the capacity to make behavioural changes in response to stimuli but marked physiological changes may restrict the capacity of animals to express the full range of natural behaviour [69]. Physiological changes have been demonstrated for several fertility control modalities, particularly those that derive their effect from endocrine suppression. Many studies on endocrine suppression methods have demonstrated weight gain in treated animals [25,27,70], but have considered the change to be positive or neutral, from a health perspective, without considering the effect of natural behaviour for a free-ranging species required to thermoregulate, forage, escape predators, compete with cohort animals or migrate. Immature male animals subjected to endocrine suppression generally do not grow as large as untreated males, as seen in white-tailed deer (*O. virginianus*; [71]) and tammar wallabies (*Macropus eugenii*; [66]). Endocrine suppression in immature male tammar wallabies (*M. eugenii*) also resulted in increased fat deposition [66]. Changes have also been demonstrated from endocrine suppression modalities to processes such as antler shedding in deer species [72]. Especially when endocrine suppression modalities are applied to juvenile animals, sex-specific phenotypes often fail to develop and male animals can become "feminized". This alteration in natural development means that these impacts are permanent [66,72]. Such altered physiology will likely reduce the capacity of such animals to express a full range of normal behaviour, and adapt to environmental changes.

3.2.2. Altered Behaviour

The importance of natural behaviour, and the ability of animals to experience positive welfare states, is a central tenet of modern animal welfare science [54]. Behavioural repertoires indicating satisfactory welfare should include the expression of normal patterns of maintenance and social behaviour, such as grooming, rest and play [52]. Deprivation of normal behaviour may compromise welfare by limiting

an animal's opportunities for positive experiences [52]. Changes in behaviour can be difficult to measure, but the importance of maintaining natural behaviour has been recognised for wildlife fertility control [73]. Of the few studies that have explicitly assessed behavioural changes in free-ranging wildlife (e.g., [74]), changes have been demonstrated to feeding, resting and dispersal behaviour.

For example, behavioural effects in free-ranging feral horses (*E. caballus*) subjected to endocrine suppression (GnRH immunocontraception) included observations that treated animals fed less, rested more, and travelled less [75]. Endocrine suppression in immature captive male tammar wallabies (*M. eugenii*) resulted in decreased agonistic (related to fighting) behaviour with intact males, lower dominance, and higher sexual interest from intact males [66]. Other behavioural changes are less predictable. For example, it has been demonstrated that male eastern grey kangaroos (*Macropus giganteus*) prefer to be associated with females that have not been subjected to endocrine suppression [76]. On the basis of such non-intuitive findings in an inherently complex field, we hypothesise that the real effect of altered social structures and disrupted social cohesiveness is not yet fully understood.

3.2.3. Extended Breeding Seasons

Beyond any welfare impact suffered by individual animals subjected to fertility control treatment, there are also often non-intuitive welfare costs to other animals of the same species, through social interactions. Specifically, fertility control modalities that prevent pregnancy occurring without preventing oestrus behaviour (physical non-endocrine and chemical non-endocrine methods) often induce a prolonged breeding season. This phenomenon has been observed for white-tailed deer (*O. virginianus*; [4,77,78]) and feral horses (*E. caballus*; [79]). The prolongation of natural breeding seasons can represent animal welfare risks for treated and untreated animals, specifically males. For example, the study of Ji *et al.* [80] demonstrates a pronounced impact on the body condition and welfare of male brushtail possums (*Trichosurus vulpecula*) with the extension of a breeding season through the presence of sterile, but hormonally competent, females. The average body condition of these males was significantly poorer than that of males in control areas. Similar effects have been observed in male deer [81]. It has been argued that extension of reproductive cycling may have important social consequences for feral horses (*E. caballus*; [79]) and African bush elephants (*Loxodonta africana*; [82]). In addition, increases in the cumulative number of mating attempts (social contacts) with non-endocrine modalities may increase infectious disease transmission risks [45].

3.3. Long-Term (Treatment) Positive Welfare States Risks

3.3.1. Deprivation of Parental Behaviour in Nurturing Species

Despite the central intended effects of fertility control agents in preventing the birth of offspring, and hence the opportunity for animals to express parental behaviour, the loss of this positive welfare state has not commonly been cited as a welfare concern for treated wildlife. In comparison, the welfare risks of deprivation of this natural behaviour have been recognised in zoo animals. The European Association of Zoos and Aquaria [83] stated: "limiting the opportunity to breed in species which display nurturing parental behaviour, by definition, reduces an individual animal's opportunity to express one of the most

important and complex sets of natural behaviour and can thus lead to a decrease in welfare". Contention surrounding the importance of this deprivation has been at the heart of many discussions of ethical practices in zoos worldwide [4,84,85].

The impact of this animal welfare risk is not easily demonstrated with empirical evidence. However, deprivation of freedom to express natural behaviour is universally considered an animal welfare concern [54] as it alters an animal's nature or "telos" [67]. Preventing such freedoms may affect animal wellbeing by reducing their ability to seek and gain pleasurable experiences and/or denying individuals the satisfaction of desires or preferences [86]. However, it must be noted that there are also welfare costs associated with reproduction, involving energetic costs and mortality risks that animals must trade-off with reproductive success [87]. Maternal behaviour in particular is complex, involving birth, nursing, and care of young. This loss of an important natural behavioural repertoire to many nurturing mammalian species warrants much greater discussion and consideration in the wildlife management sector.

3.3.2. Deprivation of Courtship and Mating Behaviour

Another positive welfare state that may be deprived of animals is the freedom to express natural reproductive behaviour, including courtship and mating. Endocrine suppression modalities often reduce or eliminate courtship and mating behaviour [81], through their mechanism of action targeting endocrine processes centrally, and depressing all reproductive hormonal activity [88]. For example, white tailed deer (*O. virginianus*) males subjected to an endocrine suppression modality (GnRH immunocontraception) display very few mating attempts with untreated (hormonally competent) females [78]. Courtship and mating behaviour are considered essential components of natural behaviour for many free-ranging mammal species and the deprivation of this natural behaviour must also be considered an important animal welfare impact, whether or not it is experienced as deprivation given the lack of reproductive hormonal stimulation for the behaviour to occur.

4. Fertility Control in Other Species

Many fertility control modalities currently applied in wildlife management were initially developed to meet the economic and emotional needs of owners of domesticated animals, such as companion or production animals. Some endocrine suppression modalities (e.g., GnRH immunocontraception) were developed for livestock species to intentionally alter physiology (to influence meat quality) and behaviour (to reduce aggression) to benefit production, rather than to prevent reproduction [89]. Fertility control of wildlife was initially performed as an extension of the practices used by veterinarians for domestic animal control and ownership, primarily gonadectomy [42,61]. However, gonadectomy is not a universally supported practice for companion animals, and the considerable welfare costs associated with the profound endocrine suppression it induces has seen its use considered unethical or even illegal in some countries (e.g., Sweden, Norway) [42,90]. The justification of the need for routine gonadectomy of companion animals is not uniform between countries, and these inconsistent views are based on value differences [42].

In livestock management, hormonally incompetent animals have been viewed as "modified" by many scientists, with concerns about the welfare of treated animals as well as human health from consuming treated animals [51]. Many studies of domestic animals subjected to endocrine suppression modalities

have demonstrated (often desired) physiological and behavioural changes, when observation methods have been intensive and long-term [89]. Despite the paucity of similar studies examining long-term changes in wildlife species, it would be naïve to assume that similar welfare costs would be unexpected in free-ranging animals that were similarly treated. It is worth considering if modalities that were originally considered humane in domestic animals should also be considered to be humane in wildlife.

5. Improving Assessment of Animal Welfare Risks

Animal welfare is complex and guidelines for how it should be assessed have evolved over time [91,92]. Modern approaches require that both negative and positive welfare states are considered [53], and that measures used to assess welfare states include both physiology ("health") and behavioural measures, as well as measures of mental state [93]. Fertility control studies have primarily focused on efficacy (reproductive output) in the short term but many studies have explicitly or opportunistically assessed health side-effects [9]. Few studies have assessed broader animal welfare impacts beyond short-term health effects, such as the impact on animal wellbeing when reproductive and social behaviour patterns are disrupted in response to altered physiology, or deprivation of positive welfare states.

5.1. Welfare Is More than Just Health

Although animal welfare is a complex entity, the Five Domains model was proposed over 20 years ago as a conceptual framework to simplify welfare considerations for research animals [94], but it has since been extended to incorporate positive welfare states [92]. The model proposes four physical/functional domains (nutrition, environment, health, and behaviour) and the fifth domain is mental state [94].

The majority of studies that have addressed animal welfare during fertility control programs have effectively restricted their assessments to domain 3 of the model; health, as typically only measures of injury rate, the presence of disease, and body condition have been examined [59]. The high frequency with which local reactions to chemical modalities at injection/insertion sites are described in the literature [9] suggest focus on this in the past has been disproportionally high given the low intensity and duration of suffering involved. However, it has been recognised for more than a decade that assessment of animal welfare must be based on a wide range of measures in addition to health indices [95]. Community debate about intensive livestock farming has demonstrated clearly the difference between concern for animal health and welfare. While intensively raised pigs and chickens demonstrate very high health outcomes (body condition, absence of parasitism *etc.*), their welfare is considered by a large majority of the general public to be severely impaired through impedance of natural behavior, and an absence of positive experiences, that is imposed by small, restrictive housing [69,96,97].

For the majority of wildlife fertility control studies, the length of time that any side-effects have been monitored for has been restricted to short time frames (*i.e.*, 2–3 years [55,77]). However, there have also been longer term studies performed [78]. The potential for, and occurrence of, long-term behavioural changes not detected by short-term studies, has been demonstrated for marine mammal marking techniques [48], and terrestrial chemical capture techniques (bear species; [98]). These studies have urged researchers to look beyond short-term health approaches for holistic assessment of welfare. While the "short-term health" approach is deemed appropriate for assessing lethal methods where death

is expected to be an acutely reached endpoint [19], it is less appropriate for non-lethal methods where duration of suffering may be chronic (years). Long-term welfare risks clearly exist for all fertility control modalities, but until evidence exists to the contrary it should be assumed that the magnitude of the impact of these effects on welfare could be significant.

5.2. Maintenance of Natural Behaviour in Free-Ranging Animals

Similar approaches have been used for fertility control in domestic and free-ranging animals, but there are important differences in society's perception of our duty of care for animals in each context. There is broad acceptance that the maximisation of health should be the focus of animal welfare for the keeping of companion animals [90], while the focus of stewardship for wildlife centres on the maintenance of natural behaviour. For this reason, practices such as supplementary feeding and veterinary treatment of wildlife populations have been consistently discouraged [99–101], For a discussion of the role of ethics in wildlife management decisions related to health and welfare, see the study of Rolston [102].

Many fertility control methods that do affect behaviour and physiology (e.g., altered fat deposition; [103]) for companion animals are considered acceptable (by some scientists [42]) because human owners take full responsibility for the care of these animals. Decreasing a companion animal's survival skills, such as the ability to hunt, survive adverse climatic conditions and defend itself against another competitor are deemed acceptable by society because humans can continue to provide food, shelter and protection for these animals. "Nuisance behaviour" in companion animals [104] such as fighting, roaming, territory marking, and oestrus calling, that are prevented or reduced by endocrine suppression, are all examples of natural behaviour essential for the welfare of free-ranging animals. This distinction between the behavioural needs of companion and free-ranging animals has not been adequately recognised by authors studying species that may exist in either state (e.g., feral/domestic cats (*F. catus*); [103,104]). We accept that the management of some free-ranging species blurs the line between domestic and wild animals (e.g., zoo animals; [84], or animals partially confined in fenced urban reserves; [21]). For these contexts, preservation of natural behaviour may be argued to be ethically less important if animals are not required to escape predation, or extensively forage. This consideration is particularly important given that many studies attempting to assess welfare impacts of fertility control modalities have provided *ad libitum* access to resources (water and feed) for study animals [105,106]. In some contexts where animals are not considered clearly domestic *or* wild (e.g., "street dogs" [42,61]), some management programs have explicitly aimed to modify natural behaviour when it creates conflict with human values (e.g., aggression leading to attacks on humans [61]).

The same logic should not apply for all wild animals, as the duty of care provided by humans often ends for managed wildlife at the point of release from restraint or administration of treatment (e.g., [107]). For this reason in feral horse (*E. caballus*) fertility control, approaches that impact upon androgenic (male) behaviour [26] are no longer considered humane or appropriate. In another context, it has been argued that the only fertility control options that should be considered for coyotes (*Canis latrans*) should be those that allow the animals to remain hormonally competent, such is the importance of the endocrine system for the maintenance of natural behaviour [108]. We argue that the preservation of hormonal competence is important to maximise the welfare of wild animals, due

to the profound effects of endocrine suppression on important behaviour such as mating, aggression and dominance.

6. Case Studies

To illustrate the dissimilarities in approaches that have been taken to fertility control in different species and jurisdictions, we present two case studies. The first describes refinement of modalities used for feral horses (*E. caballus*) in North America and the second describes the relative lack of refinement in modalities considered suitable for Eastern grey kangaroos (*M. giganteus*) in Australia.

6.1. Case Study: Feral Horses (E. caballus) in North America

Fertility control modalities for feral horses have been investigated for several decades in the USA [26,109]. While a range of modalities were used in the past (e.g., exogenous testosterone; [26], intrauterine devices; [35]; Table 1), more recently, remotely delivered GnRH immunocontraception [110] and particularly ZP immunocontraception [62,74,79,111], have become the focus. Importantly, it was recognised decades ago that the short-term welfare risks associated with immobilisation and capture necessitated that fertility control modalities were administered remotely [56]. Immunocontraception vaccines may be administered via remote delivery, removing the need for capture, anaesthesia or surgery, but the requirement for booster administration with older ZP vaccines led some authors to maintain horses in captivity between treatments [62]. However, ZP vaccines also often lead to extended breeding seasons, and therefore there are accompanying increased energetic costs and injury risks from increased mating attempts [112]. The focus on ZP immunocontraception developed from a formal process initiated by the Bureau of Land Management (BLM) [111] in the USA to identify the most humane and practical fertility manipulation options. This departure from *ad hoc* trials of many different methods has seen progress towards adoption of "best practice" methods.

6.2. Case Study: Eastern Grey Kangaroos (M. giganteus) in Australia

Many different fertility control modalities have been trialed for easily captured eastern grey kangaroos in Australia [20]. The fertility control modalities that have been applied to kangaroos and their accompanying welfare risks have recently been reviewed [113]. Much recent research has focused on the use of a range of endocrine suppression modalities. GnRH agonists [24,105], and synthetic progestins [20,63,114], have been the subject of several studies. Chemical non-endocrine (ZP immunocontraception; [115]) and physical non-endocrine (vasectomy; [21]) modalities have also been investigated less frequently. However, eastern grey kangaroos are still managed with physical endocrine suppression modalities (castration; [21]) that are rarely applied to other wildlife species. In addition, modalities that have been delivered via remote injection in other contexts (e.g., GnRH agonists; [116]) are often delivered to eastern grey kangaroos as implants, following capture, restraint and anaesthesia ([105]). There have been no formal attempts to standardise modalities (but see [113]), and no apparent progress toward development of "best practice" guidelines for kangaroo fertility control in Australia, and so *ad hoc* methods continue to be used without careful consideration of animal welfare impacts.

The management of Australian eastern grey kangaroos contrasts strongly with the management of American feral horses. In the USA, management goals have included minimising animal welfare impacts of fertility control on wildlife, particularly feral horses [4], by using methods that only rely on darting animals. In Australia, marsupials are still regularly caught, and subjected to anaesthesia and surgery to administer fertility control treatments that, when compared to ZP immunocontraception, have greater effects on their natural behaviour and physiology [21,63,66]. In Australia, similar modalities have not only been used in several kangaroo species [24], but also wallaby species [66,117] and koalas (*Phascolarctos cinereus*) [27,115,118].

These case studies have shown the different approaches taken towards fertility control in two wildlife contexts and highlight the need for increased consideration for the welfare risks of current practices. For this reason, we propose a framework to better enable researchers and managers to understand the animal welfare risks of any fertility control modality and to improve the balance between meeting animal interests with management needs.

7. Suggested Future Improvements

Given the several decades over which wildlife fertility control research has been performed, we contend that sufficient information exists to move from a *carte blanche* approach towards a "best practice" approach for refining methods to minimise animal welfare impacts. It is our contention that methods that induce endocrine suppression, and requiring repeated or multi-stage interventions, are likely to represent poorer animal welfare outcomes than those methods that do not (Table 3). We suggest where duration is similar, the modality inducing the least changes to natural behaviour and physiology will pose the least welfare risk. Likewise for administration risks, the total welfare risk should be considered as the cumulative total of the invasiveness of the procedure, and the duration and frequency of the procedure. For this reason, we propose that welfare risks associated with long-term treatment effects are considered a higher welfare priority than short-term administration risks.

We contend that the deprivation of parental behaviour is an unavoidable welfare cost common to all fertility control modalities. Beyond this cost, several studies have identified that the modalities that minimise welfare risks prevent pregnancy but do not disrupt endocrine function, thus ensuring that reproductive/social behaviour changes are minimised [39,79]. However, with the availability and marketing of modalities that induce endocrine suppression but have appealing ease of administration benefits (e.g., GonaCon™), many researchers have opted to induce endocrine suppression in treated animals despite the existence of alternative modalities that do not (e.g., ZP immunocontraception). We recognise that GnRH vaccines offer increased efficacy and convenience through their effectiveness against both sexes, single injection administration, and lack of extended breeding seasons. However, these efficacy advantages should be balanced against clearly increased welfare costs for a method that causes changes to natural behaviour and physiology [89].

Table 3. Suggested principles for assessing cumulative animal welfare impacts for wildlife fertility control modalities. Methods are listed from top (least animal welfare impact) to bottom (most impact). For example, administration methods requiring capture, anaesthesia, and surgery are given a +++ rating. ZP = zona pellucida. GnRH = gonadotrophin releasing hormone.

Mechanism	Technique	Impact of Treatment	Impact of Administration
Chemical non-endocrine	ZP immunocontraception	+	+
Physical non-endocrine	Vasectomy	+	+++
	Tubal ligation	+	+++
Endocrine suppression	Synthetic progestins	++	++
	GnRH agonism	++	++
	GnRH immunocontraception	+++	+
	Gonadectomy	+++	+++

We suggest that methods requiring capture, anaesthesia, and/or surgery, or frequently repeated administration (in long-lived species) represent poorer animal welfare outcomes when compared to remotely deliverable methods (Table 3). Given the above animal welfare risks associated with treatment (long-term) and administration (short-term), we contend that remotely deliverable non-endocrine vaccines (*i.e.*, ZP immunocontraception) are likely to be the most humane fertility manipulation methods currently available (Table 3). While welfare impacts are high for administration of physical non-endocrine modalities (vasectomy, tubal ligation), repeated treatments are not required and hormonal competence is maintained. For all non-endocrine modalities, important positive welfare states (courtship, mating) are maintained. However, the effects of extended breeding season associated with all non-endocrine modalities suggests that species specific traits need to be considered to avoid welfare impacts relating to large increases in mating attempts (e.g., [80]) or the birth of offspring in non-favourable seasons [78].

On the same mechanistic basis, we contend that gonadectomy represents the least humane of the currently available fertility control technique for wildlife. Gonadectomy requires capture, often major (abdominal) surgery, anaesthesia, and induces complete and irreversible reproductive endocrine suppression, and accompanying permanent changes in natural behaviour and physiology [42,90]. We suggest that the welfare impacts associated with gonadectomy render it appropriate only for domestic animals where a human duty of care is provided, and where behavioural changes are desired (e.g., [119]). GnRH immunocontraception and agonism both induce partial suppression of hypothalamic-pituitary-gonadal axis function [106], but the effects are not as profound as those of gonadectomy. GnRH immunocontraception is likely to pose the lowest welfare risks of all endocrine suppression modalities due to its' favourable administration method (Table 3). GnRH agonism and exogenous progestin modalities both induce endocrine suppression and require repeated administration (sometimes via remote delivery but often requiring capture), but not major surgery (Table 3). However, there has been progress toward developing remote delivery systems for both progestin implants [56] and GnRH agonists [116], reducing the short-term welfare risks associated with their administration.

Suggested Principles for Selection of Fertility Control Methods

In an attempt to improve the present approach to the choice of fertility approaches, some authors have suggested the use of formal criteria for the selection of methods that are appropriate for field applications [2]. While we support the criteria approach of Massei and Cowan [2] for assessing efficacy, we suggest that more holistic scientific rigour should be applied to assessing welfare impacts. We present Table 3 as a framework for assessing the welfare pros and cons of commonly used modalities. The challenging nature of making welfare decisions does not justify averting from the central tenets of scientific logic: observation, hypothesis and experiment. Many decisions on the choice of fertility control modalities have been *carte blanche* and this represents the antithesis of scientific reasoning. We have attempted to address the short-term and long-term animal welfare risks, as per Broom [51], in a framework using a weighting approach (as per Dubois and Fraser [101]). Table 3 summarises the parameters that we consider most important for selecting control methods, and we suggest the following principles for selecting the most humane fertility control methods for wildlife populations:

(1) It should be recognised that all fertility control methods impose animal welfare impacts. The deprivation of the opportunity to display parental behaviour in nurturing species is an animal welfare impact,
(2) Methods should be chosen that pose the lowest cumulative animal welfare cost, including capture, administration and frequency of treatment,
(3) All domains of animal welfare should be considered, including behaviour and the opportunity for animals to have positive experiences,
(4) Methods that maintain hormonal competence, thus promoting natural behaviour, should be chosen over methods that induce endocrine suppression, and
(5) Where uncertainty exists regarding welfare impacts, fertility control modalities should not necessarily be chosen over alternative lethal or non-lethal methods.

Any decisions regarding wildlife management will involve seeking a balance between the interests of the wildlife and other stakeholders. The suggested use of our framework in ethical decision-making has some unavoidable subjectivity relating to the difficulty in comparing various types of risks (e.g., long duration-low intensity welfare impacts with short duration-high intensity welfare impacts), which value-based ranking systems (e.g., [12]) may address. However, we contend that the proposed weighting approach will be more informative for wildlife managers than a list of comparative features without commentary. In addition, the weighting approach has been widely applied for welfare studies lacking comprehensive empirical evidence of all aspects (e.g., [101]).We present this framework as a provocation to wildlife researchers and wildlife managers to improve the rigour with which they assess management options, and to broaden their understanding of animal welfare.

We recognise that the range of species and contexts for which wildlife fertility control is desired makes the proposal of a single preferred method impossible [40]. We accept that efficacy, economic costs, ecological side-effects, and other factors are also important considerations for fertility control programs [2]. We present this framework to help with objective assessment of animal welfare, and to aid managers in achieving balance when weighing welfare impacts against other management considerations. It is an accepted reality that methods representing optimal animal welfare outcomes

may not be appropriate for all management contexts. We contend that understanding the complexities of both reproductive physiology and animal welfare science should be improved in wildlife management. Claims that fertility control modalities pose "no undesirable physiological or behavioural side-effects" from limited observations [105] are unrealistic and display a poor understanding of animal welfare. Likewise, the lack of consideration for the importance of depriving animals of positive experiences when comparing fertility control to other management options requires re-consideration. The lack of standardisation of wildlife fertility control modalities in many contexts is indicative of the enduring misconception that non-lethal reproductive manipulation is synonymous with benign outcomes. We contend that wildlife fertility control should not always be assumed to be humane.

8. Conclusions

Efforts to apply fertility control to wildlife have now been performed for over 30 years but there has been little standardisation of procedures over this time. Research into this area has primarily focused on the important aspect of efficacy but this has meant that the associated impacts on animal welfare have only seldom been addressed. Consequently, there is a growing need for more meaningful interchange between animal welfare scientists, wildlife biologists and wildlife managers [120]. Understanding the mechanisms of fertility control modalities and the resultant impacts on natural behaviour are important and have been under-appreciated. Broader understanding of modern animal welfare science and the importance of positive welfare indicators are required for wildlife managers. We contend that endocrine suppression in free-ranging animals is an undesirable outcome because of its long-lasting alterations to natural behaviour and physiology. Not all fertility control modalities induce endocrine suppression and these methods that do not should be treated preferentially. Likewise, administration methods that require capture, surgery, or frequently repeated treatment are similarly undesirable. Animal welfare impacts should be recognised and carefully balanced against existing management goals to ensure continuous improvement in wildlife fertility control methods.

Author Contributions

Jordan Hampton and Teresa Collins were largely responsible for the generating of ideas and writing of the manuscript. Tim Hyndman and Anne Barnes provided input and associated literature on specific topics, and reviewed the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Abstract

8 adult male beagles that had previously experienced 30-40 complete matings and 8 beagles that had experienced no copulations were given 7 series of mating tests extending for approximately 8 mo. after castration. No data from various measures of appetitive behavior and consummatory responses indicated that inexperienced Ss were inferior to experienced Ss in postcastration sexual performance. Most Ss of both groups continued to mate throughout the experiment, although a definite reduction in sexual motivation was apparent soon after castration of experienced Ss. Observations of the consummatory responses of experienced Ss revealed no reduction in duration of the intense ejaculatory reaction, but a significant reduction in the duration of the copulatory lock following castration. (PsycINFO Database Record (c) 2016 APA, all rights reserved)

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Effects of castration on sexual behavior of tropical male goats

Benjamin L Hart, T.O.A.C Jones [2]

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Abstract

This study was undertaken because comparative information regarding the role of gonadal androgen in sexual behavior of adult male mammals is notably deficient in data from ungulate species. After a series of preoperative tests, eight Red Sokoto male goats were castrated and tested for sexual behavior with receptive females at 1 to 2 wk intervals for 52 postoperative wk. Only one animal was judged to have lost the ejaculatory response; this was after 18 wk of postoperative testing. Compared with observations on other species, an unusually high percentage of these goats showed a long-term retention of sexual activity after castration. Even with this long-term retention, however, there was a significant decrement in frequency of ejaculatory responses within one week after castration. The flehmen response, which some believe to be related to detection of excreted urinary pheromones, also declined in frequency after castration.

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APPLIED ANIMAL BEHAVIOUR SCIENCE

The role of gonadal hormones in the occurrence of objectionable behaviours in dogs and cats [1]

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Abstract

The most common way of controlling or altering the behaviour of companion animals is through gonadectomy. Information about the behavioural effects of gonadectomy comes from extrapolation of findings from laboratory studies and from surveys of pet owners whose pets were castrated or spayed. The role of the placebo effect in the latter source of information is unknown. With these limitations in mind, both laboratory studies and pet owner surveys have provided information indicating that: (1) it is the sexually dimorphic behavioural patterns that are reduced or eliminated in males by castration; (2) not all males undergo a change in behaviour following castration; (3) experience and age at time of castration does not predict which males will be altered by castration; and (4) there are apparent species-specific differences between dogs and cats with regard to percentage of males showing a behavioural change related to castration. © 1997 Elsevier Science B.V.

Keywords: Gonadal hormones; Objectionable behaviour; Dogs; Cats

1. Introduction

In the course of human history with domestic animals, one of the oldest means of altering behaviour was by castration of male animals. The first animals castrated were livestock and, for thousands of years, castration of male cattle, sheep, goats and swine has been a routine husbandry practice. The fact that castration was so readily adopted by animal owners in early human societies is a testimony to the perceived, if not actual,

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effectiveness of the procedure in altering behaviour. Clearly, these ancient animal caretakers believed that castration reduced the chance of serious fighting among adult male conspecifics as well as the tendency for male animals to be aggressive towards their human caretakers (of course, a few non-castrated animals were kept for breeding). The castration of companion animals and the practice of ovariectomy has been more recent. The time in history when it became routine for male cats to be castrated is unclear, and male dogs were never routinely castrated.

Given the current widespread practice in Europe and North America of routinely castrating male cats before adulthood to prevent objectionable behaviour and the growing practice of castration of male dogs in an attempt to alter objectionable behaviour in adults, it is important to examine what is known about the effects of castration in altering male behaviour. Issues that have received some attention are: (1) which behaviours are affected; (2) the probability that a specific behaviour in an individual will be affected by castration; (3) the role of experience in the retention of a behavioural pattern after castration; (4) species differences in response to castration; and (5) a comparison of animals castrated before adulthood (i.e. before or during puberty) with those castrated as adults. Ovariectomy or ovariohysterectomy (spaying) usually results in complete and immediate elimination of female sexual behaviour, but there are questions about changes in non-sexual behaviour associated with the operation.

Clinically relevant information about alteration of gonadal hormones comes from both experimental studies of laboratory dogs and cats and surveys of pet owners whose pets were castrated or spayed. Information from surveys of pet owners necessarily involves relying upon the owner's observations. This brings up the issue of the so-called placebo effect if the owner expects gonadectomy to alter the behaviour in some way. In clinical trials in human behavioural pharmacology, it is common to use placebo treatment in double-blind studies to differentiate drug effects from placebo effects. The placebo effect may be quite pronounced resulting sometimes in as many as 30 percent of patients responding to placebo (Benkert and Maier, 1990; Rudorfer, 1993). The degree to which a placebo effect may be involved in the reports of owners of animals about the effects of castration remains to be seen. One could argue that this effect may be less important than that in human behavioural pharmacology because the animal has no expectations of a behavioural change. On the other hand, the placebo effect could be greater because the owner does not see all of the animal's behaviour and may be biased towards expecting a favorable response and/or institutes some behavioural modification procedures without investigator's knowledge (Hart and Cliff, 1996). With regard to castration, it is not feasible to have a placebo group, except perhaps with the ethically questionable procedure of using prosthetic testicles and a sham operation. Once several double-blind placebo-controlled studies are conducted with psychoactive drugs with animals, there will be some indication of the potential for a placebo effect and this could possibly be factored into the results of studies where client reports are used to create the data base. Until that time, the only alternative is to report the results from carefully conducted clinical surveys where an effort is made to acquire unbiased responses from pet owners. A placebo effect, if it does exist, is probably greater with the more subjective evaluations such as changes in aggressive behaviour than with less subjective judgements such as changes in urine deposits in the house.

In the text below, we lay some groundwork for discussion of the effects of gonadectomy on clinically relevant behaviour by covering the concept of sexually dimorphic behavioural patterns and the effects of castration on sexual behaviour as studied in the laboratory. The discussion then turns to the effects of castration on clinically relevant behaviours of adult male cats and dogs, followed by a comparison with males castrated before or at the time of puberty. Finally, there will be some mention of the effects of gonadectomy on aggressive behaviour of female dogs.

2. Concept of sexual dimorphism

A well-established concept in mammalian sociosexual development is that males experience a surge in testosterone secretion late in prenatal life, early in neonatal life, or across both periods. This surge apparently acts on the brain to bring about gender-specific cytoarchitectural changes in the hypothalamus and other areas of the brain that are related to the later display of male sex-typical behaviour (Arnold and Gorski, 1984). Actually, the mediation of the effects of testosterone in male brains is through local aromatization of testosterone to estrogen which brings about early organization of both the sexually dimorphic structures in the brain and behaviour (Hutchison and Beyer, 1994). Hypothalamic areas of the female brain are structurally organized in a feminine direction due to the lack of testosterone (and estrogen). Developmentally, the female phenotype is shaped first and through the action of androgens (aromatized to estrogen), the male phenotype is reshaped from the existing female pattern.

The above concept, developed from work on laboratory rodents and primates, presumably applies to all, or almost all, mammalian species, including dogs and cats. However, the hormonal profiles of dogs and cats during the prenatal period have not been characterized. Measurements of serum testosterone from newborn dogs from birth through 7 weeks postpartum, revealed only slightly higher levels in males than females. The levels in males were too low to represent a neonatal testosterone surge (Hart and Ladewig, 1979). Thus, assuming that a perinatal surge in testosterone occurs in dogs (and cats) as it does in other mammals studied, it would probably be prenatal.

Once the period of perinatal brain development has passed, the tendency towards masculinity in males and femininity in females seems to be permanently built into the brain. It is important to point out that both males and females appear to have the basic neural circuitry for the behavioural patterns typical of both sexes (Hart and Leedy, 1985). The difference in brain organization relates to the probability or frequency with which the masculine or feminine system is activated.

Prior to puberty, and presumably before biologically significant amounts of gonadal hormones are produced, males differ from females behaviourally. This has been documented with regard to play in rodents (Thor and Holloway, 1983; Meaney et al., 1985), primates (Owens, 1975) and ungulates (Crowell-Davis et al., 1987; Gomendio, 1988). Male and female dogs and cats may also differ in play (Deag et al., 1988), but differences have not been profiled to the same degree as rodents or primates. One prominent difference between juvenile male and female dogs is the urination posture; male dogs use a stand-lean urination posture and female dogs a squat posture.

As mentioned previously, sexually dimorphic differences between males and females are of degree or frequency, not absolute. Among sexually dimorphic traits there is also an apparent difference in the degree to which males and females differ. For example, leg lift urination is obviously a highly dimorphic trait in which males almost always differ from females. Territorial guarding, if sexually dimorphic, would appear to differ less frequently between males and females. A systematic survey of canine authorities regarding differences between male and female dogs was conducted as part of a project on behavioural profiles of dog breeds (Hart and Hart, 1985a). This survey revealed a continuum with regard to degrees of sexual dimorphism among 13 behavioural traits. Those that were considered to be most characteristic of males or females by authorities interviewed are shown in Fig. 1. Urine marking in the house and mounting other dogs or people were not included in the original survey but they are already acknowledged as highly dimorphic; these two traits are added to the data of Fig. 1.

As will be discussed below, it is the most sexually dimorphic, male sex-typical behaviour that one would expect to be altered by castration of males. Thus, in reference to Fig. 1, urine marking, mounting, aggression to other dogs and aggressive dominance towards people should be altered more readily than activity level or watchdog barking.

Some females display male sex-typical behaviour more than other females. In exploring the possible role of perinatal hormones in this difference among females, investigators have found that intrauterine position can alter the degree to which females of a number of rodent species display male-like sexual behaviour. There are two mechanisms for such intrauterine effects. One is that when females develop in-utero between two males, they are partially masculinized by diffusion of androgens through the amniotic membranes (Clemens et al., 1978; vom Saal and Bronson, 1978). This

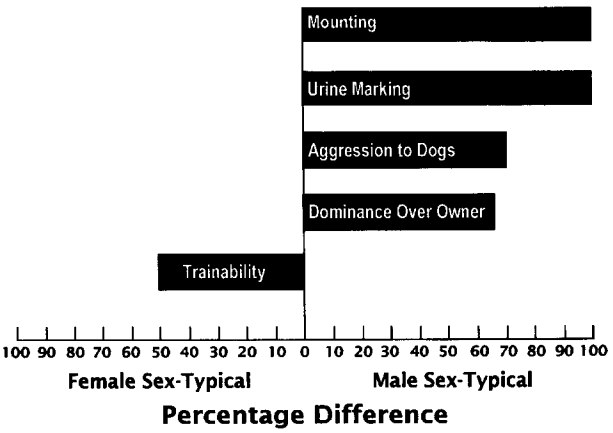

Fig. 1. Degree of sexual dimorphism of behavioural characteristics of dogs as represented by the projected consensus of canine behaviour authorities as to whether a behavioural characteristic was more prominent in male or female dogs. Data taken from Hart and Hart (1985a); Beach (1974); Beach et al., 1982 and Beach and Kuehn (1970). A rating of 100 in either the male sex-typical or female sex-typical direction implies virtually unanimous consensus; a rating of 0 implies that authorities either rated neither sex stronger on that trait or as many rated males stronger as females (see Hart and Hart, 1985a for calculating details). Only traits scoring at least 50 in either the male or female direction by this procedure are listed.

mechanism has been supported by studies on mice and gerbils (Clark et al., 1990; vom Saal, 1981). The other mechanism is that because uterine blood flows from the cervix rostrally, androgens from caudally-located male fetuses may androgenize rostrally-located females through the fetal blood supply (Meisel and Ward, 1981). Support for this mechanism exists for rats, hamsters and ferrets (Houtsmuller and Slob, 1990; Richmond and Sachs, 1984; Vomachka and Lisk, 1986; Krohmer and Baum, 1989).

These studies raise the interesting possibility that the occurrence of objectionable masculine behaviour seen in a small proportion of female cats or dogs could be due to intrauterine position. This possibility has not been tested experimentally in cats or dogs but was explored to a limited degree in a clinical survey involving interviews of cat owners who were familiar with the litters from which their cats were adopted (Hart and Cooper, 1984). In this survey of those owners who knew the littermate composition of their cats, two of the five female cats who urine sprayed frequently, and four of the 14 females that fought frequently, came from all female litters. Since all-female litters could not supply any androgen to adjacent fetuses or up-stream fetuses for masculinization, these data are not consistent with the fetal masculinization effect. Furthermore, of 22 female cats that did not spray or fight, eight came from litters in which there were three or more male littermates (average litter size of six), a condition where masculinization was at least possible. Until more information is available, these results suggest that the tendency for some cats to engage in male sex-typical urine spraying or fighting is not due to intrauterine masculinization.

3. Behavioural effects of castration on sexual behaviour

One of the behavioural patterns most readily affected by castration is sexual behaviour, including mounting, copulatory intromission, and the display of the ejaculatory pattern. These behavioural patterns are readily measured in the laboratory and can be easily evoked in experimental animals. Thus, there is considerable experimental literature on the effects of castration on sexual behaviour in a variety of mammalian species including rats, cats, dogs, rhesus monkeys, and goats. In all of these studies, changes in the behaviour, such as increased latency in response (mounting) to the female, are apparent soon after castration (Hart, 1974). The behaviour that is first lost is that of the ejaculatory pattern followed eventually in some animals by loss of mounting and even interest in females. A comparison of a variety of animals reveals considerable species differences in the rate at which the ejaculatory pattern is eliminated (Fig. 2). According to the studies that were cited in preparing Fig. 2, within 5 weeks following castration, 50% of male cats will no longer showed the copulatory pattern, whereas in male dogs, 80% still showed the pattern, at least occasionally, 15 weeks after castration. The change in sexual behaviour of male rats was about the same as male cats, whereas, goats resembled dogs; rhesus monkeys were intermediate. An evolutionary explanation of these differences in response to castration has been offered (Hart, 1974).

As is apparent from these data, there is considerable individual variation within species in the persistence of sexual behaviour following castration. Thus, in male cats, 20% of animals were still responding to females with the copulatory pattern 15 weeks

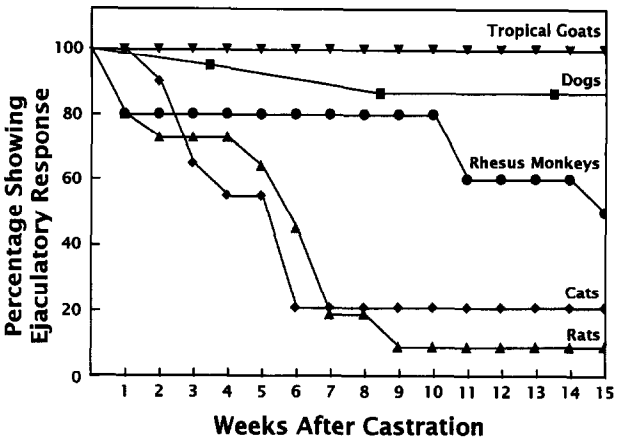

Fig. 2. Species comparisons of differences in persistence of ejaculatory response as a function of duration of time since castration (data from Hart, 1974; Hart and Jones, 1975).

after the operation while 80% no longer responded. In male dogs, 80% were still responding with the copulatory pattern 15 weeks after castration, and 60% one year later (Hart, 1968).

Finding individual differences in response to castration raises the issue of the role of copulatory experience in the loss of copulatory behaviour. This issue has been directly addressed in dogs and laboratory rodents. In a study on dogs designed to explore this issue, animals in the inexperienced group were allowed no copulations prior to castration but were habituated to females by being allowed to mount them. Animals of the experienced group were allowed 30 copulations prior to castration. In tests starting the week after castration and continuing for one year, there was no difference in any parameter of sexual behaviour between the two groups (Hart, 1968). Similar results have been reported with rodents (Hart, 1974). From these laboratory studies and data represented in Fig. 2, three important concepts are established that can probably be extrapolated to clinically relevant behaviour of companion dogs and cats: (1) there are pronounced species differences in behavioural changes after castration; (2) there are pronounced individual differences within a species after castration; and (3) experience in performing the behaviour in question appears to have no predictive ability as to whether or not the animal will change its behaviour.

4. Behavioural effects of castration on objectionable behaviour of male cats

The objectionable behavioural patterns shown by adult male cats that are generally recognized as sexually dimorphic are fighting with neighborhood males, urine spraying and roaming. A retrospective clinical survey (interview of owners) was conducted for 42 gonadally-intact adult male cats presented for one or more of these behavioural patterns (Hart and Barrett, 1973). Castration was effective in eliminating the problem behaviour in 80–90% of the animals (Fig. 3). According to the clients surveyed, in about one-half

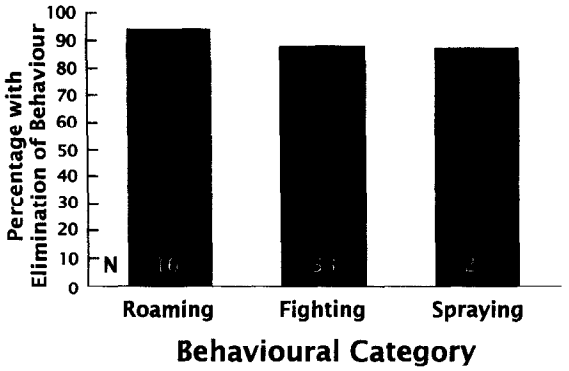

Fig. 3. Percentages of adult male cats in which objectionable roaming, fighting with neighborhood males, and urine spraying in the house were eliminated (problem resolved according to owner's report) following castration. The N is shown at base of bars (from Hart and Barrett, 1973).

of the cases involving aggression towards other males, the cats changed rapidly (within a week or two after castration) with the other half showing a more gradual change. The same was true of roaming behaviour. For cats engaging in urine spraying, most of the animals seemed to undergo a rapid change in the behaviour. Although the clients said the problem behaviour was resolved or eliminated, it is entirely possible, if not likely, that some occurrence of the behavior in question went unnoticed. When cats were presented with more than one of the problem behaviors, an elimination of one behavior did not predict a change in the other problems. The only other behavioural change reported was that 25% of cat owners mentioned their cats became more docile. There was no indication, judging by the age of the animals at the time of castration, that the animals which were older, and presumably more experienced in performing the problem behaviour, were affected any less than the younger, presumably less experienced, males. For example, the median age at time of castration of the cats which showed no decline in spraying was 12 months compared with 18 months for cats that showed either a rapid or gradual elimination of the behaviour.

5. Behavioural effects of castration on objectionable behaviour in male dogs

As mentioned above, the most dimorphic behaviour patterns in dogs are urine marking, mounting, aggressive dominance towards owners and aggression towards other male dogs. A retrospective clinical survey, similar to the one conducted for adult male cats, was conducted on 42 gonadally intact male dogs presented for: urine marking in the house; mounting other people or dogs; intermale fighting and/or roaming (Hopkins et al., 1976). According to the owners, urine marking, mounting and fighting were markedly reduced or eliminated in 50–60% of the animals (Fig. 4). Interestingly, roaming was reduced in 90% of dogs. Data were also available on eight dogs with territorial aggression and four with fear-related aggression but there was no change in any of these dogs. Aggressive dominance directed towards the owners was not included

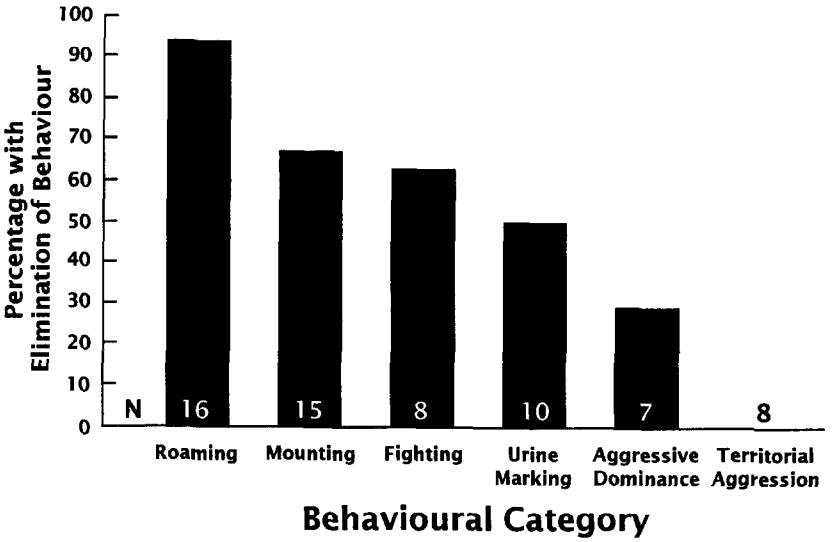

Fig. 4. Percentages of adult male dogs in which objectionable roaming, mounting of people or other dogs, fighting with non-household male dogs, urine marking in the house, aggressive dominance towards the owner and territorial aggression towards strange people were eliminated (problem resolved according to owner report) following castration. The N is shown at the base of the bars (data from Hopkins et al., 1976 and unpublished preliminary results for aggressive dominance).

in the original survey but a recent preliminary study has shown that of seven male dogs presented for this problem, castration resulted in resolution of the behaviour in 2 individuals. These recent data are included in Fig. 4. As with cats, there was no indication that dogs castrated later in life were more likely to persist in the behaviour following castration than those castrated earlier. Even with aggressive dominance involving the owner, experience seemed to play no role as a determinant in response to castration. Of the two dogs that responded to castration, the problem aggression had existed for 5–6 years; in the five dogs that did not appear to respond, the problem aggression had existed for a median of 3 years (range 2–5).

The results of the clinical survey are in line with what would have been extrapolated from laboratory studies on the effects of castration on sexual behaviour. With the exception of roaming, castration was effective in a smaller proportion of dogs than cats, and experience appeared to play no predictive role in which animals changed after castration. The rather uniform effect of castration on roaming in dogs is puzzling. Perhaps, roaming was affected in such a high proportion of dogs because it is a sexually motivated behaviour, and a decrement in sexual motivation which consistently follows castration, may result in major reduction in roaming (even though copulatory performance may still occur).

There are two possibilities for differences in the response of various males to the effects of castration, neither of which has been tested. One is simply genetic differences among individuals related to the sensitivity of neural tissue to the withdrawal of androgenic support (Hart, 1974). Some indication of this possibility might be revealed

by a study of genetic relatedness and persistence of problem behaviour after castration. A second possibility for such differences may reflect the fact that in fetal life some males experience greater exposure to testosterone from other male fetuses located more caudally in the uterus, an effect that has been shown to enhance male sexual behaviour in male rats (Houtsmuller et al., 1994).

The persistence of undesirable male behaviour following castration is apparently not due to any residual amounts of testosterone. It has been documented in a number of laboratory species (e.g. Resko and Phoenix, 1972) and cats (Hart, 1979) that testosterone is metabolized within hours after removal of the testicles. The persistence is not due to compensatory adrenal androgen secretion. Sexual behaviour, for example, persists even after adrenalectomy (Hart, 1974).

6. Behavioural effects of castration before adulthood

It is customary to castrate male cats before they reach full sexual maturity, that is, sometime between 6 and 10 months of age, to prevent fighting, roaming and urine spraying. This practice brings up the question of differences in the effects of castration performed at this earlier age versus after puberty. This question was addressed in a retrospective clinical survey of 134 male cats castrated between 6 and 10 months of age (Hart and Cooper, 1984). Spraying and fighting on an occasional basis occurred in 29 and 44%, respectively, of the cats as adults. Ten percent of the male cats became frequent urine sprayers later as adults revealing that the proportion of male cats that take up frequent spraying as an adult is about the same as the proportion of male cats that persist in urine spraying once they have started the behaviour as adults and are then castrated (Fig. 3).

The mean age of castration for those that sprayed and/or fought occasionally was 8.5 months and for those that engaged in neither behaviour 8.2 months. This small and statistically insignificant difference is consistent with the concept of no relationship within the early castrated animals of age of castration and occurrence of problem behaviour. The same study found that 5% of ovariohysterectomized female cats became frequent urine sprayers as adults, demonstrating that females do engage in male sex-typical behaviour, albeit at a lower probability than castrated males. The only study of prepubertal castration in dogs is one of sexual behaviour in males which found that males castrated prepubertally had the same frequency and tendency to mount females as those castrated as adults (LeBoeuf, 1970). The question of whether cats or dogs castrated well before puberty, for example as early as 2 months of age, will differ from those castrated at 6–10 months of age is yet to be fully tested. The preponderance of evidence revealing no effect of experience and no relationship within the 6–10 month age range between time of castration and later behavioural effects, suggests that no further effect on problem behaviour will be found with very early castration. Early castration of male cats does have permanent effects on morphology, especially in reducing the size of cheeks and jowls. Thus, cats castrated before puberty are visibly different than tom cats and Turner (1995) has suggested this may play a role in evoking

male sex-typical behaviour in males castrated as adults. As a general rule, cats should be castrated before adulthood for best management of problem behaviour.

7. Treating male sex-typical behaviour in gonadectomized males and females

A word should be said about clinical approaches to dealing with objectionable male behavioural patterns of animals previously castrated. With feline urine spraying, eliminating olfactory stimuli that might evoke the behaviour by cleaning up the marked areas with an enzymatic cleaner and blocking visual stimuli from cats outside the home may prove useful. Discouraging marking of previous target areas through the use of remote punishment, or feeding and watering the cats at target areas, has also been recommended (Hart and Hart, 1985b). Although some behavioural therapists have reported a high degree of success with such behavioural measures (Turner, 1995), others find the behaviour difficult to control (at least in the homes of most clients) and pharmacological approaches to this problem have been favored by veterinarians. This treatment has evolved over the last two decades starting with the use of progestins (Hart, 1980) and moving more recently to drugs that enhance gamma-aminobutyric acid (e.g. diazepam) or serotonin (e.g. buspirone). Both diazepam and buspirone suppressed spraying in a higher percentage of animals than progestins (Cooper and Hart, 1992; Marder, 1991; Hart et al., 1993). In prospective studies, both diazepam and buspirone resulted in 55% of cats showing an elimination or marked reduction in the behaviour, but buspirone did not have the undesirable side effects associated with diazepam of transient sleepiness, ataxia, appetite stimulation, and dependency with long-term use (Hart et al., 1993). Although with buspirone and diazepam the proportion of male and female cats responding was not significantly different, with buspirone more cats from multiple-cat households responded than did those from single-cat households. With progestin therapy a higher proportion of male cats than female cats responded.

The treatment currently recommended with buspirone is 5–7.5 mg per cat given twice daily for 1–2 weeks as a trial to see if the drug will eliminate or markedly reduce the behaviour. If the behaviour is altered, it is recommended that the cat be treated 8 additional weeks before gradually tapering off over the ensuing week or two. About half the cats can be expected to resume spraying after the drug is discontinued (Hart et al., 1993). Those that resume spraying can be placed on long-term treatment for another 6–12 months with the expectation that after the drug is then tapered off some animals will continue to be suppressed while others will resume spraying and may be continued on indefinite treatment. A major difference is seen between buspirone and diazepam in the tendency for cats to resume spraying once drug treatment is discontinued. In one study with diazepam, 90% of animals that were on 8 week treatment began spraying when the drug was tapered off (Cooper and Hart, 1992), compared with 50% with buspirone (Hart et al., 1993). The resumption of spraying with diazepam may be because diazepam produces behavioural and physiological dependency, and taking animals off the drug, even gradually, undoubtedly produces some rebound emotional effect that may predispose the animal to resume spraying.

Both buspirone and diazepam may suppress spraying in cats that were previously treated with progestin but did not respond. Based upon the fact that progestins utilize different receptors and perhaps even a different neurological pathway than antianxiety drugs, a model of urine spraying was proposed consisting of two neurological systems, one relating to male sexuality and one to anxiety. Progestin administration, which presumably suppresses neural elements that are normally activated by testosterone, may work with those cases where urine spraying is more of an expression of male sexuality. Antianxiety drugs may suppress urine spraying related to anxiety-provoking situations such as introduction of new cats or moving to a new house. With regard to treatment of objectionable male sex-typical behaviours other than spraying, there is some indication that progestin administration may alter intermale aggressive behaviour in castrated male dogs (Hart, 1981).

8. Behavioural effects of ovariectomy

There is little clinical or experimental research relevant to the behavioural effects of ovariectomy. As mentioned previously, during prenatal and neonatal development of females, there is no behaviourally significant amount of estrogen or progesterone secreted and the organization of feminine responses occurs as a function of development without testosterone.

Among dog handlers and trainers, one may hear of references to calming (or mellowing) effects of allowing female dogs to experience one or more estrus periods or even parturition and care of a litter of puppies. The degree to which removal of female gonadal hormones might result in permanent changes in behaviour was explored in a

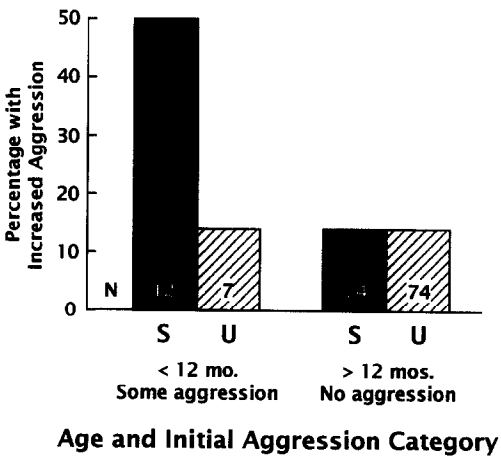

Fig. 5. Percentages of female dogs showing increased aggression after ovariohysterectomy (spay) as a function of age at time of the operation (less or greater than 12 months) and whether they showed signs of some aggression prior to the operation. Only dogs that were spayed (S) at less than 12 months of age and showing some aggression exhibited an increase in aggression compared with unspayed controls (U) who also showed some aggression (from O'Farrell and Peachey, 1990).

study of 150 spayed and 150 unspayed female dogs by O'Farrell and Peachey (1990). Owners were interviewed via questionnaire at the time the female dog was spayed and 6 months later. Owners of a control group of age-matched unspayed dogs were also interviewed twice at an interval of 6 months. In both groups there were changes over time in some dogs but the only two behaviour patterns in which spayed females showed a change that differed significantly from the control group was in aggressive dominance towards owners and in indiscriminate eating. The increase in aggression was only apparent in females spayed at less than 12 months of age and that already showed some aggression (Fig. 5). Females that were older than 12 months at time of being spayed presented no risk of increase in aggression.

One possible explanation of these results is that spaying removes the source of progestin production if done shortly after estrus. Female dogs that cycle twice a year are in a progestational state for about 2 months following each estrus period. Given that progesterone has some calming influences, removal of progesterone in some animals may increase aggressive or irritable tendencies especially if the dogs are spayed when progesterone levels are high producing a sudden change in this hormone. Once a female has gone through more estrous cycles, and perhaps been disciplined for aggressive behaviour, spaying may have less of an impact.

9. Conclusions

This review reveals the complexity of the role of gonadal hormones in companion animal behaviour and in the alteration of these hormones for changing objectionable behaviour. Two overriding concepts should be very apparent: (1) gonadal hormones have pronounced effects on behaviour and must be taken into account in any program designed to treat or manage problem behaviour; and (2) the alteration of gonadal hormones, particularly castration of males, does not affect all dogs or all cats similarly. One cannot extrapolate from experience with one or two dogs or cats in predicting the effects of castration or ovariectomy. There is a need for additional research in understanding the basis of such pronounced individual differences in the effects of hormones on behaviour.

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EFFECTS OF FERTILITY CONTROL ON POPULATIONS OF UNGULATES: GENERAL, STAGE-STRUCTURED MODELS

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Abstract: Regulating the abundance of ungulate populations using hunting can prevent populations from reaching levels that cause harm to natural and human dominated systems. However, there are an increasing number of cases where hunting is infeasible, and in such cases, fertility control has been widely advocated as an alternative means for controlling populations. Here, we develop simple analytical models offering general insight into the feasibility of using fertility control to regulate the abundance of ungulates. The models are structured in stages to represent variation in the duration of effect of fertility control agents. Analysis of these models offers several predictions, amenable to testing in field studies. (1) More than 50% of fertile females will need to be maintained infertile to achieve meaningful reductions in ungulate numbers even when fertility rates are low. (2) The relationship between the proportion of females maintained infertile and the steady state density is highly nonlinear. This means that small errors in estimating levels of infertility can lead to large errors in achieved density. It also means that managers should expect to see little change in steady-state density across a broad range of delivery rates. (3) The efficacy of fertility control as a management technique depends strongly on the persistence of the effect of the fertility control agent and the ability of managers to recognize previously treated animals. (4) Fertility control using long-lived agents can be more efficient than culling in regulating ungulate numbers. (5) Treating small populations with irreversible agents magnifies the likelihood of population extinction relative to treatment by culling. As with all techniques, managing population fertility must extend from a sound understanding of the influence of management actions on the state and dynamics of the population.

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Controlling the abundance of ungulates by regulating harvest is one of the prevailing activities of wildlife managers throughout North America. Regulated harvest offers a variety of benefits to society, including recreation for citizens and revenue for agencies and private enterprise. An additional benefit is a reduction in animal numbers, which if left unchecked, can cause significant harm to natural and human-dominated systems (Jewell and Holt 1981, Diamond 1992, Garrott et al. 1993, McCullough et al. 1997).

Although harvest can be an effective method for manipulating ungulate numbers, there are a growing number of situations where it is not feasible. The rapid expansion of urban and suburban areas during the last decade has brought populations of ungulates in close proximity to high densities of people (Adams 1994, McCullough et al. 1997). This proximity restricts the use of hunting as a management tool because it poses unacceptable risks to human safe-

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ty (Decker and Connelly 1989, Brush and Ehrenfeld 1991, McAnnich 1993, Wright 1993), and because urban residents often oppose hunting animals on ethical grounds (Decker and Connelly 1989, McCullough et al. 1997). In addition, there are many conservation areas, national parks prominent among them, where hunting is proscribed by law and policy (Leopold et al. 1963, Wright 1993, Porter et al. 1994, Wagner et al. 1995, Frost et al. 1997, Porter and Underwood 1999). In all of these situations, culling animals to regulate populations has significant liabilities, and as a result, wildlife managers are seeking innovative alternatives to lethal methods of population control (Kirkpatrick and Turner 1985, Bomford 1990, Brush and Ehrenfeld 1991, Diamond 1992, Garrott et al. 1993, McCullough et al. 1997).

Fertility control has been widely advocated as an alternative to culling (reviewed by Kirkpatrick and Turner 1985, Bomford 1990, Tuyttens and Macdonald 1998). Field and laboratory studies have evaluated efficacy of delivery of contraceptives to ungulates (Jacobsen et al. 1995, McCallum 1996, DeNicola et al. 1997, Fayrer Hosken et al. 1997, Kirkpatrick et al. 1997, Nielsen et al. 1997, Turner et al. 1997), and models have been developed to represent effects of fertility control on the population dynamics of individual species (Garrott and Siniff 1992, McAninch 1993, Swihart and DeNicola 1993, Boone and Wiegert 1994, Seagle and Close 1996). However, despite these efforts, a broad understanding of effects of fertility control on ungulate populations has failed to emerge. The absence of such understanding retards efforts to apply fertility control as a method to control ungulate numbers.

Here, we develop a general theory of regulation of ungulate populations using fertility control agents. Specifically, our objective was to develop simple analytical and simulation models offering general insight into the following questions. (1) What proportion of females in a population must be infertile to maintain the population at target densities? (2) How does fertility control compare with culling as a means to regulate ungulate populations? (3) How does duration of efficacy of contraceptives influence the number of animals that must be treated to achieve target population levels? (4) To what extent does timing of delivery (pre- or post-breeding) influence the ability of contraceptives to regulate ungulate numbers? (5) Do assumptions on the operation of density dependence modify the insights gained above? (6) Is fertility control a "safe" method for regulating small populations vulnerable to stochastic, local extinction? We provide a general mathematical basis for screening the feasibility of fertility control before developing detailed, species-specific models or conducting extensive field applications.

METHODS

Base Model

Because our purpose was to develop general insights, we used a model that was as simple as possible (Levins 1966, Starfield et al. 1990, Starfield and Bleloch 1991, Starfield 1997). The model needed to be sufficiently detailed to mimic the behavior of ungulate populations in a reasonable way, but it also needed to avoid detail peripheral to the questions posed above. To meet this challenge, we began with a simple formulation and incrementally added detail needed to meet our objectives.

Our starting point was a stage-structured model representing species where adult survivorship is high and where density dependence operates primarily on recruitment, characteristics that are particularly faithful to the life histories of many species of ungulates (McCullough 1979, Houston 1982, Skogland 1990, Bartmann et al. 1992, Gaillard et al. 1993, Putman et al. 1996). The model was formulated as

$$F_{t+1} = F_t(S + m - \beta(F_t - H_t)) - H_t, \quad (1)$$

where F_t is the density of females at time t, S is the annual per-capita survival rate of females, m is the maximum number of breeding females annually recruited to the population per breeding female, β is the linear slope of the relationship between recruitment of females to breeding age and female density, and H_t is the number of females culled at time t. The model predicts the preharvest population density of females using 3 parameters (S, m, β) and a single decision variable, H_t. Although the model represents a single stage explicitly (adult females), it implicitly represents sexually immature juveniles via the recruitment term. The explicit juvenile stage drops out of equation (1) via model reduction (Caswell 1989:100).

The model is based on several simplifying assumptions. First, we assume that the probability of survival of adults does not change with age. Thus, we treat longevity as the outcome of a

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series of independent Bernoulli trials rather than being fixed at a specific age (Gurney and Nisbet 1998:80). Culling is assumed to occur after breeding, but before recruits are born. We assume that density dependence is linear and operates entirely on recruitment. That is, the number of new breeding females recruited to the population is directly proportionate to the postharvest population density of females. Effects of nonlinear density dependence will be included as part of the analysis of the model.

It is possible to formulate the model using an explicit parameter for the density at which recruitment = 0 (i.e., $K_r = m/\beta$). Alternatively, we can represent effects of density dependence using a slope (β) and intercept (m) for the relationship between density and recruitment. We used this approach because we believe these parameters offer useful biological interpretations; the parameter β represents the "strength" of density dependence and the parameter m represents the maximum possible recruitment rate. Both of these representations prove useful when interpreting model analysis.

Although this model is extremely simple, it can represent the dynamics of ungulate populations with reasonable fidelity (Fig. 1). A more detailed model would achieve higher levels of realism, but only at the expense of reduced clarity and generality.

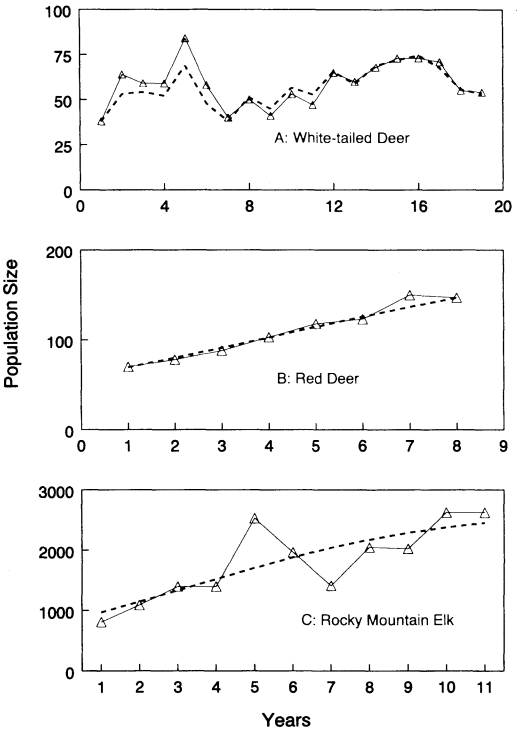

Fig. 1. Comparison of model projections (dashed line) with observed trajectories of ungulate populations (triangles). Model parameters were estimated by minimizing the squared difference between observations and predictions. A close fit between observations and predictions allows the conclusion that the model contains sufficient detail to reasonably represent trajectories of ungulate populations if its parameters are properly estimated. Data are from [A]—McCullough (1979: Table 3.2), [B]—Clutton-Brock et al. (1982), [C]—Houston 1982.

Model Development

We modified equation (1) to represent effects of fertility control as an alternative to culling. Node diagrams and the matrix form of the model are shown in Fig. 2. Here, we summarize our modifications using finite difference equations.

We defined the number of animals harvested annually as

$$H = cF_t. \quad (2)$$

Thus, c is the per capita rate of culling, the number of breeding females culled annually per breeding female in the population. It follows that the effects of culling can be represented in the model as

$$F_{t+1} = F_t(1 - c)(S + m - \beta(1 - c)F_t). \quad (3)$$

We will refer to equation 3 as the culling model.

To allow comparison with fertility control, we redefined c as the per capita rate of delivery of contraceptives. There are now 2 stages that we wish to track in the population; females that are fertile (i.e., F_t) and those that are infertile (i.e., I_t). We define fertile animals as those that are capable of breeding and carrying a fetus to term. We define infertile animals as those that cannot successfully breed. When the effect of the contraceptive persists for a single breeding season, then the dynamics of the fertile (F_t) and infertile stages (I_t) can be represented as

$$F_{t+1} = F_t(1 - c)(S + m - \beta N_t) + (1 - c)I_t, \text{ and} \quad (4)$$

$$I_{t+1} = F_t c(S + m - \beta N_t) + I_t Sc, \quad (5)$$

where

$$N_t = F_t + I_t. \quad (6)$$

However, if the effects of the contraceptive persist for more than 1 breeding season, then we must account for the time since the animal was treated. This accounting can be accomplished

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by adding stages to the model. If the duration of the contraceptive is finite, then the dynamics of the fertile and infertile stages can be represented as

$$F_{t+1} = F_t(1-c)(S + m - \beta N_t) + (1-c)I_{\tau_t} \quad (7)$$

$$I_{1_{t+1}} = F_t c(S + m - \beta N_t) + I_{\tau_t} Sc \quad (8)$$

$$I_{2_{t+1}} = I_{1_t} S, \quad (9)$$

$$I_{3_{t+1}} = I_{2_t} S, \quad (10)$$

$$I_{4_{t+1}} = I_{3_t} S, \quad (11)$$

$$I_{\tau_{t+1}} = I_{\tau-1_t} S, \quad \text{and} \quad (12)$$

$$N_t = F_t + \sum_{i=1}^{\tau} I_{i_t}, \quad (13)$$

where τ = the duration of the contraceptive in number of breeding seasons. Thus, the number of stages in the model is $\tau + 1$, 1 stage for the breeding females and 1 stage for each year that animals are infertile following treatment. The total population of females is the number of animals summed across stages (equation 13). We will refer to equations 7–13 as the fixed duration model.

It is possible to develop agents that will render animals infertile for life (reviewed by Muller et al. 1997, Tuyttens and Macdonald 1998). To represent effects of such agents, only 2 stages need to be tracked (fertile and infertile animals), and the dynamics of these stages are connected only by the rate of addition of animals to the infertile pool and the effect of total density on recruitment. Thus, the dynamics of the population are given by

$$F_{t+1} = F_t(1 - c)(S + m - \beta N_t), \quad (14)$$

$$I_{t+1} = F_t c(S + m - \beta N_t), \quad \text{and} \quad (15)$$

$$N_t = F_t + I_t. \quad (16)$$

We will refer to equations 14–16 as the lifetime duration model.

The fixed and lifetime duration models depend on the same set of assumptions described for the culling model. However, these models require the additional assumptions that treatment is applied immediately before breeding occurs and that managers can differentiate between animals that have been treated and those that have not been treated. We will examine the consequences of these assumptions as part of model analysis. Time of census in the model is

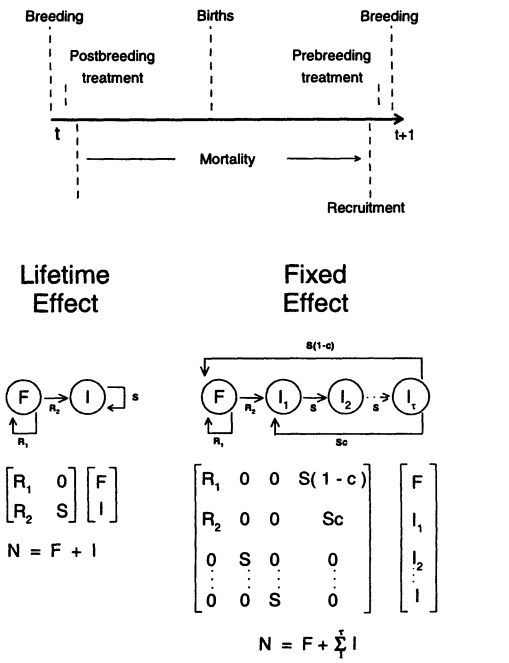

Fig. 2. Schematic of model structure and assumptions. The upper panel depicts assumptions on timing of events represented in the model. The central panel shows the structure of the model in node and matrix formats. The lower panel shows representations of assumptions about timing of delivery and the operation of density dependence.

assumed to occur annually, immediately after breeding (Fig. 2), and all fertile animals are assumed to be bred at the time of census. We chose breeding rather than births as the point of reference for census because the state of the animal that influences model behavior is its fertility rather than its age.

Model Analysis

Deterministic Analysis.—We used standard matrix techniques and algebraic manipulations (see Appendix I for details) to derive several quantities of interest. These included: K_e, the equilibrium density of animals in the absence of fertility control or culling; K_c, the equilibrium

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density of animals as a function of delivery or culling rate; c^*, the delivery rate required to maintain a target population density; P, the proportion of the population that must be infertile to maintain a target population density; and n^*, the number of animals that must be treated or culled annually to maintain a target population density. To examine the effects of timing of delivery on model predictions, we changed model formulations to represent delivery after breeding (Fig. 2). In this case, delivery of contraceptives did not prevent birth by treated animals during the current time step (because treated animals were assumed to be pregnant at the time of delivery) but did prevent births during the subsequent time step (Fig. 2). To examine effects of nonlinearity in density dependence, we reformulated the model (Fig. 2) so that recruitment remained relatively constant at low population density and then declined as threshold densities were reached (Bartmann et al. 1992). To examine consequences of relaxing assumptions on known fertility status of animals, we developed an estimator to show how many animals must be treated to achieve a treatment target, given unknown fertility status. This estimator deals with the multinomial case of sampling with replacement, and estimates necessary treatment rates, assuming that animals could be treated more than once.

Stochastic Analysis.—We performed Monte Carlo simulations to examine the relative ability of culling and fertility control to regulate animal numbers near a target density. These simulations were organized in 4 model scenarios that included 2 types of management and 2 sources of stochastic variation. Management types include static and adaptive. Static management applied the same per capita rate of delivery annually, a rate that would achieve a target population density if applied accurately. Adaptive management changed the delivery or culling rate in response to differences between realized density and an acceptable range of target densities. The acceptable range was defined as the target ±25%. If the population exceeded the target by ≥25%, then the culling or delivery rate was increased by 10% until the population returned to acceptable levels. If the population dropped below the target by ≥25%, then culling or fertility control was discontinued until the population recovered to a density within the acceptable range.

In addition to 2 types of management, model scenarios included 2 sources of stochastic variation; process and observer. Process variation was included to mimic density independent effects of the environment on population dynamics. To represent this variation, adult survival varied according to a log-normal distribution with mean 0.9 and variance 0.1. We varied the effect of density on recruitment (i.e., β) using a normal distribution with a mean of 0.009 and variance 0.01. To assure that stochastic effects on mortality and recruitment were correlated, we derived them from the same normally distributed variate at each time step. Observer variation was included to mimic the error in estimates of population size that are typical in managing ungulate populations. To represent observer variation, delivery and culling rates were based on an estimate of the current population density derived by sampling from a normal distribution with a mean equal to the model density at time t (F_t) and a standard deviation equal to 15% of the mean. Initial conditions for population density were set at the target density. Initial conditions for population composition (i.e., fertile–infertile stages) were set at levels equivalent to the deterministic steady-state at that target density. We conducted 1,000 model runs of 50 years each for 2 target densities, set as percentages (25 and 75%) of the steady state that would occur in the deterministic model when culling or fertility control was not applied (i.e., K_e). We applied 4 methods of population control: culling, and fertility control with the duration of treatment equal to 1 year, 3 years, and lifetime. For each set of model runs, we recorded the total number of animals treated or culled, the overall variance in population density, and the number of years that the population was outside the acceptable ranges. We also recorded the frequency of extinction and the persistence time of the population.

Procedures for Analysis and Plotting.—Analytical results were obtained using Maple V (Waterloo Maple, Waterloo, Ontario, Canada). All analytical results were checked for accuracy using simulation of difference equations with Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Monte Carlo simulation experiments were conducted using Visual Basic for Applications running under Excel. For the purpose of illustrating analytical results in plots, we chose parameter values that are reasonable for white-tailed deer (*Odocoileus virginianus*; $m = 0.6$, $S = 0.9$, $\beta = 0.005$) and that

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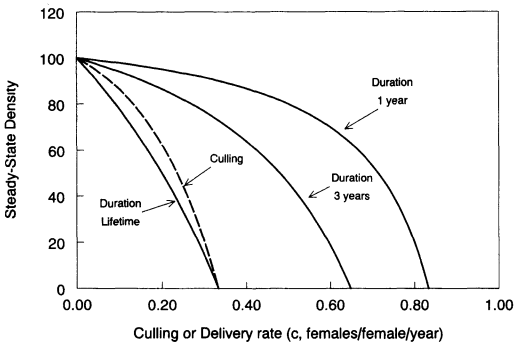

Fig. 3. Model predictions (equation 18) of steady-state density as a function of culling or delivery rate using parameter values $m = 0.6$, $S = 0.9$, $\beta = 0.005$. Different lines show differences due to variation in duration of efficacy of contraceptives.

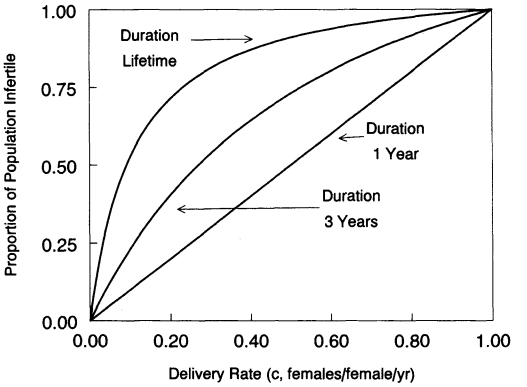

Fig. 4. Model predictions (equation 19) of effects of delivery rate on the proportion of the female population that is infertile. Different lines show differences due to variation in duration of efficacy of contraceptives. Parameter values are $m = 0.6$, $S = 0.9$, $\beta = 0.005$.

produced an equilibrium value of population density = 100 in the absence of any treatment or culling. Because density can scale to any unit area, our plots can be viewed in absolute or relative terms. For example, $N = 90$ can be accurately interpreted as an absolute density (e.g., 90 animals per unit area) or as a percentage of steady state population density that would be achieved in the absence of treatment (e.g., 90% of K_e).

RESULTS

Equilibrium Population Density and Composition

In the absence of treatment or culling (i.e., $c = 0$), all models predicted that the population would reach a steady state density, K_e,

$$K_e = \frac{S + m - 1}{\beta}. \quad (17)$$

As the rate of treatment with contraceptives increases, models predicted that steady state density declines in a nonlinear fashion (Fig. 3) according to

$$K_c = \frac{S + m + S^{\tau}c - Sc - cm - 1}{(1 - c)\beta}. \quad (18)$$

In the lifetime case, the S^{τ}_c term drops out of equation (18).

Fixed-effect models predicted a nonlinear, threshold relationship between equilibrium density and treatment rates. We found a broad range of treatment rates that exert relatively weak effects on steady state density, particularly for short-duration models (Fig. 3). Increasing duration of fertility control tended to make this relationship more linear (Fig. 3).

Fixed-effect models illustrated that when the duration of the contraceptive is 1 breeding season, the proportion of the population that is infertile (P) is equal to the treatment rate (i.e., $P = c$). However, when effects persist beyond a single season, infertile animals accumulate in the population treated annually, and as a result, $P > c$ (Fig. 4). Analysis of the model (Appendix A) suggests that the relationship between P and c depends on the duration of the effect of the contraceptive and the adult survival rate, i.e.,

$$P = \frac{(1 - S^{\tau})c}{1 + Sc - S - S^{\tau}c}, \quad (19)$$

but does not depend on birth rate (m) or the strength of density dependence (β). In the lifetime case, this simplifies to

$$P = \frac{c}{1 - S + Sc}. \quad (20)$$

The relationship between target density (N^*) and the proportion of the population that is infertile (P) is identical for all fertility control models (Fig. 5),

$$N^* = \frac{S + m - Pm - 1}{\beta(1 - P)}. \quad (21)$$

Equation 21 shows that increasing dependence of recruitment on density amplifies the effect of fertility control on the steady state population size. That is, achieving a given target density will require a smaller proportion of infertility in populations where density-dependent effects

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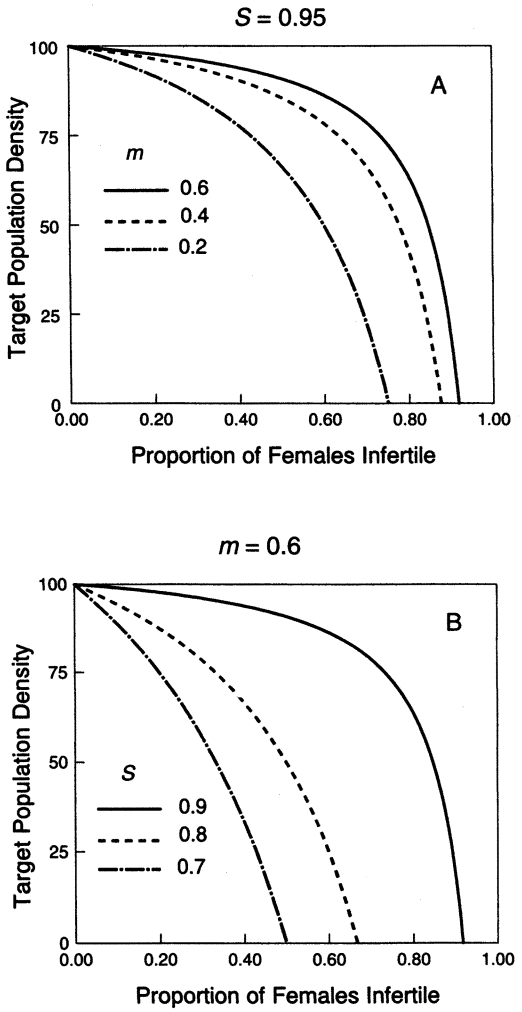

Fig. 5. Model predictions (equation 21) of the proportion of the females that must be infertile to achieve a target population density assuming a constant ecological carrying capacity of K_e = 100. Panel A shows this proportion with adult female survival rate held constant (S = 0.95) and maximum recruitment rate varying. Panel B shows this proportion with maximum recruitment rate held constant (m = 0.6) and survival rate varying.

are stronger than in populations where such effects are weak.

Population managers need a relatively easy way to estimate the proportion of the population that would need to be maintained infertile to achieve a given target density. This can be obtained as follows. By rearranging equation 21, we find P as a function of the target density:

$$P = 1 - \frac{1 - S}{m - \beta N^*}. \quad (22)$$

This result simply says that for any steady-state population density, N^*, the proportion of the population that is fertile (i.e., $1 - P$) must equal the adult per capita mortality rate divided by the per capita recruitment rate (i.e., females per female per year). When this is true, the number of animals recruited will equal the number that die.

Note that the duration of the contraceptive determines the rate of delivery that must be applied each year to achieve a given level of population infertility, (equation 19), but the duration does not affect the level of infertility required to achieve a given steady-state density (equation 22). Equation 22 also illustrates that the necessary proportion infertile will increase with increasing adult survival and maximum recruitment. It will decline with increasing "strength" of density dependence.

Equation 22 requires an estimate of β, which can be difficult to obtain. However, it is possible to derive an expression for P that does not depend on estimating β. Defining Δ as a proportion of the steady-state density (i.e., $N^* = \Delta K_e$) and substituting the right hand side of

$$\Delta K_e = \Delta\left(\frac{S + m - 1}{\beta}\right) \quad (23)$$

for N^* in equation 22, we obtain an expression for the proportion of animals that must be infertile to achieve a target density specified as a proportion of the untreated steady state, i.e.,

$$P = 1 - \frac{1 - S}{m + \Delta(1 - S - m)}. \quad (24)$$

Thus, when $\Delta = 1$, there is no reduction in density; when $\Delta = 0$, the population goes extinct. Equation 24 is an important result because it provides a general rule of thumb for estimating the effort required to regulate a population using fertility control without knowing the strength of density dependence. This rule requires estimates of only 2 parameters (adult female survival rate and maximum recruitment rate of females) and a decision on the targeted reduction, Δ.

Vital Rates and Population Composition at Equilibrium

Equation 24 also reveals that a relatively large proportion of the population must be infertile in order to achieve meaningful reductions in density (Fig. 5). When adult survival is high (>95%),

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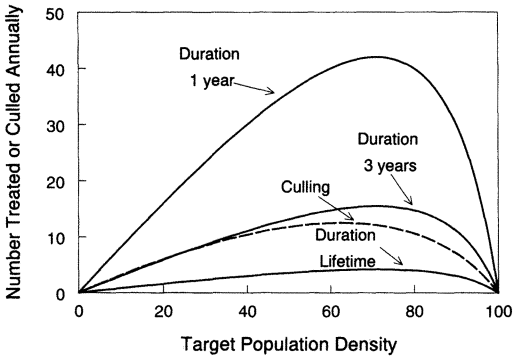

Fig. 6. Model predictions (equations 25–28) of the number of animals that would need to be treated or culled to maintain a population at the specified target density. Parameter values are $m = 0.6$, $S = 0.9$, $\beta = 0.005$. Different lines show differences due to variation in duration of efficacy of contraceptives.

it is reasonable to assume that >60% of the breeding females will need to be infertile to achieve meaningful reductions in population density, even for species with very low intrinsic rates of increase (e.g., $m = 0.2$, Fig. 5). The shape of the relationship between the proportion of the population that is infertile and steady-state density is more sensitive to differences in adult survival than to differences in maximum rates of recruitment. This relationship remains strongly nonlinear as long as adult survival is high. However, as adult survival declines, the relationship approaches linearity (Fig. 5).

Number of Animals Treated or Culled at Equilibrium

When effects of fertility control agents persist for only 1 year, then the proportion of the population that is infertile equals the delivery rate. Moreover, in this case, the number of animals that must be treated the first year to achieve a given target is the same as the effort required in subsequent years. However, when regulating populations with fertility control agents that persist for >1 breeding season, there are 2 types of effort required: the effort required to achieve a specified level of infertility in the population, and the effort required to maintain that level of infertility. Here, we examine this maintenance effort and compare it with culling.

In populations regulated with culling, the number of animals harvested annually (n) to maintain a given target density, N^*, at steady state is simply

$$n = c^*N^*, \quad (25)$$

where c^* is the culling rate needed to maintain a population at the target density. For the culling model, c^* is given by

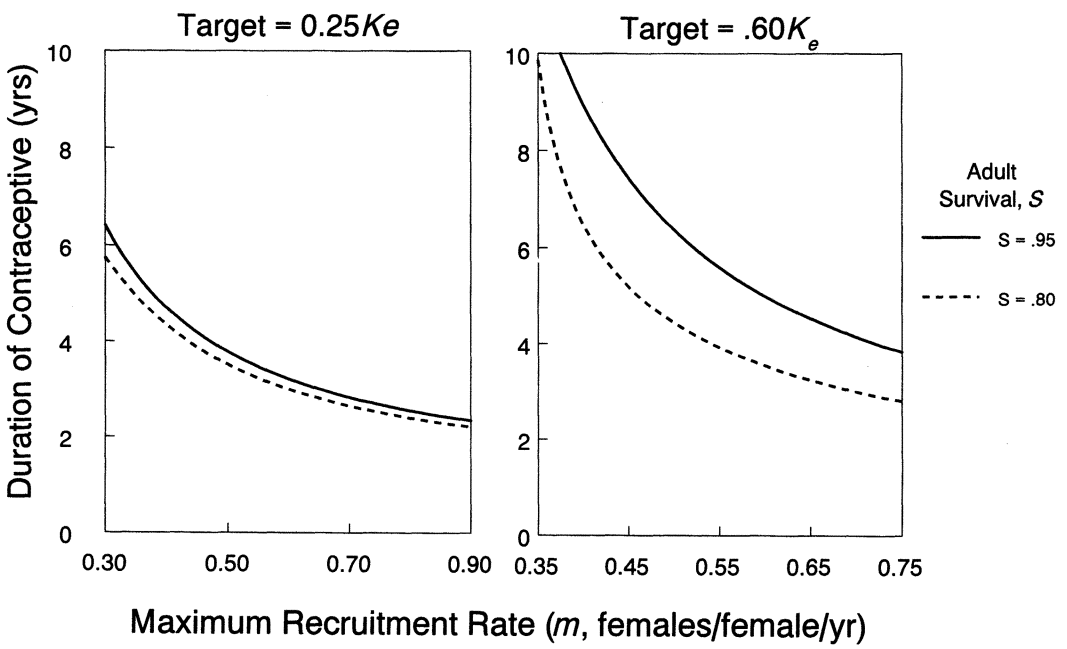

Fig. 7. Model predictions of the duration of contraceptive delivered to an animal needed to cause the same effect on population growth as culling the animal (equations 25 and 28).

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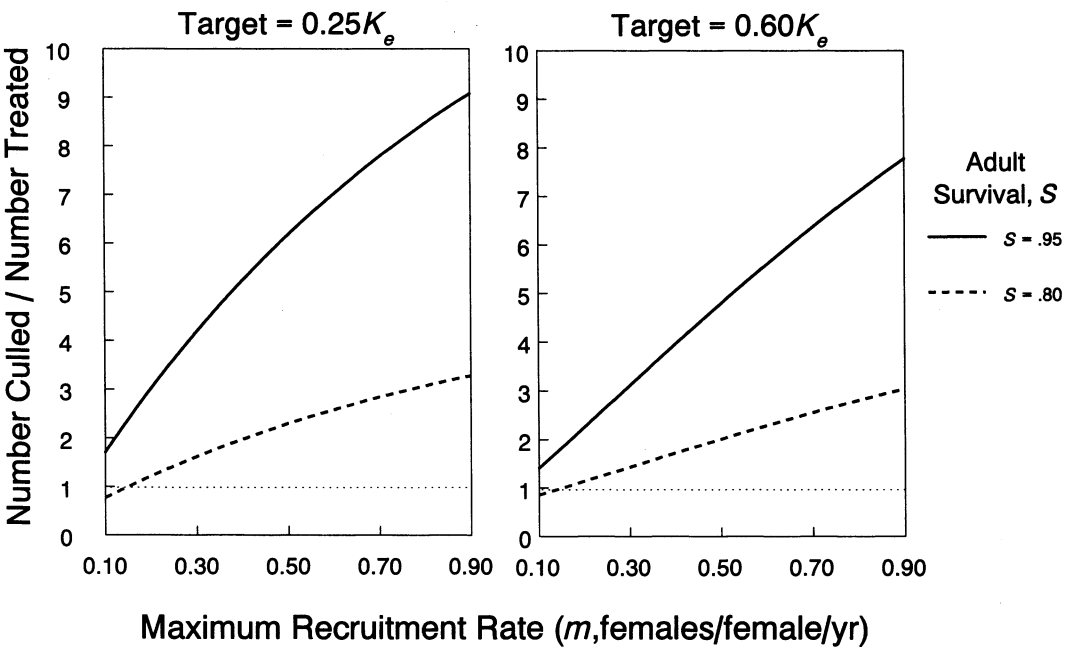

Fig. 8. Model predictions of the ratio of the number of animals that would need to be culled relative to the number treated with contraceptives to maintain steady states at target densities. The horizontal dotted line shows where the number culled equals the number treated (equations 25–28).

$$c^* = \left(2N^*\beta - S - m + \sqrt{S^2 + 2Sm + m^2 - 4N^*\beta}\right)/(2N^*\beta). \quad (26)$$

In populations regulated with fertility control, there are 3 cases relevant to the number of animals treated annually (n) to maintain a given target density. In the case of fixed duration agents lasting a single year, the delivery rate at equilibrium (c^*) is the same as the proportion of the population that is infertile, so using equation 22, we calculate the number treated as

$$n = c^*N^* = PN^* = \left(1 - \frac{1 - S}{m - \beta N^*}\right)N^*. \quad (27)$$

In the case of agents with duration >1 year and greater than a lifetime, the number that must be treated annually is

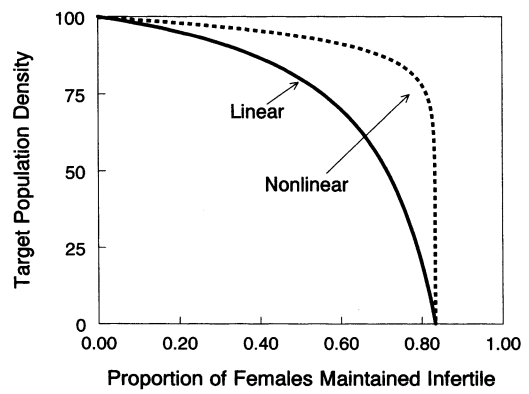

Fig. 9. Comparison of model predictions of the relationship between the target density and the proportion of the population that is infertile, assuming linear (solid line) and nonlinear (dashed line) representations of the effects of density dependence on recruitment.

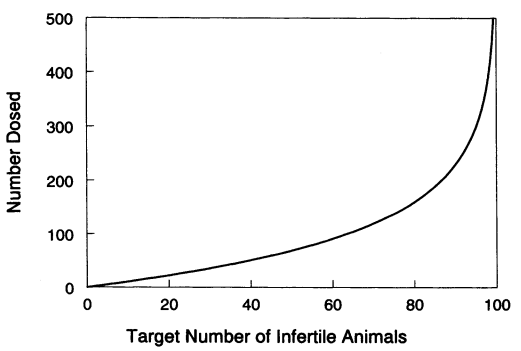

Fig. 10. Predictions of the number of animals that would need to be dosed to achieve a given level of infertility in a population, assuming animals are not marked and are subject to treatment more than once. The axes can be interpreted as a number of individuals in a population of 100, or as percentages.

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Table 1. Results of 1,000 model runs of 50-year duration with stochastic variation. Static management applied a constant, per capita culling or delivery rate needed to achieve the target density. Adaptive management adjusted the culling or delivery rate to keep the population within ±25% of the target density. Process variation mimicked stochastic environmental effects on adult survival and juvenile recruitment. Observer variation mimicked uncertainty in estimating population size. Control methods included culling and 4 contraceptives differing in duration, compared with the no management case (i.e., "Natural"). Results include average population size (*N*) and its coefficient of variation. We also report percentage of runs for which population density dropped below 1 (% extinct) and mean time to extinction averaged across model runs (persistence time in yr).

Management type	Variation type	Control method (duration of contraceptive)	Target density							
			25				75			
			N	CV	% extinct	Persistence time	*N*	CV	% extinct	Persistence time
Static	Process	Culling	22.1	51.3	0.5	49.9	69.1	31.7	0.2	49.9
		1 yr	23.2	46.3	0.2	49.6	71.3	28.4	0.1	50.0
		3 yr	23.3	49.3	2.6	49.5	71.8	28.8	0.1	50.0
		Lifetime	19.7	61.3	37.6	41.7	69.2	33.4	3.2	49.3
		Natural	105.5	25.2	0.0	50.0	108.0	23.0	0.0	50.0
Static	Process and observer	Culling	21.6	65.4	1.3	49.4	68.7	32.2	0.0	50.0
		1 yr	29.9	56.2	0.5	49.8	71.2	31.0	0.0	50.0
		3 yr	23.3	85.1	12.3	45.0	72.3	41.8	1.3	48.7
		Lifetime	17.6	87.1	40.1	34.2	69.2	34.7	3.8	49.2
		Natural	105.4	25.7	0.0	50.0	108.1	23.0	0.0	50.0
Adaptive	Process	Culling	27.3	37.2	0.0	50.0	75.8	50.6	0.2	49.9
		1 yr	28.9	29.4	0.5	49.9	79.0	29.2	0.0	50.0
		3 yr	31.0	35.8	0.6	49.8	79.0	43.4	0.0	49.9
		Lifetime	22.7	76.7	10.1	37.7	74.2	37.5	1.0	49.6
		Natural	105.5	25.7	0.0	50.0	108.1	29.4	0.0	50.0
Adaptive	Process and observer	Culling	27.3	36.6	2.5	49.5	75.7	31.3	0.2	49.9
		1 yr	31.8	37.0	1.0	49.8	80.5	29.2	0.0	50.0
		3 yr	34.9	56.44	9.5	47.8	84.5	43.4	5.9	48.7
		Lifetime	27.0	62.26	29.4	44.8	75.2	37.6	1.4	49.7
		Natural	103.4	34.23	0.1	50.0	108.9	29.4	0.0	50.0

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Table 2. Modeling estimates of proportion of the target sex that must be infertile to achieve target population objectives. Assumed annual growth rate in absence of treatment = 1.49.

Species	Population objective	% infertile	Reference
White-tailed deer	Negative growth rate[1]	90%	(Swihart and DeNicola 1993)
Feral horses	Reduce population from 600 to range of 300–600	68–82% depending on treatment regime	(Garroutt et al. 1992)
White-tailed deer	Maintain at ½ carrying capacity	80%	(Boone and Wiegert 1994)
Feral horses	Reduce growth rate (i.e., lambda) to 1	90–95%	(Garrott and Siniff 1992)
White-tailed deer	Reduce population size	>50%	(Seagle and Close 1996)
White-tailed deer	Maintain at ½ carrying capacity	96%	(Nielsen et al. 1997)

$$n = c^*(1 - P)N^*(S + m - \beta N^*) + c^*\rho N^*, \quad (28)$$

where c^* is the treatment rate required to achieve a given target density, N^*P is the portion of the population that is infertile at time t as a result of applying c^*, and ρ is the proportion of the population that becomes fertile during $t \rightarrow t + 1$ given a treatment rate = c^*. The components of equation 28 are as follows. The variable P is given by equation 22. The equilibrium treatment rate is

$$c^* = \frac{S + m - 1 - \beta N^*}{S + m - S^\tau - \beta N^*}. \quad (29)$$

The proportion of the population that will become fertile during a time step is

$$\rho = \frac{S^{\tau-1}(1 - S)c^*}{1 + Sc - S - S^\tau}. \quad (30)$$

In the lifetime duration case, the S terms with exponents drop out of the expression for c^* and $\rho = 0$.

Equations 25 and 28 allow comparison of the effort required to control populations using culling or fertility control. Because these equations predict the number of animals that must be treated or culled to maintain populations below K_e (Fig. 6), they are analogous to classical production functions, also known as yield curves (Roughgarden 1997).

Effect of Duration of Contraception

We asked the question, "On average, how long must the effect of a contraceptive persist in an animal to exert the same effect on population-level production as culling that animal?" To address this question, we chose a broad range of parameter values to represent diverse life-history characteristics of ungulates, and applied those values to equations 25 and 28. Models suggested that for a broad range of assumptions about survival and maximum recruitment, delivery of a contraceptive to an individual animal would have the same effect as culling that animal if the effects of the contraceptive persisted for 2–5 years (Fig. 7). Briefer duration is needed when adult survival rates are low. Increasing strength of density dependence reduces the required duration; weakening it increases the required duration.

When contraception persists for the lifetime of the animal, models predicted that in most cases, the effort required to regulate a population at a specified density using fertility control will be less than the effort required by culling (Fig. 8). Exceptions to this result occur when adult survival is low and recruitment is low, that is, when populations grow slowly (Fig. 8). For species with high rates of adult survival and high recruitment rates, the number of animals culled could be almost an order of magnitude greater than those treated with contraceptives at the same target density (Fig. 8). Thus, if we define efficiency of control in terms of the number of animals that must be culled or treated annually to maintain a population at a specified target, then culling will be more efficient than lifetime duration contraception only for slowly growing populations. In all other cases, models suggest that treatment with lifetime effect contraceptives is likely to be more efficient than culling. However, it must be understood that these estimates of effort specify the number of animals that must be treated annually to maintain a steady state population with a proportion infertile, P (equation 22). These are not estimates of the effort required to achieve P in the pretreatment population.

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Consequences of Changing Model Assumptions

Assumed Pre-breeding Delivery.—In all results above, we assumed that contraceptives were delivered to females before breeding occurs. It is possible to change the model to represent delivery after breeding by assuming that treated animals give birth during the current time step but are infertile during the subsequent one (Fig. 2). An important result emerged from this analysis. We found that when contraceptives are delivered postbreeding, there is a fundamental limit to the magnitude of the reduction in population density that can occur using fertility control,

$$N^* \geq \frac{m - 1}{\beta}, \tag{31}$$

or, solving in terms of a proportionate reduction,

$$\Delta \leq \frac{m - 1}{S + m - 1}. \tag{32}$$

The right-hand-side of equation 32 gives the steady-state density of females that occurs when the delivery rate = 100%. At this density, each fertile female produces exactly 1 female offspring surviving to reproductive age and the proportion of fertile females in the population equals the adult mortality rate.

Equations 31 and 32 are important because they reveal that it may not be possible to achieve meaningful regulation of populations where contraceptives are delivered after animals have been bred, even when the entire population of fertile females is treated. If animals can breed at 1 year of age, then a cohort of animals always escapes the postbreeding treatment, and this escape may allow populations to grow to levels that are close to K_e, if recruitment rates are sufficiently high. Equations 31 and 32 raise serious doubt about the efficacy of fertility control as a means of regulating populations whenever yearlings contribute significantly to breeding and contraceptives are delivered after they are bred.

Assumed Linear Density Dependence.—Changing the form of the relationship representing density dependence from linear to nonlinear did not change the qualitative results obtained above (Fig. 2). The composition of the population at equilibrium (i.e., equations 19 and 20) does not depend on the form of density-dependent relationships. However, the relationship between equilibrium population size and the proportion of the population infertile changed when we changed the formulation for density dependence. In the case of nonlinear density dependence,

$$N^* = \frac{\ln\left(\frac{S + m - Pm - 1}{1 - S}\right) + \alpha}{\theta}, \tag{33}$$

where α and θ are parameters controlling the shape of the nonlinear relationship between recruitment and density.

Note that this expression does not depend on the duration of the effect of the contraceptive, as we saw earlier for the linear case. Plotting equation 33 shows that nonlinearity in density dependence can sharpen the threshold relationship between N^* and P (Fig. 9). In the neighborhood of this threshold, small errors in estimating the proportion of the population that is infertile can lead to very large errors in equilibrium population size. Nonlinear density dependence amplifies the consequences of such errors. Nonlinearity in density dependence also expands the range of levels of infertility in the population (i.e., P) over which we expect virtually no change in equilibrium density (Fig. 9).

Assumed Knowledge of Fertility Status.—One of the assumptions of our base model is that managers can differentiate between fertile and infertile animals when delivering fertility control treatments. There are cases where this assumption is reasonable, for example, when treated animals can be visually marked, or when it is possible to achieve the desired delivery rates by treating young of the year. However, there will be many cases where the fertility status of animals is unknown. This means that some animals are likely to be treated more than once, thereby increasing the number of "doses" of fertility control agents that must be delivered to achieve a target delivery rate.

This is essentially a problem in multinomial sampling with replacement. That is, after an animal is given a dose of contraceptives, it is released into the population unmarked, and is subject to "resampling" a later time. Because it is unmarked, its fertility status is unknown, and it will be redosed if it is resampled. We are interested in estimating the number of doses (d) that must be delivered in order to treat a target number of animals (η) in a population of size

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N. We first consider the case where the duration of contraceptives is a single breeding season and all infertile animals must be dosed annually to maintain infertility. In this case, it can be shown (Appendix B) that the number of animals (d) that must be dosed to expect η animals to be infertile at the end of the treatment campaign can be estimated as

$$d = \frac{\ln\left(\frac{N-\eta}{N}\right)}{\ln\left(\frac{N-1}{N}\right)}. \quad (34)$$

Alternatively, when the duration of contraceptives persists for more than a year, there will be some animals in the population that will remain infertile and do not need to be retreated. Define I as the number of animals in the population that are infertile at the start of a treatment campaign and that remain infertile during the subsequent year. In this case the number of animals that must be dosed to expect that η additional animals are infertile is given by

$$d = \frac{\ln\left(\frac{N-I-\eta}{N-I}\right)}{\ln\left(\frac{N-1}{N}\right)}. \quad (35)$$

Equations 34 and 35 portray the large inefficiencies associated with treating a significant proportion of a population whenever treated animals are not easily distinguished from untreated ones (Fig. 10). For example, a population of 100 fertile animals would require dosing 160 animals to reasonably expect 80 infertile animals in the population after treatment (Fig. 10). A population of 100 animals, 80 of which are infertile at the time of delivery, would require dosing 69 animals to expect that only 10 additional animals would be infertile during the subsequent year. This result illustrates that the feasibility of treating populations with fertility control agents depends in a fundamental way on knowing the fertility status of individual animals at the time of treatment. In the absence of such knowledge, achieving meaningful reductions in steady state density could require delivering doses in excess of the total number of females in the population.

Stochastic Models

We compared the ability of different control regimes to maintain populations at target densities in the face of environmental stochasticity and error in estimation of population size. For all model runs, simulations of fixed-effect fertility control with single year duration showed the lowest coefficients of variation around target population densities, lowest likelihood of extinction, and longest persistence times (Table 1). Results from the culling model resembled those of single-year duration fertility control, but tended to show slightly higher variability and slightly greater probability of extinction. Lifetime effect fertility control models predicted the highest levels of variability and greatest chance of extinction. In the extreme case, extinctions occurred in >40% of all model runs for lifetime effect representing static application of fertility control with process and observer variation to populations at 25% of K_e (Table 1). Population variability and indicators of extinction risk for models of fertility control with 3-year duration showed effects intermediate to the culling model and the lifetime model. For all models, simulations of adaptive management scenarios increased variability in population density (as measured by coefficients of variation), but reduced probability of extinction and increased persistence time.

DISCUSSION

Relationships to Earlier Models

The models we offer differ in 4 fundamental ways from previous modeling efforts directed at understanding effects of fertility control on vertebrates (Garrott 1991, Garrott and Siniff 1992, Swihart and DeNicola 1993, Boone and Wiegert 1994, Seagle and Close 1996, Pech et al. 1997, White et al. 1997). First, our models are structured in stages where each stage represents the time since a female was treated. Earlier models have been structured by animal age, irrespective of time since delivery. As a result, previous efforts failed to consider effects of duration of contraceptives or the interaction of duration with other model parameters. Stage structure allows us to offer the first analysis of the consequences of variation in the duration of fertility control agents. As a result, we are able to show that animals with long lifetimes (i.e., high adult survival rates) can be effectively controlled with long duration contraceptives, even

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when they have relatively low fertility rates. This contrasts with earlier efforts that argued that r-selected species with short lives and high fertility were most appropriate targets for regulation by controlling fertility (e.g., Hone 1992).

Second, we offer closed-form solutions for relationships among delivery rate, proportion of the population infertile, number of animals treated, and target population densities. In the past, estimating these relationships demanded laborious trial and error using simulations (Garrott et al. 1992, Boone and Wiegert 1994, Seagle and Close 1996). Unlike previous results, our models enjoy analytical solutions that provide a rapid and general way to approximate the effort required to stabilize a population using fertility control, without developing detailed simulation models.

Third, our models are the first to consider effects of different fertility control regimes on risks of extinction of small populations. We demonstrate that small populations regulated with long-lived contraceptives will be vulnerable to stochastic extinction.

And finally, we are the first to explicitly consider the inefficiencies associated with resampling unmarked animals when delivering fertility control agents. We show that failing to know the fertility status of individuals can create large inefficiencies in achieving desired levels of infertility in real-world populations.

Although our models differ in important ways from earlier ones, we also reinforce previous findings. Similar to other modeling results, we found that more than half of the breeding females must be infertile to achieve meaningful reductions in population density (Fig. 5). It is true that a smaller infertile fraction may be required for species with low adult survival and low recruitment rates (Fig. 5), but these are not usually the species that cause problems of overabundance. These general results are consistent with several, more detailed studies of effects of fertility control on ungulates (Table 2), as well as, field tests in small mammals (McCallum 1996). It follows from these results that there may be situations where it is logistically impossible to treat a sufficient portion of the population to achieve population objectives. This may explain, in part, why field studies have failed to demonstrate that fertility control can maintain populations at lower densities (McCullough et al. 1997). We should not expect any change in steady state when the proportion of the population treated is small (McCullough et al. 1997).

Our models reveal that this problem is exacerbated by the nonlinear relationship between the proportion of the population treated and the steady-state density (Fig. 4). That is, there is a broad range of levels of infertility producing essentially no change in population equilibrium. This relationship could make it very difficult to assess the effect of different levels of population infertility in field studies because widely differing levels of infertility could result in very similar steady-state densities. Similar nonlinear relationships have been identified in other modeling efforts (Boone and Wiegert 1994, McCallum 1996, Seagle and Close 1996). This problem is magnified in populations where the fertility status of animals is not known (e.g., equations 34 and 35, Fig. 10). In this case, the number of doses that would need to be delivered to achieve a meaningful reduction in population density could easily exceed the total number of animals in the population.

Efficiency of Fertility Control Relative to Culling

There is broad disagreement about the relative efficiency of lethal and nonlethal means of controlling animal abundance. It has been argued that fertility control is more efficient than culling because infertile animals remain in the population, maintaining density-dependent feedback to recruitment and survival (Knipling 1959, Sturtevant 1970, Knipling and McGuire 1972). In contrast, others contend that there are virtually no circumstances in which fertility control will be more efficient than lethal methods (Bomford 1990, Garrott 1995, Nielsen et al. 1997, Swinton et al. 1997). Some workers even assert it is not possible to reduce population numbers with nonlethal control (Seal 1991).

The models we offer can resolve these differences of opinion. There are 2 types of efficiency in population regulation. The first type assesses the amount of time required to bring an overabundant population to an acceptable lower density. There is no question that culling is more efficient than fertility control when efficiency is defined in these terms. Using lethal methods, it is theoretically possible to bring a population to a desired lower density in 1 season of effort. In contrast, the maximum rate of reduction of a population regulated by fertility control is simply $1 - S$, the natural adult mor-

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tality rate. Assuming that all fertile females are treated ($c = 1$), our models show that the annual growth rate of the population (λ) is S. Because $S < 1$, the population will decline when $c = 1$, but it may decline very slowly if the adult survival rate is high.

In contrast, it is also possible to define efficiency in terms of the number of animals that must be treated annually to maintain a population at a specified target density. By defining efficiency as the number of animals that must be treated, we deliberately ignore the time required to treat them or the monetary expense of treatment. These costs are highly variable and situation specific. Thus to achieve generality, we will restrict our discussion to the number of animals that must be treated or culled and will allow the reader to apply cost estimates to weight efficiency in a case-specific fashion.

When efficiency is defined in terms of the number that must be treated annually, our models reveal that long-duration fertility control agents can be more efficient than lethal methods if the fertility status of treated animals is known. Although this result may be surprising at first glance, a small thought experiment reveals the intuitive basis for our result. Assume that you have 100 breeding animals with a survival rate of 0.9. On average, each of these animals produces a single female offspring every year. Nine of these offspring must enter the population to offset the losses of adults who die annually. It follows that 91 recruits must be culled to maintain the population at 100 animals. Now, presume instead that the population is stabilized by controlling fertility. Ninety animals are sterilized, leaving 10 fertile. One of the fertile animals dies each year after reproducing. Thus, there are 10 fertile offspring, replacing 9 infertile deaths and 1 fertile death. Only 9 of the recruits must be sterilized to maintain a level of infertility in the population = 90%. However, this result does not hold for short-duration agents. In this case, the 90 fertile adults and 90 recruits must be treated to maintain 90% infertility in the population.

Obviously, this is a grand oversimplification of population dynamics, but it illustrates the fundamental logic underpinning the efficiency of fertility control relative to culling. The number of females that must be treated or culled to maintain a given density depends on the difference between the total number of births and the total number of natural deaths. When a large proportion of long-lived species is sterile, this difference will be likely smaller than in fertile populations that are culled because very few animals will be reproducing. Reducing the duration of the fertility control agent increases the number that must be treated annually until this number eventually exceeds the number culled.

MANAGEMENT IMPLICATIONS

We can provide 4 general insights for implementing fertility control relative to culling. First, the efficacy of fertility control as a means to regulate populations pivots on the ability to develop agents with persistent effects. Second, we also point out that the expense of fertility control will never compete favorably with the revenue that can be provided by licensed hunting, regardless of the duration of contraceptives. Fertility control will only be cost-effective for situations where recreational hunting isn't feasible and where population control can only be achieved by professional treatment, lethal or nonlethal. Third, the feasibility of achieving target levels of infertility in the population will require knowledge of fertility status of individuals. In cases where this status is not known, the inefficiencies associated with redosing animals can increase the number of animals that must be treated by several fold. Finally, the greatest efficiency can be obtained by combining culling with contraception such that an initial reduction in animal numbers is maintained by annual treatment of marked animals with long-acting fertility control agents.

The reason that lifetime duration fertility control can be an efficient method for maintaining a population at target densities also explains why the approach is difficult to implement initially and why it poses grave risks to the persistence of small, closed populations. Our models suggest that it is relatively easy to maintain a population near a given steady state, once a sufficient number of animals have been sterilized. Achieving this initial level of infertility in the population will often be a formidable task, however, and accomplishing that task means that the breeding population will be small. Such breeding populations are subject to stochastic risks of loss of genetic variability and even extinction, as our models illustrated. It follows that while fertility control may not affect survival of individuals, it can easily be lethal to populations.

This creates a genuine dilemma for manag-

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ers. Small, closed populations are the most likely candidates for regulation by fertility control. This is simply because delivery of fertility control agents to a sufficient number of animals is most likely to be logistically feasible when populations are small and bounded in space. However, these populations are also the most prone to extinction if managers make mistakes in applying fertility control agents. The nonlinearity in many of the relationships we observed magnifies the likelihood of such errors. It follows that although fertility control can be an efficient means of regulating animal numbers, that efficiency creates risk for the population manager. As with all other techniques, managing population fertility must be grounded in a fundamental understanding of the state of the population and the role of management in influencing its dynamics. This simple fact reinforces the conclusions of Garrott (1995) that careful field studies applying control techniques over long time periods are needed to thoroughly understand effects of fertility control.

We offer a suite of predictions that can be usefully tested in field studies. Our models provide a sketch of the dynamics of populations regulated by fertility control. Offering a sketch rather than a blueprint requires that we avoid many details. Understanding those details will no doubt prove necessary to minimize unpleasant surprises in managing fertility of ungulate populations (Caughley et al. 1992, Nettles 1997). However, by offering general rather than detailed models, we provide broad insights useful as a starting point for future studies.

We identify 2 urgent needs for future work on fertility control agents. Development of techniques that render animals infertile with a single dose offer a promising alternative to culling in situations where it is feasible to mark animals to know their fertility status. Thus, work at the level of the individual animal should emphasize increasing the duration of effect of fertility control agents. Studies at the level of the population must demonstrate that fertility control can achieve meaningful reductions in rates of increase in the field. Although models like ours can suggest situations where fertility control could be efficacious in controlling abundance of ungulates, such efficacy remains in doubt until field studies demonstrate such control in real-world populations.

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APPENDIX A

Example Derivation of Deterministic Results

We used a standard series of steps to analyze all models. Here, we illustrate those steps using fixed-effect model, assuming prebreeding delivery of contraceptives. We begin with a state vector $[F\ I_1\ I_2\ I_3]$ for the case where duration of fertility control persists for 3 years. The corresponding projection matrix is

$$\begin{bmatrix} (1-c)(S+m-\beta N) & 0 & 0 & S(1-c) \\ c(S+m-\beta N) & 0 & 0 & Sc \\ 0 & S & 0 & 0 \\ 0 & 0 & S & 0 \end{bmatrix} \quad (36)$$

We obtain the characteristic polynomial of the matrix:

$$0 = \lambda^4 - \lambda S^3 c - S\lambda^3 - m\lambda^3 + \beta N\lambda^3 + Sc\lambda^3 + cm\lambda^3 - c\beta N\lambda^3. \quad (37)$$

By iterating over several values of τ, we observe a general series in characteristic polynomials for all $(\tau + 1 \times \tau + 1)$ matrices:

$$0 = \lambda^{\tau+1} - \lambda S^\tau c - S\lambda^\tau - m\lambda^\tau + \beta N\lambda^\tau + Sc\lambda^\tau + cm\lambda^\tau - c\beta N\lambda^\tau. \quad (38)$$

When the population is at equilibrium, $\lambda = 1$. Therefore, to find a general expression for the equilibrium density as a function of delivery rate we set $\lambda = 1$ and solve equation 38 for N, obtaining:

$$N^* = \frac{S + m + S^\tau c - 1 - Sc - cm}{\beta(1-c)}. \quad (39)$$

Solving for c, we find the delivery rate (c^*) needed to achieve a specified target density (N^*),

$$c^* = \frac{S + m - N^*\beta - 1}{S + m - N^*\beta - S^\tau}. \quad (40)$$

To find the composition of the population (i.e., the proportion of each stage of infertility in the population) we obtain the eigenvectors of several projection matrices corresponding to different values of τ. For each matrix, we extract the first element ($v[1]$) of the normalized vector (corresponding to an eigenvalue of 1), which gives the proportion of the population that is fertile,

$$v[1] = \frac{1-c}{(1-c)/c + 1 + S + S^2 + S^3 \ldots + S^\tau}. \quad (41)$$

To find the infertile proportion (P), we subtract $v[1]$ from 1 and simplify,

$$P = \frac{(1 - S^\tau)c}{1 + Sc - S - S^\tau c}. \quad (42)$$

In a similar way, we observe the last normalized element of the eigenvector for several values of τ and find that the proportion of the population that is in the final stage of infertility (ρ) is given by a series which simplifies to

$$\rho = v[\tau + 1] = \frac{(1-S)S^{\tau-1}c}{1 + Sc - S - S^\tau c}. \quad (43)$$

We then derive a general expression for the proportion of the population (P) that must be infertile to achieve a target density N^*. We substitute the right hand side of equation for c in equation 42, and after some algebra, obtain

$$P = 1 - \frac{1-S}{m - N^*\beta}. \quad (44)$$

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To remove β from the denominator, we observe from equation 39 that the equilibrium density in the absence of fertility control (i.e., $c = 0$) is

$$K = \frac{S + m - 1}{\beta}. \quad (45)$$

Defining Δ as the proportional reduction in population density below K (i.e., $N^* = \Delta K$), it follows that

$$N^* = \Delta K = \frac{\Delta(S + m - 1)}{\beta}. \quad (46)$$

Substituting the right hand side of equation 46 for N^* in equation 44,

$$P = 1 - \frac{1 - S}{m + \Delta(1 - S - m)}. \quad (47)$$

To derive an expression for the number of animals treated (n) to maintain a target density, we observe from the projection matrix and the state vector that the number of animals treated at equilibrium is

$$n = c^*F_t(S + m - \beta N^*) + c^*I_{t_\tau}, \quad (48)$$

which is simply the treatment rate multiplied by the steady state number of fertile females at time t plus the number of females that are recruited plus the number that regain fertility during $t \rightarrow t + 1$. Substituting $(1 - P)N^*$ for F_t and ρN^* for I_{t_τ} we have

$$n = c^* (1 - P)N^* (S + m - \beta N^*) + c^* \rho N^*, \quad (49)$$

Substituting the right-hand-side of equation 40 for c^*, rhs equation 44 for P and rhs equation 43 for ρ provides the needed expression for n in terms of the decision variable N^* and the parameters m, S, and β.

APPENDIX B

Derivation of an Unbiased Estimator for the Number of Doses that must be Delivered to a Population of Unknown Fertility to Achieve a Target Level of Infertility

We assume that a simple random sample of d animals is selected with replacement from a population of N animals. All of the animals in the sample are dosed. At the beginning of the sampling effort, the population contains I infertile animals. In order to establish the results shown in equations 34 and 35 we note that equation 35 is a special case of equation 36 where $I = 0$. We define t_i as an indicator random variable that equals 0 if the *ith* fertile animal is still not dosed after d selected animals are treated and equals 1 if it has been dosed at least once for $i = 1, 2 \ldots, N - I$. We define a as the number of additional infertile animals that are added to the population after d doses are delivered,

$$a = \sum_{i=1}^{N-I} t_i. \quad (50)$$

The expected number of additional infertile animals is given as

$$\eta = E(a) = \sum_{i=1}^{N-I} E(t_i) = (N - I)E(t_i). \quad (51)$$

Define $P(t_I = 1)$ as the probability that $t_i = 1$; then,

$$E(t_i) = P(t_i = 1) = 1 - P(t_i = 0) = 1 - \left(\frac{N - 1}{N}\right)^d. \quad (52)$$

Thus,

$$\eta = (N - I)\left(1 - \left(\frac{N - 1}{N}\right)^d\right). \quad (53)$$

Solving equation 53 for d gives equation 35. The variance of a can also be easily determined.

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EFFECT OF OVARIECTOMY ON PREGNANCY IN MARES

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Summary. Pony mares were bilaterally ovariectomized at different stages of pregnancy between Days 25 and 210. Abortion or fetal resorption occurred within 2 to 6 days after operation in all 14 mares ovariectomized between Days 25 and 45 and after an interval of 10 to 15 days in 9 of 20 others ovariectomized between 50 and 70 days. All 12 mares ovariectomized on either 140 or 210 days carried their foals to normal term. The termination of early pregnancy was preceded by a loss of uterine tone and of a palpable uterine bulge. The mean length of gestation in all mares in which pregnancy was not interrupted by ovariectomy was not significantly different from that in a group of contemporary control mares. Plasma progestagen concentrations dropped to <2 ng/ml after ovariectomy, whether or not pregnancy was maintained. Mares ovariectomized on Day 25 and injected with 100 mg progesterone daily for 10 or 20 days remained pregnant during treatment but showed a loss of uterine tone and the fetal bulge disappeared within 4 to 6 days after the end of treatment. Non-pregnant ovariectomized or intact seasonally anoestrous mares injected i.m. with 50 or 100 mg progesterone daily for 8 weeks showed changes in uterine tone, length and thickness similar to those occurring in mares during early pregnancy.

INTRODUCTION

It has been well accepted that some source of progesterone is necessary for maintenance of pregnancy in mammals. The maternal ovaries with a functional corpus luteum (CL) appear necessary almost throughout gestation in the rabbit, rat, sow, cow, goat and bitch but for a much smaller proportion of pregnancy in the ewe, guinea-pig, mare and primates, the latter species deriving their progesterone from the placenta (Gomes & Erb, 1965). Although the mare placenta contains high levels of progesterone (Short, 1957), peripheral blood levels reflect ovarian sources of the hormone during early pregnancy when CL are functional (Squires, Wentworth & Ginther, 1974b;

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Holtan, Nett & Estergreen, 1975). The placenta presumably takes over progesterone production at some stage during the first half of gestation (Squires & Ginther, 1975) but it is not known exactly when the ovaries become dispensable. Early reports indicated that ovariectomy at Days 150 (Amoroso, 1955) and 200 (Hart & Cole, 1934) of gestation does not lead to abortion.

The present experiment tested the effects of ovariectomy performed between Days 25 and 210 gestation on the maintenance of pregnancy in Pony mares. The effects of daily injections of progesterone on peripheral blood progesterone levels and on the tone, thickness and diameter of the uterus were also studied.

MATERIALS AND METHODS

Mature Pony mares weighing 150–300 kg were routinely examined for the occurrence of oestrus and ovulation as described by Ginther, Whitmore & Squires (1972). Following either natural service or artificial insemination, pregnancy was diagnosed by rectal palpation of the uterus as described by van Niekerk (1965).

A total of 50 mares were bilaterally ovariectomized at selected times between 25 and 210 days of gestation (Day 0 = ovulation) spread over 3 separate years. Mares were anaesthetized, placed in dorsal recumbency and the ovaries exposed via a midline laparotomy as described by Ginther & First (1971). The ovarian pedicle, including the ovarian artery, was ligated and the ovaries removed. The location and size of the fetus and the sizes and number of follicles and CL were noted. After the operation the uterus was palpated daily for 4 days, on alternate days for the next 8 days and once weekly thereafter. Termination of pregnancy was designated as the first day the fetal bulge was not palpable since fetal resorption could not be distinguished from abortion during early stages of gestation. The stage in pregnancy when ovariectomy was performed and the number of mares operated are given in Table 1. During the third year, another 4 mares were ovariectomized on Day 25 and at the same time were treated with progesterone (100 mg, i.m. daily) for 10 or 20 days (2 mares each). Peripheral plasma samples were collected at 4-day intervals following ovariectomy and assayed for total progestagen concentrations as described by Squires *et al.* (1974b). The sensitivity of the assay was 0·15 ng/ml plasma and the inter- and intra-assay coefficients of variation were 19·1 and 9·9% respectively.

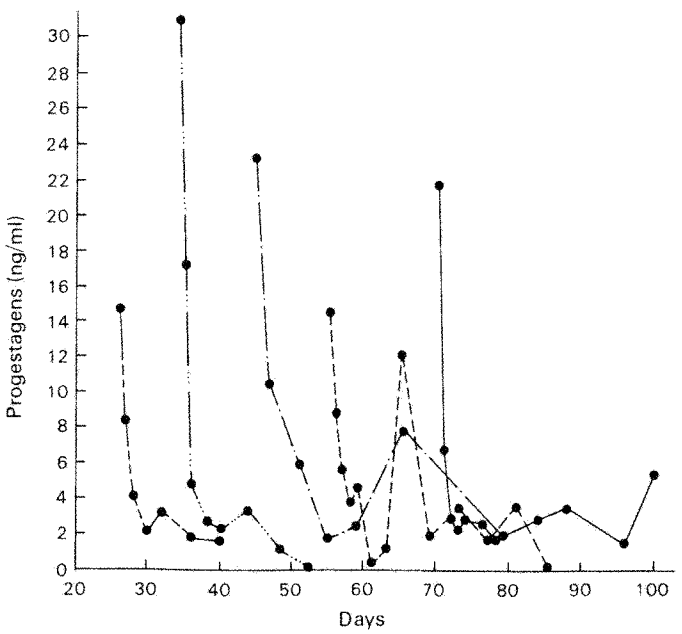
Six non-pregnant ovariectomized mares were injected i.m. daily with 50 or 100 mg progesterone for 8 days and plasma progesterone concentrations monitored. Subsequently, 17 seasonally anoestrous mares were treated similarly with progesterone for 8 weeks; half of these animals were treated for another 2 weeks with 250 μg oestradiol-17β i.m. daily) and the same regimen of progesterone. Ovarian changes and the occurrence of oestrus and the tone, length and thickness of the body and horns of the uterus were assessed twice weekly by rectal palpation by an individual who was unaware of the treatments being administered.

Table 1. Effect of ovariectomy on the maintenance of pregnancy in Ponies during the 3-year experimental period

	Year of ovariectomy and Day of gestation									
	First			Second		Third				
	70	140	210	50	70	25	35	45	55	70
No. of mares	2	6	6	1	7	4	5	5	5	5
Pregnancies maintained	0	6	6	1	5	0	0	0	2	3

RESULTS

Table 1 shows that abortion (or resorption) of the conceptus occurred in all 14 mares ovariectomized before Day 50 of gestation, that pregnancy was maintained in 11 of 20 mares after ovariectomy between Days 50 and 70 and that pregnancy was not interrupted in any of 12 mares ovariectomized on Days 140 or 210. Since there were no sham-operated controls the possible effect of surgery alone remains unknown. Text-figure 1 shows peripheral plasma progestagen concentrations in individual mares after ovariectomy on Days 25, 35, 45, 55 and 70. These mares each had a single CL which is considered to be the primary CL of ovulation, according to previous work in this laboratory (Squires, Garcia & Ginther, 1974a). The precipitous decline in progestagen levels preceded loss of uterine tone in all cases and the pregnancy bulge disappeared between 2 and 6 days after ovariectomy on Days 25 and 35 and between 10 and 15 days following ovariectomy at later stages of gestation. There appeared to be a slight rise in progestagen concentration after the initial



Text-fig. 1. Plasma progestagens after ovariectomy at 25, 35, 45, 55 and 70 days gestation. One mare each with a primary corpus luteum only.

decline which roughly coincided with the first noted absence of a pregnancy bulge. However, mares which did not abort showed similar progestagen patterns but exhibited only a slight loss of uterine tone. Pregnancy was maintained in 4 mares ovariectomized on Day 25 and treated daily with 100 mg progesterone for 10 or 20 days (2 mares each) but was terminated in all 2–6 days after treatment ceased.

Gestation length (mean ± s.e.m., number, range) was not significantly altered ($P > 0{\cdot}05$) by ovariectomy (328·3 ± 4·0, 6, 317–347) compared to contemporary intact controls (330·2 ± 5·7, 6, 309–350). When data for all 3 years were compared, there was no effect of day of ovariectomy on gestation length in those mares which carried their pregnancies to term. All foals were born healthy and fully developed, and no differences were noted in placental retention, mammary development or milk production between ovariectomized and intact mares.

Among the groups of mares ovariectomized during the third year (see Table 2) only those operated on Days 25 and 35 had only one CL and their number in mares operated later in pregnancy was significantly greater. There was no significant difference between the total number of follicles or the number of small, medium or large follicles and the days on which ovariectomy was performed except on Day 45. There was, however, a difference ($P < 0{\cdot}05$) between the average numbers of small (6·0), medium (2·4) and large (1·4) follicles when totalling the figures obtained over all days of ovariectomy (Table 2).

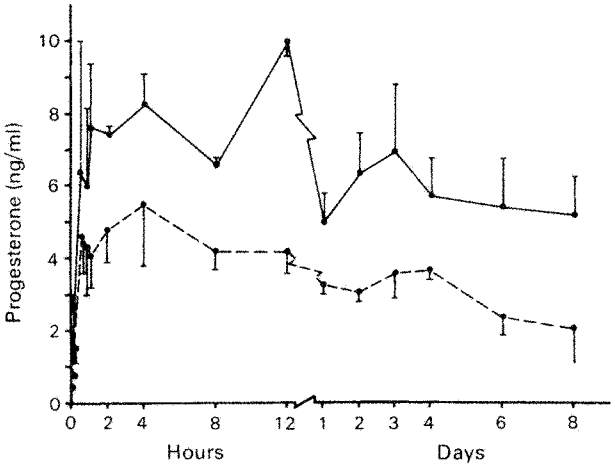
Table 2. Numbers of follicles and CL found at different days of ovariectomy

Day of ovariectomy	No. of mares	Mean no. of follicles				Mean no. of CL
		Small (<10 mm)	Medium (11–20 mm)	Large (>20 mm)	Total	
25	4	4·2	2·2	1·8	8·2	1·0
35	5	5·6	2·6	2·2	10·4	1·0
45	5	4·4	2·2	0·4	7·0	1·4
55	5	8·8	2·4	1·2	12·4	2·6
70	5	6·8	2·8	1·4	11·0	4·8

Mean number of follicles within size was different ($P < 0{\cdot}05$) while mean number between days was not ($P > 0{\cdot}05$).

The incidence of side of primary ovulation (12 left, 12 right) and side of placentation (10 left, 14 right) was not different ($P > 0{\cdot}05$). However, the conceptus was situated in the uterine horn contralateral to the side of ovulation, indicating transuterine migration, in 15 of 24 mares (7 from left to right and 8 from right to left). There was no apparent relationship between the side of placentation and the locations and numbers of accessory CL.

The effect of daily injections of progesterone on peripheral blood levels in 6 non-pregnant ovariectomized mares is shown in Text-fig. 2. Progestagen concentrations of >2 ng/ml were maintained during injections of 50 mg and >5 ng/ml during injections of 100 ng. A subjective increase in uterine tone was also noted at the end of the treatment (Day 8) in both groups. Subsequently, in



Text-fig. 2. Mean ± s.e.m. plasma progesterone concentrations in 3 ovariectomized mares following daily i.m. injections of 50 (– – –) or 100 (——) mg progesterone.

a well controlled long-term trial in which daily intramuscular injections of 50 and 100 mg progesterone were given to seasonally anoestrous mares, a significant increase ($P < 0{\cdot}05$) in mean uterine tone, measured in arbitrary units, occurred by the third week (untreated controls, 1·4; 50 mg/day, 2·2; 100 mg/day, 2·5) and was maximum by the 5th week (controls, 1·1; 50 mg/day, 2·8; 100 mg/day, 2·9) after the start of treatment. Both treated groups of mares exhibited tense and turgid uteri at this time which were similar in texture to the uteri in mares between 18 and 25 days of pregnancy. Length of the uteri decreased ($P < 0{\cdot}05$) by 2 weeks while diameter of the horns and thickness of the body of the uterus increased ($P < 0{\cdot}05$) by 3 weeks after the start of treatment. Subsequent treatment of some mares with progesterone and 250 μg oestradiol-17β daily for 2 more weeks did not significantly change either uterine or ovarian parameters; behavioural oestrus was initiated in 2 of 3 controls treated with oestradiol-17β alone. Following withdrawal of treatment uterine tone declined rapidly and was similar to that of untreated control mares by 1 week. The length and diameter of the uterine horns also could not be distinguished from those of controls by 2 weeks after treatment. Total ovarian size and the numbers and sizes of follicles were not significantly different between control or treated mares, either during or after progesterone treatment.

DISCUSSION

The present study indicates that the ovaries of the mare are dispensable between Days 50 and 70 of gestation, considerably earlier than the figure of 150 given by Amoroso (1955) or 200 days by Hart & Cole (1934). Our findings of a precipitous decline in peripheral plasma prostagen after ovariectomy on Days 25–70 (Text-fig. 1) indicate that the ovaries are the primary source of progestagens during this time. This agrees with Squires & Ginther (1975) who found uterine vein progesterone concentrations significantly higher than those

in jugular vein plasma at Days 80 and 100, thereby indicating that a non-ovarian source may be of major importance in maintaining pregnancy after this time. This non-ovarian source, possibly the placenta (Short, 1957) or conceptus, is evidently not sufficiently established until after Day 45, as ovariectomized mares maintained with exogenous progesterone daily from Days 25 to 45 subsequently aborted following treatment.

These data question the necessity and function of PMSG in accessory follicle and CL formation. Some mares with only a single CL (Text-fig. 1) had progestagen concentrations higher than those in mid-dioestrus. Thus PMSG may only be luteotrophic as might be suggested from the in-vitro work of Squires, Stevens, Nett & Pickett (1978). However, high progestagen concentrations on Days 25 and 35, before the expected appearance of PMSG in the peripheral circulation, may suggest that the conceptus (Flood & Marrable, 1975) or gonadotrophins other than PMSG may be stimulatory before Day 40. It is tempting to speculate that PMSG may be more important for its anti-immunological properties (Allen, 1975) than its gonadotrophic effects.

The fact that exogenous progesterone can increase uterine tone in non-pregnant anoestrous mares and maintain tone and pregnancy after ovariectomy would suggest that it is a major component of early pregnancy. However, progesterone is obviously not totally responsible for increased tone as non-pregnant mares with a prolonged CL maintain progesterone concentrations without increased uterine tone (Hughes, Stabenfeldt & Evans, 1975). In the present experiments using Pony mares daily injections of 0·25 mg oestradiol-17β, in addition to 100 mg progesterone, failed to stimulate uterine tone further. In later work Berg & Ginther (1978) found that twice this dose of oestradiol-17β stimulated uterine tone to the condition seen during early pregnancy. During early pregnancy (i.e. Days 18–30) peripheral plasma progesterone actually decreases (Holtan *et al.*, 1975) during the very time increasing uterine tone is noted. There may be an additive effect of hormone production and or metabolism by the conceptus during this period (Flood & Marrable, 1975). In any event, the reported short half-life of progesterone (Ganjam, Kenney & Flickinger, 1975), along with the results of the present study, would question the efficacy of less than daily therapy of exogenous progesterone to prevent abortion during early pregnancy in mares.

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Effects of bilateral ovariectomy via colpotomy in mares: 23 cases (1984–1990)

R. Neil Hooper, DVM, MS; Tex S. Taylor, DVM, MS; Dickson D. Varner, DVM, MS; Terry L. Blanchard, DVM, MS

Summary: *Postoperative performance and behavioral patterns were investigated retrospectively in 23 client-owned mares after bilateral ovariectomy via colpotomy. The interval from surgery to postoperative inquiries ranged from 9 to 67 months. Information obtained from review of the medical record and client interviews included the reason for ovariectomy, postoperative complications, problems identified by owners after discharge of the mare from the hospital, postoperative level of athletic performance, postoperative signs of estrus, and overall owner satisfaction. Reasons given by owners for having mares ovariectomized were behavioral modification (16 mares), use as embryo-transfer recipients (3 mares), use as mount mares for collecting semen (2 mares), elimination of chronic colic during estrus (1 mare), and sterilization for registration (1 mare). Postoperative complications developed in 4% (1/23) of the mares; however, problems were noticed by the owners of 4 other mares after discharge from the hospital. Continuing signs of behavioral estrus were detected in 35% (8/23) of the mares, but in only in 9% (2/23) was the behavior judged to be objectionable by the owner. Of 12 mares used in performance events prior to bilateral ovariectomy, 10 were judged to be competing at greater than preoperative levels, 1 was judged to be competing at the same level, and 1 was judged to be competing at less than preoperative level of performance. Of 18 owners, 14 were satisfied, 2 were undecided, and 2 were dissatisfied with their mare after it had had bilateral ovariectomy.*

Horse owners tend to discriminate against the use of mares with intact ovaries for performance events because of undesirable behavior during estrus. Two methods typically used to minimize estrus behavior in mares are long-term administration of exogenous progestins[1-4] and bilateral ovariectomy.[5-7] Use of exogenous progestins may be less desirable than ovariectomy, unless mares are eventually to be used for breeding. Commercially available progestins are expensive when administered orally on a long-term basis and can induce myositis if repeated injections are given IM.

Use of ovariectomized mares in performance events has increased in popularity.[7] Although colpotomy has been used for ovariectomies throughout the 20th century, this technique is gaining renewed interest among veterinarians because of the development of improved analgesics that facilitate implementation of the procedure.[7] Owners prefer the reduced cost of surgery and more rapid postoperative return of mares to use in bilateral ovariectomy via colpotomy, as opposed to via celiotomy.[5,7]

Review of the literature revealed several reports describing ovariectomy by colpotomy and potential complications,[5-11] but few reports incorporate a large number of cases and specific postoperative problems[7] with changes in estrus behavior and level of athletic performance. The primary objective of the study reported here was to examine the effects of bilateral ovariectomy via colpotomy on subsequent estrus behavior and athletic performance in mares.

Criteria for Selection of Cases

A computer search of the veterinary medical record system indicated that 69 mares had undergone ovariectomy between 1984 and 1990. All 23 mares that had had a bilateral ovariectomy via colpotomy during the study period were included. Information was obtained by review of medical records and by phone contact with owners, trainers, or referring veterinarians. Time from surgery to postoperative inquiries ranged from 9 to 67 months. Information obtained from medical records or owners included the reason for ovariectomy, postoperative complications that developed prior to discharge from the hospital, postoperative problems detected by the owner after discharge of the mare from the hospital, change in performance of the mare, detection of signs of estrus after ovariectomy, and overall owner satisfaction with the mare after surgery.

If objectionable behavior (ie, excessive estrus

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behavior) was given as the reason for ovariectomy, owners were asked whether behavior of the mare prior to surgery was constant or cyclic (ie, associated only with physiologic estrus). Owners who indicated a mare's behavioral problems had been associated only with estrus were asked whether behavior of the mare was still a problem after bilateral ovariectomy. In addition, owners were asked what they considered objectionable about their mare's behavior prior to surgery.

Owners of mares that competed in performance events (ie, competed for a purse) were asked whether they believed their mare performed at a lower, equivalent, or higher level after bilateral ovariectomy, compared with preoperative performance. Owners who indicated their mare had been observed showing signs of estrus after ovariectomy were asked to rate the signs as mild (ie, infrequent signs of estrus, but not in a cyclic or repetitive pattern), moderate (ie, intermittent signs of estrus, sometimes in a cyclic or repetitive pattern), or strong (ie, consistent signs of estrus, in a cyclic or repetitive pattern). Owners also were asked to indicate whether they found postoperative signs of estrus to be objectionable (ie, interfered with training, performance, or general use). Assessment of owner satisfaction was based on owner perception of the mare after ovariectomy and on willingness to have another mare ovariectomized under similar circumstances.

Ovariectomies had been performed by use of the technique described by Walker and Vaughan.[8] Food had been withheld, and mineral oil (4 L) had been administered by nasogastric intubation, 24 hours prior to surgery. Procaine penicillin G (20,000 U/kg of body weight, IM, q 12 h) had been administered 2 hours prior to surgery and had been continued through the third day after surgery. After ovariectomy, each mare had remained tied for at least 5 days to discourage recumbency. The incision site had been examined through a vaginal speculum every other day, beginning on the fifth day after surgery. When the diameter of the incision had decreased to < 2 cm, the mare had been released. Owners had been asked to turn the mares out for a minimum of 2 weeks before starting to ride them again.

Results

Four owners had had more than 1 mare ovariectomized during the period studied. Reasons given by owners for ovariectomizing their mare were behavioral modification to correct objectionable estrus behavior (16 mares), use as embryo-transfer recipients (3 mares), use as mount mares for collection of semen (2 mares), elimination of chronic colic detected only during estrus (1 mare), and sterilization as required for registration (1 mare). Postoperative complications were reported in the medical record of only 1 of the 23 mares. That mare developed clinical signs of peritonitis (ie, fever, lethargy, and anorexia) 1 week after surgery, but bacteria were not detected in peritoneal fluid by histologic examination or by culture. That mare responded to administration of trimethoprim/sulfamethoxazole (30 mg/kg, PO, q 12 h), and problems have not recurred during the 2-year period after surgery. Problems reported to have developed in the interval between discharge from the hospital and follow-up interview were episodes of mild to moderate colic that recurred every 2 to 3 months in 2 mares, and signs of chronic lumbar pain in 1 mare and bilateral hind limb pain in another mare that, in each owner's opinion, limited athletic performance of each mare.

Seventy-four percent (17/23) of the mares were bilaterally ovariectomized to alter behavior. All 15 owners indicated that objectional behavior occurred primarily during estrus. When asked specifically what behaviors were found objectionable, 11 owners (with 11 mares) indicated inconsistency in performance during estrus, 2 owners (with 3 mares) objected to aggressive behavior toward the owner and toward other horses during estrus, 1 owner (with 2 mares) objected to outward signs of estrus, and 1 owner (with 1 mare) objected to recurrent episodes of colic during estrus. After the mare was ovariectomized, 13 owners believed behavior was no longer a problem (in 14 mares), whereas 2 owners believed behavior was still a problem (in 3 mares). One owner objected because 2 of these mares still showed signs of estrus, whereas the other owner objected because the third mare began to perform erratically 6 months after ovariectomy.

Signs of behavioral estrus were still detected in 35% of the mares (8/23) after bilateral ovariectomy. Of the mares showing signs of estrus after surgery, 5 mares displayed signs of estrus classified by the owners as mild, 1 mare displayed signs classified as moderate, and 2 mares displayed signs classified as strong. However, only the owner of the 2 mares that had strong signs described the postoperative intensity of signs of estrus as objectionable, because the mares showed signs of estrus and would stand to be mounted when confined in a pasture with a sexually aggressive gelding.

Twelve owners had used mares in performance events prior to bilateral ovariectomy. When owners were asked to compare the preoperative and postoperative level of performance in these mares, 10 owners believed that performance was improved after surgery (in 8 mares), 1 owner believed that performance did not change (in 1 mare), and another owner believed that performance was worse (in 1 mare). The reason given for improvement after ovariectomy was the elimination of fluctuations in behavior and performance associated with estrus, which resulted in more consistent performance. The mare in which performance was the same after surgery was ovariectomized because of recurrent bouts of colic associ-

ated with estrus. After ovariectomy, recurrent colic returned in 6 months, and the mare was euthanatized 2 months later. Necropsy to determine the cause of colic was not done. The owner who believed that performance was worse after surgery indicated that the mare performed as desired for 6 months, then began to misbehave and perform as erratically as before bilateral ovariectomy.

Two additional mares were intended for use as performance horses, but had not entered training prior to surgery; therefore, the owners could not comment on the effects of bilateral ovariectomy on performance of these mares. One owner was pleased with the way the mare performed after it was trained. The other owner sold the other mare because of poor performance during race training.

Of the 18 owners, 14 indicated that they would consider having another mare ovariectomized, 2 were undecided, and 2 would not choose to have another mare ovariectomized. Reasons given for owner dissatisfaction with ovariectomy included poor performance in 1 mare and continuing display of estrus behavior in 2 mares. Repeated colic after surgery in 2 mares and failure to meet the owners expectation of racing performance in 1 mare were reasons given by the 2 owners for being undecided about having additional mares ovariectomized.

Discussion

Primary indications for ovariectomy in mares have traditionally been ovarian tumors and nymphomania (ie, continuous estrus behavior),[6,7,12,13] but bilateral ovariectomy continues to gain popularity among owners as a method to alter undesirable behavioral patterns of mares in estrus. Additionally, ovariectomized mares have been shown to be beneficial for research, for collection of semen from stallions, and as embryo-transfer recipients.[7,14,15] Colpotomy is well suited as an approach for ovariectomy in performance mares because of reduced postoperative recovery time and decreased expense, compared with that of celiotomy.[5,7]

Behavioral changes associated with estrus in mares can interfere with training and performance. In theory, bilateral ovariectomy should eliminate behavioral signs of estrus by eliminating the primary source of estrogen production; however, some mares continue to exhibit estrus behavior (albeit of reduced intensity) after ovariectomy. It has been suggested that the production of estrogens by the adrenal cortex may be sufficient to cause mild signs of estrus.[16] In support of this concept, administration of glucocorticoids (which would likely suppress adrenal production of steroid hormones) to ovariectomized mares is reported to suppress behavioral signs of estrus.[1] However, a study[17] involving ovariectomized mares suggested that the adrenal glands do not produce detectable amounts of estradiol in response to stimulation with ACTH. Therefore, in ovariectomized mares, estrogen either does not play a role in the exhibition of behavioral estrus or there is another source of estrogen production. Conversely, removal of the major source of progesterone (ie, corpora lutea), which inhibits the outward expression of estrus, could be the primary reason that some ovariectomized mares continue to show mild signs of behavioral estrus.[18] In support of this concept, ovariectomized mares are successfully used as mount mares in horse breeding operations.

Most ovariectomized mares fail to exhibit signs of estrus unless they are teased by a stallion or sexually aggressive gelding. A few ovariectomized mares occasionally exhibit mild signs of estrus without being teased.[1] Ovariectomized mares rarely exhibit strong signs of estrus behavior that would be considered objectionable in performance horses. Ovariectomy may improve performance by promoting more consistent behavioral patterns. Changing economics of the horse industry may lead owners to consider ovariectomy to modify behavior and maximize performance of mares not valued as breeding prospects.

Mares that exhibit extreme aggression toward other horses and human beings are rarely treated effectively by ovariectomy.[1,5-7,19] Strong consideration should be given to euthanatizing these potentially dangerous mares.[5,19]

Problems after ovariectomy via colpotomy in this study were minimal. Postoperative complications that have been reported after bilateral ovariectomy via colpotomy include peritonitis, evisceration, abscess or hematoma formation at the incision site, abdominal adhesions, and delayed healing of the incision.[5-7] Evaluation of peritoneal fluid from mares that have undergone bilateral ovariectomy via colpotomy revealed development of a mild subclinical peritonitis, but bacteria were usually not detected during cytologic examination of the peritoneal fluid.[5-7] Although 5 mares in our study had problems (repeated colic in 2 mares, signs of lumbar pain in 1 mare, signs of bilateral hind limb pain in 1 mare, and clinical signs of peritonitis in 1 mare) after surgery, evidence is inconclusive in each as to the role played by surgery.

Intestinal adhesion to the incision site after ovariectomy could predispose to recurrent colic.[5,7] The 2 mares that experienced repeated episodes of colic did not develop these problems until 2 years after ovariectomy. Both mares belonged to the same owner and were managed similarly. One mare was subsequently euthanatized during severe colic; necropsy revealed entrapment of the small intestine in the epiploic foramen. Adhesions were not found involving the gastrointestinal tract or urogenital tract.

Ovulation has been reported as a cause of cyclic colic in mares.[20] Confirmation of this observation was based on elimination of signs of colic when mares were administered altrenogest daily. The mare in our study that had repeated bouts of colic

had been treated repeatedly by the same veterinarian for > 2 years. On the basis of historical data and repeated clinical observation by the veterinarian, colic appeared to develop in association with estrus. To confirm the diagnosis, the mare was administered altrenogest[a] (0.44 mg/kg, PO, q 24 h) for 60 days prior to bilateral ovariectomy and did not have signs of colic.

The cause of the signs of lumbar pain (in 1 mare) and of bilateral hind limb pain (in 1 mare) observed by 2 owners is unknown. One mare had a history of signs of back pain prior to admission. The owner and referring veterinarian believed the pain originated from the ovaries because of misbehavior and erratic performance when the mare was in estrus. After ovariectomy in this mare, performance met the owner's expectations for 6 months before the same behavioral problems recurred. In the owner's opinion, surgery was not successful because the mare began to perform erratically again after surgery. The other mare was 15 months old at surgery and was ovariectomized to be eligible for breed registration, and was subsequently trained for racing. Performance of this mare never met the owner's expectations, and the mare was sold prior to completion of race training.

On the basis of discussions with owners, trainers, or referring veterinarians, bilateral ovariectomy via colpotomy generally did not adversely affect ability to perform in athletic events, nor did it totally eliminate all signs of estrus in mares. Our study suggested that use of ovariectomized mares in performance events should continue to gain popularity among horse owners. Whereas bilateral ovariectomy usually does not eliminate all signs of estrus behavior in mares, the attenuation in signs is generally beneficial to performance because it promotes more consistent behavior.

[a]Regu-mate, Hoerchst-Roussel Agri-Vet Co, Somerville, NJ.

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Correction: Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats

In "Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats" (*JAVMA*, Aug 1, 1993, pp 396-405), the plot labeled Figure 3 should be Figure 5, that labeled Figure 5 should be Figure 6, and that labeled Figure 6 should be Figure 3. The *JAVMA* regrets the error.

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Characterization of Bone Mineral Composition in the Proximal Tibia of Cynomolgus Monkeys: Effect of Ovariectomy and Nandrolone Decanoate Treatment

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Like postmenopausal women, ovariectomized cynomolgus monkeys (*Macaca fascicularis*) experience accelerated loss of bone mass. Treatment of ovariectomized monkeys with nandrolone decanoate results in an increase in bone mass to levels comparable to those of intact animals. The changes in bone composition that occur with these treatments, however, are less well characterized. In the present study, we used synchrotron Fourier-transform infrared microspectroscopy (FT-IRM) and curve-fitting methods to monitor specific changes at cortical, subchondral, and trabecular bone regions in the proximal tibia. Four groups were studied: (1) sham-operated (sham); (2) ovariectomized and treated with placebo for 2 years (ovx); (3) ovx + nandrolone decanoate for 2 years (NAN); and (4) ovx + nandrolone decanoate beginning 1 year after ovx (dNAN). The results demonstrate that ovariectomy and nandrolone treatment did not affect the degree of mineralization as defined by the phosphate/protein ratio, but acid phosphate content (HPO_4^{2-}) in cortical and subchondral bone was increased by ovariectomy, suggesting this bone to be less mature due to increased remodeling that occurs after ovariectomy. In the subchondral and cortical bone regions, ovariectomized monkeys showed a lower total carbonate content (CO_3^{2-}/matrix ratio) than sham controls, specifically due to the decrease in labile carbonate content. In the trabecular region, no change of carbonate content was observed. Treatment with nandrolone decanoate was found to restore the loss in carbonate, where the resulting mineral had a larger quantity of type B carbonate. Finally, we correlated carbonate content with dual-energy X-ray absorptiometry measurements, and found a positive correlation between bone mineral density and type A carbonate in bone, which is stoichiometrically related to the amount of calcium in bone. Therefore, the results presented herein identify significant differences in bone chemistry after ovariectomy and nandrolone treatment, which may help explain previous findings that, although nandrolone decanoate treatment increased bone mass, it could not reverse the decrease in bone strength due to ovariectomy (Aerssens et al., *Calcif Tissue Int* 53:269–277; 1993). (Bone 30:492–497; 2002) **© 2002 by Elsevier Science Inc. All rights reserved.**

Key Words: Ovariectomy; Infrared microspectroscopy; Bone mineral; Nandrolone decanoate; Postmenopausal osteoporosis; Cynomolgus macaques.

Introduction

Osteoporosis is characterized by low bone density and microarchitectural loss of bone tissue leading to increased susceptibility to fracture.[9] The association of estrogen deficiency with postmenopausal osteoporosis has long been recognized. Increased activity of several mediators secondary to estrogen deficiency results in increased osteoclast activity and a higher bone resorption rate, resulting in loss of bone.[11] However, decreased bone density alone cannot explain the change in bone strength, because the correlation between bone density and bone strength is only moderately strong.[19] Thus, it is likely that chemical and morphological differences, such as mineral apatite composition and organic matrix maturity and organization, may contribute to bone strength and disease severity.[5,10,18,19]

Infrared (IR) spectroscopy has been widely used to examine bone composition because it can detect alterations in several major biological constituents of bone.[31,32] In synthetic and animal bone IR studies, bone of greater maturity is associated with a higher mineral/matrix (phosphate/Amide I) ratio,[6,26,28] a higher degree of crystallinity,[4,6,23,26,29] and a lower acid phosphate (HPO_4^{2-}) content.[23,26,30] Osteoporotic bone has a lower mineral/matrix ratio than normal bone, suggesting that it is less mature than normal bone.[3,25,30] However, when comparing the degree of crystallinity of osteoporotic bone to that of normal controls, some investigators have reported a higher degree,[2,3,12,25,30] whereas others observed a lower degree[14,30] or no difference.[24] These conflicting results may have arisen due to the variability among human bone specimens,[2,12] as well as different methods of inducing osteoporosis, including nutritional deprivation,[30] ovariectomy,[24] and biological menopause.[2,12]

The most common method of examining bone samples with IR spectroscopy is by homogenization of the bone. However, diseased bone is affected differently at distinct anatomical sites,[1,17] which may respond to drug treatment differently.[7,15] Localized effects in bone disease are particularly relevant in osteoarthritis (OA), where subchondral bone thickening and

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sclerosis parallel disease severity.[8] Treatments for osteoporosis that increase bone mass may also increase the potential for subchondral bone thickening, which may predispose the individual to the development of osteoarthritis (OA). Thus, examination of treatment effects on different regions of bone (i.e., cortical, trabecular, and subchondral bone) is important. With the combined advantages of infrared spectroscopy and optical microscopy, Fourier-transform infrared microspectroscopy (FT-IRM) has been used to examine bone samples in situ, with a spatial resolution near the diffraction limit (3–20 μm).[20]

In the present study, tissues from ovariectomized cynomolgus monkeys (*Macaca fascicularis*) were used to examine the mineral and matrix chemistry of bone under conditions of chronic estrogen deficiency. Ovariectomized cynomolgus monkeys are a well-recognized model of postmenopausal bone loss in women.[15,16,27,33] Two ovariectomized groups of monkeys received nandrolone decanoate, an anabolic steroid hormone that enhances bone density in osteoporotic women[27] and inhibits bone turnover in ovariectomized monkeys.[8,15,16,33] Bone from the proximal tibia of animals in these two treatment groups was analyzed at three distinct anatomical regions: cortical, subchondral, and trabecular bone. The results were compared with those in the same location from nontreated intact and ovariectomized control animals.

Materials and Methods

Animals and Study Design

Fifty-nine young adult female cynomolgus monkeys were group-housed and fed identically for the study, which was conducted at the Comparative Medicine Clinical Research Center, Wake Forest University School of Medicine. The details of the trial have been described previously.[16] Briefly, the subjects were randomly assigned to four treatment groups for the 24 month study: (1) sham-operated (sham; n = 15); (2) ovariectomized, treated with placebo for 2 years (ovx; n = 15); (3) ovx + nandrolone decanoate for 2 years (NAN; n = 15); and (4) ovx + nandrolone decanoate beginning 1 year after ovx (dNAN; n = 14). The treated groups (NAN and dNAN) were given 25 mg nandrolone decanoate intramuscularly every 3 weeks, and the nontreated groups (sham and ovx) were given sterile vehicle intramuscularly every 3 weeks. At the time of ovariectomy (or sham operation), 1 year after surgery, and 2 weeks before necropsy, all animals were administered fluorochrome labels (tetracycline HCl, calcein, and alizarin complexone, respectively) intravenously on a 1-7-1-7 schedule.[16] One tibia from each animal was obtained at the termination of the study. This site exhibits changes in bone mineral content and bone mineral density in response to hormonal therapies that are similar to those occurring in the vertebrae.[7] A midcoronal section from each proximal tibia was embedded in methylmethacrylate/dibutylphthalate. The blocks were sectioned at a thickness of 5 μm with a sledge microtome, and tissue sections from five SHAM, seven OVX, six NAN, and six dNAN animals were air-dried and mounted in an IR sample cell.

Spectral Data Collection

Infrared spectra were collected using a FT-IR microscope (Spectra Tech, Shelton, CT), equipped with confocal 32× Schwarzchild objectives, a motorized x-y stage, and an external MCT/B detector (Belov Technology, New Brunswick, NJ) cooled to 77K. The microscope was attached to a Nicolet Magna 860 spectrometer that receives its light source from Beamline U2B (National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY). For data collection, the bone section was secured in a compression cell (Spectra Tech) with the center portion exposed to the beam path. A video camera was mounted on the microscope to capture visible light images before each data collection. An adjustable aperture was placed before the objective to limit the IR illumination area of each data point to 20 μm × 20 μm, calibrated using a visible light image at its focal point. Absorption due to water vapor and CO_2 was minimized by purging the system with nitrogen gas. For each animal, 900, 600, and 400 spectra were collected from randomly chosen cortical, subchondral, and trabecular regions, respectively. At all chosen regions, data acquisition was done in a rectangular-sized map format using ATLμS software (Nicolet Instruments, Madison, WI) with 20 μm steps using the x-y motorized stage. A spectrum was acquired for each data point using 64 coadditions and Happ–Genzel apodization at 4 cm^{-1} resolution. Prior to starting a map, a light image of the map area was taken using the same coordinate as the map for visual guidance in later data filtering, and a background spectrum through air (no sample) was collected for an absorbance reference.

Bone Density Measurements

Bone mineral content (BMC) and density (BMD) of whole body, lumbar vertebrae L2–4, and knee (distal femur and proximal tibia) were measured under anesthesia at the termination of the study using dual-energy X-ray absorptiometry (DEXA; Norland Model XR-26, Fort Atkinson, WI), and these results have been reported previously.[7,16]

Data Analysis

Curve-fitting and area integrations were carried out on two spectral regions: $\nu_2CO_3^{2-}$ (905–825 cm^{-1}) and $\nu_4PO_4^{3-}$ (650–500 cm^{-1}) using GRAMS32 v5.1 (Galactic Industries Corp., Salem, NH) software. Both spectral regions were baseline-corrected by a cubic function to account for the underlying broad peaks.[23] The integrated Amide I band (1600–1700 cm^{-1}) was used to represent the total protein content in the bone, where >90% is collagen. The center positions for each subpeak were determined based on previous assignments[23,31,32] and confirmed by using second-derivative peak analysis. All peaks were fitted by a Gaussian function with the center position limited to ±2 cm^{-1} from the initial position and the width limited to 30 cm^{-1} (for broad bands, 40 cm^{-1} was used) to avoid alternative convergent solutions. Macro routines were developed to process all spectra using the same initial fit parameters. The convergence criteria were determined by reduced chi-square statistics and the root-mean-square (RMS) noise level. A maximum of 50 iterated optimization algorithms were done in each fit routine until the convergence criteria were met. Nonconverging spectra were discarded. Several initial conditions were tested to ensure reproducibility and validity of the final fit. Spectra from nonbone regions were identified and filtered out by their higher absorption intensity from 1700 to 1760 cm^{-1} due to the presence of embedding material. All peaks and subpeaks of interest were area-integrated and expressed as ratios to account for sample thickness heterogeneity. The calculated ratios were tabulated into histograms and then fitted to a normal distribution curve. The center and width values from all normal fits were analyzed by one-way analysis of variance (ANOVA) followed by pairwise multiple comparisons using Fisher's exact test. $p < 0.05$ was considered statistically significant. Adequate transformation was applied to each data set if it did not meet the assumptions for ANOVA. The variances of two data sets (see *Results*) were inhomogeneous and resistant to the available transformations, so a pooled *t*-test was applied to the ovx and sham groups. Paired

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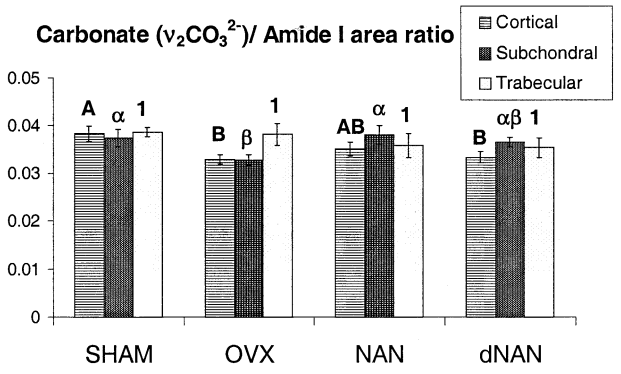

Figure 1. The column height represents the mean values of each test group and the error bar indicates the standard error of the mean (SEM) where the number of subjects is between five and seven in each treatment group (600 spectra from subchondral, 400 from trabecular, and 900 from cortical region were collected for each subject). The letter or number above each column indicates that, for any two groups that have no letter in common, a statistical difference between these two groups was detected after ANOVA with multiple comparisons ($p < 0.05$). For example, in the cortical region, sham (A) differs from ovx (B) and dNAN (B), but not NAN (AB). Ovx differs from sham, but not NAN or dNAN. Comparisons were only made among groups in the same bone region.

t-tests were used to compare means from cortical regions with those from subchondral regions for each animal. The mineral(including carbonate and phosphate components)-to-matrix ratios were compared and also to the DEXA data obtained previously (see earlier) using correlation analysis. All statistical analyses were done using Microsoft EXCEL 97 with an ANALYZE-IT v1.50 add-in.

Results

Mineral-to-Matrix Ratios

The peak assignments for the carbonate ($\nu_2CO_3^{2-}$) and phosphate ($\nu_4PO_4^{3-}$) bands have been characterized.[23,31,32] The mineral/matrix ratios, $\nu_2CO_4^{3-}$/Amide I and $\nu_4PO_4^{3-}$/Amide I, are indices to represent the degree of mineralization.[3,23,25] The $\nu_4PO_4^{3-}$/Amide I ratio among the four groups was the same in all three bone regions (data not shown). Conversely, the $\nu_2CO_3^{2-}$/Amide I values for the ovx animals were significantly lower than the sham controls ($p < 0.05$) in the cortical and subchondral bone regions (**Figure 1**). Nandrolone treatment for 2 years restored these ratios to sham levels ($p < 0.05$ in the subchondral region). In the trabecular region, no significant differences in $\nu_2CO_3^{2-}$/Amide I values were detected among the four groups (Figure 1).

Composition of $\nu_2CO_3^{2-}$ Carbonate Peak

Curve-fitting of the $\nu_2CO_3^{2-}$ (905–825 cm^{-1}) region resulted in three subpeaks, at 878 cm^{-1}, 872 cm^{-1}, and 866 cm^{-1}. The peaks at 878 cm^{-1} and 872 cm^{-1} were assigned respectively to the apatitic carbonate that occupies the OH^- (type A carbonate) and PO_4^{3-} (type B carbonate) sites in the hydroxyapatite lattice. The peak at 866 cm^{-1} was the remaining, nonapatitic or "labile" carbonate.[26] The labile carbonate content (866 cm^{-1}/total $\nu_2CO_3^{2-}$) was higher among sham controls compared with the ovx group in the cortical and subchondral regions (+15%, $p < 0.05$), but this difference was not seen in the trabecular bone region (**Figure 2**A). The two treated groups were not compared due to their larger variances. The ratio of type A/type B carbonate was unchanged between SHAM and OVX animals in all three bone regions, but both treated groups had lower ratios ($p < 0.05$ for all regions except for the NAN group in the trabecular bone region) than the two nontreated groups (Figure 2B).

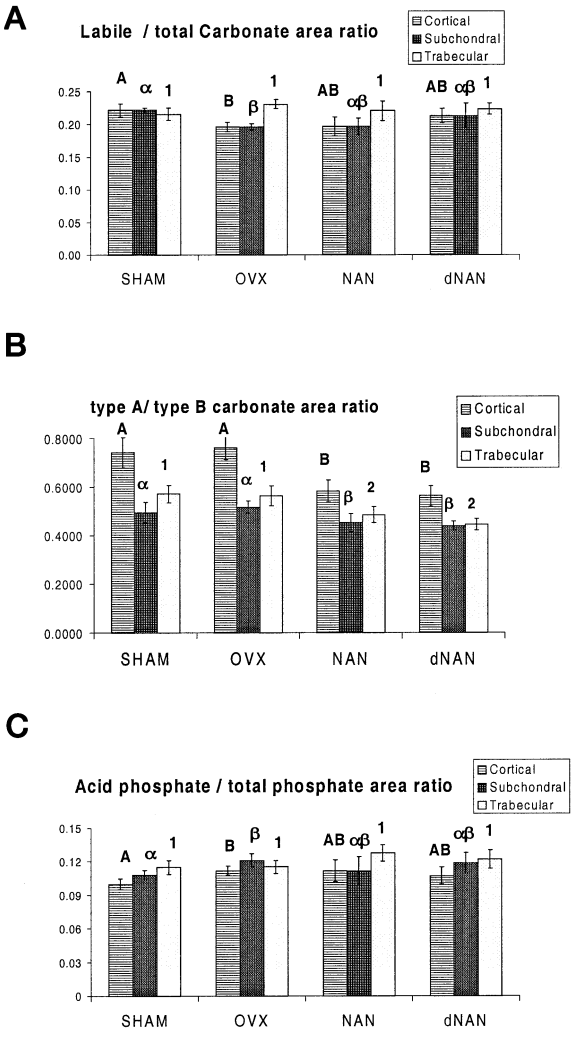

Figure 2. The results from curve-fitting of $\nu_2CO_3^{2-}$ and $\nu_4PO_4^{3-}$ were compared among the four test groups: (A) labile carbonate/total carbonate; (B) type A carbonate/type B carbonate; and (C) acid phosphate/total phosphate. The error bar represents the standard error of the mean (SEM). The letter designation above each column is described in Figure 1. (A) and (C) were tested by pooled *t*-test between ovx and sham, because the treated groups had a significantly larger variance that could not be tested by ANOVA.

Composition of $\nu_4PO_3^{2-}$ Phosphate Peak

Curve-fitting of the $\nu_4PO_4^{3-}$ band (650–500 cm^{-1}) resulted in five components that have been assigned previously.[23,32] The peaks at 613 cm^{-1} and 536 cm^{-1} represent labile phosphate, with the latter more closely related to labile acid phosphate (HPO_4^{2-}). The other three peaks, at 603 cm^{-1}, 578 cm^{-1}, and 563 cm^{-1}, correspond to apatitic phosphate. We used the ratio of integrated 536 cm^{-1} peak to total $\nu_4PO_4^{3-}$ to represent relative amount of labile acid phosphate content vs. total phosphate content.[23] In the subchondral and cortical regions, but not the trabecular region, the ovx animals had a higher acid phosphate content than sham controls (+12%, $p < 0.05$) (Figure 2C).

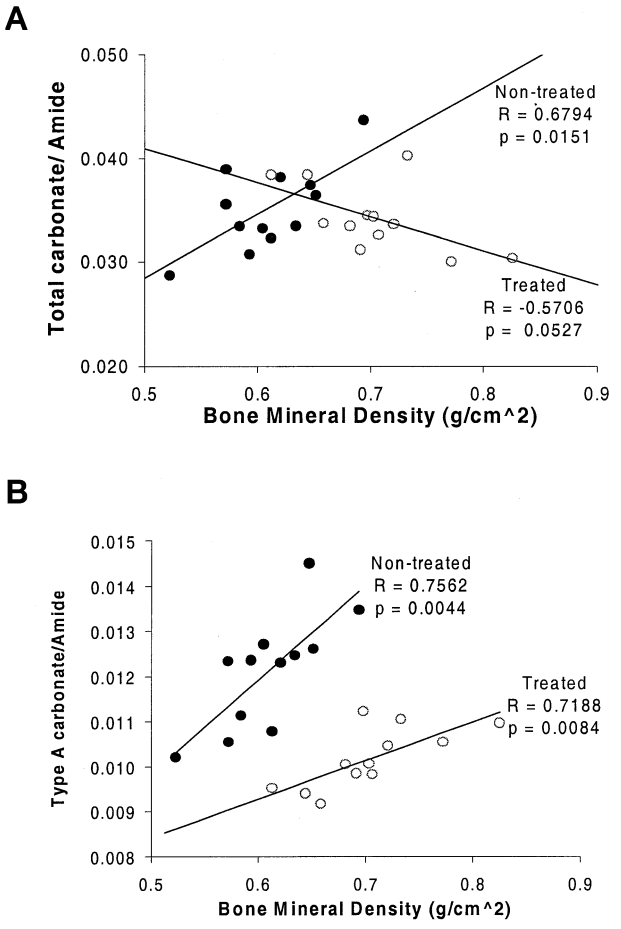

Figure 3. Whole body bone mineral density using DXA was correlated with (A) total carbonate ($\nu_2CO_3^{2-}$)/Amide I and (B) type A carbonate/Amide I. Pearson's correlation coefficient (R) and p value were calculated and are shown in each plot.

Correlation of Mineral-to-Matrix Ratios With Bone Mineral Density

No correlation was found between $\nu_4PO_4^{3-}$/Amide I and bone mineral content (BMC) or density (BMD) in any of the sites among any of the treatment groups (data not shown). Conversely, **Figure 3**A illustrates the correlations of $\nu_2CO_3^{2-}$/Amide I with whole body bone mineral density (WBBMD). The $\nu_2CO_3^{2-}$/Amide I ratio positively correlated with whole body bone mineral density and whole body bone mineral content ($R = 0.679$, $p < 0.05$) for both ovx and sham groups, but not in the two treated groups, which showed a weak negative or no correlation. Figure 3B shows positive correlations between type A carbonate/Amide I and whole body bone mineral density (WMBMD) in both treated and nontreated groups. Correlations were significant between type A carbonate and whole body bone mineral content (WBBMC). BMC and BMD measurements of proximal tibiae and lumbar vertebrae (L2–4) also correlated with type A carbonate/Amide I (**Table 1**).

Discussion

The results presented herein demonstrate that the average phosphate/matrix ratio is indistinguishable between normal and osteopenic bone, and is not altered by treatment with nandrolone decanoate. These findings are consistent with results obtained by Bohic et al.[3] using global spectroscopy and biochemical methods in a rat model of osteoporosis. In contrast, a recent FT-IR study of lumbar vertebrae from similar monkeys demonstrated a lowered phosphate/Amide I ratio in the trabecular bone after ovariectomy.[13] This discrepancy may have been due to differences in tissue sampling. In the present study, a large number of spectra (400–900) were randomly collected from a larger number of animals,[5–7] minimizing the effect of variations in bone composition at distinct locations within an osteon.[21,25] In the previous study, the small number of spectra (15 per animal) and animals (3 per control group) were not sufficient to demonstrate a statistical significant difference.

The results of the present study, however, do not exclude the possibility of an increase in one type of phosphate (e.g., apatitic phosphate) with a simultaneous decrease in another (e.g., labile phosphate), with the total phosphate remaining unchanged. This hypothesis was examined by studying the individual components of $\nu_4PO_4^{3-}$. By curve-fitting the $\nu_4PO_4^{3-}$ contour, we observed a significant increase in the amount of acid phosphate (HPO_4^{2-}/total phosphate) in the cortical and subchondral bone, but not the trabecular bone, of the ovx animals. Acid phosphate has been shown to be abundant in young bone, and the content decreases as bone matures.[32] Thus, high acid phosphate content in the bone of ovx animals suggests that the mineral apatite in these animals is similar to that of young bone. Most likely this related to increased bone turnover occurring after ovariectomy, and has been supported by morphometric studies done on these animals.[17] The mineral apposition rate (MAR) in the periosteonal and osteonal regions of the cortical bone[17] of the second lumbar vertebra and in the subchondral bone (unpublished data) of the proximal tibia increased significantly after ovariectomy, whereas there were no significant differences in the mineral apposition rate in the trabecular bone of the second lumbar vertebrae.[17]

Unlike the phosphate/matrix ratio, the $\nu_2CO_3^{2-}$/Amide I ratio indicates a significant decrease in carbonate content after ovariectomy in the cortical and subchondral bone regions. This de-

Table 1. Whole body, (WB) tibial bone, and L2–4 bone mineral content (BMC) and bone mineral density (BMD) measurements as correlated with $\nu_2CO_3^{2-}$/Amide I and type A carbonate/Amide I by Pearson's correlation coefficient (R)

	$\nu_2CO_3^{2-}$/Amide I	Type A carbonate/Amide I
WBBMC		
Nontreated	0.6240[a]	0.5997[a]
Treated	−0.5635	0.6539[a]
WBBMD		
Nontreated	0.6794[a]	0.7599[a]
Treated	−0.5706	0.7157[a]
L2–4 BMC		
Nontreated	0.7270[a]	0.5302
Treated	−0.5068	0.6462[a]
L2–4 BMD		
Nontreated	0.6404[a]	0.7134[a]
Treated	−0.4472	0.6174[a]
Tibial BMC		
Nontreated	0.5604	0.5539
Treated	−0.3277	0.4350
Tibial BMD		
Nontreated	0.5050	0.5730
Treated	−0.6574[a]	0.2563

Treated and nontreated groups presented separately for each set of measurements.
[a]Statistically significant at $p < 0.05$.

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crease in carbonate content is in sharp contrast to the abnormally high carbonate content in the subchondral bone of monkeys with severe osteoarthritis.[22] As bone ages, the carbonate content increases.[28] Thus, the lower carbonate content in the bone of the ovx animals supports the conclusion from the acid phosphate content result that the bone is more newly formed in these animals. The trabecular bone region had no change in carbonate content with ovariectomy, similar to the acid phosphate results and concurring with a recent study on ovariectomized rats in which Bohic et al. also demonstrated, using FT-IR spectroscopy and microspectroscopy, that the carbonate content in trabecular bone was unaffected by ovariectomy.[3] Although chemical analysis of the whole ovx rat vertebrae demonstrated a decrease in overall carbonate content, this is most likely due to the fact that the samples examined contained areas of cortical bone, which would have contributed to a decrease in carbonate content. By curve-fitting the $\nu_2 CO_3^{2-}$ band, we found that the decrease in total carbonate in the ovx group at cortical and subchondral bone regions was attributed to the loss of labile (surface) carbonate (Figure 2A). These findings are consistent with a similar study performed on ovariectomized rats.[3]

Although the nature of the apatitic carbonate (i.e., types A and B) was not affected by ovariectomy in our study, the two nandrolone-treated groups had significantly lower type A/type B carbonate ratios than the two untreated groups (Figure 2B). Because the overall carbonate/matrix ratio increases with nandrolone treatment, this observation suggests that nandrolone treatment results in an increased formation of apatite with carbonate occupying the phosphate position of hydroxyapatite (type B carbonate). Furthermore, treated animals showed greater spatial as well as intersubject variations in their labile carbonate, possibly explained by a nonuniform drug response at different anatomical regions (i.e., drug diffusion rate and selective uptake), which has been documented previously, albeit at a macroscopic level.[8,15,33] Taken in concert, these findings suggest that ovariectomy results in a decrease of carbonate content in the subchondral and cortical regions without alteration of the ratio between the amount of type A and type B carbonate. Nandrolone decanoate treatment restores the total carbonate content to levels almost as high as those of sham controls by introducing more type B carbonate.

Bone mineral density (BMD) is significantly decreased with ovariectomy, and is restored with nandrolone treatment.[17] To determine how bone composition may be related to this important disease marker, we correlated our FT-IR data with bone mineral density measurements from the same animals. We found that, for the sham and ovx animals, there was no correlation between BMD and phosphate/matrix ratio (measured at several anatomical sites), but there was a positive correlation between BMD and carbonate/matrix ratio. The latter finding is consistent with earlier evidence that the carbonate content in bone increases as bone ages and mineralization increases.[28] However, we did not observe a correlation between BMD and carbonate/matrix ratio in the nandrolone-treated animals (Figure 3A). The nature of the different correlations between treated and nontreated animals was investigated by plotting the type A carbonate/Amide I ratio vs. BMD (Figure 3B). Positive correlations were seen in both the treated and nontreated groups with similar strength ($R =$ 0.759 and 0.715, $p < 0.05$), indicating that, in general, high BMD values in bone are also associated with increased type A carbonate content in bone. However, when the type A carbonate/Amide I ratios of all four treatment groups were plotted together against bone mineral density, the correlations were lost because the treated groups had a flatter slope compared with the nontreated groups. This result is evidence that, although a correlation was observed between BMD and mineral stoichiometry, they are not necessarily dependent variables. For example, BMD is affected by bone porosity, whereas FT-IR microspectroscopy is not. Thus, changes in BMD with treatment may also occur from altered bone porosity, independent of altered mineral/matrix ratios or stoichiometry. However, mineral stoichiometry may also be directly related to altered BMD; for example, when bivalent CO_3^{2-} ions substitute into the hydroxyl position in hydroxyapatite (type A carbonate), the resulting net gain of a negative charge will require the addition of cation (i.e., a calcium ion) to maintain electroneutrality. Thus, type A carbonate substitutions into the apatite lattice could increase the "true density" of the bone. Although BMD and the "true density are not equivalent (because BMD takes into account factors such as porosity), it is possible that an increased "true density" of the bone can also result in an increase in BMD.

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Fertility control of rodent pests

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Abstract

Ricefield rats (*Rattus argentiventer*) in south-east Asian rice fields and house mice (*Mus domesticus*) in Australian grain fields are major pest species. They cause damage before and after harvest and carry zoonotic diseases. For both species, management techniques have been pursued using the approach of immunocontraceptive vaccination. We review results from a series of enclosure and field studies conducted with these species to assess the effects of fertility control in small rodents. In the experiments, fertility control was simulated by tubal ligation, ovariectomy or progesterone treatment. A once-off sterilisation of 50–75% of enclosed founder females considerably reduced reproductive output of ricefield rat populations until the end of the reproductive period. In house mice, similar success was achieved when a sterility level of 67% of female founders and offspring was maintained. Repeated antifertility treatments are required because of the much longer breeding period of house mice versus ricefield rats. Comparing the results of enclosure trials with the outcome of simulation models suggests that partial compensation of treatment effects can occur through enhanced reproduction of the remaining fertile females and improved survival of juveniles. However, such compensatory effects as well as behavioural consequences of sterility in field populations are not likely to prevent the management effect at the population level. The challenge for effective fertility control of small rodents in the field is the wide-scale delivery of an antifertility treatment to founders at the beginning of the breeding season and to fertile immigrants that are recruited into the population, which otherwise contribute to the reproductive output at the population level. Future research efforts should focus on species-specific techniques and on agents that can be effectively delivered via bait.

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Laboratory Investigations

Decreased Bone Mass and Strength in Ovariectomized Cynomolgus Monkeys (*Macaca fascicularis*)

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Abstract. Agents for prevention or treatment of osteoporosis must now be tested in a large animal species that exhibits bone remodeling. Ovariectomized, nonhuman primates provide one such model, and they consistently develop osteopenia accompanied by high bone turnover rates. The goal of this study was to further characterize this model, and particularly to determine the effect of ovariectomy on bone strength in vertebrae and femoral necks. Longitudinal evaluations of spinal bone mass and serum markers of bone turnover were performed in 19 sham-ovariectomized (SHAM) and 18 ovariectomized (OVX), domestically reared cynomolgus monkeys, aged >9 years. OVX monkeys lost bone relative to both baseline values and SHAM controls. Serum markers of bone turnover were increased by OVX. After 72 weeks, both vertebral bone compressive strength and femoral neck breaking strength were significantly decreased in OVX animals compared with SHAM. Ovariectomized cynomolgus monkeys, like postmenopausal women, develop accelerated bone loss, increased bone turnover, and reduced bone strength, and provide a suitable large animal model for efficacy studies with agents for prevention or treatment of osteoporosis.

Key words: Osteopenia — Bone density — Nonhuman primates — Osteoporosis — Bone biomechanics.

Recent recommendations and draft guidelines for drug registration require that agents for prevention or treatment of osteoporosis be tested in the ovariectomized rat model and in one larger remodeling species [1, 2]. The primary purpose of these studies was to provide direct evaluation of bone quality, because validated techniques for noninvasive assessment of bone strength in humans are not currently available. The rat model has been extensively validated, and like postmenopausal women, develops high bone turnover rates and osteopenia after ovariectomy. Relatively few studies of the effect of ovariectomy have been done in the larger species, which include dogs, pigs, sheep, ferrets, and nonhuman primates. Consequently, there is little information available to guide selection of species or experimental design for regulatory studies.

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Several studies in dogs were reviewed, with the conclusion that mild osteopenia develops after ovariectomy, but the bone dynamic changes may not model those occurring in postmenopausal women [3]. Ovariectomized, moderately calcium-restricted minipigs developed spinal osteopenia, cancellous microstructural changes, and reduced normalized maximum compressive load of lumbar vertebral cancellous bone, but no increase in bone turnover rates was observed at 6 months after ovariectomy [4]. Sheep developed spinal osteopenia after ovariectomy, but the effect was dependent on both site evaluated [5] and on the time of year at ovariectomy [6]. Preliminary data from the ferret suggest that they may be a useful model [7].

More studies have been done in nonhuman primates than in any other species except rats and mice, and those studies have consistently demonstrated development of osteopenia accompanied by high bone turnover rates after ovariectomy. In monkeys ovariectomized for 2 years, spinal osteopenia ranged from 11% to 15% lower mean bone mass in ovariectomized animals than in intact animals [8–10]. Bone turnover rates were increased for up to 2 years in ovariectomized monkeys as evidenced by increased serum and urine markers [8–11] and increased bone formation rates measured histomorphometrically [8, 9, 11–13].

The goal of this study was to further characterize the nonhuman primate model of estrogen deficiency bone loss, and particularly to determine the effect of ovariectomy on bone strength by mechanical testing of vertebrae and femoral necks. The intent was both to further validate the model, and to provide information to guide experimental design such as appropriate sample size, experimental conditions, and duration. The experiment was designed to provide favorable conditions for bone loss by selection of older female animals and provision of a diet lower in calcium than usually provided to captive monkeys (but still comparable to human calcium intake). Bone mass data for the first year of this study have been reported previously [14].

Materials and Methods

Animals and Experimental Design

From a breeding colony of domestically reared cynomolgus monkeys, 48 colony-born animals aged 9–15 years and 5 domestically reared, imported animals housed in the colony at least 7 years, sexually mature upon importation, and estimated at 11–15 years of

age were selected. They were housed in one-third acre outdoor pens containing up to 25 animals. Although the pens usually provide abundant space, the animals cannot be allowed outside when the temperature drops below 40°F, and the indoor pen size is limited (9′ × 12′ × 12′). In the case of this experiment, formation of new social groups in these pens coincided with the onset of cold weather. This combination of relative confinement and social upheaval resulted in weight loss early in the experiment.

The 48 animals were assigned to one of two groups with equivalent group means and variances for body weight and spinal bone mass. Twenty-four animals were bilaterally ovariectomized (OVX), and the remainder were sham-ovariectomized (SHAM). All surgeries were done under surgical anesthesia; SHAM animals had both ovaries exteriorized and replaced. One animal designated OVX was eliminated from the experiment when endometriosis was diagnosed on laparotomy. Two SHAM and three OVX animals died due to fight trauma during formation of the social groups, and two SHAM and one OVX animal died subsequently. One animal from each group was excluded from final analysis due to obesity (terminal body weights $\geqslant$7.12 kg relative to overall mean ± SD for all animals surviving at end of study of 4.23 ± 1.21 kg). Thus, there was a final total of 18 OVX and 19 SHAM animals.

All animals were fed purified diets containing 0.8% Ca and 0.7% P initially, switching to a diet containing 0.14% Ca and 0.14% P at approximately 3 months (mean: 11.7 weeks, range: 9.0–14.7 weeks) after OVX or SHAM and remaining on this diet for the rest of the experiment. All animals were necropsied for collection of tissues 69–76 weeks (mean: 72 weeks) after OVX or SHAM. Some data from this experiment have been reported previously [14].

Dual Energy X-Ray Absorptiometry (DXA)

Bone mineral content and density of lumbar vertebrae 2-4 (LVBMC, LVBMD) were measured using a Norland XR26 DXA [15] before and at 17, 34, 47, and 72 (mean) weeks after OVX or SHAM (maximum deviation from mean time at any timepoint was 5 weeks). Precision of the measurements was determined by measuring one adult cynomolgus monkey six times in 1 day with repositioning, and 6 times at weekly intervals. The coefficients of variation (CV) for LVBMC and LVBMD on the same day were 1.7% and 1.3%, respectively, and at weekly intervals were 2.2% and 2.1%.

Serum and Urine Biomarkers

Serum was collected at SHAM or OVX and at 10, 23, 37, 52, and 72 (mean) weeks after OVX or SHAM (maximum deviation from mean time at any timepoint was 4 weeks). Serum total alkaline phosphatase and tartrate-resistant acid phosphatase were assayed using standard reagents and methods for the COBAS FARA analyzer (Roche Diagnostic Systems, Inc., Branchburg, NJ). Serum bone-Gla protein (osteocalcin) was assayed by radioimmunoassay using bovine standards (Diagnostic Systems Laboratories, Inc., Webster, TX).

Ash Weights

Lumbar vertebra 1 (LV1) was removed at necropsy, cleaned of soft tissue, and frozen at −20°C pending further analysis. The posterior elements were removed by cutting the neural arches at their attachment to the body using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL). The anterior and posterior portions were ashed at 900°C overnight (or until constant weight was obtained) and then weighed.

Mechanical Testing of Bone

Lumbar vertebrae 3 (LV3) and 4 (LV4) and the right proximal femur were removed at necropsy, frozen at −20°C, and shipped on dry ice from Bowman Gray School of Medicine to Indiana University Medical Center for mechanical testing. The specimens were kept at −20°C up to the time of testing.

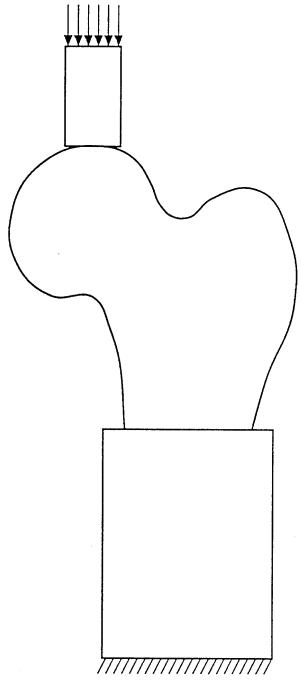

Fig. 1. Diagram of mechanical loading for femoral neck strength tests. The femoral shaft was mounted vertically in a chuck and a downward force was applied on the femoral head to cause fracture.

Immediately before mechanical testing, the vertebral specimens were thawed, cleaned of soft tissue, and the posterior elements were removed using a low-speed diamond wafering saw (Isomet, Buehler). The cranial and caudal ends of each vertebral body were removed using the wafering saw to obtain a 6–8 mm-thick specimen with two parallel surfaces. Width in the medial-lateral (a) and anterior-posterior (b) directions and thickness were measured using digital calipers. Cross-sectional areas of the vertebrae were estimated from the medial-lateral and anterior-posterior widths using the formula area $= \pi ab/4$. The specimens then were mechanically tested in compression between pivoting platens in a 37°C saline bath. Loading was applied using a servo-hydraulic testing machine (MTS 810, MTS, Minneapolis, MN) at a rate of 50 N/second until failure occurred. The maximum load at failure and stiffness were measured from the load-displacement curve using a commercial software package (Sigmascan, Jandel Scientific, San Rafael, CA) and the toughness (energy to failure) was measured as the area under the stress-strain curve [16]. Mean values for LV3 and LV4 from each animal were calculated and used for statistical analysis.

The femoral specimens were thawed and cleaned of soft tissue before testing. The femoral shaft was mounted vertically in a chuck, and a downward force was applied on the femoral head to cause fracture (Fig. 1). Force was applied using an MTS 810 servo-hydraulic machine at a stroke rate of 1 mm/second. All tests were done at room temperature (approximately 23°C). The maximum load at failure was measured from the load-displacement curve using Sigmascan.

In five animals (one OVX and four SHAM), the femoral specimens broke just proximal to the gripping chuck, so femoral neck strength could not be calculated.

Statistical Analysis

All data were tested for normality and equality of variances and transformed if necessary before further analysis. Longitudinal data (body weight, DXA, bone biomarkers) were analyzed by repeated measures analysis of covariance (ANCOVA), where baseline data were used as covariates and multiple post-baseline data were re-

Table 1. Baseline data from SHAM and OVX animals

	SHAM		OVX		
	Mean ± SEM	n	Mean ± SEM	n	*P*
Age (year)	12.3 ± 0.3	19	12.0 ± 0.4	18	0.59
Body weight (kg)	4.97 ± 0.23	19	4.94 ± 0.30	18	0.94
Bone mass					
LV2-4 BMC (g)	6.06 ± 0.17	19	6.25 ± 0.22	18	0.51
LV2-4 BMD (g/cm^2)	0.60 ± 0.01	19	0.61 ± 0.02	18	0.45
Serum measurements					
BGP (ng/ml)	6.13 ± 0.78	19	8.01 ± 0.95	17	0.13
ALP (U/liter)	193 ± 15	19	222 ± 19	17	0.24
TRAP (U/liter)	4.71 ± 0.19	19	5.05 ± 0.35	17	0.39

ALP = alkaline phosphatase; BGP = bone-Gla protein; BMC = bone mineral content; BMD = bone mineral density; TRAP = tartrate-resistant acid phosphatase

peated measures. For these analyses, the significance (*P*) of the group effect (OVX versus SHAM), time effect, and group X time interaction are reported. Terminal data (bone ash weights, mechanical data) were analyzed using Student's *t*-test, or in some cases by ANCOVA, using appropriate baseline data as indicated in the results. All tests were 2-tailed. Significance values ≥ 0.1 are reported as not significant (NS) and values <0.1 are indicated in the results. All data are presented as means ± SEM except as indicated. Statistical power calculations were performed using an iterative method [17], with $\alpha = 0.05$ and $\beta = 0.2$.

Statistical Power and Sample Size

Statistical power calculations were done to determine sample sizes required to detect the observed differences in bone mass and strength between intact and OVX monkeys. For bone mass, a difference of 12%, with a CV of 17% (the averages for a number of experiments) were used, whereas in the present experiment, there was a mean 15% difference in fracture force for lumbar vertebrae and femoral neck, with a mean CV of 16%. For good statistical power ($\beta = 0.2$), a sample size of 29 is required to detect bone mass differences, and 19 to detect differences in bone strength.

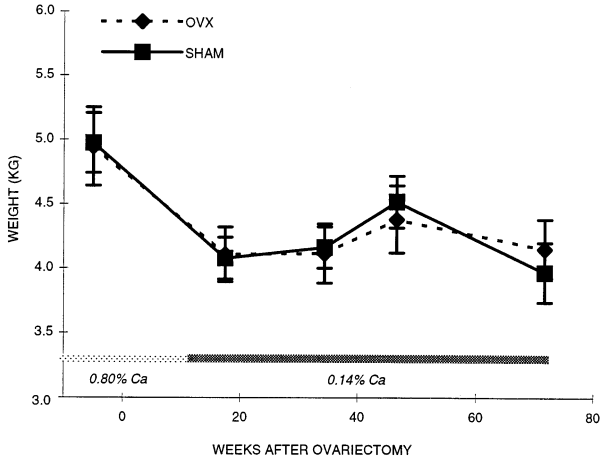

Fig. 2. Body weight (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8% or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) not significant (NS); effect of time $P < 0.0001$; interaction effect not significant.

Results

Baseline Values

There were no significant differences between groups for any variables at baseline (Table 1).

Body Weight

There was no significant effect of OVX on body weight, but both groups changed in body weight over time (Fig. 2, Table 2). Both groups lost weight during the first 4 months (SHAM group, −16.71 ± 2.44%; OVX group, −15.17 ± 2.59%), but subsequently stabilized.

Table 2. Results from analyses of covariance[a]

	Group	Time	Group x time
Body weight	NS	<0.0001	NS
Bone mass			
LV2-4 BMC	<0.0001	<0.05	<0.05
LV2-4 BMD	<0.0001	<0.0001	<0.05
Serum measurements			
BGP	<0.0005	NS	<0.01
Alkaline phosphatase	<0.1	<0.0001	<0.05
TRAP	<0.1	<0.0001	NS

[a] *P*-values are given for the effects of group, time, and interaction between group and time

DXA and Vertebral Ash Weight

Ovariectomy decreased spinal BMC (Fig. 3, Table 2) and BMD (Fig. 4, Table 2). There was a slight drop in spinal BMC and BMD in SHAM monkeys during the experiment (final BMC relative to baseline = −2.14 ± 1.49%; final BMD = −4.55 ± 0.99%), but OVX monkeys lost more (final BMC = −10.34 ± 1.65%; final BMD = −11.45 ± 1.45%).

Mean ash weight of the anterior portion of LV1 was significantly lower in OVX (633 ± 25 mg) than in SHAM (718 ± 28 mg, $P < 0.05$). Mean ash weight of the posterior

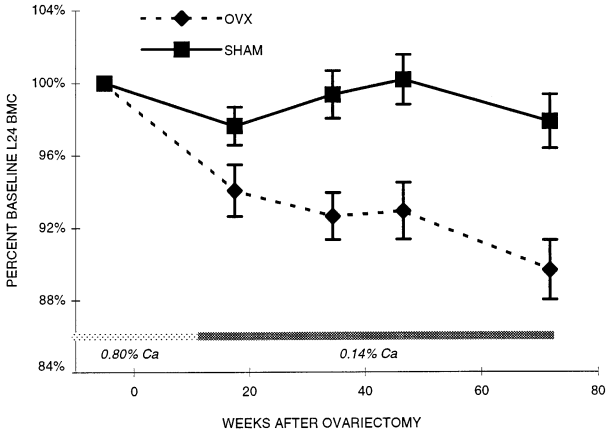

Fig. 3. Lumbar vertebrae 2-4 BMC as percent of baseline value (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8 or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) $P < 0.0001$; effect of time $P < 0.05$; interaction effect $P < 0.05$.

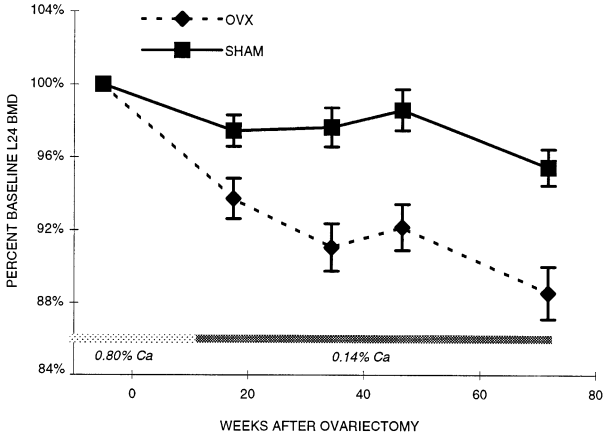

Fig. 4. Lumbar vertebrae 2-4 BMD as percent of baseline value (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8% or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) $P < 0.0001$; effect of time $P < 0.0001$; interaction effect $P < 0.05$.

portion was nonsignificantly lower in the OVX group (1,008 ± 53 mg) than in the SHAM group (1,062 ± 38 mg). The anterior portion of the vertebra contained approximately 40% of the total vertebral ash.

Serum Biomarkers

Serum bone-Gla protein (BGP) was significantly increased by OVX (Fig. 5, Table 2). There was a trend toward increased serum total alkaline phosphatase, with a significant group × time interaction (Fig. 6, Table 2), and serum tartrate-resistant acid phosphatase also tended to be increased (Fig. 7, Table 2). Both alkaline phosphatase and tartrate-resistant acid phosphatase rose in both SHAM and OVX animals after reduction of dietary calcium.

Bone Strength

All parameters of bone strength were reduced in OVX ani-

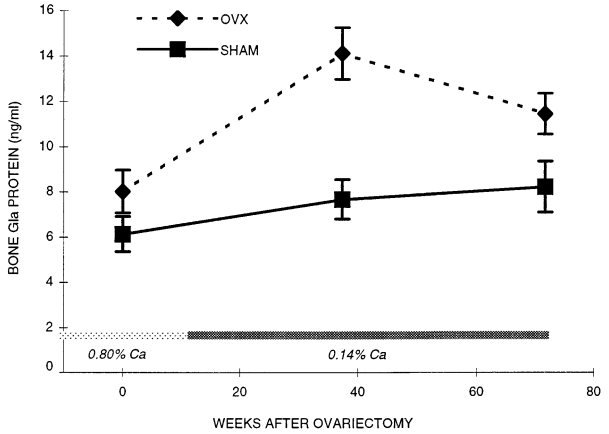

Fig. 5. Serum bone-Gla protein (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8% or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) $P < 0.0005$; effect of time NS; interaction effect $P < 0.01$.

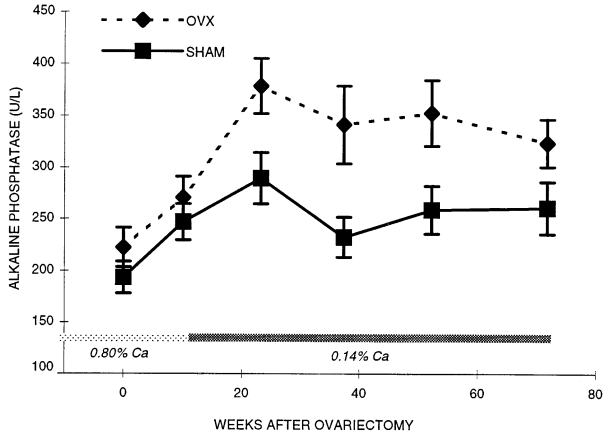

Fig. 6. Serum total alkaline phosphatase activity (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8% or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) $P < 0.1$; effect of time $P < 0.0001$; interaction effect $P < 0.05$.

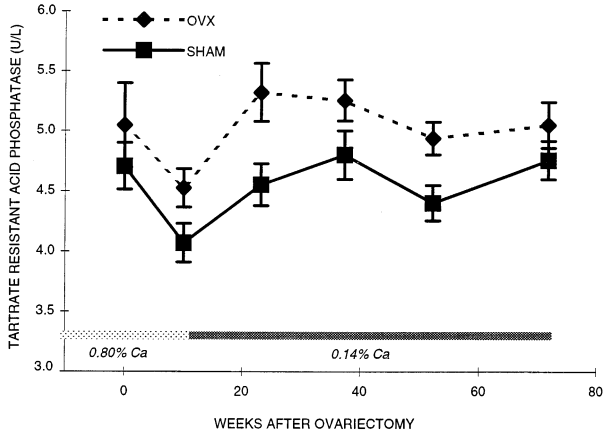

Fig. 7. Serum tartrate-resistant acid phosphatase activity (mean ± SEM) before, and throughout 72 weeks after SHAM or OVX. Horizontal bar represents time during which 0.8% or 0.14% Ca diets were fed. Effect of treatment (SHAM versus OVX) $P < 0.1$; effect of time $P < 0.0001$; interaction effect NS.

Table 3. Bone strength in SHAM and OVX animals 18 months after ovariectomy

	SHAM (n = 19)	OVX (n = 18)	*P*
Femur neck[a]			
Fracture force (N)	1386 ± 51	1226 ± 43	<0.05
LV 3-4 (mean)			
Fracture force (N)	2587 ± 82	2127 ± 75	<0.001
Stiffness (N/mm)	10018 ± 507	8669 ± 491	<0.1
Toughness (MPa)	1.30 ± 0.12	0.947 ± 0.074	<0.05

[a] For this parameter, n = 15 in Sham group and n = 17 in OVX group

mals (Table 3). Force required to fracture was significantly lower in OVX than in SHAM animals for both femoral neck and lumbar vertebrae. Analysis of these data covarying for BMC at baseline of the same vertebra or of whole body did not affect the results of these analyses. Vertebral fracture force correlated with BMC and BMD of the same vertebrae measured just prior to necropsy ($r = 0.5$, $P < 0.01$ for both). Vertebral toughness correlated with BMC ($r = 0.4$, $P < 0.05$) and BMD ($r = 0.5$, $P < 0.01$), but vertebral stiffness did not correlate with BMC or BMD.

Discussion

Following natural or surgical menopause, women experience a period of rapid bone loss, accompanied by high bone turnover rates [18–21]. This phenomenon precedes the development of osteoporotic fractures, which can be prevented by estrogen therapy [22]. Thus, estrogen deficiency in women causes accelerated bone loss, increased bone turnover, and reduced bone strength. The results of the present study demonstrate that the OVX cynomolgus monkey models each of these features of postmenopausal osteoporosis.

This is the first study to demonstrate decreased vertebral and femoral neck strength in OVX monkeys. There have been few previous evaluations of bone strength in OVX monkeys. Elastic modulus, shear modulus, and failure shear stress of whole tibiae were significantly lower in ovariectomized than in intact animals, but trabecular bone from the femoral head of the same animals showed no differences between intact and OVX monkeys [23]. In a previous study, the mechanical properties of femoral diaphysis, cortical beam specimens, femoral neck trabecular cylinders, vertebrae, and vertebral trabecular cylinders were not different between intact and OVX baboons, although the sample size (seven per group) was small [8].

This also is the first study to demonstrate unequivocal bone loss relative to baseline in OVX animals. In previous longitudinal studies, relative spinal osteopenia was due at least in part to failure to gain bone in OVX animals [8, 10, 13, 24], with a component of absolute spinal osteopenia in two studies [13, 24]. Based on this and other experiments, we believe that absolute bone loss after OVX occurs only in animals that have reached stable bone mass prior to OVX. Stable bone mass is partly a function of age. Growth plates close in cynomolgus monkeys at approximately 6 years, whereas peak bone mass is achieved at 9 years in domestically reared animals [25]. However, wild-caught animals with radiographic and dental characteristics consistent with ages >9 years are osteopenic relative to domestically reared monkeys, and gain bone for approximately 1 year after importation [14]. We hypothesize that if OVX were performed after 1 year of captivity, absolute bone loss would occur. Such studies are rarely performed, but preliminary data from one experiment in our laboratory in which animals were captive for 2 years before OVX do show significant bone loss. The animals in the present study were known to have reached stable bone mass prior to OVX, being both domestically reared and >9 years of age.

On a caloric basis, we estimate that 0.14% Ca fed to a monkey is approximately equivalent to 750 mg of Ca consumed by a woman, which implies that normal laboratory diets grossly oversupplement with Ca. The relatively low dietary Ca fed during most of the present study does not appear to have contributed significantly to the rate of bone loss, although the rise in serum alkaline phosphatase and TRAP after the switch to low Ca is suggestive of an increase in turnover rate. Rapid bone loss occurred in OVX animals during the first 4 months, during which time 0.8% Ca was fed. Conversely, SHAM animals maintained or slightly gained bone after initiation of the 0.14% Ca diet. Most previous studies of bone loss in OVX nonhuman primates have used dietary Ca levels of 0.6% or greater. The single exception is a study in which 0.1% Ca was fed to intact and ''chemically ovariectomized'' monkeys; in that study, intact animals continued to gain bone on the low Ca diet [24]. Therefore, it appears that Ca levels of 0.1% or higher permit bone gain in intact animals, and that levels as high as 0.8% do not prevent bone loss in OVX animals. It also has previously been shown that 0.14% Ca supports normal skeletal development in young baboons [26].

The findings in this study provide some additional information pertinent to the use of this model. Careful attention must be paid to the social situation of nonhuman primates. Though it is highly desirable to house monkeys in pairs or groups, group housing can also engender problems. All-female groups in particular tend to fight upon initial group formation, and high morbidity and mortality can result. Also, as in this study, social upheaval can result in loss of body weight, which can in turn potentially confound a study. Steps should be taken to minimize fighting during formation of social groups, including provision of adequate space and of perches, barrels, and other large objects which permit vertical and/or visual separation of animals within the cage. We have also found that formation of pairs rarely results in conflict. Fighting is substantially reduced if larger groups are formed from pairs which have been co-housed for a month or longer.

The statistical power calculations suggest that a sample size of 19 could be used for future experiments where bone strength is the primary outcome. A larger sample is required to detect differences in bone mass (in a single timepoint comparison), because the effect of OVX is less dramatic and the CV is slightly higher. For the time being, we suggest

using the more conservative estimate of sample size required to detect the observed differences in bone mass, until more data are available to confirm the magnitude of the effect on bone strength.

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Ad libitum feeding following ovariectomy in female Beagle dogs: effect on maintenance energy requirement and on blood metabolites

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Summary

The aim of this study was (i) to evaluate the effect of ovariectomy on daily energy requirement in Beagle dogs, and (ii) to evaluate the effects of *ad libitum* feeding with a high energy diet on energy consumption, body weight gain and blood metabolites in these spayed dogs. Four young adult female Beagle dogs were used. Ovariectomy induced a significant decrease of daily energy requirement in dogs. *Ad libitum* feeding, initiated 6 months after spaying, induced a significant increase in consumption in spayed dogs. This overconsumption was greatest during the first month of *ad libitum* feeding but continued for the entire 4 months of this period. When fed *ad libitum*, dogs gained excess body weight without significant effect on plasma cholesterol, triglycerides, glucose and insulin, at short term. These data suggest that energy intake should be strictly controlled to avoid excess weight gain in spayed dogs.

Introduction

Obesity and excess body weight (BW) are estimated to affect approximately 25–44% of dogs receiving veterinary cares in Western countries (Lewis, 1978; Norris and Beaver, 1993). It is the more common nutritional disease. It is agreed that a lot of factors contribute in the development of obesity. Obesity is more frequent in females than in males (Krook et al., 1960), except in older dogs (Lewis, 1978) and is nearly twice as high in spayed females as in the total population (Anderson, 1973; Edney and Smith, 1986).

Ovariohysterectomy can result in significant weight gain after surgery in bitches fed *ad libitum* (Houpt et al., 1979) whereas ovariectomized bitches fed a fixed amount of food and exercised regularly gained no weight (Le Roux, 1983). The aim of this study was to determine the daily energy requirement to maintain stable BW after ovariectomy in adult Beagle dogs (experiment 1) and to determine the effect of *ad libitum* feeding on weight gain and blood metabolites in spayed female Beagle dogs (experiment 2).

Materials and methods

Animals and experimental set-up

Experiment 1

Four young adult 2-year-old female Beagle dogs were ovariectomized. The four dogs were maintained together in their usual kennel and were fed individually. The size of the kennel

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was 4 m × 3 m. The BW at the day of sterilization was considered as the optimal BW, based on body condition scoring in a 9 points scale (optimal 5/9) (Laflamme et al., 1994). Food consumption and BW were determined weekly during 32 weeks, 6 weeks before surgery (period I) and 26 weeks after surgery (period II). After surgery, the amount fed was reassessed each week and adjusted if necessary to maintain optimal BW by a reduction or an increase of 5% of the amounts offered. Dogs received a maintenance diet (Premium Croc; Royal Canin, Aimargues, France, crude protein 24.0%, fat 16.1%, calculated metabolizable energy 15 605 kJ/kg as fed) during the experiment. Metabolizable energy was calculated using modified Atwater factors (NRC, 1985). The room temperature was maintained at (mean ± standard error of the mean) 15.1 ± 0.4 °C.

Experiment 2

The same four spayed female Beagle dogs received a high-energy commercial dog food (Energy Croc; Royal Canin, Aimargues, France, crude protein 30%, fat 20%, calculated metabolizable energy 16 284 kJ/kg as fed) during 16 weeks (period III) after a transition period of 3 weeks. During the transition period, dogs received high energy food at maintenance level. During period III, the amount offered was twice the amount that maintained BW. Dogs were fed once a day. Food left uneaten after 1 h was collected and weighted. Plasma cholesterol, triglycerides, glucose and insulin were measured at the end of the transition period between periods II and III and then every 2 weeks during period III (cholesterol reagents, triglycerides reagents and glucose reagents; Technicon RA, Bayer Diagnostics, Puteaux Cedex, France; INS-IRMA; Biosource Europe, Nivelles, Belgium). Period III could be divided into four subperiods of 4 weeks. This division of the data was performed to facilitate a quantitative comparison of the changes in the measured parameters as the trial progressed. For statistical analysis, each subperiod was compared with mean values of period II for energy consumption and BW or with values at the end of the transition period between periods II and III for cholesterol, triglycerides, glucose and insulin.

Statistical analysis

All values are expressed as mean ± standard error of the mean. Data were normally distributed and were submitted to a one-way analysis of variance (anova) for repeated measurements with an autoregressive structure of first order (SAS Proc Mixed; SAS Inst. Inc., Cary, NC, USA). Differences were considered statistically significant at $p < 0.05$.

Results

Experiment 1

The mean BW of the Beagles at the day of the sterilization was 13.7 ± 0.8 kg. Mean BW during period I was 14.0 ± 0.3 kg. During period II, mean BW was 13.7 ± 0.1 kg which corresponds to optimal BW (Fig. 1). Before ovariectomy, dogs received 720 ± 13 kJ/kg $BW^{0.75}$. After surgery, energy offered had to be significantly decreased to maintain ideal BW. During period II, dogs received 506 ± 8 kJ/kg $BW^{0.75}$, which corresponds in a 30% decrease (Table 1).

Experiment 2

When a high energy food was allowed in double amounts, dogs spontaneously ate significantly more food with mean food amounts corresponding to 761 ± 21 kJ/kg $BW^{0.75}$. During the first 4 weeks of period III, mean energy consumption was 883 ± 38 kJ/kg $BW^{0.75}$ and dogs consumed almost all the proposed food. Then, the ingested amounts

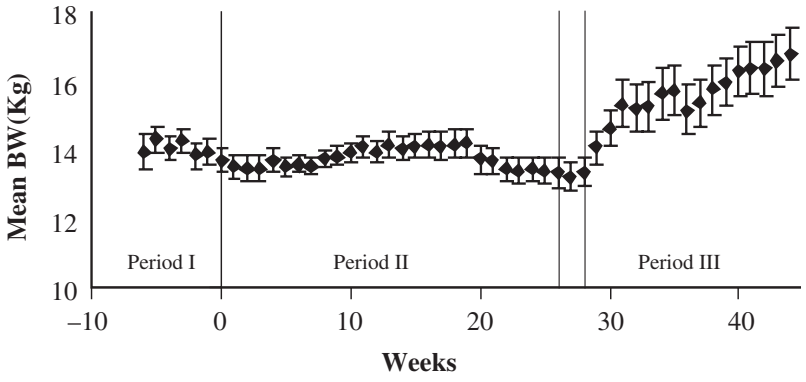

Fig. 1. Evolution of mean body weight (kg) during periods I, II and III.

Table 1. **Mean energy consummations (kJ/kg body weight$^{0.75}$ and kJ) of dogs during periods I, II, III and subperiods III.1, III.2, III.3, III.4**

	Period I	Period II	Period III			
KJ/kg BW$^{0.75}$	720 ± 13	506 ± 8	761 ± 21			
KJ	5209 ± 59	3602 ± 29	5866 ± 96			
P-value		<0.01[1]	<0.01[2]			
			III.1	III.2	III.3	III.4
KJ/kg BW$^{0.75}$			883 ± 38	757 ± 50	736 ± 42	669 ± 29
KJ			6611 ± 138	5728 ± 222	5749 ± 197	5372 ± 176
P-value			<0.01[3]	<0.01[4]	<0.01[5]	<0.01[6]

The p-values give the significant level of differences between periods I and II[1], between periods II and III[2], and between period II and different subperiods of period III, respectively[3,4,5,6]. P-values were the same for kJ/kg body weight$^{0.75}$ and kJ. Values are expressed as mean ± standard error of the mean

decreased spontaneously. Mean energy consumption was 757 ± 50, 736 ± 42 and 669 ± 29 kJ/kg BW$^{0.75}$ for each following subperiod. These increases were all significant when compared with period II. As BW changed during period III, it was interesting to present total intake as well as intake per BW. Statistically, the results were the same. Mean consumption of the dogs was 5866 ± 96 kJ for the whole period III and 6611 ± 138, 5728 ± 222, 5749 ± 197 and 5372 ± 176 kJ for each subperiod of period III. For period II, energy consumption was 5209 ± 59 kJ (Table 1). The BW increased once dogs were fed *ad libitum* (Fig. 1). At the end of period III, mean BW was 16.7 ± 0.8 kg, which represents a significant increase of BW of 22%. All dogs became overweight or obese (body condition score of 7 or 8/9). No significant modification was observed for plasma cholesterol, triglycerides, glucose or insulin during period III (Table 2).

Discussion

Before ovariectomy, dogs received 720 kJ/kg BW$^{0.75}$. It was a high energy intake to maintain BW but dogs were housed together, that could induce a higher activity level. Moreover, temperature was of 15.1 °C, which was not very high. It seems that ovariectomy could induce a significant decrease of daily energy requirement in female Beagle dogs. This

Table 2. **Mean values of plasma cholesterol, triglycerides, glucose and insulin at the end of the transition period between periods II and III and during period III, expressed as mean ± standard error of the mean**

	End of the transition period High energy diet at maintenance level	Period III High energy diet *ad libitum*	Reference values (Kaneko et al., 1997)
Cholesterol (mmol/l)	5.22 ± 0.33	5.07 ± 0.15	3.5–6.99
Triglycerides (mmol/l)	0.42 ± 0.08	0.63 ± 0.05	0.2–1.3
Glucose (mmol/l)	5.92 ± 0.06	5.86 ± 0.11	3.61–6.55
Insulin (pmol/l)	56.10 ± 8.71	85.79 ± 6.09	36–144

decrease was 30%, as approximately observed in cat (Flynn et al., 1996; Harper et al., 2001). The underlying mechanism is unknown, but authors suggest a decrease in physical activity in dog (Le Roux, 1983; Sibley, 1984; Sloth, 1992), which was not confirmed in cat (Flynn et al., 1996).

Ad libitum feeding a high energy diet induced overconsumption in spayed Beagle dogs. Overconsumption was maybe due to a decrease in the oestrogen level in spayed dogs. Indeed, oestrogen has been shown to inhibit food intake in a variety of species (Houpt and Hintz, 1978). However, oestrogen implication is not obvious because more than 6 months run between ovariectomy and *ad libitum* feeding and overconsumption was observed during 16 weeks, which was the length of the study, but was more important the first 4 weeks. A second hypothesis was the inability of spayed dogs to control caloric intake at short term, as observed in cats (Goggin et al., 1993; Flynn et al., 1996). It would have been of great interest to use a control group of intact females fed *ad libitum* with a high energy diet and a spayed group fed *ad libitum* with a maintenance diet to conclude that weight gain is unique to spayed females or to high energy food. Indeed, even if the calorie difference between the maintenance diet and high energy diet was not large, the higher content in protein and lipids in the high energy diet could increase the palatability and thus the consumption. At the end of the study, all dogs became obese. Weight gain was probably a consequence of the overconsumption but the importance of the activity level, the metabolic rate and the efficiency in the utilization of nutrients were not evaluated. In neutered cats, increased food consumption appeared to be a major cause of the weight gain (Flynn et al., 1996; Fettman et al., 1997). Indeed, no effect was observed on activity level (Flynn et al., 1996) or on nutrient digestibility, but a decrease in fasting metabolic rate was observed in spayed female cats (Fettman et al., 1997). It seems that *ad libitum* feeding induced increased plasma triglycerides and insulin concentrations but these increases were not significant. Obesity has been shown to cause insulin resistance in dogs (Mattheeusws et al., 1984). Two phases in the development of obesity have been recognized: an initial growth phase followed by a static phase. During the initial phase, energy intake exceeds utilization and the excess calories are deposited as fat. It is during this phase that the dogs gain excess BW. The static phase begins when excess fat has been deposited. Several separate feedback mechanisms reduce dietary energy intake but BW remains constant in the obese state (Sibley, 1984). Dogs in our study were in the initial phase and it is maybe why we observed no significant effect on the measured blood metabolites. But conclusions are difficult to draw because the number of dogs was very small and moreover blood values of period III were compared with only one value for each dog at the end of the transition period between periods II and III.

In conclusion, a control of the food intake seems necessary to maintain ideal BW after gonadectomy in female dogs. Spayed female dogs seem unable to control food intake, at least at short term, when a high energy food is allowed *ad libitum*. Our data suggest that obesity has no significant consequence at short term on cholesterol, triglycerides, glucose and insulin.

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ORIGINAL ARTICLE

Effect of ovariectomy and *ad libitum* feeding on body composition, thyroid status, ghrelin and leptin plasma concentrations in female dogs*

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Summary

The objective of this study was to evaluate the effects of ovariectomy (i) and *ad libitum* feeding (ii) on energy intake, body weight (BW), body composition, thyroid status, leptin and ghrelin plasma concentrations. Four young adult female Beagle dogs were fed a maintenance diet for 6 weeks prior to ovariectomy, then 6 months after. Food allowance was adjusted in order to maintain optimal BW. Then, a diet slightly higher in energy concentration was fed *ad libitum* for 4 months. The maintenance diet was then fed *ad libitum* for one additional month. The maintenance of optimal BW after ovariectomy required a significant decrease in energy allowance. No increase in fat mass was observed. Ghrelin concentration remained unchanged. During the first month of *ad libitum* feeding, plasma ghrelin concentration and energy intake increased, then they decreased. Mean BW, plasma leptin, thyrotropin (TSH), total triiodothyronine (TT3) and total thyroxine (TT4) concentrations significantly increased over the study. The BW increase was exclusively due to an increase in body fat. In conclusion, energy allowance should be strictly controlled in spayed female dogs. The results suggest that in dogs, thyroid hormones, leptin and ghrelin concentrations change in response to a positive energy balance in an attempt to limit weight gain. However, the significant weight gain shows that this goal was not achieved.

Introduction

Obesity and excess body weight (BW) are estimated to affect approximately 25–44% of dogs receiving veterinary care in Western countries (Norris and Beaver, 1993). Two phases in the development of obesity have been recognized: an initial growth phase followed by a static phase. During the initial phase, energy intake exceeds utilization and the excess energy is deposited as fat. It is agreed that a lot of factor contribute to the development of obesity. Obesity is more frequent in females than in males dogs except in older dog and is nearly twice as high in spayed female than in the total population (Edney and Smith, 1986; Robertson, 2003). Ovariohysterectomy can result in significant weight gain after surgery in bitches fed *ad libitum* (Houpt et al., 1979) whereas ovariectomized bitches fed a fixed amount of food and exercised regularly gained no weight (Le Roux, 1983). The circulating peptides

leptin and ghrelin have actions that include reciprocal effect on appetite-regulating neurons in the hypothalamus. In addition to its growth hormone (GH)-releasing properties, exogenous ghrelin injections stimulate food intake and adiposity in rodents (Tschop et al., 2000; Wren et al., 2001a), in humans (Wren et al., 2001b) and in dogs (Yokoyama et al., 2005). In human as in dog studies, ghrelin was found to decrease in case of obesity (Tschop et al., 2001; Shiiya et al., 2002; Jeusette et al., 2005). Leptin is a protein synthesized and secreted primarily by adipocytes, and the circulating leptin concentration is elevated in obese humans and rodents. Plasma leptin concentration is a good index of adiposity in dogs (Ishioka et al., 2000). The increase in thermogenesis observed after thyroid hormones administration has led to speculate whether thyroid, acting on energy expenditure, could affect BW (Silva, 1995). The aim of this trial was first (i) to study the effects of ovariectomy on energy requirement and body composition and secondly (ii) to study the effects of *ad libitum* feeding on BW, body composition, thyroid hormones, leptin and ghrelin concentrations in plasma.

Materials and methods

Animals and experimental set-up

Four young adult, 2 years of age, female Beagle dogs were used. The four dogs were maintained together in their usual kennel and were fed individually during all the experiment. The size of the kennel was of 4×3 m. Before the study, the dogs were trained to be fed once a day and to finish their meal in 10 min.

The schedule of the experiment is presented in Fig. 1. The study was divided in four phases, one before ovariectomy (P0) and three after ovariectomy (P1, P2 and P3).

During the 6 weeks of P0 and the 6 months of P1, the dogs received, in order to maintain stable BW, a maintenance commercial extruded (dry-type) diet (Premium Croc; Affinity Petcare, Barcelona, Spain) that was formulated to contain 24.0% (wt/wt) crude protein, 16.1% fat, 38% starch, 2.5% crude cellulose, 6% ash, 17.1 kJ/g measured metabolisable energy (MME). The amount fed was reassessed each week and adjusted, if necessary, by 5% reduction of the amount offered. The BW was considered as the optimal BW, based on body condition scoring (BCS) on a 9-point scale (optimal 5/9) (Laflamme et al., 1994).

During P2, the four spayed female Beagle dogs received a high-energy commercial extruded (dry type) diet (Energy croc; Affinity Petcare, Barcelona, Spain) that was formulated to contain 30% crude protein, 20% fat, 18.0 kJ/g MME during 4 months. The amount offered was twice the amount that maintained BW. Dogs were fed once a day and food left uneaten after 90 min was collected and weighed. P2 could be divided into four sub-periods of 1 month. This division of the data was performed to facilitate a quantitative comparison of the changes in the measured parameters as the trial progressed.

In P3, the four dogs were fed *ad libitum* during 90 min the maintenance diet again during 1 month.

Individual food intake was recorded daily and BW weekly. Body compositions were determined at P0, at the end of P1 and P3. Total body water was measured using the isotopic dilution of ^2H_2O. On the day of the measurement, 24-hour food deprived dogs were placed in individual cages for water restriction and body water equilibration 4 h after the tracer injection. Dogs were injected with ^2H_2O (99.9% $^2H/H$, Leman, Saint-Quentin-en-Yvelines, France) sub-cutaneously (1.5 g/kg BW). Blood samples were collected from the cephalic vein before and 4 h after the tracer injection. Deuterium was assayed by Fourier transform infrared spectroscopy. Total body water was estimated from the deuterium pool value. Fat free mass was obtained from total body water divided by 0.744 (Ferrier et al., 2001). The body fat content was further calculated from BW and fat free mass. Fat free mass and fat mass were expressed in kg.

Blood samples for measurement of ghrelin, leptin, total thyroxine (TT4), total triiodothyronine (TT3), reverse triiodothyronine (rT3), free thyroxine (FT4) and thyrotropin (TSH) serum concentrations were taken at the beginning and at the end of P1, at the beginning and the end of P2 and at the end of P3. Blood samples from all the dogs were collected between 9 and 10 AM after a 24-hour fast on tubes

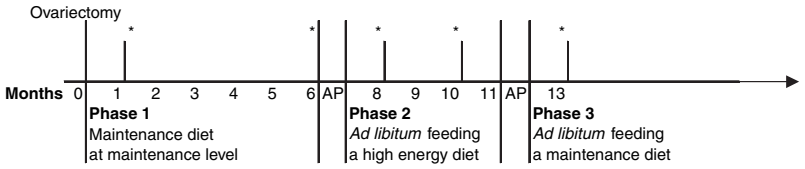

Fig. 1 Schedule of the experiment, indicating the diet fed in each phase and the timing (month) of blood samples (*) in each phase.

with clot activator (thyroid hormones) or EDTA (leptin and ghrelin). Serum or plasma aliquots were frozen at –20 °C (thyroid hormones) or –80 °C (leptin and ghrelin) in plastic tubes until assayed. Total plasma ghrelin concentrations were measured using commercial radio-immuno assay (RIA) human kits performed for dogs (Total ghrelin RIA kit, Linco research, MO, USA). Leptin plasma concentrations were assayed using a canine specific ELISA method validated by Iwase et al. (2000). Determinations of TT4 and TSH serum concentrations were performed through previously validated RIA (Clinical Assays Gammacoat M Total T4 125I RIA Kit, Diasorin, Stillwater, MN, USA; Coat-A-Count canine TSH IRMA, Diagnostic Products, Los Angeles, CA, USA) (Sauve et al., 2003). Reference ranges for TT4 and TSH serum concentrations were 15–67 nmol/l and 0–0.68 ng/ml respectively. Determination of FT4 serum concentrations after equilibrium dialysis (FT4D) was performed using a commercial assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) (Daminet et al., 1999). The reference range for FT4 serum measurements was 6–42 pmol/l. Total T3 concentrations were measured through a validated in-house charcoal separation assay (Panciera, 1994). The reference range for TT3 serum concentrations was 1.0–2.4 nmol/l. Reverse T3 serum concentrations were measured through a validated RIA method (Biodate S.p.A., Rome, Italy) (Center et al., 1984). Reference range for rT3 was 0.41–0.95 nmol/l. All the thyroid assays were performed at the Endocrine Section, Animal Health Diagnostic Laboratory, Michigan State University.

The room temperature was maintained at [mean ± standard error of the mean (SEM)] 59.18 ± 1.57 F (15.1 ± 0.4 °C) during the experiment. Drinking water was provided *ad libitum* during all the experiment. The activity level was not measured. The protocol was approved by the Animal Use and Care Advisory Committee of the University of Liège.

Statistical analysis

All the values are expressed as mean ± SEM. Data were normally distributed and were submitted to an univariate analysis of variance (ANOVA) for repeated measurements. A compound symmetry or an autoregressive structure of first order was assumed to account for the tendency between repeated measures (SAS Proc Mixed). A Pearson's correlation analysis (SAS Proc Corr) was performed on the data. Differences were considered statistically significant at $p < 0.05$.

Results

Results are presented in Tables 1 and 2.

At the beginning of the study (P0), all the parameters were within reference ranges in all four spayed dogs.

BW, body composition and energy intake

The mean BW of the Beagles at the day of the sterilisation was 13.7 ± 0.8 kg. Mean BW during P0 was

Table 1 Mean body weight, energy intake, ghrelin and leptin concentrations in the four spayed female Beagle dogs during phase 1, 2 and 3*

	Body weight (kg)	FFM (kg)	FM (kg)	Energy intake (kJ/kgIBW$^{0.75}$)	Ghrelin (pg/ml)	Leptin (ng/ml)
P0						
Weeks-6-0	14 ± 0.3[a]	ND	ND	807 ± 13[b]	ND	ND
Day 0	13.6 ± 0.8[a]	9.4±0.6[a]	4.2 ± 0.2[b]	ND	ND	ND
P1						
Months 0–6	13.7 ± 0.1[a]	ND	ND	573 ± 13[a]	3513 ± 773[a]	ND
Month 0	13.6 ± 0.8[a]	ND	ND	ND	3480 ± 1220[a]	ND
Month 6	13.6 ± 0.8[a]	10.8 ± 0.4[b]	2.7 ± 0.5[a]	ND	3546 ± 1142[a]	4.8 ± 2.1[a]
P2						
Months 7–11	15.7 ± 0.3[b]	ND	ND	912 ± 13[b]	7222 ± 3184[a]	ND
Month 7	14.8 ± 0.5[a]	ND	ND	1033 ± 21[c]	13400 ± 4666[b]	ND
Month 10	16.7 ± 0.7[b]	ND	ND	820 ± 29[b]	1047 ± 320[a]	10.7 ± 4.4[b]
P3						
Month 12	17.8 ± 0.6[c]	10.9 ± 0.5[b]	6.9 ± 0.8[c]	862 ± 17[b]	867 ± 188[a]	10.9 ± 3.7[b]

FFM, fat free mass; FM, fat mass; IBW, ideal body weight; ND, not determinated.

*Data are expressed as mean ± standard error of the mean.

[a,b,c]Differing letters indicate significant ($p < 0.05$) differences in parameters between the different phases.

Table 2 Mean thyroid hormones and TSH serum concentrations in the four spayed female Beagle dogs during phase 1, 2 and 3*

	Total T4 (nmol/l)	Total T3 (nmol/l)	FT4D (pmol/l)	rT3 (nmol/l)	TSH (ng/ml)
P1					
Month 6	42.5 ± 2.1[a]	1.48 ± 0.06[a]	30.08 ± 2.4[a,b]	0.75 ± 0.04[a]	0.13 ± 0.05[a]
P2					
Month 7	40.3 ± 4.8[a]	1.75 ± 0.12[a,b]	23.5 ± 2.8[a]	0.77 ± 0.93[a]	0.32 ± 0.04[b]
Month 10	51.3 ± 4.7[a]	1.95 ± 0.16[b,c]	27.0 ± 2.8[a,b]	0.81 ± 0.05[a]	0.23 ± 0.02[a,b]
P3					
Month 12	65.8 ± 5.1[b]	2.30 ± 0.15[c]	34.8 ± 5.0[b]	0.88 ± 0.09[a]	0.28 ± 0.07[b]
Mean P2 + P3	52.4 ± 4.0[a]	2.00 ± 0.10[b,c]	28.4 ± 2.4[a]	0.82 ± 0.04[a]	0.28 ± 0.02[b]

T4, thyroxine; T3, triiodothyronine; FT4D, free thyroxine by equilibrium dialysis; rT3, reverse triiodothyronine; TSH, thyrotropin; ND not determinated.

*Data are expressed as mean ± standard error of the mean.

[a,b,c]Differing letters indicate significant ($p < 0.05$) differences in parameters between the different phases.

14.0 ± 0.3 kg. During P1, mean BW was 13.7 ± 0.1 kg which corresponds to ideal BW (IBW) (Table 1). Before ovariectomy, dogs received 807 ± 13 kJ/kg $IBW^{0.75}$. After surgery, energy offered had to be significantly decreased to maintain ideal BW. During P1, dogs received 573 ± 13 kJ/kg $IBW^{0.75}$, which corresponds to a 30% decrease (Table 1). A decrease in fat mass was observed at the end of P1. The ratio fat free mass on fat mass was improved.

When a high-energy food was allowed in double amounts, dogs spontaneously ate significantly more food with mean food amounts corresponding to 912 ± 13 kJ/kg $IBW^{0.75}$. Mean ingested food amounts during P2 increased with 59%, when compared to P1. However, the increase in ingested food amounts was of 80% during the first month of P2 and dogs consumed almost all the food that was offered. Then, the ingested amounts decreased spontaneously during the following months of P2 and P3 although the total energy intake remained statistically higher when compared with P1 (Table 1). The BW increased once dogs were fed *ad libitum*. At the end of the 4 months of P2, mean BW was 16.7 ± 0.8 kg, which represents a significant increase of BW of 22%. At the end of P3, mean BW was 17.8 kg (+30%) (Table 1). All the dogs became overweight or obese (BCS of 7 or 8/9). The weight gain during P2 and P3 consisted exclusively of fat gain (+156%).

Blood parameters

Ghrelin plasma concentration remained constant during the 6 months of P1. When comparing mean values of the entire P1 and the entire P2, the increase in ghrelin plasma concentration was not significant. By contrast, when comparing mean ghrelin plasma concentration during P1 and at the beginning of P2, a very high increase was observed (+281%). Then, ghrelin plasma concentration decreased at the end of P2 and P3. When compared with P1, the decrease in ghrelin plasma concentration at the end of P2 and P3 was not significant (Table 1).

When compared with P1, plasma leptin concentration was increased more than twofold at the end of P2 and this increase was maintained in P3 (Table 1).

One month after the beginning of P2, when compared with P1, no significant modification was observed for thyroid parameters except a mild but significant increase in TSH serum concentration (Table 2). At the end of P2, mild non-significant increases in TT4 and TT3 concentrations were observed. However, at the end of P3, when compared with P1, significant increased serum concentrations were observed for TT4, TT3 and TSH. No changes were found in rT3 and FT4D serum concentrations (Table 2).

Discussion

The first objective of this study was to assess the effects of ovariectomy on energy requirement and body composition. A first study (Houpt et al., 1979) showed that during a period of 3 months after surgery, a 20% increase in food consumption of four-female dogs was observed, resulting in BW gain. The aim of the present study was to determine the energy requirement to maintain IBW after ovariectomy. The energy requirement before ovariectomy was high. A possible explanation is that experimental dogs are different from pet dogs; moreover, these experimental dogs were housed together

that can increase physical activity. A 30% decrease in energy consumption was necessary to maintain IBW after ovariectomy. This restriction seems to be high, but Beagle are prone to obesity. Two hypothesis can be proposed to explain this observation: a decrease in basal metabolism, as observed in female cats (Fettman et al., 1997) or a decrease in physical activity as suggested in dogs by Le Roux (1983) and Sloth (1992). Indeed, spayed female fed a fixed amount of food and exercised regularly gained no weight (Le Roux, 1983). The present study cannot validate one of these hypothesis, physical activity or basal metabolism being not measured. The control of food intake in order to maintain IBW resulted in an improvement of the ratio fat free mass to fat mass. No increase in fat mass was observed. The increase in fat mass after ovariectomy is not an inevitable result.

The second objective of this study was to assess the effects of *ad libitum* feeding in spayed dogs on BW, body composition and blood parameters. *Ad libitum* feeding of a high fat, high protein, and presumably more palatable, diet resulted in over consumption and weight gain in spayed Beagle dogs. Over consumption could be due to the decrease in the oestrogen level in spayed dogs. Indeed, oestrogens have been shown to inhibit food intake in a variety of species (Houpt and Hintz, 1978). This over consumption was more important during the first month and decreased thereafter. The same phenomenon has been previously reported in male or female cats (Flynn et al., 1996; Kanchuk et al., 2003) and in one study in female dogs (Houpt et al., 1979). Weight gain – consisting exclusively of fat – was probably a consequence of the over consumption but the importance of the activity level, the metabolic rate and the efficiency in the utilization of nutrients were not evaluated. Dogs have continued to gain weight when they were fed their maintenance diet in P3. This implies that there is little difference in the response of the dogs to the two levels of energy in the two diets. A higher difference in energy concentration between the two diets should have been used to compare the effects.

Simultaneously to BW and body fat gain, increases in plasma leptin, TSH, TT3 and TT4 concentrations were observed. The increase in leptin level probably reflected the increase in body fat because leptin concentration is highly correlated with body fat in dogs (Ishioka et al., 2002; Sagawa et al., 2002). Plasma leptin concentration could be one of the first parameters to be modified during weight gain, before plasma insulin, cholesterol or triacylglycerols (Jeusette et al., 2004). As expected, leptin in our study correlated positively with BW. Leptin plays a role in energy intake and energy expenditure. In dogs, leptin injections inhibit food intake (Lebel et al., 1999). In rodents, leptin increases also energy expenditure (Pelleymounter et al., 1995). Therefore, the increase in leptin concentrations could be an attempt of the organism to limit weight gain. Another attempt to counteract the development of obesity is the increase in TSH serum concentration. Most studies in obese humans report normal basal TSH serum concentration although a recent study reported high TSH level in some obese children and adults. High leptin levels observed in obese humans might contribute to this finding through stimulation of thyrotropin-releasing hormone (TRH) release (Stichel et al., 2000). An increase in TT3 and TT4 concentrations was observed with weight gain. The increase in TSH serum concentrations most likely contributes to the increase in TT4 observed in our study in overfed obese dogs. Increased total binding globulin concentrations have also been suggested in older studies to explain higher TT4 levels in obese humans (Scriba et al., 1979). However, increased TSH concentrations remained in the reference range and therefore would not lead to misdiagnosis of primary hypothyroidism in overfed obese dogs. In obese humans, rT3 levels remained unchanged in most studies (Roti et al., 2000), which corresponds to our findings. In our study, the concentration in FT4D was not influenced by weight gain. Therefore, FT4D is reliable to evaluate thyroid function in dogs that gain weight, which is in accordance with several studies showing that FT4D is less influenced by non-thyroidal factors than TT4 measurements (Scott-Montcrieff and Guptill-Yoran, 2000).

Ghrelin is also an important hormonal mediator. Recent studies in humans, rodents and dogs suggest that ghrelin may provide a peripheral signal to the brain to stimulate food intake and adiposity (Tschop et al., 2000; Wren et al., 2001b; Yokoyama et al., 2005). A decrease in ghrelin concentration is observed in obese humans, in fatty rats, and in obese dogs (Tschop et al., 2001; Shiiya et al., 2002; Jeusette et al., 2005). In the present study, over consumption resulted in a very high increase in plasma ghrelin concentration during the first month after which ghrelin concentration decreased. So, it seems that the change for a high palatable diet, after a period of restriction, promoted food intake in dogs. The objective could be to store fat when energy is available. However, it would have been of great interest to use a similar group of dogs fed *ad libitum* the

maintenance diet to determine whether the observed modifications were unique to *ad libitum* feeding compared to *ad libitum* feeding a high energy diet. *Ad libitum* feeding induced weight gain. After the initial increase, plasma ghrelin concentration decreased with weight gain although not significantly. The lack of significance of the decrease in ghrelin concentration was maybe due to the small number of dogs. Indeed, in a previous study with a larger effective, the decrease in plasma ghrelin concentration in obese dogs was significant (Jeusette et al., 2005). Another hypothesis could be that there was no modification in ghrelin concentration with obesity on the short term while in the previous study, dogs were obese for at least 1 year before entering the study and they were not in an overfeeding state.

In conclusion, the restriction of energy intake seems necessary in dogs after ovariectomy. Indeed, the BW of spayed female dogs increases rapidly once fed *ad libitum* and the BW gain is exclusively a fat gain. The control of food intake after ovariectomy allows the dogs to have an optimal body composition. During the dynamic phase of obesity, thyroid hormones, leptin and ghrelin plasma concentrations change as a result of counteract weight gain. However, the significant weight gain suggests that this goal is not achieved. In dogs, leptin is one of the first parameters to be modified during weight gain.

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Survival and behaviour of castrated Soay sheep (*Ovis aries*) in a feral island population on Hirta, St. Kilda, Scotland

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(With 5 figures in the text)

The free-living population of Soay sheep on the island of Hirta, St. Kilda, in the Outer Hebrides, has been intensively studied since 1959. The present study was initiated to throw light on the causes of the high mortality rate of adult rams in comparison to that of ewes. In 1978, 1979 and 1980, a total of 72 male lambs was castrated within a day or two of birth. The survival of these castrates has been much longer than that of the entire rams, marked as controls, and longer than that of ewes of the cohorts of the same years. The daily activity pattern of the castrates was similar to that of ewes rather than that of rams. In particular, during the rut the castrates spent most of the daylight hours grazing, in contrast to the rams who were continuously moving and involved in agonistic and sexual encounters. This study substantiates the earlier assertion that the costs in energy of reproduction are a major cause of mortality in temperate zone ungulates. The social organization of some castrates was similar to that of females in that they remained with the home-range group of ewes into which they were born, but other individuals resembled males in that these castrates clubbed together in their own groups. These patterns of behaviour persisted throughout life.

Introduction

Many species of ungulates exhibit marked sexual dimorphism, and there is a higher mortality rate among males than among females; there is intense competition between males to become successful sires (Jewell, 1976). In these ungulates, the ratio of males to females at birth is usually close to 1:1 (Parkes, 1926) but, in the adult breeding population, females may greatly outnumber males. The feral Soay sheep that occupy the island of Hirta, St. Kilda, the most westerly of the Outer Hebrides in Scotland, show this condition to a marked degree. This population, showing cyclic fluctuations around 1200 head, has been studied continuously since 1959 (Jewell, Milner & Boyd, 1974; T. H. Clutton-Brock *et al.*, 1991, 1992; Jewell, 1995), and it is recorded that mortality among rams is very high with a heavy incidence in ram lambs and yearling rams: the ratio of adult rams to ewes varies from 1:3 to 1:12 through the phases of the cycle (see Fig. 2). Most sheep die in late winter when all suffer from inanition but it is presumed that the events of the rut, occurring in November, further reduce the chances of males surviving over winter. In the rut, not only are males in physical competition with each other for access to females, but they spend much less time feeding (Grubb & Jewell, 1974). In Soay rams, as the mating season approaches, testosterone levels rise and there is a marked increase in aggressive behaviour (Grubb & Jewell, 1973; Lincoln & Davidson, 1977). It is well known that castration reduces libido and aggressiveness (Mattner, 1980; Wodzicka-Tomaszewska, Kilgour & Ryan, 1981), and that castrated rams (also commonly called wethers) are placid animals, although sexual drive can be restored in them by the injection of testosterone (Fulkerson, Adams & Gherardi, 1981). In an attempt to reveal some of the sex-specific factors involved in the differential mortality of males, a number of male lambs on Hirta was castrated in the years 1978, 1979 and 1980. It

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was hypothesized that the castrates would not attempt to participate in the rut and that their survival rate would be much better than that of 'entire' (uncastrated) rams.

One of these castrates of 1980 is still to be seen alive today, nearly 17 years later (spring 1997)*, but all the entire rams of the same cohort are long since dead. It seems an appropriate time, therefore, to record the results: a preliminary note on the greatly enhanced survival of castrates has been published (Jewell, 1986). Intriguingly, there was an early observation on the survival of castrates on Hirta: when Soay sheep were introduced to Hirta from the adjacent island of Soay, in 1932, the consignment of 107 sheep included 22 wethers, these being male lambs of the year that had been castrated. Sixteen years later, James Fisher visited Hirta (Fisher, 1948) and afterwards remarked to Morton Boyd (Boyd, 1953) that he had seen some castrates still alive, recognizable by their 'knob horns'.

There is a very extensive literature describing the behaviour of sheep (recently reviewed by Lynch, Hinch & Adams, 1992), mostly relating to husbanded animals. While much quantitative ethology has been performed on ewes and lambs, there is little specifically about wethers. Much research has been carried out on the relationship between ewes and lambs, and on the sexual behaviour of rams (Lindsay & Pearce, 1984), but in commercial flocks the age structure and sex ratio are strikingly dissimilar to those in wild populations. The feral Soay sheep have afforded an opportunity to study behaviour in free-living conditions and with a full complement of rams (Grubb & Jewell, 1966, 1973; Jewell, Milner & Boyd, 1974; Jewell, 1995), and it is in this context that the behaviour of the castrates is particularly interesting. I know of no other study of a large mammal in which a group of castrates has been liberated into a free-living, unmanaged population in this way.

Methods

Soay sheep

Soay sheep are a relic population of primitive domestic sheep similar to those that were widespread in Britain in the Bronze Age (Ryder, 1983). In their feral form they survived only on the small island of Soay in the St. Kilda group, but Soays were being brought to parks in mainland Britain at the beginning of the century (Elwes, 1913). After the evacuation of people from the nearby larger island of Hirta in 1930, a founding population of Soay sheep was established there. Over the years their numbers fluctuated between 600 and 1800 on the whole island, but the study was carried out on the sub-population that occupied the Village Glen and the nearby promontory of Ruaival (see Fig. 1), numbering 300 to 500 animals.

Soay sheep are polymorphic for fleece colour and horns (Boyd *et al.*, 1964). The 2 basic fleece colours are light fawn (L) and dark brown (D) and, as with species of wild sheep, the fleece is usually cast completely every summer. The rams usually carry heavy horns in a close spiral, although occasionally crumpled horns are to be seen, and, very rarely, polled. Almost half the ewes have horns that are finer and more upright than the rams horns and almost half are completely polled (without horns); the remainder have small knobs of horn, or very poorly developed horns, called scurs.

A description of home-range organization among Soay sheep is given in Jewell (1966) and Grubb & Jewell (1966), and the social behaviour of Soay sheep is described in detail in Jewell, Milner & Boyd (1974).

Castration

In 1978, in a pilot study, 14 male lambs were castrated within 1 or 2 days of birth using the usual method for husbanded sheep of applying a rubber ring round the scrotum ('Elastrator', Elastrator Co., Blenheim,

*This castrate died on, or a day or two before, 23 March 1997. He was born on 22 April 1980 and so was one month short of 17 years old.

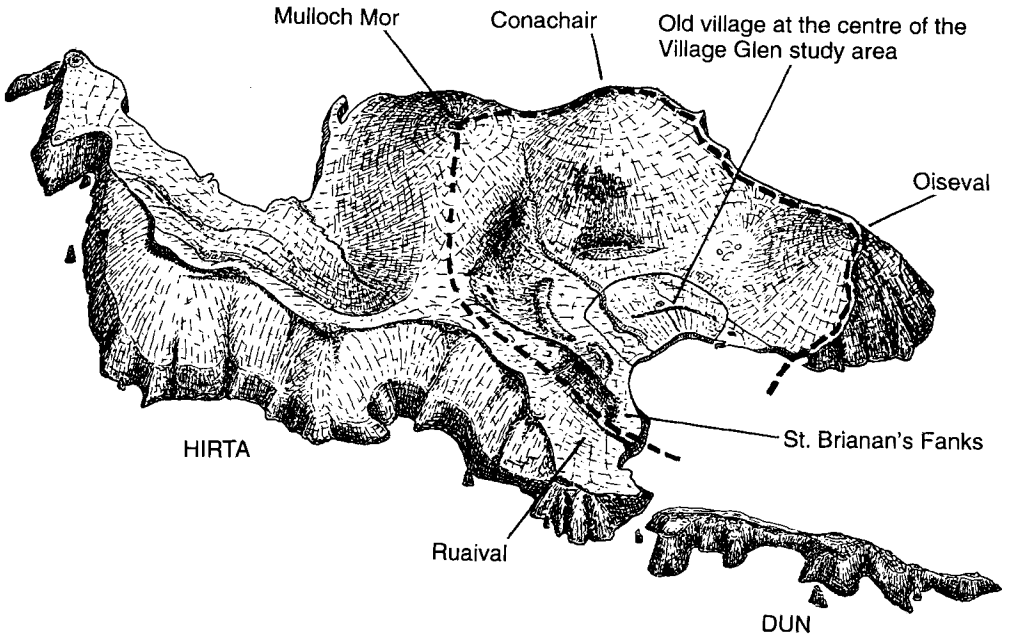

FIG. 1. Block diagram of Hirta and Dun giving an oblique aerial view from the south. A scale is provided by the island of Dun which is 1425 m long (after Macgregor, 1960). The dashed line encloses the Village Glen study area.

New Zealand). Fourteen other male lambs, caught concurrently, were tagged but not castrated to serve as controls (entire rams) see Table I. These lambs were caught over what proved to be the peak of the lambing period between 14 April and 22 April (median date April 19/20, see Jewell, 1989). An additional 16 male lambs (not shown in Table I) and 54 ewe lambs were tagged.

No problems having arisen in the pilot study, a second visit was made in 1979 . This proved to be a crash year for the sheep population, however (see Fig. 2), and there had been heavy mortality in February and March. The surviving ewes were in poor condition, the lambs were light in weight, and many died before the end of the lambing period. No attempt was made, therefore, to castrate a large sample and only 8 castrates and 8 controls (3 of which died neonatally) were tagged, together with 33 other ram lambs and 44 ewe lambs.

The following year, 1980, was one of recovery for the sheep (see Fig. 2). No dead sheep were found in the village area. A hundred male lambs were caught of which 50 were castrated and 50 became controls; 83 ewe lambs were caught . The male lambs were assigned to be castrated at random and the colour and horn types into which they grew had to be recorded later when they became mature. Forty-two castrates were seen in July 1983 and categorized in this way (see **Results**).

Tagging

Lambs were given small Dalton plastic ear-tags each with a unique number and of a different colour for each year. The size of the numbers on these tags is too small to be read at a distance, even with field glasses, but small tags were used for aesthetic reasons as many National Trust parties visit the island.

The island of Hirta

The island (Fig. 1) lies at 57° 49′ N lat., 08° 34′ W long., and is 637 ha in extent (Macgregor, 1960). It is treeless but provides productive grazing and abundant shelter for the sheep. The sheep feed on the old pastures in the

TABLE I

The numbers of castrated male lambs, entire male lambs, and female lambs entering the population and surviving through subsequent years, starting in the year 1978. Gaps in the table signify no information, 0 = class of sheep never seen again, but year of demise unknown

Year Tag#	Class of Lamb	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97
Pink 1978	Castrate	14	10	10			6	6				4				1	0	0	0	0	
	Control ram	14	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Female	54	22	22				14			1			1	1	1	1	0	0	0	
Orange 1979	Castrate		8	7			6	4				2			1		0	0	0	0	
	Control ram		5	1	1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Female		44	40			12	10			5	4		1	1	1		0	0	0	
Blue 1980	Castrate			50				33				32			23					1	
	Control ram			50	11	6	5	4		4		3	1	1	0	0	0	0	0	0	
	Female			83				33			11	9	2	2	2	1		1	0	0	
Totals entering population in 1980	Castrate			67				43		42		38	31	28	26		15	11	4	1	
	Control ram			52	12	6	5	4		4		3	1	1	0		0	0	0	0	
	Female			145				57			17	14		4	4		3	1	0	0	

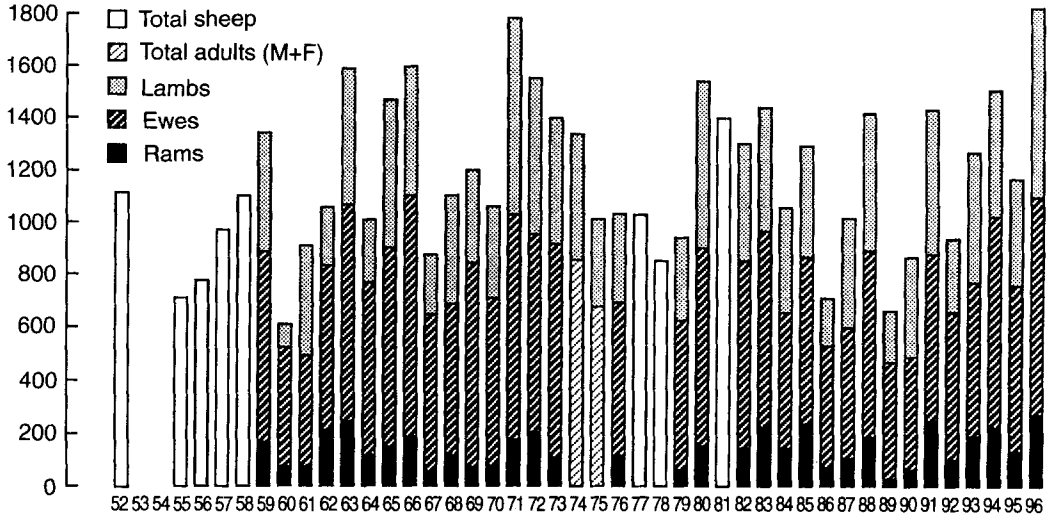

FIG. 2. Year-to-year fluctuations in numbers of Soay sheep on Hirta, St. Kilda, taken from the summer census in May or June from 1952 to 1996. In some years a breakdown into the several categories of sheep was not achieved, and some counts were made by inexperienced volunteers.

deserted village, on the heather-dominated hill slopes, and on cliff ledges and gulleys. A standard annual census of the sheep on the whole island was, and continues to be, made every year in early summer (Fig. 2) but intensive study is limited to the village area, Fig. 1 (T. H. Clutton-Brock *et al.*, 1991). Detailed accounts of the environment and the general methods of study are given in Boyd *et al.* (1964), and Jewell, Milner & Boyd (1974) where a large-scale fold-out vegetation map is also to be found.

Visits

Following the last year in which castrations were carried out (1980), I visited the island in October–November 1981, July–August 1983, October–November 1984, April 1985, August 1987, and July 1990. Other people from the Soay Research Project and the Large Animal Research Group at Cambridge have been on the island in every year from 1985 to the present.

Recording behaviour

Focal animal follows were carried out on selected castrates and entire rams. When possible, 2 observers worked together, one following a castrate and the other following a control ram; activity was recorded every 5 minutes. *Activity surveys*: to compare the activity of rams, ewes, and castrates, all visible animals were recorded as grazing, resting, or engaged in some other activity (standing, walking, sexual, or agonistic encounters) in surveys through the village at half-hour intervals through the daylight hours. *Continuous watches* of castrates and rams provided details of behaviour; when a castrate who was a member of a group was selected, information on the behaviour of the whole group could be obtained as they remained together.

Results

Physical types

The range of colours seen in the fleeces of castrates was the same as in other Soays, but in many

castrates, as they grew older, the fleece was not fully cast and old matted fleece was retained. Many of the castrates, despite being male, failed to grow horns and remained polled (P); others grew small crumpled horns (Crump), rather larger than the scurs of ewes; most of the castrates, however, grew horns (H) that were similar to the horns of ewes (see photograph in J. Clutton-Brock *et al.*, 1990). In a check of 42 castrates seen in July 1983, the categories recorded were: L,11; D,31 and of these LH,6; LCrump,3; LP,2; DH,19; DCrump,3; DP,9. Castrates are much larger than ewes, and are taller and less stocky than rams and have longer limbs (for skeletal measurements see J. Clutton-Brock *et al.*, 1990).

Social organization

The small numbers of castrates and controls of the 1978 cohort of lambs were observed in April 1979, one year after birth. Their numbers had been reduced by the heavy mortality in the whole population in the winter of 1978/79 (see Fig. 2) and only six castrates and five controls out of the original 14 in each category were seen. These young animals, just becoming yearlings, were still in their maternal home-range groups and no unusual behaviour was recorded.

Observations between 29 October and 9 November 1981 found many of the castrates still with females, presumably adhering to those groups into which they had been born. Typical, and persistent, grazing groups comprised one or two castrates, three or four adult ewes and the several lambs of these ewes. This behaviour of the castrates was not different from that of some young rams of the same age and many female groups had one-and-a-half-year-old rams among them: some groups had both castrates and entire young rams. The behaviour of the few older castrates (surviving from the 1978 and 1979 cohorts) was of great interest in being of two different kinds, some showing persistent association with particular female groups whilst others had associated together in 'castrates only' groups, none having joined existing ram groups. This behaviour was in contrast to that of rams of the same age many of whom had joined groups of older rams. Three such groups of castrates were regularly seen in the meadows of the village, one group of six individuals and two of four. (A few of the younger castrates of 1980 also adhered to these groups.)

Observations in July–August 1983 revealed several stable groups of castrates as a conspicuous feature of the village sheep. One group of eight individuals that moved in the centre of the village and on the slopes of Oiseval in the east comprised two five-year-olds, one four-year-old, and five three-year-olds. This group was seen on almost every day of the visit and they were always together; they moved freely through the home ranges of ewes and the central village rams and no unusual interactions were observed. Another group of four moved at the east end of the village also using Oiseval, and a group of seven moved between the west meadows and the hill slopes to the west of the village. Yet another five castrates occupied a home range at the west end but they were often accompanied by ewes and a ram who behaved as an adherent to the group. Similarly, five castrates on Ruaival, further west, associated in a group that included ewes and their lambs. Notwithstanding the formation of these distinctive home-range groups, there were several castrates who did not associate with others of their kind but who remained as members of ewe home-range groups. The castrates moved freely through the home ranges of ewes and the rams that lived in the central village and no interactions were observed.

A visit to Hirta between 23 October and 21 November 1984 covered the first half of the rut (as judged from the median date of lambing, 23 April 1985, see Jewell, 1989). The castrates were still associated in the conspicuous all-castrate home-range groups. All eight members of the central village group (all could be recognized individually) had survived and were still together. They had recruited another 1980 castrate making a group of nine. A second group of eight that ranged over the mid-village comprised one from 1978, one from 1979, and six from 1980. A group of seven castrates (two born in

1978, one in 1979, four in 1980) was frequently seen on the slopes above the west end of the village. A large number of castrates moved between St. Brianan's Fanks (fanks are old stone-walled exclosures formerly used for the growing of crops) further to the west and the promontory of Ruaival (see Fig. 1). They were seen most often as two distinct groups of four (one of 1989, three of 1980) and of six (one of 1978, five of 1980). Other castrates using the west side of the village appeared not to be adherents to stable peer groups but associated with ewe groups and occasionally with young rams.

The alternative type of social organization also persisted. Certain castrates remained with ewe home-range groups and, in at least one instance, the castrate's dam was known individually and she was a stable element of the group. Four castrates were known to have spent their entire lives in this way.

The persistence of stable home-range groups, of castrates only, also remained a feature of this class of sheep throughout their lives. As castrates died, and the original groups became smaller, so an amalgamation of the survivors was noted, as in groups of entire rams.

Survival

The persistence of the castrates in the population is a striking phenomenon (Table I). Their survival rate and longevity are evidently greater than that of entire rams. Although only one castrate is still alive today (spring 1997), 26 castrates were still alive in April 1991 at a time when all the rams from the same cohorts were dead.

Quantifying the sequence of mortality has not been simple because of the impossibility of making a complete census during a short visit to the island. The sheep move widely and are often out of sight behind rocks or walls, or are out on the cliff ledges. Moreover, 10 lambs had originally been caught on Ruaival outside the area subsequently used for intensive studies. In addition, some wethers and entire rams born in the western meadows of the village may have taken up residence beyond the study area. Not all dead animals are found, but regular intensive searches at the end of March ensures that most deaths, which occur in late winter, are enumerated. Very few adult animals die between April and January. In any survey of the living animals, only a proportion of the tagged ones are seen. Although some wethers lost ear-tags, their distinctive appearance ensured that, with persistent counting, the total number of survivors (from the three cohorts combined) could be recorded (Table I). These total numbers are dominated by the large cohort of 1980, so it has proved practical to take the numbers present in that year as the starting point for the graph (Fig. 3) comparing the relative survival of rams, castrates and ewes.

Longevity

The longest-living ram amongst the controls was 10 years old. The longest living ewes in the cohorts under consideration here were one from 1978 who died at 15 in 1993, one from 1979 who died at 14 in 1993, and one from 1980 who died at 14 in 1994. In July 1983, I saw a 13-year-old ewe who was successfully rearing twins. The longest-lived castrate is the one seen alive in March this year (1997) who was born in 1980 and so is over 16 years old. It will be recalled (see **Introduction**) that James Fisher in his visit to Hirta in 1948 saw some castrates that were by then 16 years old.

Reproductive behaviour

The visit to Hirta in October–November 1981 coincided with the early phase of the rut.

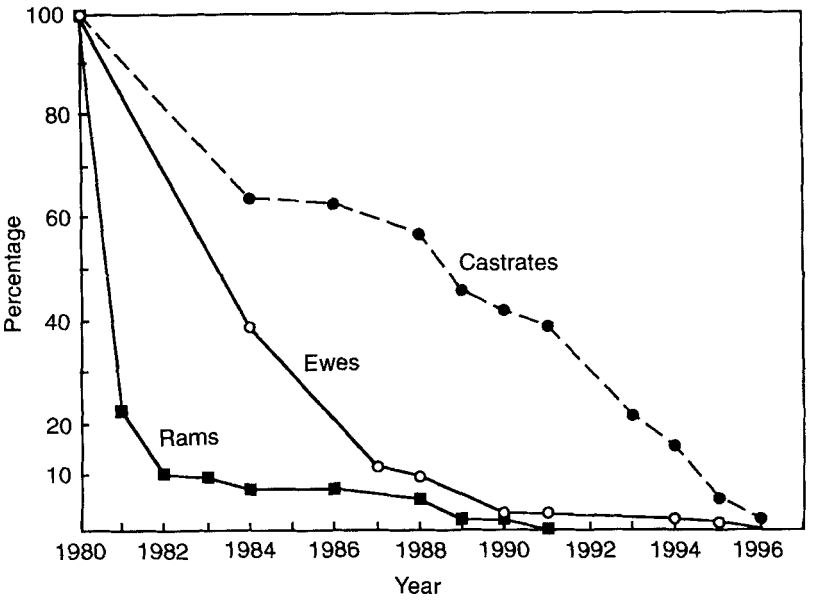

FIG. 3. The survival of castrates (-●-) compared with that of entire rams (-■-) and ewes (-○-) standardized as a percentage of the starting numbers (given in Table I).

Occasionally, rams threshed clumps of vegetation and showed low-level aggression among themselves. Rather perfunctory investigation of ewes by rams and ram lambs was seen with frequent flehmen, but with no attempts to mount. No investigation of ewes by castrates was seen. The similarity of appearance of castrates and ewes was notable. There was some low-key interaction between rams and castrates: rams occasionally sniffed castrates and showed flehmen and head-twist towards them. Usually they ignored each other, although the castrates sometimes contested rams with head-butting intention movements. Ram lambs provoked reaction from castrates by approaching them and sniffing as if the castrates were ewes. The castrates either walked away or rebuffed the ram lambs by butting them. This could develop into chasing, reminiscent of the play of lambs. Sometimes mature rams also appeared to mistake castrates for ewes. A six-year-old ram made a low-stretch approach to the rump of a three-and-a-half-year-old castrate. The castrate stood still briefly and the ram then sniffed the ground just behind the place where the castrate had been standing and exhibited flehmen. This was the typical reaction shown to ewes who would normally urinate on the ground behind themselves following such an approach (see description and photographs in Jewell & Grubb, 1974).

The visit in October–November 1984 covered the first half of the rut and at this time the castrates were between four-and-a-half and six-and-a-half years old, ages at which rams are in their prime. Courtship of ewes by rams and mating activity increased in intensity throughout the period; the first mounting was seen on 2 November, a few mountings were seen daily after this and from 16 November onwards seven or eight mountings, between different ram and ewe pairs, were seen each day. Consortships were numerous, the rams making frequent testing of the urine of the ewes.

In most respects, the activities of the castrates could not have been more different. Indeed, the group of nine castrates that moved in the central to east side of the village was seen out on the cliffs of Oiseval on 6 November and was not seen in the village again during the remainder of our visit. These castrates had distanced themselves from the main mating arena of the village. The castrates that

remained in the village seemed relatively indifferent to the mating activity that was going on around them. Castrates continued lying, cudding, or grazing despite agonistic encounters between rams, or mating activity, nearby; such circumstances usually provoked entire rams to participate, or join in the chasing of ewes. Nevertheless, a few encounters involving castrates were noted. A castrate did nudge a ram, and some castrates on seeing young rams blocking nearby ran over and broke up the confrontation. Two castrates engaged in a head to head confrontation before lying down. Only once was a castrate seen to investigate a ewe and to nudge her from both sides and rise to mount. Many of the encounters were provoked by the activity of entire males. A large ram (6 years old) sniffed castrates and performed kicks and twists towards them; on this occasion the castrates simply moved on. Occasionally castrates did urinate before moving off and then the ram would sniff the urine patch and make flehmen. Ram lambs were persistent in approaching castrates and 'annoying' them; these young males were butted and shown off by the castrates. The castrates were larger (at least taller) than most rams and so could hold their own and carry on with their own preferred activities.

Activity patterns

The proportions of sheep engaged in different activities over the daylight hours is set out in Fig. 4. Activity on five selected days in 1984 is presented extending from the end of October (pre-rut) to 20 November (near the peak of the rut). It will be seen that the ewes, as expected, spent most of the time grazing, and the castrates presented a similar pattern: throughout November the wethers spent between 60 and 70% of the daylight hours grazing. The rams, in contrast, show a progressive reduction in the time spent grazing and, by mid November, this proportion had been reduced to about 25% of the daylight hours (a result similar to that recorded in November 1964, see Grubb & Jewell, 1974, fig. 7.4).

Continuous records of the activity of selected individuals illustrate these activity patterns in more detail (Fig. 5). The distribution of bouts of grazing through the daylight hours demonstrate how intensively a ewe grazed (Fig. 5a). The castrate of Fig. 5b only differed in spending more time resting. The continuation of grazing through the afternoon and on into darkness characterizes the ewes and wethers. The record of a mature ram, however (Fig. 5c) shows how little time is spent grazing, most time being spent in moving or in social and sexual encounters. A castrate (Fig. 5d), recorded concurrently with the ram, was relaxed at midday, even apparently being asleep with its head on the ground, and showed the characteristic period of continous grazing as darkness supervened.

Discussion

This study was initiated to throw light on the marked difference in survival rate of rams and ewes in this feral population. The population is limited by food and experiences intermittent 'crashes' in numbers followed by recovery over a few years (Boyd, 1974); analysis reveals an exaggerated cycle of 'boom and bust' (T. H. Clutton-Brock *et al.*, 1991), but ewes survive better and live longer than rams despite the demands of pregnancy and lactation.

It was postulated in earlier research (Boyd & Jewell, 1974) that a key factor bringing about this difference was the feeding behaviour of the two sexes during the rut in late autumn. Support came from the observation that rams spend much less time grazing than ewes during the whole period from late October to early December (Grubb & Jewell, 1974). The hypothesis proposed at the outset of the present research was that castrated males should not participate in the rut and should not reduce the time spent feeding. This is exactly what has been found. The feeding pattern of castrates was similar to

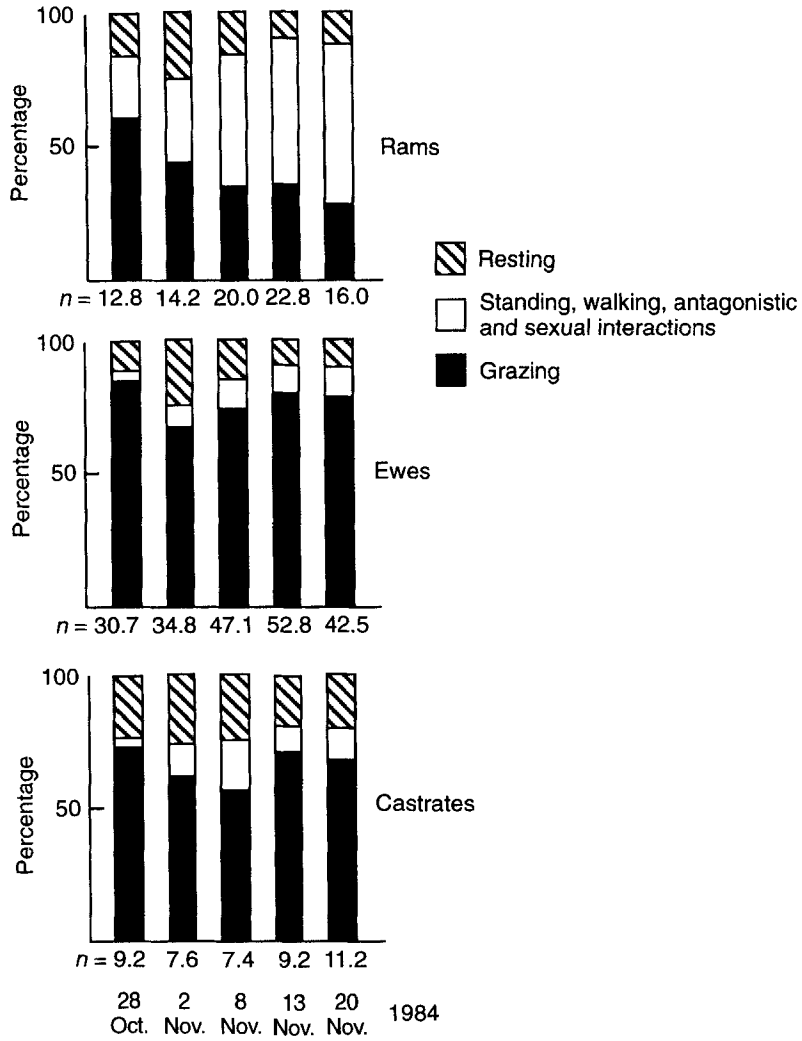

FIG. 4. The proportionate activity of sheep through the daylight hours, based on half-hourly scans, on selected days leading up to the peak of the rut (22 November) in 1984. *n* = the mean numbers of individuals seen at each half-hour scan.

that of ewes and their survival was even better. Stephenson (1994) has been able to support this interpretation by showing that ram lambs 'hormonally castrated' by the injection of a progestagen (which only lasts for the duration of the rut) spent more time feeding and survived better than intact ram lambs.

The effects of overpopulation are so severe that the prospects of survival of animals born into successive cohorts in pre-crash, crash, and post-crash years are markedly different (T. H. Clutton-Brock *et al.*, 1991, 1996). The castrates have survived four crash years and so have experienced several kinds of cohort- and age-related privations. This is a particularly revealing outcome of castration that permanently deprives males of the hazards and misfortunes of masculinity.

A notable feature of male life histories on Hirta is the precocity of rams. Ram lambs, at seven

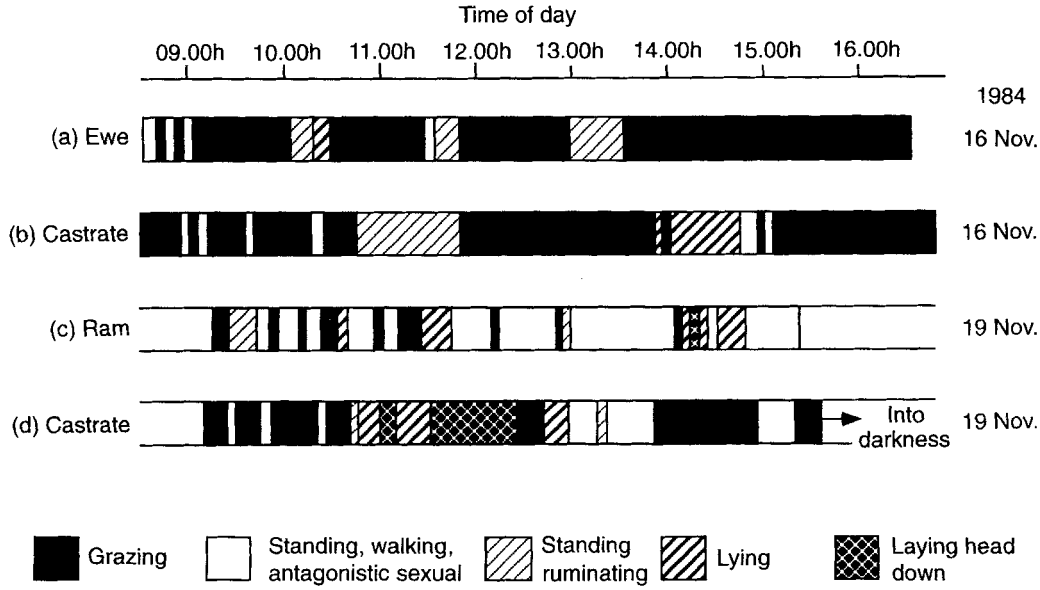

FIG. 5. Continuous activity records of sheep through the daylight hours in the first part of the rut in 1984.

months of age, join in the rut, and are even more persistent in their endeavours to mate as yearlings at 19 months old. I had thought (Boyd & Jewell, 1974) that the experience gained in this way, and possibly the improvement of their position in the hierarchy of young rams, conferred reproductive success that compensated for heavy mortality. But a further factor was identified when it became clear that the mortality of mature rams, in the winter that terminates in a crash, can be so severe that the young rams form the majority of the male population in the next rut (Jewell, 1989). The direct reproductive success that the young rams gain in this way has been established (Bancroft, 1993; Stephenson & Bancroft, 1995). Notwithstanding this benefit, it may be noted that the survival rate of castrates as lambs and yearlings was high, demonstrating the benefit of not participating in the rut at that age.

Physical castration alters many aspects of the anatomy and physiology of the male mammal affecting, amongst other things, the immune system, parasite load, and fat storage (Parkes, 1966; Glucksmann, 1978). (I observed castrated Soays, kept near Cambridge, to have considerable fat reserves in the omentum, and in commercial breeds of sheep, wethers are known to be relatively fatter than entire rams [Ritar *et al.*, 1988; Thompson & Butterfield, 1988]. Conspicuous deposits of fat, however, may give a false impression of adequate reserves since it has been shown that even the abundant fat reserves of reindeer suffice to meet only a small part of energy requirements in winter [Tyler, 1987*a*, *b*].) It must be noted that through most months of the year the survival rate of entire males and castrates is similarly good, and that deaths are concentrated in late winter in the months of February and March, when inanition takes its toll. Castration averts this disaster by promoting a continuous intake of energy through the late autumn and early winter. This results from the elimination of libido (Wodzicka-Tomaszewska *et al.*, 1981) and reduced aggressiveness of wethers (Mattner, 1980) and is not due to a difference in appetite because it has been shown in research on housed sheep (Gordon, 1964; Brown *et al.*, 1979) that wethers exhibit a reduction of voluntary food intake in winter in the same way as rams.

The longevity of the Soay castrates, at nearly 17 years, has been greatly superior to that of entire rams at 10 years (Grubb, 1974, also recorded a few rams surviving to this age). There is little published work that permits comparison with other mammals. Parkes (1966), quoting several authors, concluded that "in animals castration does not seem to have a beneficial effect on longevity". Data from humans are conflicting: Hamilton & Mestler (1969) showed that eunuchs living in an institution for the mentally retarded had better survival than entire men, but Darwin, in 1856, noted: "Yet Eunuchs nor cut Stallions nor nuns are longer lived" (Barrett *et al.*, 1987), and Nieschlag, Nieschlag & Behre (1993), in an analysis of historical documents on the longevity of male singers, found that the mean life span of castrati was not significantly different from normal singers. In cats (Hamilton, Hamilton & Mestler, 1969) and in rats (Talbert & Hamilton, 1965), castration does prolong life, and in observations on bank voles (*Cleithrionomys glareolus*) living in a natural outdoor enclosure it was shown that castrated males survived better than entire males (Gipps & Jewell, 1979).

The present observations comprise the first experiments in which a substantial number of castrates has been released into a free-living population. Short (1979), working on the island of Rum, observed two castrated male red deer calves (castrated within a week of birth) and these animals remained closely attached to their mothers, within their respective groups of hinds, for the rest of their lives. An unexpected and interesting outcome of the present study is that the wethers displayed two distinct types of social cohesion. Some individuals stayed with females, known to be, or presumed to be, their mothers, in their natal home-range groups, and behaved socially very like females throughout their lives. Other castrates associated together in their own permanent castrate groups and in this respect were like normal males. These groups were apparently given cohesion by mutual recognition and acceptance.

In physical appearance the castrates looked like ewes, even to the extent of showing similar horn types ranging from polled, through scurred (or crumple-horned), to horned. This effect of castration has long been known (Seligmann, 1906; Marshall & Hammond, 1914), but it is also certain that the odour of the castrates was changed (Izard, 1983). The different physical responses to castration have a genetic basis and it can be asserted that the different behavioural types, revealed by castration, have a genetic basis too (McGill, 1978). These novel observations would not have been revealed if adequately large numbers of lambs had not been castrated.

In their review of the ethology of free-ranging domestic animals, Arnold & Dudzinski (1978) make only passing reference to wethers, and they have not been the subject of any previously published studies of behaviour. Yet wethers have been of very great importance in animal husbandry from ancient times (Trow-Smith, 1957; Killen, 1964), through the Middle Ages (Lloyd, 1977–78; Ryder, 1983) and on into the present century (Fraser & Stamp, 1957). Even today, on the island of North Ronaldsay in Orkney, where the sheep are traditionally confined to the foreshore (Hall, 1975) and eat only seaweed, I have observed that a high proportion of the sheep are wethers and serve as a 'walking larder' (J. Clutton-Brock, 1989). The data presented here provide a new dimension to the many factors considered by Boyd & Jewell (1974) for the management of the Soay flock on Hirta.

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REFEREED

ORIGINAL RESEARCH

Clients' Perspectives on the Effects of Laparoscopic Ovariectomy on Equine Behavior and Medical Problems

J. Lacy Kamm, DVM, and Dean A. Hendrickson, DVM, MS, DACVS

ABSTRACT

Because laparoscopic surgery eliminates the side effects of general anesthesia, the success and therefore the use of laparoscopic surgery continues to broaden. Laparoscopic ovariectomy, once only used to treat medical problems such as granulosa cell tumors, is now being expanded to treat behavioral problems in mares. Thirty-five mares with behavioral or medical problems were treated with ovariectomies, and their owners were later contacted and questioned concerning the effect of ovariectomy on their mares.

Behavioral improvement was seen in 83% (19/23) of mares treated for behavior-related problems. Aggression problems improved in 86% (12/14) of cases, general disagreeable demeanor improved in 81% (17/21), and excitability improved in 75% (12/16) of cases in which these behaviors were previously observed. Kicking and biting improved in 73% (8/11), problems in training improved in 72% (13/18), and frequent urination and problems with other horses improved in 64% (7/11 and 9/14, respectively) of cases.

Owners with nonovariectomized mares also were contacted to compare the normal progression of mare behavior over time with the behavioral change in ovariectomized mares. A statistically significant change in behavior of ovariectomized mares over nonovariectomized mares was seen in the categories of decreased aggression ($P < .01$), decreased training problems ($P < .025$), decreased frequency of problem behaviors ($P < .001$), and improved overall behavior ($P < .01$).

In cases in which medical problems such as granulosa cell tumors, ovarian cysts, and cycle-related colic were treated with ovariectomy, all of the clients were very satisfied with the treatment, whereas clients attempting to treat cycle-related laminitis were dissatisfied.

Keywords: Laparoscopic ovariectomy; Estrus; Mare; Behavior; Granulosa cell tumor

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INTRODUCTION

Various techniques of standing equine laparoscopic ovariectomy have been described for the removal of equine ovaries by Dechant and Hendrickson,[1] Palmer,[2] Ragle and Schneider,[3] Hand,[4] and Hanson and Galuppo.[5] Standing laparoscopic surgery reduces complications associated with general anesthesia, which reportedly causes death in 0.12% to 0.9% of equine patients.[6,7] Laparoscopy requires multiple small incisions, with one to two incisions enlarged only enough to remove the ovary, thereby decreasing scar size and recovery time compared with other flank and ventral approaches. The approach described by Dechant and Hendrickson[1] uses three 1-cm incisions on the right, two 1-cm incisions on the left, and one incision on the left side large enough to remove both ovaries. The surgery can be performed in less than 1 hour using a localized bilateral flank block and an epidural between the first and second coccygeal vertebra.[1] Intraoperative pain can be decreased using a mesovarian injection of 10 ml 2% lidocaine before operating on the ovary.[8]

Complications associated with laparoscopic surgery are generally related to procedural errors.[1] These include injury to the sublumbar muscles, spleen, gastrointestinal organs, or kidney and can be reduced with careful technique. Other complications include incisional infection, inappetence, lack of hemostasis when using only one staple on the ovarian pedicle,[5] mild tachycardia postoperatively relating to abdominal discomfort,[1] and mild fever.[4]

Bilateral equine ovariectomy was first described for use in mares with behavioral problems in 1993.[9] The commercial success of mare hormonal supplements documents the strong desire for suppression of problem behaviors such as aggression, disagreeable demeanor, excitability, kicking and biting, training problems, problems with other horses, frequent urination, or problems during estrus. Removing the ovaries eliminates the production and release of estrogen because this hormone is primarily made in the theca and granulosa cells of developing ovarian follicles.[10] Estrogen is released during estrus, a potential time of increased aggression and behavioral problems, and has been found to be a contributing factor to mare behavioral problems.

The purpose of this report is to evaluate the clients' perception of the effectiveness of laparoscopic ovariectomies in correcting behavioral and reproductive medical problems in the mare. This study delineates the different types of mare behavioral problems that cause owners to seek an

ovariectomy and evaluates the likelihood that the frequency of these behaviors will be decreased after surgery. The benefits and consequences of ovariectomy by laparoscopic surgery versus the conventional ventral midline laparotomy are discussed.

CRITERIA FOR SELECTION OF CASES

Records of 80 horses were evaluated when either a unilateral (in most medical problem cases) or bilateral ovariectomy (in behavioral problem cases) had been performed between 1996 and 2005. Of 80 horses, 35 of the owners were available to survey, and all responses were included in the study. Because earlier cases were more commonly unavailable for follow-up, the average time between surgery and evaluation was 3.6 years. The longest follow-up was 9 years, and the shortest was 12 months. Seventeen owners of nonovariectomized mares were randomly selected and contacted. Temperaments of these control mares were unknown to the researchers before contacting their owners. The average length of ownership for control group mares was 5.4 years.

METHODS

A questionnaire was developed that contained questions relating to the overall satisfaction level of the entire experience, reasons for surgery, effectiveness of the surgery in resolving the primary behavioral (aggression, disagreeable demeanor, excitability, kicking and biting, training problems, problems with other horses, frequent urination, overall demeanor, and frequency of behavioral problems) or medical problems, healing time (incisional healing, return to work), and appearance of incision sites. Clients were contacted by telephone and asked 10 to 20 questions, depending on the primary problem. Owners of mares treated for behavioral problems were questioned on changes in the following behaviors after surgery: duration of heat (based on owner's perception), aggression to both people and other horses, training problems (such as disobedience), problems around other horses, frequency of urination, kicking and biting, excitability, frequency of problem behaviors, and overall demeanor. Owners of nonovariectomized mares were contacted and asked similar questions about changes in their mare's behavior. Clients chose answers from predetermined answer categories of return to activity, issues surrounding heat cycle, aggression to people and other horses, response to training, problems with other horses, overall satisfaction with experience, frequent urination, kicking or biting, excitement, overall demeanor, time to behavioral or medical change, and satisfaction with medical improvement. Responses were set at: very satisfied, satisfied, impartial, dissatisfied, or very dissatisfied. The mares were housed in whatever environment they were accustomed to after the second postoperative week.

Data were compiled using the answer choices of the clients and univariant descriptive statistics including chi-square tests, odds ratios, and confidence intervals were performed. Statistical significance in this study was defined as $P \leq 0.05$.

RESULTS

Of the 35 ovariectomized mares, overall satisfaction levels for behavioral and medical problems (granulosa thecal cell tumor, ovarian follicular cysts, cycle-induced colic, laminitis) was 57% (20/35) very satisfied, 20% (7/35) satisfied, 9% (3/35) impartial, 6% (2/35) unsatisfied, and 9% (3/35) very unsatisfied. Client satisfaction with ovariectomy as a treatment for behavioral problems was 52% (12/23) very satisfied, 26% (6/23) satisfied, 13% (3/23) impartial, and 9% (2/23) very unsatisfied. The most common source of dissatisfaction in behavior patients was a lack of behavioral change after surgery. Aggressive behavior and general disagreeable demeanor were most likely to be improved with 86% (12/14) and 81% (17/21), respectively, of cases showing these improvements. Aggressive behavior included threatening gestures and actions whereas a disagreeable demeanor included behavior such as ear-pinning and pawing. Excitability, kicking and biting at horses, and training problems including resistance to training, bucking, and other behaviors related to estrus were only slightly less likely to improve after surgery, with scores of 75% (12/16), 73% (8/11), and 72% (13/18), respectively. Problems with other horses and frequent urination were less likely to be altered, with 64% (9/14 and 7/11, respectively) improved in each category.

Behavioral changes in ovariectomized mares were compared with the changes observed over time in nonovariectomized mares. Owners of nonovariectomized mares were asked whether change had occurred in levels of aggression, disagreeable demeanor, excitability, kicking and biting, training problems related to estrus behavior, problems with other horses, frequent urination, frequency of problem behavior, and overall demeanor over the time of ownership. Problem behaviors were all of those listed previously, with additional behaviors not given as options. Changes in temperament were compared between ovariectomized mares and nonovariectomized mares to eliminate the variable of improvement with time rather than the improvement with surgery alone. Control mares were less likely to have behavioral problems than mares presented for ovariectomy, and therefore, they were less likely to show behavioral improvement.

In all categories studied, ovariectomized mares were more likely to have a decrease in the adverse behavior than nonovariectomized mares. Statistically significant (greater than 95% confidence interval) improvements of ovariectomized versus nonovariectomized mares were seen in the categories of decreased aggression ($P < .01$), decreased training problems ($P < .025$), decreased frequency of problem behavior ($P < 0.001$), and improved overall behavior ($P < 0.01$) (Table 1).

Table 1. A Comparison of Behavioral Changes between Ovariectomized and Nonovariectomized Mares over Time

Improved Behavior	Odds Ratio	95% Confidence Interval
Duration of heat	1.5	0.1–18.3
Aggression	17.5 (*)	1.1–154.4
Training problems	6.1 (*)	1.4–27.1
Problems around other horses	1.5	0.4–5.9
Frequency of urination	18.1	1.0–344.6
Kicking/biting	8	0.9–72.1
Excitability	3.5	0.9–14.2
Frequency of problem behaviors	21.3 (*)	3.7–121.6
Overall demeanor	6.8 (*)	1.6–28.9

NOTE. The statistics show the likelihood that a problem behavior will decrease in duration or frequency in an ovariectomized mare as compared with a nonovariectomized mare. Average follow-up for ovariectomized mares was 3.6 years after surgery, and average time of ownership for nonovariectomized mares was 5.4 years. Categories marked with a (*) are significant ($P \leq .05$).

Medical problems treated with ovariectomy in this study included eight mares with granulosa cell tumors, one with ovarian follicular cysts, one with follicular cysts and cycle-induced colic, and two mares with cycle-related laminitis in which the horses would show signs of acute laminitis when in heat. This did not appear to be associated with increased exercise. Mares with granulosa cell tumors had only one ovary removed, whereas the others had a bilateral ovariectomy. Ovarian follicular cysts were diagnosed on histopathology, and owner complaints were inability to become pregnant and cycle-induced colic. No research has been performed on cycle-related laminitis, and its existence is anecdotal. Overall satisfaction levels were 67% (8/12) very satisfied, 8% (1/12) satisfied, 17% (2/12) unsatisfied, and 8% (1/12) very unsatisfied. Sources of dissatisfaction were related to price of treatment and inability of the surgery to treat the problem. Eighty-three percent (10/12) of the clients with mares with medical problems were very satisfied with the treatment of the medical problem. The other 17% (2/12) of clients were very unsatisfied with the treatment. These two patients with very unsatisfied results were being treated for cycle-related laminitis. The owners of all mares (8/8) treated for granulosa cell tumors were very satisfied with the outcome of the procedure.

Age of the equine patient at the time of surgery played a role in the overall satisfaction levels of customers when treating medical problems. Owners of mares younger than 10 years that were treated for medical problems were unanimously very satisfied (4/4) with the outcome of the surgery. For mares 10 to 14 years of age and treated for medical problems, satisfaction scores were 67% (2/3) very satisfied and 33% (1/3) unsatisfied. Patients older than 14 years had a score of 40% (2/5) very satisfied, 20% (1/5) satisfied, and 40% (2/5) unsatisfied.

For behavioral problems, satisfaction and age of mares were not related. Mares younger than 10 years at surgery had scores of 42% (5/12) very satisfied, 42% (5/12) satisfied, and 17% (2/12) impartial. Mares aged 10 to 14 years had scores of 57% (4/7) very satisfied, 14% (1/7) impartial, and 29% (2/7) very unsatisfied. Patients over 14 years of age had scores of 75% (3/4) very satisfied and 25% (1/4) satisfied.

Healing time for laparoscopic ovariectomies was quantified by the time it took for the patient to return to full activity. Owners were asked to limit activity of mares for 2 weeks after surgery or longer if the incision sites appeared to be healing slowly. Owners gradually returned the mares to activity according to a schedule that they believed suitable. Fifty-four percent (15/28) of mares returned to their preoperative work level within 2 to 4 weeks of surgery. Another 29% (8/28) were in full activity between 1 and 2 months of surgery. The remaining 18% (5/28) took longer than 2 months. Cases of prolonged healing were attributable to infection of the incision site or inability of the owner to provide activity for the animal.

For the 83% (19/23) of clients who observed a behavioral change after surgery, most (16/17) of the clients noted the change in behavior occurred less than 1 year after the surgery. Forty-seven percent (8/17) observed the behavioral change less than 6 months after surgery, and another 47% (8/17) observed the behavioral change between 6 months and 1 year from the surgery date. Twenty-nine of 30 patients undergoing laparoscopic surgery were very satisfied with the appearance of the incision sites after healing. Almost all claimed that the sites were not visible through the horse's coat. One owner stated that white hairs appeared at the incision sites.

DISCUSSION AND CONCLUSION

The possible benefits of ovariectomy on behavioral problems has previously been reported by researchers at Texas Veterinary Medical Center.[9] This study found that 83% (10/12) of mares treated with a bilateral ovariectomy via colpotomy for behavioral problems obtained a higher level of performance after the ovariectomy was performed. Eighty-two percent (14/17) of the mares no longer had behavioral problems after the ovariectomy as reported by their owners, trainers, and referring veterinarians. When comparing the results of the earlier study with this study, satisfaction levels are comparable.

Based on the results of these studies, laparoscopic ovariectomy in the mare is a practical treatment for behavioral and medical problems. Bilateral laparoscopic ovariectomy proved successful in more than 75% of the mares with the behavioral problems studied. Most clients were very satisfied with the change in behavior that followed the surgery.

Some clients noticed only small changes in behavior, whereas a few clients were disappointed with the complete lack of improvement of behavior.

More generalized behavioral problems were more likely to be corrected with an ovariectomy than more specific behavioral issues. Aggressive behavior and a generalized disagreeable demeanor were most commonly corrected, followed by excitability, kicking and biting, and problems during training that related to estrus-like behavior. Problems with other horses and frequent urination were less likely to be improved after surgery; nevertheless more than half of the mares with these problems improved after surgery.

The results of this study were comparable with previous results, which stated that 20 to 35% of mares continued to have estrus-like behavior after having an ovariectomy performed.[2,9] It is generally accepted that ovariectomized mares can continue to be used for stimulating stallions for semen collection. In cases in which ovariectomy did not correct the behavioral problem, the inappropriate behavior can likely be explained as a learned response instead of one that is triggered by estrogen. However, in most cases, blood work was not performed before or after surgery to evaluate hormonal levels.

When comparing data between ovariectomized and nonovariectomized mares, ovariectomy proved favorable to time in improving behavioral problems. By studying the behavioral changes over time for nonovariectomized mares, variability for normal behavioral change with aging was taken into account. Data comparing the mares with a decreased frequency of a particular behavioral problem are listed in Table 1. The number of mares with an increased frequency of the behavioral problem was negligible in nonovariectomized mares and was nonexistent in ovariectomized mares. Behaviors that were most likely to decrease because of ovariectomy rather than aging were aggression, training problems, and frequency of problem behavior. Overall behavior also was improved.

Cycle-related laminitis was the only medical problem not helped by ovariectomy in this study. In both cases, the lameness associated with laminitis became more evident after estrus. Because only two patients with this problem were included, and the relationship between estrus and laminitis is only anecdotal, little can be concluded.

In this study, clients were satisfied with laparoscopic ovariectomy as a treatment for behavioral and medical problems, appearance of incision sites after surgery, and recovery time after the surgery. This study was based only on clients' opinions of the surgery and can be used to guide the veterinary practitioner to the likelihood that a client will be satisfied with the procedure. Standing laparoscopic ovariectomy can be considered a beneficial and safe method to treat mares with behavioral and reproductive medical problems.

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Causes of natal dispersal and emigration and their effects on harem formation in Misaki feral horses

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Keywords: horse; harem formation; Misaki; feral; behaviour; ethology

Summary

Misaki feral horses were separated into 2 herds and the difference between dispersal from natal group (natal dispersal) and dispersal from natal area (natal emigration) was studied. The causes of dispersal and emigration and their effects on harem formation were studied 1979–1994. The number of horses ranged from 73 (mature males: 8, mature females: 26, young males: 8, young females: 3, colt foals: 6, filly foals: 10 and geldings: 12) in 1979 and 86 (mature males: 14, mature females: 37, young males: 12, young females: 7, colt foals: 5, filly foals: 7 and geldings: 4) in 1994 when the present study ended. All 29 males which survived to age 4 years and 58 females which survived to age 3 years left their natal or mother groups at age one to 3. Seventeen of 22 dispersing males and 29 of 39 dispersing females left their natal groups around the birth of their siblings and significant correlations were found between natal dispersal and birth of a sibling. The number of emigrating young males correlated negatively and significantly with the total number of young males in another herd and the number of emigrating young females correlated positively and significantly with the total number of young females in the natal herd. All 13 emigrating stallions which survived to age 5 years formed stable harem groups and a significant correlation was found between natal emigration and harem formation. Twenty-three of 35 resident mares formed stable consort relations with harem stallions and a significant correlation was found between residence and formation of stable consort relations.

Introduction

Dispersal is a widely observed behaviour in many species. A number of proximate factors have been hypothesised, but these causes are not consistent even in equids (Rutberg and Keiper 1993). One of the important factors related to female dispersal is sexual maturity and first oestrus (Berger 1986; Tyler 1972; Penzhorn 1984; Rutberg and Keiper 1993). Oestrous mares are abducted from their family group by other stallions (Klingel 1969; Penzhorn 1984) or young mares in oestrus are sometimes met with aggression from older mares (Klingel 1982). Young males are often reported to leave voluntarily from their natal band (Klingel 1967, 1969; Tyler 1972; Welsh 1975). Prior to the birth of the next foal, a zebra mare becomes aggressive towards her previous foal and invariably threatens if it approaches her (Penzhorn 1984). However, when they do not foal or have lost their newborn foal most mares do not change in their behaviour towards their yearling (Klingel 1969; Feist and McCullough 1975, 1976; Kaseda *et al.* 1984; Tyler 1972). It seems that birth of a sibling is one of the proximate causes of natal dispersal. Dispersal of young males is affected by the presence or absence of peers in the natal group (Klingel 1969; Tyler 1972; Harris and White 1992). Rutberg and Keiper (1993) observed that male dispersal age was correlated with the number of peers in the natal group.

Greenwood (1980) stated that natal dispersal is a permanent movement from birth site to first breeding or potential breeding site. Bekoff (1977) proposed that dispersal is the movement of an individual from its natal site and out of the home range of its parent(s) to another site where it breeds. Berger (1986) stated that in equids it is important to distinguish between dispersal from the natal band and the natal area. In nonterritorial and harem-dwelling equids, 'dispersal from natal band' is easily understandable because it means separation from the parent's band, but 'dispersal from natal site' is difficult to define if the border of the natal band is not clear. Klingel (1968) reported that there was no evidence of any social organisation above the level of the families and stallion groups. However, Miller and Denniston (1979) and Miller (1982) proposed that 'a herd' is defined as a structured social unit made up of groups of horses that follow similar movement patterns within a common home range (McCort 1984). Assuming this social unit of 'a herd', dispersal from natal site can be considered to be a movement from the natal to another herd. The Misaki feral horse lends itself to a study of the difference between dispersal from natal band

TABLE 1: Change in number of males in 5 group types relating to age

	Age (year) of male					
Group type	0	1	2	3	4	5
Natal harem	17	9	2	1		
Mother[a]	12	7	3	2		
Other harem[b]		5	2	1		
Mixed[c]		8	22	25	19	4
New harem					6	18
Died					4	3
Total	29	29	29	29	29	25

[a]dam or dam and her offspring.
[b]dam and other stallion than natal harem stallion.
[c]nonreproductive young males and/or females.

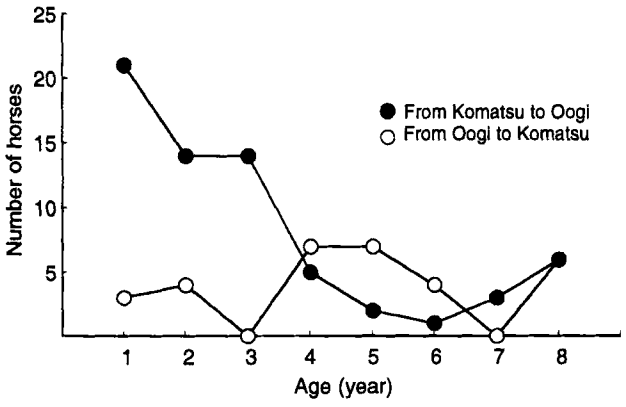

Fig 1: Changes in the total number of horses which migrated between Komatsu and Oogi relative to age between 1979 and 1994.

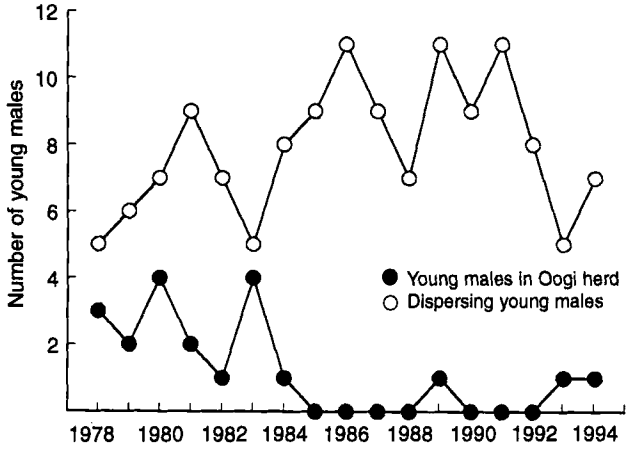

Fig 2: Change in the total number of young males under age 3 years in the Oogi herd and the total number of young males which moved from Komatsu to Oogi between ages one and 3 years. A negative and significant correlation was found between them (r_S = 0.517, n = 17, P<0.05).

(hereafter defined as natal dispersal) and that from natal site (hereafter defined as natal emigration), because the population is separated into 2 herds mainly by topographical conditions.

The purpose of the present study was to establish the causes of natal dispersal and emigration and their effects on harem formation in Misaki feral horses.

Materials and methods

Study area

The Misaki horse range covers an area of about 5 km^2 in Cape Toi, the southeast end of Kyushu Island. There are 2 hills in the centre of the Misaki range, Komatsu and Oogi, about 290 m above sea level. They are separated by 2 valleys which run down from the 2 mountains into the sea dividing the range into 2 areas, Komatsu and Oogi, which are similar in size, topographical and ecological characteristics. Komatsu and Oogi hills are covered with various kinds of native grasses, and other areas are occupied by artificial forests with Japanese cedars and a variety of copses.

Study animals and population characteristics

Misaki horses have been living freely in the range since 1697. Management practices are minimal and a small amount of

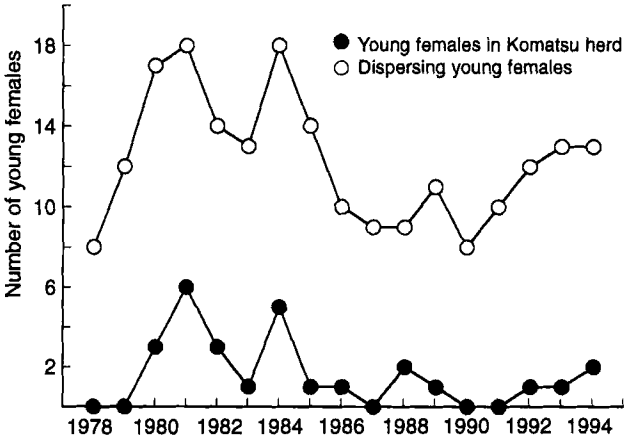

Fig 3: Changes in the total number of young females under age 3 years in Komatsu herd and the total number of young females which moved from Komatsu to Oogi between ages one and 3 years. A positive and significant correlation was found between them (r_S = 0.786, n = 17, P<0.01).

supplementary grass is fed during severe winters only. Since 1972, the range has been patrolled daily, in order to record any births, deaths or accidents; and the population dynamics of the horses and individual reproductive success and home range of each animal have been recorded. The number of horses ranged from 73 (mature males, 8; mature females, 26; young males, 8; young females, 3; colt foals, 6; filly foals, 10; and geldings, 12) in 1979 and 86 (mature males, 14; mature females, 37; young males, 12; young females, 7; colt foals, 5; filly foals, 7; and geldings, 4) in 1994, when the present study ended (Kaseda 1981, 1983, 1991).

Most animals were observed to move between Komatsu and Oogi from late September to late March, but very little movement between the 2 areas was seen during the breeding season from early April to late August (Kaseda 1983). It is, therefore, possible to separate Misaki horses into 2 herds (Komatsu and Oogi), according to their home range, during the breeding season. A herd in this context is defined as a structured social unit made up of harem groups which followed similar movement patterns within a common home range, as proposed by Miller (1981).

During the study period the number of stallions (≥ 4 years) ranged from 3–6 in Komatsu and from 2–7 in Oogi. The number of mares (≥ 2 years) ranged from 16–30 in Komatsu and from 8–20 in Oogi. About one half of animals (1–3 years) which left their natal groups or their dams and most geldings migrated from the Komatsu area to the Oogi area in the nonbreeding season. Some harem groups which were formed by all stallions with mature mares and maternal groups, which consisted of dams and their offspring, were found in the Komatsu area, while some harem groups, some mixed peer bands which consisted of young males and females, and a few gelding groups were seen to live in the Oogi area during the study period (Kaseda 1991). In Misaki feral horses, almost all mature mares were found to have stable consort relations with their harem stallions. However, some mares had no stable consort relations with any particular stallion and were observed to wander solitarily or with their offspring, remaining with particular stallions in order to mate during oestrus only transiently for a few hours or days (Kaseda *et al.* 1995). Maternal groups were usually formed by such wandering mares and their offspring. A few males were castrated because of their mixed breed during their third autumn from 1973 to 1979.

TABLE 2: Change in number of females in 5 group types relating to age

	Age (year) of female			
Group type	0	1	2	3
Natal harem	48	19	5	1
Mother[a]	10	14	3	
Other harem[b]		13		
Mixed[c]		12	30	25
New harem			20	30
Died				2
Total	58	58	58	58

[a]dam or dam and her offspring.
[b]dam and other stallion than natal harem stallion.
[c]nonreproductive young males and/or females.

Such geldings survived longer than intact males and had totalled 14 (60% of mature males) in 1980 and decreased gradually to 4 (22%) in 1994. Numbers of investigated young animals were 29 males which had survived to the age of 3 years and 58 females which had survived to the age of 2 years during the study period.

Field observation of social behaviour

Misaki horses were identifiable by their coat colour, sex, body size, markings and the number branded on the hip (Kaseda 1981). Every animal had been recorded individually in the stud book since 1972. Field observations were made twice or more a month between 1979 and 1987 and once a week between 1988 and 1994. When a horse was sighted in the field, the location and branded number of the animal, and its behaviour were recorded on field maps. In this way, data on members of social groups were collected throughout the study period. Detailed observations were made on the social behaviour, such as social relationships between stallions and mares and their offspring, group membership, and harem group formation of young stallions and mares.

Definitions of stability of consort relations between harem stallions and mares.

The horses were observed at least 8 times in the breeding season from early April to late August (at least once in April and August, and at least twice in May, June and July). A consort relation between a harem stallion and a mare was defined as stable if a particular mare was seen with the same stallion on every observation throughout the breeding season of one year. Consort relations were defined as unstable if a mare was found to change stallions or if she was seen to live solitarily or with her offspring on every observation.

Statistical analysis

Data are presented as means ± s.d. We analysed the data with 'Statview' and employed Spearmans rank correlations (r_s), Chi-squared tests on 2 x 2 tables and Fisher's exact probability test to estimate the relationship between 2 variables. The significance of all tests were established at $P<0.05$.

Results

Natal dispersal

Almost all foals were born in harem or maternal groups consisting of dams and their offspring. Out of 29 males which survived to the age of 4 years, 8 left their natal groups or dams at age one year, 14 at age 2 years and 3 at age 3 years, and all joined mixed groups. Six and 12 young stallions formed their stable harem groups at age 4 and 5, respectively (Table 1). Seven young stallions died between age 4 and 5 years. Out of 58 females which survived to age 3 years, 12 left their natal groups or dams at age one year and 18 at age 2 years (Table 2). Twenty 2-year-old females joined newly formed harem groups or existing harem groups and kept stable consort relations with their harem stallions. Thirty young mares (53%) became stable harem members at age 3 years. Eight females did not leave their natal or maternal groups at age 2 years and one female remained within her natal group until age 3 years, but no males and females were found to remain in their natal or maternal groups after age 4 years. All of the mares which remained in their natal groups after maturity (age 2 years or older) did not foal in their natal groups. Sex difference in age of dispersal from natal or maternal groups was not significant (males, 2.0 ± 0.86 years, n = 28; females, 1.98 ± 0.10 years, n = 58; t = 0.102, df = 84, $P>0.05$).

There were significant correlations in dispersing males and females, age one and 2 years, and both of siblings (males: 17 out of 20, $Ch^2 = 13.487$, df - 1, $P<0.001$. Females: 29 out of 39, $Ch^2 = 4.963$, df = 1, $P<0.05$).

Natal emigration

Investigation of movements of young animals between Komatsu and Oogi during the study period showed that animals under age 3 years moved more frequently from Komatsu to Oogi; conversely, horses age 4–6 years moved more often from Oogi to Komatsu (Fig 1). Five stallions which moved from their natal area (Komatsu) to Oogi between ages 1–3 years developed to their sexual maturity and then returned to their natal area and successfully formed new harem groups. The total number of young males which moved from Komatsu to Oogi between ages 1–3 years was found to correlate negatively and significantly with the total number of males under the age of 3 in the Oogi herd ($r_s = 0.517$, n = 17, $P<0.05$), but not with the total number of young males under the age of 3 in the Komatsu herd ($r_s = 0.455$, n = 17, $P>0.05$) (Fig 2). The total number of young females which moved from Komatsu to Oogi between age 1–3 years was found to correlate positively and significantly with the total number of young females under the age of 3 years in the Komatsu herd ($r_s = 0.786$, n = 17, $P<0.01$).

Differences in the age at which males (mean ± s.d. 1.93 ± 0.884 years, n = 15) vs. females (mean 2.04 ± 0.903 years, n = 22) first emigrated from their natal area were not significant (t = 0.362, df = 35 $P>0.05$). A significant difference was found for males in the ages at which young animals under age 3 years left their natal groups between nonemigrants (mean 2.57 ± 0.507 years, n = 14) and emigrants (1.60 ± 1.01 years, n = 15) (t = 3.292, df = 27, $P<0.05$), but not for females (mean 2.05 ± 0.695 years, n = 38) and (1.85 ± 0.587 years, n = 20) (t = 1.110, df = 56, $P>0.05$). Two of 15 emigrating males and 5 of 14 nonemigrating males died at age 4 or 5 years, but there was no significant correlation between emigration and death ($Ch^2 = 1.981$, df = 1, $P>0.05$) (Fig 3).

Relationship between emigration and harem formation

All 13 stallions which dispersed and survived to age 5 years successfully formed stable harem groups, while only 5 of 9 stallions which stayed in their natal area formed stable harem

groups by age 5 years. A significant correlation was found between emigration and harem formation ($Ch^2 = 7.062$, df = 1, $P<0.01$).

Seventeen mares emigrated from their natal area at age one year and only 2 of these joined existing harem groups, while 18 of 41 mares which stayed in their natal area until age 2 years joined existing or newly formed harem groups. Moreover, only 7 of 21 mares which emigrated at age 3 years were able to join existing or new harem groups, while 23 of 35 mares which stayed in the natal area until age 3 years formed stable consort relations with new harem stallions or existing harem stallions. There was a significant correlation for mares between residence in their natal area and formation of a stable consort relation ($Ch^2 = 5.494$, df = 1, $P<0.05$ at age 2 years; $Ch^2 = 5.533$, df = 1, $P<0.05$ at age 3 years).

For 2- to 3-year-old mares, a positive and significant correlation was found between stability of consort relation between mares and harem stallions and foaling rate in the next year ($Ch^2 = 7.999$, df = 1, $P<0.05$).

Discussion

Cause of natal dispersal

Individuals are generally born in permanent stable harem groups in wild and feral equids (Klingel 1969; Feist and McCullough 1975, 1976; Salter and Hudson 1982), but Misaki feral horses are sometimes born not only in harem groups but also maternal groups which consist of a dam and/or her offspring. Natal dispersal should therefore be observed in maternal in addition to harem groups. Many authors report various kinds of causes for dispersal from natal groups, but they are not always consistent even in equids. It is often reported that oestrus or onset of sexual maturity relates to female dispersal (Klingel 1969, 1982; Tyler 1972; Penzhorn 1984; Berger 1986; Rutberg and Keiper 1993). It seems that young oestrus mares are abducted by stallions other than their natal stallions, or that they often meet with a certain amount of aggression from their dams (Klingel 1982). However, some authors report that yearlings or 2-year-olds left their natal bands (Salter and Hudson 1982; Keiper 1986) and joined other mixed groups. Twelve one-year-old females were observed to leave their natal or maternal groups and join other mixed groups in the present study. Dispersal from natal groups is reported to be attributable to the birth of new siblings (Klingel 1969; Penzhorn 1984).

Almost all one- to 2-year-old males and females left their natal or maternal groups around the birth of their siblings and significant correlations were found between natal dispersal and birth of siblings in the present study. Detailed observations on the behaviour of dispersers around the birth of their siblings is needed to make clear the relationships between dispersal and birth of a sibling. However, it is probable that dispersal from the natal group may be affected, to a certain extent, by the birth of a sibling. Most mares are found either to threaten or avoid their yearlings or older offspring immediately before parturition when they approach to suck (Tyler 1972), but yearlings are sometimes allowed to suck if the mare failed to breed or lost its foal at an early age (Tyler 1972; Kaseda *et al.* 1984). These threatening or avoiding behaviours of mothers toward their offspring may be in preparation for their newborn foals and have the function of weakening or cutting the bond between dam and offspring (Klingel 1969).

It is reported that the number of intraband playmates influenced the ages at which dispersal occurs in Plain's zebra (Klingel 1969), but there was no association between the number of peers and dispersal age for feral horses in the Great Basin (Berger 1986). However, Rutberg and Keiper (1993) reported that male dispersal age was strongly and significantly correlated with the number of peers in the natal group and males dispersing with peers were significantly older than males dispersing without peers. On the other hand, some males who remained in their natal groups after the birth of their siblings were thereafter observed to leave with the siblings (Kaseda *et al.* 1984). However, the relationships between dispersal and number of peers in the natal group were not difficult to analyse because of very few examples in which dispersers had their playmates in the same group. Eighty-six per cent of females and males left their natal groups by age 2 and 3 years, respectively, but no definite proximate causes were found to explain every dispersal from the natal group. It is possible in wild and feral equids that the attachments or bonds of almost all young animals with their parents or dams may be gradually weakened by the mother's rejection of nursing or antagonistic behaviour of parents at the births of their siblings and their dispersals may be caused by such triggers as inducement of young stallions or playmates.

Cause of natal emigration

Dispersal from the natal group does not always mean dispersal from the natal area, because the natal area is usually in the home range of their parents in nonterritorial harem-dwelling equids. About a half of one- to 3-year-old males and females emigrated from their natal area to another area during the period of study. Young animals were mainly observed to move from Komatsu to Oogi because the number of foals in the Komatsu herd exceeded that in the Oogi herd until 1986, but only a few migrations between 2 areas were found thereafter because of the nearly equal number of young animals in both herds (Kaseda et *al.* 1991).

The highly negative correlation between total number of migrating males from the Komatsu herd and total number of young males in the Oogi herd indicates that migration of young males may depend on their density in the destination, in other words, that young males may emigrate when there are a few rival young males in their destination. On the other hand, a highly positive correlation was found between total number of emigrating young females from the Komatsu herd and the total number of young females in the Komatsu herd. This indicates that female emigration may depend on the density of the young of the same sex in a natal herd, and some of them may emigrate when density increases in their natal herd. Many authors report that dispersal is density-dependent and is related to regulation of population and improvement of reproductive success (Greenwood 1980; Reiter *et al.* 1981; Berger 1986; Flowerdew 1987; Clarke and Low 1992). Both sexes of the young animals seem to disperse in accordance with the density of same sex animals in their natal herd or destination (Kaseda 1991).

Effects of natal emigration on harem formation

Some remarkable sex differences were found in dispersal from the natal area in Misaki horses. More dispersing young males left their natal groups at a younger age and formed a new harem groups in their destination than did residents who did not disperse. Male dispersers exceeded residents in survivorship and ability to form harem groups. To summarise, it is possible that young potential males may actively leave their natal area for another area where they have a few rivals over mates and where

there is a high possibility of forming new harems, while 2- and 3-year-old resident females more easily joined existing or new harem groups and formed more stable consort relations with their harem stallions than dispersing females. It seems likely that young females who are socially subordinate and less potential in their natal herd may tend to disperse from their natal area to avoid competition for stallions in the existing or new harem groups, because individuals forced to disperse are frequently the socially subordinate, younger and weaker members of the population (Greenwood 1980).

Since harem stability directly affects the reproductive success of both sexes (Berger 1986; Stevens 1990; Kaseda *et al.* 1995), it is possible that natural selection would favour advantageous behaviour for the formation of stable harem groups: e.g. emigration of males from the natal area or residence of females in the natal area as observed in Misaki feral horses.

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Effect of natal dispersal on the reproductive strategies of the young Misaki feral stallions

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Abstract

We have reported previously that there are two remarkable stages between the separation from the mother and harem formation for male Misaki feral equines. The first stage, in which the young stallion commits more time to social play than to aggressive interactions, starts after the separation and is called the developmental stage. The second stage begins immediately before the young males succeed in forming their own harems, and is called the pre-harem formation stage. The present study was carried out to investigate the behavioral characteristics of the young male Misaki feral horses in the pre-harem formation stage. We observed the behavior of a total of 12 young male Misaki feral horses once a week from January 1988 to March 1997. Seven young males started the pre-harem stage in the non-breeding season, and the duration of time spent at this stage ranged between 0.08 and 0.6 years, with an average of 0.3 years. The other five started in the breeding season, and the duration of time spent at this stage ranged between 0.01 and 3 years, with an average of 1.3 years. The difference between these two averages was significant ($P < 0.05$). Seven of 12 stallions formed harems at about same age as a result of the effect of the female-biased sex ratio which makes it easier for all stallions to obtain harems at 5 years old. During this pre-harem stage, the young males have been shown to join wandering female groups more frequently than mixed sex groups, other harem groups and bachelor groups. Hence, the young males have a good chance of picking wandering females with which to establish their own harems. The results suggest that the age of separation has a great effect on the reproductive strategies in males after separation through its effect on the duration of the period between

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Keywords: Feral horse; Young stallion; Pre-harem formation stage; Reproductive strategies

1. Introduction

Misaki feral horses live in a wild-like state and have occupied parts of Southern Japan for up to 300 years. The Misaki population is far from having been unmanaged. From 1697 to 1953, the breeding sex ratio was kept at 1 male to 20–30 females, almost all colts being removed (Kaseda, 1981). But until recently, little was known about their ecology and behavior. Attention has been paid to some aspects of the social behavior of young group-living equids in different areas of the world, such as their separation from their native harem groups and the causes underlying that separation (Klingel, 1969; Tyler, 1972; Welsh, 1975; Berger, 1986; Rutberg and Keiper, 1993; Kaseda et al., 1995; Khalil and Kaseda, 1997); other studies have been carried out on the reproductive success of the young stallions after they form their own harem groups (Kaseda and Khalil, 1996; Tyler, 1972). However, few investigations have been made on the period between the separation from their natal harem groups and subsequent harem formation for feral equids. Our investigations have shown that there are two remarkably basic stages during this period. The first stage starts just after the separation from the mother in which the young stallion commits more time to social play than to aggressive interactions: this is called the developmental stage (Khalil and Kaseda, 1998). The second stage, upon which no previous investigations have been made, begins immediately before the young males succeed in forming their own harems; this is called the pre-harem formation stage. In other words, all young males eventually separate from their natal bands or mothers, but the age of dispersal can vary considerably depending on the environmental circumstances (Waring, 1983). For example, young males may leave their natal groups voluntarily and earlier, when there are few peers in the group (Klingel, 1969; Rutberg and Keiper, 1993), or the birth of a sibling may be one of the main causes of the separation of young males in Misaki area (Kaseda et al., 1984). Finally, another proximate cause of male natal emigration is forced eviction by the stallion of the natal group (Berger, 1986; Rutberg and Keiper, 1993). Almost all young males leave their natal band or mother at between 1 and 4 years old, with the average age being 2.3 years old (Khalil and Kaseda, 1997). Therefore, most stallions form harem groups at the age of 4–6 years in the Misaki area (Kaseda and Khalil, 1996), and at 6–8 years in Great Basin horses (Berger, 1986). The Misaki feral horses lend themselves to the study of the possible causes for the differences in harem formation age among the young stallions due to the uneven breeding sex ratio and because they could be observed individually as a result of the small area of Cape Toi Island.

The objective of the present study was to provide an overview of some of the behavioral characteristics of the pre-harem formation stage. We analyzed the correlation between the different stages of the young males and their separation from their natal band until they formed their own harems, the effect of the female-biased sex ratio on the

behavior of the young stallions and suggest that the time of separation and the reproductive strategies of males for harem formation are related.

2. Materials and methods

2.1. Study location

Cape Toi, at approximately 5 km^2, is located at the southeastern end of Kyushu Island. There are two hills in the center of the Misaki range; Komatsugaoka hill (287 m above sea-level) and Oogiyama hill (295 m above sea-level). Numerous valleys (both small and large) are found throughout the range, dividing it into two areas, the Komatsu and Oogi areas, which are similar in size, and topographical and ecological characteristics. These hills are covered with various (kinds of) native grasses, and other areas of the range are mostly occupied by 'artificial' forests with Japanese cedars and a variety of copses (Kaseda and Ogawa, 1982).

The climate is moderate, with temperatures ranging from 4°C to 15°C in winter and from 24°C to 31°C in summer. June and July are rainy periods. Fresh water ponds are plentiful in the Misaki range. Generally, the warm climate and abundant rain provide lush vegetation in the summer.

2.2. Study animals and population characteristics

Misaki horses have been living freely in the range since 1697. Management practices are minimal and a small amount of supplementary grass is fed only during severe winters. Since 1972, the range has been patrolled daily in order to record any births, deaths or accidents, and the population dynamics of the horses and individual reproductive success and home range of each animal have also been recorded (Kaseda, 1981). This study was conducted from January 1988 to March 1997 and 12 young Misaki males were observed during this time. The number of horses ranged from 88 (mature males, 13; mature females, 43; young males, 9; young females, 6; colt foals, 5; filly foals, 5; and geldings, 7) in 1988 to 99 (mature males, 22; mature females, 41; young males, 11; young females, 7; colt foals, 5; filly foals, 10; and geldings, 3) in 1997, when the present study ended. In other words, Misaki female horses outnumbered males as adults and the differences of the sex ratio at birth were not significant when compared to the adult sex ratio which skewed more towards females (Table 1). Before 1980, almost all males other than males-to-be stallions had been castrated in their third autumn and left together with other horses on the pasture (Kaseda, 1981). Such geldings survived longer than intact males and had totalled 14 (60% of mature males) in 1980 and decreased gradually to 3 (14%) in 1997. In Misaki feral horses, almost all mature mares were found to have a stable consort relation with their harem stallions. However, some mares had no stable consort relations with any particular stallion and were observed to wander alone or with their offsprings, remaining with particular stallions in order to mate during estrus only transiently for a few hours or days (Kaseda et al., 1995). In the present study, every young male has been recorded individually on sketch maps since

Table 1
Sex ratio at birth (filly/colt) and adult sex ratio (mares[a]/stallion[b]) from 1988 to 1997

Sex ratio	Year									
	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Birth sex ratio	5:5	12:8	2:4	5:5	4:4	9:6	7:5	6:11	7:3	8:8
Adult sex ratio	43:13	42:13	38:13	40:12	35:13	37:13	36:14	33:16	37:18	41:22

[a]Age 2 years and older.
[b]Age 4 years and older.
There was no significance at the birth sex ratio ($P > 0.05$), but a significant correlation was found in adult sex ratio ($P < 0.05$).

1988. Almost all the horses spent a majority of their time on the grasslands from early April to late September. During the other seasons, they lived in the forests or in the weedy grasslands that face south (Kaseda and Ogawa, 1982). Individual horses were identified by their unique markings, such as coat color, sex, body size, marking, age [recorded as foal, yearling, immature (2 to 3 years old) and adult; each animal was considered to be in only one age class throughout a particular calendar year, and the age of all animals of the population is known through a birth date certificate] and the numbers branded on their hips. Because the horses were accustomed to the presence of people, they could be observed on foot from a distance of 5–15 m without affecting their normal behavior.

2.3. Field observation of social behavior

Observations of every animal had been recorded individually in the stud book since 1972. Field observations were made once a week or more during the study period. During the breeding season (from the end of March until the end of August), most of the males were found on top of the hill. Detailed observations were made on the social behavior of each group when the young males were allowed to remain within the group and to determine whether they would remain or enter any other particular group. During the non-breeding season (from September until the beginning of March), locating and observing the horses were not as easy because most of the groups had separated into small groups and then moved into the forests. Data on the behavior and movements of the young males were gathered by traversing routes through the study area on foot and by car. So when the known young males were sighted in the field, the location and branded number of the animal and its behavior were recorded on field maps. In this way, data on members of social groups were collected throughout the study period. The following behavioral categories were recorded on hand-held maps for each sighting: (1) the stability of the group and the amount of time each horse stayed in a particular group were recorded by observing their branded numbers during the association; (2) each time horses were observed associating with a particular group, the members of that group were checked to see if they were the same or different than at the last time of observation; (3) observing the stability (consort relationship) of the joining between the young stallion and the mare or mares during the breeding season. If this stability were

established, then the pre-harem formation stage was considered to have been completed from the first sighting of the joining between them. Young males which are known to have died before completing this stage were excluded from the data.

2.4. Definition of the stability of the consort relationship between the harem stallions and the mares

A consort relationship between a harem stallion and a mare was defined as stable if a particular mare was seen with the same stallion at least eight times in the breeding season of 1 year from early April to late August (at least once in April and August, and at least twice in May, June and July). Consort relationships were defined as unstable if a mare was found to change stallions or if she was seen to live solitary or with her offspring on every observation. Some mares were named wandering mares because they lived very often solitarily or with their offspring (Kaseda et al., 1995).

2.5. Demarcation of the commencement and completion of the pre-harem formation stage

The criteria used to decide whether the pre-harem formation stage had begun are as follows. Preliminary observations on the social organization of the males were made during their stay with their mothers or inside their natal band. Follow-up observations were made after their separation to make sure that separation was final (i.e., that they did not rejoin their mothers or natal band). From our investigation, the period between this separation and formation of new harems by the young males could be divided into two stages: the developmental stage and the pre-harem formation stage (Khalil and Kaseda, 1998). The developmental stage begins just after the separation of the young males from their natal band or mother. At the beginning of the development stage, according to the social groups in which they lived before they separated, the young males associated with three types of social groups. These were bachelor groups, harem groups and wandering female groups. During this period, males joined the three groups, mixed sex groups and sometimes were solitary. It was considered that these associations provided a good opportunity for males to acquire different behavioral patterns and experiences before they entered the next stage. At the end of the developmental stage, all young males become solitary for a time, suggesting the existence of a distinct phase between the developmental stage and the pre-harem formation stage, (3) the pre-harem formation stage, which starts after the males complete their transition period, and finishes when they form their own stable harems with their new mares.

2.6. Statistical analysis

The non-parametric Spearman rank correlation coefficient test was used for the correlation between the separation age and the number of days spent by the young males for the period between separation from the natal band and harem formation. Fisher's Protected LSD was used to evaluate differences in birth sex ratio and adult sex ratio,

average duration of the pre-harem formation stage in the breeding and non-breeding season. The significance of the test was established at $P < 0.05$.

3. Results

3.1. Starting season of pre-harem formation stage

As shown in Table 2, among all males, the duration of the pre-harem formation stage varied between 0.08 and 3.0 years. This variation was attributed to the season in which this stage commenced. Hence, of the 12 male horses living in the Misaki range, the period being from 1988 to 1997, seven started this stage in the non-breeding season, which began in late August and lasted until late March. Their pre-harem formation stage was characterized by a short duration which ranged between 0.08 and 0.6 years, with an average of 0.3 years. The other five male stallions commenced this stage in the breeding season, and their pre-harem stage was characterized by a long duration of time which ranged between 0.01 and 3 years, with an average of 1.3 years. The difference between the average duration of the pre-harem stages of the two season was significant ($P < 0.05$).

3.2. Social grouping association

In the pre-harem formation stage, the male horses associated with different social groups which were recognized as follows. (1) Bachelor groups which consist of two or three horses. The largest of these bachelor groups was composed of six horses. These

Table 2
Season and duration of the pre-harem formation stage

Male name	Season (beginning month)	Duration (year)	Average (year)
M23	NBS[a] (March)	0.08	0.3
M74	NBS (February)	0.1	0.3
M20	NBS (January)	0.2	0.3
M2	NBS (January)	0.2	0.3
M67	NBS (September)	0.6	0.3
M36	NBS (September)	0.6	0.3
M42	NBS (September)	0.6	0.3
M57	BS[b] (April)	0.01	1.3
M12	BS (April)	3.0	1.3
M61	BS (May)	0.9	1.3
M6	BS (May)	0.9	1.3
M16	BS (July)	1.8	1.3

[a] Non-breeding season.
[b] Breeding season.
The average age difference between the non-breeding and breeding season was significant ($P < 0.05$).

groups were formed by young males that had been separated from their natal band or their mothers, and/or by older males that had lost their harem members. (2) A harem group that consisted of a stallion and his harem. A harem consists of mares, juveniles and foals. (3) A wandering female group that is formed by young females separated from their natal band at adolescence and adult mares who had separated from their harem bands after their stallion had died. (4) A mixed sex group that consists of one or more young males, one or more young females and adult mares and their offspring. During the pre-harem formation stage, the number of days that the male horses spent associating with different social groups differed from male to male. In addition, these associations changed randomly from time to time. Furthermore, the young males associated with the same social group many times throughout this stage. Members of the same social group either remained unchanged, or were different at every observation time. The most time that young males spent during this stage was with wandering female groups, 1557 days (50.8%). The next was for associations with mixed sex groups, 843 days (27.5), then associations with other harem groups, 368 days (12%), and the shortest being the association with bachelor groups, 298 days (9.7%) (as shown in the Fig. 1).

3.3. Effects of natal separation on the reproductive strategy

The separation age among the 12 males studied here ranged between 0.7 and 3.9 years. After they separated, they began their developmental stage which was followed by the pre-harem formation stage which lasted until the males could form their own stable harems. In the present study, we found that the age of separation was correlated negatively but not significantly with the duration of the period between natal emigration and harem formation among all young stallions (Spearman $r_s = -0.492$, $n = 12$, NS). For example, the young males, M67, M16, M20 and M61, separated from their natal band at less than 2 years old and they formed their own harems at the average of 3.8 years after their separation. Other young males, M36, M74 and M42 separated at between 2 and 3 years old but the average duration spent until they had their harem formation was 2.9 years. The last five young males, M12, M57, M2, M6 and M23,

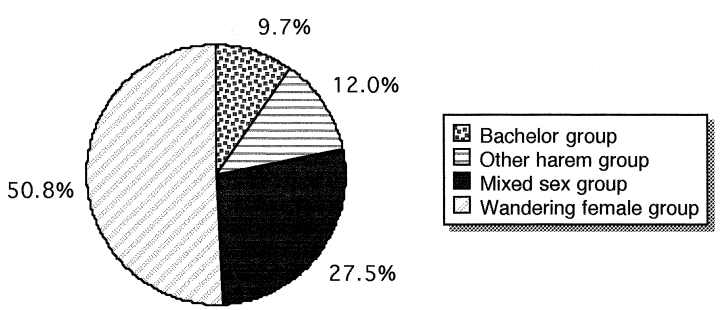

Fig. 1. Duration of the association between young males from different social groups during the whole period of the pre-harem formation stage.

Table 3
The effect of the separation age on the period between natal separation and harem formation of the young stallions

Age (years)	Male name	Separation age (year)	Harem formation age (year)	Period between separation and harem formation (year)
0–2	M67	0.7	4.9	4.2
	M16	1.3	4.9	3.6
	M20	1.5	5.9	4.4
	M61	1.7	4.7	3
	Average*	1.3	5.1	3.8
2–3	M36	2.7	4.9	2.2
	M74	2.7	4.8	2.1
	M42	2.8	7.2	4.4
	Average*	2.7	5.6	2.9
3–4	M12	3.2	6.9	3.7
	M57	3.2	3.8	0.6
	M2	3.8	4.9	1.1
	M6	3.8	7.6	3.8
	M23	3.9	4.9	1.0
	Average*	3.6	5.6	2

*The average period between the separation and harem formation among the different classes of age was not significant ($P > 0.05$).

separated at more than 3 years old and formed their harems at an earlier average age of 2 years. Also, after separation, we find that regardless of age of separation and other variables, 7 of 12 stallions formed harems at about the same age (Table 3). Moreover, during our study period from 1988 to 1997, we found that more than 70% of the young stallions formed their harems at the age of 5 years old (Table 4).

Table 4
The appropriate age of the new harem formation by the young stallions from 1988 to 1997

Year	Number of young stallions at different age (year)			Young stallions succeed in acquiring harems at different age (year)		
	4	5	6	4	5	6
1988	1	1	2	–	–	1
1989	2	1	1	1	1	1
1990	3	1	–	–	1	–
1991	1	3	–	–	2	–
1992	4	1	1	–	1	–
1993	5	1	–	–	1	–
1994	1	5	1	–	4	1
1995	3	1	1	–	–	–
1996	2	3	1	–	1	–
1997	5	2	1	–	1	1
Total	27	19	8	1	13	4

4. Discussion

4.1. Starting season of pre-harem formation stage

Some researchers report that some harem groups become unstable and break into smaller units during the late fall and winter (Keiper, 1976; Miller, 1979). Furthermore, in the Misaki area, since the grass becomes poor in both quality and quantity and the grazing capacity tends to decrease after September, most horses are forced to transfer from the grasslands to other areas where there is enough food for the winter, in addition to water and shelter. Therefore, Misaki horses are usually forced to separate into numerous small groups and then move to their winter habitats (Kaseda, 1983; Kaseda and Khalil, 1996). Moreover, some mares have different winter habitats from their harem stallions, and live alone or with their offspring in their winter home range (Kaseda et al., 1995). So, 7 of 12 young Misaki feral stallions observed in the present study began their pre-harem formation stage in the non-breeding season which began in early fall and ended in late spring. During this time, there were many wandering females who had become separated from their harems, and joined mixed sex and wandering female groups, thus giving the young stallions a good chance to associate with a wandering mares to begin their own harems. Consequently, these young stallions spent only a short time in this stage, with the average time being 0.3 years for acquiring mares as the nucleus of a new breeding herd. These new herds established a stable consort relationship between the young stallions and their mares during the following breeding season. By contrast, the other five young stallions began this stage in the breeding season. During the breeding season, these young stallions tried to establish their own harems by stealing females from neighboring harems and mating with them, regardless of whether the females were in estrus or not, or of whether they were associated with other mares. However, these types of newly formed breeding bands are often unstable and the young stallions were displaced after only a few weeks or months. These young males spent a long time in the pre-harem formation stage, with the average being 1.3 years until they were successful in forming their own new harems.

4.2. Social grouping associations

During the pre-harem formation stage, the young stallions had many social associations with the different basic types of social groups (see Section 3), and they joined and then separated from these groups several times. Sometimes, the group consisted of the same members and sometimes of different members, giving each stallion a good chance to have various experiences that may have allowed him to bluff his way through fights and control his harem in future. Moreover, among these associations, the most frequently observed throughout this stage was that between the young male and the wandering female groups and the lowest one with the bachelor groups. Otherwise, in the developmental stage which starts just after separation, the young males have neither the prowess nor fighting skills needed to dominate stallions and achieve successful mating. So, they join bachelor groups more than other social groups to develop skills. However, in the pre-harem formation stage and after the young stallions have completed their

previous stage, they usually attempt to herd various mares before eventually succeeding in establishing a herd of their own through joining wandering female groups. In addition, throughout this stage, mutual recognition may have contributed to the attraction between two animals because the males were seen with the same groups with the same members many times during this stage.

4.3. Effects of natal separation on the reproductive strategy

Parental care of foals is basically given by the mother. Quickly after birth, the mother develops a close association with her foal and then provides it with its nutritional and productive needs (Waring, 1983). Moreover, the mare has a uniquely close relationship with the newborn, refusing to leave it from the moment of birth (Fraser, 1992). Our observations indicated that the duration of the period between emigration of the young males from the natal groups or mothers and harem formation is affected by the age of separation. For example, the males who leave their natal groups at less than 2 years of age are obviously not yet adult (Berger, 1983). Thus, they had less experience as to how prepare themselves to acquire mares by short and easy ways and spent a longer time until they successfully established their own harems than other males that separated later. Commonly, after weaning, the mares and her offspring maintain some degree of companionship that may last into adulthood or only until the offspring becomes sexually mature. Therefore, the young stallions acquire many essential ingredients for normal development such as, survival, play fighting, engagement in mutual grooming, mounting attempts, skills they will need to be successful adults, and were able to exhibit a strong attraction to any available mares (Tyler, 1972; Waring, 1983; Kiley-Worthington, 1987). Furthermore, remaining with the mother for a prolonged period is a good opportunity to play more with numerous peers in the same natal band. These results are also consistent with the proximate hypothesis of Bekoff (1977) that young animals with the least interaction with peers develop the weakest ties to the group, and disperse earliest, suggesting that peer interactions may be an essential component to the development of fighting skill and hence, the achievement of a harem after short time. In addition, in Misaki area, the young stallions formed harem groups between 4 and 6 years of age (Kaseda and Khalil, 1996), and the population of Misaki horses is highly female-biased sex ratio, suggesting that the skewed sex ratio may make it easier for all stallions to obtain harems at the same age. Hence, we found that the relationship between mares and their colts before separation had a great effect on the reproductive strategies in males after separation which leads to shortening of the period until they acquire their own new harems.

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Relashionship between Plasma Testosterone Concentrations and Age, Breeding Season and Harem Size in Misaki Feral Horses

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ABSTRACT. Jugular vein blood samples were collected from 23 young and sexual mature feral stallions to examine the relationship between plasma testosterone concentration and age, breeding season or harem size. Testosterone concentration increased with the age of the stallions until they formed their own harems, at about 4 to 6 years old. Seasonal variations in testosterone concentrations were observed, and found to be significantly higher ($P<0.001$) throughout the breeding season than non-breeding season, from 3 years of age. Testosterone levels were correlated with harem size for individual stallions. It can be inferred from these results that there is a relationship between plasma testosterone concentration and age, breeding season and harem size. — KEY WORDS: equine (wild), harem, testosterone.

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Testosterone is the hormone responsible for libido in mature stallions. Domestic stallions are sexually mature at about 16 months or older [6], while wild horses take at least 3 years [10]. It has been demonstrated, in domestic horses, that immature stallions have lower testosterone concentrations than the mature stallions, and that blood testosterone levels are higher during the breeding season than non-breeding season when they are mature [1, 4, 15]. These finding have also been observed in the wild and feral horses [9, 10, 15]. High testosterone levels during the breeding season leads to seasonal changes in libido [1], followed by a marked increase in the sperm and seminal fluid production rate for increased copulation and mating in the breeding season [1, 7]. When they have formed a successful harem, changes or individual differences in harem size between stallions may possibly be influenced by some factors which affect their reproductive success such as age [9], body weight and fighting abilities [2], or dominance and aggressiveness behavior [13]. Previous studies have concentrated on seasonal changes in the testosterone levels of domestic and wild stallions, but there have been no investigations into its role in harem formation and consequent effect on changes in harem size. In this study, therefore, we at first measured testosterone concentrations before and after sexual maturation or beginning of harem formation. Then we compared levels between breeding and non-breeding season in Misaki feral horses and examined the relationship between plasma testosterone levels and harem size in individual stud horses, by sampling over two consecutive years.

The study area is located on the Misaki range (about 500 ha) where the horses live in natural conditions, without human management, throughout the year. The Misaki range is divided into two areas: the Komatsugaoka area (287 m above sea-level) and the Ogiyama area (295 m above sea-level), and numerous small and large valleys that run down in all directions from them. The climate is moderate with the temperature ranging from 4°C to 15°C in winter and 24°C to 31°C in summer. June and July is the rainy season. Fresh water ponds are plentiful in the range. The grasslands are restricted to the upper area (about 40 ha) half way up the two hills (about 290 m above sea level) which are located in the center of the range and covered with various kinds of native grasses. Other areas of the range are mostly occupied by artificial forests with Japanese cedars and a variety of copses [8]. Generally, the warm climate and abundant rain provide a lot of vegetation in summer. In the first experiment, twenty-three feral male horses, ranging from 1 to 6 years of age, were used to determine the relationship between testosterone levels and age, or breeding season. All animals were in good physical conditions during the experiment. Individual horses were readily identified by the number branded on the hip. Almost Misaki horses were individually recorded the birth date and parents (identified by DNA analysis). Blood samples were collected four times every two weeks from 23 individual horses in May and June of 1994, and mean values of testosterone for each were obtained. These data was used to relate testosterone concentrations to age for the breeding season. Similarly blood samples were collected four times every two weeks in October and November of 1994, to provide data for the non-breeding season.

In the second experiment, we randomly selected 7 males at different ages (4–9 years old) with or without a harem, and examined the change of harem size and testosterone levels during two Breeding seasons from 1993 to 1995, in order to investigate their relationship. Blood samples were collected from the 7 individual males in May of both 1994 and 1995 and harem formation was observed. The horses were extremely dangerous to handle and were restrained with Xylazine as recommended by Dodman [5]. A dose of 4 mg/kg body weight was injected using a special syringe, fired from a blowgun at a few meters from the horse (hind quarter), for accuracy. Some of the horses never adjusted to the restraint procedures and so we tried again on other days. Blood samples were taken by jugular vene puncture into heparinised tubes and placed on ice, plasma was obtained by centrifugation and was stored at −20°C until

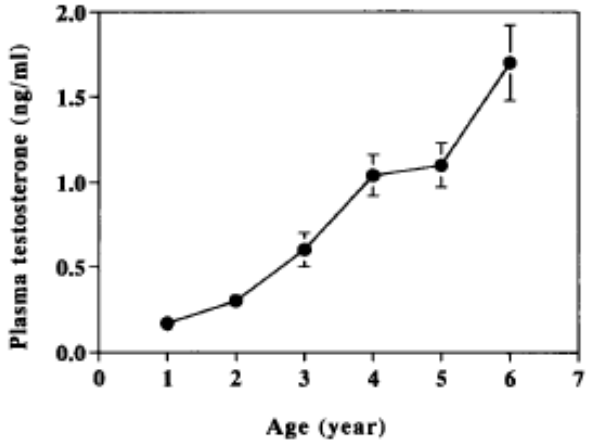

Fig. 1. Plasma testosterone concentrations at different ages in Misaki feral horses. The symbols and vertical bars represent mean ± SEM (n=4: 1, 2, 3, 4, 6, 7 years old; n=3: 5 years old). Blood samples were collected four times every two weeks in May and June from 23 individual horses and mean values were obtained, for each. Harem formation was observed in horses over 4 years old. Testosterone levels increased linearly except for in 5 years old. No significant differences were observed in analysis of values of 1 vs 2 years old, 4 vs 5 years old.

analysis.

Plasma testosterone concentration was measured by modified radioimmunoassay [14]. The specific antibody to testosterone was a gift from Dr. Etoh (Miyazaki Univ., Japan). Androstenedion (3.1%) was the only steroids, other than testosterone, with any significant reactivity. The ^{3}H-testosterone was purchased from New England Nuclear (England). The free and antibody-bound testosterone were separated by the dextran coated charcoal method. The lower and upper detection limits of the assay were 3.15 and 2,000 *n*g/tube, respectively. The intra-and inter -assay coefficients of variation for plasma testosterone were 14.5% and 5.9%, respectively. Data was evaluated by analysis of variance (Anova) and tested for significance with Duncan's new multiple range test.

The plasma testosterone of 23 young and sexually mature males in the middle of the breeding season had increased as a result of an increase in age from 1 to 6 years old (Fig. 1). These data included all samples from stallions with or without harems. However, stallions at 3 years old and younger did not appear to form harems. There were significant differences in testosterone concentration between the age groups ($p<0.001$). The testosterone levels were very low for 1 and 2 years old, and thereafter rapidly increased for 3, 4 and 6 years.

Figure 2 shows testosterone concentrations in the non-breeding and breeding season among different ages of male horses from 1 to 6 years old. Although no significant seasonal differences were observed in stallions of 1, 2 years old, all plasma testosterone levels were higher in the breeding season than non-breeding season, over 3 years of age. These differences were significant ($p<0.01$).

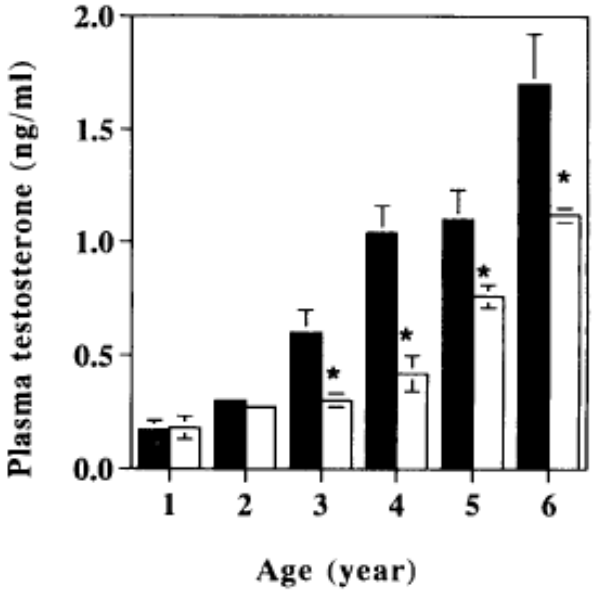

Fig. 2. Comparison of plasma testosterone levels between breeding and non-breeding season in Misaki feral horses. The black and white bars represent the mean value of breeding season and non-breeding season, respectively. The vertical bars represent the standard error. All samples were collected from the same horses as shown in Fig. 1. The blood samples for non-breeding season were collected four times every weeks in October and November. The asterisks represent the significant differences vs those of breeding season.

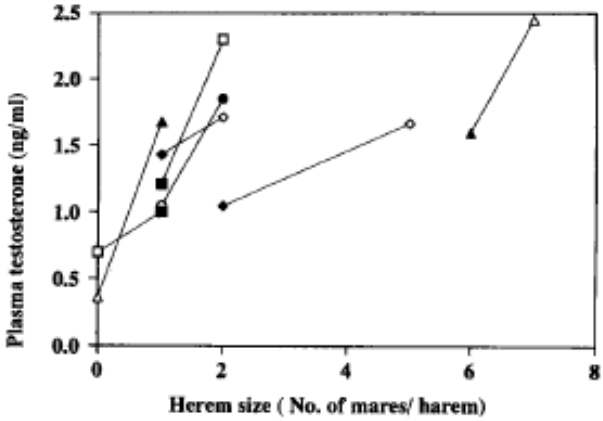

Fig. 3. Relationship between plasma testosterone levels and harem size (number of mares/ harem). Blood samples were collected from 7 individual males over 4 years old in May of both 1994 and 1995. The white and black symbols represent the testosterone value of the first and second year, respectively.

In the second experiment, 2 out of the 7 males were bachelor in the first breeding season, but acquired mares in the next season. A further 4 out of the 7 males decreased the number of mares in their harems in the second breeding season. The remaining males increased its mares from 6 to 7. In theses cases, as shown in Fig. 3, plasma testosterone concentrations increased and decreased in parallel with the

increase and decrease in the number of mares.

Generally, domestic stallions attained their sexual maturity after 16 months old [6], while the wild stallions became sexually mature at 3 years or older [11]. In both cases the testosterone concentrations in immature stallions were minimal compared to mature stallions. This is consistent with our results from feral horses which were mature at 3 years, because the young male horses had significantly lower testosterone concentrations than the mature horses ($p<0.05$). Also, the plasma testosterone concentration increased steadily with advancing age, suggesting a relationship between its concentration and the age of males up to harem formation, which generally starts from 4 to 6 years in Misaki feral stallions [9].

The finding of the present study is that the 1 and 2 year-old males had a lower serum level of testosterone and a similar level in the non-breeding and breeding season. These males were still immature with a reduced libido, which is consistent with the results of Chaudhuri and Ginsberg [3] who noted that males that have access to females, excrete more androgen than non-breeding males. Whereas, mature stallions (3 years or older), have a seasonal pattern of testosterone concentrations which were significantly higher in the breeding than the non-breeding season ($p<0.01$). These results are consistent with the hypothesis that seasonal changes in plasma testosterone can be correlated with seasonal frequencies of mating, copulation and seasonal variations in libido. It may therefore be suggested that this behavior, displayed in feral horses, may be an androgenic effect [16].

Testosterone concentrations can be shown to be closely associated with harem size, from our results. Plasma testosterone concentrations increased and decreased in parallel with an increase and decrease in the number of mares. Although there were few data, plasma testosterone was at very low levels in bachelors ($n=2$). These observations are in agreement with the data reported by Kirkpatric, *et al.* [10] who showed that testosterone concentration of bachelor stallions was significantly lower than of stud stallions. These results are consistent with the hypotheses that the presence of females may be a social stimulus influencing testosterone secretion [3], and that individual differences in harem size among feral stallions may be influenced by testosterone concentration, which affect the reproductive success of stallions. However, there may be many complex factors involved in determining harem size, such as avoidance of the parent-child relationship, strength of aggression of males, mental factor of mares. Therefore, although the testosterone levels may be associated with harem size, it is not known whether they are a predominant factor for harem size.

In this study, we used chemical immobilization to collect the blood samples for determination of the net testosterone value. However, their significance may not justify the chemical immobilization of wild creatures. We are now trying to carry the study non-invasively by means of fecal hormone measurements.

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Habitat use and spatial dynamics of takhi introduced to Hustai National Park, Mongolia

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Abstract

The successful introduction of captive bred takhi or Przewalski's horse, *Equus ferus przewalskii*, into Mongolia in the 1990s is a good example of the benefits of ex situ conservation and one of the few examples of the recovery of an animal after it became extinct in the wild. This is also particularly interesting because virtually nothing was known about how takhi lived before they died out, and the introductions have enabled us to study how they have settled, and their ecology and behaviour within their former natural range. In this paper, we describe the movement, home range size and shape, and habitat use of takhi at one of the release areas, the 570 km^2 Hustai National Park in Mongolia. Harem home ranges varied between 129 and 2399 ha, with 80% core areas of between 61 and 1196 ha. There was no relationship between range size and harem size, or length of time since release. Initially, harems stayed near their release enclosures, but over time they established home ranges further away. There was little overlap between home ranges of different harems, but neither was there evidence of exclusive range use. The more nutritious vegetation at lower elevations was preferentially selected. Thus the present situation looks good, but, as the population continues to grow, we anticipate that there will be potential problems related to intraspecific competition for water and vegetation resources, and the potential for hybridisation with domestic horses belonging to the local people. We consider the time it may take for takhi to reach carrying capacity within Hustai National Park and emphasise that continual monitoring of the population is essential because interventional management is likely to be required in the future.
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Keywords: Home range; Habitat use; *Equus przewalskii*; Takhi; Reintroduction

1. Introduction

Collections of animals and plants are increasingly being seen as opportunities for ex situ conservation, notably with respect to captive breeding programmes and subsequent release of captive-bred animals back in to the wild. However, to date there have been few successful examples of such a strategy; approximately 27% of the 116 reintroductions considered by Fischer and Lindenmayer (2000) were classified as failures, with the success of a further 47% not known. Moreover, the respective roles of ex situ and in situ conservation strategies are open to debate (e.g. Balmford, 2000; Entwistle and Dunstone, 2000). Captive breeding and reintroductions are lengthy, complex and expensive processes (IUCN, 1995; Balmford, 2000) but may be vital for the survival of a species in the wild (e.g. Spalton et al., 1999; Kleiman and Rylands, 2002).

Before reintroduction of captive bred takhi or Przewalski horses, *Equus ferus przewalskii*, into Mongolia in the 1990s, the last authenticated sighting of a takhi in the wild was in 1969, near the Tachyn-shar mountains

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in the western Gobi desert (Bouman and Bouman, 1994). The captive population of takhi in European and North American animal collections is descended from animals captured at the beginning of the 20th Century. In 1945, only 31 horses remained in captivity, and only nine of these bred. A species survival plan was set up in the USA in 1979, followed in 1986 by a European breeding programme (Europaisches Erhaltungzucht-Programm or EEP; Bouman and Bouman, 1994). By the start of the 1990s, there were more than 1500 horses in captivity. In 1992, reintroductions began to Tachyn Tal, by the German Christian Oswald Foundation and the Mongolian government, and to Hustai National Park, by the Dutch Foundation Reserves Przewalski Horse in association with the Mongolian Association for the Conservation of Nature and the Environment (Bouman, 1998).

For animals kept in captivity for many generations, there is a potential for loss of genetic variability (Foose, 1986; Ryder, 1986; May, 1991) or attenuation in survival skills (Box, 1991; Brock et al., 1994; Shepherdson, 1994; McPhee, 2003; Mathews et al., 2005). Thus there was concern that released takhi would not be able to survive after being bred in captivity for 13 generations (Klimov and Orlov, 1982). Moreover, there was no knowledge about the ecology of takhi in the wild before they became extinct, and so it was not clear how the released animals would cope in their new surroundings.

Between 1995 and 2000, we carried out a series of studies on the behaviour and ecology of released takhi in Hustai National Park. In this paper, we report on the spatial dynamics and habitat use of the horses. Against the background of changes in population size during the study period, we consider the establishment of home ranges by the harems in relation to their release point, size, interrelationship between the ranges of different harems, and utilisation of the habitat within these ranges. Finally, we consider what might happen in the future as the population continues to increase. By examining these factors we hope to be able to provide information on a reintroduction that currently appears successful, with an aim to helping future reintroduction projects.

1.1. Study site

Hustai National Park is 150 km south west of Ulaan Baatar, the capital city of Mongolia. It covers 570 km^2 along a southwestern spur of the Khentei range of mountains (47° 41′ N, 105° 54′ E). It was made a reserve in 1993 and designated a National Park in 1999. Hustai National Park borders the Tuul River, with an altitude of between 1100 and 1842 m and a continental climate (mean annual temperature = 0.2 °C and yearly rainfall = 270 mm, most of which falls in the summer; Wallis de Vries et al., 1996). During the study, horses were observed in temperatures of between −14 and +34 °C. Hustai National Park has a mountain forest steppe habitat that consists of steppe, meadow, grassland, shrub and woodland communities (Wallis de Vries et al., 1996). Water flows down most of the major valleys as streams, which are permafrost fed and covered by gravel at places along their length.

1.2. Study animals

Between 1992 and 2000, 84 horses were brought to Hustai National Park from reserves in Europe, where they had been kept in large (over 40 ha) grassy enclosures. The horses were released as a harem into acclimatisation enclosures of 40–45 ha; these enclosures were a minimum of 4 km apart and visually separated from each other. Although most of the horses had been kept together in Europe and were released together, others were introduced on arrival in Mongolia. Harems are referred to by the name of the dominant stallion (see Table 1). One harem was given a hard release (Turgen's harem), but other releases followed a 6–24 month acclimatisation period. Up to four harems other than the ones studied were also present towards the end of the study period (see Table 1).

2. Methods

Mongolian rangers and biologists gathered positional data of the harems at Hustai National Park for the full year from 1995 to 1997, and for the winter months (December to March) from 1998 to 2000. Detailed observations were carried out by the first author between June 1998 and July 2000, totalling 860 h. The standard procedure was to find a harem at dawn and follow it until 1400 h. The same harem was located at 1400 h the next day and followed until dusk. Every 10 min during an observation session the position of the harem was recorded with a Global Positioning System and marked on a large-scale map, together with weather details. Once accustomed to the presence of the observer, the horses were watched on foot from a distance of about 15 m and their behaviour recorded; these results will be considered elsewhere (see King, 2002).

The per capita growth rate per year for harems with more than three years of census data have been estimated using the formula $r = [\ln(N_t/N_0)]/t$, where N_t is the number of animals alive t years after reintroduction, and N_0 is the number of animals released (Komers and Curman, 2000). Where mares changed harems, for the purposes of this calculation they were included in the harem with which they were released. However, because mares move between harems, and the number of horses per harem is likely to decrease over time as the number

Table 1
Harem composition and harem range and core areas (ha) in Hustai National Park between 1994 and 1998

Harem	Year	Release enclosure	No. adults and juveniles	No. foals	Total harem size	No. fixes	Period	Home range	Core area
Paritet	1995	1	7	1	8	142	July–December	191	96
	1996		7	2	9	194	January–December	751	275
	1997		6	3	9	131	January–August	881	257
	1998*		7	4	11	423	June–October	1233	499
	1999*		11	5	16	1166	April–November	1223	408
	2000*		14	2	16	586	May–June	709	126
							Mean (SD)	832 (387)	277 (156)
Bayan	1998*	1	6	2	8	395	June–October	210	61
	1999*		8	2	10	628	April–October	1114	496
	2000*		9	3	12	315	May–June	748	320
							Mean (SD)	690 (455)	292 (219)
Margad	1999*	a	14	2	16	762	May–October	684	345
	2000*		12	3	15	270	May–June	277	126
							Mean (SD)	480 (288)	235 (155)
Khaan	1994	1	6	4	10		†		
	1995		9	3	12	194	April–December	1089	469
	1996		9	4	13	228	January–December	999	367
	1997		13	5	18		†		
	1998*		15	7	22	389	June–October	609	249
	1999*		7	4	11	199	April–June	1100	523
							Mean (SD)	949 (231)	402 (121)
Ares	1998*	2	6	3	9	144	July–October	744	240
	1999		9	0	9		†		
	2000		9	3	12		†		
Patron	1994	2	6	5	11		†		
	1995		9	3	12	159	April–December	2399	1196
	1996		14	4	18	156	January–December	1904	894
	1997		13	2	15	141	January–July	1653	718
	1998		12	7	19		†		
	1999		15	5	20		†		
	2000		16	5	21		†		
							Mean (SD)	1985 (380)	936 (242)
Turgen	1996	b	4	1	5	96	June–December	751	260
	1997		3	0	3	6	January/April	129	68
							Mean (SD)	440 (440)	164 (135)
Bohemian	1998	4	6	0	6		†		
	1999		3	0	3		†		
Mark	1998	5	7	0	7		†		
Manlai	1999	a	4	0	4		†		
Mangir	1999	a	4	2	6		†		
	2000		6	1	7		†		

Harem range area = 95% kernel estimates, core area = 80% kernel estimates. a = wild born bachelor stallion acquired mares. b = harem hard released from travelling crates near release enclosure 1. There was no difference in mean home range size or mean core area among years, irrespective of harem (home range: $F_{5,\ 15} = 0.56$, $P = 726$; core area: $F_{5,\ 15} = 0.78$, $P = 0.578$; these include the data for Ares). Mongolian rangers collected data prior to 1998. * = data collected by SRBK; † = no data.

of stallions competing for mares increases (Linklater and Cameron, 2000; Linklater et al., 2000), changes in numbers of individuals within harems do not reflect overall population growth. Thus, we also consider population growth based on changes in population numbers between 2002 and 2004 (see Anonymous, 2004); no further introductions took place after 2001.

Home range and habitat use data were analysed using Arcview 3.2. One hundred per cent and 95% kernel harem range areas were calculated (see Worton, 1989; Seaman and Powell, 1996; Hooge and Eichenlaub, 1997), the latter excluded occasional forays outside the areas. Utilisation curves were used to estimate core areas; in all cases these were 80% of the total range area (King, 2002).

ANOVA was used to test whether the yearly range areas since release changed in a systematic way. Paramatric or non-paramatric correlation techniques were used as appropriate to compare the area overlap of each yearly harem range with the range in its previous year, and the movement of range centres from one year to the next. Harem range use was examined according to season and temperature using Kruskal–Wallis tests, because the data were not normal. For analyses of seasonal and altitudinal effects on activity, movement, and range size, the yearly range or core areas for particular harems have been treated as independent.

Eleven vegetation categories or habitat types were distinguished within Hustai National Park (Wallis de Vries et al., 1996). The areas covered by the different habitat types within each home range, and those that were grazed or used for shelter or water, were measured, and habitat selection indices (Manly et al., 1993) calculated. Preferences were formulated using χ^2 tests. The seasonal (spring – March to May, summer – June to August, autumn – September to November, and winter – December to February) use of vegetation was based on data from all years and this was compared with the vegetation available to them within their total range area over all years.

3. Results

3.1. Numbers and density of horses

Of 115 foals born between 1994 and 2001 (Table 1), 57% were still alive at the end of the study reported here (see Anonymous, 2004, for an update of population numbers). Twenty-six per cent of deaths were caused by babesiosis (piroplasmosis), which particularly affected newly released horses (three died from this in 1998 and nine in 1999; previous deaths from this disease are also possible, but were not diagnosed). Most other deaths were foals (63% of all deaths). In each year (1995–2000), on average, foals made up 25% of the population, and 12% of the population died. The increase in births in 1998 was mostly produced by three free-ranging harems (19 of the 25 births). Predation by wolves, *Canis lupus*, was low with a mean of 2.8 horses taken each year, all one year old or less (16% of all foals born between 1994 and 1999). It appears unlikely that predation affected population growth at the time of the study.

In July 2000 there were 91 free ranging horses, a density of 0.2 horses km^{-2} over the entire Park. The yearly mean density of horses within a harem between 1995 and 2000 was 1.8 horses km^{-2} ($N = 24$ harems, SD = 1.3 horses km^{-2}, range from 0.5 horses km^{-2} for Patron in 1995 to 5.4 horses km^{-2} for Margad in 2000). In 1995, 1996 and 1998 the home range and size of every harem was known. The density of horses over the area covered by these harems was 0.8 horses km^{-2} in 1995, 1.2 horses km^{-2} in 1996 and 1.8 horses km^{-2} in 1998. In 1999, density of harems within Hustai National Park was 0.01 harems km^{-2} ($N = 7$).

The per capita growth rates (r) of three reintroduced harems with census data spanning more than three years were: Paritet 0.69 – 1995 to 2000, Patron 0.65 – 1994 to 2000, and Khaan 0.79 – 1994 to 1998. Thus, these harems were very successful and doubled in size over these time periods. However, Khaan lost half his harem in 1999 to Margad when numbers dropped from 22 to 11 (Table 1), and he subsequently lost the rest of his harem the year after. A more realistic indication of population growth rate can be obtained by considering population numbers after 2001, when no further introductions took place. In 2002 there were 142 horses in Hustai National Park, and this increased to 162 horses by June 2004, of which 84 were reintroduced (Anonymous, 2004). This gives an overall per capita growth rate (r) of 0.066 per year. This growth rate must be treated with caution since it is based on only three years of data.

3.2. Harem range and core areas

Between 1995 and 2000, 95% kernel harem range areas ranged from 1.3 to 24.0 km^2 and 80% core use areas from 61 to 499 ha (Table 1, Fig. 1). There was no difference in range area among years ($F_{5,15} = 0.56$, $P = 0.726$; note, horses were observed for different periods of time within each year). Both mean range and core areas were significantly different among harems (range area: $F_{5,14} = 6.61$, $P = 0.002$; core area: $F_{5,14} = 7.65$, $P = 0.001$, these exclude the single home range estimate for Ares, Table 1); mainly because Patron's mean range and core areas were significantly larger than those of the other harems (Table 1).

There was no correlation between number of fixes and size of harem range or core areas (Table 1; range area: $r_s = 0.035$, $N = 21$, $P = 0.881$; core area: $r_s = 0.092$, $N = 21$, $P = 0.691$). In addition, there was

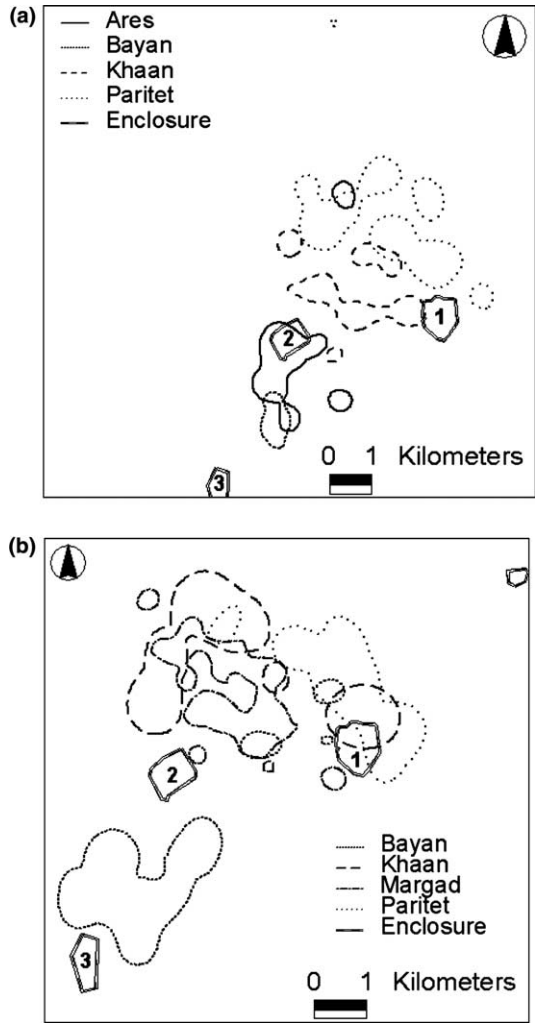

Fig. 1. Eighty percent core areas of the study harems in (a) 1998 and (b) 1999. Ranges show the core area of the entire harem, but the names of the dominant stallions are used. Paritet, Khaan and Bayan's harems were all released from enclosure 1 (in 1994, 1995 and 1998, respectively). Ares' harem was released from enclosure 2, and Margad acquired Khaan's mares in 1999.

no correlation between number of months since release and harem range ($r = 0.22$, $N = 31$, $P = 0.235$) and core area ($r = -0.01$, $N = 31$, $P = 0.976$), or between the number of adults and juveniles in each harem and range ($r_s = 0.220$, $N = 21$, $P = 0.337$) or core area ($r_s = 0.346$, $n = 21$, $P = 0.124$). Despite the small sample sizes, we tested for a year effect, but there was no significant difference in range or core area among years (range area: $H_5 = 3.56$, $P = 0.615$; core area: $H_5 = 5.09$, $P = 0.404$).

3.3. Harem range shifts between years

Overlap of individual harem ranges between years varied between 31% and 100% (mean = 59%, $N = 11$, SD = 26%) (Table 2). Overlap of yearly core areas varied between 11% and 100% (mean = 45%, $N = 11$, SD = 27%; Table 2). There was no significant correlation between the number of years since release and the degree of overlap (range area $r_s = -0.34$, $N = 11$, $P = 0.313$; core area $r_s = -0.40$, $N = 11$, $P = 0.255$;). In general the trend was for a decrease in range overlap over the years (Table 2).

The harem range centres did not move very far from one year to the next, with a minimum movement of 0.4 km and a maximum of 1.8 km (Table 3). However, distance from initial range centre after release to range centres the following years appear to show a movement away from the release enclosure. The harems tended to stay close to their acclimatisation enclosures in the year after release, then move further away to settle in a nearby valley.

3.4. Overlap between the ranges of different harems

Paritet, Bayan and Khaan were released from enclosure 1, Turgen from outside enclosure 1, and Ares and Patron were released from enclosure 2. Margad's was a naturally formed harem (Table 1). Overlap between yearly harem ranges was analysed to see whether harems moved to the same areas of Hustai National Park after release. The degree of range overlap between harems varied from 0% to 89% (mean = 39%, $N = 21$, SD = 30%); core areas overlapped 0% to 66% (mean = 18%, $N = 21$, SD = 21%) (Table 4). There was no correlation between number of years since release and amount of overlap between harem ranges ($r = -0.26$, $N = 21$, $P = 0.253$) or core areas ($r = -0.17$, $N = 21$, $P = 0.459$).

In 1995, when Paritet's harem was first released and Khaan's and Patron's had been free ranging for one year, there was no overlap between any of the core areas. Core areas overlapped in 1996 after Turgen's harem was released. Turgen's core area in 1996 and 1997 was overlapped more than any other harem except Ares' in 1998 (Table 4b). Patron's core area did not overlap with any other harem between 1995 and 1997, and did not do so during the rest of this study (SRBK, pers. obs.). The bachelor group moved among the home ranges of all (SRBK, pers. obs.).

Changes in overlap among core areas indicate range shifts and/or a change in area used. For example, Bayan overlapped with Ares in 1998, but in 1999 he used a larger area and his harem overlapped with all the other harems. The bachelor stallion Margad took most of Khaan's mares in 1999, and although they were using the same valley, their core areas only overlapped by 23%. By mid-summer, Khaan's remaining mares were taken over by another stallion and they moved to a different valley. Thereafter Margad's harem increasingly used Khaan's previous range; in 2000, 50% of Margad's core area overlapped with Khaan's 1998 core area and 57% with Khaan's 1999 core area.

Table 2
Harem home range and core area shifts expressed as a percentage of overlap from one year (column) to the previous years (row)

Year	1996	1997	1998	1999	2000
(a) Overlap of home range					
Paritet					
1995	100	93	75	89	87
1996		75	47	60	41
1997			62	64	45
1998				62	44
1999					37
Bayan					
1998				98	18
1999					33
Margad					
1999					31
Khaan					
1995	78	*	23	36	
1996		*	31	34	
1997			*	*	
1998				41	
(b) Overlap of core area					
Paritet					
1995	100	82	24	86	89
1996		79	39	78	45
1997			48	76	44
1998				47	9
1999					25
Bayan					
1998				51	0
1999					11
Margad					
1999					20
Khaan					
1995	61	*	23	4	
1996		*	34	7	
1997			*	*	
1998				19	

* = no data for Khaan harem in 1997.

In 1999 and 2000 there were four other harems in Hustai National Park (Table 1). Although there were no data for these harems, it is possible that total overlap was larger than shown in Table 4. The harems appeared to distance themselves from each other but there was no evidence of exclusive use of an area by any harem. The harems appeared to select a home range in a valley where there were no previous horses, and where the harems were separated by ridges. However, Bayan broke this trend by tending to use the same valley as Margad in 2000. Moreover, observations by the Mongolian rangers indicate that all harems sometimes use the same valley during the winter.

3.5. Habitat use within the home range

Habitat use within the home range was influenced by several factors including temperature, season, time of day and the presence of flies. Although ranges were slightly larger in winter, there was no significant difference in the size of the ranges or core areas in different seasons (range areas: $F_{3,39} = 0.49$, $P = 0.693$; core areas: $F_{3,39} = 0.62$, $P = 0.605$).

In core areas, horses were found more often at a higher altitude during the summer than in spring or autumn (there were too few data for winter; median in summer = 1500 m, spring = 1425 m and autumn = 1400 m; Kruskall–Wallis $H_2 = 613.31$, $P < 0.0001$). There was also a diurnal trend in the horses movements (Fig. 2). In the morning, when it was cool, the horses spent most of their time grazing in the valleys. As the temperature increased, the horses moved to higher elevations where they could avoid flies and direct solar radiation by standing near rocky outcrops or in forest until the weather cooled enough for them to come down to graze again. This pattern was less marked in spring

Table 3
Distance from the centre of the home range in one year to the centre in all other years since release

Year	1996	1997	1998	1999	2000
Paritet					
1995	0.5	1	2.7	1.6	1.6
1996		0.5	2.3	1.2	1.2
1997			1.8	0.8	0.8
1998				1.2	1.2
1999					0.1
Bayan					
1998				0.5	3.2
1999					2.7
Margad					
1999					1.4
Khaan					
1995	0.4	*	1.6	2	2.8
1996		*	1.3	1.7	2.5
1997			*	*	*
1998				0.9	>1.2

The arithmetic mean of all points was used to find the centre. Figures given are the distance from the centre of the row to the centre of the column.
* = no data for Khaan harem in 1997.

and autumn when the weather was cooler and the horses spent more time at lower elevations.

3.6. Vegetation use within the home range

Habitat composition of the different harems' ranges varied slightly, but all 11 vegetation classes described by Wallis de Vries et al. (1996) were grazed at some time (Table 5). However, all harems in all years did not randomly select vegetation classes (all χ^2 statistics, $P < 0.05$; Fig. 3). They most frequently selected vegetation classes from lower elevations (i.e., Meadow I, Tussock grassland and Lowland steppe; Fig. 3). Meadow I (dominated by bentgrass, sedge and *Iris lactea*) was preferred by Bayan in 1999 and 2000, and by Khaan in 1998. Although most preferred, Meadow I was not used by Khaan in 1999, and little by Margad.

Paritet's range did not include Meadow I vegetation and they preferred Tussock grassland (Fig. 3). Tussock grassland contains the needlegrass species *Achnatherum splendens* and *Stipa krylovii* which have high levels of crude protein and crude fibre (van Dierendonck and Wallis de Vries, 1993). Tussock grassland was not found in Khaan/Margad's home range.

Lowland steppe was selected significantly more than most other vegetation classes, and had a high selection index for all harems except Bayan in 1999 and Khaan in 1998 (Fig. 3). This vegetation class includes the wheatgrass *Agropyron cristatum*, which featured largely in the diets of feral horses, and is dominated by the needlegrass *Stipa krylovii*.

Both Mountain steppe vegetation classes were found in every home range, and one or both were significantly selected by the harems (Fig. 3). These were dominated by *Festuca lenensis* in Mountain steppe I and *Festuca sibirica* in Mountain steppe II. Although these species did not have the high crude protein content of needlegrass they had a higher crude fibre content (van Dierendonck and Wallis de Vries, 1993).

Grazing was observed in Woodland by most harems in most years (Fig. 3) and understorey vegetation such as *Spiraea media* and *Cotoneaster melanocarpa* was eaten rather than trees. The area covered by other vegetation classes (Upland steppe, Shrubland I, Shrubland II, Meadow II and Scrub) was small.

3.7. Seasonal use of the vegetation

The harems selected more vegetation classes in spring and autumn than in summer, and few classes were selected in winter (Fig. 4). From spring to autumn vegetation classes at mid to low elevations were used, with the exception of using woodland in the spring. In winter, vegetation at mid elevations was used. Lowland steppe was selected in every season of the year, and Shrubland I and Mountain steppes I and II were selected in every season except winter. Harems observed in winter preferred Lowland steppe. Woodland was not used at all in the autumn and winter, and Tussock grassland and Upland steppe were selected by at least two of the three harems in spring and autumn, but not in summer.

4. Discussion

4.1. Numbers

The reintroduced population of takhi increased in size during the study period, and continues to grow (Anonymous, 2004). This was despite loss of half the

Table 4
Overlap of home ranges and core areas between harems expressed as a percentage of the area covered in each year

Year	Harem	Home range area (ha)	Harem				Total % of core area overlapped
			Paritet	Khaan	Patron		
(a) Home range							
1995	Paritet	191	–	86	0		86
	Khaan	1089	15	–	4		19
	Patron	2399	0	39	–		39
1996			Khaan	Paritet	Turgen	Patron	
	Khaan	999	–	42	25	0	51
	Paritet	751	56	–	53	0	87
	Turgen	751	34	53	–	0	85
	Patron	1904	0	0	0	–	0
1997			Paritet	Turgen	Patron		
	Paritet	881	–	13	0		13
	Turgen	129	89	–	0		89
	Patron	1653	0	0	–		0
1998			Khaan	Paritet	Bayan	Ares	
	Khaan	609	–	14	0	29	35
	Paritet	1233	7	–	0	12	14
	Bayan	210	0	0	–	41	41
	Ares	744	24	19	12	–	18
1999			Paritet	Bayan	Margad	Khaan	
	Paritet	1223	–	8	6	37	40
	Bayan	1114	9	–	11	9	23
	Margad	684	10	19	–	49	66
	Khaan	1100	41	9	30	–	68
2000			Paritet	Bayan	Margad		
	Paritet	709	–	8	0		8
	Bayan	748	8	–	5		13
	Margad	277	0	14	–		14
(b) Core area							
			Paritet	Khaan	Patron		
1995	Paritet	96	–	0	0		0
	Khaan	469	0	–	0		0
	Patron	1196	0	0	–		0
1996			Khaan	Paritet	Turgen	Patron	
	Khaan	367	–	5	20	0	21
	Paritet	275	7	–	34	0	35
	Turgen	260	29	36	–	0	66
	Patron	894	0	0	0	–	0
1997			Paritet	Turgen	Patron		
	Paritet	257	–	16	0		16
	Turgen	68	59	–	0		59
	Patron	718	0	0	–		0
1998			Khaan	Paritet	Bayan	Ares	
	Khaan	249	–	4	0	0	5
	Paritet	499	2	–	0	3	5
	Bayan	61	0	0	–	33	33
	Ares	240	0	5	8	–	14
1999			Paritet	Bayan	Margad	Khaan	
	Paritet	408	–	5	0	31	35
	Bayan	496	4	–	5	1	9
	Margad	345	0	7	–	23	32
	Khaan	523	0	1	15	–	39
2000			Paritet	Bayan	Margad		
	Paritet	126	–	0	0		0
	Bayan	320	0	–	1		1
	Margad	126	0	1	–		1

Columns overlap rows. There was no difference among the harems in the overlap of home ranges ($F_{6,14} = 1.66$, $P = 0.204$), but there was a difference among the overlap of core areas ($F_{6,14} = 3.53$, $P = 0.024$).

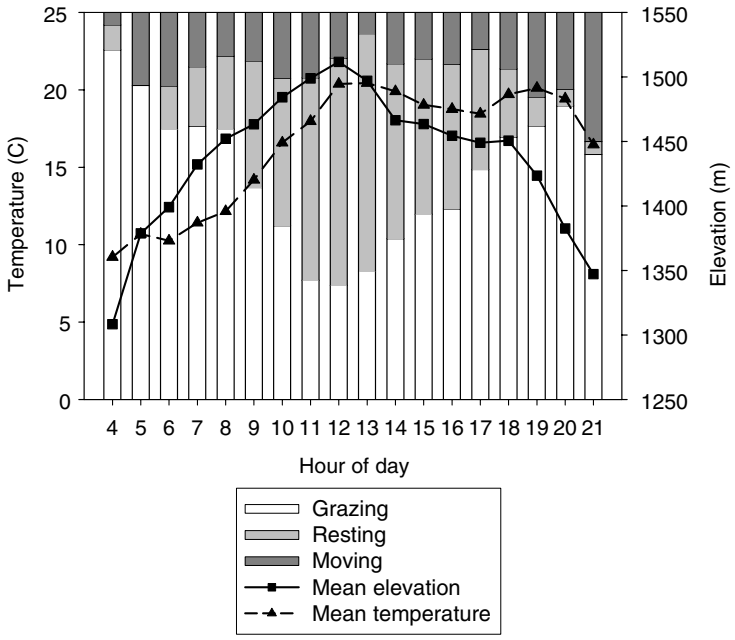

Fig. 2. Diurnal activity of the horses (% of total) according to mean elevation and temperature (SDs not included). The horses were observed in different activities at different times of day ($H = 17.38$, d.f. = 4, $P < 0.002$), and at different temperatures ($H = 577.12$, d.f. = 4, $P < 0.0001$) and different elevations ($H = 630.21$, d.f. = 4, $P < 0.0001$). The horses were found at higher elevations in the middle of the day ($H = 120.35$, d.f. = 15, $P < 0.0001$) and at higher temperatures ($H = 1279.18$, d.f. = 15, $P < 0.0001$).

Table 5
Habitat classes at Hustai National Park according to landscape features and dominant plant species (based on Wallis de Vries et al., 1996)

Habitat class	Dominant species		Landscape	Altitude	Slope
	Scientific name	Common name			
Meadow I	*Iris lactea*	Dwarf iris	Streamside	1100–1400	
Tussock grassland	*Achnatherum splendens*	Needlegrass	Valley terrace	1100–1400	
Lowland steppe	*Artemisia adamsii* *Stipa krylovii*	Wormwood Needlegrass	Footslope	1100–1400	South
Upland steppe	*Thermopsis lanceolatus* *Stipa krylovii*	False lupin Needlegrass	Footslope	1300–1500	South
Shrubland I	*Caryopteris mongholica* *Amygdalus pedunculata*	Bluebeard Sweet almond	Rocky slope	1300–1600	South
Mountain steppe I	*Festuca lenensis*	Tundra fescue	Ridge & topslope	>1300	
Mountain steppe	*Festuca siberica*	Siberian fescue	Mountain slope	>1400	North
Shrubland II	*Spiraea aquilegifolia*	Spiraea	Gully	1100–1600	
Meadow II	*Geranium pratense*	Meadow cranesbill	Combe	>1300	
Woodland	*Betula platyphylla*	Asian white birch	Mountain slope	>1400	North
Scrub	*Betula fusca*	Birch	Topslope	>1400	North

horses released in 1998 to babesiosis. *Babesia equi* and *B. caballi* are endemic to Mongolia and transmitted by the tick *Dermacentor nutalli*. Unless treated, between 25% and 100% of cases are fatal (West, 1992). However, animals can acquire immunity during the course of an infection, and mortality depends on the general immune status of the animal, and the virulence of the piroplasm (Schein, 1988). Foals can be infected during pregnancy, but gain protection from maternal antibodies (Donnelly et al., 1982). Thus local domestic horses and takhi born in Mongolia do not appear affected by the disease. In contrast, newly introduced animals with no immunity and that may become stressed for reasons such as transport, release, or poor body condition in early spring, are likely to be susceptible. In particular there is a high mortality rate during the initial infection of older animals

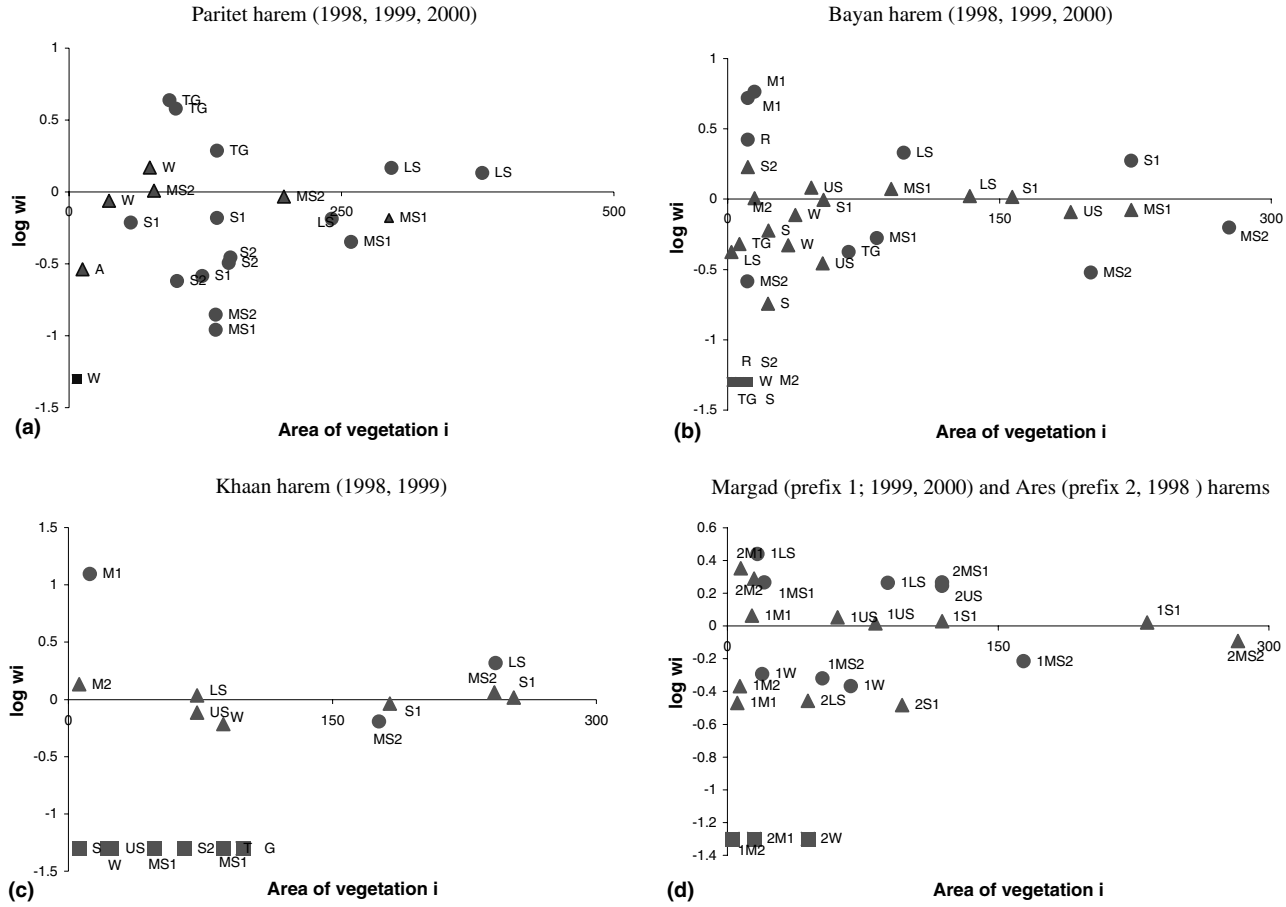

Fig. 3. Habitat selection indices (w_i, logged for presentational purposes) plotted against area of each vegetation type (i). M1 = Meadow I, M2 = Meadow II, TG = Tussock Grassland, LS = Lower Steppe, US = Upper Steppe, S1 = Shrubland 1, S2 = Shrubland 2, MS1 = Mountain Steppe I, MS2 = Mountain Steppe II, W = Woodland, S = Scrub, A = Agricultural Area, R = Rock and Stones. Habitat classification based on Wallis de Vries et al. (1996). A value of log $w_i > 0$ represents positive selection, = 0 represents no selection, <0 represents avoidance. Squares indicate habitats were not selected at all, but were arbitrarily assigned a value of 0.05 so that they appear on the figure, circles indicate w_i values significantly different to zero (χ^2 $P < 0.05$), triangles indicate insignificant w_i values or where expected values <5.

when brought from a country, such as Holland, where *B. equi* is not endemic (Schein, 1988).

Most losses to the population resulted from the death of foals. Diseases such as strangles (infection by *Streptococcus equi*) accounted for some of these deaths, as well as predation and the harshness of the Mongolian winter.

4.2. Home range use

Harem range sizes varied between 1 and 24 km^2 (mean 9.2 km^2, $N = 21$, SD = 5.64 km^2) and are similar in range to feral horse populations in Alberta, Canada (2.6–14 km^2; Salter and Hudson, 1982) and in New Zealand (1–18 km^2; Linklater et al., 2000), but smaller than those in North America (8–48 km^2; Pellegrini, 1971; Feist and McCullough, 1976; Berger, 1977, 1986; Miller, 1983a). Home ranges of horses in England (2.5–3.2 km^2; Tyler, 1972; Gates, 1979), and barrier islands (3–6 km^2; Keiper, 1976; Zervanos and Keiper, 1979; Rubenstein, 1981) were generally smaller than at Hustai National Park. In general, differences in home range size are related to vegetation quality and distribution of resources such as water and shelter (Leuthold, 1977). No trends in harem range size with time after release were found, or with harem size (cf. Zervanos and Keiper, 1979; Berger, 1986; Linklater et al., 2000), and there was no suggestion that forage was limiting. However, Mongolia suffers from periodic droughts and severe winters and these will affect the vegetation growth. It is possible that harem ranges will be smaller when forage quality is good; this was found in reintroduced Arabian oryx, *Oryx leucoryx*, whose ranges became smaller in areas where rain had fallen (Corp et al., 1998).

Similarity in size of reintroduced takhi home ranges and feral horses under similar circumstances implies not only that feral horse management techniques could potentially be applied to this population, but that they are using similar survival strategies. Thus takhi did not

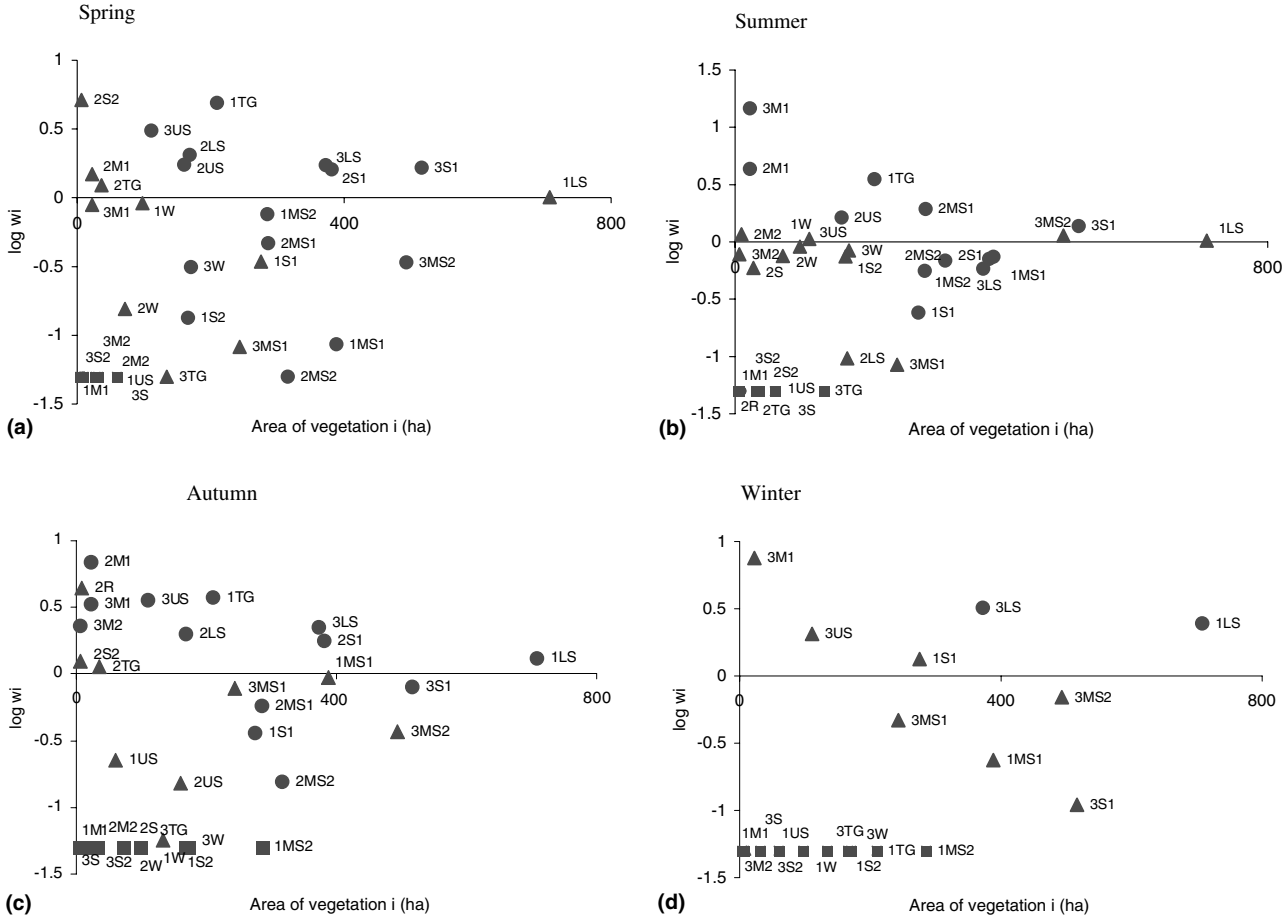

Fig. 4. Habitat selection indices (w_i, logged for presentational purposes) plotted against area of each vegetation type (i). (a) = spring, (b) = summer, (c) = autumn and (d) = winter. First number 1 = Paritet, 2 = Bayan, 3 = Khaan/Margad. M1 = Meadow I, M2 = Meadow II, TG = Tussock Grassland, LS = Lower Steppe, US = Upper Steppe, S1 = Shrubland 1, S2 = Shrubland 2, MS1 = Mountain Steppe I, MS2 = Mountain Steppe II, *W* = Woodland, *S* = Scrub, *A* = Agricultural Area, *R* = Rock and Stones. Habitat classification based on Wallis de Vries et al. (1996). A value of log $w_i > 0$ represents positive selection, = 0 represents no selection, <0 represents avoidance. Squares indicate habitats were not selected at all, but were arbitrarily assigned a value of 0.05 so that they appear on the figure, circles indicate w_i values significantly different to zero (χ^2 $P < 0.05$), triangles indicate insignificant w_i values or where expected values <5.

lose an ability to adapt to the wild despite 13 generations in captivity, just as this trait has not been bred out of horses despite 5000 years of domestication.

Characteristics of the ranges of all harems were that they included a permanent water source, an area of rocky outcrops near a ridge, and patches of forest that were used in the summer. The harems of Ares, Bayan, Khaan and Margad all overlapped at one particular place along the stream where pools were formed and the banks had been eroded to form a salt lick. However, in the main, the home ranges occupied separate valleys, defined by ridges which peaked at about 1600 m, but which also joined at passes and along watercourses.

As the size and number of harems change through time at Hustai National Park, competition for resources may increase. For example, larger harems have been shown to have priority of access to water holes (Miller and Deniston, 1979), and also tend to be found in areas of better vegetation (Miller and Deniston, 1979; Berger, 1977, 1986). Most studies on feral horses show extensive spatial overlap between ranges (Keiper, 1976; Miller, 1983a; Linklater et al., 2000), although horses appear to avoid each other temporally, as they did at Hustai National Park (King, 2002). After release, harems stayed near the release enclosure in the first year, then moved a little further away to settle in a valley. The harems selected valleys that were visually separated from each other, but as the population increases and more overlap occurs, more valleys will be colonised and smaller ridges within a valley may separate the harems. In winter harems seemed more tolerant of close proximity to each other (Mongolian rangers, pers. comm.), implying that at this time being near other harems is more important than having exclusive access to a food source. More work needs to be done in this season to examine if lack of spatial segregation between harems is for thermal reasons, or for greater protection against predation.

4.3. Vegetation use

Plants change in nutrition and palatability through the year (Novellie and Winkler, 1993), and in Hustai National Park the takhi preferred some species in their early development stages, and avoided others after they had flowered (Salter and Hudson, 1979; Duncan, 1983; Miller, 1983b; Putman et al., 1987; Enkhee, 1998). The takhi selected vegetation classes dominated by grasses and fescues which have a high fibre and crude protein content (van Dierendonck and Wallis de Vries, 1993). Although grazing was observed on all vegetation classes overall, takhi did not select all vegetation classes in every year, nor graze on all vegetation classes present.

Vegetation available to takhi affected their altitudinal movements through the year. They spent more time at low to mid elevations to shelter from inclement weather, and to feed on the most nutritious vegetation that was found in the Lowland steppe (Salter and Hudson, 1978). All the takhi had good condition throughout the year, and in the spring tended to have retained more body fat than local domestic horses (SRBK, unpubl.). As the takhi population grows there is potential for overgrazing to occur, especially on vegetation classes in the valleys that are preferred. Thus management will be needed in the future to ensure that this is kept under control.

5. Conclusions and the future of takhi at Hustai National Park

So far, the introduction of takhi to Hustai National Park seems to have been successful, although this assessment is based on a time period that only approximates one generation (see Gross, 2000). Despite this, there is little evidence that takhi have lost any ability to survive in the wild through many generations in captivity. All harems bar one were given a soft release, and maintained in acclimatisation enclosures for at least six months, thus allowing animals to get used to both the climate and disease vectors. The importance of a sufficient acclimatisation period is shown by the greater success of harems, such as Patron's, Paritet's and Khaan's, which were acclimatised for two years, compared to the those that had a hard release (Turgen's harem) or were acclimatised for only six months (Mark and Bohemian's harems). These harems suffered greater mortality, with only two horses from Turgen and Bohemian's harem surviving a year after release, and only one from Mark's. Local diseases were the main cause of mortality, suggesting knowledge of potential diseases should be obtained before animals are brought to the release site, and continuous monitoring post-release with immediate veterinary attention is desirable. Although target release sites should be surveyed for potential predators, in this case predation was not a major cause of mortality. However this is not true for all introductions (e.g., Matson et al., 2004).

The horses in this study tended to remain near their acclimatisation enclosure upon release, although they did move further away over time. Thus repeated use of a release enclosure could lead to intraspecific conflict and overexploitation of the nearby habitat. However, this does not seem to have occurred at Hustai National Park (enclosure 1 was used in 1994, 1995 and 1998, and enclosure 2 in 1994 and 1998; enclosures 4 and 5 were only used once); there was little overlap between ranges of different harems, although exclusive range use did not occur. All harem ranges at Hustai National Park contained access to a water source, and this has also been seen in feral horse studies. Releases should therefore be near a permanent water source, and this could be used to encourage animals to move further from a release enclosure. In addition shade areas and access to minerals seemed necessary for each harem. In comparison with feral horse studies, it appears that range size depends on habitat quality, and this should be taken into account in assessing target release sites. Importantly, takhi appear to use their habitat in a similar way to feral horses, so management techniques of feral populations could be applied.

It has been suggested that Hustai National Park (area 570 km^2) should be able to support a population of 400–500 horses (Bouman, 1998), equivalent to a density of 0.9 horses km^{-2} or 42 harems with the currently observed 12 horses $harem^{-1}$. However, because of the topography and availability of water, Hustai National Park may not support 500 horses. For example, in the western section of Hustai National Park there are streams in most valleys, but in the east (c. 144 km^2) there are only three ephemeral streams. Horses require a reliable water source, along with the presence of good quality vegetation and places for resting both by rocks near ridges and among trees. At present, the takhi population appears well below carrying capacity. Taking the rather tentative 2002–2004 estimate of per capita growth rate per year of 0.066, it will take 15.5 years for the population to reach a carrying capacity of 450 horses.

A future problem is the likelihood of hybridisation with domestic horses as the takhi expand beyond the Park boundary. Local people value hybrid offspring. Hybrids will need to be identified and either controlled, neutered or prevented from re-joining the population. Contraception may be a better way of limiting the population than removing animals (e.g., Gross, 2000). Future studies at Hustai National Park will provide insights into dispersal, harem formation, and the effects of intraspecific competition as the population increases. In addition studies of the horses over the winter may provide more insights into the habitat use and any

changes in social structure during this critical season. Takhi born in Hustai National Park could be used for future reintroductions in other parts of Mongolia as they will already have immunity to babesiosis and will have acquired the skills necessary to survive the varied seasons.

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Scent-marking behaviour by stallions: an assessment of function in a reintroduced population of Przewalski horses (*Equus ferus przewalskii*)

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Keywords
scent marking; Przewalski; takhi; stallion; paternity defence; Mongolia.

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Abstract

Scent marking is a common form of intraspecific communication in mammal species, and using faeces or urine is a cost-effective way of signalling competitive ability and resource holding power. Marking is ritually performed by male equids, and here we assess the function of male scent-marking behaviour in a recently introduced population of Przewalski horses *Equus ferus przewalskii* in Mongolia. Two forms of scent marking were observed: defecation on stud piles formed from repeated dunging in the same place, and overmarking of faeces and urine of mares. Stud piles were marked with dung by the harem holder and sniffed before and after dung was deposited. They were not found specifically at the periphery of harem ranges but occurred for the most part along routes used by the horses, and were more common in the core parts of harem ranges or where harem ranges overlapped. Thus, rather than being used to defend range boundaries, stud piles were placed predominantly where they would be encountered by male intruders. Mare excreta were covered with urine by the stallion, but were only sniffed before they were marked, not after. These marks appear to advertise to the mare and other, intruding stallions that the harem holder was the mare's consort and that the interloper should not risk trying to steal the mare or sneak a mating. Thus, the two forms of marking by harem holders appear to combine as first and second lines of defence of paternity rights in male intrasexual competition.

Introduction

Scent marking via the urine, faeces or scent glands (Gorman & Trowbridge, 1989) is a common form of communication in mammals (e.g. Eisenberg & Kleiman, 1972; Brashares & Arcese, 1999*a*,*b*; Begg *et al*., 2003). It is often associated with competitive ability and resource holding power (RHP) in males, but may also enable females to assess mate quality (Ralls, 1971; Trivers, 1972; Gosling & Roberts, 2001). Faecal material is particularly good for scent marking as it is relatively persistent in the environment, it has a minimal energetic cost to the signaller, and it forms a substrate for glandular secretions (Brashares & Arcese, 1999*a*). Urine is a similar cost-effective substance that can be used as a marker. Nevertheless, urine and faecal marks degrade through time and signallers must reinforce the message present by re-marking at intervals (Gosling & Roberts, 2001).

In general, scent marking occurs commonly, but not exclusively, at socially important or well-used areas, it is most often carried out by high-ranking animals, and frequently occurs during agonistic encounters (Leuthold, 1977). Marking of stud piles has been recorded in all equid populations studied (Linklater, 2000) and is normally carried out by stallions. Dung piles, also known as stud piles, are produced by repeatedly defecating at one place over a period of time. Stud piles can get quite large and it has been suggested that they act as visual signposts, especially in open areas where no other landmarks are available (Salter & Hudson, 1978). Stud piles are sniffed and marked in a ritualized manner (Turner, Perkins & Kirkpatrick, 1981): typically the stallion will sniff faeces or urine, step over it with an arched neck, deposit some dung, and then turn and sniff the excreta again. Stallions also urinate or defecate on mare eliminations, the marking of which possibly has a different function to stud pile marking. The idea that overmarking is used to eliminate the odour of a female, as suggested by Tyler (1972) and Kimura (2001), has been disputed because not all eliminations are marked (Salter & Hudson, 1982).

Other hypotheses to explain male scent marking in horses have been proposed, including the fact that it is related to male intrasexual competition (Feist & McCullough, 1976) and that it is a relict behaviour from territorial ancestors (Klingel, 1972). Although scent marking is commonly related to territoriality (Burt, 1943; Ralls, 1971; Brashares & Arcese, 1999*a*), feral horses are not strictly territorial, *sensu*

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an individual or group of individuals defending an area for exclusive use (Davies & Houston, 1984; Linklater, 2000; although see Rubenstein, 1981). However, horses exhibit female defence polygyny in the form of a harem-based social system. Each stallion dominates a group of mares or harem (multi-stallion harems have been observed; Linklater, 2000) and must defend these for exclusive mating rights; thus, similar principles to territoriality can be applied to female resource defence (Gosling, 1990; Gosling & Roberts, 2001).

Scent marks may transmit information about status and competitive ability of the resource holder (Gosling, 1982, 1990; Gosling & Roberts, 2001). It has been shown that animals have the ability to associate marks with individuals (Keverne & Kaba, 1990; Rich & Hurst, 1998; Lawson, Putman & Fielding, 2000, 2001), and this has also been observed in horses (Rubenstein & Hack, 1992). Thus, the most likely function of scent marking in stallions is a form of female resource defence, although the role of marking stud piles and marking female eliminations has not been adequately explained. We assess the function of these two types of male scent marking by studying the occurrence of scent marks in a population of Przewalski horses *Equus ferus przewalskii* reintroduced into Hustai National Park (HNP), Mongolia (van Dierendonck & Wallis de Vries, 1996; King & Gurnell, 2005). Stimuli causing scent marking are expected to alter through the year and provide information on the relative importance of female defence. By examining the spatial distribution, we aim to show the pattern of marking behaviour and whether it is used as a defensive mechanism against intruding stallions.

Methods

Study area

Studies were carried out at HNP, Mongolia between June 1998 and July 2000. HNP is situated about 150 km southwest of Ulaanbaatar, the capital city of Mongolia. It covers 570 km^2 along a south-western spur of the Khentii range of mountains (around the geographical coordinates: 47°41′N, 105°54′E). The area was made a reserve in 1993 in preparation for the first release of Przewalski horses in 1994 (Bouman, 1998) and designated a National Park in 1999. The park borders the Tuul river and has an altitude between 1100 and 1842 m. The climate is continental with a mean annual temperature of +0.2 °C and a yearly precipitation of 270 mm, most of which falls as rain in July and August (Wallis de Vries, Manibazar & Dugerlham, 1996). HNP has a mountain forest steppe habitat and water flows down most of the major valleys as streams (Wallis de Vries *et al.*, 1996).

Field observations and data analysis

Five harems were observed for a total of 860 h over three field seasons between June 1998 and July 2000. Horses were observed in temperatures of between −14 and +34 °C, but no observations were conducted between December and March. During the study period there were up to nine free-ranging harems in HNP. Harems consisted of a stallion and a group of mares, released as a unit, although the harem holder and composition of the groups changed through time. Stallions were identified individually by differences in markings and pelage.

Observations of the horses were carried out on foot from a distance of at least 15 m. We defined marking behaviour as occurring when a horse sniffed a non-food item and then proceeded to flehmen, urinate or defecate, or when excreta was placed on a known stud pile or mare excreta. All occurrences of marking behaviour were recorded on a voice recorder as they happened, even if the full ritualized sequence did not occur. The time when the marking took place, the identity of the animal carrying out the behaviour and the sequence of behaviours (e.g. whether the stimulus was sniffed before marking, after marking or both, and what form the marking took) were recorded, together with the stimulus marked and the terrain where the marking occurred. Marking during or after a confrontation with another horse was also recorded. To delineate home ranges and range use, and construct time budgets, the location and behaviour of the harem were recorded every 10 min during observation periods. Location data were entered into Arcview GIS for further analysis.

Stud piles were surveyed whenever they were encountered. A stud pile was defined as where at least two dungings had occurred on the same spot. The maximum width, length and height of the stud pile were measured and its relative age and composition were subjectively described as fresh (green and wet – just done, usually seen), new (recent – turning black and drying), old (dry, grey/brown and breaking up), new on old, fresh on old or fresh on new. The location of the stud pile was recorded as for marking events.

We combined the data from all field seasons for analyses. The frequency of marking in different months, as well as the location of marks, the type of stimulus when it could be identified, the terrain and the form of the marks were analysed using contingency tests. The sequence of events in the ritual of marking was also analysed in relation to the

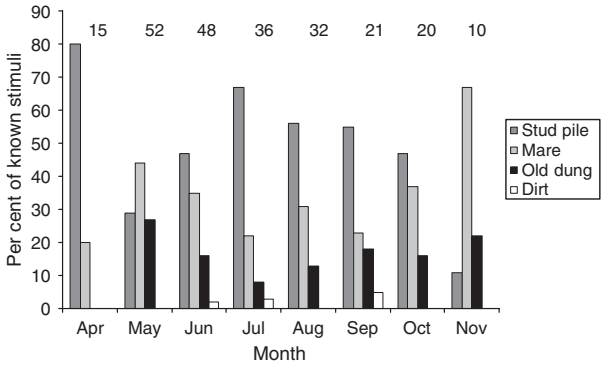

Figure 1 Per cent of stimuli marked according to month (numbers above bars = sample sizes for each month). Stimulus and month were significantly associated, excluding 'dirt' category ($\chi^2_{14} = 28.32$, $P = 0.013$).

stimulus. The sizes of stud piles of different age and form were analysed using non-parametric ANOVA. Ninety-five per cent kernel harem range areas were calculated in Arcview from the location data gathered; these excluded occasional forays outside the areas. Utilization curves were used to estimate core areas and, in all cases, these were 80% of the total range area (King, 2002; King & Gurnell, 2005). The distribution of stud piles within the ranges of the harems was examined by comparing their position within the home ranges and core areas of the harems using χ^2 goodness-of-fit tests, after first testing for heterogeneity in the data.

Results

Marking behaviour

We observed 343 marking events: 94% carried out by stallions, 6% by juvenile males (<3 years old, still within the natal harem) and 1% by mares sniffing either a stud pile or old dung. Only 11% of observed marking behaviour occurred during a confrontation between stallions; all others occurred during the course of the horses' daily movements. There was no evidence that the horses avoided eating near dung or latrine areas being formed. Excluding the very small

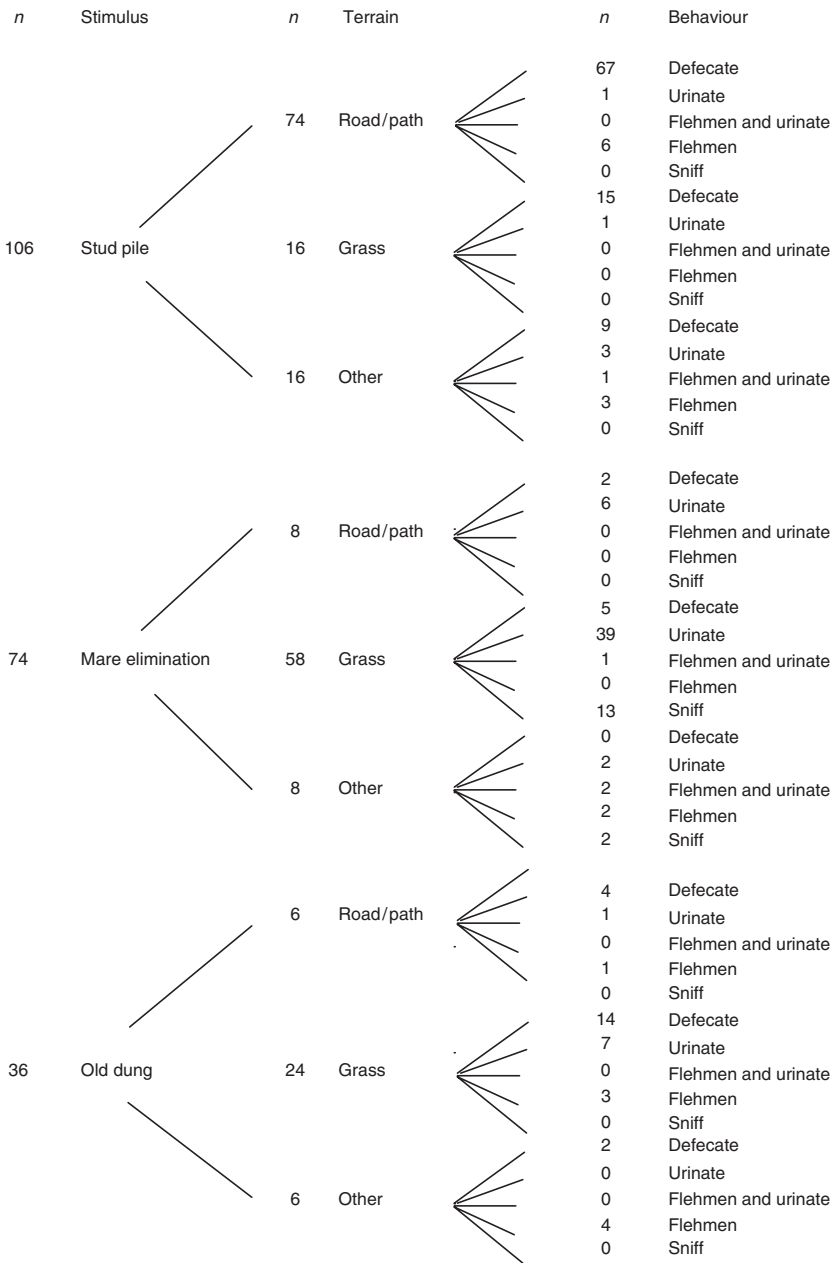

Figure 2 Tree showing the frequency of marking behaviour according to stimulus, terrain and behaviour.

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Table 1 Frequency of behaviours observed at each type of stimulus

	Behaviour				
	Flehmen	Flehmen and urinate	Sniff	Urinate	Defecate
Mare elimination	2 (6.5)*	3 (1.4)	15 (5.1)*	47 (20.6)*	7 (40.4)*
Old dung	8 (3.2)*	0 (0.7)	0 (2.5)	8 (10)	20 (19.7)
Stud pile	9 (9.3)	1 (2)	0 (7.4)*	5 (29.4)*	91 (57.9)*

*Significant cell χ^2 values, $P<0.05$.
Expected values in parentheses. Behaviour and stimulus were significantly associated ($\chi^2_8=143.6$, $P<0.0001$).

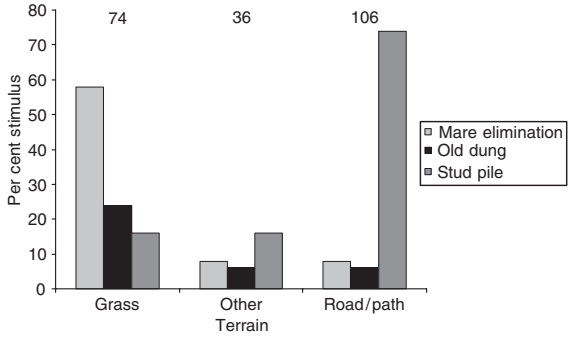

Figure 3 Per cent of stimuli on different terrains. Stimulus and terrain were significantly associated ($\chi^2_4=86.98$, $P<0.0001$).

Table 2 Frequency of behaviours observed marking each type of stimulus

	Behaviour				
Stimulus	Sniff before	Sniff after	Sniff before and after	No sniffing	Total
Stud pile	23 (49.3)*	1 (2)	82 (54.7)*	0 (0)	106
Mare elimination	62 (34)*	1 (1.4)	10 (37.7)*	1 (0)	74
Old dung	15 (16.7)	2 (0.7)	19 (18.6)	0 (0)	36
Dirt	2 (0)	0 (0)	1 (0)	0 (0)	3

*Significant cell χ^2 values, $P<0.05$.
Expected values in parentheses. Stimulus and behaviour were significantly associated ($\chi^2_4=74.54$, $P<0.0001$, excluding 'no sniffing' and 'dirt').

number of marking events on bare ground ('dirt'), there was a significant association between stimulus and month (Fig. 1). Stud pile marking was proportionally high in April before the breeding season in May and June. This dropped in May, but increased again in July. Significantly more than expected marking of mare eliminations occurred in November.

Most of the marking behaviour observed that could not be assigned to a particular stimulus occurred on grass (74%, $n=124$). The 'unknown' stimulus category has been omitted from the analyses below. Marking behaviour for known stimuli (except 'dirt', which only occurred three times) is summarized in Fig. 2. There was a significant difference in the frequency of different behaviours observed during marking events ($\chi^2_4=203.6$, $P<0.001$); defecation was the most frequent behaviour seen (55%), followed by urination (28%) and flehmen (9%). Stud piles were marked most (49%) followed by mare eliminations (34%) and old dung (16%). There was a significant association between the type of stimulus and the behaviour seen ($\chi^2_8=143.6$, $P<0.0001$; Table 1): defecation occurred more frequently on stud piles and urination on mare eliminations. Not all mare eliminations were marked, but we were not able to assess the relative frequency of this. The different stimuli also tended to be found on different terrains ($\chi^2_4=86.98$, $P<0.0001$; Fig. 3), with stud piles tending to occur on paths and mare eliminations on grass.

A sequence of behaviours was observed when the stallions marked. They would sniff the stimulus before marking, and/or afterwards, in a characteristic posture. The sequence of sniffing tended to vary with the stimulus ($\chi^2_4=74.54$, $P<0.0001$, excluding the 'no sniffing' behaviour and 'dirt' stimulus because of very low frequencies; Table 2). We observed that the stallions tended to sniff before marking on mare eliminations, but not after, and would sniff both before and after marking stud piles. Sniffing without marking occurred at 18% of mare eliminations.

Table 3 Median dimensions of stud piles of different ages and form that were mapped (interquartile ranges are shown in parentheses)

Age and form of stud pile	Length (cm)	Width (cm)	Height (cm)
New	85 (70.0–137.5)	65 (52.5–77.5)	10 (5.0–13.8)
Fresh on new	70 (55.0–85.0)	40 (37.5–102.5)	10 (5.0–16.3)
Fresh on old	140 (130.0–250.0)	90 (60.0–120.0)	10 (5.0–20.0)
New on old	200 (135.0–280.0)	110 (75.0–140.0)	10 (5.0–12.5)
Old	140 (102.5–182.5)	85 (70.0–125.0)	5 (5.0–5.0)

Size and position of stud piles

Stud piles tended to spread out and be long and wide rather than high, partly as a result of drying, weathering and insect activity, and partly because the stallions did not necessarily deposit faeces on the highest part of the stud pile. The median length of mapped stud piles was 145 cm [$n=114$, inter-quartile range (IQR) = 100–210 cm], the median width was 90 cm (IQR = 60–130 cm) and the median height was 5 cm (IQR = 5–10 cm). We found that stud piles that were used most frequently, i.e. new dung on old, were also longer (Table 3; Kruskal–Wallis $H_4=30.89$, $P<0.0001$), wider ($H_4=18.16$, $P=0.0011$) and higher ($H_4=22.23$, $P=0.0002$) than other ages and forms of stud piles.

Table 4 Area and perimeter length of home ranges of the harems (the name of the stallion is given), and position of stud piles relative to the home ranges

	80% core		95% range			Position relative to home range (%)			Position within home range (%)	
Home range of stallion	Area (ha)	Perimeter (km)	Area (ha)	Perimeter (km)	No. of stud piles	Core area	Periphery	Outside	Area of non-overlap	Area of overlap
Paritet	674	20.23	1693	33.46	21	71	24	5	45	55
Bayan	525	15.53	1117	23.74	14	79	7	14	42	58
Khaan	726	24.58	1460	19.7	25	68	8	24	5	95
Ares	240	11.02	744	32.15	21	57	38	5	45	55
Margad	345	17.62	684	32.15	7	57	43	0	14	86

Paritet, Bayan and Khaan overlapped each other in 1998 and 1999, Ares only in 1998 and Margad only in 1999.

Most stud piles were found within known home ranges (Table 4). Of those within ranges, significantly more were found in the core area (76%) compared with the peripheral area, after testing for heterogeneity among the five harem ranges studied (heterogeneity $\chi^2_3 = 5.99$, $P = 0.1118$; pooled $\chi^2_1 = 20.51$, $P < 0.0001$). The density of stud piles was also higher within core areas compared to the periphery (mean density core area = 0.025 stud piles ha^{-1}, SD = 0.010; mean density periphery = 0.004 stud piles ha^{-1}, SD = 0.004; $t_8 = 4.45$, $P = 0.007$). The data were not homogeneous to test for overall differences in the frequency of stud piles in areas of home range overlap and non-overlap (heterogeneity $\chi^2_3 = 70.68$, $P < 0.0001$). This was because most stud piles were in areas of overlap for Margad's and Khaan's ranges; these were in the valley that all other harems used, indicating that Margad and Khaan concentrated their marking in the areas of their ranges where they came across signs of other harems. However, there was no significant difference between areas of overlap and non-overlap for the other three harems (Table 4).

Twenty-five per cent of stud piles were found on ridges or saddles within the landscape and most of the rest were found on paths or tracks as described before (see also King, 2002). It therefore appears that stud piles were placed where other stallions were most likely to come across them. There was a noticeable increase in the number of stud piles along a path used by Khaan's harem after Ares' harem was released in the vicinity (S. R. B. King, unpubl. obs.), although data were not collected to quantify this.

Discussion

The stallions at HNP marked mare eliminations and formed dung piles as in most other equid populations (Turner *et al*., 1981), and adopted typical postures (Feist & McCullough, 1976; Turner *et al*., 1981; Salter & Hudson, 1982; Rubenstein & Hack, 1992). Although the same ritualized postures were observed when both stud piles and mare eliminations were marked, they were marked in different ways, suggesting different functions. Stud piles were marked with dung, and were sniffed both before and after they were marked, whereas mare eliminations were marked with urine and sniffed only before marking. The two stimuli were also found in different places, suggesting a different impact on another animal encountering the mark. Mare eliminations were normally on grass, where the mares spent most of their time feeding. This supports Lamoot *et al*.'s (2004) observations that mares do not perform latrine behaviour but defecate where they graze. However, stud piles were situated on paths or ridges and stallions would travel to a stud pile to mark it, in addition to marking them as they passed during their normal daily movements. Ridges often separated the home ranges of harems (King & Gurnell, 2005), but were also used as refugia from flies and were crossed as part of the daily movements of horses in summer.

There was no evidence that stud piles were used to mark the boundaries or peripheral areas of the home ranges. Rubenstein & Hack (1992) found that dung contained information on the identity of a stallion, and thus stud piles provide information about the horses that have passed over ridges or along paths. In addition to identifying occupancy of a range, these stud piles may provide way-points for a stallion and his consorts, and produce a familiar odour within the harem range (Gosling, 1982; Gosling & Roberts, 2001); this is possibly why stud piles are sniffed after marking as well as before. Stud piles are unlikely to be visual signposts (Salter & Hudson, 1978) due to their low profile.

Although marking stud piles during confrontations was relatively rare in this study, such marking provides an opportunity to communicate individual identity (Rubenstein & Hack, 1992; Lawson *et al*., 2000) and may also act as a release for aggressive behaviour (Turner *et al*., 1981). In other studies the order in which the stud pile is marked has been reported to indicate dominance hierarchy within the males (Miller, 1981; Turner *et al*., 1981; Redman, 1996). Conflicts between stallions can be costly (Berger, 1981), and it is expected that they should be avoided when possible. If stud piles relate to the harem's spatial position within the landscape and result in a reduction in conflict between harem holders, then their function is much more than the relict territorial behaviour hypothesized by Klingel (1975). It would be impossible to produce a line of marks sufficiently close that they would always be encountered by intruders along the boundaries of large ranges sited within a rugged, heterogeneous terrain (Gosling & Roberts, 2001). Instead stallions appear to mark in the areas they most

frequently use or in areas of spatial overlap among adjacent harems in a form of hinterland marking (Gorman & Mills, 1984). As the number of harems within the study area was relatively low when we carried out the study, there was little overlap between ranges and low encounter rates between stallions. It did not therefore appear that the number of stud piles was related to the number of stallions using an area. It is likely that as the population increases through time there will be greater competition among harems, and areas of overlap among harems would increase. It will be interesting to examine how the pattern of stud piles changes in the future.

Marking of mare eliminations most likely demonstrates the association between a stallion and a mare (Turner *et al*., 1981; Roberts & Dunbar, 2000). Most of this marking occurred during the breeding season, when comparatively fewer stud piles, but more unknown stimuli, were marked. Although not included in most of the analyses, we believe that most unknown stimuli were probably mare eliminations that were not observed being deposited. Turner *et al*. (1981) also observed more marking of mare eliminations during the breeding season than the winter. The anomalous marking of mare excreta in November is possibly due to the short time in the wild experienced by these horses; some mares may not yet have adapted to only coming into oestrus in the spring, as illustrated by foals occasionally being born in early winter in this population. The seasonal change in marking behaviour could be caused by changes in the pheromones present in mare urine and dung as they come into oestrus (Turner *et al*., 1981). Stimuli contained in female excreta, especially when detected by flehmen, have the potential to affect the sexual behaviour of animals (Eisenberg & Kleiman, 1972). The stallions behaved in a similar way to mare eliminations and unknown stimuli. Kimura's (2001) results suggest that pungent cresols within the urine of stallions may attenuate the chemical information in mare faeces, thus masking the difference between oestrous and non-oestrous mares. This might explain why mare eliminations were not sniffed after marking. However, even in the breeding season not all mare eliminations were marked, so whether there is an attempt to hide the scent of oestrous mares remains to be determined. Thus the most likely reason for marking of mare eliminations, especially when the mare is in season, is to provide information to all the other horses that this stallion is her consort, whether or not the sexual state of the mare is disguised, as was also observed in klipspringer *Oreotragus oreotragus* by Roberts & Dunbar (2000). In addition, marking may provide a sexual stimulus for the stallion and alert him to the receptiveness of the mare.

It is likely that all stallions within the small population at HNP were familiar with each other, and could connect a scent with a stallion's RHP (Smith & Parker, 1976). Thus, if an interloper can link the smell of a known stallion from a stud pile with the scent of the stallion's urine on an oestrous mare's urine, they benefit by assessing the risks involved in attempting to steal the mare or sneak a mating (Gosling, 1990; Gosling & Roberts, 2001). Marking of mare eliminations may have evolved with harem defence polygyny to benefit both the harem holder and the mare. If the stallion is in good physical condition he will be less likely to be confronted and lose the mare, and the mare will not be harassed by a large number of stallions attempting to mate with her. It is also possible that females may use the information in the scent marks to be 'reassured' that they match those of the harem holder with which they are familiar (see Gosling & Roberts, 2001).

Some studies that have dealt with marking behaviour in horses have combined both the marking of stud piles and the marking of mare eliminations (Klingel, 1972; Salter & Hudson, 1982). We believe that stud pile marking and overmarking mare eliminations have a similar but discrete function linked to intrasexual selection (Gosling & Roberts, 2001). Stud pile marking throughout the home range advertises site-specific dominance by the stallion (*sensu* Linklater, 2000) in a cost-effective manner and the fact that he is the resource holder. If interlopers enter the range of a harem holder, then overmarking of mare eliminations could advertise to intruding stallions, and the mare, that the harem holder is the mare's consort. This information can be combined with previous knowledge of the stallion's competitive ability, so that the interloper can judge the risk of trying to steal the mare or sneak a mating. Thus, we propose that the two forms of marking by harem holders combine as first and second lines of defence of paternity rights in intrasexual competition with other stallions.

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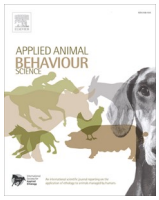

Effect of adult male sterilization on the behavior and social associations of a feral polygynous ungulate: the horse

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ABSTRACT

Castration is commonly used to control the behavior of companion animals and livestock, yet there have been few longitudinal studies of its effects. Despite the ubiquity of this surgery in ridden horses, the effects of castration (termed gelding in horses) have rarely been examined in a reproductive population. We tested effects of gelding on maintenance and social behaviors of individuals pre- and post-gelding, and in comparison to intact control adult males (2 to >16 years old) in both harem and bachelor status, we then tested how gelding affected association with mares (i.e., maintenance of a harem group) compared to intact controls, and any effects on bachelor social associations. We further explored any effects on foaling rate to assess potential impacts on population growth rate. We conducted this study over four years (2017–2020) at two Herd Management Areas (HMAs) in western Utah, USA: Conger and Frisco. We conducted demographic observations year round at both HMAs to record survival and foaling rate. We additionally recorded behavioral observations at Conger HMA. In December 2017, 27 adult males from Conger (42% of adult males in the population) were gelded and returned to the range with their social groups. Due to pre-treatment observations we were able to compare stallions of known status pre- and post-treatment (harem or bachelor), as well as gelded and intact males. We had no morbidity or mortality related to the gelding surgery and all males maintained good body condition throughout the study. There was no effect of gelding on maintenance behaviors (feeding, moving, and standing). There was no effect of gelding on frequency of agonistic behavior, and a non-significant tendency for less reproductive behavior in geldings; geldings showed more affiliative and less marking behavior. Age class and/or social status were better predictors of behavior than gelding. Over time fewer geldings maintained a harem, and their harem size declined during the study. Horses that were bachelors when gelded tended to remain as bachelors, whereas intact bachelors of the same cohort mostly attained a harem. Foaling rate at Conger was reduced in the year following treatment, but then returned to pre-treatment levels. From a welfare perspective gelding is safe to use in feral horses and has minimal effects on horse behavior and social interactions in a reproductive herd. Effectiveness for population growth control would likely require a larger proportion of males in the population to be castrated for longer-term effects on foaling rate.

1. Introduction

Castration has been used as a tool to control male behavior for millennia. It has been used to prevent reproduction and make males more tractable in all domestic mammal species, and even in humans (Zumpe et al., 1992). Sterilizing companion animals is considered the norm in many countries, with only 14% of dogs (*Canis familiaris*) remaining intact in the United States (Houlihan, 2017). Similarly the majority of domestic horses (*Equus caballus*) are castrated (Schumacher, 1996; Searle et al., 1999). This is referred to as gelding in horses, with castrates termed geldings. Despite the ubiquity of this surgery there has been surprisingly little longitudinal research on behavioral effects of castration, and even fewer studies on how castration affects free-roaming animals living in a population with intact congeners (Garde et al., 2016). In domestic dogs research has only recently started to focus on longer term effects of castration, with most studies examining health effects of castration at different ages rather than behavioral outcomes (Hart et al., 2014, 2019; McGreevy et al., 2018).

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Despite castration generally being applied to modify behavior, there is surprisingly little evidence that it affects it. Castration is expected to affect behavior through removing testicular production of androgens such as testosterone, and subsequent effects on other endocrine glands (Thompson et al., 1980; Taravat et al., 2017). The two main behaviors that are expected to be controlled by testicular removal are aggression and reproductive behaviors. Although aggression was reduced in pet domestic dogs (Maarschalkerweerd et al., 1997), there was no difference in aggression post-castration of free-ranging dogs (Garde et al., 2016). Across other species, aggression was reduced in castrates compared to intact males in rodents and Japanese macaques (*Macaca fuscata*; Christie and Barfield, 1979; Hume and Wynne-Edwards, 2005; Lofgren et al., 2012; Takeshita et al., 2017; Gale et al., 2019), but not in prairie voles (*Microtus ochrogaster*) and some other non-human primates (Wilson and Vessey, 1968; Epple, 1978; Demas et al., 1999). Effects on horse aggression have rarely been studied and are confounded by aggression sometimes being the reason for castration; one study found aggression was controlled by gelding (Smith, 1974), whereas others found it continued to be problematic (Line et al., 1985; i Rios et al., 1995; Schumacher, 1996). Studies that have examined the effect of castration on reproductive behavior in a variety of species have found that it is rarely extinguished but tends to be presented less over time after surgery (Wilson and Vessey, 1968; Phoenix, 1974). Reproductive behavior has been reported in geldings and was evident regardless of whether horses were castrated pre- or post-puberty (Smith, 1974; Line et al., 1985; Cox, 1986; i Rios et al., 1995).

Management of free-ranging feral cats (*Felis catus*) commonly uses surgery as a tool to slow population growth (Guttilla and Stapp, 2010), yet despite most male riding horses being gelded it has been little used for feral horse (henceforth "horse") management. In the American west there is currently an increasing population of approximately 90,000 horses and donkeys on public lands managed by the Department of Interior (BLM, 2020), which is considered almost three times larger than these arid rangelands can sustain (BLM, 2020). These animals are protected under the Wild Free-Roaming Horses and Burros Act, as amended (Public Law 92, 195, 1971), with management options available to management agencies currently limited to gathers and removals from public land and/or fertility control. Surgical options are allowed under the Act but have rarely been used due to public litigation when they have been proposed. Thus fertility control to date has largely consisted of immunocontraception of adult females (Kane, 2018).

Horses have a female defense polygyny social system, where typically one adult male (stallion) associates with a group of adult females (mares) and their juvenile offspring of both sexes, defending the mares from harassment by other stallions in order to maintain exclusive mating opportunities (Boyd et al., 2016; King et al., 2016). In some populations multi-male groups have been observed, although the function of additional stallions in these groups is disputed (Feh, 1999; Linklater and Cameron, 2000a, 2000b; Feh, 2001; Linklater et al., 2013). Social groups of a stallion and mares (hereafter referred to as a harem) tend to have stable adult membership over periods of months to years (Ransom et al., 2016). Equids are unusual in polygynous ungulates in that both male and female offspring disperse from their natal group around puberty (Linklater and Cameron, 2009; Debeffe et al., 2015). Dispersed males live alone or in fission-fusion groups with other males until they form a more permanent association with adult females (i.e., become harem stallions). Some males never achieve harem stallion status and thus remain as bachelor stallions (Boyd et al., 2016). While the social network of horses is underpinned by long-term relationships between mares (Sigurjónsdóttir et al., 2003; Stanley et al., 2017), stallions also have a rich social life. In a natural population it is likely that most males know each other and their relative fighting ability (Rubenstein and Hack, 1992), resulting in most stallion interactions being non-violent due to ritualized behaviors (Sigurjónsdóttir et al., 2012) or play (Feh, 2005).

Only three previous field studies have examined effects of controlling male fertility on population growth of feral horses (using microcapsulated testosterone propionate (Kirkpatrick et al., 1982; Turner and Kirkpatrick, 1982), vasectomy (Eagle et al., 1993), and gelding and vasectomy (Collins and Kasbohm, 2017)). All found reduced foaling rates in harems of treated stallions in the first year after treatment or reduced female fecundity, but none examined any behavioral effects of the treatment, or longer-term effects. Modeling approaches indicated that male-based fertility control would only be effective if 90% of males were sterilized, as one male has the potential to fertilize many females (Garrott and Siniff, 1992). However it is unknown whether this would be true in practice due to female mate choice (Pickerel et al., 1993) and the strong social bonds formed within horse groups (Cameron et al., 2009). There is little published on how geldings may behave in a reproductive herd. When in a large pasture or free-roaming with mares and their offspring, geldings have been reported to form their own groups both in the presence and absence of intact stallions (Kaseda, 1981; Kaseda et al., 1997; Sigurjónsdóttir et al., 2003; Van Dierendonck et al., 2004).

Feral horses on public lands in the western United States are managed by the agency responsible for those lands: the United States Department of Interior's Bureau of Land Management (BLM) or U.S. Department of Agriculture, Forest Service (USFS). There is intense public scrutiny on management of feral horses, with broad interest in welfare implications of any action (Scasta et al., 2018). We conducted research to address questions of interest to both management and the wider public related to castrating a proportion of males in a free-roaming horse population. Management interest relates to the utility of gelding as a potential tool for controlling population growth, either due to reduced foaling rate of females, or replacing reproductive individuals with geldings. Managers are further concerned about welfare impacts of surgery, potential behavioral impacts on individuals and the social structure of the population, with the public also being concerned about how gelding may affect perceived 'wildness' of feral horses (Bureau of Land Management, 2016). We conducted our study on two horse populations, in which 42% of males in one population were gelded and all males in the other population were untreated (intact) controls. The social status (harem or bachelor) of all males was known prior to gelding, and tracked subsequently. We tested whether there was any effect of gelding on welfare of the males, in terms of survival and body condition in comparison with intact controls. We examined behavioral effects of gelding by comparing intact and gelded males, and behavior pre- and post-treatment. We hypothesized that gelded males would quickly lose interest in females, reflected in more time spent feeding and less time moving as they would not be seeking mating opportunities, and in lower frequencies of reproductive behavior and aggression due to lack of endogenous testosterone. In harem stallions we expected those gelded (hereafter referred to as harem geldings) to quickly lose their harems due to lack of interest in reproduction. In bachelors we expected there to be little difference in behavior between gelded and intact males as both are non-reproductive. We hypothesized that gelding would reduce foaling rate as there would be fewer reproductive males and the polygynous social system would reduce pregnancy in females with a gelded harem stallion.

2. Methods

2.1. Study areas and individuals

We conducted research on horses at Conger Herd Management Area (HMA; 69,198 ha) and Frisco HMA (24,430 ha) in western Utah, USA. Both have similar habitat of valleys with sagebrush (*Artemisia* spp.) meadows and pinyon (*Pinus edulis*)-juniper (*Juniperus* spp.) covered mountain ridges, although Frisco has both more sagebrush covered flat areas and more mountainous forest. The BLM conducted management gathers at both HMAs prior to our study and all horses captured were taken to holding facilities. A randomly selected 100 of the gathered horses (50 male, 50 female) across all age classes were returned to the

range at each site. Horses were returned to Conger HMA in August 2016, and to Frisco HMA in February 2017. All individuals returned to the range were marked with a numeric freeze brand on the left hip; other individuals and those born subsequently were identified by pelage color and markings. A total of 38 adult females at Conger and 30 at Frisco were fitted with radio collars (Lotek Wireless Iridium, and Sirtrack Ltd. very high frequency collars) following methods in Schoenecker et al. (2020); we fitted telemetry tail tags (Biotrack Ltd., and American Wildlife Enterprises) on 49 adult males at Conger and 35 adult males and yearlings of both sexes at Frisco (King and Schoenecker, 2022). Between November 28 and December 1, 2017 the BLM conducted a second gather at Conger only to treat males. This gather focused on maintaining horses in their social groups: 111 horses of both sexes and all ages were captured out of an estimated population size of 157. They were kept at the BLM Delta Wild Horse and Burro Facility in their known social groups (obtained from field observations), and released in these groups post-treatment. This research was approved under Colorado State University (CSU) Institutional Animal Care and Use Committee protocol 15–6058 A, U.S. Geological Survey (USGS) FORT IACUC 2015–10, and an inter-institutional agreement between CSU and USGS. All work was conducted on wild horses managed by the Bureau of Land Management and animal handling followed their protocols (Bureau of Land Management et al., 2015) and National Environmental Policy Act (NEPA) analyses (DOI-BLM-UT-W020–2015–0017-EA).

2.2. Treatment

In 2017 there were 64 adult males at Conger (i.e., born in 2015 or before) of which 34 were captured during the treatment gather (53%). We castrated all captured males $\geq$ 2 years old (n = 27) except for 7 controls: 3 harem stallions (i.e., associating with mares and exhibiting harem tending and reproductive behaviors) and 4 bachelors (i.e., not associated with mares), identified from field observations. The 27 males gelded represented 42% of adult males on Conger, and 79% of males that were captured (Table 1). Half of the harem stallions present in 2017 (50%) and 39% of bachelors were gelded. All adult ages were represented in samples of gelded and intact stallions (Fig. 1). Castration surgery was conducted on December 6, 2017 by a BLM-contracted veterinarian at the BLM Delta Wild Horse and Burro Facility. All horses receiving surgery were first given a sedative, then anesthetized with ketamine and succinyl choline, plus given a testicular block of lidocaine. After surgery they were administered tetanus vaccine, antibiotics, and analgesics. Anesthetized horses were recumbent for 6–25 min before regaining their feet and being moved to a recovery pen. Within hours of the surgery harem stallions were put back in a pen with their social group. There was no morbidity or mortality as a result of surgery. Harem groups in which the stallion was not gelded (i.e., remained intact) were returned to the range as a group after 6–11 days at the holding facility (between December 6 and 8); harem geldings and their groups, and gelded and intact bachelor stallions were returned to the range after 14–15 days at the holding facility (5–6 days post-surgery; December 11–12). All gelded males were located in the field to confirm recovery between December 12–19, 2017, and then at least monthly thereafter for the duration of the study.

2.3. Field observations

All telemetered individuals were located at least monthly between September 2016 and September 2020 at Conger (treatment population) and between March 2017 and September 2020 at Frisco (control) so that we could determine survival, social associates, and body condition of males (using the Henneke et al., 1983 scoring system), and population foaling rate. At Conger we targeted behavioral observations before and after treatment on a focal subset of telemetered individuals (16 males: 8 who were harem stallions and 8 who were bachelors in 2017; 4 mares); no behavioral observations were conducted at Frisco. We increased our sample size of individuals in behavioral observations by recording behavior of focal horses plus any social associates at the time of the observation. In this way we were able to collect data on the focal individual plus its social group, enabling us to track behavior of focal individuals moving into a new social group, or track behavior of new individuals joining a focal individual's group. For behavioral observations we aimed to locate each focal individual in each of three time periods (0700–1100, 1100–1600, 1600–2000) twice per month between April and September each year. Observations were conducted in teams of two trained people; interobserver reliability checks were conducted periodically. We located radio marked animals using telemetry tracking by ground observers, or opportunistically. We conducted observations through binoculars (10 ×40, various manufacturers) or with a scope (Bushnell 20–60 ×80) from a sufficient distance that caused no disturbance (mean = 324 m ± 430 SD). Every 5 min during an observation hour we used scan sampling (Altmann, 1974) to record the maintenance behavior of each individual in the group (feeding, moving, lying down, standing, other, or out of sight). We used all occurrence sampling (Altmann, 1974) to record social behaviors such as agonistic, affiliative, reproductive, and marking behaviors, based on author experience (King, 2002) and ethograms from Ransom and Cade (2009) and McDonnell (2003). We recorded the actor and start time of all occurrence behaviors.

2.4. Analyses

Behavior was examined in a before-after treatment design at Conger HMA, and only data on males are presented here (Schoenecker and King, 2022). We censored data to only include males that were observed for at least 1 h in any year of the study: out of 35 males that we gathered behavioral data on, 31 (including 15 who were gelded) had sufficient data in at least one of the study years to be included in analyses (SI Table 1). Analyses were conducted on 1004 h of observations collected on these 31 males between April 2017 and September 2020 (total was 1527 animal-hours of observations). Counts of the most common maintenance behaviors (feeding, moving, and standing) collected every 5 min during the observation hour was divided by 12 to conduct analyses on rate per hour. For analyses of all occurrence behaviors we combined observations of specific behaviors to a category. Agonistic behavior consisted of bump/push, bite, kick, chase, strike, box, arched neck threat, parallel walk, head toss, naso-nasal, and herding. Affiliative behaviors were:

Table 1
Status of stallions (male horses born in 2015 or before) at Conger and Frisco Herd Management Areas in Utah, USA in 2017: harem stallions were primarily associated with mares and exhibited tending behaviors, bachelors were associated with other stallions or alone. Not gathered and gathered indicates the number of horses that were captured in a gather in November 2017, which was followed by gelding of some stallions at Conger; gelded and intact indicates whether captured horses were castrated or not. There was no 2017 gather at Frisco.

		Conger			Frisco		
		Harem	Bachelor	Total	Harem	Bachelor	Total
Not gathered		6	24	30	No gather		
Gathered	Gelded	9	18	27			
	Intact	3	4	7			
Total number of stallions		18	46	64	16	33	49

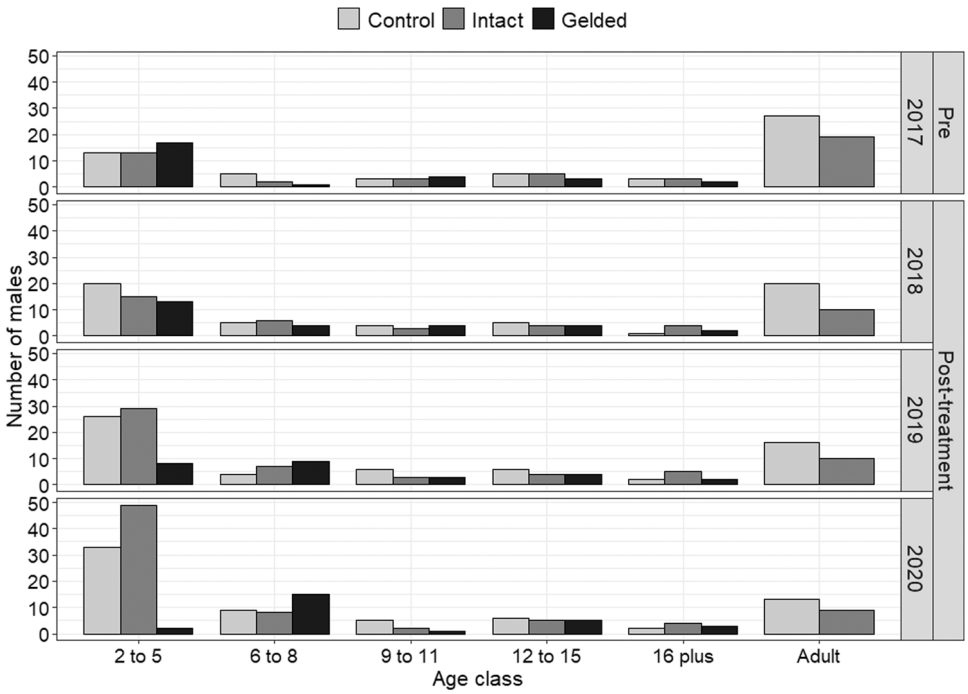

Fig. 1. Age structure of the adult male population at Conger and Frisco Herd Management Areas (HMAs), Utah, USA. Horses with no known age class (i.e., horses are those that were first seen as adults and do not have a known age) are described as "Adult". Control males were those at Frisco HMA; intact and gelded males were at Conger HMA. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year.

mutual grooming (where two individuals groomed each other simultaneously), allogrooming (where one individual groomed another without reciprocity), touch, and play. Reproductive behaviors were: sniff genitals, mounting (with or without an erection, or an erection was unknown), copulation (with or without successful intromission), and masturbation. Marking behaviors were: flehmen, sniffing feces and urine, and using feces and urine to overmark. We used all observations collected over the 1004 h of observation where one of the 31 males included in the behavioral analyses was the actor (n = 2508 events), using rate per observation hour for analyses.

All analyses were conducted in R software (R Core Team, 2017). To examine potential effect of gelding on maintenance behavior we used generalized linear mixed models with study individual as a random variable to account for repeated sampling. We used data from 2017 as the intercept in order to examine effects in relation to pre-treatment. For each model we used the behavior of interest (feeding, moving, or standing) as the response variable, year of the study, whether gelded, status (harem or bachelor), and age class as fixed variables. Prior to running models we assessed multicollinearity by fitting a global linear model for each response variable and using the package 'olsrr' (Hebbali, 2020) to calculate variance inflation factors of the fixed variables. Variance inflations factors were low (<2.2), therefore we concluded that multicollinearity was not an issue and thus using model-averaged regression coefficients to assess significance was appropriate (Cade, 2015). We used package 'MuMIn' (Bartoń, 2020) to rank models by Akaike's information criterion corrected for small sample sizes (AICc; Burnham and Anderson, 2010) and produce average coefficients of models within the top model set of delta $AIC_c < 2$. We considered parameters useful for inference if their model-averaged 95% confidence intervals (CI) did not overlap zero (Burnham and Anderson, 2010), however all parameters were additionally tested at 85% CI (Arnold, 2010); we report these results if they indicate biological significance that is not revealed with 95% CI. The nature of social behavior means that we had a zero inflated data set of all occurrence behaviors. We therefore used package 'glmmTMB' (Magnusson et al., 2021) for all models. We built models using the same factors as for maintenance behaviors, and ran them using Poisson, Conway-Maxwell Poisson and negative binomial with quadratic parameterization distributions, and hurdle models using truncated Poisson and truncated negative binomial distributions to test for the best distribution fit to the data. We determined the model with the best fit as being that with the lowest AIC using the function AICtab in package 'bbmle' (Bolker and R Core Team, 2020); for each tested behavior this was truncated negative binomial (truncated_nbinom2). We then ran all permutations of the model, using AICtab to rank models by AIC_c, and averaging models with delta $AIC_c < 2$. For all models we obtained estimated marginal means (package 'emmeans', Lenth et al., 2021) and reported odds ratio and significance level of the effect of gelding averaged over levels of other factors in the model.

To examine effects of gelding on stallion status we compiled all demographic data gathered at both Conger and Frisco between March 20, 2016 and September 23, 2020. In our populations multi-male groups manifested both as bachelors following a harem group from a distance but not integrating ("tag males"), and adult males that participated in group defense and were more integrated in the harem group but did not hold harem stallion status ("lieutenant males"; harem stallions had closer and longer association with the mares). On each occasion they were seen we determined whether a male was a harem stallion, bachelor, or a tag/lieutenant male. Tag/lieutenant males were considered bachelors for most analyses because they did not maintain a harem and most were transient members of a group. For analyses we censored the dataset to remove any males that had no known birth year, and any born in 2016 or later (i.e., horses that were foals or yearlings at the time of treatment, or not yet born). We also excluded any horse seen < 5 times in two or more years of the study. This resulted in a dataset of 49 males from Conger (25 gelded, 24 intact) and 24 males from the control population, Frisco.

We evaluated the effect of gelding on survival of males at Conger. We examined factors affecting body condition of adult males at Conger and Frisco using a generalized linear mixed model after checking for colinearity of variables: we used body condition score on each demographic observation as the response variable, with study area, year, age class, season (winter: December to February; spring: March to May; summer:

June to August; fall: September to November), harem status in the year of behavioral observation (harem stallion or bachelor), and whether the male was a Frisco control, Conger gelded or Conger intact as fixed variables, and study individual as a random variable. We ranked models by AIC_c, produced average coefficients of models within the top model set of delta $AIC_c < 2$, and considered parameters useful for inference if their model-averaged 95% confidence intervals did not overlap zero.

For analyses on harem stallions we included any horse that fulfilled those criteria (associating with mares and exhibiting harem tending and reproductive behaviors) at any period during the four years of the study. Using observations within a calendar year we classified whether stallions maintained association with mares the whole time ("kept mares"), became a bachelor and did not return to associating with mares ("lost mares"), whether they went from being a bachelor to associating with mares ("gained mares"), or whether they moved between harem and bachelor status several times during the year ("gained/lost mares") and provided these results descriptively. After testing for colinearity we used a generalized linear model to examine factors affecting the proportion of observations that an individual was the primary male seen with mares each year (Table 1). Factors included in the model were: proportion of observations stallion was with mares in a year as the response variable, and year of the study, age class, and whether a stallion was gelded, intact, or a control as fixed variables. We ranked models by AIC_c to produce average coefficients of models within the top model set of delta $AIC_c < 2$, and considered parameters useful for inference if their model-averaged 95% confidence intervals did not overlap zero. We repeated this process iteratively using each age class as the intercept to determine effect of age class. Harem size was assessed as the number of mares in the group each time a male was seen; we used linear regression to examine the relationship of harem size over time for each group of males (gelded, intact, or control), and then used Fisher's r-to-z comparison to detect any difference in slope among these groups.

For analyses, we classified bachelors as males that were not harem stallions for more than half demographic observations each year. We used a Kruskal-Wallis test to examine differences in number of bachelors in each of the three stallion groups (geldings, intact, and control males) across the years of the study. We used Wilcoxon signed rank tests to examine whether harem geldings were more likely to have tags or lieutenants, and whether the tag or lieutenant males joining the groups were more likely to be geldings.

Gestation length of horses is 11 months with most foals conceived around the summer solstice (Ransom et al., 2013), therefore most foals are born a calendar year after conception. Consequently, we assessed how gelding affected foaling rate > 1 year post-treatment. Foaling rate was calculated as the number of adult females (≥2 years old) divided by the number of foals born. Confidence intervals at 95% for foaling rate in

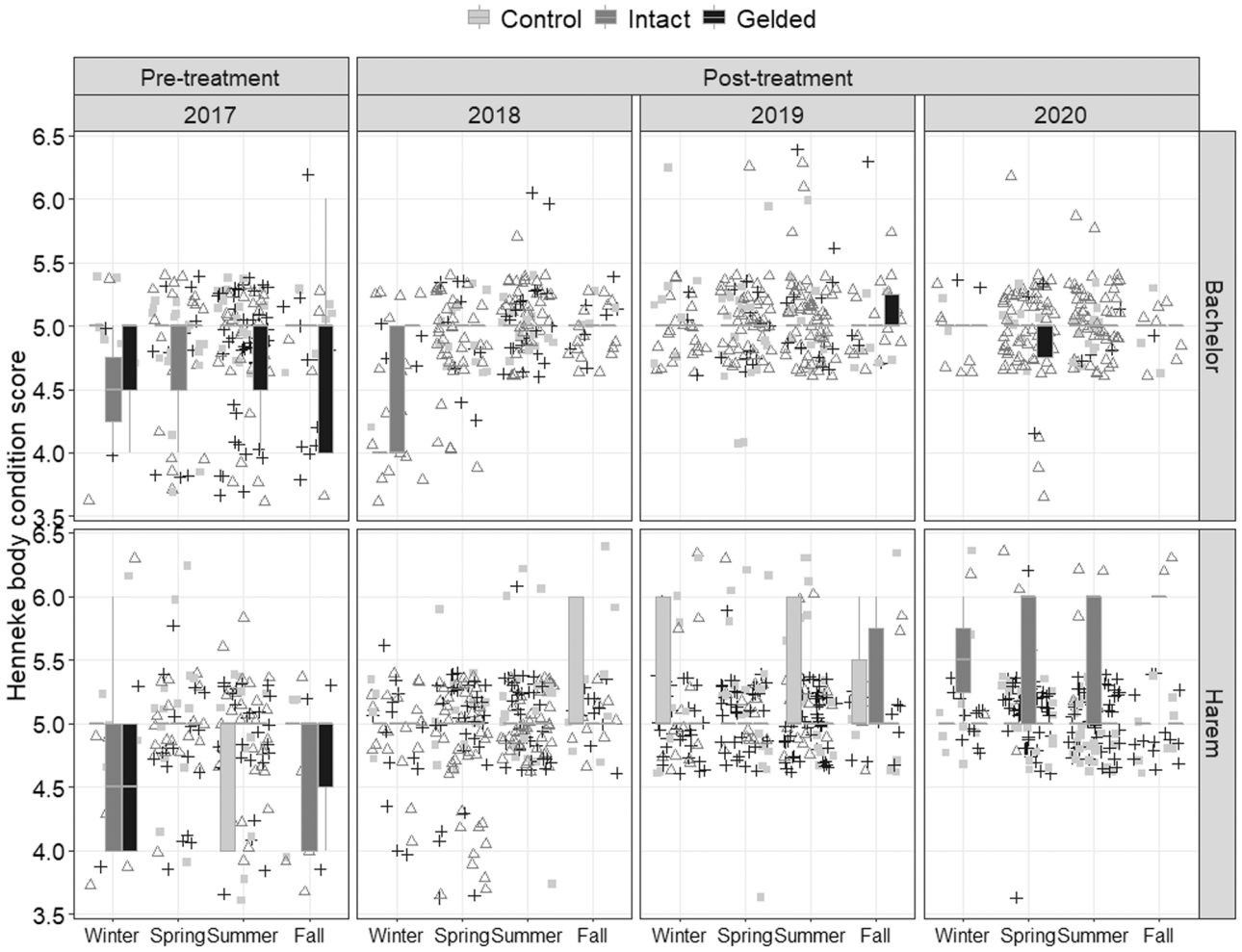

Fig. 2. Body condition score of males at Frisco (control stallions) and Conger (intact and gelded stallions) Herd Management Areas, Utah, USA across seasons between 2017 and 2020. Body condition score follows Henneke et al. (1983) on a scale of 1 = emaciated to 8 = obese. Boxplots show median values (horizontal lines), with the box limits indicating 25th and 75th percentile of the interquartile range and whiskers showing values 1.5 times the interquartile range above or below these percentiles. In most seasons median body condition was 5, meaning that no bar is displayed. Jittered data points are shown: controls in pale grey squares, intact in mid -grey triangles, and gelded in dark grey crosses. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year; they are shown separated in 2017 for comparison with post-treatment.

each year were calculated using an exact binomial test.

3. Results

3.1. Survival and body condition

Survival was not affected by being gelded. During the 4 year study the only mortality of an adult male at Conger was a 22 year old horse in 2017, the pre-treatment year, with no subsequent mortalities of gelded or intact males. Two adult males died at Frisco during the study. All males maintained good body condition throughout the study (Frisco controls: mean Henneke body condition score = 5.05 ± 0.38 SD; Conger intact: mean = 4.94 ± 0.33 SD; Conger gelded: mean = 4.96 ± 0.33 SD; Fig. 2). Body condition was not affected by being gelded: as a factor it was not included in any of the top models. Year, study area, and status were included in top models, but only year had a significant effect (0.001 – 0.033 95% confidence intervals (CI)).

3.2. Behavior

There was no effect of being gelded in models of factors affecting frequency of maintenance behaviors (feeding: model averaged odds ratio = 1.18, P = 0.2019; moving: odds ratio = 0.669, P = 0.1143; standing: odds ratio = 1.22, P = 0.730). The top models for feeding and moving only included whether a male was gelded and status (whether harem or bachelor male). For both behaviors the null model had the greatest support and neither factor was significant, indicating that they did not influence time spent feeding or moving (Fig. 3 A, B; SI Table 2a, b). The model for standing included all factors: standing was significantly affected by year, increasing after the pre-treatment year of 2017 (particularly 2019: 0.05–0.65 95% CI; Fig. 3 C; SI Table 2c), and by age class, with older males tending to spend more time standing (>16 years old: 0.08–0.85 95%CI). For both moving and standing, being gelded was significant at the 85% CI level (moving: 0.02–0.72 85% CI; standing: −0.35 to −0.05 85% CI) indicating a small effect.

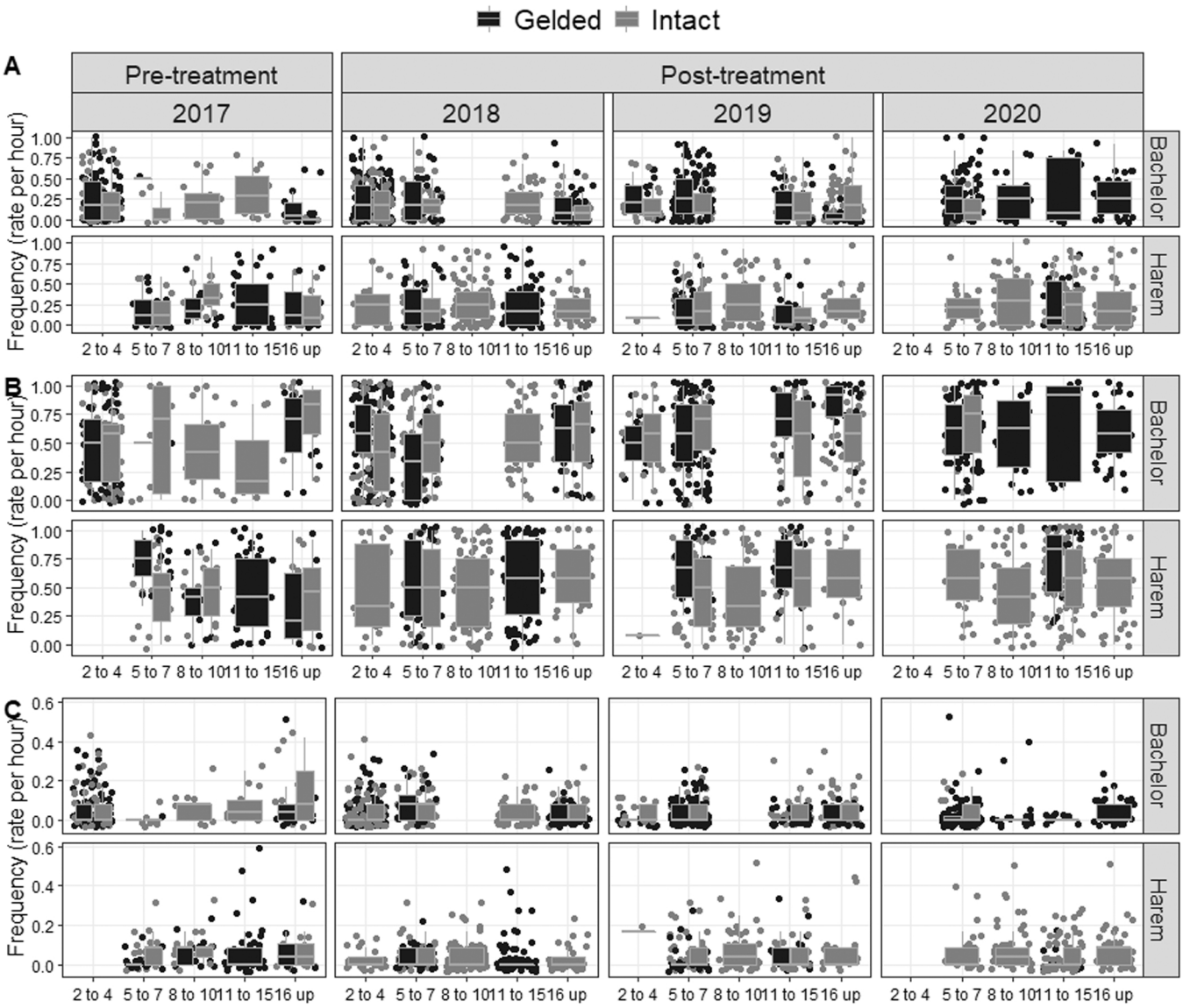

Fig. 3. Maintenance behaviors of gelded and intact males at Conger Herd Management Area, Utah, USA shown by harem status (bachelor or harem stallion) and age class in each year of the study. All data points are shown to give an idea of data spread. Maintenance behaviors presented are feeding (A), standing (B), and moving (C). Boxplots show median values (horizontal lines), with the box limits indicating 25th and 75th percentile of the interquartile range and whiskers showing values 1.5 times the interquartile range above or below these percentiles. Jittered data points are shown to give an idea of data spread: intact in paler grey, gelded in dark grey. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year; they are shown separated in 2017 for comparison with post-treatment.

Gelding was among factors in the top models for all social behaviors, but was only significant for marking and affiliative behaviors (gelded males showed more affiliative behaviors and marked less than intact males; affiliative: odds ratio = 1.74, P = 0.0254; marking: model averaged odds ratio = 0.539, P = 0.0019; Fig. 4 A, B SI Table 3a, b; Table 2), with no significant effect on agonistic or reproductive behaviors (agonistic: odds ratio = 0.838, P = 0.4769; reproductive: odds ratio = 0.524, P = 0.2009; Fig. 4 C, D; Table 2; SI Table 3c, d). Although not significant at 95% CI, gelding had biological significance at 85% CI in the model for reproductive behavior (0.42 – 1.37 85% CI; Table 2). Models for reproductive behavior and marking included status, with harem stallions having a significantly higher frequency of reproductive behavior than bachelors (Fig. 4 D; SI Table 3d). Age class was in the top models for agonistic and affiliative behaviors: 5- to 7-year-olds had a lower frequency of aggression than other age classes (Fig. 4 C SI Table 3c). Year was in the top models for all social behaviors but was not significant at 95% CI for any behavior (with the exception of reproductive behavior in 2020; SI Table 3).

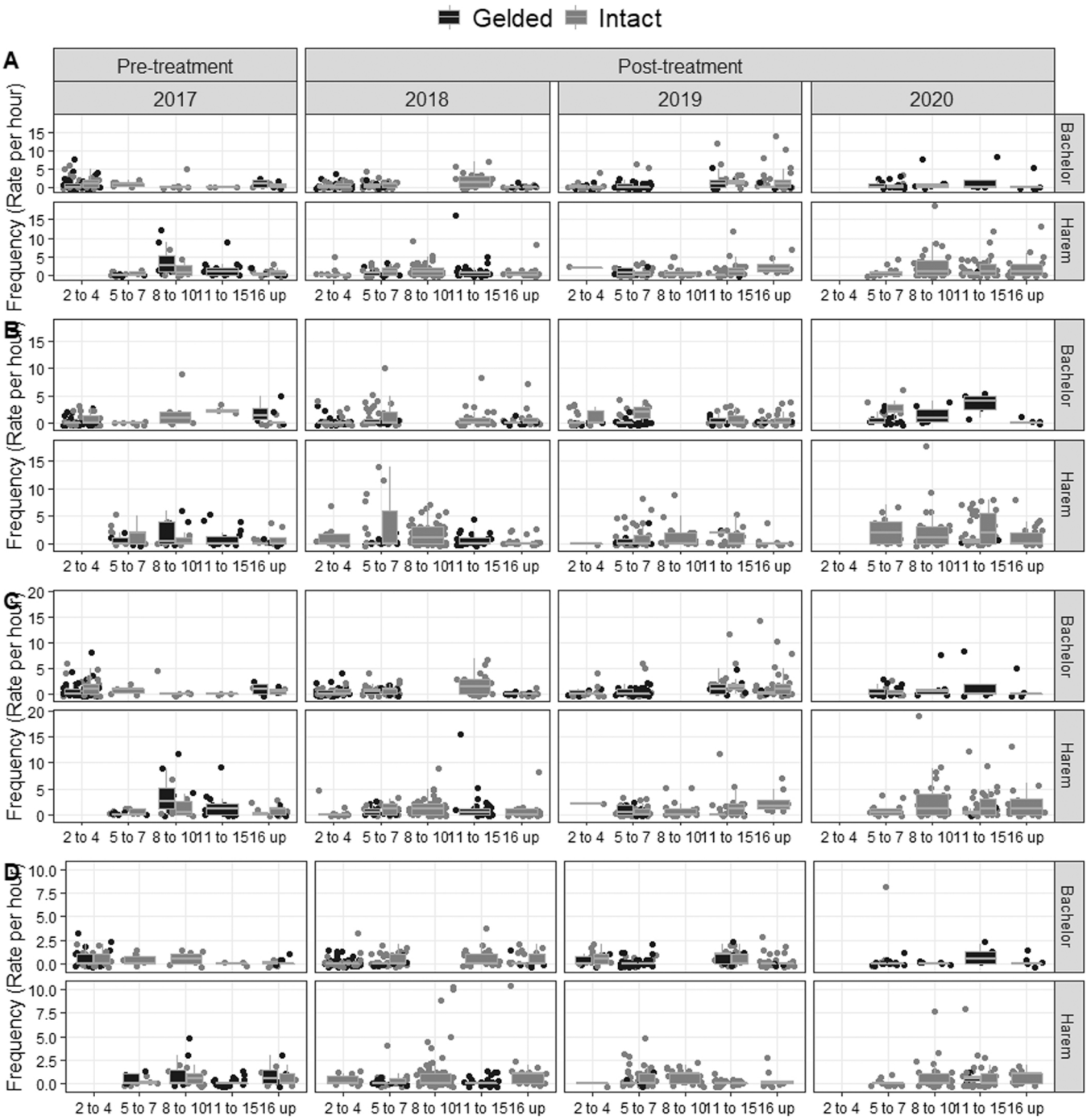

Fig. 4. Social behaviors of gelded and intact males at Conger Herd Management Area, Utah, USA shown by harem status (bachelor or harem stallion) and age class in each year of the study. All data points are shown to give an idea of data spread. Behaviors represented are affiliative (A), marking (B), agonistic (C), and reproductive (D). Boxplots show median values (horizontal lines), with the box limits indicating 25th and 75th percentile of the interquartile range and whiskers showing values 1.5 times the interquartile range above or below these percentiles. Jittered data points are shown: intact in paler grey, gelded in dark grey. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year; they are shown separated in 2017 for comparison with post-treatment.

Table 2
Frequency of social behavior per hour of male horses at Conger Herd Management Area, Utah, USA. Data were collected between April and September annually between 2017 and 2020 and summarized to show mean ± standard deviation for each behavior across all years. All males include both gelded and intact males; gelded males were intact in 2017 but data from all years is summarized.

Behavior	All males	Gelded males	Intact males
Agonistic	1.10 ± 2.04	0.86 ± 1.87	1.22 ± 2.11
Reproductive	0.53 ± 1.17	0.28 ± 0.65	0.64 ± 1.33
Marking	1.07 ± 1.89	0.54 ± 1.16	1.32 ± 2.10
Affiliative	0.77 ± 1.54	1.18 ± 2.05	0.58 ± 1.17

3.3. Harem stallions

In the pre-treatment year (2017) there was little difference in associations of harem stallions with mares between control stallions at Frisco and those at Conger (Fig. 5 A). In the first year after treatment (2018) one gelding gained a harem and 3 gained-and-lost, in 2019 no harem geldings gained mares, and 3 harem geldings gained-and-lost their harems in 2020. The number of harem geldings that kept their harem over a calendar year steadily diminished during the study, whereas the number of control stallions at Frisco and intact harem stallions at Conger that kept harems increased (Fig. 5 A).

When examining factors affecting proportion of observations harem stallions associated with mares (including data from Frisco controls; Fig. 5B), study site was not included as a factor in the averaged model, and there was no difference between intact stallions at Conger and control stallions at Frisco (0.69 to −1.59 95% CI). We ran the model a second time using only data from Conger and found gelding negatively affected the proportion of time spent with mares (Fig. 5B; −4.77 to −1.67 95% CI), but proportion of time spent with mares was also affected by age class (Table 3), with 2–4 year olds having less association with mares than all older age classes, and 5–7 year olds having less association than 8–10 year olds. There was no difference among age classes over 8 years old, and a model including an interaction term between proportion of time seen with mares and age did not converge. Year was not included as a factor in top models for time spent in association with mares. However there was a significant interaction of year and gelded status on harem size (Fig. 5 C; $F_{2,1303}$ = 13.3084, P = <0.0001), with a Fisher's r to z comparison indicating that the Pearson correlation for harem geldings (r = −0.37) was significantly different both from males at Conger that were intact (r = 0.08; z = 10.08, P = <0.0001) and controls at Frisco (r = -−0.08; z = 5.4, P = <0.0001; Fig. 5 C).

3.4. Bachelors

Bachelors in 2017 that were gelded were more likely to remain bachelors than those who were intact (Kruskal-Wallis K = 6.326, df = 2, P = 0.0423; Fig. 6A). Control males in Frisco and intact males in Conger who were bachelors in 2017 acquired harems over the years of the study. By 2020 fewer than a third remained as bachelors. There was no effect of gelded status on harem stallions that had lieutenants or tags associating with their group (intact: Wilcoxon W = 14, P = 0.1059; control: W = 6.5, P = 0.766; Fig. 6B), and there was no effect of gelded status on whether a bachelor became a lieutenant or tag (intact: W = 14, P = 0.1081; control: W = 7.5, P = 1.0).

3.5. Foaling rate

Over the 4-year study populations grew to an estimated size of 311 and 217 horses at Conger and Frisco, respectively, by September 2020. Foaling rate at Conger was reduced during the first year post-treatment

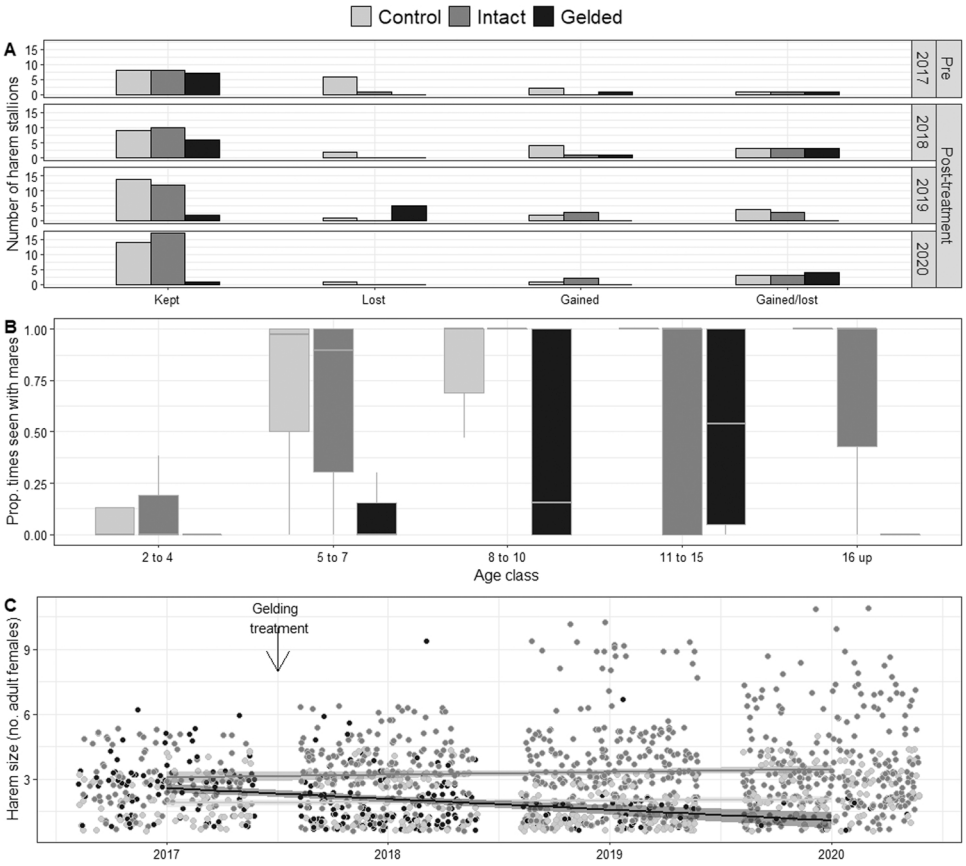

Fig. 5. Data on harem stallions at Frisco (intact controls) and Conger (gelded and intact males) Herd Management Areas, Utah, between 2017 and 2020. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year; they are shown separated in 2017 for comparison with post-treatment. A) The number of feral horse harem stallions in each year that kept their harem (i.e., were associated with mares on each observation), that lost their harem (i.e., were consistently seen with mares then were not seen with them again the rest of the year), that gained a harem (i.e., were seen alone or with bachelors, then consistently seen with mares), or that repeatedly gained or lost a harem during the year. B) Proportion of observations that males were seen with mares as harem stallions by age class. Boxplots show median values (horizontal lines), with the box limits indicating 25th and 75th percentile of the interquartile range and whiskers showing values 1.5 times the interquartile range above or below these percentiles. Instances where there is just a line at 1 or 0 indicate all observations were either of males with mares or without mares. C) Harem size over time. Regression lines with standard error are shown, together with all data points.

Table 3
Matrix of generalized mixed model estimates (beta values) and 95% confidence intervals showing the effect of age class and gelding on proportion of observations stallions were seen with mares at Conger Herd Management Area, Utah, USA. Significant effects are shown in bold.

Age class	Gelded	2–4	5–7	8–10	11–15	16 +
2–4	**-2.95 (−4.77 to −1.67)**		**2.54 (1.05–4.41)**	**4.13 (2.25–6.41)**	**3.11 (1.34–5.20)**	**2.82 (0.65–5.36)**
5–7		**-2.54 (−4.41 – −1.05)**		**1.59 (0.17–3.16)**	0.57 (−0.90 – 2.00)	0.28 (−1.65 – 2.24)
8–10		**-4.13 (−6.41 – −2.25)**	**-1.59 (−3.16 – −0.17)**		-1.03 (−2.70 – 0.51)	-1.32 (−3.49 – 0.80)
11–15		**-3.11 (−5.20 – −1.34)**	-0.57 (−2.00 – 0.90)	1.03 (−0.51 – 2.70)		-0.29 (−2.17 – 1.62)
16 +		**-2.82 (−5.36 – −0.65)**	-0.28 (−2.24 – 1.65)	1.32 (−0.80 – 3.49)	0.29 (−1.62 – 2.17)	

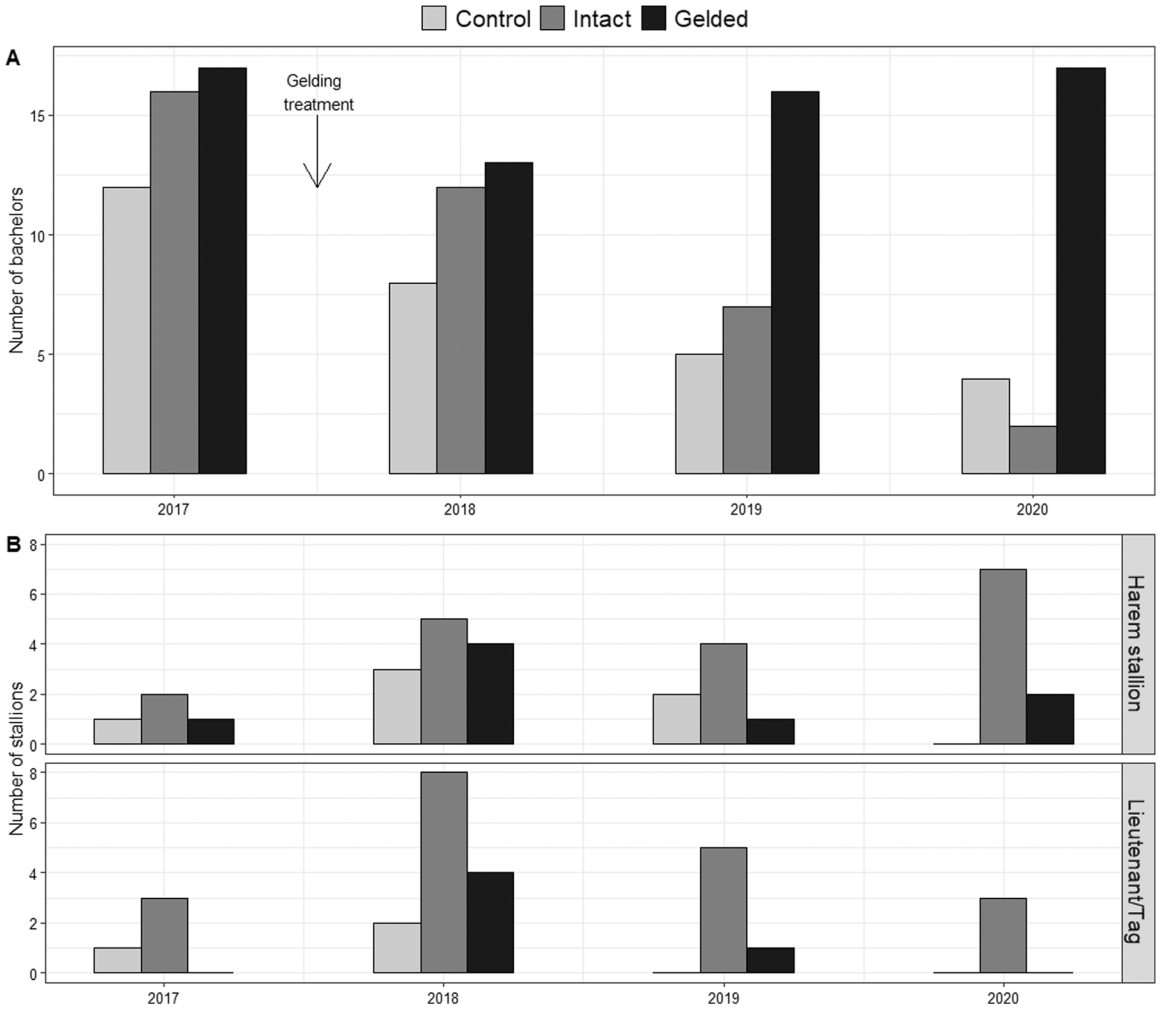

Fig. 6. Details of bachelors and multi-male groups at Frisco (intact control stallions) and Conger (intact and gelded males) Herd Management Areas, Utah, between 2017 and 2020. Twenty-seven males were gelded at Conger in December 2017 thus were intact during the pre-treatment year; they are shown separated in 2017 for comparison with post-treatment. A) Fate of males that were bachelors (i.e., not associated with mares) in 2017 across subsequent years of the study. B) Number of males that held harem status (i.e. were closely associated with mares) and that had a lieutenant or tag stallion following the harem (top panel), and number of bachelor stallions that were lieutenants or tags, following a harem (bottom panel).

Table 4
Foaling rate of feral horses at Conger (intact and gelded males present) and Frisco (only intact males present) Herd Management Areas in Utah, USA. Gestation length of horses is 11 months, thus foals are born approximately a calendar year after they are conceived. Foaling rate was calculated as the number of adult females (≥2 years old) divided by the number of foals born. 95% confidence intervals (CI) are provided. Treatment refers to castration (gelding), which occurred in December 2017.

Calendar year of birth	Conception year	Conger			Frisco		
		Females	Foals	Foaling rate (CI)	Females	Foals	Foaling rate (CI)
2018	Pre-treatment	57	44	0.77 (0.64–0.87)	42	29	0.69 (0.53–0.82)
2019	1 year post-treatment	69	40	0.58 (0.45–0.70)	46	32	0.70 (0.54–0.82)
2020	2 years post-treatment	86	61	0.71 (0.60–0.80)	57	33	0.58 (0.44–0.71)

but returned to pre-treatment levels by the second year post-treatment (Table 4). Confidence intervals were overlapping across all years for the control population at Frisco indicating there was no year effect.

4. Discussion

Our study showed that gelding is a safe potential management tool for feral horses: we had no mortality of gelded males, and no morbidity was observed. Gelded horses neither suffered loss of body condition following gelding, nor gained noticeable weight. Instead their body condition fluctuated similarly to other males on the range across seasons and years. Due to geldings' reduced testosterone (Thompson et al., 1980) we hypothesized that they would lose interest in mares and therefore devote more time to feeding and standing and less to moving. However, similar to observations on goats (*Capra hircus*; Silva et al., 2019), we found little effect of gelding on these behaviors. Instead standing was affected more by age class, with a year effect indicating environmental variables also play a role in this behavior. Our results indicated little biological impact of gelding: body condition did not differ between gelded and intact males.

No previous research has examined behavior of geldings in the presence of intact stallions. We expected gelding to reduce agonistic and reproductive behaviors due to lack of testosterone (Takeshita et al., 2017), and although there was a tendency for these behaviors to be shown less by gelded males, it was not statistically significant. Geldings have been observed to retain the full suite of equine behaviors, including reproductive behaviors (Sigurjónsdóttir and Haraldsson, 2019). We found low frequencies of agonistic behavior amongst males in our study populations, similar to those seen in other free-roaming horse populations (Fureix et al., 2012). Low levels of aggression can be simply related to space available for individuals to avoid each other (King et al., 2016), and agonistic interactions between males are moderated by ritualized displays and threat signals (Miller, 1981; Fureix et al., 2012; Sigurjónsdóttir et al., 2012). In our study age class was a major determinant of agonistic behaviors, with younger males exhibiting less agonistic behavior, and a clear shift in behavior trends once they passed 8 years old. This is considered prime of life for a stallion, when they would be most motivated to compete for a harem. Despite a lack of testicular testosterone production, geldings were still motivated to fight for access to mares, similar to free-roaming dogs that had no association between serum testosterone concentration and aggression whether castrated or not (Garde et al., 2016).

As would be expected, reproductive behavior was influenced by status, with harem stallions exhibiting more reproductive behavior than bachelors regardless of whether they were gelded or not. The persistence of reproductive behaviors in geldings likely influenced their drive to continue to seek a harem, although their ability to hold a harem decreased over time. Reproductive behaviors including herding, penile erection, mounting, and copulation have been observed in domestic geldings, and were seen whether the horse was castrated pre- or post-puberty (Line et al., 1985; Cox, 1986; i Rios et al., 1995). These behaviors were shown across age classes and harem statuses in our study, reducing the likelihood they were associated with prior sexual experience or time since castration. Bachelor males have lower levels of testosterone than harem stallions (McDonnell and Murray, 1995), so it was not surprising that they exhibited less reproductive behavior. However, demonstration of reproductive behaviors by bachelors may explain the short-term effect of gelding on foaling rate, as it is evidence of their motivation to mate. Extra-pair copulations (i.e., sneak mating by a male other than the harem stallion) have been documented in feral horse populations (reviewed in Boyd et al., 2016). Most sneak matings are considered to be done by other harem stallions or tag/lieutenant males; only one study specifically reported that sneak matings were conducted by bachelors (Ashley, 2000).

Gelding significantly affected marking behavior and affiliative behavior of males. Marking is conducted by males in response to mare eliminations, and as part of agonistic rituals when encountering other males (Rubenstein and Hack, 1992; King and Gurnell, 2007; Sigurjónsdóttir et al., 2012). Thus marking is likely affected by testosterone similar to reproductive behaviors; we saw a similar pattern of significance of status in addition to gelding affecting marking behavior, with harem stallions performing this behavior more than bachelors. Feces encodes information about familiarity, and stallions are undoubtedly aware of relative fighting ability with familiar males (Rubenstein and Hack, 1992). Thus geldings marking less may be related to loss of their harems.

While crucial for forming strong social bonds (Cameron et al., 2009), affiliative behavior has rarely been quantified in feral horses, particularly in relation to males (Costa et al., 2019). This is probably both because it is relatively rare and is often less obvious than agonistic interactions, however there is evidence that affiliative behavior is important for the cohesion and stability of horse social groups (Heitor et al., 2006; Costa et al., 2019; Sigurjónsdóttir and Haraldsson, 2019). Affiliative rates among males in our study were lower than those seen in Icelandic ponies of both sexes (including geldings) in a large pasture (Granquist et al., 2012; Sigurjónsdóttir and Haraldsson, 2019), but higher than those observed in a Przewalski's horse population (King and Gurnell, 2019), although generally consistent with rates in the literature (summarized in King et al., 2016). Notably, our results were only for males and included several behaviors, including play. As in Feh (2005), we observed play by both harem stallions and bachelors. We found that affiliative behavior was more frequent in both geldings and in younger horses, meaning that although geldings did not show any less agonistic behavior, they were possibly more inclined to reconcile or calm intense interactions (Feh and de Mazières, 1993).

We are confident that any differences in social associations observed post-treatment are a result of gelding, due to our field observations that social groups were maintained during and after the gather. As indicated by continued reproductive behavior and contrary to our expectations, not only did harem geldings not lose interest in mares but many continued to fight for and attain a harem throughout the study. By the end of the study only one gelding maintained a harem (a 14 year old who had a harem throughout the study), although four others gained and lost a harem during the final year. Harems of gelded stallions did become smaller over time, but geldings maintained similar aggression levels to intact stallions suggesting smaller harems were unlikely due to lack of fighting ability, but possibly a greater inclination for mares to change groups. In populations where mares are administered the fertility control immunocontraceptive porcine zona pellucida (PZP), mares continue to come into estrus but do not conceive; these treated mares change groups at a higher rate than untreated mares (Nuñez et al., 2009, 2017). A similar mechanism in which mares repeatedly come into estrus without conceiving could be also causing mares with harem geldings to change groups. It is notable that harem stallions did not immediately lose their harems after gelding, indicating that mares did not detect any changes that affected their social bond.

The biggest difference between geldings and intact males was among bachelors. Of the cohort of males who were bachelors in the pre-treatment year, all gelded individuals were bachelors in the final year of the study, compared to only a few intact males. As these gelded bachelors did not associate with mares prior to castration, this suggests that social or sexual experience may play a role in the drive to attain or maintain a harem, which could additionally be affected by lower testosterone levels in bachelors (McDonnell and Murray, 1995). We do not know whether bachelors who were older at the start of the study had a harem prior to our observations. Gelding treatment did not affect likelihood of a harem having multiple males. Both geldings and intact harem stallions were joined by lieutenants and tags, and both geldings and intact bachelors became lieutenants or tags. Formation of multi-male groups therefore seems independent of the presence of geldings in the population and is more likely due to other reasons beyond the scope of this study.

As a population growth management tool, gelding stallions had limited efficacy. At the levels applied in our study, it only reduced foaling rate in the first season after treatment. Statistical modeling of a horse population predicted that more than 90% of the male population would need to be sterilized in order to achieve meaningful population growth suppression (assuming that mares would be bred by any male; Garrott and Siniff, 1992). A higher proportion of males gelded would therefore be expected to show different outcomes for population control. Previous studies examining male fertility control also found short-term reductions in foaling rate, with treated stallions continuing to maintain a harem (Kirkpatrick et al., 1982; Eagle et al., 1993). In stable horse populations, most foals are sired by the harem stallion due to mare fidelity (Feh, 1990; Kaseda and Khalil, 1996; Gray et al., 2011) and the risk of infanticide and harassment (Cameron et al., 2003; Gray et al., 2011), but where group instability is high due to disturbance or other factors more extra-pair copulations are likely (Nelson, 1980). Both gelding and two gathers in two years likely contributed to some group instability at Conger, which combined with a cohort of males who were too young to geld in 2017 reaching maturity during the study likely resulted in increased sneak mating. A forthcoming study examining paternity of foals in this population will address this question. Gathering free-roaming horses over large areas is difficult. In our study population of known individuals in which groups could be located due to the presence of radio collared individuals it was still only possible to capture 53% of the males. This limits the ability to sterilize high percentages of males, and suggests gelding may be most effective when used in conjunction with other population control tools.

5. Conclusions

This study can allay public concern about welfare of gelded stallions – we found no difference in body condition or mortality, and geldings continued to exhibit the same suite of behaviors as pre-treatment, maintaining similar levels of aggression, and with ability to maintain a harem only slowly diminishing over time. In our study age class and/or harem status were often bigger predictors of behavior than gelding, and the lack of larger or longer impact on population growth may have been due to an insufficient proportion of males being gelded and due to young horses of both sexes achieving maturity during the study. Gelding has the potential to be effective as a management tool that reduces mare fertility rates only if a large proportion of males can be gathered and castrated, and is likely to be most effective for this when used together with other population control strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105598.

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Sworn Statement of Dr. Jay Kirkpatrick

1. I hold a B.S. in Biology from East Stroudsburg State College, and a Ph.D. in Reproductive Physiology from The College of Veterinary Medicine at Cornell University.

2. I currently work as the Director of The Science and Conservation Center in Billings, Montana. Prior to this position, I was an Associate Adjunct Professor in the Department of Population Health and Reproduction within the School of Veterinary Medicine at University of California, Davis. Concurrently, I was a Senior Staff Scientist for the Deaconess Research Institute in Billings, Montana. I also spent more than two decades working as an Associate Professor of Physiology in the Department of Biology at Montana State University-Billings, Montana. I also spent thirty years working with the U.S. Department of Interior, National Park Service, Bureau of Land Management (BLM), researching reproductive biology and chemical fertility control in wild horses.

3. For the past forty years I have dedicated my work as research scientist to studying wildlife fertility control. I have conducted research in a variety of areas including: reversible contraception in free-roaming herds, ovarian function in mares chronically treated with porcine zonae pellucidae (PZP), long-term effects immunocontraception of wildlife, and mechanisms of reproductive self-regulation in free-roaming ungulates. I was also integrally involved in the construction of a laboratory for wildlife immunocontraception research. In addition to this work, I have published more than seventy peer reviewed articles and peer reviewed book chapters on these topics.

4. Throughout my career I have been actively involved in many professional organizations. Since 1999, I have served as a Professional Fellow for the Association of Zoos and Aquariums (AZA), and for the past twenty years I have been a Member of the AZA

Contraceptive Advisory Group and the American Association of Zoo Veterinarians. In addition to these positions, I was a long-time member of the Society for Experimental Biology and Medicine and the Society for Study of Reproduction. A copy of my curriculum vita is attached, as Attachment A.

5. I have spent considerable time living, recreating, and working on the public lands in the Western United States, including six years working as a Park Ranger at Rocky Mountain National Park in Estes Park, Colorado. The wild horses that roam public lands are an integral part of the landscape and the ecological systems that comprise these areas.

6. I am familiar with the proposed BLM roundup in the Jackson Mountains Herd Management Area ("HMA"), which includes a proposal to return 44 geldings (castrated male horses) to the HMA. Based on my review of the pertinent scientific literature, I oppose this approach to herd management. It is extremely intrusive for the individual horses and not viable for the herds as a whole.

7. If stallions are sterilized as proposed by BLM, there will be massive changes in social organization and behavior. As a research scientist, current collaborator with the National Park Service with regard to wild horse management, and former BLM contractor who worked extensively on chemical fertility control in wild horses, I believe capturing and castrating – a non-reversible sterilization technique – wild male horses, and then releasing them back into the herds, is incompatible with the BLM's mandate to protect and retain the free-roaming nature of wild horses. This is true anywhere that BLM manages wild horses, meaning that geography plays no role in the level impact that would occur to horses and their herds as a result of this irreversible approach, because the impacts would be uniform across their range.

8. The very essence of the wild horse, that is, what makes it a wild horse, is the social organization and social behaviors that differ significant from domesticated horses. Geldings (castrated male horses) no longer exhibit the natural behaviors of non-castrated stallions. We know this to be true from hundreds of years' experience with gelded domestic horses. Furthermore, gelded stallions will not keep their bands together, which is an integral part of a viable herd. These social dynamics were molded by millions of years of evolution, and will be destroyed if the BLM returns castrated horses to the Jackson Mountains HMA.

9. Reproduction in male horses is a complex cycle that moves from the horse's brain to the testes and back again. Reproductive steroids, such as testosterone [T], affect the higher levels of the brain and cause a variety of a stallion's behaviors. These higher levels of the brain also send neural messages to the hypothalamus. The hypothalamus, in turn, stimulates the production and release of a protein hormone known as gonadotropin releasing hormone (GnRH). The GnRH, in turn, stimulates the anterior pituitary to produce and release follicle stimulating hormone (FSH) and luteinizing hormone (LH). The protein hormones stimulate the testes. Testosterone causes the aggressive behavior associated with successful stallions. These higher levels of the brain signal the hypothalamus to release GnRH, which in turn signals the pituitary to secrete FSH and LH. The FSH causes sperm production and the LH causes the testes to produce testosterone, which then feeds back to the brain, and on and on. This is a serious and significant alteration of normal physiological and behavioral functions for horses so treated and again, inconsistent with the law.

10. A major flaw in the plan to geld stallions and return them to the range both generally and at the Jackson Mountains HMA, is the fact that this will have absolutely no effect on reproductive rates of mares on the same range. As long as the harem band is intact and a

fertile stallion is at the head of it, mares will get pregnant regardless of the number of gelded stallions on the range. Indeed, the proposed action calls for 78 stallions (in addition to 44 geldings) – which is many more than is necessary to impregnate the 52 mares that will remain in the HMA. Further, gelded male horses will not become harem stallions, affecting social dynamics and behavior. But, beyond that, as explained above, the gelded stallions no longer even represent wild horses; they will quickly become the biologic anomaly that domestic horses have become. Finally, an emphasis on male fertility control to attempt to limit reproduction and "manage" any population of wildlife (deer, elk, horses) is superfluous; females have offspring – not males. Thus, as BLM has long recognized, population growth suppression must focus primarily, if not only, on limiting female reproduction. Since gelding does not further that goal, there is no scientific basis for returning geldings to the Jackson Mountains HMA under the circumstances.

11. The age priority given in the EA for removal actually runs counter to the objective of slowing reproduction. The removal of young animals from mares simply increases reproductive performance of those mares, a phenomenon known as compensatory reproduction (see Kirkpatrick and Turner 1991, *Compensatory reproduction among feral horses*. J. Wildlife Manage. 55:649-652). This action simply exacerbates the situation. Instead removal should focus on mares in the primary reproductive age class of five to ten years.

12. Castrating horses will effectively remove the biological and physiological controls that prompt these stallions to behave like wild horses. This will negatively impact the place of the horse in the social order of the band and the herd. For this reason, I oppose the BLM's proposed method of managing the Jackson Mountains HMA. There are less intrusive, more humane, and more sustainable forms of fertility control available to the BLM, which find

much more support in the scientific literature, and which comport with BLM's past understanding and handling of these issues with respect to wild horse population control.

Pursuant to 28 U.S.C.§ 1746, I hereby declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Jay F Kirkpatrick

Jay Kirkpatrick

Executed this 25th day of April, 2012.

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Achieving population goals in a long-lived wildlife species (*Equus caballus*) with contraception

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Abstract. The ultimate goal of any wildlife contraceptive effort is some alteration of the target population, either through a slowing of growth, or stabilisation or reduction of the population. Early population models suggested that short-term contraceptive agents applied to long-lived species would not achieve significant population changes. Native porcine zona pellucida antigen (PZP), a short-term contraceptive vaccine, was applied to a herd of wild horses inhabiting Assateague Island National Seashore, MD, USA, over a 13-year period, with an immediate goal of achieving zero population growth, a secondary goal of reducing the population from 175 to 150 and a tertiary goal of reaching 120 individuals, all without the physical removal of animals. Contraceptive efficacy ranged from 92 to 100% on an annual basis (96.28 ± 2.49%), and the percentage of adult females that was treated on any given year ranged from 42 to 76% (67.78 ± 18.21%). The goal of zero population growth was achieved in 2 years, an initial decline in the population became apparent in 8 years and by Year 11, the population declined to 135, a decrease of 22.8%. The lengthy period required for achieving a population decline was caused by increasing body condition scores, reduced mortality and significantly increased longevity among treated females.

Additional keywords: equids, fertility control, horses, immunocontraception, population, zona pellucida.

Introduction

Contraceptive research aimed at managing populations of wild horses did not begin until the late 1970s (Kirkpatrick *et al*. 1982; Turner and Kirkpatrick 1983). These early and subsequent studies (Plotka *et al*. 1988; Kirkpatrick *et al*. 1990, 1991; Eagle *et al*. 1992; Willis *et al*. 1994; Daels and Hughes 1995; Goodloe *et al*. 1997; Turner *et al*. 1996; Turner *et al*. 1997, 2001, 2002; Cameron *et al*. 2001; Stafford *et al*. 2001; Killian *et al*. 2004) were all directed at testing the efficacy and safety of various antifertility compounds, vaccines, devices and delivery systems and/or the effects on social behaviour.

The actual management of wild horse populations by means of fertility control was initiated in 1995, on Assateague Island National Seashore (ASIS), MD, and represented the first attempt at actually altering an entire population and attempting to achieve a discrete population goal exclusively with fertility control. Shortly thereafter, additional attempts at managing entire populations were initiated on Carrot Island, NC, for the Rachel Carson National Estuarine Reserve, on the Shakleford Banks of Cape Lookout National Seashore, NC, and Return-to-Freedom Wild Horse Sanctuary, CA, and are ongoing. Two smaller and limited trials for the contraceptive management of wild horse populations were initiated in 2001 and 2002, on the Pryor Mountain National Wild Horse Range, MT and the Little Book Cliff National Wild Horse Range, CO respectively. The antifertility agent of choice for these initial attempts at population management was porcine zona pellucida (PZP) vaccine, first described as an inhibitor of fertility in horses by Shivers and Liu (1981) and Liu *et al*. (1989) and first applied to free-ranging wild horses by Kirkpatrick *et al*. (1990).

The onset of serious wildlife contraceptive efforts, in the early 1980s prompted the growth of population modelling with which to assess the theoretical effectiveness of contraceptive efforts. Several models were developed with which to determine the various levels of effort and the concomitant effects of contraceptive management on entire populations of large ungulates, and specifically, wild horses (Garrott 1991; Garrott and Siniff 1992; Hobbs *et al*. 2000). These various models examined possibilities within the parameters of (1) duration of contraceptive effect, (2) reproductive rates, (3) mortality rates and (4) contraceptive efficacy. The early predictions, although interesting, lacked a sound empirical database derived from actual population management trials, yet provided some direction for researchers in the field (McCullough 1997). For example, before the late 1990s, most models suggested that contraception would only be effective in species with short life spans and high reproductive rates (Hone 1992). Other models suggested that only contraceptives with multi-year life spans (Garrott 1991*a*) or actual sterilising agents (Garrott 1995) would be effective in population management. Still other complicating factors in early models included reproductive and growth rates of herds. Garrott (1997) suggested that only herds with less than 15% growth rates and the application of contraceptives with pharmacological life spans of 3–10 years

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would lend themselves to successful contraception. Almost all, however, made it clear that generalisations based on data that were not site specific with regard to herd age class profile, reproductive and mortality rates were fraught with risk.

The purpose of this research report is to provide empirical data regarding the management of a wild horse population exclusively by means of contraception, from which even more accurate models might be developed, and for other ungulate species that are candidates for contraceptive management. The hypotheses put forth at the beginning of this study were, that contraceptive management of a herd of barrier island wild horses, using a remotely-delivered short-term fertility control agent, could (1) stabilise the population rapidly and (2) reduce the herd size from ~175 animals to 150 over a longer period of time, and in a second phase, further reduce the population to 120, without the need to remove animals. The time elements necessary to achieve these goals were undefined at the initiation of the study.

Methods

Population

The study population consisted of wild horses inhabiting ASIS. The species has inhabited the island for 330 years and originated from 28 wild horses inhabiting the Maryland portion of the island (16 085 ha) at the time of the creation of the national seashore, in 1968. This population expanded to a high of 175 in March of 2001. The nature and circumstances surrounding this population, and the need for managing it in a manner ensuring acceptable ecological impacts and the maintenance of a healthy and viable herd have been described in detail elsewhere (Kirkpatrick 1995; Kirkpatrick and Turner 2007).

In 1988, the managing authority for horses, the National Park Service (NPS), authorised research aimed at evaluating the use of immunocontraception as a potential population management tool, and from 1988 through 1993, 46 different mares were intermittently treated with PZP vaccine (Kirkpatrick *et al*. 1990). Research during this period showed that the vaccine was effective and safe (Kirkpatrick *et al*. 1990, 1991, 1992, 1995). Beginning in 1994, with the herd size at 166, the NPS authorised actual management of the ASIS herd through PZP contraception. In phase I, and under the provisions of a written management plan and required environmental assessment, every untreated mare 2 years or older and which had never been treated before was given a single 'primer' dose of PZP. The purpose of this phase was to cause antigen recognition in the herd and thereby create a 'one-inoculation' population that would only require a single booster inoculation in subsequent years. In 1995, with the March herd size at 166 and the environmental assessment completed, phase II of the management plan was initiated, with an immediate goal of stabilising the population, a secondary goal of reducing the population to 150 animals and a tertiary and subsequent goal of reaching 120. For the first 3 years, the management plan allowed the inoculation of all 2-year old mares and booster inoculations with PZP at ages 3 and 4, followed by withdrawal of treatment until the mare produced three living offspring or a second generation, after which the mare would be treated with the PZP annually until her death. The underlying philosophy behind this plan provided for a delay in the reproductive age of mares, but a continuous contribution of genes from all mares on the island. This plan was modified in 1998 to allow only two foals after withdrawal from treatment, and again in 2000 to allow only a single foal, largely because there was no apparent decrease in the population as a result of contraception. This plan was maintained through 2007.

Vaccine application

The vaccine was prepared according to the modified method of Dunbar *et al*. (1980) at the University of California, Davis from 1988 through 1997 and at the Science and Conservation Center thereafter. Delivery of the vaccine was by 1.0 cc Pneu-Darts, at doses of 100 μg, along with an appropriate adjuvant, as described by Kirkpatrick *et al*. (1990, 1991), at ranges of 10–55 m and authorised under the Food and Drug Administration Investigational New Animal Drug Exemption File 8857-G0002. From 1994 to 2001 the adjuvant used for all initial inoculations was Freund's complete adjuvant (FCA), and since 2002, Freund's modified adjuvant has replaced the use of FCA. All booster inoculations for the entire duration of this study utilised Freund's incomplete adjuvant. No horses were captured or handled during the course of this study.

Data collection

All animals in the Assateague population are monitored every 2 months by an ASIS employee (AT) and data collected include: (1) identification of individual animals by unique markings; (2) band affiliation; (3) band location; (4) band habitat; and (5) dates of births and deaths. Numbers presented in this study are not estimates, but actual numbers based on bi-monthly data collection.

Results

The ASIS wild horse population profile is illustrated in Fig. 1. Systematic and regular population counts were initiated in 1975 and the period between then and 1987 indicates a growth curve that represents an approximate 8% annual increase. The dramatic drop between 1987 and 1990 was associated with the initiation of contraceptive research, which removed 26 mares from the reproductive pool, and an epidemic of eastern equine encephalitis (EEE) that caused, at a minimum, the death of at least another 14 horses. By 1997, 2 years after the initiation of management-level contraceptive control, the population was stabilised and continued within a range of nine animals over the ensuing 7 years. The March population began a decline to 158 animals in 2005, 138 in 2006 and 135 in 2007. During the 11 years following the initiation of management-level contraception, foaling rates dropped to $6.9 \pm 2.30\%$ annually (an approximate 88% reduction) for mares 3 years or older. Thus, the first two population goals of stabilisation and a reduction to 150 were achieved and significant progress has been made towards the goal of 120.

The age profile of the herd changed coincident with the intensive contraceptive control and is illustrated in Fig. 2, which provides a comparative view from 1990, before management, to April 2007. This change in age profile is characterised by (1) fewer young animals, (2) an increase of animals in older age classes and (3) the appearance of entirely new older age classes, primarily among mares. Sex ratio also changed

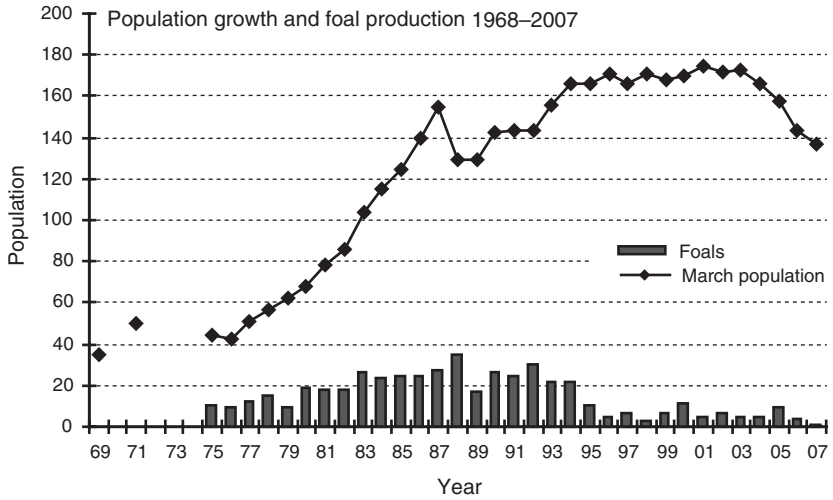

Fig. 1. Population changes of the Assateague Island National Seashore (ASIS) wild horse herd over a 38-year period. Contraceptive management began in 1995.

during the contraceptive management of the herd. Historically, the ASIS sex ratio has been ~1 : 1, but after 12 years of contraceptive management, the ratio has changed to 51 stallions : 84 mares, or 1 : 1.68 (Keiper and Houpt 1984).

The effort required, and the contraceptive efficacy necessary to bring about this level of population change, in this particular herd, is shown in Table 1. The percentage of adult mares treated annually ranged from a low of 52% to a high of 95% (67.78 ± 18.21). The latter high figure represents an effort in 1994, the year before the actual management effort went into effect, to treat all previously untreated mares with a 'primer' inoculation, not for purposes of contraception that year, but rather to establish the population as a 'one-inoculation' herd thereafter. The annual efficacy of the contraceptive vaccine ranged from a low of 92% to a high of 100% (96.28 ± 2.49). As time increased, so did the amount of time necessary to dart the target mares. Table 2 indicates this trend.

Discussion

The application of a short-acting immunocontraceptive to certain wild horse herds has the potential to reduce populations of long-lived wild horses given sufficient time; however, the application must be fairly intense over time. The change in population associated with contraceptive management provides some cause for optimism with regard to wider applications elsewhere. Mortality, outside of episodic disease and weather catastrophes, has been historically low in this population, yet the attainment of near-zero population growth was possible in only a few years.

At the outset of this research, historic data for population dynamics of this herd included a $57.1 \pm 3.9\%$ foaling rate (among adult mares), an annual 5% mortality rate and an overall annual 8% population growth rate (Keiper and Houpt 1984). The average age of a mare in 1994, before management-level contraception, was 8.19 ± 5.6 (s.d.) years. The 88% reduction in foaling rate during the 11 years following the initiation of management-level contraception, for mares 3 years or older, was expected; however, mortality rates also dropped until recently. From the onset of contraceptive management, in 1995, to 2003, the annual mortality rate was $3.3 \pm 1.0\%$ and the average age of mares increased to 14.8 ± 6.4 years. This was a period of population stabilisation characterised by an ever-increasing longevity among treated mares (Kirkpatrick and Turner 2002, 2007). As treated mares reached a new 'age of mortality', from 2004 to 2006, the mortality rate increased to $9.0 \pm 1.0\%$. The increased longevity has previously been shown to be directly related to contraceptive treatment (Kirkpatrick and Turner 2007), with the average age at death reaching >20 years for mares treated for 3 years or more.

In a recent critique of immunocontraception in wildlife, Cooper and Larsen (2006) suggested that it may be impossible to evoke a strong enough immune response against the contraceptive antigen to cause contraception in a large enough proportion of the target animals for effective population control. Much earlier, Seal (1991) suggested that any effort to successfully manage free-ranging populations would require an agent with 100% efficacy. The concept embedded in that concern confuses two elements of wildlife contraception. Virtually all contraceptives have differing efficacies (see Asa 2005; Frank *et al*. 2005), but the more important question of relevance in wildlife contraception is to what proportion of the target population the contraceptive can be delivered. It is intuitive that a contraceptive with an overall efficacy of 92–100%, as in the case of PZP and wild horses, or any contraceptive for that matter, would not be acceptable for human contraception. However, if a 92% effective contraceptive was applied to 48 to 76% of the human population, as it has been in the ASIS horses, there would be a significant decrease in fertility rates of humans. Obviously, the more effective the contraceptive, the more effective is the population control that can be achieved, but efficacy alone is not the determining factor in achieving success with wildlife.

The dramatic increase in the average age of the female population would be expected as a result of fewer young animals; however, those average ages alone mask the more significant trend of a shift to older age classes. In 1994, the

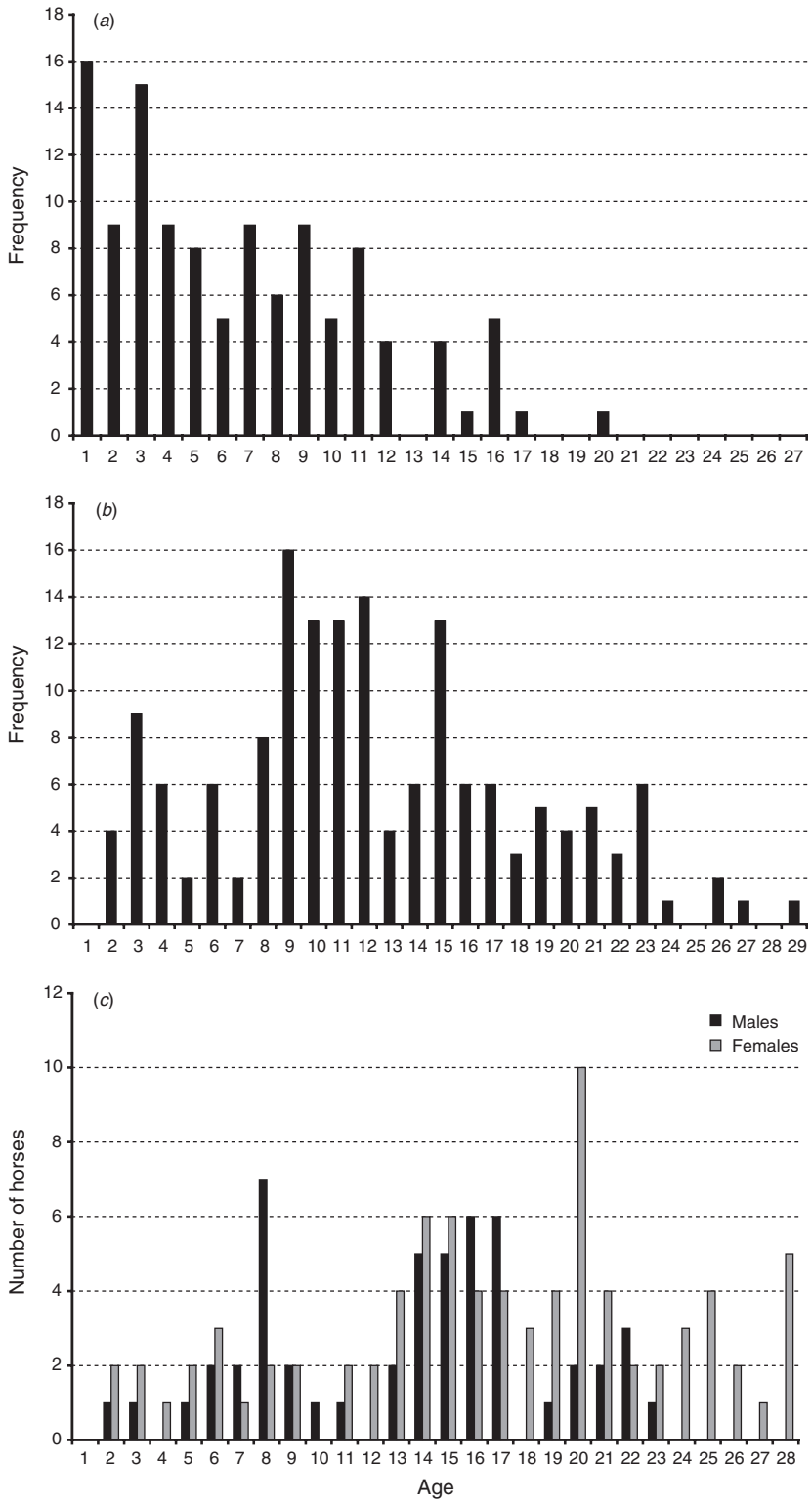

Fig. 2. Age-class structure for the Assateague Island National Seashore (ASIS) wild horse herd in (*a*) 1990, (*b*) 2002 and (*c*) 2007. Part (*a*) describes the age-class structure before contraceptive management, and it reflects a 'typical' structure profile for most wild horse herds. Part (*b*) indicates a shift in the structure after 7 years of management-level contraception, with increasing longevity being the major feature. Part (*c*) gives the structure for both males and females after 12 years of contraceptive management, reflecting the major increase in longevity among females.

Table 1. Percentage of mares treated and contraceptive efficacy, 1993–2006

Year	Spring population	Mares treated (%)	Foals	Foals from treated mares	Foals from untreated mares	Efficacy
1993	156	15 (20)	22	4	18	94%
1994	166	76 (95)	22	0	22	100%
1995	166	68 (83)	10	1	9	99%
1996	171	72 (84)	5	2	3	97%
1997	166	54 (59)	7	2	5	97%
1998	171	49 (52)	3	1	2	98%
1999	168	48 (52)	7	3	4	94%
2000	170	64 (68)	11	1	10	98%
2001	175	75 (79)	5	0	5	100%
2002	172	66 (69)	7	5	2	93%
2003	173	66 (70)	5	4	1	94%
2004	166	74 (76)	5	3	2	95%
2005	158	69 (71)	9	5	4	93%
2006	143	65 (71)	4	3	1	96%

Table 2. Number of days spent darting, 1994–2007

Year	No. of mares treated	Total days darting
1994	76	15
1995	68	14
1996	72	12
1997	54	17
1998	49	16
1999	48	17
2000	64	20
2001	75	25
2002	66	25
2003	66	28
2004	74	26
2005	69	34
2006	65	29
2007	55	25

percentage of mares between the ages of 15 and 19 years and >20 years were $9.8 \pm 1.8\%$ and $3.7 \pm 0.9\%$ respectively. By 2006, those same age-class cohorts had increased to $26.1 \pm 2.7\%$ and $23.8 \pm 2.5\%$ respectively. Thus, historic population dynamic data alone will not provide the necessary information to develop a sound and effective contraceptive management plan. Normally, 20 years represents an extreme old age among wild horse herds. Eberhardt *et al*. (1982) considered 20 years the maximum age for wild horses inhabiting ranges in Oregon, and Garrott *et al*. (1991) found that among 14 788 wild horses across Nevada, only 115 (0.007%) were older than 20 years. Linklater *et al*. (2004) found that among New Zealand's Kaimanawa wild horses, only an average of 1.0% would reach 20 years. Thus, the dramatic departure from these age restrictions among the ASIS horses represents a new understanding of the effects of contraception.

A major effect of the shift to older age classes among mares on ASIS is the change in the sex ratio from the historic 1 : 1 (male female) to 1 : 1.67, post-contraception. This change is clearly the result of mares living longer than before contraceptive management. There is no expectation that this change will widen in favour of mares, as mortality rates have now increased, once the mares reach a new 'age of mortality'. Normally, sex ratios for foals among most wild horse herds is near parity but in some cases it changes in favour of males in older age classes (Garrott 1991*b*), but this is not a hard and fast rule. The male : female sex ratio for a Nevada wild horse herd was 0.78 : 1.0 (Greger and Romney 1999). Indeed, a National Academy of Sciences study found a wide range of sex ratios among wild horses (NAS 1982). Other management options that involve removal of animals may also skew sex ratios. In the Pryor Mountains of Montana, the adult sex ratio in 1970 was close to 1 : 1 (48 males : 52 females) (Feist and McCullough 1975), but within the ensuing 8 years, the ratio changed to 1 : 2, largely because of the bias in removing males through removal programs (Perkins *et al*. 1979). Given the stabilisation of the sex ratio on ASIS, as a result of more mares dying now, the current sex ratio should pose no serious problem and should shift back towards parity.

Used to slow or stabilise population growth among a long-lived species such as the horse, rather than to decrease numbers, PZP immunocontraception can have a rapid and significant effect. A reduction in growth rates and stabilisation of the population can occur in as little as 2 years if the intensity of the treatment is high enough. Indeed, such stabilisation of population growth has even been reported for African elephants treated with PZP (Delsink *et al*. 2006, 2007). If the management goal is reduction in herd size, on the basis of contraception alone the time necessary will be significantly longer and, it is now clear, even longer than historic models predicted, based on historic foaling and mortality rates. Both of the latter parameters, foaling and mortality rates, will change as the length of treatment time for individual animals increases, as a result of significantly lower mortality, increased body condition and increased longevity. Thus, population models will have to account for these changes in population dynamics, or contraceptive management must be flexible and adaptive.

Some concern might arise over the genetic effects of intense contraception. Studies were conducted on the genetic diversity of

the ASIS population (Eggert *et al*. 2005) using faecal mtDNA haplotypes and nuclear genotypes at eight polymorphic loci. The results indicated that the relative genetic contributions of the founder animals were well represented and consistent with a high level of nuclear genetic diversity. Even at numbers as low as 100 horses, the population will not accumulate significant loss of genetic diversity sufficient to result in any inbreeding depression effects, nor will kinship increase, over time, and lead to line breeding potential (CBSG 2006).

Despite these empirical data, concerns about genetic effects of wildlife immunocontraception persist in some quarters (Nettles 1997; Cooper and Larsen 2006). In the case of the ASIS population, contraception has eliminated the use of gathers and removals for population control. As conducted by other agencies, for other wild horse herds, gathers and removals most often focus on young horses that are popular for adoption by the public. These removed animals have never bred or entered the gene pool, thus it is intuitive that the potential for genetic effects of the alternative management approach is far greater than from a simple delaying of reproduction and decreasing the number of foals produced by each mare.

It is now clear that contraception is associated with significant downward population trends and changes in long-lived wildlife species. The requirements for achieving these changes are both species specific and site specific, but careful analysis of all demographic and contraceptive parameters for any given species and agent, based on the data derived from the Assateague studies, suggests that this approach is viable and can be effective.

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DIRECTOR'S REPORT

Advancing Science within the Bureau of Land Management

February 2012

DIRECTOR'S REPORT

ADVANCING SCIENCE WITHIN THE BUREAU OF LAND MANAGEMENT

INTRODUCTION

The Bureau of Land Management (BLM) has a critical and compelling mission: *to sustain the health, diversity, and productivity of America's public lands for the use and enjoyment of present and future generations.* Distinctive to its mission within the Department of the Interior (DOI) are the BLM's core purposes of multiple use and sustained yield. Resource development, restoration, and conservation are all essential activities of the BLM.

Fulfilling its mission requires leadership and field managers to make important decisions about natural and cultural resources under BLM's management. These decisions often impact adjacent lands, waters, and communities. They often impact other federal and state resource management agencies. They take place within a broad and complex environmental, legal, socioeconomic, scientific, and political context. Hence, effective BLM decision-making must reflect best available sound science, accurate fidelity to the law, and long-term public interest.

The BLM must be a "science-informed" bureau. Science for the BLM includes the full range of disciplines within the biophysical and socioeconomic sciences relevant to the multiple-use mission of the BLM. It includes monitoring, research, syntheses of existing science, and application of findings to meet BLM resource management needs. BLM science (science conducted by the BLM and/or for the BLM) should be mission-oriented, place-based, high-quality, objective, and result in usable knowledge for BLM managers and stakeholders.

Given the extent of current and future environmental and socioeconomic change—including energy development, demographic trends, climate change, and water demands—the scientific needs of the BLM are necessarily increasing. Like other federal land management agencies, the BLM must employ science as it "manages for change".

To prepare the BLM to effectively manage now and in the future, the science it uses must be expanded, improved, and more deeply integrated into the bureau's culture and decision-making

processes. In May 2012, BLM Director Abbey commissioned a Director's Report that would assist in advancing science within the BLM. Director Abbey asked that the BLM's National Landscape Conservation System (NLCS) serve as a pilot in this effort.

The BLM has produced several strategic plans for science. These documents include detailed objectives, research agendas, processes for priority setting, and implementation actions. The development of an additional strategic plan or an update of existing on-the-shelf plans is unlikely to have significant impact on the bureau's approach to and use of science. Rather, this Director's Report focuses on specific recommendations for: a) advancing science within the BLM, b) integrating science into BLM decision making, and c) accelerating the progress of advancement and integration of science for the BLM.

How this Director's Report was Prepared

BLM Director Bob Abbey requested that Dr. Gary Machlis, Science Advisor to the Director of the National Park Service, prepare the report. Dr. Machlis co-leads the DOI Strategic Sciences Group, and is a professor of conservation at the University of Idaho. Dr. Machlis formed a core group that included Dr. Matt Preston, NLCS National Science Coordinator, and Dr. Healy Hamilton, an independent scientist with experience working with BLM managers. An executive committee of BLM field and national leadership was formed to provide guidance, assistance, and review of the Director's Report.

The core group conducted a detailed review of over 75 laws, regulations, policies, and plans related to science within the BLM. In addition, they conducted over 40 detailed interviews with a range of individuals including: a) BLM leaders and managers at the national, state, and field levels, b) BLM scientists and professionals, and c) external scientists with knowledge of BLM science, such as USGS scientists and university faculty. These interviews were not a formal representative sample, but did provide a range of insights regarding advancing science in the BLM. The core group met with BLM managers within the NLCS at their 2012 management conference in Albuquerque, NM. They traveled to the BLM National Operations Center (NOC) in Lakewood, CO and met with a range of group leaders and staff. A draft of this report was reviewed by the executive committee and comments were provided to the core group. A final

draft was completed, reviewed by the executive committee, and prepared by Dr. Machlis and other members of the core group for delivery to the BLM Director.

BACKGROUND

Authorities and Mandates

The BLM has legal, regulatory, and policy authorities and mandates to conduct science. The Federal Land Policy and Management Act of 1976 (FLPMA) provides the BLM explicit authority to conduct management-related science and requires the systematic consideration of scientific information when developing land use plans. Other authorities require scientific investigation and consultation before BLM actions (e.g. NEPA, Endangered Species Act), while some legislation requires scientific tasks on specific topics (e.g. inventory and monitoring of archaeological resources). More generally, the DOI policy manual (e.g. §305 DM 2) describes how science shall be integrated and used in management practices and decisions. Similar mandates for incorporating science into management are in several other DOI and BLM policy documents (e.g. BLM Manual §1601), including those related to the NLCS (e.g. Secretarial Order 3308; BLM Manual §6100).

The bureau also has an obligation to ensure that BLM lands remain a resource for the scientific community. FLPMA, the NLCS' Organic Act, and several individual laws creating NLCS units call for the protection of the scientific values of BLM lands, i.e. the protection of resources deemed to be of importance to scientific research.

Organizational Support for BLM Science

BLM scientific expertise is spread throughout the bureau. In the Washington Office there are several individuals tasked with providing scientific advice and/or coordination; most do not work full-time on science projects or policies. Many are affiliated with specific programs and focus on applying relevant science to these programs. There are also scientists assigned to multi-state zones who provide expertise to BLM offices.

In State and Field Offices, many resource staff members have scientific backgrounds. The majority of BLM's field-level scientific expertise is focused on interpretation and application of available science, rather than the conduct of scientific research as part of their workload.

The NOC's Division of Resource Service houses a group of BLM's senior technical specialists, including some who conduct scientific research. The Washington Office uses their expertise to conduct and support national-level projects. Some State and Field Offices utilize NOC technical expertise as well.

The BLM has outsourced substantial scientific work to partners that include local, tribal, state and federal governments, academic institutions, professional societies, industry groups, entities under land use authorizations, non-governmental organizations, citizen groups, and contractors.

Scientific funding is spread throughout the bureau. In FY11 about $15 million was spent on applied research projects across the BLM States. Some scientific activities, such as monitoring, are not included in this amount. Funding does not come from a centralized pool and is spent on an *ad hoc*, office-by-office basis.

The BLM chartered an external Science Advisory Board under the Federal Advisory Committee Act (FACA) in the late 1990s and early 2000s to provide scientific advice to the BLM Director. It is no longer active. Various versions of an internal National Science Committee have been formed to coordinate, prioritize, and communicate scientific endeavors. At times the Director has had a science advisor. Current and developing science-related committees include the BLM Science Committee/Core Science Team (the most recent version of a National Science Committee), the NLCS Science Advisory Team, the Data Advisory Committee/Working Group, and the Socio-economic Oversight Committee.

BLM Science Strategies

The BLM has prepared several overarching science strategies, including versions in 1991, 1996, 2000, and 2008. A science strategy for the NLCS was prepared in 2007. These strategies share several themes and goals. Each of the science strategies recognize the need to: a) systematically identify research needs; b) engage with science partners; and c) transfer research results to

managers and to the public. Implementation plans, or draft implementation plans, have been written for several of the strategies.

Other currently available science-related strategies include a socioeconomic strategic plan, a geo-spatial strategic plan, the joint fire science plan, and NLCS unit science strategies. The latter has been completed in a few NLCS units and a new template for unit strategies was recently developed that includes the unit's scientific background, scientific needs, engagement with partners, and more.

While not a formal "science strategy", the BLM's recent adoption of a landscape approach is a framework for science-informed management, with scientific data, assessments, and projections forming the base from which to develop management direction and decisions. It builds upon previous BLM field applications, such as the Northwest Forest Plan and the Interior Columbia Basin Ecosystem Management Project. This approach connects regional and local decisions to landscape-scale understanding (e.g. BLM's rapid ecoregional assessments, REAs). The landscape approach requires standardization of assessment, inventory, and monitoring (AIM) efforts by instituting common indicators, statistically-valid data collection, and centralized data storage (i.e. BLM's AIM Strategy).

Results of Interviews with BLM Staff and Partners

Amongst interviewees there was widespread but not universal agreement that the primary obstacle to integrating science into BLM decision making is organizational culture. The BLM is viewed as a decentralized agency with inconsistent direction from leaders at multiple levels concerning the integration of scientific information into decision making. Pressure to incorporate available science in the planning and use authorization processes is often viewed as arising from external forces, such as litigation. There was general recognition that the need for science to become a more ingrained component of BLM decision-making has never been greater, as the multiple, complex, and interacting forces of environmental and socioeconomic change (e.g. intensified fire regimes, resource development, invasive species, and climate change) erase the concept of "business-as-usual" for the BLM.

Interviewees felt that the range of disciplines that provide information potentially useful to BLM decisions is broad and diverse, including life, earth, and socioeconomic sciences, and the pace of discovery and advancement is fast and increasing. There was recognition that it is impractical for BLM to maintain internal scientific expertise in all these fields with responsibilities to conduct research, assist managers in translating data to decisions, and keep abreast of the latest advances in their respective fields. Those interviewed frequently mentioned the resulting emphasis on the essential role of science partners. A recurring theme emerged that existing partnerships are often underperforming relative to their potential. For example, perception of the quality, timeliness, and accountability of science requested and funded by the BLM and conducted by USGS greatly varied. While some interviewees were satisfied, the majority of BLM-USGS interactions were not regarded as a productive return on investment or as an effective agency-wide strategy for injecting usable science into management decisions. The Cooperative Ecosystems Studies Units were repeatedly mentioned as logical partners to more actively cultivate as science providers to the BLM. Establishing closer ties to relevant professional societies (e.g. Society for Range Management) was consistently mentioned as another potential underutilized avenue.

The interviews highlighted specific scientific needs required to support BLM activities at different scales. The most consistent need involved the translation of available scientific data into usable knowledge that managers can easily and rapidly incorporate in the process of decision making. In addition to science translation, there was a recognized need to organize and increase accessibility to existing data. Another science need identified by interviewees was better understanding of the implications of the forces of global change at multiple scales, from the project up to the landscape, and the incorporation of this understanding into decisions across these scales.

Finally, the NLCS was widely regarded by interviewees as a valuable locale for advancing BLM science, though it was often viewed as being isolated from the rest of the bureau's activities and core mission. Many interviewees saw the NLCS as a reference point for understanding the impacts of change drivers in the relative (but not complete) absence of extractive practices, as well as a location to conduct science in support of adaptive management. The NLCS was often

viewed as a system that could be utilized for experimental testing of alternative methods for achieving desired conditions. The degree of public visitation on the NLCS was recognized as an opportunity for increased application of social science research on recreation impacts, cultural and historic values, citizen science, and community engagement.

FINDINGS ON THE CHALLENGES FACING SCIENCE IN THE BLM

Like all federal land management agencies, the BLM faces challenges (real and perceived) in meeting the ambitious goal of becoming a "science-informed" organization. Some challenges are inherent to the bureau's complex mission; other challenges stem from organizational structure and culture. Additional challenges are related to work processes and the delivery of science to managers and decision makers. In addition, there are specific challenges to advancing science within the NLCS.

A key challenge facing the BLM is that its mandate for science is fragmented and sometimes not clearly understood or consistently applied by BLM managers. Flowing from this key challenge are several other organizational barriers to advancing science within the BLM: a lack of integration of science across BLM programs and directorates, inconsistent integration of science into BLM decision-making, and lack of consistent policies concerning science. Morale amongst BLM science professionals varies, as does the degree of scientific training of BLM managers.

There are also challenges with BLM work processes related to science. A major issue is improving the scientific foundation of environmental impact statements and assessments (EIS and EA). BLM analyses are often single-dimensional and species-specific, rather than integrative and systems-based. Similarly, the temporal and spatial scales of analyses are often too narrow; e.g. Field Offices' geographic boundaries are typically not the appropriate scale for addressing cumulative impacts and future scenarios. The BLM's reliance on *ad hoc* and reactive scientific efforts limits scientific integration. In addition, there are significant time and resource constraints that hinder the ability of BLM professionals to conduct and apply best available sound science.

Another key challenge is an often slow and less than adequate information transfer—including slow delivery of research results, presentation of results in formats that are of limited used to managers, limited access to data and relevant scientific literature, and lack of advanced

information technology. Inadequate information transfer may in part stem from BLM's often disconnected relationships with science partners, in which BLM lacks clear communication of needs and has limited engagement. The BLM's lack of a workable and consistent system of scientific peer-review for conducted and sponsored research limits the assurance of the scientific quality of products and affects manager's trust in using the scientific products in decisions.

There are also science challenges specific to the National Landscape Conservation System. While conserving "scientific values" is part of the NLCS mission, this mandate is ill-defined and hence not yet fully implemented. Science has a low profile with many NLCS managers and stakeholders. Often, NLCS science is perceived as "marginalized" or separate from broader BLM needs. There is significant lack of awareness of the NLCS value and opportunity amongst the general scientific community.

These challenges, while substantial, are tractable and create opportunities to advance the role of science within the BLM.

RECOMMENDATIONS FOR ADVANCING SCIENCE WITHIN THE BLM

The following recommendations are derived from the review of written documents, the interviews and site visits, and the core team's experience with advancing scientific programs. The recommendations are organized around three categories: a) organizational structure and culture, b) work processes and delivery, and c) distinctive challenges and opportunities for NLCS science.

Organizational Structure and Culture

1. *The BLM should revise appropriate sections of the BLM Manual and/or consider adding a new section in order to explicitly recognize and require that BLM resource decisions should be based on best available sound science, accurate fidelity to the law, and long-term public interest.* This policy will advance science as an essential input into decision-making. The manual should make clear that "science-informed" decision-making is the required standard for managers, and the BLM should invest in the necessary training and preparation of managers to enable this standard to be met.

2. *The BLM should establish a chief scientist position (at the ST level), reporting to the Deputy Director for Operations.* The chief scientist would provide critical science leadership. The BLM chief scientist would lead the development and implementation of science-related policies, processes, and priorities. Additionally, the chief scientist would provide advice, coordinate science across BLM states and directorates, and represent BLM science to other federal land management agencies, the broader scientific community, and stakeholders. The chief scientist should be a member of the Executive Leadership Team (ELT). The chief scientist would lead the implementation of several of the recommendations identified in this report. Line authority over individual scientists should remain within existing structures.

3. *The BLM should re-establish an external national BLM Science Advisory Board.* The board would provide necessary, independent advice on science and science policy to the Director. The board should operate under a FACA charter. It should include outstanding scientists from the external scientific community representing a broad range of relevant disciplines. The chief scientist should serve as liaison to the group.

4. *The BLM should eliminate the multiple and overlapping science committees and replace them with a single internal Science Coordinating Group (SCG).* A single coordinating group would increase efficiency, reduce overlap and unnecessary administrative burden, improve internal communication of science priorities, and focus responsibility for advancing science. The SCG would work with the chief scientist to develop BLM's science-related policies, processes, and priorities. It would include senior scientists and managers from Field Offices, State Offices, Centers, and Washington Office directorates, and be chaired by the Chief Scientist. Identified science leaders from each of BLM's three key work areas (conservation, development, and restoration) should be represented on the SCG. The Chief Scientist would be responsible for gaining support and approval for SCG outputs from the ELT and the Deputy Director. Technical work groups could be temporarily established for specific tasks (e.g. standardizing data, calculating ecosystem services).

5. *The BLM should clarify and communicate the scientific roles and responsibilities of the National Operations Center's Division of Resource Services*. This would improve the effectiveness of the division, clearly connect its work to the BLM mission, and increase awareness of its

services throughout the BLM. The role of the division should be developed collaboratively with BLM offices. Annual or multi-annual scientific responsibilities should be developed in consultation with the Science Coordinating Group.

6. *The BLM should institute ecoregion-based teams of scientists to facilitate and/or conduct scientific efforts for and with BLM State Offices, Field Offices, and NLCS units within that ecoregion.* With limited resources, ecoregion-based teams would enable efficient, coordinated, and relevant science to be proactively identified, conducted, and delivered to decision-makers. Collaboration with other DOI bureaus should be encouraged. Five-to-ten year work plans should be developed for these teams in collaboration with regional BLM leadership and the Science Coordinating Group.

7. *The BLM should accelerate development of a diverse set of qualified scientific partners, and reduce administrative hurdles associated with science partnerships.* Connection to the external scientific community is vital to ensure the application of modern scientific practices for BLM resource management. Collaboration with other DOI bureaus should be encouraged and supported with resources. Staff time needs to be allocated to developing quality partnerships and to working closely with partners before, during, and after projects.

8. *The BLM should invest in maintaining and improving the understanding of science and its application by BLM professionals*. To ensure that scientific understanding is a core tenet of BLM's work, BLM professionals need to be conversant in science and its application to BLM decision-making. Options include increased scientific training, scientist/manager meetings, and the publication of a quarterly science magazine for BLM professionals.

Work Processes and Science Delivery

9. *The BLM should examine each of its work processes and establish specific policies to ensure that science is integrated at relevant junctures*. This is a necessary and essential step to provide clear guidance on the practical actions that can advance science within the BLM. This effort should be the Science Coordination Group's first assignment, with draft policy statements submitted to the Deputy Director. For example, the SCG should develop a set of policies and procedures for the scientific activities required of permittees for each type of land use

authorization. More broadly, the SCG should consider policies that promote integrative, systems-based, multi-scale analyses of resources and impacts to resources; promote communication of uncertainty surrounding data or forecasts used in analyses; and for NLCS units, require the consideration of impacts to designated NLCS values.

10. *The BLM should establish and implement a scientific peer-review policy that applies to BLM funded science products, both internal and external.* Peer-review increases the quality of scientific products, and makes science-informed decisions defensible. The peer review policy should be consistent with OMB requirements currently in place and with the DOI peer review policy now in development. Implementing the policy should be based on producing best available sound science, meeting DOI information quality standards, and operating a peer review system that is efficient, cost-effective, and practical.

11. *The BLM should periodically and systematically identify and prioritize scientific needs, with a focus on supporting future management decisions*. In a period of limited resources, such assessments focus effort, increase effectiveness, and reduce unnecessary costs. Scientific needs should be identified at all levels of the organization, with input from the Science Advisory Board. For example, every NLCS unit should identify its scientific needs. The Science Coordinating Group should propose priorities for these scientific needs and then communicate them internally and externally. In addition, bureau-wide scientific needs that could be best answered in the NLCS should be identified (e.g. experimental testing of best management practices in a limited use environment).

12. *The BLM should provide operational support for rapid adoption of the Assessment, Inventory & Monitoring (AIM) Strategy protocols on all BLM lands.* This will allow systematic assessment of resource conditions at multiple scales and improve the quality of data for decision-making. Bureau-wide adoption of this standardized, quantitative approach should occur as soon as possible. The value of the AIM Strategy protocols should be enhanced by continued development of explicit, consistent, measurable standards for describing resource trends and conditions. Funds from across the bureau that have been historically used for ad hoc assessment, inventory, and monitoring efforts should be redirected towards this standardized approach.

13. *The BLM should ensure that geospatial data is properly collected, curated, analyzed, and distributed in order to support the systematic use of spatial data in decision-making, especially at local levels.* The availability of high quality geospatial data allows for large amounts of disparate information to be incorporated in the decision-making process, as well as communicated to diverse, non-technical audiences. Such data increase the defensibility of BLM decisions and potentially reduce BLM's exposure to litigation. In some cases, interagency collaboration will be the most efficient and cost-effective way to make geospatial data available to BLM managers.

14. *The BLM should establish a centralized, online science permitting and tracking system for both BLM funded and external projects.* This system would ensure research activities are appropriate and maximize the utility of the scientific projects being conducted on BLM lands. The NLCS would be an appropriate pilot location for this system. To properly permit research projects, the BLM should establish guidelines for appropriate scientific use, with specific and distinctive consideration for NLCS units (e.g. ensuring the preservation of NLCS units as benchmark sites). Guidelines should include reporting requirements, such as a brief manager's summary and a full scientific report.

15. *The BLM should enhance delivery of scientific information via a searchable, web-based, data and report portal.* This portal would greatly increase the communication of scientific information. The National Operations Center should develop this portal in consultation with State and Field Offices; the NLCS could pilot its use. Full potential of a web-based data and report portal would be realized if coupled with a modernization of the bureau's information technology infrastructure and policy (e.g. data sharing technology and policy).

Distinctive NLCS Recommendations

16. *The BLM should collaborate in the integration of the NLCS into a landscape-scale conservation network in partnership with the other federal land management agencies*. This network would help to ensure protection for all US ecosystem types, provide a venue to increase collaboration between and amongst agencies, and increase public understanding of federal conservation efforts.

17. *The BLM should systematically identify the "scientific values" of each NLCS unit and ensure the protection, conservation, and restoration of these values.* This would be an important step toward fulfilling the legislative mandate to protect, conserve, and restore scientific values in the NLCS. The BLM should issue guidance directed at NLCS units for identifying scientific values and ensuring their conservation (e.g. via management prescriptions provided in land use plans). Following the identification of NLCS scientific values, the BLM should communicate them to the internal and external scientific communities.

18. *The BLM should establish a standardized framework for periodically assessing how well NLCS units' designated values are protected, conserved, and restored.* Periodic assessment of conditions will improve the NLCS' management and funding effectiveness at both the unit and system level. In addition, these assessments can contribute to increasing public awareness and gaining public support. The assessments should be based on scientific data, collected at both the landscape-scale and the local-scale. Results from these assessments will aid in determining how compatible certain uses are with the NLCS' conservation mandate.

19. *The BLM should invest in a strategic NLCS communications campaign that brands the NLCS as "America's Outdoor Laboratory"*. A communications campaign would expand the scientific community's recognition of the NLCS as a well-known, respected, and useful place for conducting science. Communication techniques could include participation in appropriate scientific meetings, modern web-based communication efforts that describe priority NLCS research topics, and incorporation of scientific findings into outreach materials.

CONCLUSION

The BLM has a critical role in the future of American energy development, resource management, restoration of lands and waters, and conservation. Science is essential to fulfilling these responsibilities. The BLM must advance the role of science within the bureau, improve the integration of science into BLM decision-making, and accelerate the process of accomplishing both these goals. The NLCS, as a valuable national resource and laboratory for science, is a strategic and appropriate element of the BLM to pilot several of this report's recommendations.

BLM-relevant applied science and the usable knowledge that it creates has significant potential return on investment. It can improve decision-making, empower managers at the field, state, and national levels, engage citizens and communities, and increase the efficiency and effectiveness of the bureau. Hence, the need for action is also an opportunity for the BLM to manage its future.

Confronted with heightened resource demands and unprecedented and increasing environmental and socioeconomic change, there is great urgency in BLM acting boldly and decisively. The recommendations in this Director's Report, if implemented, will accelerate the advance of science within and for the BLM.

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A Review of Equine Standing Laparoscopic Ovariectomy

Monika Lee and Dean A. Hendrickson, DVM, MS

INTRODUCTION

Equine ovariectomies are performed for various reasons. The most common indications are to prepare a mount mare for semen collection, eliminate estrus behavior and colic signs associated with estrus,[1,2] sterilize the mare for registration purposes, and to prepare recipient mares for embryo transfer.[2] Other reasons are associated with removing pathologically abnormal ovaries in the form of granulosa–theca cell tumors.[2] Regardless of the reasons for performing the surgery, it has developed into a regularly requested procedure.

Different surgical approaches and amputation techniques have been developed to remove equine ovaries. Horses can be operated on while in a standing or dorsally recumbent position. The standing technique can be performed using sedation and local anesthesia,[3] whereas dorsally recumbent procedures require administration of general anesthesia.[2] General anesthesia has been shown to cause hypoxemia and hypoventilation that may necessitate a ventilator.[1] The surgery can be done as a ventral midline celiotomy, flank laparotomy, or colpotomy.[1,2] Colpotomy complications include unidentified and potentially fatal hemorrhage of the mesovarium caused by poor hemostasis, intestinal and mesenteric trauma, peritonitis, adhesions, and possibly death.[2] The flank approach is favorable because visibility is improved, but it is still difficult to exteriorize the ovary without making large incisions.[2] Consequently, standing laparoscopic ovariectomy has become a more common technique to remove ovaries in mares.

Advantages of laparoscopic techniques include the reduction of complications through full observation of the operative field, minimal invasiveness, a shortened convalescent time with fewer postsurgical complications,[1,2] and improved cosmetics after surgery.[1] It also allows for tension-free ligation of vessels in the mesovarium.[2] Concerns and limitations associated with laparoscopic techniques include the necessity and cost of specialized equipment, technical difficulty of the procedures and required training to conduct them, and the fact that a lack of familiarity with procedures can dramatically increase operating time.[2,3]

Performing laparoscopy in standing horses adds the advantage of avoiding the risk and expense of general anesthesia while providing easier access to the ovaries because of the location of the reproductive anatomy.[3] Limitations of standing procedures include horses with unsuitable temperaments for standing sedation surgery, the size of the horse, and the availability of appropriate facilities for restraint.[3]

MATERIALS AND METHODS

Patient Preparation

Feed but not water is withheld from the horses for 18 to 24 hours to reduce bulk in the peritoneal cavity and improve the working space. The use of prophylactic antibiotics is left to the surgeon's discretion; however, at most only perioperative antibiotics should be used. Preoperative nonsteroidal anti-inflammatory drugs should be given to reduce pain and inflammation associated with surgery. The horses are sedated, a catheter is placed, and surgical sedation can be obtained with intravenous boluses of detomidine, epidural detomidine (40 μg/kg brought to a total volume of 10–12 mL with sterile saline), or titrated to effect intravenous infusion of detomidine (20 mg/L liter saline or polyionic replacement fluids). Both flanks for bilateral ovariectomy, and the ipsilateral flank for unilateral ovariectomy, should be clipped and aseptically prepared for surgery. The surgical sites can be anesthetized with local anesthesia in either an inverted "L" block, a line block, or, as the authors prefer, individual portal blocks (Fig. 1). The first portal is placed at the level of the base of the tuber coxae, midway between the tuber coxae and the last rib. A 10- to 12-mm-diameter, 15- to 20-cm-long cannula with a blunt trocar is advanced through a 12-mm incision in the skin and external abdominal oblique fascia and the rest of the abdominal musculature into the peritoneal space. The trocar is then replaced by a laparoscope connected to a 300-watt xenon light source and laparoscopic camera, which is used to confirm penetration through the parietal peritoneum. Abdominal insufflation with carbon dioxide is to an intra-abdominal pressure of 8 to 12 mmHg initially. The second portal is made just cranial and 10 cm proximal to the first cannula, and the third portal is made 10 cm ventral to the first cannula[3] (Fig. 2).

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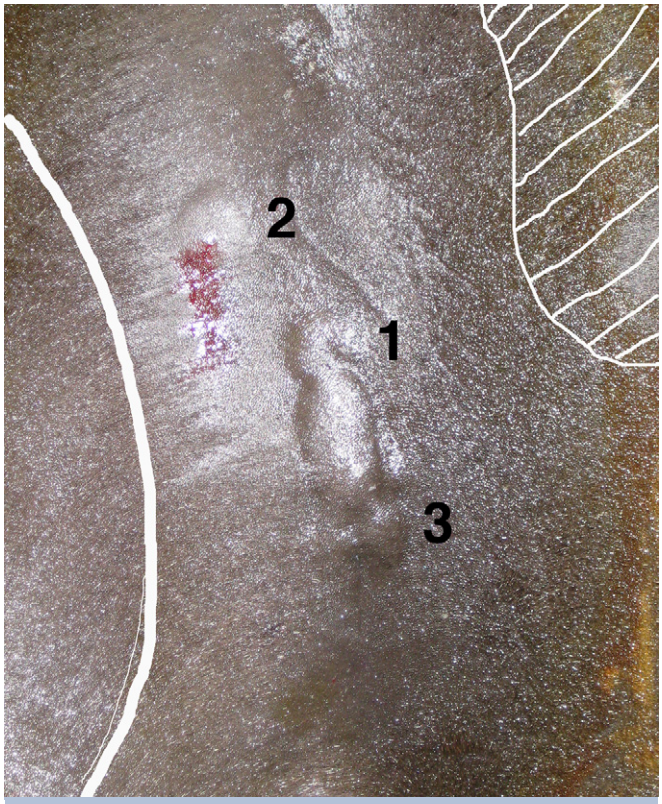

Figure 1. Photograph of left flank showing portal sites: 1, first portal; 2, second portal; 3, third portal. Note the local anesthetic between portals 1 and 3 to facilitate enlargement of portals for ovary removal. White line on left corresponds to last rib. White line with crosshatches on right corresponds to tuber coxae.

Throughout the procedure, all laparoscopic instruments are inserted through the instrument portals as required. The mesovarium should be infiltrated with 10 to 15 mL 2% lidocaine before resection.[4] If a single ovary is removed, the ventral incision is enlarged for removal of the ovary. For bilateral ovariectomy, both are resected; the right ovary is passed under the descending colon to the left side of the abdomen and removal is via the left flank. Only the skin is closed in the small incisions using size 2–0 nylon in a cruciate or simple interrupted pattern. The external abdominal oblique muscle and fascia are closed in the single enlarged incision using size 0 polyglyconate (Maxon, Covidien) in a simple continuous pattern and size 2–0 nylon in the skin in a simple continuous pattern. The horses are generally kept in a stall for 1 week and a stall/run for 1 week, and then they are returned to work.

Various techniques have been used to amputate the ovary in standing laparoscopic ovariectomy. The main variations are the methods with which hemostasis is achieved. Reported methods include hand-tied ligating loops, pre-tied ligating loops, bipolar and monopolar electrosurgical forceps, ultrasonic cutting/coagulating devices, radiofrequency devices, surgical staples, and lasers. The ultimate objective in ovariectomy experiments is to find a method that facilitates the ovarian resection and removal with minimal hemorrhage and ovarian manipulation.[5]

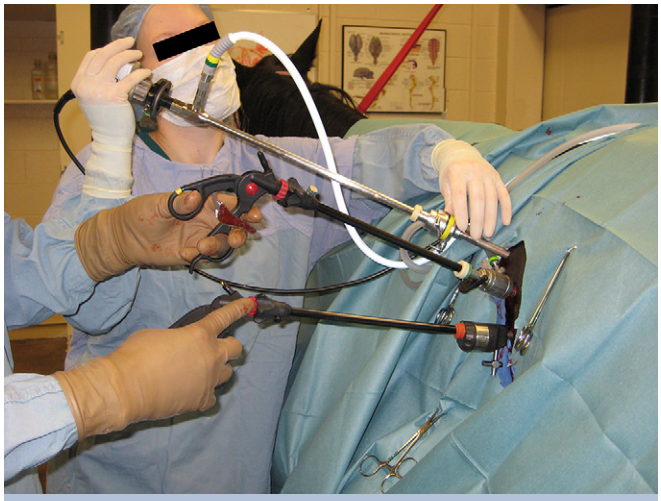

Figure 2. Portal placement in left flank.

Ligating Loops

Intracorporeal suturing in laparoscopic surgery requires extensive skill and practice. Consequently, other suture techniques have been developed to provide a simpler ligation technique using loop sutures. The most common suture combinations are size 2 or 3 polyglactin 910 (Vicryl, Ethicon) in a modified Roeder knot,[1] size 1 polyglyconate (Maxon, Covidien) using a 4-S modified Roeder knot,[3,6] or the pre-tied Endoloop (size 0 polyglactin 910 or polydioxinone, Ethicon Endosurgery). All of these knots are designed to slip while advancing but to lock into place when tightened. In the study by Shettko et al,[6] the combination of the 4-S modified Roeder knot, and size 1 Maxon was significantly stronger than all other suture and knot combinations tested.

The caudal pole of the ovary is sharply dissected to reduce the pedicle size (Fig. 3), and two ligating loops are placed on the ovarian pedicle (Fig. 4). The ovary is grasped with forceps through the ligature loop and then manipulated to tease the loop around the newly created ovarian pedicle. It is generally effective to twist the ovary through the loop. Once the pedicle is snared, the loop is tightened by advancing the knot pusher while pulling the tail end of the ligature. The knot pusher is then exchanged for scissors, and the tail of the ligature is cut and removed; this is repeated. The ovarian pedicle is then carefully transected with laparoscopic scissors between the ovary and ligature and checked for bleeding (Fig. 5). Specific equipment for this technique

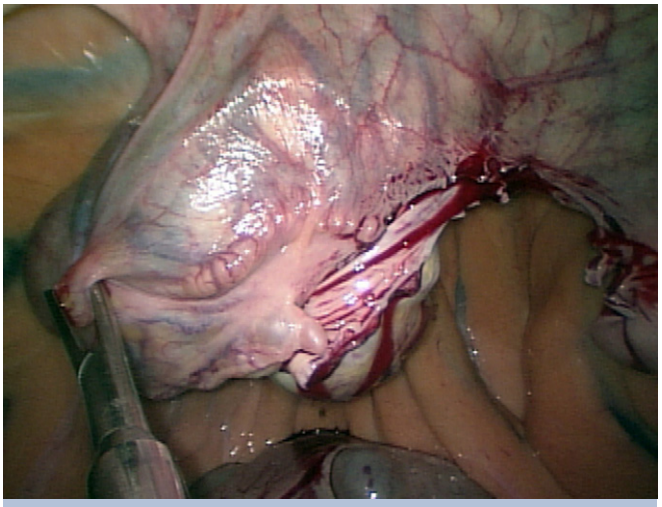

Figure 3. Photo showing dissection of the caudal pole of the left ovary.

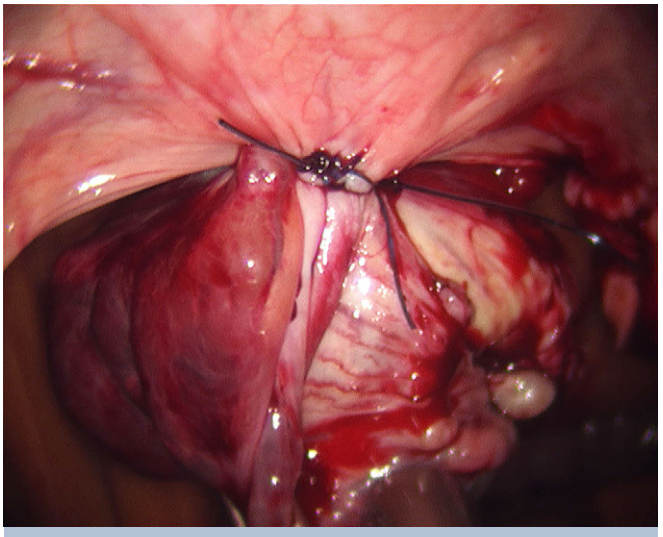

Figure 4. Photo showing two ligating loops on the left ovarian pedicle.

includes a knot pusher, a 5-mm reducer, a suture scissors, and a tissue scissors.

No major complications have been associated with this technique. In a few circumstances, incomplete hemostasis occurs, which is very easily resolved with an additional ligature or by placement of a ligating clip. Hemostasis also can be achieved without ovarian pedicle dissection, but it often results in reduced ligature security. Limits to this technique occur when the ovaries are larger than 15 to 18 cm in diameter, because it is difficult to control such a large loop in the abdomen.

Polyamide Tie-Rap

Another technique used to perform mesovarian ligation is through the use of a white-colored, commercially available polyamide tie-rap, more commonly known as a zip-tie.[7] The tie used is 500 mm long, 4.8 mm wide, and 1.2 mm thick. Sterilization of the tie-rap can be done using gas plasma sterilization, steam, ethylene oxide (which minimizes elongation of the material), and autoclave techniques. The tie is manually marked every 20 mm before initiation of the surgery to ensure that the tie-rap tightens adequately during surgery. The polyamide tie-rap is prepared by creating a loop and connecting it to a hooked metal bar. The metal bar tie-rap unit is inserted in the metal tube, and cervical forceps are inserted to grasp the ovary and provide tension on the mesovarium. Curved Metzenbaum scissors are inserted through the second portal and are used to slightly transect the mesovarium, on the caudal and cranial ends. The scissors are then removed, replaced by tie-rap unit, and the loop is set horizontally and under the ovary. The loop typically slides quite easily over the ovary due to the stiffness of the polyamide tie. With the traction from the cervical forceps on the ovary, the loop

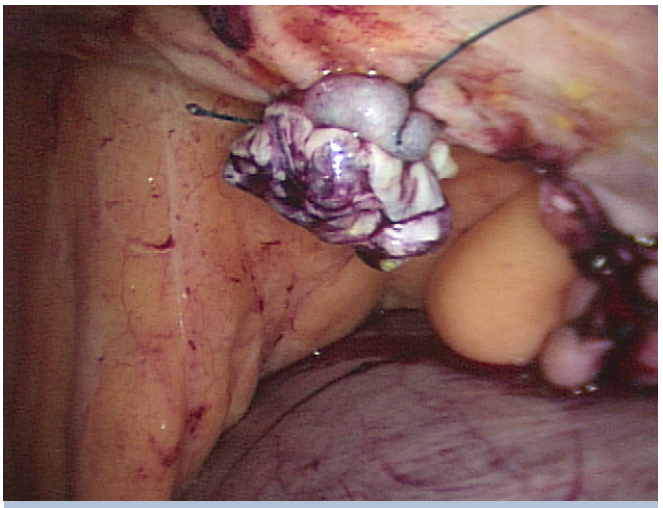

Figure 5. Photo showing transected stump after amputation of left ovary.

is positioned as high as possible on the pedicle and then tightened by pushing up on the buckle of the tie rap with the metal tube while pulling on the end of the tie-rap with the hooked metal bar. The tie-rap is tightened until the surgeon sees the buckle rest between marks 3 and 4. The metal bar is then removed and replaced by tissue forceps, which is used to completely tighten the loop. The cutter–sleeve unit is inserted to cut off the end of the tie rap, and the ovary is then completely transected from the mesovarium with laparoscopic scissors.

Few complications were noted in this report. Mesovarial hemorrhage can occur if the vessels are not entirely occluded, which is easily fixed with the application of a second

tie-rap proximal to the first. Two weeks after surgery, the tie-rap and mesovarial stump is partially encapsulated with yellowish fibrovascular tissue, at 4 weeks encapsulation is complete, and at 12 weeks, the stump is entirely encapsulated with organized fibrous tissue.

The polyamide tie is inexpensive and widely available at any hardware store. Because the polyamide polymer is a nylon monofilament material, it is biologically inert and noncapillary. Its melting point is 250°C, which is important to remember when sterilizing. The suture material is nonabsorbable and can elicit an inflammatory reaction but typically does not cause problems. The major advantage of using the polyamide tie-rap is that the loop to be placed around the ovary is rigid and is therefore easier to apply in comparison with a ligature. The 14-cm-diameter loop can be a limitation when it is insufficient to allow passage of a large ovary. Larger tie-raps are available, however, which minimize this problem.

Electrocoagulation

Bipolar cautery can be used to provide hemostasis of the ovarian pedicle.[2] This procedure involves the use of basic laparoscopic tools and bipolar forceps to provide hemostasis of the mesovarium. Bipolar cautery provides a safer environment than monopolar cautery, because there is a reduced likelihood of collateral damage from aberrant currents.

Laparoscopic grasping forceps are inserted through one instrument portal to provide traction on the ovary. The bipolar electrosurgical instrument is inserted through either the most cranial or the most dorsal instrument portal. Pedicle dissection is initiated when the electrosurgical forceps are placed across the cranial aspect of the mesovarium approximately 1 cm proximal to the ovary. The cranial mesovarium is coagulated until blanching and shrinkage are observed. The laparoscopic scissors then replace the atraumatic grasping forceps, and coagulation and transaction is repeated caudally until the ovary is only suspended by the tubal membrane, oviduct, and proper ligament of the ovary. The electrosurgical instrument is replaced with grasping forceps to grab the ovary. Laparoscopic scissors are used to transect the remaining mesovarium, which now lacks any major blood vessels. Once the ovary is transected, the mesovarium is observed for bleeding before laparoscope removal.

The advantages of electrosurgery are that it is less technically demanding than ligature placement; however, it does require more equipment to purchase and maintain. It can generally provide adequate hemostasis of the mesovarium and consequently a dry surgical field. However, it is generally limited to coagulating vessels of 3 mm in diameter or less. Thermal injury to bowel from stray currents can lead to perforation and subsequent life-threatening postoperative peritonitis. This can occur because of insulation failure in the active electrode, direct coupling, or capacitive coupling. These complications can be reduced by avoiding the use of high-power settings, energizing the active electrode only when it is in contact with the target tissue, keeping the active electrode in the field of view, not inserting metal cannulas through plastic devices, avoiding touching the active electrode to other metal instruments, and avoiding long activation of the active electrode. The standing position helps minimize injury because the ovary and mesovarium are located dorsal to abdominal viscera; however, distention of the bowel can cause problems, which emphasizes the importance of withholding feed before surgery.

Ultrasonic Cutting and Coagulating Devices

Ultrasonic energy is delivered to the ovarian pedicle to coagulate vessels and cut the tissue of the pedicle.[8,9] Standard laparoscopic instrumentation along with a device for providing the ultrasonic energy are required for this procedure. Two main devices are available, the Harmonic Scalpel (Ethicon Endosurgery) and the Autosonix (US Surgical, Covidien). The units consist of a generator, a foot pedal, and hand piece with a connecting cable, and a blade system. A transducer in the hand piece converts electrical energy from the generator into ultrasonic vibration, 55,000 Hz, which is transmitted along an extending rod to the active blade tip. This energy is sufficient to cause protein disorganization and denaturation, resulting in a sticky protein coagulum capable of sealing vessels up to 3 to 5 mm in diameter. The devices use multiple power settings to empower a blade system that allows a surgeon to control the balance between cutting and coagulation by varying power settings, changing blade configurations, and varying the grip force.

The ovary is stabilized by grasping the infundibulum with laparoscopic claw forceps while the shears are inserted. When using the Harmonic Scalpel (Ethicon Endosurgery), the coagulating shears transect the mesosalpinx, uterine tube, and proper ligament with a sharp blade and the number 5 setting while simultaneously coagulating blood vessels. The vertical part of the ovarian pedicle is transected using the blunt blade and number 3 setting, and the first cuts are made on the most cranial part. The shears are exchanged for laparoscopic scissors, and the remainder of the ovarian pedicle is separated into medial and lateral components by blunt dissection. The laparosonic coagulating shears are reintroduced into the craniodorsal portal, and the lateral aspect of ovarian pedicle is transected followed by the medial aspect.[8] A similar technique is performed using the Autosonix (US Surgical, Covidien) except that a single pair of shears is used[9] (Fig. 6).

The advantages of using an ultrasonic device are that it is simple to use and it is capable of grasping, coagulating, and cutting tissue simultaneously. It minimizes the number of

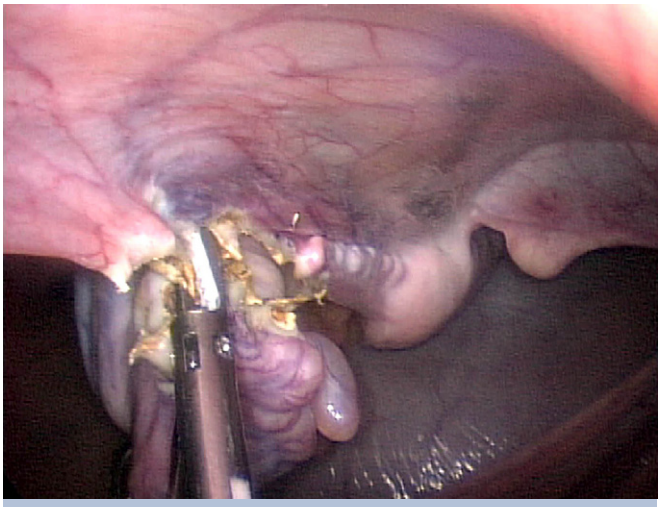

Figure 6. Intra-abdominal view of cauterization and amputation of the left ovary using the ultrasonic cutting/coagulating shears (Autosonix, US Surgical, Covidien).

Figure 7. Intraabdominal view of cauterization and amputation of the left ovary using a radiofrequency wand (LigaSure, Covidien).

instrument exchanges needed to complete the procedure, and the surgeon can control the balance of coagulation and cutting by varying the power setting, blade configuration, and tissue pressure. The shears are easy to use and achieve reasonable hemostasis of the ovarian pedicles. In the report by Alldredge and Hendrickson,[9] many of the pedicles required further treatment in the form of ligating clips for hemostasis. The shears can be used to offer further hemostasis. The equipment can be expensive, and the shears are designed to be single use.

Radiofrequency

Radiofrequency can be used to cauterize the ovarian pedicle.[10] The LigaSure (Covidien) device is a feedback-controlled bipolar vessel sealing device used to establish hemostasis. The feedback-controlled electrothermal scaler applies the precise amount of energy to produce a seal of the vessel walls in the form of partially denatured protein. The integrity of the seal is independent of a proximal thrombus and resists dislodgment because the seal is inside the vessel wall structure. Unlike electrocoagulation device, the LigaSure minimizes thermal spread to adjacent tissue and reduces sticking or charring at the seal sites.

The use of the LigaSure does not require the dissection of the mesovarium to create a smaller ovarian pedicle. The instrument is inserted through the appropriate portal and is positioned to begin from either the caudal or the cranial pole of the ovary. The Atlas wand incorporates a cutting blade so that the tissue can be coagulated and cut sequentially without changing instruments. This process is repeated until the ovary is removed, which takes approximately five to seven applications of the LigaSure (Fig. 7).

A technical problem of spark emittance has been reported with mechanical operation of the device when discharging it across tissue. This could be attributable in part to too much tissue forced into the instrument's jaws, or sterilizing and re-using a device designed for single use. The LigaSure is advantageous to use because it does not require dissection of the mesovarium, there is no likelihood of ligature slippage, and no foreign-body reaction occurs because of the suture material. The instrument is effective for vessels up to 7 mm in diameter, and the translucent appearance of the seal indicates a complete lack of blood flow to the cut edge of the pedicle. Although designed to be a single-use instrument, the wand can be sterilized for cost-effectiveness in a hydrogen peroxide/plasma sterilizer.

Endoscopic Stapling

Stapling devices have been part of equine surgery since the 1970s. Recently they have been adapted for endoscopic procedures. The endoscopic stapling devices generally require a 12- or 15-mm-diameter cannula. The Endo-GIA II (US Surgical, Covidien) stapling device is designed to seal ovarian vessels and transect the mesovarium all in a single application. It also is possible to use a disposable specimen pouch for removal of the ovaries from the abdomen.[5]

The mesovarium is dissected to isolate ovarian vessels, creating a pedicle capable of containment within the stapling device. The ovary is manipulated with grasping forceps to incorporate the entire pedicle into both arms of the stapler. The distal aspect of the stapler is typically the most common site of incomplete occlusion, which is

important to consider when performing this procedure. Once the pedicle is completely encased in both arms of the stapler, the blade within the stapler is advanced, causing release of the staples and subsequent severing of the pedicle. Any residual attachment of the pedicle that is not incised is transected using laparoscopic tissue scissors. It is critical that the transected pedicle be evaluated for bleeding before removal of the ovary. The detached ovary can then be placed in the EndoCatch II (US Surgical, Covidien) disposable specimen pouch, previously inserted into abdomen through the dorsal instrument portal. In the report reviewed, the right ovary was placed into the bag and then the left ovary directed toward the caudal aspect of the right abdomen by passing it between the bladder and the body of the uterus. To perform this step more carefully, the camera is moved to the left flank for better visualization and then moved to the right flank for removal. Once both ovaries are in the pouch, it is closed with a drawstring and pulled tightly against the flank. The right abdominal incision is then enlarged to pull the bag through the body wall, and all incisions are closed.

Bleeding from the pedicle can occur when the initial dissection is too close to the ovary. The advantages of using this technique are the simultaneous hemostasis of the ovarian vessels and transection of the ovary. The disadvantage is the cost of the staple cartridge and bag if used. The main advantages of using a laparoscopic stapling device technique compared with a suture are the shorter surgical time, that the ovary is resected from the mesovarium immediately without the need for transaction after individually ligating vessels, and that there is no risk of inadvertently cutting the suture or displacing the knot during amputation. The primary advantage of using the EndoCatch II device is the avoidance of losing the ovary during transfer to another grasping instrument, given there is sufficient space in the bag for both ovaries. The disadvantage associated with this technique is the increased surgical time needed to control bleeding when ovarian vessels are inadvertently transected, which can be corrected with electrocautery or laparoscopic clips.

CONCLUSIONS

The primary advantages of standing laparoscopic surgeries are excellent intraoperative visibility, secure hemostasis, reduced surgical and postoperative morbidity, decreased postoperative discomfort, rapid and uncomplicated healing, reduced quantity of medication needed, shorter postoperative management, and less expense than for ovariectomies performed under general anesthesia.[3]

There have been few reported significant complications associated with standing laparoscopic ovariectomy. Choosing the appropriate animals to undergo standing surgery under sedation is the most important first step. If the mare is not amenable to a standing surgical procedure performed in the stocks, it should be anesthetized. Bleeding from the pedicle can be managed immediately because the procedure allows direct visualization of the surgical site. It is possible to drop the ovary into the abdomen after amputation; it is generally considered important to find the ovary and remove it from the abdomen. In most cases, this can be done using laparoscopic instruments. In some cases, it requires an enlarged incision and identifying the ovary manually for removal.[2] Sometimes ovaries with large follicles are difficult to exteriorize and are incised and drained in the abdomen to decrease ovarian size and avoid slippage during removal.[7] Most mares are clinically normal immediately after surgery. The tension-free amputation is believed to be less painful than traditional techniques. There are reports of mares that have a slight decrease in appetite and increase in temperature for the first 12 hours postoperatively or that experience mild incisional edema and subcutaneous emphysema, which often resolves in 3 to 5 days.[1] Mares often show mild signs of colic and are given a single dose of flunixin meglumine, which has been shown to be useful.[2]

All of the reported techniques are useful in equine ovariectomy. Of the techniques discussed in this review, the ligating loop is the least expensive but requires the most skill. The LigaSure device requires the least skill and is similar in cost to the other techniques. The authors recommend using the ligating loops for normal-sized ovaries and the LigaSure device for ovaries larger than 15 to 18 cm. In a recent retrospective study evaluating the clients' perspective on standing laparoscopic ovariectomy, behavioral improvement was seen in 83% (19/23) of mares treated for behavior-related problems, aggression problems improved in 86% (12/14) of cases, general disagreeable demeanor improved in 81% (17/21), and excitability improved in 75% (12/16) of cases where these behaviors had been previously observed. Kicking and biting improved in 73% (8/11), problems in training improved in 72% (13/18), and frequent urination and problems with other horses improved in 64% (7/11 and 9/14, respectively) of cases.[11]

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Laser Ablation of Endometrial and Lymphatic Cysts

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This article discusses uterine cysts (endometrial and lymphatic), their diagnosis, and therapeutic management using neodymium: yttrium-aluminum-garnet laser ablation as directed by hysteroscopic visualization. The technique used in our clinical setting is described along with a discussion of the follow-up results in mares treated by the Sarkey's Surgical Laser Laboratory at Oklahoma State University, Stillwater, OK, over the past 7 years.
Key Words: Cyst, cysts, endometrium, uterus, laser, neodymium:yttrium-aluminum-garnet, hysteroscopy, mare, equine.
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UTERINE CYSTS are of 1 of 2 types: endometrial (glandular) or lymphatic. Endometrial cysts are typically smaller in dimension (1-10 mm) and are dispersed multifocally throughout 1 or more areas of the uterus. They result most commonly from chronic degenerative changes in the endometrium leading to periglandular fibrosis and glandular lumen restriction with dilatation of the glandular lumen resulting in its cystic distension. Lymphatic cysts are typically larger (>10 mm) and usually are isolated to 1 to 3 locations. These result from lymphatic outflow stasis; surrounding endometrial tissues may have chronic inflammatory infiltrates or may be normal histologically. Either condition requires use of either ultrasonography or hysteroscopy for identification; however, the larger lymphatic cysts may be identified by careful rectal palpation. The relationship between presence of cysts and fertility is still as yet unclear.

Most clinicians believe that large cysts influence early embryonic mobility in the uterine lumen (after day 7 or 8 and before day 15 or 16), thus threatening maternal recognition of pregnancy, if not jeopardizing embryonic nutritional support because of restriction of uterine histotroph absorption. There are also exceptions to this, in that many clinicians have also observed normal embryonic development and pregnancy maintenance in the presence of 1 or more large uterine cysts. Despite their potential indication of significant underlying endometrial pathology, their presence also confuses the naive diagnostician, as well as many of the most experienced clinicians, at the time of initial pregnancy diagnosis. Mistaken identity of an embryo for a cyst, and vice versa, is a common challenge. Most cysts are also observed in older brood mares. One report[1] found most cysts in 11 of 73 mares whose mean age was 13.4 years, whereas 62 of 73 mares without cysts were of a mean age of 6.9 years. A Swiss study[2] in thoroughbred mares reported that pregnancy rates at days 14 and 40 were significantly ($P < .01$) lower in mares with cysts (77.6% and 71.4%) compared with mares without cysts (91.5% and 88.0%). This would suggest that the presence of uterine cysts may play an important role in the reduction of fertility. However, in this study when all mares were assigned to 3 age groups, less than 7 years (n = 116), 7 to 14 years (n = 117), and older than 14 years (n = 26), a significant ($P < .01$) increase in the number of endometrial cysts was observed with advancing age (4.3%, 29.1%, and 73.1%, respectively). In a postmortem examination of the genital tracts of 104 mares in Germany, endometrial cysts were identified in 13% of cases.[3] In clinical examination of 82 mares, endometrial cysts were recorded in 13.4% of mares of various breeds.[4] These investigators found no evidence of cysts in mares less than 10 years of age, and mares in their study that had cysts in the endometrium had a 10% higher incidence of disturbed fertility. They reported that 7 of 9 mares having evidence of endometrial cysts became pregnant and continued their gestation without complication. Therefore, the true relationship between presence of cysts and age-related reduction in pregnancy maintenance is difficult to sort out.

For these reasons, and the general lack of truly successful treatment options, most clinicians will recommend breeding the mare with uterine cysts on 1 or more cycles in an attempt to see if she will carry her pregnancy in spite of them. If this approach is taken, it is very prudent to record and map on a uterine chart the presence and size of all endometrial or lymphatic cysts identified before each attempted breeding. Cysts will appear and disappear occasionally throughout an estrous cycle or between an ovulation and an initial pregnancy diagnosis at 14 to 15 days postovulation. When several attempts to induce a successful pregnancy have failed, it would be advised then to obtain an endometrial biopsy (or biopsies) to determine the histomorphologic status of the endometrium in general. Treatment plans directed solely at the elimination of a cyst or many cysts will not be fruitful should the uterus in general have significant endometrial fibrosis. In many mares with uterine cysts, there may also be intraluminal adhesions present that cannot be detected by any means short of direct intrauterine palpation or visualization by hysteroscopy.

Treatment options have included electrocoagulation, direct puncture by biopsy forceps, manual rupture, and various intrauterine infusions with saline, hypertonic saline, and magnesium sulfate. Cysts often have either failed to be removed by these attempts, or when successfully resolved, have returned in 1 or 2 cycles. Electrocautery for cyst removal has been used via hysteroscopy.[5,6] Results were similar in these 2 reports in that 3 of 5 treated mares became pregnant in the subsequent estrous period after cyst removal,[5] and 3 of 6 mares in the other report either produced a live foal or became pregnant.[6] Cyst ablation by hysteroscopic neodymium:yttrium aluminum garnet (Nd:

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Fig 1. Ultrasonographic image of the base of the left uterine horn in an 11-year-old thoroughbred mare. The anechoic structures are lymphatic cysts with the more echodense linear to curvilinear lines between them representing cyst wall interfaces.

YAG) laser irradiation has been reported previously[7]; 3 of 6 treated mares had positive outcomes after these photoablative treatments. The following is a description of the methodology developed and a report of some of the outcomes associated with laser ablation of equine uterine cysts at the Sarkey's Surgical Laser Laboratory, College of Veterinary Medicine, Oklahoma State University. This work represents the clinical research effort and experience of multiple clinicians as well as these authors over the past 7 years.

Diagnosis and Case Selection

Clinical cases with uterine cysts were identified or diagnosed by referring practitioners and confirmed at the Oklahoma State University Teaching Hospital, Equine Clinic, by using palpation and ultrasound examination (Fig 1). The cysts' exact location and number were determined by ultrasonography before treatment. Mares with cysts greater than 20 mm in diameter or those with multiple cysts less than 20 mm, which were cylindrical and covered more surface area, were considered candidates for laser ablation. Mares received a complete breeding soundness examination including endometrial biopsy to evaluate the presence and degree of endometrosis and to provide some prognostic value to the mare owner with respect to the value of the laser ablation procedure.

Surgical Procedure

Horses were tranquilized with 20 to 40 μg/kg detomidine HCl administered intravenously (Pfizer, Exton, PA). Restraint was by use of examination stocks, and the room or area in which the laser ablation was performed had its access restricted. The mare was prepared with a full tail wrap; tail tied to the side; and given a perineal scrub using water, cotton, and a nondisinfectant, mild soap or chlorhexidine scrub; thoroughly rinsed again with clean water; and then dried with clean paper towel. The operator wore a sterile shoulder length plastic obstetrical sleeve, and a sterile, nonbacteriostatic obstetrical lubricant was placed on the back of the gloved hand. Before the introduction of the endoscope, a thorough vaginal and cervical examination was performed digitally to evaluate the cervix for patency, absence of fibrotic scars, adhesions, or other damage.

Examination of the vaginal mucosa, cervix, uterine endometrium, and visualization of the cysts was accomplished with a Welch Allen laser-safe flexible endoscope (model 81201; Welch Allen, Skaneateles Falls, NY) attached to a Welch Allen video processor (model 86000; Welch Allen, Skaneateles Falls, NY). A commercially available RCA (Thomson Multimedia, Indianapolis, IN) 4-head VHS video tape recorder was used to document the procedure. The tip of the endoscope was guarded by the sterile gloved hand and guided through the vulva, into the vagina, avoiding the urethral opening ventrally, and advanced cranially until the cervix was identified. The external cervical os was gently invaded and the full length of 1 or 2 fingers were inserted through the cervix stopping at the body of the uterus. The endoscope (1 cm × 1 meter) was then carefully guided between the fingers and placed into the uterus, the lumen of which was then insufflated with air. Once the uterus was properly insufflated, large lymphatic cysts were easily identified (Fig 2). The laser fiber was then advanced through the biopsy channel of the endoscope until the tip was visualized. A suction apparatus was attached to facilitate the removal of carbonized tissues and released cyst contents as needed.

A Sharplan model 2100 Nd:YAG laser (Sun Medical, Inc, Arlington, TX) with peak energy at 1064-nm wavelength was operated at a power setting of 25 to 30 W and a pulse duration of 7 seconds. Energy was delivered through a Sharplase (Sun

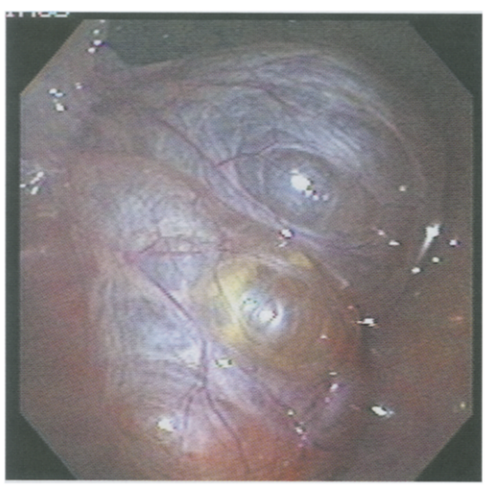

Fig 2. Hysteroscopic view of a large, thin-walled, multiloculed lymphatic cyst. Once adequate insufflation of the uterus has been achieved, these cysts are easily identifiable. (Courtesy of S. Palmer.)

Medical Inc, Arlington, TX) frosted conical 600-μm diameter fiber (model 25581) producing approximately 175 to 210 J of energy per pulse (Fig 3). This conical fiber provided a higher degree of localized energy dispersion than did a flat-ended fiber. This allowed the cyst wall to encompass the fiber and keep the laser energy localized to the cyst wall. Protective eyewear appropriate for use with the Nd:Yag laser was used by all persons in the vicinity of the operating laser. Smoke evacuation was accomplished by a filtered house vacuum attached to the endoscope. The typical total energy delivered ranged from approximately 1,500 J to over 5,000 J. This variability was dependent on the number of cysts present and their type. The energy required to rupture a cyst was quite variable and depended greatly on the nature of the cyst wall. Thin, friable cysts usually ruptured at 25-W energy levels and fibrous walled cysts ruptured around 30 W (Fig 4).

Postoperative Therapy

After laser ablation of the cysts, the uterus was flushed with a large volume of sterile lactated ringers solution (1-3 L). Follow-up uterine examinations were performed by using transrectal ultrasound at days 1, 3, 14, and 21 to 30. On rare instances, treated mares returned for ablation of additional cysts not treated on the first attempt or for ablation of additional cysts that developed after the first treatment was completed.

Discussion

On gross hysteroscopic examination of the mare's endometrium, large lymphatic cysts often occluded the uterine lumen until insufflation allowed uterine distention and greater visualization of the cyst's margins. On vaporization of the cyst's wall, the fluid contents poured into the uterine lumen and were subsequently aspirated, leaving the collapsed structure, which could be further reduced by vaporizing the remaining redundant tissue. Large lymphatic cysts were often pedunculated, and vaporization of these cysts at their base allowed quick drainage and tissue excision. This method appeared to be the most desirable approach for complete elimination of the structure. Cysts with large bases often required multiple passes for complete ablation (Fig 3). Cysts embedded deeper in the endometrium were usually small and were possibly cystic dilations of degenerated endometrial glands. These could be ablated by linear vaporization, thus allowing evacuation of the contents and dissolution after necrosis of the margins. The laser was fired when the quartz fiber was being drawn across the cysts or toward the endoscope channel and not while being pushed away. Perforation of the uterus will not occur if this rule is observed. Fluid-filled cysts absorbed more thermal energy than less or noncystic structures because the fluid acted as a transmitting medium that aids in dissipation of heat produced during the treatment. Because of this dissipation effect, multiple passes had to be performed so that less heat penetrated into the underlying endometrium and myometrium. It is our general impression that cysts containing fluid of higher viscosity required treatments of higher wattage for ablation. It was necessary to destroy the endometrial gland dilations within the stratum compactum or cause coagulation of the lymphatic lacuna to prevent recurrence of endometrial cysts and lymphatic cysts, respectively.

Fig 3. Hysteroscopic view of laser tip being applied to a lymphatic cyst. Below the tip of the laser fiber is a blackened or charred area in which photoablation has just occurred.

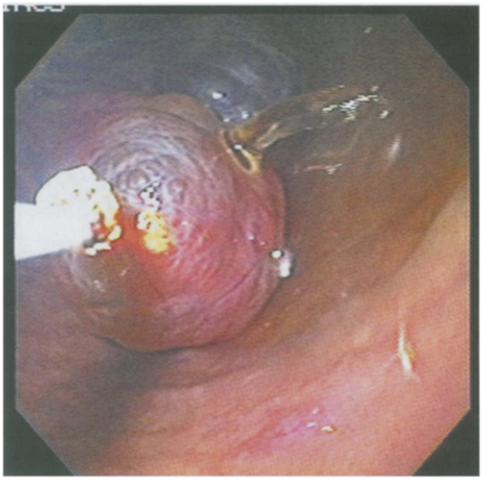

Fig 4. Hysteroscopic view of the laser being used to puncture the wall of a large, thin-walled, multilobular lymphatic cyst. Note the clear yellow fluid draining from the cyst onto the floor of the uterus. (Courtesy of S. Palmer.)

The Nd:YAG laser produced tissue coagulation necrosis for about 2 to 4 mm in all directions around the zone of vaporization. This caused localized destruction of the endometrium to the level of the superficial myometrium without threatening the integrity of the deeper myometrium. Immediately after laser ablation of large lymphatic cysts or detectable endometrial cysts, the target structure was reduced to a small area of carbonized or blanched tissue with a concomitant radius of erythematous tissue.

The endometrium possesses amazing powers of regeneration. Within 14 days of laser treatment, often the only marker remaining was a small (<2 mm) foci of carbon. By 28 days posttreatment, nothing more could be found other than a focal area of white scar tissue usually 1 to 2 mm in diameter. In some cases, no scar was present at all. When comparing histopathologic samples of endometrial biopsies taken 14 days and 28 days

posttreatment, preliminary results indicate no concomitant increase in endometrial fibrosis or glandular degeneration in the area of cyst ablation over that found in the uterus before laser surgery (W.B.L., R.G.H., and G.R.H., unpublished data, 2001). There was no indication of cyst redevelopment in the site of the previous cyst. However, some mares may develop both lymphatic and endometrial cysts in other areas of the uterus. The exact mechanism(s) behind this has not been elucidated.

Laser therapy involves invading the uterine lumen, the evacuation of cyst contents into the lumen, and the destruction of endometrial and lymphatic tissue. This will cause an acute inflammatory response that peaks within 24 to 48 hours. Immediately after laser surgery, the uterus was lavaged with 2 to 3 L of lactated ringers solution. Generally, 1 L was infused into the uterus through gravity flow and then siphoned off. A second liter was then infused in a similar manner; if the evacuated fluid returned clear, then a broad spectrum antibiotic was infused prophylactically. The subsequent inflammatory response probably helps to clear necrotic debris by forming a mucopurulent accumulation within the uterine lumen 2 to 3 days posttreatment. To clear the lumen of this material, the uterus was lavaged for 2 to 3 days posttreatment and reevaluated ultrasonographically to assess the need for further uterine treatment. To assist in uterine emptying, these mares can be administered oxytocin (10 units subcutaneously every 4 to 6 hours) or prostaglandin F_2 alpha to increase myometrial activity and tone. Uterine lavage was continued until there was no further fluid accumulation within the uterine lumen, usually 1 to 2 treatments 1 to 2 days apart were all that was needed.

Although we have ample anecdotal evidence of improved fertility after laser ablation of uterine cysts, until our current study is completed, we cannot say that pregnancy rates will be significantly increased. However, we have had mares come into our program with a history of being barren that have successfully become pregnant and carried foals to term after laser ablation of cystic structures in the uterine lumen. It would appear that larger cysts, greater than 20 to 25 mm in diameter, present a challenge for the embryo to circumnavigate during the prefixation mobility phase of its development. Location of these larger cysts is most commonly observed at the base of the uterine horns[8] and thus may affect proper fixation and embryonic orientation. Further study in this area is warranted.

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Effect of prepubertal versus postpubertal castration on sexual and aggressive behavior in male horses

Scott W. Line, DVM; Benjamin L. Hart, DVM, PhD; Linda Sanders, BA, MA

SUMMARY

Male horses castrated prepubertally occasionally display stallion-like sexual and aggressive behavior as adults. A retrospective survey of 140 horse owners was undertaken to compare the effectiveness of prepubertal versus postpubertal castration on objectionable sexual and aggressive behavior. Of 94 geldings that had been castrated prepubertally (before 2 years of age), 20% to 30% displayed stallion-like sexual behavior and aggression toward horses, and 5% were aggressive toward people in a stallion-like manner. This occurrence of problem behavior was not significantly different from the occurrence of these behavioral patterns in 46 geldings that had been castrated as stallions (over 3 years of age). When castration was examined as a therapeutic measure to eliminate sexual and aggressive behavior in stallions in which the behavior had become objectionable, it was found that the operation was effective on sexual behavior and aggression toward people in 60% to 70% of horses and on aggression toward horses in 40% of horses.

MALE HORSES NOT intended for breeding usually are castrated before puberty in an attempt to prevent development of objectionable sexual libido and the aggressive temperament characteristic of stallions. Nevertheless, geldings occasionally display stallion-like sexual behavior or aggressiveness toward horses or people.[1,2] In 2 studies, stallion-like behavior in geldings was found not to be attributable to residual testosterone or the production of testosterone by nongonadal tissue.[3,4] Although castration of juveniles for behavioral purposes is common, its effectiveness in preventing objectionable sexual or aggressive behavior is unknown. A related question is whether castration before puberty is more effective in preventing objectionable behavior in adults than is castration after puberty. An answer to this question would be useful in advising clients who might wish to delay castration beyond puberty. Information about the effectiveness of castration on objectionable sexual and aggressive behavior, once it appears in stallions, would be useful in formulating prognoses in using castration therapeutically. The purpose of the study in this report was to obtain quantitative data on the aforementioned issues by interviewing a large number of owners whose horses had been castrated either prepubertally or postpubertally.

Methods

Selection of cases—Two groups of horses were selected from the January 1976 to June 1982 records of horses castrated at the Veterinary Medical Teaching Hospital, University of California at Davis. Those designated prepubertal (juvenile) castrates were less than 2 years old when castrated. Those designated as postpubertal (adult) castrates were older than 3 years at the time of castration. All horses were at least 5 years old and had been castrated for at least 1 year at the time of the study.

Interviews of horse owners—Telephone interviews of the owners were completed for 46 adult castrates and 94 juvenile castrates. Owners were told the study was being conducted to determine behavioral effects of castration on horses. Questions pertained to duration of ownership, type of housing, presence or absence of aggressive or sexual

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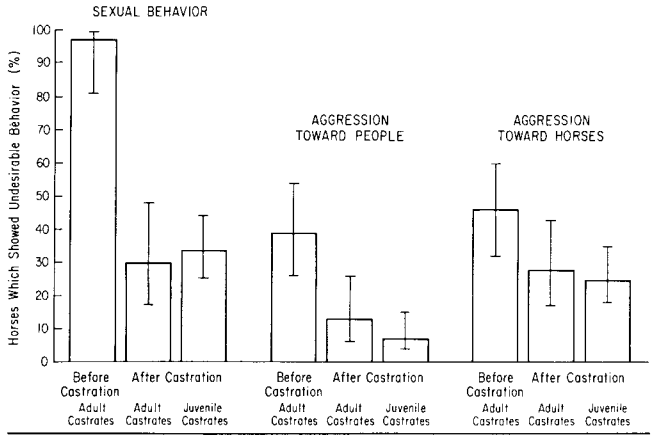

Fig 1—Sexual and aggressive behavior in postpubertal male horses before and after castration and in prepubertally castrated males. Vertical lines represent 95% confidence intervals.

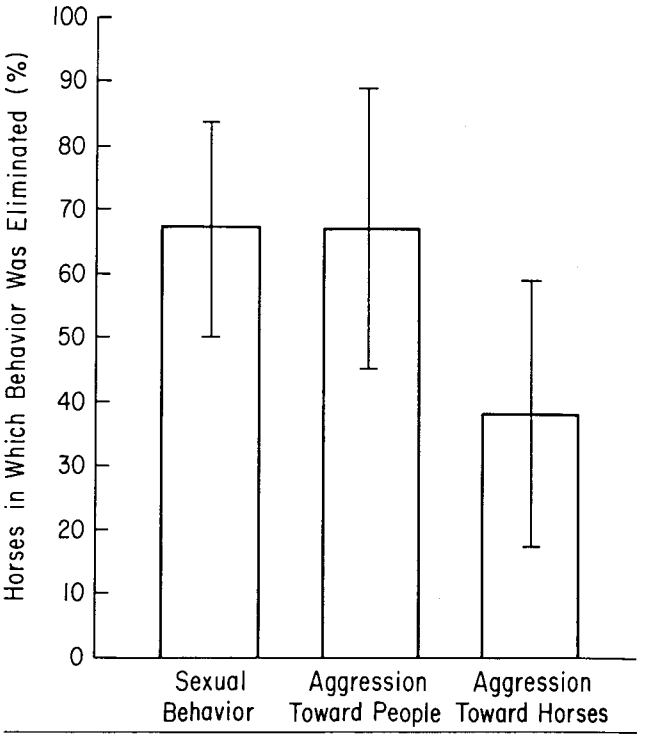

Fig 2—Percentage of male horses in which sexual behavior (n = 30), aggression toward people (n = 18), or aggression toward horses (n = 21) was eliminated after castration. Vertical lines represent 95% confidence intervals.

behavior, and the main reason for castration. Specific questions were asked about biting, kicking, or rearing directed at people and horses, and about sexual interest in mares in estrus, which included displaying erection, mounting, and genital investigation during attempts at courtship, as well as copulation itself.

Statistical methods—A 2-sample binomial comparison (95% confidence intervals) was used to compare the occurrence of sexual and aggressive behavior in juvenile and adult castrates and the pre- and postcastration occurrence of sexual and aggressive behavior in adult castrates. A logit transformation was used to correct for distortion of the confidence interval for each proportion less than 0.20 or greater than 0.80.

Results

Of the 94 horses castrated prepubertally, 33% displayed stallion-like sexual interest (erection, mounting, genital investigation, or copulation), 25% were reported aggressive toward horses, and 5% were aggressive toward people in a stallion-like manner. When compared with the occurrence of these behavioral patterns in stallions castrated in adulthood, there was no statistical difference (Fig 1). When the behavior of stallions was examined before and after castration, it was found that sexual behavior and aggression toward people declined significantly ($P<0.01$), as did aggression toward horses ($P<0.05$; Fig 1). Combining the data for the adult and juvenile castrates, it was found that the prevalence in all castrates was 31%, 25%, and 8%, respectively, for objectionable sexual behavior, aggression toward horses, and aggression toward people.

The survey data for the effects of castration in stallions may be looked at in a different manner. If one is interested in the effectiveness of castration for stallions in which castration would be performed as a therapeutic measure for objectionable behavior, one would want to look at the alteration of behavior for just those stallions that displayed the problem behavior before castration. Of the 18 stallions reported to be aggressive toward people before castration, 6 still were aggressive after castration, and of these 6, 3 were reported to be less aggressive. Of the 21 stallions reported to be aggressive toward other horses before castration, 13 were still aggressive after castration, and 6 of these 13 were reported to be less aggressive. The analysis of the effects of castration on sexual behavior is more problematic because many male horses are not given an opportunity to express sexual interest toward mares in estrus after castration. In the survey, there were 30 stallions that copulated with mares before castration and also were observed in the presence of mares in estrus after castration. Of these horses, only 2 still copulated a year or later following castration and an additional 7 displayed some interest in the form of mounting, erection, or attempts to genitally investigate mares. Thus, the probability of eliminating sexual behavior or aggression toward people in stallions was found to be between 60% and 70%, and the probability of eliminating aggression toward horses was about 40% (Fig 2).

Discussion

It has been theorized that geldings with stallion-like behavior may have been castrated improperly, such that some androgen-producing tissue was left intact; these animals were often referred to as "proud cut." However, analyses of blood testosterone concentrations of geldings with stallion-like behavior revealed that testosterone concentrations were not different from those of more typically behaving geldings.[3] Our study indicates that about 20% to 30% of horses castrated at less than 2 years of age can be expected to display stallion-like sexual interest in

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mares and aggression toward horses and that about 5% can be expected to be aggressive toward people in a stallion-like manner. These percentages must be considered the normal prevalence of stallion-like behavior in geldings and not an abnormal occurrence due to factors such as hormonal imbalance or extragonadal production of testosterone.

The appearance of stallion-like behavior in geldings that had been castrated prepubertally is consistent with what is known about the effects of prepubertal castration on behavior in other mammalian species. Mounting by prepubertally castrated male cattle[5] and dogs[6] has been noted. Recently it has been documented that urine spraying and aggressive behavior in prepubertally castrated male cats[7] occurs at the same rate as the persistence of urine spraying and aggression in male cats that are castrated in adulthood after the behavior has appeared.[8]

The effectiveness of castration as a therapeutic measure in stallions to eliminate objectionable sexual behavior and aggressive behavior toward people was found to be between 60% to 70% and about 40% for aggression toward horses. This finding is along the lines reported for behavioral changes in adult males of other species. In adult male cats, castration is effective 80% to 90% of the time in reducing objectionable spraying, roaming, or fighting behavior.[8] In dogs, castration of adult males is 50% to 60% effective on mounting, aggression toward other dogs, and urine marking in the house.[9] Our results from this survey of stallions are therefore consistent with what has been reported in other species, and the percentages we present for the effectiveness of castration can be used as guidelines in giving clients prognoses. It should be noted that the percentages we report were for those cases in which the owners reported an elimination of the behavior rather than just a reduction. Since some clients may feel that a reduction in the objectionable behavior may be considered a favorable outcome of the castration, the effectiveness is probably underestimated.

An important result is that the data reveal no behavioral differences between male horses castrated as adults or juveniles. This information may be useful in advising clients who wish to delay castration of their males until they have obtained the more stallion-like morphologic characteristics. Presumably such a delay would not increase the chance of the animal retaining objectionable stallion-like behavior once it is castrated at a later age.

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Cutaneous innervation of the thorax and abdomen of the dog

The anatomy of the cutaneous nerves innervating the canine thorax and abdomen was investigated by gross dissection of 38 dogs. Additionally, the cutaneous areas innervated by the thoracic and abdominal cutaneous nerves were mapped in a 2nd group of 33 barbituate-anesthetized male dogs, using electrophysiologic techniques. The skin of the thorax was innervated by dorsal cutaneous branches, lateral cutaneous branches, and ventral cutaneous branches of the spinal nerves. The dorsal cutaneous branches were branches of the dorsal primary branches of spinal nerves C6 and T2 through T11. The lateral cutaneous branches were branches of the ventral primary branches of spinal nerves T2 through T12. The ventral cutaneous branches were branches of the ventral primary branches of spinal nerves T2 through T10. The skin of the abdomen was innervated by dorsal and lateral cutaneous branches of spinal nerves T12 through L3 (and occasionally L4). The cutaneous areas of the dorsal cutaneous branches occupied the dorsal half of the scapular and thoracic regions and the dorsal 2/5 of the abdominal region. The cutaneous areas of the lateral cutaneous branches covered the major portion of the ventral half of the thorax and the ventral 3/5 of the abdomen. The cutaneous areas of the ventral cutaneous branches occupied the axilla and the ventral part of the thoracic wall.—*C. S. Bailey, R. L. Kitchell, S. S. Haghighi, et al in Am J Vet Res, 45 (Sept 1984): 1689.*

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Tests for cooperative behaviour between stallions

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Breeding groups with multiple stallions occur sympatrically with single-stallion breeding groups in feral horse, *Equus caballus*, populations. Mutualism and reciprocal altruism between stallions have been proposed to explain the origin and functioning of multistallion bands. However, empirical support for these hypotheses is contradictory and incomplete. Furthermore, there are no explicit tests of the predictions that each hypothesis makes about stallion behaviour and social structure. We compared nine multistallion and 18 single-stallion bands in the Kaimanawa Ranges, New Zealand. Compared with agonistic behaviours, affiliative behaviours were relatively unimportant in the relationships between stallions within bands. The number of stallions in the band did not have a positive influence on mare group size, stability, home range quality or reproductive success in bands. Furthermore, there was a positive relationship between aggression ('intolerance') by the dominant towards subordinate stallions and the subordinates' effort in mare group defence ('helping') but a negative relationship between helping effort by subordinates and their proximity to, and mating with, the bands' mares. Therefore, the predictions of the mutualism and reciprocal altruism hypotheses were not supported. Indeed, for some of the predictions we found the opposite outcomes to be true. Multistallion bands had significantly poorer reproductive success, and dominant stallions were less tolerant of subordinates that helped most and reduced their access to mares. Nevertheless, in all other respects Kaimanawa stallions in multistallion bands behaved like those described elsewhere. Thus, we reject cooperative hypotheses for multimale breeding groups in horses and discuss the mate parasitism and consort hypotheses as better alternatives.

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Animal breeding groups sometimes contain individuals that appear superfluous to reproduction and whose behaviour may even be costly to the Darwinian fitness of other group members, if not their own. For example, the breeding groups or resource territories of polygynous ungulates sometimes contain more than one breeding male (e.g. waterbuck, *Kobus ellypsiprymnus*: Wirtz 1982; oribi, *Ourebia ourebi*: Arcese 1999; white rhinoceros, *Ceratotherium simum*: Owen-Smith 1975; horse, *Equus caballus*: Miller 1981; Linklater et al. 1999). The mating system of the feral horse is described in detail in Linklater et al. (1999) and Linklater (2000). Briefly, it is characterized by polygynous breeding groups, called bands, with 1–26 mares and one to five stallions with declining frequency. Males that are not part of a band live alone or in all-male (bachelor) groups with ephemeral membership. Both male and female offspring disperse from natal bands and so kinship relationships between band members are rare.

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The presence of multimale breeding groups that are sympatric with the more common single-stallion bands have been hypothesized to result from cooperation between stallions for mutual benefit (Miller 1981; Rubenstein 1982; Berger 1986; Franke Stevens 1990; Feh 1999). However, there are flaws in the approach, data and logic that have been used to support cooperative hypotheses such as mutualism and reciprocal altruism between stallions in bands (Linklater & Cameron 2000). Also, Linklater et al. (1999) showed that multistallion breeding groups were actually deleterious for the body condition and fecundity of mares. In multistallion bands poorer foaling rates and higher offspring mortality (fetal to yearling) are attributed to greater harassment of mares by resident stallions (Linklater et al. 1999) and possibly infanticide (Cameron 1998; see also Duncan 1982; Berger 1983; Kaseda et al. 1995). Therefore, it seems unlikely that the relationship between stallions in multistallion bands is a cooperative adaptation.

Resolving the controversy requires explicit tests of the null model (that stallions do not cooperate) by testing for the predictions that each cooperative model makes about stallion behaviour and social structure. Such tests must measure differences in the rates of primary stallion

behaviours that are defined operationally, for example, kicking behaviour as a measure of aggression, mutual grooming for affiliation, or interindividual distance for association. Tests of the cooperative models must avoid seeking differences in derived or secondary behaviours (e.g. 'helping'), where the definition of the behaviour is conceptual and presupposes its cause (Linklater & Cameron 2000; see Tinbergen 1963 on 'neutral terminology').

We compared the behaviour and ecology of Kaimanawa feral horses in single- and multistallion breeding groups with those described elsewhere, and tested the following predictions made by authors invoking mutualism and reciprocal altruism hypotheses for multistallion bands. (1) Via mutualism, each stallion in a multistallion band receives a net fitness gain from their relationship by being able to maintain a larger and more stable mare group (Miller 1979; 1981; Franke Stevens 1990), dominating limiting resources and better-quality habitat (Miller 1981) and obtaining greater reproductive success (Feh 1999). (2) Stallions in a multistallion band have a relationship based on reciprocal altruism whereby inferior stallions trade their help in mare group defence with the dominant stallion for his tolerance and, therefore, band membership, greater access to mares, and more reproductive opportunities (Berger 1986; Feh 1999). In the light of our results we discuss the limitations of cooperative hypotheses for multistallion bands and alternatives such as the mate parasitism and consort hypotheses.

METHODS

Study Site and Population

The origins, range use and demography of Kaimanawa wild horses, and the extent, vegetation, topography and climate of their range, as well as the study area and focal population, are described by Linklater et al. (2000). Comparisons in Linklater (2000) show that the population structure, behaviour and ecology of Kaimanawa feral horses are identical to those of feral horses throughout the world. We noted the membership and locations of 27 bands that inhabited the central study area (listed in Table 1) in all 32 months from August 1994 to March 1997. The Four-male and Shoehorn bands are excluded from some analyses because of the difficulty of locating the former during winter and the formation of the latter less than 5 months before the end of the study. The majority of adults in all bands were identified by freeze brands. (Horse capture and marking are described in Cameron & Linklater 2000.) The remainder were identified by permanent and unique differences in their colour marks. Where necessary, observations of bands and individuals were made with binoculars (× 10–15) and field telescopes (× 15–60) but often we were able to approach marked individuals and bands to identify them without visual aids because they became habituated to us. The minimum age of branded individuals was estimated by tooth eruption and wear patterns (Hayes 1968; Fraser & Manolson 1979) and their front shoulder height measured (Cameron 1998; Linklater 1998).

Band Size, Structure and Membership Stability

We calculated average band size, and the number of mares and stallions in bands, from the monthly modal number of adults. We recorded the membership of bands often enough to estimate membership changes and foaling reliably (Linklater 1998; Cameron et al. 1999). All changes in band membership by mares moving into or out of bands, whether temporary or permanent, were termed join or leave events, respectively. A permanent change in band membership by a mare was termed a dispersal event. There was a positive correlation between the number of join and leave events per year and mare group size (Linklater et al. 2000). Therefore, we adjusted the rate of join and leave events for mare group size so we could compare bands. Measures of membership stability for each band are expressed as rates (i.e. number of dispersal or join and leave events per mare per year). A stable core membership group was defined by the individuals that were members of their bands from the beginning of observations or the band's formation to the end of observations or the mare's death.

Home Range Size, Structure and Quality

We recorded the locations of bands often enough to estimate home range size and structure reliably (Linklater 1998). We calculated band home range and core use areas from the coordinates derived by marking the location of the bands on 1:25 000 scale topographical and vegetation maps (Linklater et al. 2000). To determine home range, we used 95% minimum convex polygons (WildTrak software, Todd 1992). Core areas and core area fidelity were calculated from 50% adaptive kernels (Worton 1989) and the ratio of the 50 to 95% adaptive kernels, respectively (Home software, Taborsky & Taborsky 1992). Home range size per adult and core use area fidelity were considered coindicators of home range quality given that there was a significant positive relationship between band size and home range size (Linklater et al. 2000). Thus, we inferred that bands in poor-quality habitat would have larger home ranges per adult and show reduced fidelity to small core areas within their home range.

We selected seven single- and four multistallion bands with a representative range of mare group sizes from the existing population in spring 1994 (Table 1). The 11 bands were located in a random sequence at intervals between 3 and 21 days from 29 November 1994 to 4 December 1995 to provide 36–44 independent locations per band. The sites occupied by the 11 bands, when located, were described from the slope (degrees from flat), altitude (above sea level to nearest 10 m), and aspect (i.e. N, NE, E, SE, S, SW, W, NW), and from ground cover within the area enclosed by the spread of their adult members. Ground cover was described from vegetative (i.e. species or taxonomic group) or other (i.e. bare earth, mud, rock, scree, gravel, sand) items observed at the site.

Table 1. The size, membership composition and history of 9 multistallion and 18 single-stallion Kaimanawa feral horse bands in the study area from August 1994 to March 1997

Band type	Band name	Adult band size	Mares				Stallions					Band age††	History	
			N	Range†	Core‡	Ages§	*N*	Range	Core	Ages§**	Height** (cm)		Formed	Disbanded
MS	Shoehorn	2.9	1.0	1	1	5	1.9	1–3‡‡	1	**8**, ND	**148**, ND	1	Nov. 1996	—
MS	Four-male	3.8	1.0	1	1	ND	2.8	1–4§§	1	ND	ND	4	—	Dec 1996
MS	Punks	4.2	1.2	1–3	1	9	2.9	2–3	3	**5, 6, 5**	**143, 140, 145**	2	Nov. 1995	—
MS	Georgy	4.2	2.3	1–3	2	4, 4	1.9	1–2‡‡	2	**6, 4**	**148, 135**	2	Oct. 1995	—
MS	27	5.8	2.3	1–3	1	8	3.5	1–4	2	**11**, 8, ND	**145**, 148, ND	4	—	—
MS*	Raccoon	5.6	3.6	2–5	2	5, 5	2.0	1–2‡‡	2	**8, 9**	**140, 148**	4	—	—
MS*	Rust	6.1	4.1	1–6	2	4, ND	2.0	1–2‡‡	2	**8, 5**	**145, 143**	3	Aug. 1994	—
MS*	W.f.m.	8.1	5.0	4–5	5	12, 9, 9, ND	3.2	3–4	3	ND	ND	4	—	—
MS*	Black	9.0	7.0	3–9	5	ND	2.0	1–2‡‡	2	ND	ND	4	—	—
SS*	Mule	2.0	1.0	1	1	6	1.0	1	1	**9**	**143**	3	Sep. 1994	—
SS	Th'	2.0	1.0	1	1	3	1.0	1	1	**4**, ND, ND	**ND**, 148, ND	2	Mar. 1996	—
SS	Rob Roy	2.0	1.0	1	1	12	1.0	1	1	**ND**	**ND**	4	—	Sep. 1995
SS	M&M	2.3	1.0	1	1	10	1.3***	1–3***	1	**4**	**ND**	1	Oct. 1996	Feb. 1997
SS	Hillbilly	2.4	1.4	1–4	1	8	1.0	1	1	**9**	**145**	4	—	—
SS	Zig zag	2.6	1.6	1–3	1	9	1.0	1–2***	1	7, **8**	143, **150**	4	—	—
SS*	Alaskans	2.8	1.8	1–3	1	11	1.0	1	1	**9**	**143**	4	—	—
SS*	Mary	3.3	2.3	1–4	1	11	1.0	1	1	**8**	**148**	4	—	—
SS	Ice cream	3.6	2.6	1–6	0	—	1.0	1	1	**ND**	**ND**	2	Mar. 1996	—
SS	W	3.8	2.8	2–3	2	8, 9	1.0	1	1	**12**	**144**	4	—	—
SS	Electra	4.6	3.6	3–6	3	9, 7, 5	1.0	1	1	**10**	**148**	4	—	—
SS*	Victor	4.6	3.6	3–5	3	6, 10, ND	1.0	1	1	**11**	**143**	4	—	—
SS	Imposters	4.9	3.9	3–5	3	5, ND	1.0	1	1	**10**	**145**	4	—	—
SS*	Henry	5.5	4.5	3–5	3	9, 11, 10	1.0	1	1	**11**	**150**	4	—	—
SS*	C	6.0	5.0	3–8	3	5, 5, 6	1.0	1	1	**10**	**150**	4	—	—
SS	Lumps	6.6	5.6	3–8	4	6, 5, ND	1.1***	1–2***	1	**ND**, ND	**ND**, ND	4	—	—
SS	Canadians	8.3	7.3	6–9	4	4, 9, 5, 10	1.0	1	1	**7**	**143**	3	Oct. 1994	—
SS*	Ally	8.4	7.4	6–11	6	9, 6, 10, 9, 10, 7	1.0	1	1	**11**	**145**	4	—	—

MS: Multi-stallion band; SS: single-stallion band; ND: no data.

*Each of the 11 focal bands located in a random sequence between November 1994 and December 1995 to determine ground cover categories and topographical variables that were selected or avoided by bands.

†Range refers to the range in the size of the mare or stallion membership of each band during the study period.

‡Core refers to the stable mare or stallion membership that was in the band at the beginning of the study or the band's formation and still in the band at the end of the study or the individual's death.

§All ages are minimum estimates and given as of summer 1996–1997 at the end of observations.

**The stallions are listed in order from dominant to most subordinate, left to right, from observations of the outcome of agonistic interactions. The ages and heights of stallions in bold type indicate the core stallion group.

††Bands were placed in the following age categories: 1: formed during 1996–1997 year (1 September to 31 August); 2: formed during 1995–1996 year; 3: formed during 1994–1995 year; and 4: were extant bands when observations began in August 1994.

‡‡These bands had only one stallion during brief periods when the stallions were separated either each with some of the mares or one with mares from other bands.

§§This band lost the first of its four stallions in spring 1995 and only one remained by spring 1996.

***These bands had more than one stallion during brief periods in which a bachelor successfully challenged the resident stallion and resulted in loss of mare(s) or the permanent displacement of the resident stallion from all resident mares.

Table 2. Results of the logistic regression analysis (backward selection procedure, SAS Institute 1990) to determine the topographical variables and ground cover categories present in home ranges more or less than expected from their contribution to the entire range of 11 of the 26 focal bands in the study area

Order of removal	Topographical variables and catagories of ground cover	Wald chi-square statistic	*P*	Coefficient estimate
Nonsignificant variables ($P \geq 0.1$) sequentially removed from the model				
1	Tertiary woody	0.01	0.94	—
2	Litter	0.03	0.86	—
3	Primary woody	0.04	0.85	—
4	Altitude	0.23	0.63	—
5	Secondary woody	0.54	0.46	—
6	Aspect SE	0.53	0.47	—
7	Aspect SW	0.25	0.61	—
8	Aspect E	0.23	0.63	—
9	Aspect NE	1.74	0.19	—
10	Aspect N	1.42	0.23	—
11	Sedges, reeds, rushes etc	1.06	0.30	—
12	Aspect W	1.23	0.27	—
13	Aspect NW	1.21	0.27	—
14	Aspect S	1.17	0.28	—
15	Bracken and ground ferns	2.68	0.10	—
16	Slope	2.46	0.12	—
Significant variables ($P<0.05$) remaining in the model				
	Short green grasses	35.68	0.00	2.833
	Tussock grasses	18.32	0.00	0.695
	Leguminous herbs	30.48	0.00	3.173
	Other dicotyledonous herbs	8.27	0.00	4.125
	Moss and lichen	5.26	0.02	0.587
	Bare ground	5.82	0.02	0.592
	Intercept	38.20	0.00	−6.183

We ranked the five that contributed most from 8 (most common) to 3; any other items that were judged common scored rank 2, all items present but not already scored ranked 1, and items not seen at the site but found elsewhere in other samples ranked 0 (see Scott 1989, for plotless vegetation description by the ordinal ranking of species). We combined data from all sites ($N=432$) to provide a sample of habitat selected by bands in the study area.

We drew 10 parallel lines running magnetic north to south on a 1:25 000 topographical map at 375-m intervals across the study area between the borders of the minimum convex polygon that contained all 11 bands' location coordinates. Points were prescribed at 250-m intervals along each line from one side of the polygon to the other. Using a compass and topographical map as guides, we visited the coordinates prescribed by each point. Once at a coordinate, we walked a random number of steps from 0 to 999 to the north or south to the sample site. At the resulting sample site we measured slope, altitude, aspect and ground cover as for the sites selected by the 11 bands. Data from all sample sites ($N=189$) were combined to provide a sample of the habitat available to the 11 bands.

The average score for the ranks of taxonomic or morphologically similar groups of ground cover species or items (Table 2) were obtained for all selected and available sites. We determined the selection or avoidance of topographical variables and ground cover categories by a logistic regression (logistic, backward selection procedure, SAS Institute 1990) of data from selected and available sites, as described in Manly et al. (1993). Variables whose coefficients of selection were least significant were sequentially removed until only significant variables ($P<0.05$) and their coefficient estimates remained. The estimates of significant coefficients of selection were used to construct a resource selection probability function (Manly et al. 1993).

We combined available sample sites that fell within the home range of each band to give average scores for the ranks of the ground cover categories within each band's home range that had significant selection coefficients. We then entered the average scores of these ground cover categories into the resource selection probability function to obtain a 'probability of selection' value for each home range. The probability of selection value was used as a relative index of home range quality with possible values that could range from 0 (extremely poor-quality home range) to 1 (an extremely good-quality home range).

Stallion Behaviour in Multistallion Bands

We conducted continuous focal behaviour samples on seven of the multistallion bands from November 1995 to March 1997. Samples ranged from 30 to 150 min during daylight hours to provide an average of 29 h per band (range 7–38 h). During samples, we recorded agonistic and affiliative interactions that involved the stallions

whenever observed. Agonistic interactions were defined as those that involved overt, threat, or display aggressive behaviours. Aggressive behaviours (e.g. overt and threatened bites, kicks, arched neck threats and head threats with ears laid back) and avoidance, retreat and chase behaviours were as described and defined by Feist & McCollough (1976) and McDonnell & Haviland (1995). An agonistic interaction was classified as a win if the other stallion retreated first. The stallion winning the most dyadic agonistic interactions was considered dominant. Defence events of mare groups are defined as those in which agonistic interactions occurred between resident stallions and other stallions or bachelor males when the latter approached the mare group. An affiliative behaviour was an approach between stallions that culminated in an attempt to groom or a mutual grooming event as defined by Feist & McCollough (1976) and McDonnell & Haviland (1995). We recorded all mounting of mares by stallions that included intromission whenever observed. We could not always determine if ejaculation also occurred with intromission. Every 4 min during focal behaviour samples we visually estimated the distance from each stallion to the centre of the mare group in adult body lengths. Average stallion distance to the mare group centre was calculated from averages of each sample. Focal behaviour sampling took precedence over 4-min samples of stallion distances if they occurred simultaneously.

Hypothesis Testing

We tested two hypotheses. First, we tested whether the number of stallions in a band had a positive influence on the reproductive success, size and stability of the mare group, and the band's home range quality, as predicted by the mutualism hypothesis. We used a multivariate analysis of variance (MANOVA, SAS Institute 1990) to test the influence of additional stallions on band and home range measures, and a chi-square test for differences in the foaling rate of mares in single- and multistallion bands. Second, we tested whether there was a negative relationship (regression, SAS Institute 1990) between the frequency of defence behaviours by subordinate stallions in mare groups (i.e. 'helping') and (1) the rate of agonistic interactions that the dominant stallion directed towards the subordinate stallion (dominant stallion 'intolerance') and (2) the distance of the subordinate stallion from the mare group ('reproductive opportunity') as predicted by the reciprocal altruism hypothesis.

RESULTS

Band Composition and Structure

Band stallions varied in age from 4 to 12 years (minimum estimate) at the completion of observations. The oldest known male was a bachelor aged at least 14 years. The average age and height of stallions in those bands already formed when the study began (i.e. band age category 4, Table 1) were not different between single- and multistallion bands (age: multistallion: $\bar{X} \pm \mathrm{SE} = 9.0 \pm 0.7$; single stallion: 9.7 ± 0.4; Mann–Whitney U test: $U=17.5$, $N_1=4$, $N_2=12$, $P=0.42$; height: multistallion: 145.3 ± 1.9 cm; single stallion: 146.2 ± 0.8 cm; Mann–Whitney U test: $U=21.5$, $N_1=4$, $N_2=12$, $P=0.76$). Core groups of mares were evident in both single- and multistallion bands (Table 1). Core group mares ranged in age from 4 to 12 years in those bands that had already formed when the study began (i.e. band age category 4). The average age of core group mares was not significantly different between single- and multistallion bands (multistallion: $\bar{X} \pm \mathrm{SE} = 8.0 \pm 1.1$; single stallion: 8.1 ± 0.4; Mann–Whitney U test: $U=75.5$, $N_1=6$, $N_2=27$, $P=0.79$; Table 1). In conclusion, the age and size of the members of single- and multistallion bands were similar.

Table 3. The percentage (with numbers in parentheses) of interactions won and lost by stallions in multistallion bands during dyadic agonistic interactions

	Won		
Lost	Alpha	Beta	Gamma
Beta	98.7 (154)	—	—
Gamma	95.7 (23)	68.2 (22)	—
Delta	100 (20)	100 (28)	92.3 (13)

Stallions are ranked from most dominant (alpha) to most subordinate (delta).

Affiliative behaviour between stallions was extremely rare. We observed only two mutual grooming events and both occurred between the alpha and gamma stallions of Punks band. In comparison, agonistic behaviours between stallions were frequent. We recorded 7–90 agonistic interactions between stallions in each multistallion band. From 13 to 71% of agonistic encounters between stallions in each band had no clear outcome because both stallions withdrew after an aggressive display sequence (see Feist & McCollough 1976; McDonnell & Haviland 1995). Of the remaining interactions for which we could determine a winner or loser, stallions showed a consistent dominance relationship with one of the stallions causing the retreat of the other in greater than 90% of encounters, with the exception of beta and gamma stallions in bands with three or four stallions (β won 68% of the time against γ, Table 3). Furthermore, in four of the seven multistallion bands, one of the stallions won all agonistic encounters. Therefore, there was a stable hierarchy between the stallions in multistallion bands: alpha (α)>beta (β)>gamma (γ)>delta (δ).

A stallion's rank determined his position relative to other stallions and the mare group in multistallion bands and the number of matings they obtained. Subordinate stallions were more likely to be further from the centre of the mare group than stallions dominant to them (paired t test: $t_{15}=3.16$, $P=0.01$) and dominant stallions mounted and achieved intromission with band mares more often than stallions subordinate to them (paired t test: $t_5=2.61$, $P=0.048$). Furthermore, of the subordinate stallions, we

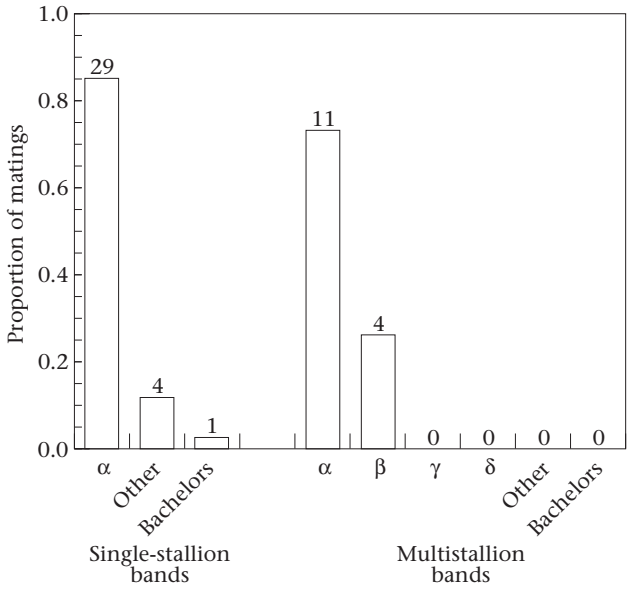

Figure 1. The distribution of observed matings between stallions (of different rank, i.e. α>β>γ>δ, from other bands and bachelors) and the mares in single- and multistallion bands. The numbers of matings are given above the bars.

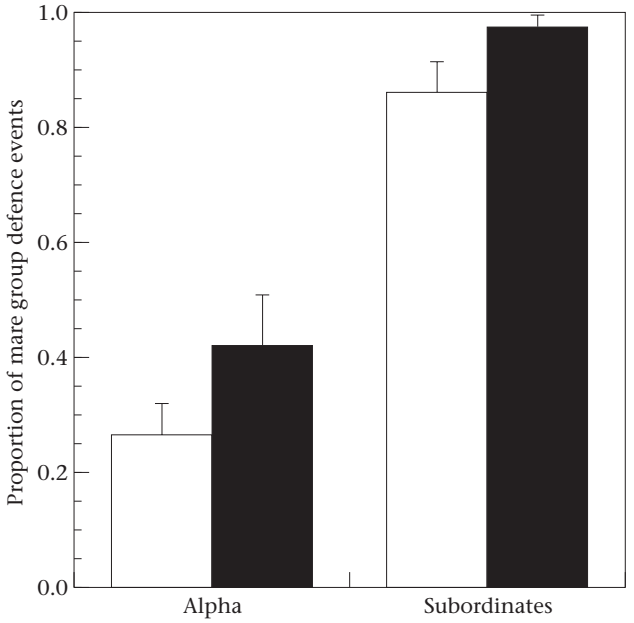

Figure 2. The average proportion of mare group defence events (N=88) that the alpha and subordinate stallions initiated (□) and contributed to (■) in multistallion bands. Vertical bars indicate 1 SE.

observed only beta stallions mating, never gamma and delta stallions. We observed 34 matings with mares in single-stallion bands and 15 with mares in multistallion bands. Mating of a band's mares by the stallions of other bands and bachelor males was observed only in single-stallion bands (Fig. 1).

Dominant stallions were significantly less likely to initiate and contribute to mare group defence events and subordinate stallions contributed to most mare defence (paired t test: initiated: t_6=5.73, P<0.001; contributed: t_6=6.06, P<0.001; Fig. 2).

Table 1 shows the age and height of member stallions in multistallion bands in their order of dominance. In multistallion bands where more than one of the stallions was aged and height measured, the dominant was not significantly younger, older, or taller than the subordinate stallion (paired t test: age: t_6=1.53, P=0.18; height: t_6=0.00, P=1.0; Table 1). Dominant stallions were on average only 1 year older (range 1 year younger to 3 years older) than the subordinate and there was no difference in the average height of member stallions (range dominant 8 cm shorter to 13 cm taller). Therefore, the age and size of stallions of different rank in multistallion bands were similar.

The Mutualism Hypothesis

The average size and range in size of multistallion and single-stallion bands were almost identical (multistallion: 2.9–9.0; single stallion: 2.0–8.4 adults), as was the number of mares in them (multistallion: 1.0–7.0; single stallion: 1.0–7.4 mares; Fig. 3a). The rate of join and leave and dispersal events by mares in single- and multistallion bands, were also indistinguishable (Fig. 3b, c). Furthermore, for mares that remained in the same band for each entire year (1 September to 31 August), those in multistallion bands had significantly poorer foaling rates than those in single-stallion bands (multistallion: 0.54; single stallion: 0.70 foals/mare; chi-square: χ^2_1=4.8, P<0.05). Therefore, stallions in multistallion bands did not each obtain a net fitness gain by increasing the size, stability or reproductive success of their mare group compared with stallions that defended a mare group alone.

Of the topographical variables and vegetation classes, only short green grasses, tussock grasses, leguminous herbs, other dicotyledonous herbs, mosses and lichens, and bare ground categories were significant indicators of site selection by the 11 bands for which it was measured. Therefore, we used estimates of these selection coefficients (Table 2) to construct the resource selection probability function.

Selection probability (home range quality)= exp(2.833 (short green grasses)+0.695(tussock grasses)+ 3.173 (leguminous herbs)+4.125(other dicotyledonous herbs)+0.587(moss and lichen)+0.592(bare ground)).

Of the 27 bands, 21 were observed for more than 1 year and had home ranges that were contained entirely within the area in which available habitat was measured. We calculated the values for the resource selection probability function for the home range of each of the 21 bands. They varied from 0.42 (relatively poor-quality home range) to 0.68 (relatively good-quality home range) and were almost identical for single- and multistallion bands (Fig. 3d). Furthermore, band home range size/adult and core area fidelity were similar for single and multistallion bands (Fig. 3e, f) and there was no relationship between band size and home range quality (Pearson correlation: r_{23}=0.354, P>0.05). Therefore, additional

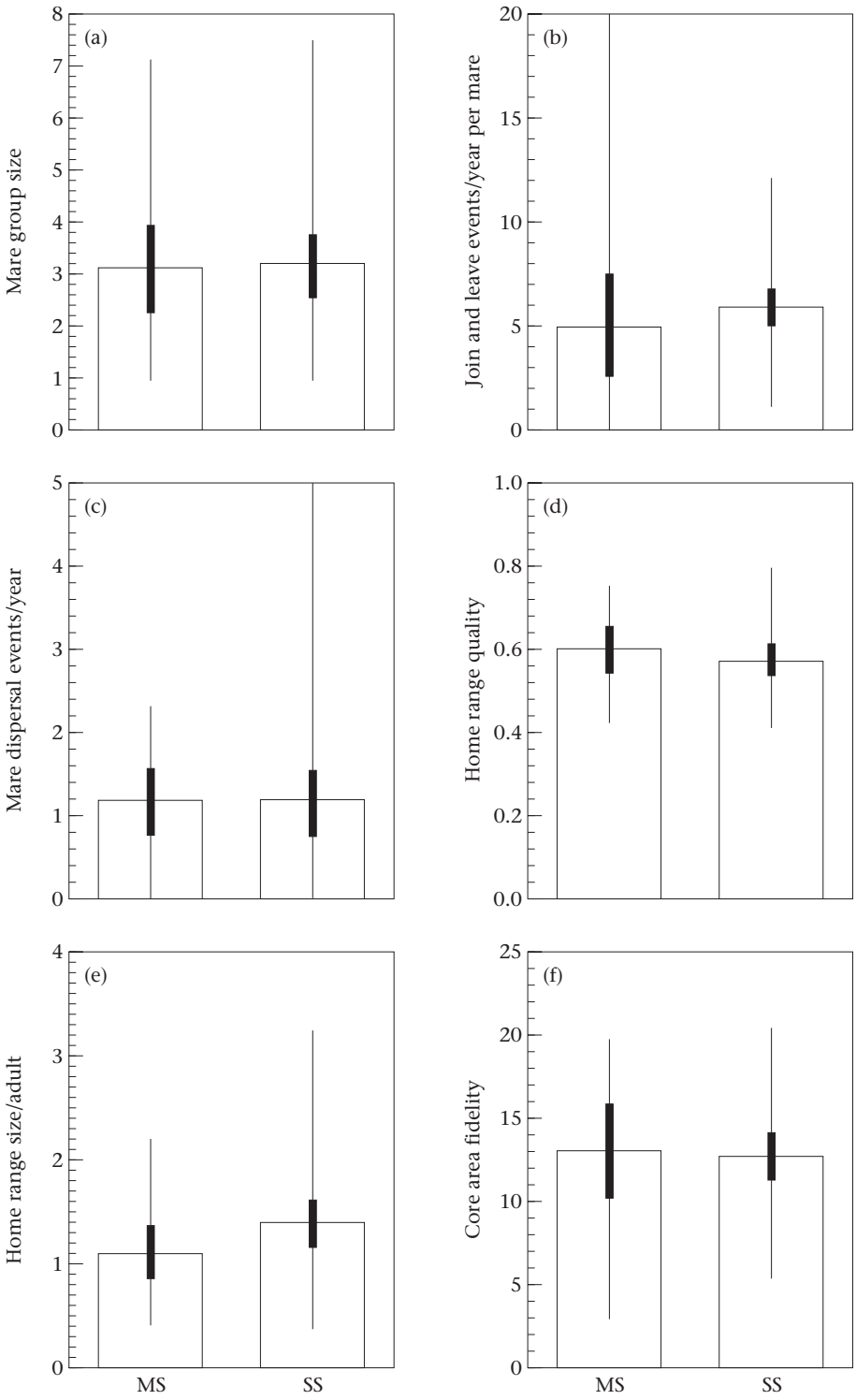

Figure 3. A comparison of (a) mare group size, (b) rate of mare join and leave events, (c) rate of mare dispersal, (d) home range quality, (e) home range size and (f) core area fidelity for single- (SS, *N*=18) and multistallion (MS, *N*=8) bands. The bar denotes the average figure, the black vertical rectangle denotes ± 1 SE, and the vertical line the range of values.

stallions in bands did not improve a band's access to better-quality habitat.

The two measures of band stability were significantly correlated with each other (Pearson correlation: r_{25}=0.53, P<0.01) and therefore only one, mare dispersal rate, was entered into the MANOVA. Additional stallions did not alter the size or stability of their mare groups, the size or quality of their home ranges, or their fidelity to core areas

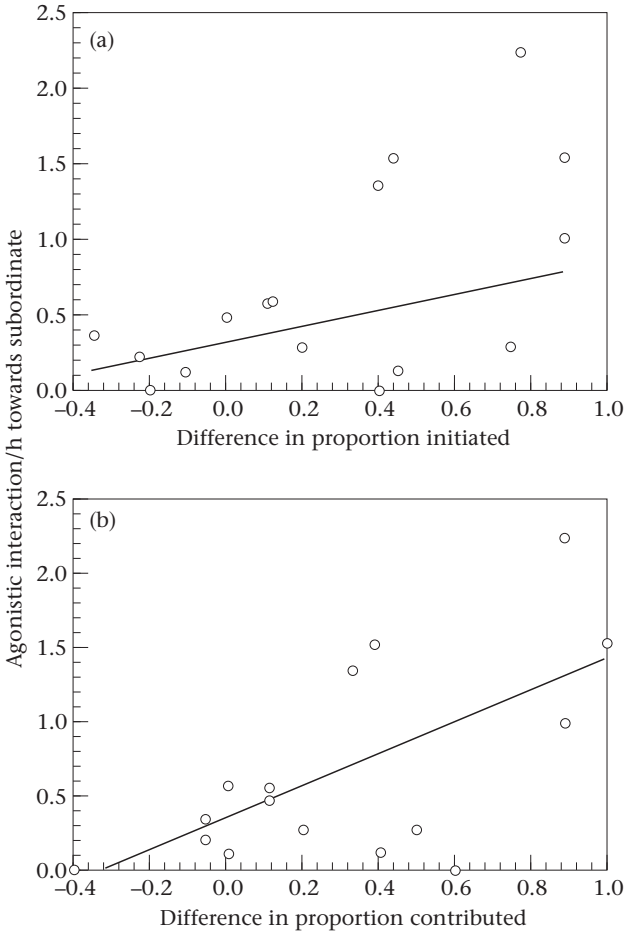

Figure 4. The relationship between the difference in the proportion of mare group defence events that stallions (a) initiated and (b) contributed to (subordinate's–dominant's proportion) and the rate of aggressive interactions by the dominant stallion that caused the subordinate to retreat.

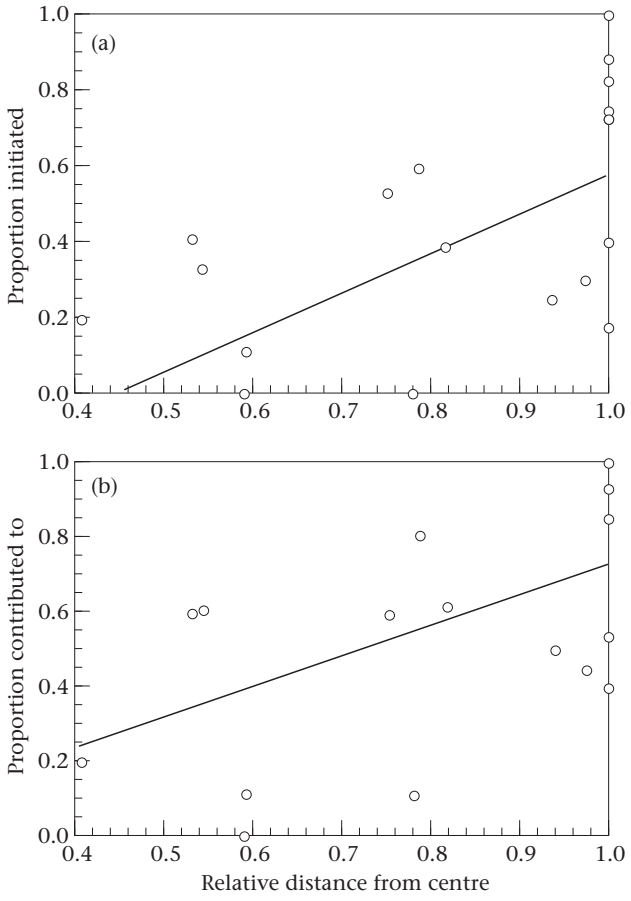

Figure 5. The relationship between the average distance of a stallion from the centre of the mare group relative to the average distance of the most peripheral stallion and the proportion of mare group defence events that they (a) initiated and (b) contributed to. A distance of 1.0 is equivalent to the distance of the most subordinate stallion from the mare group's centre.

within their home ranges (MANOVA: $F_{6,17}$=0.19, P=0.96; Fig. 3).

Reciprocal Altruism Hypothesis

There was a significant positive, not negative, relationship between the difference in the proportion of mare group defence events that subordinate and dominant stallions initiated and contributed to and the rate of aggressive interactions directed at the subordinate by the dominant stallion (regression: initiated: $F_{1,14}$=8.10, R^2=0.32, P=0.013; contributed: $F_{1,14}$=9.60, R^2=0.36, P=0.008; Fig. 4). Furthermore, there was a positive relationship between the relative distance each stallion was from the mare group's centre and the proportion of band defence events that he initiated and contributed to (regression: initiated: $F_{1,16}$=6.34, R^2=0.24, P=0.02; contributed: $F_{1,16}$=7.28, R^2=0.27, P=0.016; Fig. 5). Thus, dominant stallions were less, not more, tolerant of subordinate stallions that 'helped' most, therefore ensuring that subordinate 'helpers' were more, not less, peripheral to the mare group. The marginalization of subordinates from the mare group was reflected in the number of mating opportunities they obtained. Beta stallions obtained approximately a quarter of observed matings in multistallion bands while gamma and delta stallions obtained none (Fig. 1), even though they contributed most to mare defence in their respective bands. Therefore, subordinate 'help' was not reciprocated with dominant stallion tolerance or greater mare access and mating opportunities, contrary to the prediction of the reciprocal altruism hypothesis.

DISCUSSION

The stallions in multistallion bands had a strongly hierarchical relationship that was not subject to change in the medium term relative to a stallion's reproductive life. Agonistic encounters between stallions were frequent and affiliative behaviours rare. The dominant stallion maintained proximity with the mare group while successively subordinate stallions were increasingly peripheral to it. Consequently, more dominant stallions were on average

closer to the mares in a band and conducted the majority of matings even though more subordinate stallions contributed most to mare group defence. Similar observations have been reported in other populations (Miller 1981; Berger 1986; Feh 1990; Franke Stevens 1990). Furthermore, our observations of mating by stallions conform to expectations from previous comparative analyses of paternity in single- and multistallion bands (Bowling & Touchberry 1990; Eagle et al. 1993; Asa 1999; Feh 1999). The alpha stallions in multistallion bands obtained slightly fewer matings and less paternity in their bands than stallions in single-stallion bands because of some successful mating by the subordinate(s). Subordinate stallions in multistallion bands obtained few matings: the lower their rank the fewer they obtained. The gamma and delta stallions were not observed to mate. Also, approximately half of the matings and paternity gained by the beta stallion would have been lost by the alpha stallion to stallions from other bands or bachelors if he was the sole stallion in a single-stallion band anyway. Thus, the alpha stallions in multistallion bands are largely successful at limiting the paternity lost to subordinate(s) (see also discussion of comparative paternity analyses in Linklater & Cameron 2000).

Thus, our results confirm previous observations about the social and spatial structure of the relationships between stallions in multistallion bands. However, although Kaimanawa multistallion bands were outwardly identical in these ways to those in other populations (see also Linklater 2000), we found no indication that their structure or functioning could be explained by mutualism or reciprocal altruism as they have been applied previously to the problem of multistallion bands.

Mutualism?

Miller & Denniston (1979) found that large bands generally displaced smaller bands at water holes and Miller (1981) found multistallion bands to be larger than single-stallion bands in the Red Desert, Wyoming, U.S.A. Consequently, Miller (1981) suggested that multistallion bands gained better access to limited resources such as water in the Red Desert. He concluded that these factors meant that collaborating stallions had greater lifetime reproductive success than stallions in single-stallion bands. However, although band size was an indicator of dominance, Miller & Denniston (1979) found no correlation between the number of stallions in the band and its dominance relationship with other bands. Second, as we found in the Kaimanawa population, there was near complete overlap in the sizes of single- and multistallion bands (single stallion: 2–21, multistallion: 3–17 members, Miller 1979; single stallion: 1–16, multistallion: 6–16 members, Miller & Denniston 1979). Third, Berger (1986) suggested that it was single-stallion, not multistallion, bands that were more likely to occupy high-quality home ranges in the Great Basin. Lastly, Franke Stevens (1988, 1990) found no difference in the success of single- and multistallion bands defending access to a water hole and recorded no significant difference in mare group size between single- and multistallion bands. She found that the band occupying the water hole won agonistic encounters most often and independently of its size or number of stallions. Therefore, proposed relationships between the number of stallions in bands, mare group size and band dominance are at best weak, if they exist at all.

Miller (1979) found more stable mare membership in multistallion bands and concluded, without behavioural measures of the effort stallions took in maintaining the band, that 'the presence of subordinate males probably contributes to the greater stability and larger average size of multiple male bands' (Miller 1981, page 346). Furthermore, of the 10 mares Franke Stevens (1990) observed changing bands during 7 months of observation, all came from single-stallion bands. Franke Stevens (1990) showed that in winter the mare groups of single stallions spread further, and suggested that a single stallion may be less able to retain mares in winter than multiple stallions. Consequently, she suggested that the dominant stallion's lifetime reproductive success was improved if he was able to prevent mare loss by accepting a subordinate stallion's 'help', while the subordinate stallion(s) stood to inherit the mare group from the dominant stallion at the end of the latter's reproductive life. Unfortunately, Franke Stevens (1990), like Miller (1981), assumed that additional stallions contributed to mare group stability, even though her measures of band maintenance activities by stallions (e.g. herding) in winter were not significantly greater in multistallion bands than in single-stallion bands. In addition, she measured no increase in reproductive success in multistallion bands or inheritance of mare groups by subordinate stallions (Franke Stevens 1990), and Berger (1986) showed that it was bachelor males, not subordinate stallions, that were more likely to inherit mares. We found no relationship between the number of stallions and mare group stability in the Kaimanawa population. Therefore, while a relationship between the number of stallions in bands, mare group size and stability, and band dominance in preferred habitat makes an elegant hypothesis, there are clearly significant inconsistencies and contradictions in it.

Lastly, and most crucially, we must consider reproductive success directly. Mutualism predicts that individual stallions will make a net gain in reproductive success by cohabitating with other males in the same mare group. This prediction is best illustrated from the work on lions, *Panthera leo* (Bygott et al. 1979; Packer et al. 1988; Grinnell et al. 1995) where the reproductive success of males that live together is improved by better control of access to females. Prides with more male lions have more offspring. Feh (1999) showed that even the alpha stallions in multistallion bands do not obtain greater paternity than stallions who defend a mare group alone partly because they lose paternity to the subordinate stallions. Only if both the dominant and subordinate stallions in multistallion bands obtain even less paternity without their relationship can it be regarded as mutualistic. Without this evidence the pattern of paternity between stallions, as Feh (1999) presented it, is not evidence for

mutualism and is open to alternative explanation (see Linklater & Cameron 2000).

Therefore, there is still considerable work to be done here. In particular, paternity measures should be conducted in populations from which males are not artificially and selectively removed, and where multistallion bands include more than just two stallions. Adult sex ratio parity, large variation in male quality, and bands with as many as four, and sometimes five, stallions are typical of unmodified and free-roaming feral horse populations throughout the world (Linklater 2000) and are thus unlike the Camargue population of horses (Feh 1999). Consequently, the information available at this time is clearly not strong enough to invoke mutualism for the origin and functioning of multistallion bands in feral horse populations. This conclusion and recent work that suggests multistallion bands may be deleterious for the body condition and reproductive success of mares (Linklater et al. 1999) do not support the mutualism hypothesis, that stallions cohabit in the same band to gain a net reproductive advantage.

Reciprocal Altruism?

Subordinate stallions put disproportionately more effort into mare group defence than dominant stallions (Berger 1986; Feh 1999), despite having fewer matings (this study) and significantly less paternity (e.g. Bowling & Touchberry 1990; Eagle et al. 1993; Asa 1999; Feh 1999). Asymmetrical effort in mare group defence by resident stallions indicates that they do not reciprocate in kind for contributions to mare group defence. However, Berger (1986) and Feh (1999) suggested that by helping in mare group defence the subordinate stallion's membership in the band is tolerated by the dominant stallion. Thus, help from a subordinate is reciprocated with tolerance by the dominant and vice versa. The subordinate stallion gains greater access to mares and improved paternity but costs the dominant stallion some paternity. The dominant stallion benefits from the subordinate's assistance with mare defence and therefore longer tenure with the mare group. In this way both may gain fitness benefits (Feh 1999). This is a compelling idea that, until now, has not been tested by checking for a negative relationship between subordinate effort in mare defence (i.e. 'help') and aggression by the dominant towards the subordinate (i.e. 'intolerance').

If reciprocal altruism is occurring, then subordinate stallions that contribute more to mare group defence than the dominant stallion should receive less aggression from the dominant stallion, be more proximal to the band's mares, and obtain more matings. In the Kaimanawa population we observed the reverse to be true. Dominant stallions directed significantly more aggression at subordinates that helped most and subordinates that helped relatively more were significantly less proximal to the mare group. Furthermore, although the most subordinate and, therefore, peripheral stallions helped most, they obtained few matings and in bands with three or four stallions the gamma and delta stallions were never observed to mate.

In support of the reciprocal altruism hypothesis, Berger (1986) and Feh (1999) described a pattern in which the subordinate more often met an intruding stallion while the dominant more often herded the mares away, and where the dominant stallion came less to the aid of subordinates in band defence than vice versa. Berger (1986) and Feh (1999) attributed this pattern to subordinate stallions helping with mare defence in return for tolerance by the dominant stallion, subordinate band membership and access to mares. We suggest that this pattern is more simply explained by the competitive and spacing relationship between stallions, rather than reciprocity. The pattern may result from (1) the different probabilities with which dominant and subordinate stallions encounter foreign stallions and are near mares during these encounters, (2) the dominant stallions' reluctance to leave the mares with the subordinate stallion, and (3) the subordinate stallions being aggressively dissuaded, by the dominant stallion, from approaching the band's mares.

Subordinate stallions were more peripheral to the mare group and their marginalization increased with increased aggression from the dominant stallion. Consequently, when a multistallion band is approached by a foreign stallion, the subordinate is disinclined to respond by moving towards the mares to herd them away because he is aggressively deterred from approaching them by the dominant stallion. His only alternative course of action is to meet the foreign stallion to stem his approach. Furthermore, being peripheral means that the subordinate stallion is more likely than the dominant to identify and encounter foreign stallions first. If the dominant stallion is the first to identify an approaching foreign stallion, he will be disinclined to meet the intruder first because that would necessitate leaving his mares open to an approach by the subordinate stallion. When the subordinate stallion identifies and encounters the foreign stallion first then the dominant stallion, who is proximal to the mares and already recognizes the subordinate stallion as an intruder and persistent challenger, will be disinclined to meet with two competitors for his mares at the same time, and his best strategy is to retreat, taking his mares with him, if he responds at all. Therefore, the dominant stallion is more likely to respond to the intrusion of yet another stallion by herding his mares away. Dominant stallions use herding behaviour to move mares away from both the subordinate and foreign stallions who approach their mares (Linklater et al. 1999). Their herding response is, therefore, a generalized response to avoid potentially successful and/or multiple competitors, rather than a specific response made possible by a collaborating subordinate(s). In many cases the dominant stallion will not respond and contribute to the defence event because the foreign stallion does not proceed past the subordinate towards the mare group and so the interaction does not escalate. In conclusion, the disparities in mare defence and herding behaviours between dominant and subordinate stallions, and differences in the frequency with which they defend mares at the same time (i.e. 'come to each other's aid'), may be an artefact of their competitive and spacing relationship that results in the subordinate

being peripheral to the mare group, rather than a cooperative relationship between stallions based on reciprocity.

Future Directions

Clearly there are still gaps in our understanding of multistallion bands in horses. In particular, there is the need for experimental stallion removals from multistallion bands to test for a mutually dependent relationship between stallions that share a mare group (e.g. Linklater 1998; Linklater et al. 1998). The absence of experimental manipulations and the lack of well-designed comparative paternity data is the weakness of this and previous studies. Nevertheless, this study describes inconsistencies and contradictions in previous evidence for stallion cooperation. Furthermore, it provides results on the behavioural structure and functioning of single- and multistallion bands in the Kaimanawa population that are different enough from the expectations of mutualism and reciprocal altruism to suggest that cooperation hypotheses cannot be applied generally to explain variation in the number of stallions in feral horse bands. In addition, multistallion bands are found in all free-roaming populations despite extreme differences in their habitat, density and adult sex ratios (Linklater 2000). Only when people have removed so many stallions that some mare groups were without one, have multistallion bands not formed. Multistallion bands, therefore, cannot be explained as an adaptive behavioural response or as a behavioural adaptation to differences in the resource or social environment. In feral horses, valid explanations for variations in social and spatial organization should apply to unmodified populations throughout the world since extreme regional differences in environment and demography have a negligible influence on the social organization and mating system of horses (Linklater 2000). Cooperative and socioecological explanations for multistallion bands have poor cross-populational generality and so we hope that this analysis will stimulate effort towards new explanations for the multistallion band phenomenon.

One alternative hypothesis that may prove useful is mate parasitism (Taborsky 1997) or a best-of-a-bad-job strategy (Dunbar 1982) by subordinate stallions as also suggested by Berger (1986). Indeed, the positive relationship between aggression by dominant stallions and defence of the mare group by subordinate stallions may be interpreted as tentative support for this hypothesis. If subordinate stallions are mate parasites then dominant stallions should be less tolerant of the most capable subordinates (i.e. those that 'helped' more) as we observed in this study. Perhaps this relationship is evidence that dominant stallions are making greater efforts to oust the most effective mate parasites from their bands or maybe they are coercing the mate parasite to 'help'? Our record of matings indicates that although mate parasitism is likely to be a less successful strategy for gamma and delta stallions than remaining a bachelor male, the beta stallion does achieve matings, particularly those normally lost to stallions from other bands or bachelors when stallions defend a band alone. Feh (1999) also showed this pattern in bands from the Camargue that had two stallions. Therefore, the mate parasitism hypothesis appears prima facie to warrant further investigation.

There are, however, some problems with the mate parasitism hypothesis for multistallion bands. For example, multistallion bands may contain up to five successively subordinate stallions and sometimes with only one mare as in the population described here (Table 1). The selective advantage of mate parasitism by the most subordinate stallion in such circumstances appears negligible given the absence of mating by gamma and delta stallions and the success of alpha stallions in limiting even the beta stallions to a small fraction, and sometimes none, of the band's total paternity (Bowling & Touchberry 1990; Feh 1999). Even when the dominant stallion is vasectomized, he appears to control the paternity of subordinate stallions (Eagle et al. 1993; Asa 1999). Therefore, although mate parasitism may explain the behaviour of beta stallions it cannot account for additional subordinates that behave like the beta stallion. Furthermore, and perhaps most importantly, during this 32-month study we observed nine new bands form from bachelor males and dispersing mares, including four multistallion bands (Table 1). However, on no occasion did a multistallion band form by a bachelor male attaching himself permanently to an existing single-stallion band to become a subordinate stallion (mate parasite), as one would predict if mate parasitism is the route for the origin and functioning of multistallion bands.

Linklater et al. (1999) and Linklater & Cameron (2000) suggested that an alternative explanation for the existence of multistallion bands may be found in the ontogeny of breeding groups that involves variation in the frequency of critical events during the short period of band formation (i.e. the consort hypothesis). Where, during band formation, there are dominance reversals between stallions after courtship and mating, and courtship and mating stimulate mate loyalty by consorting stallions (mate loyalty or 'guarding' reduces harassment of mares and prevents infanticide, see Linklater et al. 1999; Berger 1983; Kaseda et al. 1995), then multistallion bands may result from mares soliciting more than one stallion. Thus, multistallion bands may be an unselected artefact of the process by which mates are chosen and breeding relationships form, which normally results in the formation of single-stallion bands, but occasionally in bands with from two to five stallions with increasing rarity. The number of stallions in a newly formed band is determined by the number of stallion dominance reversals and consort relationships formed with a receptive mare after she disperses from her previous band. Our preliminary data (Linklater 1998; unpublished) suggest that this is the route of band formation. This approach offers new ways of understanding the origins and structure of horse breeding groups and new predictions to test. Furthermore, it may have utility in many other species with breeding groups of multiple males and females in birds and mammals where, because of mate loyalty, events during the ontogeny of new breeding groups may have long-term implications for breeding group structure. We advocate a greater awareness of the

potential role of breeding group ontogeny in variation in mating systems.

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Surgical Approaches to Ovariectomy in Mares

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KEY FACTS

- When performing ovariectomy by a colpotomy (vaginal) approach, proper positioning of the vaginal incision is necessary to prevent fatal complications.
- A colpotomy approach should not be used to remove ovaries larger than 8 to 10 cm in diameter; ovaries up to 15 cm in diameter can be removed safely through a flank approach; and larger ovaries should be removed through a celiotomy incision.
- Laparoscopic ovariectomy techniques allow optimal visualization and tension-free ligation to maximize hemostatic security.
- A wide variety of complications of differing severity (from mild to fatal) can follow any ovariectomy technique.

ABSTRACT: The preoperative considerations, surgical techniques, postoperative care, and complications associated with the surgical techniques available to perform unilateral or bilateral ovariectomy in horses are described. Standing techniques described include the colpotomy (vaginal), flank laparotomy, and laparoscopic approaches. Procedures that require general anesthesia, including flank laparotomy, ventral midline celiotomy, paramedian celiotomy, diagonal (oblique) paramedian celiotomy, and laparoscopic approaches, are also discussed. Complications following surgery can range from mild incisional swelling and pain to fatal intraabdominal hemorrhage or eventration. Careful consideration of the advantages and disadvantages of each procedure allow equine surgeons to select the most appropriate approach for each patient.

Equine ovariectomy is a commonly performed elective surgical procedure. Various surgical approaches are used for unilateral or bilateral ovariectomy. The surgical approaches described include vaginal or colpotomy, flank, diagonal or oblique paramedian, ventral midline, caudal paramedian, and numerous laparoscopic techniques. The decision as to which approach to use for a particular case depends on the following factors:

- Specific indications for ovariectomy
- Size of the affected ovary
- Surgeon's preference
- Financial constraints imposed by the client
- Temperament of the mare
- Equipment available
- Client expectations

An understanding of the benefits and disadvantages of all approaches can aid the clinician in selecting the appropriate surgical approach for each patient. This article reviews the surgical approaches used to perform unilateral and bilateral ovariectomy in mares.

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PREOPERATIVE CONSIDERATIONS

Special preoperative preparation of the mare should be instituted before performing ovariectomy. Food should be withheld for 12 to 24 hours before surgery to help decrease the amount of ingesta and gas within the gastrointestinal tract, thereby making it easier to exteriorize the ovary and suture the abdominal wall incision. Depending on patient positioning, laparoscopic techniques require that food be withheld for 12 to 48 hours prior to surgery[1,2] to improve visualization of intraabdominal structures and to decrease the likelihood of penetrating a viscus when the laparoscopic instruments are introduced into the abdomen.[3–7]

Abdominal palpation per rectum (with or without ultrasonographic evaluation) is useful in detecting abnormalities associated with the reproductive tract and should be performed in all mares presenting for ovariectomy regardless of the reason. Results of this evaluation may help dictate the necessary surgical approach based on the palpable size of the ovary to be removed. In addition, identifying pathology, such as adhesions or abscessation associated with the reproductive tract, may provide valuable information regarding the optimal surgical approach.

The rationale for and use of antibiotics to treat horses undergoing ovariectomy vary among surgeons. If antibiotics are used, they should be administered preoperatively to ensure that adequate systemic concentrations are present at the time of surgery. Administration of broad-spectrum antibiotics should be continued postoperatively if a break in aseptic technique occurred during the procedure. Tetanus prophylaxis should be administered routinely when performing ovariectomy.

SURGICAL PROCEDURES

Vaginal Approach (Colpotomy)

In 1903, Williams first described a vaginal approach, or colpotomy, using an ecraseur to ovariectomize mares.[8] The vaginal approach is now commonly used to perform a bilateral ovariectomy in normal mares, but unilateral removal of a suspected granulosa–theca cell tumor less than 8 to 10 cm in diameter can also be performed using this approach.[9,10] There are several advantages to the colpotomy technique, including the fact that it is performed as a standing procedure, minimal instrumentation is required, the procedure can be performed quickly, excellent cosmetic results can be expected, and the convalescent period following surgery is relatively short.

Ideally, mares should be in diestrus or anestrus because ovarian vasculature is believed to be minimized at these times.[11] Mares are restrained in standing stocks and sedated. Sedation is most commonly accomplished with an α_2-agonist and butorphanol to provide analgesia as well as profound sedation. Acepromazine can be administered for tranquilization as well. Administration of caudal epidural anesthesia is unnecessary but is recommended by some surgeons to help prevent the mare from straining during the procedure.[12,13] The tail is wrapped and secured away from the perineal region, and all manure should be evacuated from the rectum to prevent contamination of the external genitalia. Routine aseptic preparation of the external genitalia and perineal region is performed, and the vagina is lavaged with sterile saline or dilute povidone–iodine solution. In some mares, catheterization of a large urinary bladder may facilitate the procedure and may help reduce the risk of inadvertent injury during the procedure.[14–16]

The location of the initial incision in the cranial fornix of the vagina is very important. The incision must be placed in either a craniodorsal (at the 2- or 10-o'clock) or a cranioventral (at the 4- or 8-o'clock) position. Potential complications of a misplaced incision include entering the rectum if the incision is placed too dorsal, injuring the urethra or bladder if placed too ventral, and incising the caudal uterine branch of the urogenital artery if too medial or lateral (at the 3- or 9-o'clock position).[11,17,18] The incision should be started 3 to 5 cm caudal to the os cervix to avoid disruption of the cervical musculature.[12,14,16] Using a scalpel blade, scissors, or mosquito hemostat, a small 1- to 3-cm vaginal incision is sharply/bluntly created. This initial incision should penetrate the full thickness of the vagina and peritoneum to prevent the peritoneum from lifting away from the underlying tissues during blunt dissection. The incision is then bluntly enlarged digitally, and the peritoneum is perforated to allow the surgeon's hand to enter the abdomen. The ovary and associated mesovarium are isolated by direct manual palpation. Anesthesia of the mesovarium can be attempted using gauze sponges soaked with local anesthetic, which are held around the mesovarium for 30 seconds to 2 minutes. To prevent loss of the sponges within the abdomen, a long suture or strand of umbilical tape should be secured to the sponges.[13,16,19]

To transect the ovary from the mesovarium, a chain ecraseur is used to slowly crush and cut the mesovarium. The chain ecraseur should be carefully placed around the mesovarium, ensuring that bowel, intestinal mesentery, or a portion of the uterine horn is not encircled by the chain.[12,17,18] Once the chain is properly positioned, it is tightened slowly (over 1 to 4 minutes) using the ratchet system of the ecraseur. Hemostasis of the mesovarium depends on crushing the vascular structures and the resulting vasospasm. While the ecraseur is being tightened around the ovary, the ovary

should be held within the surgeon's hand to prevent losing it within the abdomen. The contralateral ovary may be removed via the same vaginal incision if a bilateral procedure is to be performed. The vaginal incision is left open to heal by second intention. Optionally, an episioplasty (Caslick's procedure) may be performed to complete the surgery in an effort to decrease the risk of ascending infection.[11]

Tie stall restriction for 2 to 7 days postoperatively has been advocated to prevent recumbency and reduce the risk of evisceration.[13,19,20] However, some authors suggest that this is unnecessary and report no adverse effects with routine stall confinement.[11,17] Exercise restriction is employed for 1 to 3 weeks following surgery, depending on the rate of healing of the vaginal incision. During this time, limited hand walking or small paddock turnout is important to reduce the potential formation of adhesions to the ovarian stump.[11,15,18] Light riding and a slow return to normal work are allowed after this time.

The primary disadvantage to the colpotomy approach is the lack of visualization.[21] By palpation alone, the ovary must be differentiated and isolated from the omentum, local mesentery, loops of intestine, and fecal balls within the small colon.[12,22] Hemorrhage from the mesovarium may be difficult to determine and control due to the lack of visualization with this approach.[13,21] Postoperative administration of broad-spectrum antibiotics may be indicated in mares undergoing a vaginal approach because it is difficult to adequately prepare the vagina for aseptic surgery. This approach is not recommended in mares that pool urine or in mares with vaginal, cervical, or uterine infections.[14,18,22] Ecraseur transection of large ovaries (larger than 8 to 10 cm in diameter) should not be attempted due to the size of vaginal incision needed to remove the ovary, the possibility of dropping the ovary within the abdomen, and the enlarged vascular supply associated with larger ovaries.[10,11,13,18,23] Even with adequate restraint and heavy sedation, this approach poses a risk to the surgeon due to positioning behind the mare.[12,22] Thus only tractable mares are good candidates for this procedure.

Flank Approach

The flank ovariectomy approach can be performed with the mare in the standing or recumbent position. To perform a standing flank laparotomy, the mare's temperament must be amenable to standing surgery. When performing a standing flank laparotomy, the mare is sedated, restrained in standing stocks, and the tail is wrapped and secured away from the surgical site (as described for the colpotomy procedure). For the recumbent technique, mares are placed in lateral recumbency so that the ovary to be removed is uppermost. The recumbent flank technique requires general anesthesia, resulting in a slightly greater cost to the client and potentially increased risk to the mare. The recumbent flank technique is generally only used for unilateral ovariectomy, as it offers easy access to only one ovary.[14]

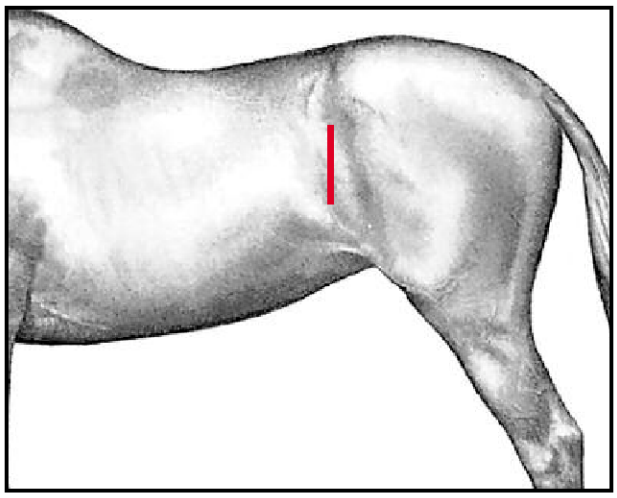

Figure 1—Position of paralumbar flank incision for ovariectomy between the 18th rib and tuber coxae. This incision position can be used in both standing and recumbent horses.

Compared with the colpotomy approach, both the standing and recumbent flank laparotomy approaches enable the surgeon to remove larger ovaries and give better exposure of the mesovarium, thus potentially providing superior hemostasis.[13,19,22] The ovary is normally situated beneath the paralumbar fossa. In horses, the limited size of the paralumbar fossa (compared with bovines) and the thickness of the body wall in the flank region may limit the ability to easily exteriorize large ovaries.[10,11,24] Ovaries up to 15 cm in diameter can be removed easily through a flank approach.[9,18,23] Bilateral ovariectomy typically requires a second incision through the opposite paralumbar fossa to remove the contralateral ovary. In the standing mare, bilateral ovariectomy can be achieved through a single flank incision, but the contralateral ovary must be excised blindly within the abdomen using a chain ecraseur.[12,14,19]

Regardless of positioning, the paralumbar fossa is clipped, aseptically prepared, and draped. Regional anesthesia in the form of an inverted L-block or local infiltration of the proposed incision site is required in standing mares.[11,12,24] The incision is started 5 cm ventral to the lumbar transverse processes between the 18th rib and the tuber coxae and is extended ventrally 10 to 15 cm as needed, depending on the size of the ovary to be

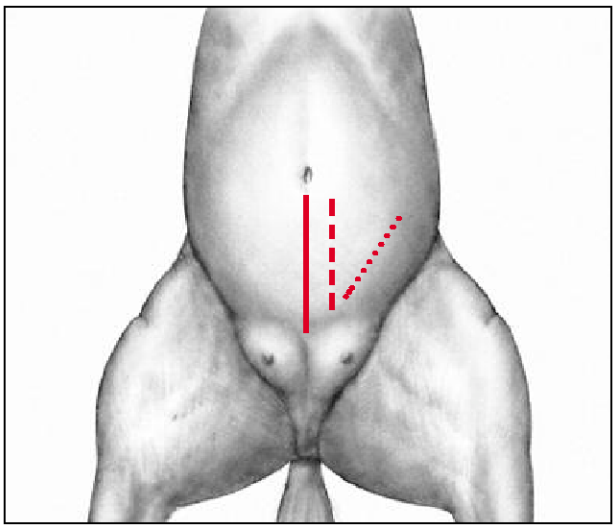

Figure 2—Position of traditional celiotomy incisions performed under general anesthesia with the horse in dorsal recumbency. *Solid red line* = ventral midline approach; *dashed line* = paramedian approach; *dotted line* = diagonal (oblique) paramedian approach.

removed (Figure 1). Following incision of the skin and subcutaneous tissues, a grid or modified grid approach may be used to incise the abdominal musculature. The grid approach creates a slightly smaller opening into the abdomen and should only be used for ovaries less than 10 cm in diameter; larger ovaries will necessitate use of the modified grid technique.[13,14,19] A grid approach involves separating the external abdominal oblique muscle along the direction of its fibers, whereas a modified grid technique involves incising this muscle along the line of the skin incision.[14] For both techniques, the internal abdominal oblique and transversus abdominis muscles are bluntly separated along the direction of their fibers or incised to expose the peritoneum. The peritoneum may be incised or bluntly perforated to allow access to the abdomen. The ovary is then identified and isolated. In standing mares, the mesovarium is anesthetized by topical administration of anesthetic, similar to that described for the colpotomy procedure, or by direct injection of anesthetic into the mesovarium, if visualization permits. The ovary may be transected within the abdomen or after exteriorization. Aspiration of cystic cavities within the ovary may reduce the overall ovarian size and facilitate exteriorization.[9,10,18] The means to achieve hemostasis and transection of the mesovarium depend on the ability to exteriorize or visualize the mesovarium through the flank incision. Possible choices include the use of a chain ecraseur, emasculator, surgical stapling device, or transfixing ligatures.[11,14,16,19,24] Following removal of the ovary, the mesovarium is observed for hemorrhage and oversewn if desired, when adequate visualization permits. Oversewing the mesovarium with 2-0 absorbable suture material may reduce possible intraabdominal adhesions of a segment of bowel to the transected mesovarium.[14,18] Closure of the laparotomy incision is routine.

Postoperative pain and discomfort may be observed in association with the flank incision. Incisional swelling and discharge may be noted 24 to 72 hours after surgery.[24,25] Incisions created in a more ventral position on the flank tend to develop more swelling and cause increased postoperative pain and discomfort.[24] Incisions in the flank have been associated with incisional discharge and partial dehiscence.[13,14,22] Occasionally, this approach results in a poor cosmetic outcome if scarring of the incision site occurs.[10-12] Exercise should be restricted to hand walking or turnout in a small paddock for 4 to 6 weeks following surgery, after which light riding and a slow return to normal work may be allowed, provided the incision has healed without complications.

Diagonal (Oblique) Paramedian Approach

The diagonal (oblique) paramedian approach has been reported to be superior to other approaches for ovariectomy, especially for the removal of ovaries up to 20 to 25 cm in diameter.[26,27] Because the incision is created so close to the intraabdominal position of the ovary, exteriorization through a diagonal paramedian incision generally results in less traction being placed on the mesovarium. In addition, the body wall is thinner at this location, as compared with other approaches, which allows greater flexibility in retracting the wound edges. Improved visualization of the ovary and mesovarium can often be achieved with this approach. Bilateral ovariectomy can be performed; however, two incisions are generally required. General anesthesia is necessary to perform an ovariectomy with this approach; therefore, the cost and risks associated with recumbency are also associated with this technique.

Perioperative preparation and care is similar to that for the previously described approaches. Following induction of general anesthesia, the mare is placed in dorsal recumbency. Slightly tilting the mare toward the side opposite the affected ovary helps minimize the tendency for bowel to protrude from the incision. Following routine aseptic preparation of the ventral abdomen, an incision is started approximately 5 to 10 cm cranial to the ipsilateral mammary gland and extended approximately 20 cm (or as needed, depending on the size of the affected ovary) cranially and laterally toward the fold of the flank[11,15,16] (Figure 2). The incision is extended through the underlying external rectus sheath,

parallel to the initial skin incision. The rectus abdominis, internal rectus sheath, and peritoneum are bluntly divided in the direction of their fibers or incised (depending on surgeon preference) to allow access to the abdomen.[13,18,26] The ovary is located and exteriorized. Local anesthesia of the mesovarium, either by topical application of anesthetic-soaked gauze sponges or direct injection of local anesthetic, may be used to diminish the pain response secondary to traction on the ovary.[16,28] Fluid within cystic cavities may be aspirated to reduce the size of the ovary and facilitate its removal from the abdomen.[16,23,27] Large suture material placed as stay sutures within the ovary and retraction on the incision edges may aid the surgeon in exteriorizing large ovaries. Overlapping transfixation ligatures of No. 2 absorbable suture material or application of a TA-90 stapler (US Surgical) may be used for hemostasis prior to transection of the mesovarium.[15,18,26,29] Ligatures should be tightened and staples should be applied to the mesovarium in a relaxed position; application under tension may result in failure to provide adequate hemostasis.[13,19,28] Following complete transection of the mesovarium, closure of the abdominal incision is routine. Due to the location of the incision on the ventrum, a good cosmetic outcome usually follows ovariectomy by the diagonal paramedian approach.[11]

Exercise restriction during the early postoperative period should consist of stall confinement with hand walking for the first 2 to 4 weeks following surgery.[11,27] After this time, small paddock turnout may be allowed for an additional 2 to 4 weeks of confinement,[11] the actual length of restriction depending on the individual patient's wound-healing progress. Return to a full exercise schedule and/or the allowance of natural service should not be allowed for at least 8 to 12 weeks postoperatively.[27] Mares may be bred by artificial insemination when the estrous cycle occurs following surgery.[15]

Ventral Midline Celiotomy Approach

A ventral midline celiotomy approach offers good exposure of the ovaries in the majority of mares.[14,15,28] The ventral midline incision can easily be extended as necessary, depending on the individual case, making it the technique of choice for removal of extremely large, tumorous ovaries.[13,19] The ventral midline approach is generally used to remove very large granulosa–theca cell tumors but can also be used to perform a bilateral ovariectomy.[11,15,16,23,25] A good cosmetic outcome is expected with this approach, provided no complications with incisional healing occur.

Perioperative preparation and care are similar to previously described approaches. For a ventral midline technique, general anesthesia is required and the mare is placed in dorsal recumbency. A caudal ventral midline linea incision is created, beginning at the mammary gland and extending cranially 25 to 35 cm as needed for adequate exposure[13,14,29] (Figure 2). The ovary is located by manual palpation and exteriorized. Local anesthesia of the mesovarium can be used if desired. Topical application of anesthetic-soaked gauze sponges or direct injection of the mesovarium with local anesthetic may decrease the pain response related to traction on the mesovarium.[16,28] Intraoperative hypotension believed to cause myopathies and neuropathies has been associated with excessive traction on the mesovarium, and these complications may be reduced by the application of local anesthetic.[28] Hemostasis and transection of the mesovarium are similar to what was described for the flank and diagonal paramedian approaches. Closure of the ventral midline incision is routine.

Postoperatively, care is similar to the other ovariectomy approaches. Stall confinement with hand walking should be enforced for the first 2 to 4 postoperative weeks, at which time sutures or staples used to close the ventral incision should be removed.[19] Small paddock turnout may be allowed after this, for an additional 2 to 4 weeks of confinement, after which light riding or pasture turnout may be allowed.[14] Full exercise should not be allowed for a minimum of 8 to 12 weeks after surgery, depending on the progress of incisional healing.[13,14]

Paramedian Approach

The paramedian approach can be used to remove large pathologic ovaries or to perform bilateral ovariectomy through one (contralateral ovary must be transected blindly) or two incisions.[18,19,23,25,28,30,31] The paramedian approach is similar to the ventral midline approach except for the location of the incision. The incision is made 4 to 8 cm lateral to midline and extends cranially from the level of the mammary gland for 25 to 35 cm or as needed for adequate exposure[16,19] (Figure 2). Methods to exteriorize the ovary and provide hemostasis of the mesovarium are similar to those described for other approaches. Postoperative care following a paramedian celiotomy is similar to the ventral midline and diagonal paramedian approaches.

Laparoscopic Techniques

In the past 10 years, laparoscopic ovariectomy techniques have been described for mares in the standing[3,4,32–34] and dorsally recumbent[2,4,5,21,34,35] positions. Laparoscopic techniques can greatly improve visualization of the ovary and mesovarium, potentially decrease postsurgical complications, and allow tension-free ligation of the vessels within the mesovarium.[2,3,21,32,33] How-

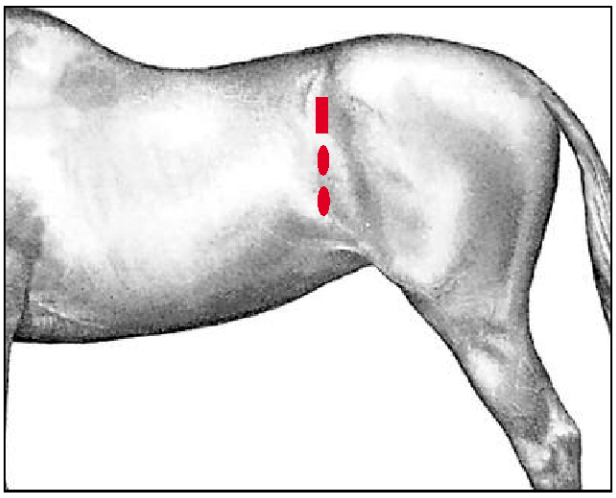

Figure 3—Position of the laparoscope *(rectangle)* and instrument portals *(ovals)* for laparoscopic ovariectomy in the standing horse. The laparoscope portal is positioned between the 18th rib and the tuber coxae, just dorsal to the crus of the internal abdominal oblique muscle. Instrument portals are created 4 to 8 cm ventral to the laparoscope portal.

ever, important considerations when performing equine laparoscopic ovariectomy include the requirement of specialized equipment, the technical difficulty of certain procedures, and the potential for anesthetic complications in horses placed in the Trendelenberg position.[4,21,33] Additionally, the use of laparoscopic equipment may be cost prohibitive in certain situations.[3,21]

Laparoscopic Ovariectomy in the Standing Mare

Laparoscopic ovariectomy in the standing mare avoids the need for general anesthesia, eliminates the cardiovascular derangements associated with the Trendelenberg position, and shortens the required preoperative fasting time (12 hours instead of 24 to 48 hours).[1,3,32] Mares are sedated using either xylazine hydrochloride or detomidine hydrochloride in combination with butorphanol tartrate. For bilateral ovariectomy, both paralumbar fossae are prepared for aseptic surgery and draped. The paralumbar fossa is desensitized using regional anesthesia or by direct infiltration of the proposed laparoscope and instrument portal sites.[4,5,36] The abdominal cavity can be insufflated with carbon dioxide through either a Verres-type needle inserted dorsally in the paralumbar fossa or a teat cannula inserted ventrally, as if performing abdominocentesis.[7,37] As an alternative (to avoid the potential complication of inadvertently insufflating the retroperitoneal space), the trocar–cannula can be inserted through the paralumbar fossa prior to insufflation of the abdominal cavity.[6,32,33,38]

A 15-mm skin incision is made at the dorsal border of the internal abdominal oblique muscle, and the sharp laparoscopic trocar–cannula is introduced into the abdominal cavity perpendicular to the paralumbar fossa[1,3,4,39] (Figure 3). The trocar is replaced by the laparoscope, and the caudal portion of the abdomen is examined to identify the ovary. The first instrument portal is made 4 to 8 cm ventral to the laparoscope portal, and a second instrument portal is made 4 to 8 cm ventral to the first[4] (Figure 3). Trocar–cannula units are passed through each instrument portal perpendicular to the flank musculature. Using a long spinal needle inserted separately through the flank musculature or a laparoscopic injection needle placed through a cannula, the mesovarium can be infiltrated with local anesthetic.[5,31,38] Hemostasis of the mesovarium can be achieved using suture ligatures, staples, laser energy, electrosurgical instrumentation, a vessel-sealing device, or a harmonic scalpel.[1,3,32,33,36,38–41] The ovary is transected using laparoscopic scissors distal to the site of ligation or coagulation. The ovary is removed by enlarging one of the instrument portals or by connecting the two instrument portals.[5,36] After removing the ovary, the abdomen is deflated through a laparoscopic cannula. The superficial abdominal fascia and skin at the portals are closed separately. The same procedure is then performed through the opposite paralumbar fossa to remove the contralateral ovary in bilateral procedures.

A hand-assisted laparoscopic ovariectomy technique in standing mares can be used to remove granulosa–theca cell tumors as well.[42] A standard flank approach is used, but the process of injecting local anesthetic into the mesovarium, application of a surgical stapling device, and transection of the mesovarium is performed intraabdominally with digital manipulation while using laparoscopic observation of the procedure.[42] After transecting the ovary from its mesovarium, the ovary is placed within a sterile plastic bag and sharply transected. Placing the ovary within the bag facilitates removal through a smaller incision and prevents abdominal and body wall contamination from the ovarian cystic fluid.[35,42] The standing, hand-assisted, laparoscopic ovariectomy technique is technically easy to perform, can be used for large pathologic ovaries (up to 30 cm in diameter), allows accurate placement of the staple line, and eliminates the potential risks and costs associated with general anesthesia.[42]

Laparoscopic Ovariectomy in the Anesthetized Mare

To perform unilateral or bilateral ovariectomy in an anesthetized mare using laparoscopy, the horse is anesthetized and positioned in dorsal recumbency, and the

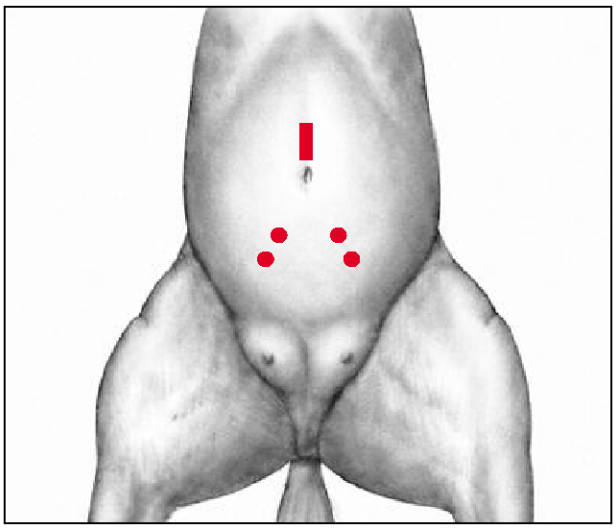

Figure 4—Position of the laparoscope and instrument portals for ovariectomy in the dorsally recumbent horse under general anesthesia. The laparoscope portal *(rectangle)* can be positioned just cranial to the umbilicus or just lateral to the umbilicus. Instrument portals *(dots)* are created between the laparoscope portal and the ipsilateral mammary gland.

tail is secured to the surgery table. The caudal abdomen is aseptically prepared and draped. To improve visualization of the caudal abdomen, a urinary catheter can be passed to decompress the urinary bladder.[35,43] A 10-mm skin incision is made just cranial or lateral to the umbilicus (Figure 4). The abdomen is insufflated with carbon dioxide through a teat cannula to a pressure of approximately 15 to 20 mm Hg.[2,4,39] A laparoscopic trocar–cannula unit is introduced into the abdomen, and the trocar is replaced with the laparoscope. In the recumbent technique, patient positioning becomes important for adequate visualization of the caudal abdomen. In routine dorsal recumbency, the female reproductive tract is obscured by intestinal viscera, so the surgical table must be elevated in such a way that the mare's head is lower than the hindquarters. For removing ovaries, an angle of inclination of approximately 30° from horizontal (Trendelenberg position) is generally required.[4,21] Two instrument portals (cranial and caudal) are created on both the left and right ventral abdomen (Figure 4). The cranial instrument portals are located midway between the ipsilateral mammary gland and the umbilicus. The caudal instrument portals are placed midway between the ipsilateral mammary gland and the cranial instrument portal.[35] After creating the four instrument portals, the ipsilateral uterine horn is elevated using a Chambers catheter (Jorgensen Laboratories) inserted through the contralateral caudal instrument portal. A knot push rod equipped with a modified Roeder knot or a commercial suture loop is inserted through the ipsilateral caudal instrument portal (left caudal instrument portal for removing a left ovary).[2] Sharp-toothed laparoscopic grasping forceps are passed through the cranial instrument portal on the same side as the suture loop. The jaws of the forceps are passed through the suture loop and used to grasp the ovary. The suture loop is passed over the ovary and tightened around the mesovarium. The mesovarium is then transected distal to the suture ligature.[21] The transected ovary is maintained in the jaws of the grasping forceps while the same series of steps are reversed to allow removal of the opposite ovary. The abdomen is deflated and the ovaries are removed by enlarging one of the cranial instrument portals. For the enlarged incision, the external fascia of the rectus abdominis, subcutaneous tissue, and skin are closed separately.[21] All remaining incisions are closed by simply apposing the skin.

Because most laparoscopic ovariectomy techniques can be accomplished through small incisions, the mare can be returned to exercise shortly after the procedure is performed. Postoperatively, mares should be confined to a stall for the first 24 hours followed by stall or small paddock confinement for 2 to 3 weeks before they are returned to unrestricted exercise.[33,35,38]

Laparoscopy can be used to remove large ovarian granulosa–theca cell tumors. One report described two mares that had a granulosa–theca cell tumor removed using a recumbent laparoscopic ovariectomy technique.[2] The mares were placed in Trendelenberg position, and the maximum diameter of the ovaries was estimated to be 20 cm in diameter.[2]

Complications that may occur following laparoscopic ovariectomy are similar to those seen with other approaches. Hemorrhage from the mesovarium has been reported following ligature slippage.[1,21] Subcutaneous emphysema may be observed postoperatively if the abdomen is not decompressed adequately prior to closure of the incisions.[32,43]

COMPLICATIONS

Although great advances have been made in the routine use of general anesthesia and surgical technique in horses, ovariectomy remains a procedure in which potential complications may occur, regardless of the approach used. In general, ovariectomy has been associated with greater postoperative morbidity and mortality than for other elective procedures.[10,13,15,22,25] Postoperative hemorrhage from the mesovarium can occur if hemostasis of the mesovarium fails.[3,22,23,44,45] Intraabdominal hemorrhage from branches of the ovarian artery is a serious and possibly fatal complication that

may go undetected at the time of surgery. Therefore, mares should be confined to a stall for the first 24 hours after surgery. Clinical signs associated with blood loss include tachycardia, pale mucous membranes, weakness or ataxia, weak thready pulse, and poor jugular distention.[13,18,22] Hemorrhage must be controlled, and initial medical therapy should be aimed at replacing the lost blood volume by intravenous administration of fluids and whole blood.

The vaginal approach also has the potential risk of inadvertently incising the caudal uterine branch of the urogenital artery when making the incision into the abdomen.[11,15] Care to avoid the 3- and 9-o'clock positions will help prevent this potentially fatal complication. Other potential complications reported when performing a vaginal approach include pain and discomfort; injuries to the cervix, bladder, or a segment of bowel; delayed vaginal healing; eventration of bowel, incisional site hematoma, or abscess; intraabdominal adhesions to the vagina; and chronic lumbar or bilateral hindlimb pain.[14,17,19,45]

Reported complications with the other celiotomy approaches vary depending on position. Intraoperative hypotension, myopathies, and neuropathies have been associated with traditional ovariectomy approaches performed under general anesthesia to remove granulosa–theca cell tumors.[13,22,25,45] Tension placed on the mesovarium during the process of exteriorizing an ovary is speculated to cause a decrease in arterial blood pressure and potentially lead to inadequate peripheral circulation.[25]

Cardiopulmonary derangements have been observed during laparoscopic procedures with horses placed in the Trendelenberg position.[21,32,39,46] This positioning exaggerates the force of abdominal insufflation and the weight of abdominal viscera on the diaphragm, decreasing the horse's ability to adequately ventilate without mechanical assistance and potentially compromising venous return to the heart.[21,32] Metabolic acid–base disturbances may also occur following prolonged abdominal insufflation with carbon dioxide, which diffuses easily into the systemic circulation.[39,46] As with traditional surgical approaches, myopathies and neuropathies can be a consequence of prolonged dorsal recumbency.[21] Therefore, proper anesthetic patient monitoring is required when performing laparoscopic ovariectomy with the mare in the dorsal recumbent position.

Regardless of whether the surgery is performed with the mare standing or recumbent, postoperative pain, anorexia, depression, incisional swelling, incisional infections, incisional dehiscence, eventration, peritonitis, intraabdominal adhesions, and death have been reported following ovariectomy in mares.[10,11,13,17,22,25,44,45] Incisional complications have been associated with ovariectomy techniques to remove granulosa–theca cell tumors.[25] A higher incidence of incisional complications have been observed with approaches through the paralumbar fossa.[14,24,47] This may be associated with the increased amount of dead space and possible muscle necrosis that may occur with the paralumbar approach.[47]

Proper aseptic techniques must be employed when performing ovariectomy procedures, or postoperative septic peritonitis may result.[12,24,25,44] This potential complication can be prevented by adhering to proper aseptic technique throughout the procedure and by the administration of perioperative antibiotics. Intraabdominal adhesions can develop after any abdominal procedure; however, the use of proper aseptic technique and ensuring minimal trauma to gastrointestinal serosal surfaces can help prevent formation of adhesions.[14,28]

Ovariectomized mares may continue to display signs of estrus after ovariectomy. It has been reported that 60% of ovariectomized mares will cease estrous behavior following surgery.[19] If previous hormonal therapy has been successful in altering the mare's behavior and/or performance favorably, then bilateral ovariectomy is likely to be successful at meeting the client's expectations. Prospective "jump" mares to be used for stallion collection should stand well during estrus as an intact mare; otherwise, the individual is not likely to be a good candidate for ovariectomy for this purpose.[48]

CONCLUSION

The equine surgeon presented with a mare requiring ovariectomy has numerous approaches available. The specific approach to be used should be chosen carefully to minimize traction on the pedicle yet allow adequate exposure and visualization of the ovarian pedicle for secure hemostasis. Standing techniques include colpotomy (vaginal), flank laparotomy, and laparoscopy. Traditional techniques that require general anesthesia are flank, paramedian, diagonal (oblique) paramedian, and ventral midline celiotomy approaches as well as laparoscopic procedures. Laparoscopic techniques are superior in providing visualization of the ovaries and tension-free ligation for maximal hemostatic security. However, laparoscopy requires specialized instrumentation and surgical knowledge, which may increase operative time, at least initially, until experience is gained. Each technique for equine ovariectomy has associated advantages and disadvantages, and as such, there is no single "proper" technique to be used for every case. When deciding which approach to use, clinicians should be intent on completing the procedure in the most efficient way, while at the same time minimizing patient discomfort and postoperative complications.

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ARTICLE #5 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. *Choose the best answer* to each of the following questions; then mark your answers on the postage-paid envelope inserted in *Compendium.*

1. Mares undergoing ovariectomy by colpotomy should be in what stage of the estrous cycle?
   a. immediately after ovulation
   b. diestrus or anestrus
   c. actively cycling
   d. pregnant
   e. seasonal transition

2. The initial vaginal incision for the colpotomy approach to ovariectomy should be in which position?
   a. directly dorsal
   b. on the medial wall
   c. on the lateral wall
   d. cranioventral or craniodorsal
   e. directly ventral

3. Oversewing the mesovarium following transection is recommended for which of the following reasons?
   a. decrease adhesion formation
   b. prevent eventration
   c. provide analgesia
   d. ensure adequate hemostasis
   e. complete sterilization

4. Which of the following is considered the main advantage to the ventral midline celiotomy approach to ovariectomy?
   a. little interference from abdominal viscera
   b. ease of performing a bilateral procedure
   c. optimal visualization of ovaries on short pedicles
   d. most tension-free ligation
   e. the ability to extend the incision as needed

5. Which of the following is generally not used for hemostasis when performing laparoscopic ovariectomy?
   a. suture ligatures
   b. staples
   c. chain ecraseur
   d. laser energy
   e. electrosurgical instrumentation

6. What is the main theory for why intraoperative hypotension is associated with traditional ovariectomy procedures performed under general anesthesia?
   a. Tension on the mesovarium decreases arterial blood pressure.
   b. Blood loss from the surgical incision causes hypovolemia.
   c. Hypoventilation causes decreased arterial oxygen content.
   d. Anesthetic agents cause decreased systemic blood pressure.
   e. Abdominal viscera interfere with venous return to the heart.

7. Which incision location has been associated with a higher incidence of complications?
   a. ventral midline
   b. paramedian
   c. laparoscopic
   d. flank
   e. diagonal paramedian

8. What percentage of mares stop showing estrous behavior following bilateral ovariectomy?
   a. 100%
   b. 60%
   c. 75%
   d. 50%
   e. 30%

9. Which of the following are advantages to laparoscopic ovariectomy approaches compared with the traditional celiotomy approaches?
   a. smaller incisions for access to the abdomen
   b. visualization of the ovary and mesovarium
   c. tension-free ligation of the mesovarium
   d. shorter, less complicated postoperative recovery
   e. all of the above

10. Which of the following is not a reported complication following equine ovariectomy?
    a. septic peritonitis
    b. eventration
    c. neurologic deficits
    d. hemorrhage
    e. hindlimb pain

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BONE AND MINERAL

The long-term effect of ovariectomy on the quality and quantity of cancellous bone in young macaques

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Abstract

The effect of ovariectomy on the quality and quantity of cancellous bone using the young cynomolgus monkey was evaluated after a 2-year period. The bodies of the second lumbar vertebrae were analyzed for changes in bone mineral quality using density fractionation, chemical analysis, and X-ray diffraction techniques. Changes in bone tissue quality and quantity were evaluated using bone histomorphometry and image analysis. The experimental group ($n = 14$) was made surgically menopausal (bilaterally ovariectomized), compared with intact controls ($n = 16$), and then sacrificed after a 2-year period. There was a non-significant shift in the mineralization profile towards less dense bone in the ovariectomized (OVX) vertebrae compared with controls. Physical characteristics of the bone mineral in terms of crystal size or strain were unaffected by OVX. There was a parallel increase in mineral content with fractions of increasing density, however there was no difference in mineral content or the Ca/P ratio in each fraction between treatment groups. Histomorphometric analysis for structural parameters demonstrated no difference in bone volume between control and OVX groups. There was no significant change in trabecular width in the OVX vertebrae compared with controls. There was a significant increase in both osteoid volume and osteoid surface in the OVX vertebrae ($P < 0.001$). Trabecular architecture as measured by image analysis was unchanged. There was a significant increase in eroded surface in the OVX vertebrae ($P < 0.03$) compared with the controls. In conclusion, young, ovariectomized female cynomolgus macaques do not appear to be a useful animal model for the study of postmenopausal bone loss, however they may be a useful model to evaluate skeletal pathology which might be observed after surgical ovariectomy in young human females.

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1. Introduction

Understanding the mechanism by which the quality and/or quantity of bone is affected by surgical or physiologic cessation of ovarian function has challenged many in recent years. In spite of these efforts, the evidence for altered quality and distribution of mineral in bone is largely restricted in humans, at least, to non-invasive measurement findings, or at best, biopsy from bony sites such as the iliac crest which display inherent functional differences from the vertebrae where changes are so remarkable at the time of the menopause. The multifactorial nature of bone mass regulation in humans makes it difficult to study the relative contribution of a single factor and as a consequence, the changes that occur with ovariectomy are complicated and difficult to qualify. Results of studies in the acute phase post-ovariectomy are well documented across numerous animal models [1–4] as well as in humans [5]

It is generally accepted that in the human menopausal period of approximately 5–7 years duration, there is altered (increased) turnover and up to 2% annual loss of bone which predominantly affects trabecular bone of the vertebrae, with an eventual decline in bone loss occurring at the end of this period [6]. It has been argued that bone behaviour in premenopausal oophorectomized females is not qualitatively different from that occurring in postmenopausal females, and that any differences in bone loss between postmenopausal and ovariectomized premenopausal females are only quantitative [7]. In a study where premenopausal oophorectomized females were reviewed after a follow-up period of 9 years, both peripheral and axial bone mineral density measurements were decreased in a placebo treated group, while estrogen treatment was seen to prevent bone loss at both sites over this same time period [7].

In another study, the estrogen deficiency associated with both premenopausal OVX and the menopause determined that estrogen deprivation rather than the effects of aging was the most important determinant of bone loss at least in the axial compartment under both conditions [8]. Few studies assume an observation period that is of sufficient length to capture any true effort on the organism's behalf at achieving homeostasis over the long-term, as would be experienced over the natural course of events in a lifetime. In addition to the temporal limitations of studies, the species chosen for experimentation typically differs from the human in their response to or creation of a sex steroid hormone climate, making it less likely that the results are comparable with the human condition. Numerous short-term, cross-sectional studies have been conducted in ovariectomized rats, where increased bone turnover is detected within 2 weeks, and bone loss is demonstrable after 8 weeks [2,3,9] as well as in dogs of different ages [10–13]. Regardless of the model used, there is consistent agreement that a loss of bone and an increase in bone turnover occurs soon after cessation of ovarian function, however the long-term manifestations are heterogeneous or remain unexamined.

The early histomorphometric observations of cancellous bone loss following

ovariectomy (OVX) has been characterized in dogs by Faugère et al. [10] which has provided advanced information about the sequence of events following OVX within a 4-month period, however similar studies which might capture further processes beyond the acute phase in non-human primates or other animal models are few and contradictory. There are conflicting results in the literature characterizing the changes occurring in bone after ovariectomy using the non-human primate model. In this regard, findings by Miller et al. [14] revealed a significant loss (42%) in trabecular bone volume (TBV) in OVX cynomolgus monkeys after 22 months, while Jayo et al. [15] did not observe these changes in a similar model and over a period of similar duration. In baboons, increased bone turnover and bone loss were noted as early as 6 months [1]. A study by Thompson et al. [16] employed OVX adult female baboons and their data showed no actual difference in BMC of the radius or femoral neck regions between OVX and control groups after a 1-year period. Shaw et al. [17] found no significant difference in the TBV in the vertebrae of young OVX dogs compared with controls after 12 months. A recent 6-month study by Shen et al. [18] identified a lack of sizable responses in histomorphometric, bone mass and biochemical parameters which may serve to limit the utility of dogs for the study of cancellous bone loss in ovarian-dysfunction osteoporosis.

The purpose of this paper was to specifically examine the nature of both the quality and quantity of cancellous bone after ovariectomy in a young non-human primate. Techniques to study the quality of bone mineral, such as density fractionation (to obtain a profile of mineral of different maturities) can help to capture events involving turnover of bone [19]. Quantity of bone can be determined and its respective constituents further qualified using histomorphometric techniques, demarcating bone tissue remodelling activity. Trabecular architecture can be determined using image analysis of histological sections. Non-human primates have been used extensively in studies on reproductive biology, in part, because in the female the menstrual cycles are similar in length and control to those in human females. The dynamics of androgen and estrogen metabolism and production in the cynomolgus monkey show a pattern that is, in general, similar to those in humans, with minor variations in the interconversion of androgens and estrogen [20]. Additionally, 1 macaque year is equivalent to 3 human years in biological terms, making a 2-year study potentially useful for simulating conditions affecting the human skeleton involving a length of time such as the 5–7 year period of menopause. The goal of this study was to identify whether a 2-year period post-OVX allows sufficient time for bones to reach a new equilibrium. A young OVX adult primate model observed over a 2-year period, as well as specific and thorough examination using multiple techniques to determine quality and quantity of the cancellous bone in the vertebrae were employed to identify the events during and after high-turnover bone loss known to occur after ovariectomy.

2. Materials and methods

2.1. Animals

A group of 30 feral young adult female cynomolgus macaques (*M. fascicularis*) (mean age, 9.5 years as estimated by dentition: age range, 7–12 years) imported from

Jakarta, Indonesia were used in this study. All the animal experiments were conducted at the primate colony in the Department of Comparative Medicine, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. The animals were randomly selected from a group of monkeys involved in a large 2-year experiment designed to study the inhibition of diet-induced coronary artery atherosclerosis by different hormonal therapies [21]. Fourteen animals were made surgically menopausal (bilateral ovariectomy) with sixteen left intact as controls, observed and cared for as outlined by Carlson et al. [22], and sacrificed after a 24-month period. At sacrifice, the second lumbar vertebrae were removed and cleaned of soft tissues, stored at −70°C, and sent to our laboratory for structural histomorphometric and chemical analysis of cancellous bone.

2.2. Bone histology

A 2-mm mid-coronal section was cut using the Isomet saw through the central body of the second lumbar vertebrate from each monkey. Sections were fixed in 10% buffered formalin, dehydrated through graded alcohols to acetone, infiltrated in increasing strengths of spurr/acetone solutions, and embedded undecalcified in polymerized plastic spurr blocks. Also, 5-μm thick coronal sections of the vertebrae were cut using the heavy duty Jung K microtome with a carbide type knife, oven-dried for 12 h, then stained undeplasticized using a modified Masson-Goldner trichrome technique which permits optimal discrimination between mineralized and non-mineralized (osteoid) bone [23].

Structural parameters of trabecular bone were analyzed by histomorphometric methods which complied with the nomenclature, and were calculated according to the ASBMR histomorphometric nomenclature committee [24]. Sections were viewed at 150 × magnification using a 100-point integrating eye piece and a semi-automatic image analyzer (Leitz ASM, Wetzlar, FR). The sections were examined quantitatively using an IBM-PC microcomputer and Bioquant image analysis software.

The structural parameters were measured by static analysis as dynamic labeling information was not available. These morphometric parameters included cancellous bone volume (BV/TV) (percentage of endosteal bone and marrow tissue occupied by osteoid and mineralized bone); the mineralized trabecular bone volume (percentage of endosteal bone and marrow tissue occupied by mineralized bone alone); the osteoid volume (OV/BV) (percentage of endosteal bone and marrow tissue occupied by osteoid); the osteoid surface (OS/BS) (percentage of total endosteal surface covered by an osteoid seam); the endosteal mean osteoid seam width (O.Wi.) was determined indirectly by surface/area measurements [25]; the eroded surface (ES) (percentage of mineralized endosteal bone surface displaying evidence of erosion); and trabecular width (Tb. Wi.) was determined indirectly by using the area and length method (Ar/0.5 Pm) [26]. In addition, we calculated trabecular separation and trabecular number using the formulae of Parfitt and co-workers [27] and Birkenhager-Frenkel et al. [28]:

Trabecular separation: Tb. Sp(μm) = 1000/Tb.N. − Tb. Wi.

Trabecular No. (No./mm) = % Trabecular Volume/Trabecular Width × 10

An average of 24 fields of pure cancellous bone was analyzed per sample at 150 × magnification, allowing an average area of 20×10^6 μm^2 to be examined per specimen. Each specimen was measured 5 mm down from the superior and lateral surfaces of the vertebral body, taking care to avoid the mid-central portion of the vertebrae known to be supplied by the nutrient arteries.

2.3. Image Analysis

Photographic images of the bone sections used for histomorphometry were scanned to obtain TIFF images files accessed by a Quantimet 570 (Leica) image processing and analysis system. Mathematical morphology operations [29] together with standard image processing tools were used to analyze the images. After thresholding the grey level images, we obtain binary images which were then skeletonized in order to obtain connectivity and orientation parameters [30]. The skeleton of the trabeculae obtained by the thinning transformation was pruned to remove parasitic branches. Using the multiple point function, the connection pixels between vertical and horizontal trabeculae were found. The end-point function was used to mark the free ends of the skeleton on the binary images. The images containing triple points and end points were used to obtain the connectivity parameters: average number of node/trabecula [30] and the parameters described by Mellish et al. [31] and Parisien et al. [32] in their trabecular strut analysis. The parameters used were: the number of nodes (No. Nd/mm^2), the number of terminus (No. Tm/mm^2), the total strut length (TSL/mm^2), and the node-to-node (Nd.Nd), node-to-terminus (Nd.Tm) and terminus-to-terminus (Tm.Tm) strut lengths. Finally, a rose of direction was obtained for each binary image and the ratio between the trabeculae in the 90° direction (vertical) and these in the 0° direction (horizontal) was called the anisotropy ratio [30].

2.4. Bone mineral studies

Density fractionation. The remainder of the vertebrae were crushed, tris-washed to rid them of bone marrow, lyophilized and then defatted in 2:1 v/v chloroform/methanol. The bone pieces were then pulverized in a percussion mill (Spex Freezer Mill, Meteuchen, NJ), cooled in liquid nitrogen, and sieved in a sonic sifter to separate bone particles less than 20 μm in size. The bone powder was then chemically 'fractionated' ($<1.8->2.2$ g/ml) in a bromoform-toluene mixture by the stepwise centrifugation method of Grynpas and Hunter [19]. Approximately 200 mg of sieved bone was added to a polyallomer centrifuge tube containing 35 ml of a 2.0 g/ml density solution (calibrated with sink floats). The tubes were sonicated to achieve a homogeneous suspension of the powder which subsequently was centrifuged at 10 000 RPM for 30 min. The density of the supernatant was adjusted to 1.9 g/ml via the addition of toluene, and the modified solution was re-centrifuged. In a similar way, each precipitate obtained from a solution of progressively decreasing density (at 0.1 g/ml steps) was collected. For a range of mineral densities greater than 2.0 g/ml, the precipitate obtained from the initial 2.0 g/ml density solution was resuspended in a solution with a density of 2.2 g/ml. Similarly, successive centrifugation of the precipitate at progressively decreasing densities (0.1 g/ml steps) determined the higher density fractions. Removal of organic solvents was achieved by a 'rinse'

centrifugation of the series of specific gravity fractions in 100% ethyl alcohol. The samples were then dried in a dessicator at room temperature. The contribution of each fraction relative to the original weight of unfractionated bone was calculated to determine a mineralization profile for each group.

X-Ray diffraction. Samples of bone powder from the unfractionated samples were analyzed with a Rigaku diffractometer using Cu-Kα radiation and a highly crystalline mineral fluorapatite as a standard. The values of $B½$ (002) and (130), the widths at 50% the maximum height of the hydroxyapatite reflections, were measured using a step-scanning procedure with 0.4° 2θ per step and 100 s of counting. Because the instrumental broadening was small compared with sample peak breadths, the measured half-widths were corrected for instrumental broadening by subtracting the square of $B(002)$ and $B(130)$ for the standard (fluorapatite) from the square of the bone value and taking the square root of the difference. D values, which are related to the crystal size\strain in the long dimension (002) and the cross section (130) of the apatite crystal, were calculated from the corrected $\beta½(002)$ and $\beta½(130)$ values using the Sherrer equation [33]:

$$D = \frac{K\lambda \text{ radian}}{\beta½ \cos \theta}$$

where λ is the X-ray wavelength, $\beta½$ the breadth at half the height of the 002 and 130 peaks, and θ the diffraction angle. K is a constant varying with crystal habit and chosen as 0.9 for the elongated crystallites of bone. Each measurement was repeated three times and the results averaged.

Chemical studies. Samples of bone powder (10 mg) from the three largest fractions as determined by the density fractionation technique were washed in a 4:1 v/v nitric/perchloric acid mixture in order to digest the organic matrix. A blank containing only acid mixture was used as a control for the procedure. Two external standards were used: 10 mg of phosphate rock (NBS Standard #1206) and 10 mg of animal bone from the IAEA (International Atomic Energy Association). The samples, contained in covered teflon beakers, were placed on low heat and then brought to a boil for approximately 1.5 h. The samples were then uncovered and allowed to evaporate (about 0.5 h) to a small volume (1 ml). The digested sample was then brought to a volume of 25 ml with double deionized water, brought to a pH range between 2.5 and 2.9 using 1 and 10 M NaOH or HCl stock solutions accordingly and transferred to a dust-free container. From each sample, a single 2-ml aspirate was drawn and analysed for calcium (Ca) and phosphorus (P) using the Beckman Synchron CX4 chemistry analyser [34]. Quantitation of the elements calcium (Ca) and phosphorus (P) using Arsenazo II dye and phosphomolybdate as reagents, respectively, was determined by timed end-point reactions and then expressed in mM. Final concentrations were expressed as %Ca and %P of the dry weight of bone. Additionally, the Ca/P molar ratio and the mineral content (Ca + PO_4) were calculated for each of the three largest fractions recovered from the density fractionation technique.

Data analysis. Results are expressed as the mean ± S.D., with the exception of the mineralization profiles and image analysis which are presented as means ± S.E.M. Comparison between means was performed using the Student's two-tailed t-test,

with a level of acceptance of $P < 0.05$. Most computations were performed using the PowerStat software package (PowerStat version 1.04, Analytical Engineering Corporation, Toronto, Canada).

3. Results

3.1. Bone histomorphometry

Table 1A gives the results of the measured structural parameters as evaluated by static morphometric analysis. There was no difference in trabecular bone volume (TBV) in the young ovariectomized non-human primate compared with controls. There was a significant increase in osteoid volume (OV/BV) compared with the controls ($P < 0.001$). In addition to an increased volume of osteoid, an increase in osteoid-covered endosteal surface (OS/BS) in the ovariectomized group was noted ($P < 0.001$). There was a significant increase in eroded surface (ES/BS) ($P < 0.03$) in the treatment group compared with controls. The control group demonstrated a normal eroded surface value of $\approx 4.76\%$, indicating a relatively active remodelling surface in the young cynomolgus monkey. Osteoid width (O.Wi.) indices were augmented, but remained non-significantly different in the experimental group compared with controls which was most likely a reflection of the increase in both osteoid surface and volume values. Interestingly, the contribution to osteoid volume in the OVX group was in part achieved by the occupation of large erosion pits by osteoid. There was no significant decrease in trabecular width (Tb. Wi.) in the OVX group compared with controls. There was no significant difference between trabecular separation and number values between the OVX group and controls.

3.2. Image analysis

Table 1B gives the results of the trabecular connectivity and orientation as evaluated by image analysis. There was no significant difference in the average number of nodes per trabecula or in the connectedness of the trabecular network as shown by strut analysis: node number, terminus member, total strut length, terminus-to-terminus strut length, terminus-to-node strut length and node-to-node strut length. Also, when we examined the ratio of vertical trabeculae to horizontal trabeculae (anisotropy ratio) we found no difference between the control and the ovariectomized group.

3.3. Bone mineral studies

Using a logit function [ln(proportion < 1.9 mg/ml per proportion ≥ 1.9 mg/ml)] we were able to compare shifts in the distribution of mineralization between the experimental and control vertebrae. The mineralization profile of the vertebrae of the OVX monkeys after the 2-year period demonstrated a shift to lower density fractions, which indicates a lower mineralization pattern compared with controls; however, this shift did not reach significance (Fig. 1). The X-ray diffraction data demonstrate no significant changes in crystal size/strain in the mineral of the vertebrae of the experimental group compared to controls (Table 2). There was a parallel increase in mineral content with fractions of increasing density in both groups, however, there was no significant difference in mineral content or in the Ca/P ratio in each fraction between treatment groups (Table 3).

Table 1A
Histomorphometric parameters of cancellous bone in control and ovariectomized monkeys: (values expressed as mean ± S.D.)

Group	Trabecular bone volume (TBV)	Mineralized trabecular bone volume (MTBV)	Osteoid volume (OV)	Trab. Width (Tb. Wi.) (μm)	Trab. Sep. (Tb. Sp.) (μm)	Trab. No. (Tb. No.) (No./mm)	Osteoid Surface (OS) (μm)	Eroded surface (ES) (μm)	Osteoid Wi. (O. Wi.) (μm)
	% Bone tissue						% Endosteal surface		
Control $n = 16$	23.8 ± 5.0	23.1 ± 4.6	1.5 ± 1.4	125 ± 30	412 ± 64	1.9 ± 0.3	9.4 ± 7.7	4.8 ± 2.6	8.8 ± 3.8
OVX $n = 14$	22.9 ± 4.8	20.9 ± 5.2	4.3 ± 2.5	123 ± 19	412 ± 70	1.9 ± 0.5	24.4 ± 12.7	7.3 ± 3.0	11.5 ± 5.0
P value	—	—	$P < 0.001$	—	—	—	$P < 0.001$	$P < 0.03$	—

Table 1B
Image analysis of cancellous bone in control and ovariectomized monkeys (values expressed as mean ± S.E.M.)

Group	Control $n = 14$	OVX $n = 14$
Average no. node/trabecula	1.12 ± 0.22	1.00 ± 0.22
No. Nd (/mm^2)	1.60 ± 0.20	1.51 ± 0.16
No. Tm (/mm^2)	4.09 ± 0.32	3.95 ± 0.20
TSL (mm^2)	2.18 ± 0.13	2.21 ± 0.11
Anisotropy ratio	1.20 ± 0.02	1.29 ± 0.03
Nd.Nd (mm/mm^2)	0.71 ± 0.10	0.63 ± 0.08
Nd.Tm (mm/mm^2)	1.05 ± 0.08	1.18 ± 0.07
Tm.Tm (mm/mm^2)	0.42 ± 0.06	0.41 ± 0.03

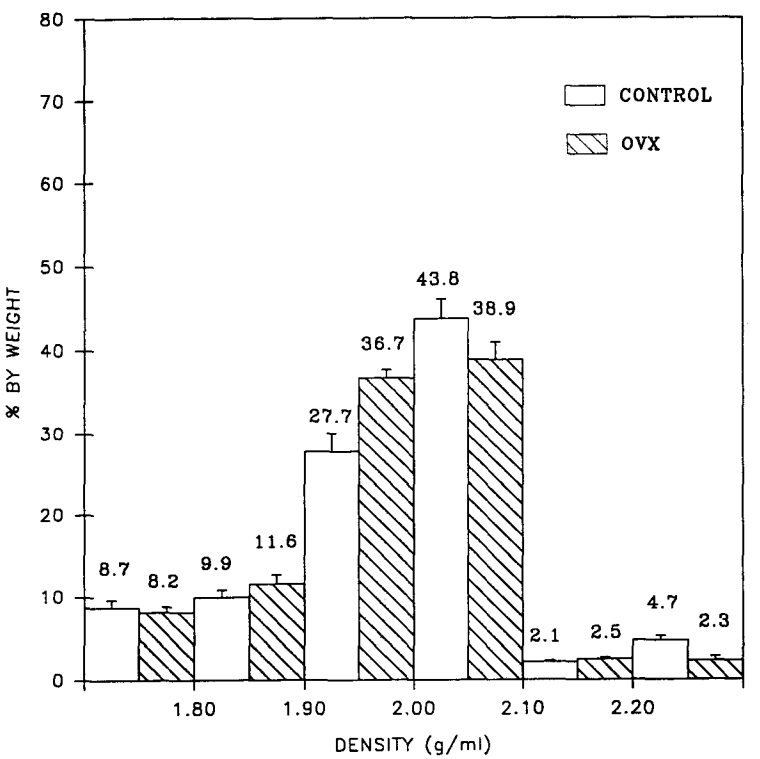

Fig. 1. The distribution of mineralization between the experimental and control groups can be observed using a logit function [ln(proportion < 1.9 mg/ml/ proportion ≥ 1.9 mg/ml)]. The mineralization profile of the vertebrae of the OVX monkeys after the 2-year period demonstrated a non-significant shift to lower density fractions which indicates a lower mineralization pattern compared with controls.

Table 2
Long-term effects on cancellous bone post-ovariectomy: X-ray diffraction (values expressed as mean ± S.E.M.)

Control				Experimental			
D-002		D-130		D-002		D-130	
β½	Å	β½	Å	β½	Å	β½	Å
0.42 ± 0.02	196 ± 12	1.28 ± 0.02	66 ± 1	0.45 ± 0.02	182 ± 10	1.31 ± 0.04	65 ± 2

4. Discussion

Our findings indicate that in the young non-human primate model (*M. fascicularis*) minimal changes in mineralization are evident 2 years after OVX in cancellous (trabecular) bone. There is no difference in trabecular bone volume of the OVX model versus controls, however, there are indices of persistent and increased formation and resorption activity in trabecular bone 2 years post-OVX. There have been few studies examining the long-term effects of ovarian dysfunction on the quality and quantity of bone mineral/tissue in non-human primates [1,14–16]. There is even less representation in the literature defining these events using a young non-human primate model, which would have the added advantage of avoiding confounding age-related variables known to affect the skeleton.

There is some disagreement about the effects of ovariectomy (OVX) or ovariohysterectomy (OVH) on cancellous bone across species of different ages and over different lengths of time. In the dog, a decreased [10,11] or unchanged [13,17,18] volume defect has been shown. It is of interest that those reporting no change in unqualified total bone volume are the long-term studies [17,18], while rapid loss of cancellous bone volume with evidence of a later initiation of formation indices is a reported feature of the short-term studies [10]. The observations of Danucci et al. [13] of increased osteoid surface, resorption surface, and bone formation rate after

Table 3
Chemistry: long-term effects on cancellous bone post-ovariectomy

Fraction	%Ca	%P	Ca/P	Ca% + PO_4%
A Control vertebrae				
1.8–1.9	18.7 ± 0.8	8.1 ± 0.4	1.8	43.4 ± 1.9
1.9–2.0	19.8 ± 1.7	8.4 ± 0.7	1.8	46.5 ± 2.2
2.0–2.1	22.4 ± 1.2	9.4 ± 0.6	1.8	51.3 ± 2.9
B Experimental vertebrae				
1.8–1.9	17.8 ± 0.8	8.2 ± 0.4	1.7	42.8 ± 1.7
1.9–2.0	19.6 ± 0.6	8.8 ± 0.2	1.7	46.5 ± 1.0
2.0–2.1	21.2 ± 0.9	9.6 ± 0.8	1.7	50.7 ± 1.9

a 6-month period post-OVX in the dog is in agreement with ongoing high remodelling activity. In concert with the failure to observe a significant decrease in cancellous bone volume, to which osteoid was reported to contribute at least in part as a surface phenomenon, may indicate a certain recovery effect has been initiated after acute loss of surface cancellous bone in the dog model.

Limited studies of cessation of ovarian function and the long-term effects on the skeleton have employed non-human primate species [1,14,15]. A non-human primate model (cynomolgus monkey) offers the advantage of being physiologically similar to the human especially in terms of reproductive cycles and endocrine profiles [20] making continued efforts at analyzing the quality and quantity of bone in this model worthwhile. A significantly decreased BMD was observed in the lumbar spine in a group of young ovariectomized adult monkeys, however, there was no report of increased incidence of crush fractures in this population [35,36].

In a study of the effects on bone of surgical menopause and hormone replacement therapy in adult cynomolgus monkeys, there was in fact no difference in trabecular bone volume between the monkeys that were oophorectomized and those that received either estrogen-replacement therapy alone, or estrogen replacement plus progesterone after 25 months. This was found despite a reported significant decrease in BMD between the oophorectomized and the estrogen-replacement therapy plus progesterone treated group [15]. The lack of a difference in cancellous bone volume between oophorectomized and the control group of monkeys is similar to that which was observed in the present study, and in fact does not support the use of this young cynomolgus monkey as a model for postmenopausal bone loss. Of great interest and support to our findings in the present study were the results of the effects of alendronate on the trabecular bone (iliac crest) in the OVX baboon [16]. After a 12-month period, a significant increase in osteoid surface and volume measurements was observed as early as 6 months in the OVX model compared with the controls, despite a significant decrease in mineralized trabecular bone volume compared with controls. An increase in resorption parameters (osteoclast number) was significantly elevated at 6 and 12 months, coincidental with the significantly increased values of formation indices (osteoid volume and surface measurements, as well as serum alkaline phosphatase levels). Bone mineral content of the radius and femoral neck regions as measured by DPA in this study reported a significant difference in BMC in the femoral neck region between the control and OVX group when compared relative to time 0, however, there is no significant difference between the effects of OVX and controls at the end of the 12-month period.

An interpretation made retrospectively, could be that the presence of decreased TBV followed shortly by evidence of bone manufacturing indices of increased osteoid volume and surface measurements suggest attempted recovery of some kind, providing mineralization ensues. If indeed an OB insufficiency is the explanatory variable for the decreased bone volume seen relatively soon after cessation of ovarian function, it would seem that this phenomenon is arrested at least in the young non-human primate model by some means thereafter (within the 2-year period, the equivalent of 6 human years). The fact that recovery of bone volume was achieved in this study through/by both osteoid volume and surface parameters is a novel finding, having been reported only as an initial effort in the short-term study by

Faugère et al. [10] and in the long-term by Thompson et al. [16]. Any kind of impressive bone loss must have occurred (and it must have, based on the amount of osteoid) well before the 2-year post-OVX period was terminated.

At some time during this time course a return to normal bone volume values was established and future sequential studies over a 2-year period are required to capture the temporal profile of these events, as are longer ones to observe whether this new bone mineralizes normally. Because of the significant amount of osteoid volume and surface characteristics in the OVX group indicating ongoing formation, a significantly increased eroded surface, and essentially intact mineral quality, there is a powerful suggestion of ongoing/persistent high turnover rate (HTR) changes rather than a mineralization deficit occurring in the experimental group even after a 2-year period. The image analysis results show that this remodelling did not affect the cancellous bone architecture as shown by strut analysis [31,32] or the orientation of the trabecula as shown by the anisotropy ratio [30]. Alternatively, because the monkeys used (human equivalent 20–30 years) endured surgical menopause, a climate was created that is not dissimilar to that created by amenorrhea or other ovulatory disturbances, where tolerance of decreased bone mass exists [37].

Of additional interest is that the mechanical properties measured in terms of elastic modulus and maximum stress in compression were not significantly different in cores of cancellous bone obtained from the femoral head in the same group of monkeys. Apparent density as measured by water displacement was not significantly different between OVX and control groups (Kasra, pers. commun.). It is not out of order to hypothesize that in the young female adult there may be other physiological compensatory mechanisms which may be more at play in younger organisms than in older ones, making this inherently a poor model for postmenopausal osteoporosis. This model may be an ideal one to study the long-term effects on the quality of cancellous bone of premenopausal females who have undergone OVX or suffer from acute ovulatory dysfunction, a population in whom only a quantitative decline in BMD affecting both cancellous [7,8] and cortical compartments [7] has been previously reported.

The bone mineral studies support lower mineralization occurring in the ovariectomized model vertebrae than controls reflected by a mineralization profile shifted to less dense mineral. Based on past findings using the density fractionation technique, bone that is less mineralized usually is less mature and has been formed more recently in comparison with bone that is more highly mineralized [19]. Our findings identified trabecular bone mineral that was slightly less dense in the experimental group compared with controls, and based on these principles most likely represented newly formed bone indicating recent turnover activity. The X-ray diffraction and chemical data determined that the physical characteristics of the mineral in the OVX vertebrae did not differ from the control group.

The results of the present study indicate that TBV is not different between experimental and control groups due to the contribution of substantial osteoid to this volume in the OVX group. This suggests that some strong effort at recovery has ensued beyond the acute stages post-OVX. This observation becomes particularly intriguing when a study involving similar bone mineral studies and histomorphometry addressing the effects of ovariectomy in the same monkey population identified the

cortical bone (femora) as being victim to this treatment in terms of having less dense bone and demonstrating active high turnover activity characteristics [38]. That bone tissue is targeted differentially by the same treatment, in the same population, and over the same course of time may bring us closer to understanding the pathophysiology of bone loss in trabecular bone compared with cortical bone, which to date has been classified as Type 1 and Type 2 osteoporosis.

This study provides novel quantitative and qualitative information about trabecular bone tissue and the status of the bone remodelling system as affected by a lack of ovarian derived female sex steroid hormones over a 2-year period (equivalent to 6 human years) in a young non-human primate. Characterization of the physical-chemical qualities of the mineral phase of trabecular bone, in addition to detailed histomorphometric findings shows that the trabecular bone in the vertebrae of the young, non-human primate is susceptible to the acute effects of ovariectomy but has demonstrated the potential to return to values similar to controls in terms of bone mineral quality and quantity.

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Normal masticatory function partially protects the rat mandibular bone from estrogen-deficiency induced osteoporosis

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ABSTRACT

Background/aim: In a previous study we showed that mandibular alveolar (trabecular) bone appears to be less sensitive to estrogen deficiency than the proximal tibia spongiosa. We hypothesized that the mechanical loading of the alveolar process during mastication may protect the alveolar bone from the detrimental effects observed in other skeletal sites. To test this hypothesis we compared the effect of ovariectomy on the mandibular alveolar bone and the proximal tibia spongiosa of rats fed either a normal (hard) or a soft diet.
Methods: Forty six-month-old female Sprague–Dawley rats underwent trans-abdominal ovariectomy (OVX) or sham operation (SHAM). Half of the animals received their food in the usual form of pellets (hard consistency), while the other half received a soft, porridge-like, isocaloric diet of identical composition (soft consistency). Micro-computed tomographic histomorphometry was used to evaluate the trabecular micro-architecture. A two-factor analysis of variance was used to test for effects and interaction of ovariectomy and/or soft diet.
Results: OVX had a significantly negative effect on the proximal tibia spongiosa (all parameters under study except trabecular thickness; $p<0.001$) and on the mandibular alveolar bone (trabecular number and spacing; $p<0.05$). Soft diet led to a further decrease of mandibular BV/TV ($p<0.01$), trabecular thickness ($p<0.05$) and number ($p<0.05$), as well as increase of separation ($p<0.001$). A significant interaction was observed between OVX and soft diet concerning the mandibular BV/TV, as well as trabecular thickness and spacing ($p<0.05$).
Conclusion: Normal (hard) diet limited significantly the negative effects of estrogen deficiency on mandibular alveolar bone micro-architecture four months after ovariectomy.
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1. Introduction

Ovariectomy (OVX) in the rat results in bone loss and micro-architecture deterioration at various skeletal sites containing trabecular bone (Ammann et al., 1996) and is the most commonly used animal model of post-menopausal osteoporosis (for a review see Turner et al., 2001). As far as mandibular bone is concerned, there are some studies that have demonstrated a negative effect of OVX on the mandibular body (Elovic et al., 1995), condyle (Tanaka et al., 2000; Sakakura et al., 2001), and the alveolar process (bone surrounding the teeth) (Hara et al., 2001; Tanaka et al., 2003). However, many studies have failed to detect any effect or concluded that the effect of OVX on the mandibular condyle (Fujita et al., 2001; Yamashiro and Takano-Yamamoto, 1998; Tanaka et al., 1999) or the alveolar process (Johnson et al., 2002; Jiang et al., 2003) was significantly weaker in comparison to the long bones. Rat strain and site of measurement might partially explain these discrepancies (DeMoss and Wright, 1998).

Mastication is the first step in the process of digestion and is meant to prepare the food for swallowing and further processing in the digestive system (Van der Bilt et al., 2006). During mastication the bite force is regulated by central control mechanisms and sensory feedback from peripheral mechanoreceptors according to the size and the texture of food (Woda et al., 2006; Kohyama et al., 2003; Van der Bilt, 2010) in order to produce a smooth and efficient break down of the bolus before swallowing (Peyron et al., 2004; Mishellany et al., 2006). Food hardness is sensed during mastication and affects the masticatory force (Kohyama et al., 2004), the jaw muscle activity (Agrawal et al., 1998), and the

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mandibular functional movements (Peyron et al., 1997). Animal experimental studies have shown that changes of the masticatory muscle function induced by soft diet lead to a reduction of masticatory muscle strength and functional capacity (Kiliaridis and Shyu, 1988). The masticatory muscles have broadly distributed origins and insertions on the skull and mandible, while most muscle fibers attach to internal aponeuroses rather than to the bones directly (Herring, 2007). Masticatory hypofunction has been shown to result in changes of the mandibular morphology in both growing (Mavropoulos et al., 2004a, 2004b) and adult (Mavropoulos et al., 2004a, 2004b) rats.

There is significant evidence that the association between systemic and oral bone loss is very weak (Kribbs et al., 1989; Krall et al., 1996; Streckfus et al., 1997; Jeffcoat et al., 2000; Bollen et al., 2004; Wactawski-Wende et al., 2005; Ejiri et al., 2008). In a previous study we demonstrated that mandibular alveolar (trabecular) bone is less sensitive than the proximal tibia to estrogen-deficiency induced osteoporosis in the rat (Mavropoulos et al., 2007). We hypothesized that this might be due to the mechanical loading of the mandibular bone during mastication, which has been shown to influence the mandibular bone density and micro-architecture in the growing (Mavropoulos et al., 2004) and the adult rat (Mavropoulos et al., 2010). To test this hypothesis we compared the effect of estrogen depletion (ovariectomy) and reduced masticatory loading (soft diet), as well as their interaction, on the mandibular alveolar bone and the proximal tibia spongiosa in adult rats.

2. Materials and methods

2.1. Animals and protocol

Forty six-month-old female Sprague–Dawley rats (Novartis, Basel, Switzerland) underwent trans-abdominal ovariectomy (OVX) or sham operation (SHAM), and were pair-fed isocaloric hard or soft diet (Novartis Nutrition, Bern, Switzerland). For half of the animals the food was given in the usual form of pellets (hard consistency), while for the other half it had a soft, porridge-like, consistency. The experiment lasted 16 weeks. Body weight was recorded every week. Blood was collected at the tip of the tail and 12 hours urine collections were obtained at the end of the study in metabolic cages. The study received the approval of the Animal Ethics Committee of the Faculty of Medicine of the University of Geneva.

2.2. Microtomographic histomorphometry by microcomputed tomography (μCT)

Micro-computed tomographic histomorphometry of the secondary spongiosa of the proximal tibia and the mandibular alveolar process was performed with a high-resolution μCT system (μCT 40; Scanco Medical AG, Bassersdorf, Switzerland) as previously described (Muller et al., 1996; Mavropoulos et al., 2004a, 2004b). Three-dimensional images of each proximal tibia and hemi-mandible were acquired with a voxel size (nominal resolution) of 16 μm in all spatial directions. Samples were secured in a cylindrical sample holder in air. Resolution was set to medium (500 projections with 1024 samples each). The trabecular and cortical parts of the tibia were separated with semi-automatically drawn contours. For the rat hemi-mandible (Fig. 1A), the volume of interest (VOI) was drawn on a slice-based method starting from the first slice containing the crown of the first molar and moving dorsally one hundred slices in the area of the alveolar process between the roots of the molars and the root of the incisor. Trabecular bone was carefully contoured on the first and the last slice, while the intermediate slices were first interpolated by morphing (Fig. 1B). Each slice was subsequently visually inspected and the contour was modified where necessary.

Micro-tomographic histomorphometric indices were calculated directly from the binarized VOI. Total volume (TV) was the volume of the whole sample examined. Bone volume (BV) and surface (BS) were calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Mean trabecular thickness (Tb.Th.) was determined from the local thickness at each voxel representing bone. Trabecular number (Tb.N.) was calculated by taking the inverse of the mean distance between the middle axes of the structure. The complete acquiring procedure was repeated a further four times with four mandibles chosen at random to calculate the coefficient of variation (CV; range, 1.57–2.51% for Tb.Th and BV/TV, respectively).

2.3. Biochemical parameters

Plasma osteocalcin and IGF-I were measured using radioimmunoassay with reagents from Biomedical Technologies (Stoughton, MA, USA) for the former and with a kit from Nichols Institute (San Juan Capistrano, CA, USA) after extraction by acid-ethanol and cryoprecipitation for the latter. Total urinary deoxypyridinoline was determined after acid hydrolysis using a kit from Metra-Biosystems (Mountain View, CA, USA).

2.4. Statistical analysis

All data are presented as mean and standard error of the mean (in parentheses). A two-way analysis of variance was employed to investigate the main effects of both experimental factors (OVX and soft diet) as well as their interaction. All statistical analyses were performed using the SPSS statistical package (SPSS 19.0.0, SPSS Inc., IBM Corp., USA). A result was considered as statistically significant at $p < 0.05$.

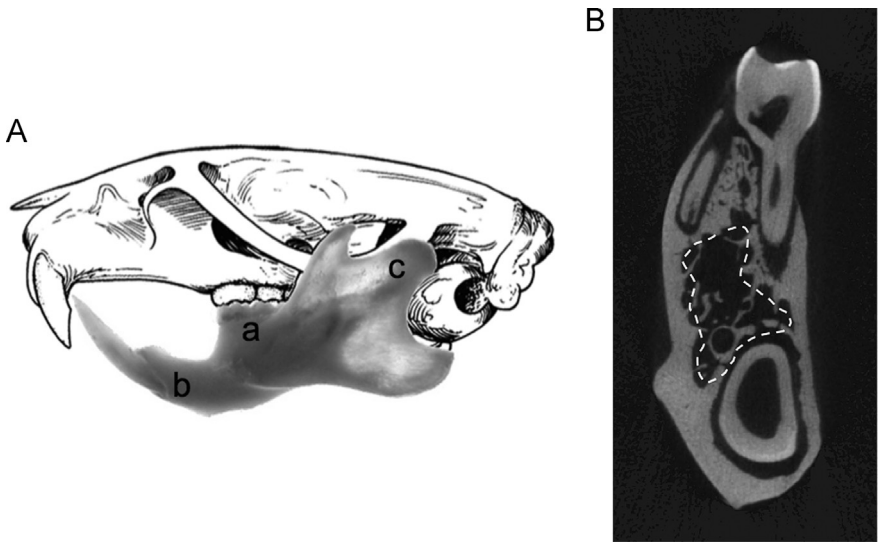

Fig. 1. (A) Lateral photograph of a rat mandible superimposed on a drawing of the skull (a) the alveolar process that houses the molars and is the equivalent of the alveolar process in humans; (b) not to be confused with the incisor alveolar process, which does not exist in the human mandible; and (c) the condylar process, which articulates with the skull, and (B) micro-CT scan (the dashed line represents the volume of interest (VOI) as it appears in one layer).

3. Results

3.1. Body weight and biochemical parameters (Table 1)

At the end of the experiment no weight difference was observed between the animals fed the normal (hard) and those fed the soft diet, indicating a similar nutrient absorption with both diets. OVX was associated with higher weight ($p=0.001$) at the end of the experiment despite a strict pair feeding, as expected.

Ovariectomy led to increased levels of serum IGF-I ($p<0.001$), serum osteocalcin ($p<0.01$), and urinary deoxypyridinoline ($p<0.001$). Soft diet was associated with lower levels of serum IGF-I ($p<0.05$) and serum osteocalcin ($p<0.01$). Plasma IGF-I at sacrifice was significantly correlated to the weight of the animals ($R^2=0.57$; $p<0.001$).

3.2. Micro-tomographic histomorphometry of the proximal tibia spongiosa (Table 2)

Ovariectomy led to a deterioration of all histomorphometric parameters of the proximal tibia spongiosa under study ($p<0.001$) with the exception of trabecular thickness that increased ($p<0.001$). Soft diet did not have any effect on the proximal tibia spongiosa.

Table 1
Main effects and interactions of ovariectomy and soft diet on body weight and biochemical parameters.

	Normal (hard) diet		Soft diet		*Two-way anova*		
	SHAM	OVX	SHAM	OVX	*Effects*		*Interaction*
					OVX	*SD*	*OVX × SD*
Body weight (g)	294.4 (19.4)	328.3 (16.2)	295.0 (29.4)	334.2 (21.9)	***⇧	ns	ns
IGF-1 (ng/ml)	745.1 (179.4)	1074.6 (116.0)	581.0 (129.6)	974.6 (194.2)	***⇧	*⇩	ns
Serum osteocalcin (ng/ml)	44.2 (12.4)	56.9 (15.6)	34.0 (8.3)	46.2 (6.3)	**⇧	**⇩	ns
Urinary deoxypyridinoline (nmol/24 h)	2.7 (1.2)	4.6 (2.3)	2.6 (1.2)	6.1 (1.2)	***⇧	ns	ns

OVX, ovariectomy instead of sham operation for the controls; ***SD***, soft diet instead of the normal (hard) diet in the form of pellets for the controls.
BV/TV, bone volume fraction; ***Tb.Th.***, trabecular thickness; ***Tb.N.***, trabecular number; and ***Tb.Sp.***, trabecular separation.
The arrows denote the direction of change.

* Significant effect at $p<0.05$.
** Significant effect at $p<0.01$.
*** Significant effect at $p<0.001$.

Table 2
Main effects and interaction of ovariectomy and soft diet on proximal tibia micro-computed histomorphometric parameters.

	Normal (hard) diet		Soft diet		*Two-way anova*		
	SHAM	OVX	SHAM	OVX	*Effects*		*Interaction*
					OVX	*SD*	*OVX × SD*
BV/TV (%)	0.16 (0.03)	0.03 (0.02)	0.17 (0.06)	0.02 (0.01)	***⇩	ns	ns
Connectivity density	68.1 (32.7)	4.1 (4.0)	80.6 (40.1)	2.6 (1.9)	***⇩	ns	ns
Tb.Th. (μm)	0.068 (0.004)	0.075 (0.014)	0.072 (0.010)	0.073 (0.006)	***⇧	ns	ns
Tb.N. (1/mm)	3.68 (0.97)	1.43 (0.35)	4.02 (0.93)	1.33 (0.20)	***⇩	ns	ns
Tb.Sp. (μm)	0.29 (0.14)	0.73 (0.17)	0.25 (0.10)	0.78 (0.14)	***⇧	ns	ns

OVX, ovariectomy instead of sham operation for the controls and ***SD***, soft diet instead of the normal (hard) diet in the form of pellets for the controls.
BV/TV, bone volume fraction; ***Tb.Th.***, trabecular thickness; ***Tb.N.***, trabecular number; and ***Tb.Sp.***, trabecular separation.
The arrows denote the direction of change.

*** Significant effect at $p<0.001$.

Table 3
Main effects and interaction of ovariectomy and soft diet on mandibular alveolar bone micro-computed histomorphometric parameters.

	Normal (hard) diet		Soft diet		*Two-way anova*		
	SHAM	OVX	SHAM	OVX	*Effects*		*Interaction*
					OVX	*SD*	*OVX × SD*
BV/TV (%)	0.45 (0.06)	0.46 (0.04)	0.42 (0.06)	0.36 (0.05)	ns	**⇩	*
Connectivity density	27.2 (4.9)	25.8 (3.6)	26.1 (4.5)	24.9 (4.1)	ns	ns	ns
Tb.Th. (μm)	0.148 (0.025)	0.153 (0.017)	0.146 (0.021)	0.132 (0.017)	ns	*⇩	*
Tb.N. (1/mm)	2.99 (0.16)	2.91 (0.11)	2.93 (0.18)	2.74 (0.19)	*⇩	*⇩	ns
Tb.Sp. (μm)	0.19 (0.02)	0.20 (0.01)	0.21 (0.03)	0.24 (0.03)	*⇧	***⇧	*

OVX, ovariectomy instead of sham operation for the controls and ***SD,*** soft diet instead of the normal (hard) diet in the form of pellets for the controls.
BV/TV, bone volume fraction; ***Tb.Th.***, trabecular thickness; ***Tb.N.***, trabecular number; and ***Tb.Sp.***, trabecular separation.
The arrows denote the direction of change.

* Significant effect at $p<0.05$.
** Significant effect at $p<0.01$.
*** Significant effect at $p<0.001$.

Table 4
Delta percentages of ovariectomized animals (OVX) compared to the controls (SHAM) for normal and soft diet.

	Normal (hard) diet OVX (%)	Soft diet OVX (%)
BV/TV (%)	2.2	−14.3*
Connectivity density	−5.1	−4.6
Tb.Th. (μm)	3.4	−9.6*
Tb.N. (1/mm)	−2.7	−6.5*
Tb.Sp. (μm)	5.3	14.3*

OVX, ovariectomy instead of sham operation for the controls; ***Soft diet***, soft diet instead of the normal (hard) diet in the form of pellets for the controls.
BV/TV, bone volume fraction; ***Tb.Th.***, trabecular thickness; ***Tb.N.***, trabecular number; ***Tb.Sp.***, trabecular separation.

* Significant difference at $p < 0.05$.

3.3. Micro-tomographic histomorphometry of the mandibular alveolar bone (Tables 3 and 4)

Overall, ovariectomy had a negative effect on trabecular number ($p < 0.05$) and spacing ($p < 0.05$). Soft diet led to a deterioration of mandibular BV/TV ($p < 0.01$), as well as trabecular thickness ($p < 0.05$), number ($p < 0.05$) and spacing ($p < 0.001$).

A significant interaction (synergy) was observed between OVX and soft diet concerning the mandibular BV/TV ($p < 0.05$), as well as trabecular thickness and spacing ($p < 0.05$). The delta percentages of all histomorphometric parameters under study between the sham and the ovariectomized animals for both normal and soft diet are presented in Table 4. Ovariectomy led to a deterioration of BV/TV and trabecular thickness, number and spacing, only in those animals fed the soft diet.

4. Discussion

In the present study, we showed that normal (hard) diet protected (at least partially) the rat mandibular alveolar bone from estrogen-deficiency induced osteoporosis. Among the ovariectomized animals, those fed a soft diet (masticatory hypofunction) underwent a significant deterioration of the mandibular alveolar bone architecture, while those fed a normal (hard) diet preserved their mandibular alveolar bone almost completely four months after the operation.

During the same period of time the proximal tibia spongiosa in both groups of animals displayed a substantial and similar bone loss. As expected, there was no effect of the diet consistency at this skeletal site, which indirectly attests to the quality of pair feeding and nutrient absorption. Ovariectomized animals were found, as expected, to have higher body weight, serum IGF-I and osteocalcin, as well as urinary deoxypyridinoline. This IGF-I increase has been repeatedly observed after ovariectomy in both rodents (Ammann et al., 1996) and humans. The effect of ovariectomy on tibia bone microarchitecture and plasma IGF-I is fully in agreement with previous studies (for a review see Turner et al., 2001). These effects of ovariectomy observed at the level of the proximal tibia are in contrast with the moderate effects observed at the mandibular alveolar bone of the same animals.

In the present study, OVX provoked only a small reduction of alveolar trabecular thickness and a small increase of alveolar trabecular spacing. In the rat, the trabecular bone of the alveolar process is approximately three times thicker compared to the proximal tibia (Mavropoulos et al., 2007). This possibly means that further bone loss is needed before thinning of the trabeculae leads to structural deterioration of the mandibular alveolar bone. This experiment lasted 16 weeks. It is possible that a longer exposure to OVX might have had a greater impact on the alveolar bone. In humans, trabecular bone of the alveolar process is approximately two times thicker than that of the proximal tibia (Moon et al., 2004; Ding and Hvid, 2000). It is noteworthy that the mandibular bone arises from a different embryonic germ layer (neuroectoderm) compared to bone of the axial and appendicular skeleton, which arise from mesoderm.

The dentition is an integral part of the masticatory system, which prepares food for deglutition. The teeth form the occlusal area where the food particles are physically broken down into smaller particles to facilitate swallowing and digestion (Van der Bilt et al., 2006; Buschang, 2006). The number of processing cycles increases as foods become more difficult to chew. Adding fluid to a solid dry food has been shown to reduce both the muscle activity and the number of chewing cycles until deglutition (Van der Bilt et al., 2007). The teeth are subjected to the masticatory forces and they transmit them to the bone of the alveolar process through the periodontal ligament (PDL), which functions as a shock absorber. It is generally accepted that the PDL plays a crucial role in transferring occlusal forces from the teeth to the alveolar bone. In a recent study using finite element analysis it has been shown that the PDL affects not only the deformation and thus strain magnitudes of the alveolar bone around the teeth, but also the deformation of the whole mandible under masticatory stress (Groning et al., 2011). This unique biomechanical configuration is entirely different from any other skeletal site of the body. Of note, rat molars erupt normally, exactly like human molars, in contrast to rat incisors, which erupt continuously (Wise et al., 2001).

The alveolar bone, the bone process of the mandible housing the dentition, is subjected to heavy forces during mastication. The correlation between mandibular alveolar trabecular architecture, bone mineral density, and masticatory mechanical loading has been established in a previous study, where masticatory forces were modified through the alteration of food consistency in the adult rat (Mavropoulos et al., 2010). It was suggested that a reduction of the masticatory loading of the alveolar bone (soft food) leads to a reduction of bone mineral density, trabecular bone volume and thickness, as well as narrowing of the alveolar process itself. It seems that the mechanical loading during mastication directly influences the alveolar bone macro- and micro-architecture. In the present study, we clearly demonstrate that the mandibular alveolar bone resists more than the tibia spongiosa to estrogen deprivation, because of the anabolic effect of the mastication-induced bone strain. In a previous study on growing rats on a calcium deficient diet, it was concluded that normal masticatory function (hard diet) led to a higher bone apposition rate and better-organized trabecular system compared to animals fed a soft diet (Kiliaridis, 1989). The protective effect of exercise during estrogen deprivation has been demonstrated in a number of previous studies (for a review see Iwamoto et al., 2005).

In the rat, the forces developed during incision have been estimated to range from 2 to 25 N (but mainly below 8 N) depending on the hardness of the food, but are of relatively short duration (40–160 ms). During chewing (and swallowing), however, forces are of lower magnitude (4 N) but of a relatively long duration (160–680 ms) (Robins, 1977; Nies and Ro, 2004). In normal function the mechanical loading of the tibia is inferior to that of the alveolar bone during mastication. For most vertebrates, long bone peak functional strains range from less than 1000 microstrain (με) during walking to between 2000 and 3200 με for more vigorous activities (Ehrlich and Lanyon, 2002). Finite element data suggest that alveolar bone strain during mastication can reach up to 4000–6000 με depending on food consistency (Knoell, 1977; Daegling and Hylander, 1997).

Bone formation is influenced by strain rate, frequency, amplitude, duration, and interpolation of rest periods (Ehrlich and Lanyon, 2002). It is known, for example, that bone cells respond better to a mechanical environment dominated by high strains changing at fast rates and presented in unusual distributions (Mosley and Lanyon, 1998; Nordstrom et al., 1998; Skerry, 2008). During each chewing session that lasts a few minutes, alveolar bone strain goes from almost zero to 4000 με and back again to almost zero in 160–680 ms. When the food is available ad libitum, eating frequency has been estimated to be around 30 times per day in the rat (Konig et al., 1968).

A recent study on Sprague Dawley rats investigated the effect of estrogen deficiency (OVX) on the indentation viscosity of mandibular and vertebral bone tissue using nanoindentation (Kim et al., 2013). Indentation viscosity was higher for the mandibular bone compared to the vertebral bone. The OVX mandibular bone tissue had a greater viscosity than both the sham groups and the OVX vertebral bone. It has been suggested that bone tissue with high indentation viscosity can absorb more energy under loading (Isaksson et al., 2010). Ovariectomy has been shown to result in a reduction of mineralization and osteocyte density, in contrast to soft diet, which led to a significant increase of mineralization density in Sprague Dawley rat alveolar bone (Rawlinson et al., 2009; Kingsmill et al., 2010). Although one should be cautious interpreting these findings, they provide an insight into why estrogen deficiency has less effect on mandibular bone than on other load-bearing bones.

The reason why osteocalcin and IGF-I plasma levels were lower in those rats fed a soft diet is not clear. A different protein intake between the two groups is excluded since the rats were pair fed. One hypothesis is that mechanical stimulation of the mandible during mastication may induce a production of osteocalcin that influences its plasma levels. It has been demonstrated that mechanical loading results in both an increase of bone formation (Chambers et al., 1993) and of bone IGF-I mRNA expression (Lean et al., 1995). Another hypothesis is related to the fact that the duration of food consumption is longer to those animals fed the hard diet. Since IGF-I was measured only 4 hours after feeding the rats, its plasma levels might be differently influenced. It is possible that its plasma levels peaked earlier in the soft diet group.

In the present study, normal (hard) diet limited significantly the negative effects of estrogen deficiency on mandibular alveolar bone micro-architecture four months after ovariectomy. Combining this finding with the results of previous studies conducted by the same research group, it can be deduced that the alveolar bone is partially protected due to the anabolic effect of high bone strain during normal masticatory function. In those animals with a masticatory hypofunction (soft diet), ovariectomy provoked a significant amount of bone loss and micro-architecture deterioration. This study underlines the specificity of the alveolar bone behavior to classic bone loss stimuli and helps better understand the pathophysiology of mandibular osteoporosis.

Conflict of interest statement

The authors have no conflict of interest.

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Mare and Foal Behavior

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Inadequate maternal behavior of mares and mare and foal bonding problems are rare in natural social environments. Management that best accommodates natural behavior can help avoid or overcome problems in domestically managed horses. Author's address: University of Pennsylvania School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348; e-mail: suemcd@vet.upenn.edu. © 2012 AAEP.

1. Introduction

A basic understanding of normal and problem mare and foal behavior under natural and domestic social conditions can be valuable to the veterinary practitioner for (1) education of clients to manage foaling to best support normal mare and foal bonding and foal development, for (2) providing professional assistance in instances of inadequate or aberrant maternal behavior or mare and foal bonding problems, as well as for (3) advising on common questions concerning normal and abnormal foal behavior during development, early intensive handling, and least-stress weaning of foals.

Parental Behavior in Horses

Studies of mare and foal behavior under natural social and environmental conditions as observed in various free-roaming and semiferal populations with original references have been summarized elsewhere.[1] In brief, care and protection of the neonate and developing foal involves principally the dam and her harem stallion but also includes some contributions as needed from other adults and even older juveniles within the natal band in the form of assisting with retrieval of a lost or stranded foal or directing a neonate to the dam, should it attempt to nurse a herd mate.

Normal maternal behavior includes attention to fetal fluids and membranes during and immediately after parturition and positive attention to the neonate, including olfactory investigation and Flehmen response, licking, nuzzling, avoiding walking on, keeping intruders from approaching or interacting, allowing and facilitating sucking, and standing guard near or with head and neck over the foal whenever it is recumbent. Attention to the fetal membranes and fluids is common, but ingestion of the afterbirth as is common in some species is rare.[2] Under natural conditions, the strategy of horses for dealing with the predator-attracting birth fluids and membranes is to move away from the birth site as soon as possible. The harem stallion can often be seen encouraging the foal and mare to move on to a fresh site as soon as the foal stands, even before nursing.

The foal's behavior appears to play a role in stimulating the dam's response and vice versa. In its first coordinated movements immediately after birth, even before standing, the foal appears to orient toward the dam, reaching for her head and appearing to seek nose-to-nose contact at first. Upon standing, the foal appears to be instinctively attracted visually to follow horizontal edges at nose height, which in nature is usually only the dam's

NOTES

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abdomen. Along with assistance of the dam's reverse parallel orientation to the foal and likely olfactory cues as the foal approaches the udder, this instinctive behavior brings the foal's lips to the flank and udder.

Under domestic management, care of the foal is generally limited to the dam. Detailed descriptions of mare and foal behavior under domestic conditions can be found in publications of Dr. Katherine Houpt[3] and her student Dr. Sharon Crowell-Davis.[4] When isolated or when housed or pastured with unfamiliar horses, some individual dams appear hypervigilant and demonstrative in their role of protection of the neonate, perhaps due to lack of the social support for this role within a band under natural social conditions. In pasture groups of domestically managed horses, the behavior of other mares is typically either ambivalent or positive toward a dam and neonate. Gelding herd mates may show protective behavior of the dam and/or foal, as under natural social conditions a harem stallion would guard and protect his band. Most geldings, like stallions, are especially protective and tolerant of foals.

Mare and foal bonding problems have been detailed in Grogan and McDonnell.[5] Briefly, there are at least six distinct types of problem.

(1) Good maternal protectiveness misunderstood as aggression toward foal. Probably because of the domestic practices of isolation and confinement for foaling, some dams appear especially stressed concerning foal protection and care. When confined in small or "trapping" spaces with poor footing and obstacles, foals can easily be injured by a mare trying to position herself between her foal and a threat. For example, if her hay is fed at the rear of a stall, a dam with strong instantaneous reaction to threats may inadvertently trample or bump a foal into an object in the process of responding to a threat at the front of the stall. Adequate space and facilities with minimal provocation of protective behavior is recommended until protectiveness usually naturally wanes over the period of 3 days to 2 weeks.

(2) Ambivalence. Primiparous mares, as well as weak or painful mares, may have delayed interest and response to the foal. The onset of intensely protective behavior is typically not immediate and may be first seen as late as 18 to 36 hours after parturition. Under natural conditions, other adults, including the harem stallion, appear to assist such dams with protection of the foal and with keeping the foal with the dam. Support of the foal in staying near the dam and supervised nursing is recommended, with typically a good prognosis for onset of adequate to excellent maternal behavior and bonding with the foal within 2 to 5 days.

(3) Fear of the foal. Some mares, almost always primiparous, react to their neonate with fear behavior as some horses may react to species such as swine or camelids, with which they have not been exposed. Positive reinforcement–based systematic acclimation, similar to what is effective in helping a horse overcome fear of other new objects, procedures, or situations, is recommended, with typically a good prognosis for eventual acceptance of the foal adequate for maintenance of the pair.

(4) Nursing only avoidance or aggression toward foal. Most often this is judged to be due to sensitivity at the flank or udder, sometimes in association with obvious udder discomfort. Relief of udder discomfort and positive reinforcement–based acclimation to touch of the flank and udder as well as supervised nursing or milking and bottle-feeding at the udder are recommended until the foal is accepted.

(5) Savage attack. Just as some stallions intermittently attack people, some mares that show otherwise good maternal behavior, intermittently attack their foal. Attacks can involve biting, kicking, thrashing by the crest of the neck, and stomping in a sustained manner similar to that of a horse killing a small mammal. If the foal survives, the mare typically returns to good maternal behavior and then repeats at some later date. In confirmed cases of savage attack, immediate separation is recommended. It can be expected that the mare may repeat with subsequent foals.

(6) Foal stealing. A near-term pregnant mare may steal a neonate from a weaker mare. When the thief mare foals, she typically abandons the stolen foal in favor of her own. Foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling, for example, induced parturition in feed-lot conditions.

Orphan Foals

Use of a foster mare is generally considered the best method for meeting the nutritional and behavioral needs of an orphaned foal. Foster mares can include local mares that have recently (within 24 hours) lost a foal or occasionally one that will accept a second foal, open mares hormonally prepared to lactate and to accept foals,[6] and professional "nurse" mares managed by farms with systems of hand-rearing the foals of the nurse mares. If a horse or pony mare is not available, nanny goats typically accept nursing of a foal (Fig. 1), and typically provide better for the nutri- F1
tional and behavioral needs better than hand-feeding. Most foals can be trained to drink from a bucket or tub (Fig. 2). Training can be accom- F2
plished by drawing the foal's muzzle into shallow pail of milk, with a finger or nipple in the foal's mouth. In healthy foals, withholding feedings for several hours before bucket feeding attempts may increase appetite and acceptance. Groups of foals have also been successfully fed using an automatic calf-feeding machine, which provides a constant supply of fresh milk replacer through a nipple mounted on the wall.[7] To avoid behavior problems in hand-reared foals, which can be generally described as overly bonded to people to the extent of sexual response to humans, it is recommended to

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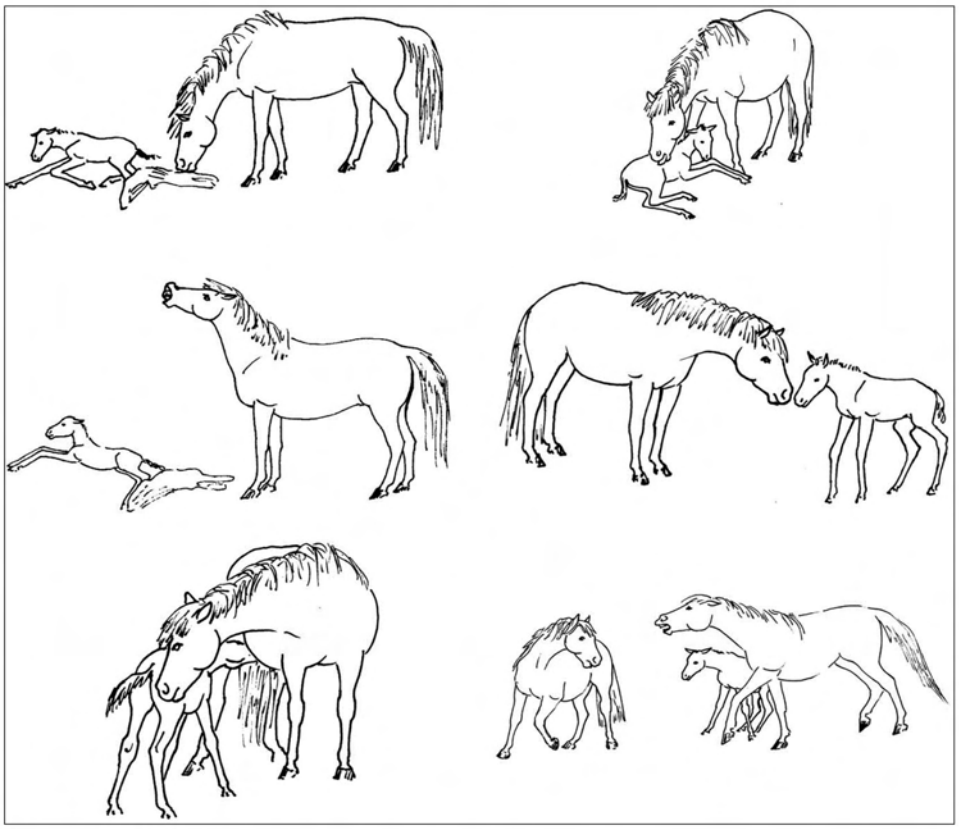

Fig. 1. Key elements of normal early maternal behavior (from McDonnell,[1] *Inadequate Maternal Behavior and Bonding*).

provide as much social interaction with horses (or other animal companions such as donkeys or goats) and as little human contact as possible. Particularly, to the extent possible, feeding should not be done in the presence of people. Therefore bucket-feeding is far superior to hand-held bottle-feeding. Orphans reared together and bucket-fed in a "kindergarten," as shown in Fig. 3, typically develop normal behavior. Older foals can be observed performing some of the protection and emotional sup- F3

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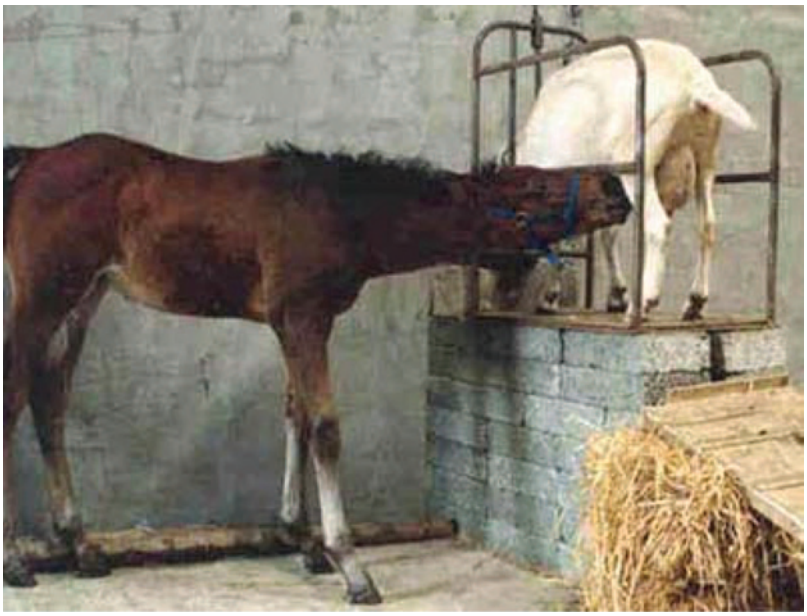

Fig. 2. Nanny goat using inexpensive set-up for nursing a foal. http://dianabuja.wordpress.com/tag/boer-goat/. Accessed December 11, 2011.

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Fig. 3. Orphan foals feeding from buckets and playing in a commercial kindergarten at Justaplain Farm in Cochranville, Pennsylvania, which has "graduated" eventual stakes race winners. Photo by Elkanah Grogan of the Equine Behavior Lab at New Bolton Center, reprinted with permission from Grogan and McDonnell.[5]

COLOR

port responses typical of dams. Geldings or open mares without foals in nearby paddocks or within a kindergarten can provide additional social support for orphan foals.

Early Intensive Handling Protocols for Foals

A program of early organized positive handling of foals to introduce them to human contact and basic handling equipment and techniques, including transport, while they are young, is considered generally to be a wise practice. Considerable debate continues on the best timing and the most efficient and effective procedure with least likelihood of adverse effects due to "technique failures." In general, the younger and less strong the foal, the easier to safely confine and restrain it for organized and positive introduction to human handling and acclimation to restraint and specific procedures. There is evidence that the foal's experience as a bystander when its dam is handled in a positive manner can be a positive influence on its own acceptance of human handling (Henry et al[8]).

Normal Early Foal Behavioral Development

Based on observations of both semiferal and domestic foals, Grogan and McDonnell[5] proposed a scoring system for early foal development based on an expanded list of behaviors reflecting early development. Table 1 outlines their neonatal early development (NED) scoring system. Foals born in more natural environments such as at pasture and in semiferal social conditions generally score higher than foals born indoors, isolated from the stimulation of herd mates or on artificial surfaces that obviously delay the ability to stand and move. T1

Least-Stress Weaning

In recent decades, evidence has accumulated that early or abrupt weaning poses considerable risk to the foal of developing gastric ulcers, losing condition, and showing behavioral signs of stress that together can result in interruption of thriving and in some instances can predispose the foal to life-long behavior problems such as stereotypies and separation anxiety (Waran et al[9]). Current recommendations for least-stress weaning include separation from the dam no younger than 5 or 6 months of age, ensuring that the foal has developed behavioral signs of independence from the dam and social interest in other horses, and ensuring that the foal

Table 1. Neonatal Early Development (NED) Scoring Inventory

Behavior	Slower Than Usual = 0	Average to Low Normal = 0.5	High Normal = 1
Sternal righting	>5 min	3–5 min	<2 min
Shake response (head or body)	>10 min	3–10 min	<3 min
Attempts to stand begin	>30 min	10–30 min	<10 min
Stand successfully (with steps)	>60 min	30–60 min	<20 min
Standing udder-seeking	>10 min	3–10 min	<3 min
Suckle	>90 min	45–90 min	<45 min
Locomotor burst of speed	>2 h	1–2 h	<1 h
Circle dam at speed	>2 h	1.5–2 h	<1.5 h
Organized recumbency	>3 h	2–3 h	<2 h
Autogrooming	>3 h	2–3 h	<2 h
Organized gaits (walk, trot, plus)	>4 h	2–4 h	<2 h
Retreat from approaching human	>4 h	2–4 h	<2 h

From Grogan and McDonnell.[5]
Total score of greater than 10 is high normal, 4 to 6 is average to low normal, and less than 4 is slower than usual.

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is nutritionally independent from the dam before weaning. Methods that temporarily separate the foal from access to the udder while allowing direct contact or nearby visual contact with the dam for "emotional" support during moments of stress, typically appear least stressful to the foal. Udder covers or gradual introduction to separation from the dam at increasing distances and durations within sight and with known companions and/or some remaining adult mares is considered less stressful than abrupt permanent separation in isolation from known herd mates or from other horses.

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When asked by Juan and Jon to join them in editing this *Current Therapy* I accepted without much thought. The chance to work with authors, learn from reading and editing their manuscripts, and to publish a book with editors who were employed in private practice was a temptation too much to deny. Apart from the edification, it emphasized to me that specialization in private practice was so well accepted that authors and editors need not be restricted solely to those institutions of higher learning.

The *conception* and *gestation* of this *Current Therapy* was clearly Juan's domain. He decided on the necessity of this, *his progeny* to be introduced. He provided *the gametes* with the outline and list of authors and Jon and I were co-opted for the *fertilization* process. Early in gestation a problem with this *pregnancy* was identified wherein, after narrowly avoiding *early embryonic death*, it began to look like *elective termination or even abortion* was a possibility. After identifying and rectifying the problems and deficiencies, the gestation then became somewhat *prolonged*.

At last, successful delivery!

Angus O. McKinnon

We all owe a great deal to Jolynn Gower who was our managing editor from Elsevier. How she handled the difficulties of delinquent authors and editors with such level-headed aplomb was a surprise and an inspiration to us all. The only reason the project eventually came together was through her untiring efforts to keep us all on track.

Juan C. Samper
Jonathan F. Pycock
Angus O. McKinnon

CHAPTER 24

Selected Reproductive Surgery of the Broodmare

ANGUS O. MCKINNON
JAMES R. VASEY

The notes herein are a compilation of some of the techniques that are used at the Goulburn Valley Equine Hospital and are not intended as an exhaustive, heavily referenced treatise of all available techniques. No attempt has been made to discuss all reproductive techniques or conditions requiring intervention. The procedures herein work very well for us. However, readers should be aware that different methods are employed successfully by others. We would be grateful to those who have different ideas and experiences if they would communicate them by e-mailing Dr. McKinnon at aom@iinet.net.au. Many of the techniques presented are modifications of existing techniques, newer techniques, and/or areas we feel warrant special attention. We have assumed that readers are at least familiar with both relevant anatomy and standard documented surgical correction of reproductive-related problems.

Topics discussed have been dictated by our experience and preference for an individual or alternate technique when many techniques exist. A busy equine practitioner can expect to encounter most of these problems in any individual breeding season. Most of the techniques relate to restoring or improving fertility, and most are elective and require experience to make the correct diagnosis and therapeutic approach.

PATIENT SELECTION

In consideration of the client, patient, and general perception of our professional abilities as well as the merit of our interference, care should be exercised to select only those patients that are likely to respond favorably to surgery (Box 24-1). For example, it is pointless to perform a sophisticated, expensive surgery for vesico vaginal reflux if the mare has sustained chronic uterine damage and fibrosis that will render her infertile despite an excellent surgical outcome. Full reproductive evaluation is necessary before surgery in any candidate subjected to physical insults such as recto-vaginal lacerations and fistulas for longer than 1 year.

SEDATION AND ANESTHESIA

Most procedures are performed with the mare standing, using local infiltration or epidural anesthesia. Apart from minor procedures (Caslick's vulvoplasty), we prefer to have mares tranquilized and restrained in appropriate stocks such as facilities for cross tying and/or tail elevation. Tranquilization and analgesia are provided most commonly with intravenous (IV) detomidine (mg) and butorphanol (mg). Longer surgeries may require the repeated IV use of low doses of xylazine (100 mg). Cost and length of tranquilization/analgesia are important considerations. Some have other physiological effects, such as increased urinary output with xylazine. Detomidine provides excellent tranquilization and analgesia; however, effects are often still apparent hours after administration. Epidural anesthesia may be extremely effective; however, variation in response and individual susceptibility and time before appropriate anesthesia is obtained occasionally make it less rewarding than tranquilization and local infiltration for some of the procedures discussed here. Techniques for epidural anesthesia have been discussed elsewhere.[1] One word of warning: epidural administration of more than 7 to 9 ml of 2% local anesthetic to a 500 kg mare can be associated with loss of motor control to the hind limbs and recumbency. These mares are difficult to manage and may require support for 2 to 4 hours.

Epidural injection of xylazine for perineal analgesia has been reported to be very efficacious. A dose of 0.17 mg/kg (around 75-100 mg in an average horse) was as effective as local anesthetic agents for analgesia and had nil or minimal effects on depression of motor nerves in the lumbosacral intumescence. Another advantage was demarcation of the area of analgesia with an area of sweating (dermatome) that was consistent both temporally and topographically.[2] In our experience the perineal dermatome is not always obvious. In addition it takes around 35 to 40 minutes for good analgesia to be recognized associated with xylazine epidural anesthesia. Currently we prefer to use a combination of xylazine (70 mg) and lidocaine (60 mg [3 ml]), q.s. saline to 10 ml for most horses. With this regime, loss of tail tone has occurred associated with a successful "block" within 5 to 10 minutes and surgery generally has begun by approximately 30 to 45 minutes post administration. Our most common method of epidural anesthesia is to begin with the infusion of 1 ml of local anesthetic through a 25 gauge needle subcutaneously above the first moveable coccygeal/sacral joint (usually C1 and C2). Next, a 38-mm, 18-gauge needle (bevel forward and introduced at 45 degrees) that has a small amount of fluid left to create a meniscus on the needle hub is introduced through the

Box **24-1**

Considerations for Favorable Response to Surgery

The probability of a successful outcome (i.e., pregnancy, live foal, etc.) must be evaluated with regard to the following:

- Severity and nature of the problem
- Breeding history of the mare
- Value of the mare and/or offspring (commercial or sentimental)
- Cost of the procedure
- Mare age
- Fertility of the stallion
- Availability and suitability of sophisticated breeding techniques such as AI, ET, etc.
- Long-term value of interference (i.e., temporary or permanent improvement)
- General health of the candidate
- Perpetuation of heritable conditions
- Insurance and informed client consent
- Ethical considerations
- Experience of the veterinarian
- Quality of on-farm management

local bleb. Recognition of the fluid being aspirated into the needle infers correct placement, and the appropriate amount of solution is injected slowly into the epidural space whilst continually evaluating the ease of administration to ensure that the needle tip is still in place.

Occasionally epidural anesthesia is difficult to achieve (infusion outside the spinal cord before proper epidural placement, hemorrhage through the needle and tissues, anatomic abnormalities and fat horses). Provided local anesthesia can be obtained by infiltration without adverse affects on wound healing, we prefer to perform surgery without the epidural and provide incremental IV administration of xylazine (i.e., 50-100 mg to already tranquilized patients when or if minor surgical discomfort is recognized).

In some instances we have been able to identify correct epidural placement by injecting the anesthetic slowly while moving the needle until there is an obvious change in the amount of pressure needed to advance the solution. These situations are usually associated with a mare with abnormal anatomy (i.e., after a traumatic foaling) and lack of movement of fluid in the needle hub.

VESTIBULE AND VULVAL LIPS

Pneumovagina

There are 3 barriers to aspiration of air and contaminants into the cranial reproductive tract.

1. The vulval/vaginal lips
2. The vestibular sphincter
3. The cervix

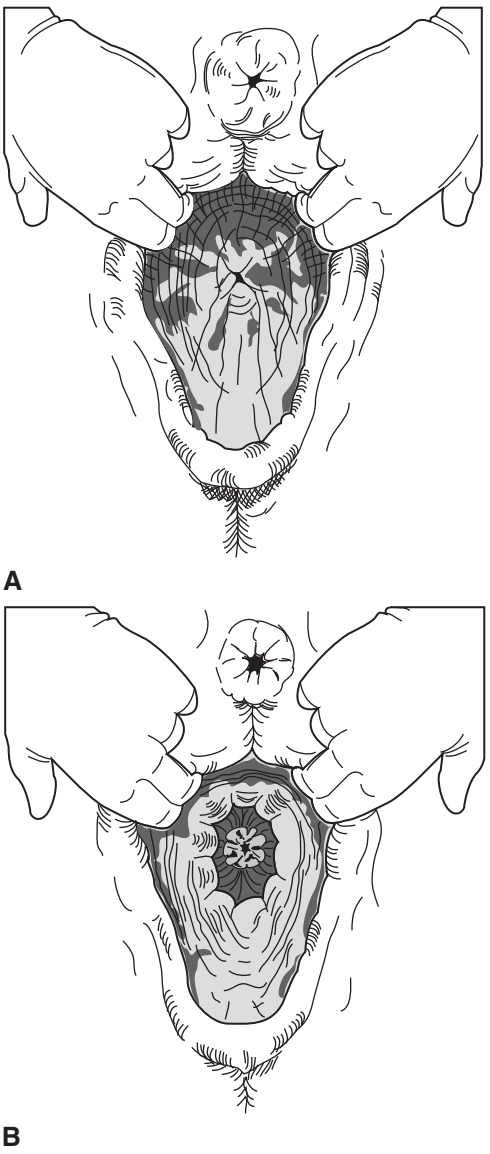


Figure 24-1 **A,** Retraction of vulval lips reveals an intact vestibular sphincter. **B,** Retraction of vulval lips reveals an open vestibular sphincter that is likely associated with pneumovagina.

It would appear that the vestibular barrier is more important than previously recognized; however, when any of these protective barriers are rendered incompetent, contamination and concomitant vaginitis, cervicitis, and metritis may result. Pneumovagina can result from faulty perineal conformation, previous injury to perineal tissues, or effects of poor body condition. Pneumovagina can also be iatrogenic (i.e., reproductive examination or breeding).

Recognition of potential candidates for pneumovagina are made from anatomical observations[3] (i.e., predisposition is associated with a low pelvis, tilted vaginal lips, and a sunken anus) (Figure 24-1). If possible, mares should be examined during estrus when the reproductive tract is relaxed. Parting the vulval lips results in loss of integrity of the vestibular sphincter and an audible ingress of air

to the cranial vagina. Recognition of foamy exudates from the vaginal lips may often be noted during rectal examination. In addition, ultrasonography has been used to visualize air in the uterus.[4] If in doubt after clinical evaluation regarding a mare's potential to aspirate air into the vagina, we recommend treating by surgery all mares that have been barren one or more breeding seasons, unless other causes of reduced fertility are obvious (i.e., stallion, farm management).

Caslick's Vulvoplasty

Caslick's vulvoplasty was first described by Dr. E. A. Caslick in 1937 and involves apposition of the vulval lips.[5] Local anesthesia of vulval lips, removal of a thin portion of mucosa from the mucocutaneous junction, and apposition of the cut edges is simple, quick, and effective. Length of apposition is determined by height of pelvis relative to vulval lips. In general the join should continue to a point at least 3 to 4 cm below level of the pelvis, and approximately 3 cm should be left unopposed to allow for urination. If this is not possible, other surgical techniques (see below) may be necessary.

Points for consideration

- For suture material we use 0 catgut (Metric 4) on a continuous spool. The benefit of gut is that it is gone or degraded by either pregnancy testing (~15 days) or the next breeding cycle (if the mare is empty). Other materials that are nonabsorbable or delayed-absorbable may damage the stallion if the mare is rebred.
- For suture pattern, a simple continuous pattern is quicker and just as effective as a simple pattern interrupted. However, failure to appose the deepest layers of the exposed tissues may result in fistula formation. Failure to exit/enter in the middle of the cut tissue caused by taking bites too deep will result in wound breakdown.
- Method of mucosa removal:
  - Tissue should be removed from the mucosal surface of the vestibule, not the skin of the vulva lip.
  - Removing too much will result in fibrosis and difficulty for the next veterinarian (Figure 24-2, *A*).
  - If multiple procedures are performed in 1 season, freshening with a scalpel blade is sufficient, provided a careful suture pattern is placed (Figure 24-2, *B*).
  - Incision with a scalpel blade rather than mucosal removal is recommended for maidens (i.e., race mares) (Figure 24-2, *C*). This results in a thinner join that is easily opened at the breeding farm.

Postoperative treatment

- Frequent rectal palpation within 2 weeks will result in fistula formation.
- Remove (open) 2 to 3 weeks before anticipated foaling.

Variations. Placement of a breeding stitch (usually doubled heavy gauge suture material or umbilical tape) will save unnecessary replacement and facilitate breeding and some manual vaginal manipulations for treatment (Figure 24-2, *D*). Care is needed with natural service to prevent penile trauma. We routinely treat or bred (AI) mares by passage of an infusion pipette into the vagina (past the vestibular sphincter) and use rectal manipulation of the pipette through the cervix similar to, but with a little more difficulty than cattle.

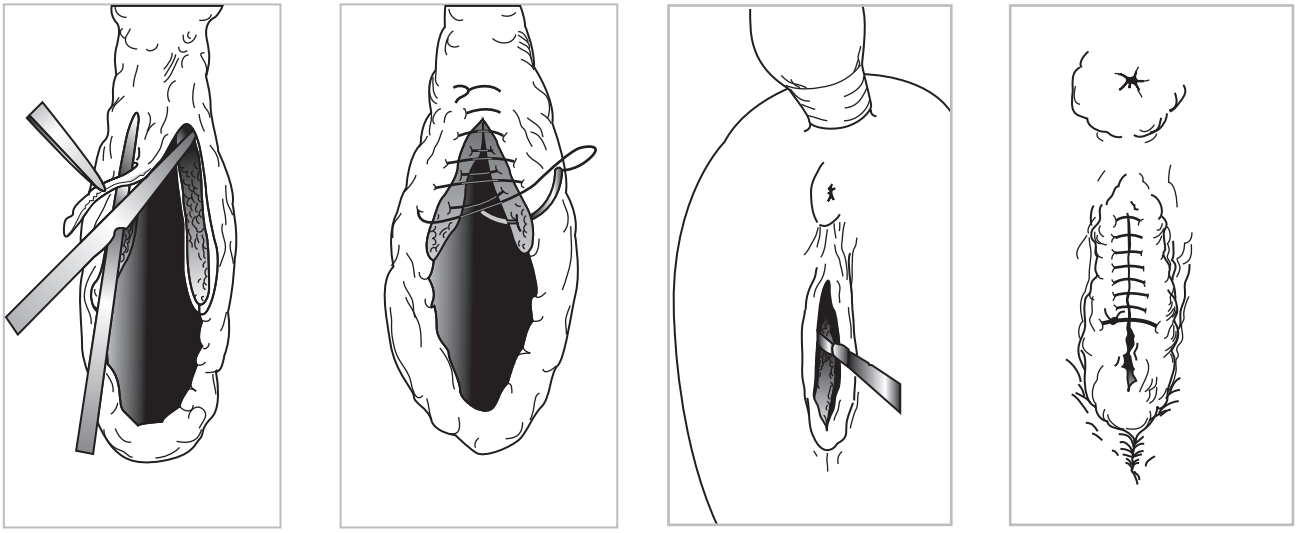


Figure 24-2 **A,** Careful trimming of the mucocutaneous junction without removing skin from the vulval lips facilitates many more identical surgeries. **B,** Simple continuous suture is all that is necessary, but suture placement will help avoid fistulas. **C,** A scalpel blade is recommended for the first Caslick a mare has, especially maidens in work. **D,** A breeding stitch may be useful in preventing the Caslick from opening with vaginal examination or natural service.

Episioplasty

This procedure is used in cases with more severe anatomic abnormalities where the Caslick vulvoplasty is ineffective and in mares with extensive or repeated second-degree perineal lacerations. Technically the previous surgery is an episioplasty as well; however, we use the term to describe the more extensive procedure previously referred to as (1) a deep Caslick, (2) the Gadd technique, or (3) a perineal body reconstruction.[6] The procedure is designed to restore some degree of function to the perineal body. (Figure 24-3)

The surgery is performed on the standing mare with local anesthesia. Visualization is improved by good retraction and light sources. We prefer to perform all perineal surgeries using a centrally mounted beam as the light source.

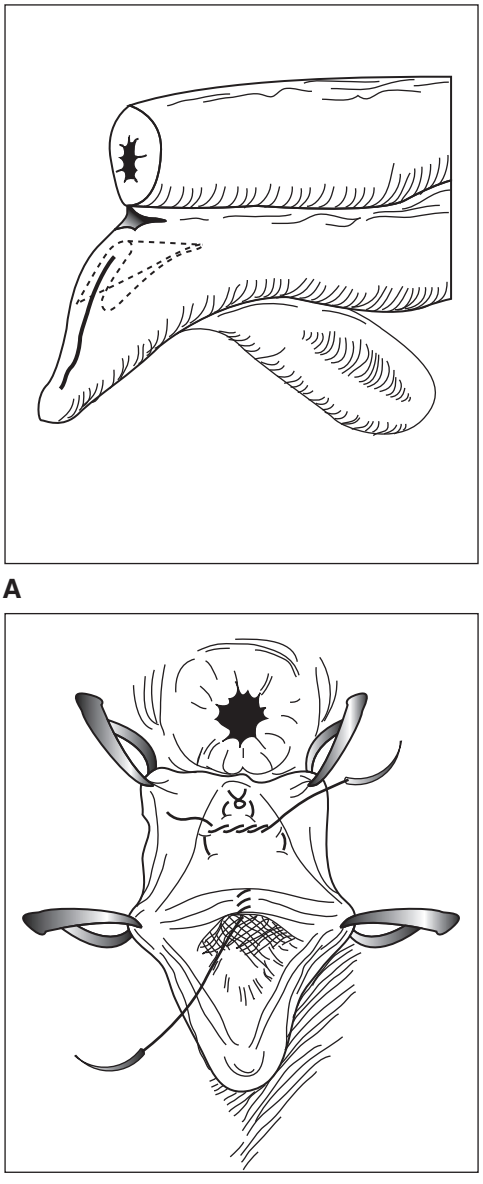


Figure 24-3 **A,** The *dotted lines* outline the area to be apposed with the formation of a new perineal body. **B,** The ventral layer of the perineal body consists of the vaginal mucosa. The perineal body is created from apposition of the vaginal mucosa where the tissue has been denuded.

A triangular portion of the dorsal caudal vestibule is removed from both sides. Apposition of the ventral borders of the incision and obliteration of the potential space above result in an increase in the size of the perineal body and a decreased propensity of the vestibule to create negative pressures.

The ventral borders are apposed with a continuous absorbable suture (2/0 vicryl) and dead space with single interrupted sutures. The mucocutaneous junction is closed similar to the Caslick procedure.

Points for consideration

- The procedure appears most beneficial when combined with a perineal body transection (see below).
- Failure to "open" the dorsal vestibule and vulval lips before foaling (2-3 weeks) may result in severe perineal laceration.
- The surgery usually takes longer (30-40 minutes) than expected (10-15 minutes) and is sometimes associated with significant and annoying obscuring hemorrhage.

Urovagina

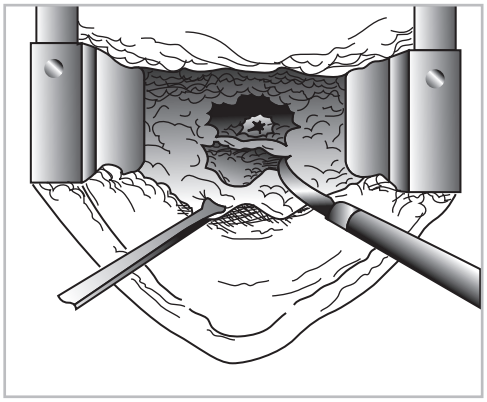
Pooling of urine in the vagina is a problem for a variety of reasons. In 1985 Jay Belden, then of Fossil Creek Farms, showed me his technique for urovagina. Before this time, both of us had tried other described techniques and were not particularly happy with the outcome. After approximately 34 surgeries between us using Jay's technique, we decided the technique was efficacious and should be presented. The resultant publication is summarized below.[4]

The midline caudal border of the transverse vaginal fold is grasped with Allis tissue forceps and retracted caudally toward the surgeon 2 to 4 cm cranial from the caudal border of the transverse fold. A horizontal transverse incision to the submucosa is made with a No. 12 Bard Parker blade (Figure 24-4). The incision is continued laterad and then slightly dorsad to the vaginal wall. The vaginal retractors are then repositioned, and a No. 20 Bard Parker blade is used to extend the incision from the lateral border of the transverse folds caudad. This incision is made approximately one half to two thirds of the distance between the vaginal floor and roof and is extended to the vulvar labia. The caudal cut edge from the transverse fold incision and the ventral cut edge of the vaginal wall incision are dissected so that the free tissue flaps are reflected caudad and mediad, respectively. This causes vaginal mucous membranes of the free tissue flaps to be reflected ventrally and the underlying cut surface to be exposed dorsally. Next, the retractors are repositioned and an identical incision is made on the opposite vaginal wall, extending from the lateral extremity of the transverse fold caudally toward the surgeon.

Dissection of the tissue flap from the transverse fold is continued until the caudal cut edge can be reflected approximately 3 to 6 cm toward the surgeon. The dissection of both vaginal wall flaps is continued ventrally until the cut edges can be reflected without tension past the midline (Figure 24-4, *C*). The suture pattern used to appose the submucosal tissue layer is a continuous

modified Connell's, using 2-0 gut (Figure 24-4, *D*). The final configuration is in the shape of a Y, with the apex pointing caudally. The first suture line begins cranially and laterally at the junction of the transverse fold and vaginal wall incisions and is ended at the midpoint of the transverse fold reflection. Cut edges of the transverse fold and vaginal wall are inverted so that denuded tissues are in apposition. The second suture line begins on the opposite side, at the junction of the transverse fold and vaginal wall incisions, and is continued to the end point of the first suture pattern and then on the midline, toward the surgeon, to the caudal end of the vaginal wall reflections. The result is an extended tunnel from the urethral orifice (under the transverse fold) to the caudal vaginal vault. It is important to have minimal tension associated with the suture line and have all apposed edges inverted so that dissected or denuded tissues are in apposition (Figure 24-4, *E* and *F*)

The denuded tissues created dorsally by the dissection of the transverse and vaginal folds are allowed to heal by second intention. If necessary, an episioplasty is performed after surgery.



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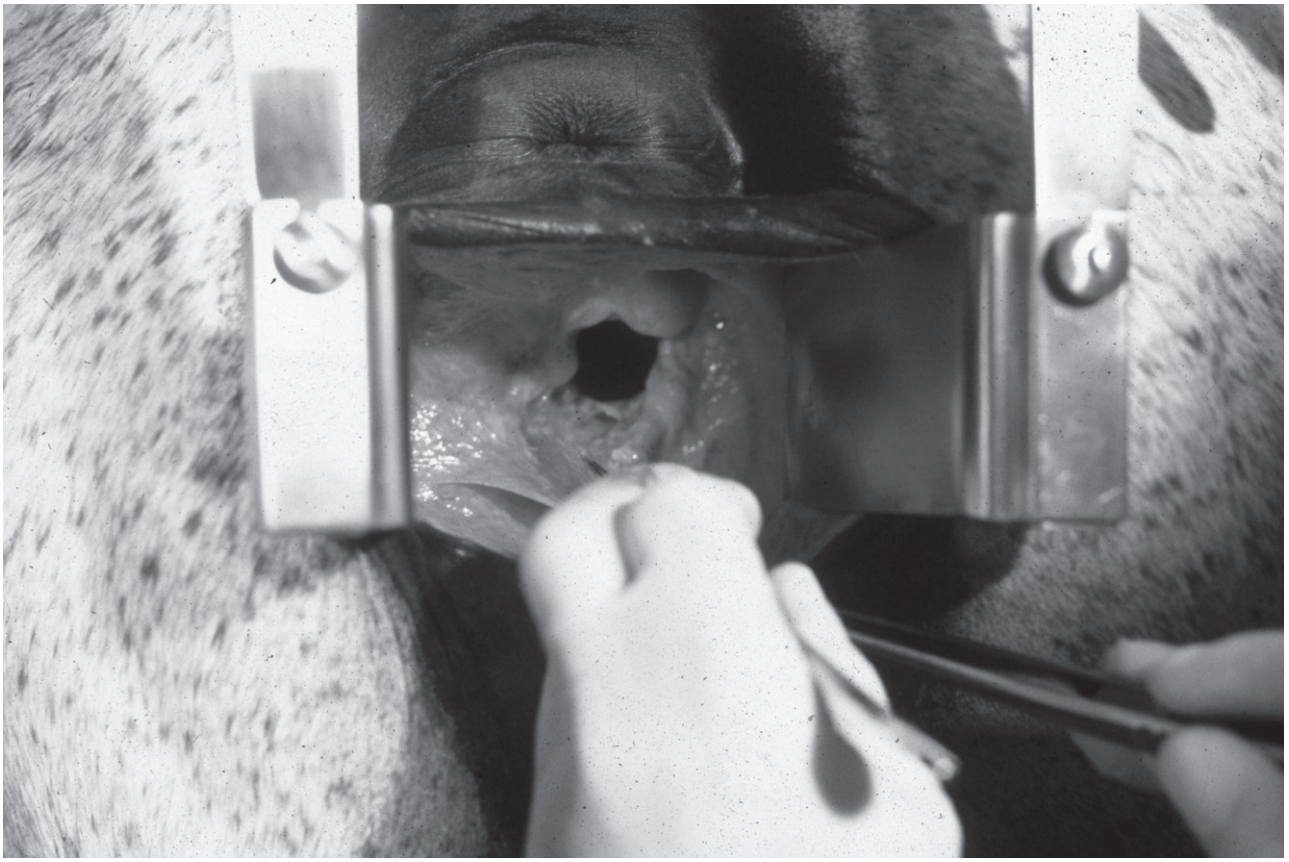
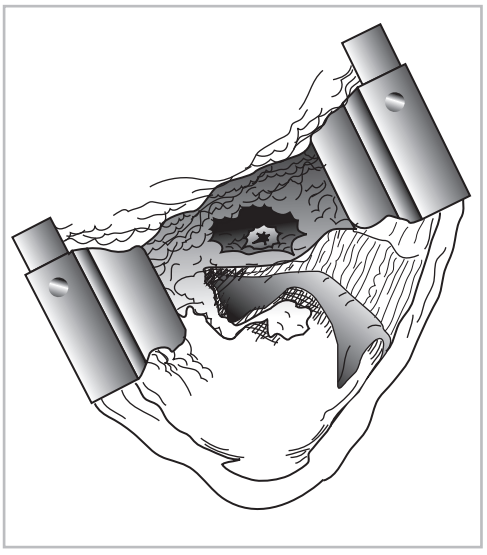


B

Figure 24-4 **A,** The first incision is along the transverse fold of the vestibular sphincter. **B,** A No. 12 blade (hook blade) facilitates the undermining of the transverse fold.

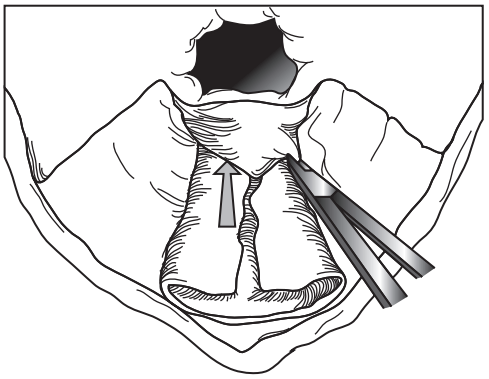
Continued



C

D

Figure 24-4, cont'd **C,** The lateral vaginal wall is dissected after the retractors are repositioned. **D,** The vaginal wall is dissected from between one half to two thirds of the wall until the free mucosal flap can be passed across midline without undue tension.



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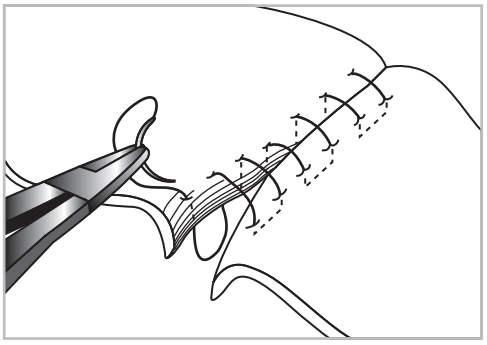

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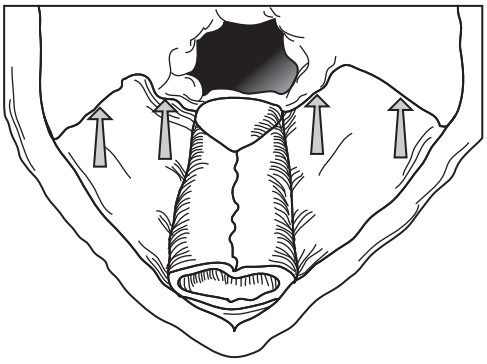


G

H

Figure 24-4, cont'd **E,** The reflected two vaginal walls and the reflected undermined transverse fold are all brought together and inverted, leaving the denuded surface in the vestibule. **F,** The suture pattern is a modified Connell. **G,** The finished urethral tunnel. **H,** There is not much tension on the newly created urethra.

Additional points

- The technique described is our technique of choice for treatment of all mares with urovagina. Other treatments such as perineal body transection or caudal relocation of the transverse fold are often only temporarily effective.
- Episioplasty performed at the same time may result in excessive ablation of vestibule.
- In some mares, urine will pool in the caudal relocation of the urethral tunnel and it will be voided during exercise, commonly resulting in urine staining of the perineum. While this does not affect fertility, it is unsightly and may be treated by incision into the new urethral tunnel to a point 2 to 4 cm cranial to the vulval lips but not far enough to allow urovagina to recur.

Perineal Body

Surgeries of the perineal body are necessary to correct severe anatomic defects (perineal body transection [PBT]) or injuries due to foaling (perineal lacerations and fistulae).

Perineal Body Transection

Perineal body transection has been described to be beneficial for both pneumovagina and urovagina.[7] Our experiences suggest definitely the former but only occasionally the latter.

The primary indication for PBT is mares with such external reproductive conformation that we are not able to prevent pneumovagina after either Caslick's vulvoplasty or episioplastly (i.e., mares with a severely sunken anus and most or all of the vulval lips positioned above the brim of the pelvis (Figure 24-5, *A* and *B*).

The procedure is performed with local anesthesia with or without tranquilization. Local anesthesia (40-70 ml) is liberally infused into the perineal body and laterally to include the vaginal walls to a depth of 8 to 14 cm.

Towel clamps positioned just ventral to the anal sphincter and dorsal to the dorsal commissure of the vulva are used to provide retraction and tension while a 4- to 6-cm horizontal incision is made midway between the anus and dorsal vulva. The incision is continued for a short distance (2-5 cm) along either side of the vulva. Sharp and blunt dissection are used to completely transect the muscular and ligamentous supporting tissues between the rectum and vestibule (Figure 24-5, *C*, *D*, and *E*). Depending on the individual mare, the dissection proceeds cranially for 8 to 14 cm and finishes before entering the peritoneal cavity. The aim of the surgery is to allow the vulval lips to assume a more horizontal position by freeing them from attachments to the rectum. Generally the surgery is finished when the desired external conformation is achieved after allowing for wound contraction.

Closure of the skin incision was originally recommended[7]; however, we commonly leave the incision open. Closing the skin occasionally results in dehiscence and also seems to influence the result after wound contracture occurs. The wound heals by second intention, which is surprisingly rapid (2 to 3 weeks).

Points for consideration

- Placing a hand in the vestibule enables accurate dissection. Penetration of the vagina or rectum should be avoided.
- The wound is unsightly while healing, and we recommend careful client communication and/or hospitalization for 1 to 2 weeks.
- The benefits from corrective surgery are immediate, and mares can be bred at the first opportunity, provided that qualified personnel are available. Natural service is generally delayed 2 to 3 weeks to allow strengthening of the dorsal vestibule and vagina.
- Despite the surgery causing moderate hemorrhage, we have not had to take any special precautions or protective measures.

Perineal Lacerations

Most perineal injuries occur at the time of foaling and are associated with a mal-presented or oversized fetus, extensive, vigorous, inappropriate or sometimes unavoidable manual manipulation during parturition and/or violent expulsive efforts of the mare. Maiden mares are by far the most commonly injured with third degree perineal lacerations.

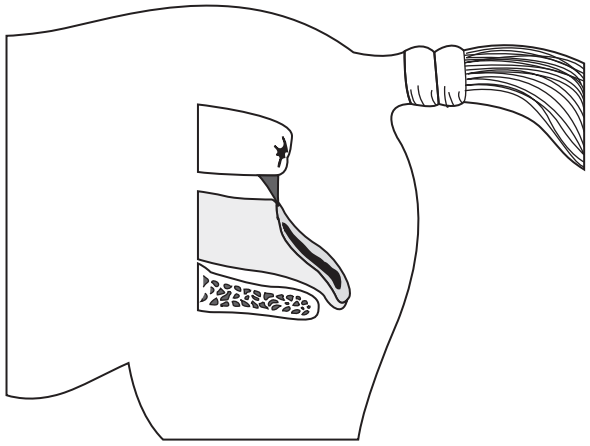
The injuries are commonly referred to as first, second, or third degree perineal lacerations (PL). First degree PL involve only the mucosa of vestibule and skin of the dorsal commissure of the vulva. Minor first degree PL may require no treatment. Extensive lacerations may require episioplasty or perineal body reconstruction. If tissue damage results in significant edema, inflammation, and infection, then surgical correction is often delayed for 2 to 3 weeks.

Second degree PL involve both the mucosa and submucosa of the dorsal vulva, and some of the musculature of the perineal body, in particular the constrictor vulvae muscle. There is no damage to rectal mucosa.

Third degree PL result in tearing of the vestibular and sometimes vaginal wall and disruption of the perineal body, anal sphincter, and rectal wall. This results in a common opening between the rectum and the vestibule.[8] The constant presence of feces in the vestibule and occasional unpleasant sound from air movement make repair imperative for breeding and recommended for future riding horses.

Immediate care for the mare at foaling involves antibiotics, anti-inflammatories, and protection against tetanus. The surgical correction is delayed at least 4 weeks to allow initial second intention wound healing to occur. Heroic repair at the time of foaling is almost never successful.[8] The longer the injury is left untreated, the more opportunities for continual contamination of the reproductive tract; however, this is related to functional capabilities of the vestibular sphincter, and many people will wait until weaning if a live foal was delivered. The cervix must be examined before surgical correction of PL, and if a prolonged time between foaling and repair has occurred, a full reproductive evaluation including biopsy is warranted.

Surgical technique. There are many methods described. Most importantly, the procedures are modifications of either a single- or two-stage repair. A modification of the single-stage repair is the technique we prefer.[9]



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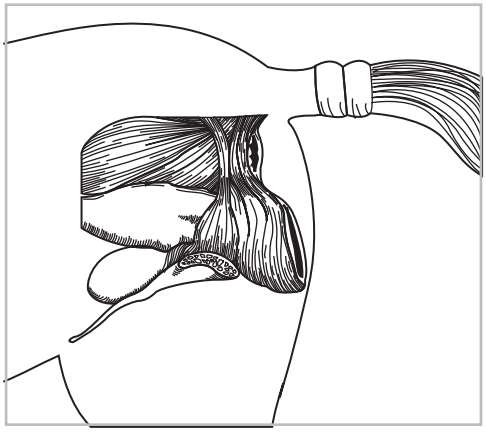

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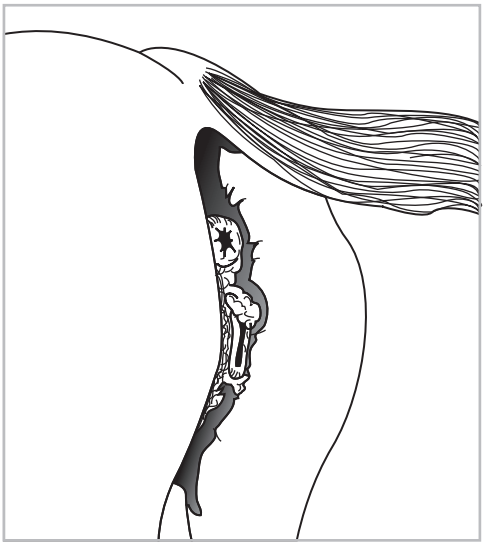

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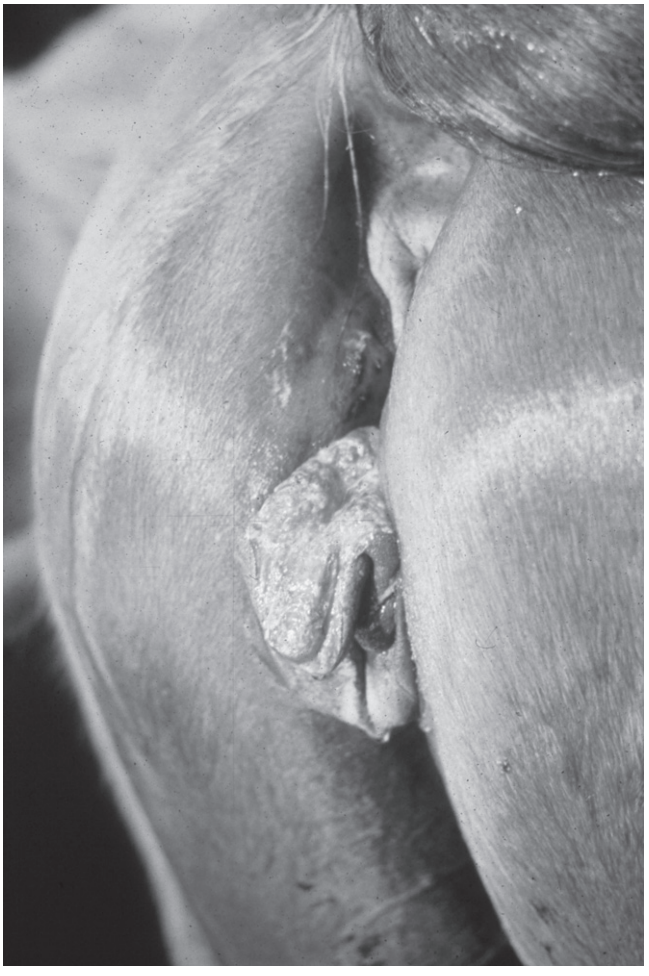


E

Figure 24-5 **A,** The ageing process of the mare and her conformation has allowed the vestibule and vagina to be positioned mostly above the pelvic brim. **B,** This conformation results in pneumovagina and occasional urine pooling. **C,** The musculature associated with the rectum and vestibule/vagina must be transected to allow the vulval lips to relax caudal. **D,** The surgery often results in the tip of the vulva relaxing to create a shelf if the incision is left not sutured. **E,** The final result, about 48 hours after surgery.

Before surgery vigorous efforts are made to modify the consistency of the feces. Mares are held off feed for at least 24 hours and given a mineral oil drench (2-3 liters) before beginning surgery. After surgery mares are placed on pasture if available, and mineral oil is administered by stomach tube daily for 3 days as necessary to maintain a soft fecal consistency.

Mares are restrained standing, tranquilized, and epidural anesthesia is employed.

Fecal material from the rectum is removed as far cranial as possible. Large wads of cotton are inserted into the cranial rectum to absorb fecal fluid and prevent fecal contamination of the surgical site. Tissues are cleansed and prepared for aseptic surgery. Towel clamps are inserted into the ventral anal sphincter in a configuration that when apposed represent the ideal surgical apposition point. In addition, towel clamps are placed on the dorsal vulval commissure and then retracted to provide visualization for surgical access or, alternatively, stay sutures are used to retract the vulva. An incision is made along the scar tissue line marking the junction between the vestibule/vagina and rectum. Tissues ventral to the incision are dissected to create mobilized vestibular mucosa and submucosa that when apposed from side to side will form the ventral border of the perineal body. Tissues dorsal of the incision are debrided, and rectal submucosa is undermined to allow sufficient mobilization to invert by side-to-side apposition the ventral border of the terminal rectum. All tissues are then sutured concurrently and incrementally from cranial to caudal. The rectal and vaginal reflections are apposed by a continuous modified inverting Connell suture. Suture material preferred is a No. 1 delayed absorbable (i.e., PDS) for the fibrous layer, 0 PDS for the vagina, and 2/0 for the rectal mucosa. When the suture pattern of the rectal submucosa has proceeded 2 to 3 cm, the suture material is retracted and then the vaginal or vestibular submucosa is similarly apposed. The resulting dead space in between, the *recreated perineal body*, is obliterated by multiple interrupted or continuous apposition sutures. The three areas of apposition are then alternatively progressed caudally, 2 to 4 cm each time, diverging at the perineal body, until the ventral vestibular submucosa stitch can be tied and vulval lips closed by a Caslick. The apposing suture of the rectal submucosa is terminated at the dorsal perineal body at the level of the defect in the anal sphincter. The anal sphincter may or may not be apposed at all. Adequate dissection of vestibular and rectal submucosa is necessary to prevent undue tension on the continuous suture patterns.

Postoperatively, apart from antibiotics, anti-inflammatories, and protection against tetanus, the management of fecal consistency is most important (Figure 24-6).

Two-stage repair. In the first stage the aim is to recreate the rectovestibular shelf (i.e., close the vestibular cavity and recreate a portion of the ventral perineal body). Because the rectal mucosa is not apposed and anal sphincter not repaired, there is less tension on the sutures, minimal straining, and potentially less dehiscence.

The surgical technique is described elsewhere; however, it is very similar to the single-stage repair except the rectal mucosa and dorsal perineal body are not apposed until 3 to 4 weeks later (second stage).[8,10]

Points for consideration

- Techniques are largely dictated by experience. We believe inexperienced surgeons will have less difficulty with the two-stage repair.
- The better the surgeon's anatomical and functional understanding of the perineal body, the more likely a favorable outcome will follow.
- If a two-stage repair is attempted, economic consideration for the client appears warranted as single-stage repair is just as successful with experienced surgeons.
- Fertility is good after successful repair. A slightly higher incidence of perineal trauma is expected with these mares.

Rectovestibular Fistula (RVF)

RVF most commonly results from foaling injuries, although it can occur from breeding, sadism, or other accidents. Some will heal without surgical intervention, so surgical correction is attempted at least one month or more after the injury. In the last five years we have been breeding the mares on an induced (prostaglandin) second postpartum estrous period and then immediately (within 2 days) performing the fistulae repair. The fertility of mares undergoing this technique has been excellent (~75% per cycle). For this to be successful, as much fecal material as possible is removed immediately before breeding (either natural or AI). After breeding the uterus is lavaged at least daily until repair is performed.

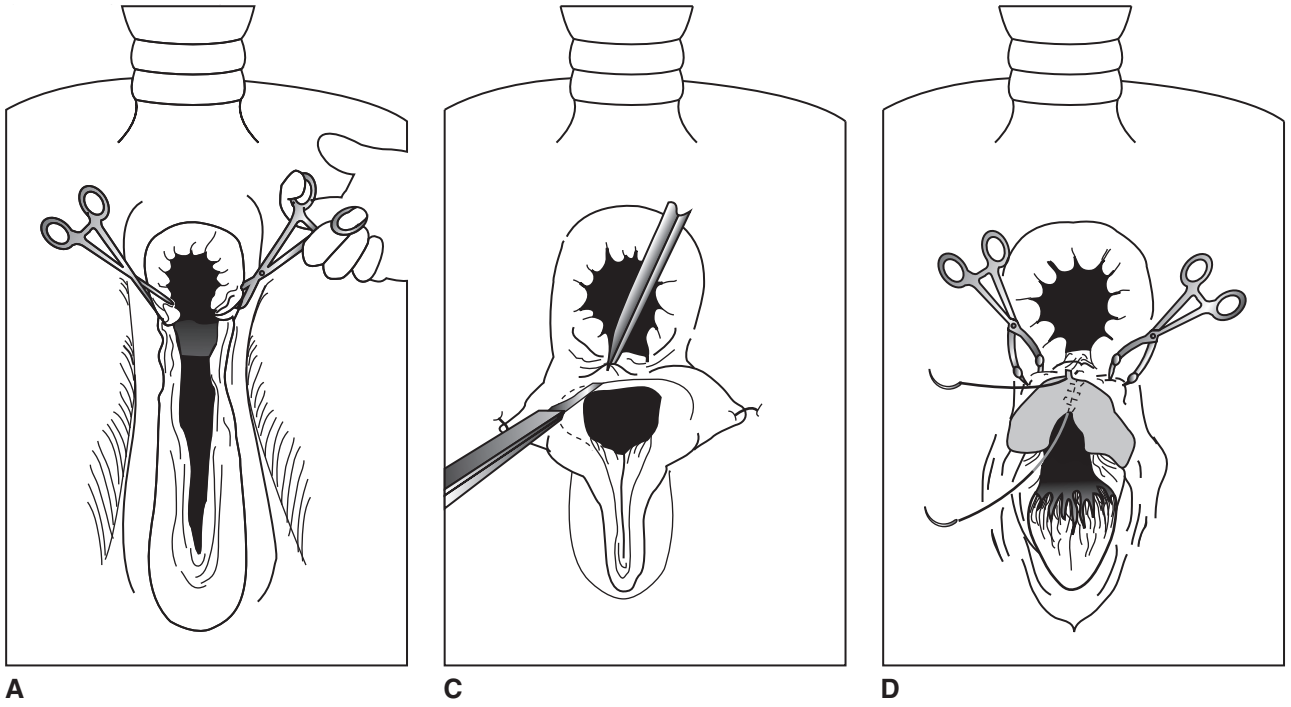
Most surgeons treat RVFs by converting them to third degree PL and repairing as previously described, either standing or under general anesthesia.[11] For deep (cranial) RVFs, a perineal body transection has been utilized.[9]

During the last 15 years we have repaired RVF with a transrectal approach, which we first described in 1991.[12] A similar technique was described in the 1996 AAEP proceedings by Dr. Stephen Adams.[13] In our hospital, modified Finochetto retractors (Scanlan Instruments, Englewood, Colorado) are inserted into the rectum through the anal sphincter.[9] These retractors make the surgery quite simple. The fistula is debrided and closed in three layers, with continuous suture patterns and without conversion into a third degree PL. The vestibular portion may be closed through the vagina or rectum and is inverted into the vestibule. The middle layer (perineal body) is closed from the rectum, and the rectal portion inverted into the rectum. When we previously described the surgical correction, it was recommended to alternate the orientation of the closure.[12] Now we recommend that all three layers are closed transversely, not longitudinally. The fistula middle layer is closed with 0 or 1 PDS, the vagina with 0 PDS, and the rectum with 0 or 2/0 PDS (Figure 24-7).

Restraint and postoperative care is similar to mares with perineal lacerations; however, not as much attention is given to fecal softening.

Points for consideration

- The caudal border of the fistula may be difficult to debride and is most commonly rotated toward the surgeon by grasping it with forceps (from the rectum) and everting it caudally. On occasion it may be helpful to have the surgeon's hand in the vagina while working on this part of the fistula. The use of a No. 12 blade greatly facilitates dissection of the fistula ring.



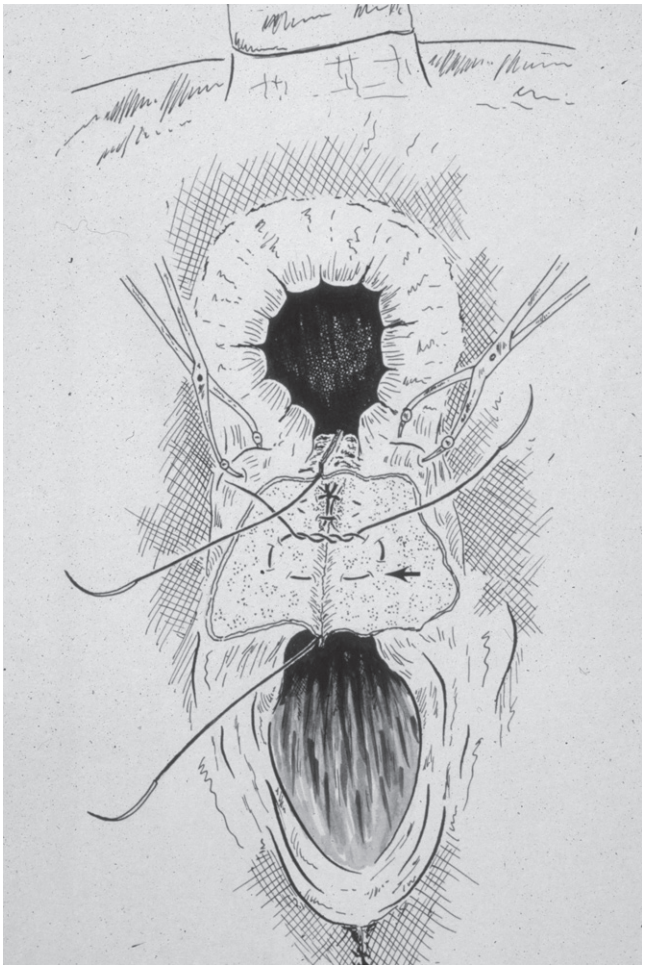


Figure 24-6 **A,** Towel forceps are used to identify the ideal position of the rectum at the end of the surgery. **B,** Typical appearance of a mare before surgery. **C,** The incision before beginning dissection. **D,** After dissection of the scar tissue, the tissues are sutured according to their origin (rectal mucosa, perineal body, and vaginal wall). **E,** The three suture patterns are incrementaly increased to ablate dead space in the perineal body and provide integrity of the rectal and vaginal layers.

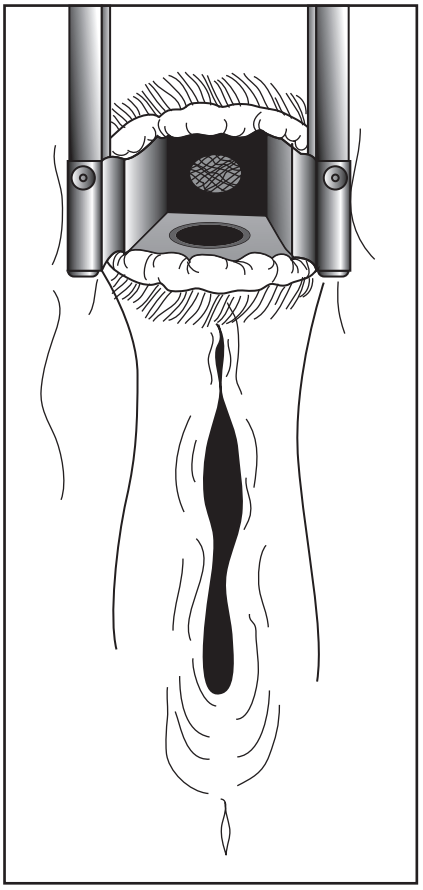
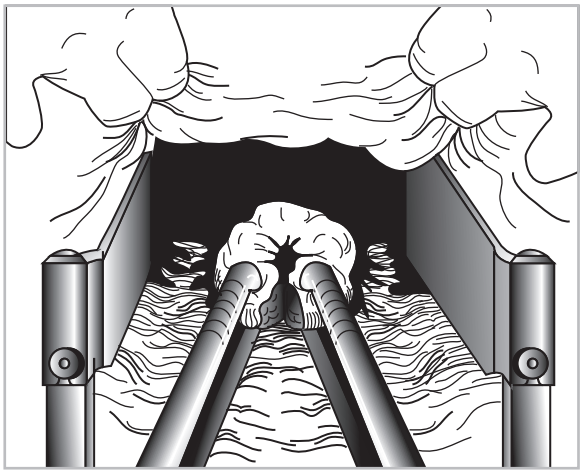


Figure 24-7 The fistula is easily approached per rectum if good retractors are available



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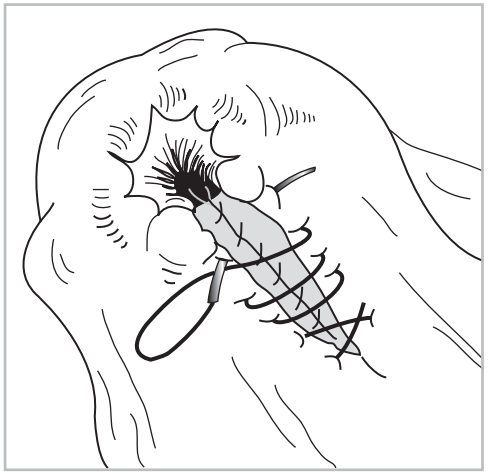

B

Figure 24-8 **A,** Knowles forceps applied to either side of the laceration facilitate the retraction and identification of the best place to debride and suture. **B,** Suture pattern recommended for cervix surgery.

Cervix

Cervical Lacerations

Injuries to the cervix most commonly occur at foaling. Many are not associated with a recognized dystocia. Assessing degree of compromise to the cervix as a barrier to intrauterine infection may be difficult. In general, examination immediately after foaling will reveal only the most major defects. Most cervical lacerations are identified later and are best evaluated in diestrus because increased tone facilitates assessment of degree of apposition of cervical folds and damage to the muscular layer. Manual palpation is essential for accurate diagnosis. Many mares with major cervical defects are still able to conceive and carry a foal to term.

In general only those mares with a cervical defect that progresses cranially to involve the junction of the external Os with the vagina are candidates for surgery at our practice. In addition, only mares with obvious subfertility are usually considered (i.e., uterine infection, barren one or more years, etc.)

Surgical technique. Mares are restrained standing and tranquilized. Local anesthesia is preferred with 40 to 60 ml of 2% lidocaine infused dorsally and laterally deep in the vaginal tissues around the cervix.

The most difficult aspect of cervix surgery in the mare is adequate exposure. Modified Finochietto retractors are preferred, and the cervix is drawn caudally by Knowles forceps.[9]

The forceps used must have large teeth that facilitate retraction with vigorous caudal pressure without tearing out of the cervix. With this technique it is generally not necessary to use specially extended cervical instruments (Figure 24-8).

The forceps are placed about 1 to 2 cm on either side of the defect or laceration. Slight rotational movement provides excellent access to the fibrous scar of the defect. The scar is debrided to the level of the cervical musculature, and the external vaginal mucosa of the cervix is slightly undermined. The internal cervical mucosa is undermined and trimmed. Original reports of cervical closure describe a three-layer closure.[9] We prefer two. The internal layer is closed with a 1 PDS with a cutting needle by a continuous pattern through the muscular layer. The external layer is closed with a simple continuous layer using 2/0 or 0 PDS. Occasionally it is necessary to trim excessive cervical folds that prolapse into the suture pattern and result in fistula formation; however, care in

not removing too much is recommended to avoid creating a cervix with major orientation deviations. In addition, it is easy to inadvertently include cervical mucosa on the wrong side of the lumen if the cervical forceps are not positioned well. Patency of the cervical Os is continually confirmed during placement of sutures.

Postoperative treatments for the mare are antibiotics and protection against tetanus. After 21 days, patency of the lumen is assessed by careful manual examination. Frequently, mild adhesions attempt to form. Their occurrence is of no major concern, and gentle manipulation and daily application of a corticosteriod, antibiotic, and oil-based cream, after the surgical site has healed, result in return to normal function.

Points for consideration

- The most craniad portions of the external cervical Os appear to be the most important barriers to infection. Overzealous surgical apposition of the caudad cervix results in a very small external Os that broadens cranially and does not create a good enough seal in diestrus or relax enough during estrus to allow adequate drainage of uterine contents.
- Previously repaired cervix problems often are injured again at breeding and/or foaling. AI is recommended, or a breeding roll at the very least, if natural service is necessary.

Ovaries

Ovariectomy

Some indications for ovariectomy include: mares displaying continual annoying nymphomania; ovarian neoplasia; use of ovariectomized steroid treated mares as recipients for embryo transfer; or mares to be used as tease/jump mares (with or without exogenous hormone administration).

Most techniques describe ovariectomy by a ventral midline approach with ovarian pedicle ligature before excision. However, this is expensive and in our experience occasionally unnecessary. Ovariectomy by colpotomy (vaginal approach) with hemostasis and excision performed with an ecraseur ("crusher") is an approach not often utilized, despite having been described centuries before the advent of powerful restraining pharmaceuticals. Of course there are many occasions when flank or ventral midline approaches will be necessary, particularly for mares with ovarian neoplasia.

Colpotomy

Mares are not fed for 24 hours before surgery. Mares are restrained standing and tranquilized. The tail is secured dorsally. The external perineum is cleansed, and the vaginal cavity is lavaged with sterile saline (1 liter). No epidural anesthesia is necessary. Blunt/Blunt scissors (Metzenbaum) are used to puncture the cranial vaginal wall in a position between 4 and 5 o'clock relative to the external Os of the cervix and near the border of the pelvic canal. The scissors are advanced 4 to 6 cm, then opened and withdrawn through the vaginal wall in the opened position. The resultant hole enables a single finger to bluntly enlarge the opening to admit a hand. The peritoneum is punctured with a single digit, and ovaries and uterus identified and palpated. Swabs that have been previously sewn together and then sterilized are soaked with 50 ml of local anesthetic and applied circumferentially to each ovarian pedicle for at least 1 minute each. A "Hauptner" ecraseur is used to remove each ovary individually. Frequently the ovarian attachments to the ovary have to be manually stretched to allow ovarian removal. The vaginal incision is not closed; however, we recommend a Caslick be performed.

Mares are treated with anti-inflammatory medication (i.e., phenylbutazone, 2 g IV) approximately 10 to 20 minutes before beginning surgery. Delaying phenylbutazone administration or use of oral preparations may result in moderate abdominal discomfort manifested by sternal recumbency. Other prophylactic administrations are antibiotics and protection against tetanus. Mares are not cross tied but usually are boxed overnight and then turned out onto pasture the next day.

We have used this technique in many mares, including mares with small granulosa theca cell tumors up to approximately 10 cm, without complication.

Reported complications are evisceration, hemorrhage, removal or penetration of bowel, fatal peritonitis, and local infection. The incidence of these problems is likely related to the experience of the surgeon and speed of the surgery.

Clearly the technique has potential drawbacks (i.e., size of ovary or structure is limited to less than approximately 10 cm). However, the procedure is safe and efficacious in many instances and able to be performed expediently by personnel experienced with examination of female reproductive tract.

Points for consideration

- Occasionally some mares strain during the procedure. Allowing more time for improved analgesia or additional analgesia appears effective.
- Many of the complications such as evisceration and infection appear to be minimized by presurgical administration of IV phenylbutazone to prevent postsurgical recumbency; a Caslick; and prophylactic antibiotics.
- The procedure is safe, expedient, and efficacious, and the complication rate is similar to or less than male castration.
- Mares with uterine infection or urovagina are not considered for this technique.

Uterus

Uterine Torsion

Uterine torsion is an infrequent but serious complication of the late gestation mare. Most occur in the last two months of gestation and are recognized by mild to moderate colic that responds temporarily to analgesics. Unlike cattle, most uterine torsions of the mare are preterm and not associated with parturition. Definitive diagnosis is based on careful rectal examination. Identification of the ovarian pedicle and uterine ligaments should suggest whether the rotation is clockwise (to the right and downwards when viewed from behind) or counterclockwise. The uterine ligament on the side to which the rotation

has occurred is pulled directly downward and under the uterine body. The other uterine ligament is displaced medially and runs over the uterine body toward the side of rotation and then downward. The greatest tension is on the ligament that the rotation is turned toward. Occasionally diagnosis of direction of torsion is difficult per rectum.

Technique for correction depends on stage of gestation and value of the animal. At term, manual rotation of the fetus through the cervix is often possible. Before imminent parturition, nonsurgical repositioning (rolling) is possible but may occasionally result in uterine rupture.

The mare is positioned in lateral recumbency on the side to which the uterus has rotated (i.e., clockwise torsions result in the mare being placed in right lateral recumbency). The mare is quickly flipped over to her other side and hopefully uterine weight allows the mare's body to pivot about the fetus and reposition the problem. Degree of success can be ascertained by repeated rectal palpation. Another approach is to place a board with weight (i.e., a person) on the upper flank and turn the mare slowly. In this case the board keeps the foal in position during rotation.

Alternative techniques for correction include standing flank laparotomy and ventral midline approach. Standing flank laparotomy has been our preferred method for correction if the mare and/or foal are valuable and there is no evidence of uterine rupture. This technique has a lower probability of creating uterine rupture and avoids the stress of anesthesia.

Standing Flank Laparotomy

For standing flank laparotomy, the mare is moderately sedated, and the paralumbar fossa on the side toward which the rotation is directed is clipped and prepared for aseptic surgery. Local anesthetic (40-80 ml) infused in a vertical line is administered at or slightly cranial to the anticipated incision in the middle of the paralumbar fossa. The skin and paniculus (cutaneous) muscle are incised vertically, and external and internal abdominal oblique muscles are separated along their direction of orientation (grid approach). The transverse adominus is bluntly dissected and the peritoneum punctured with a single finger. The direction of the torsion is confirmed, and a prominent part of the foal under the uterus is grasped and used to gently rock and lift the uterus toward the surgeon. Resolution of the torsion is usually immediately obvious; however, on occasion, lengthening the incision to allow two hands to enter the abdomen or a bilateral flank incision with two surgeons is sometimes necessary. The external and internal abdominal oblique muscles and subcutaneous tissues are apposed by a simple continuous suture pattern with a No. 1 or 2 absorbable material. The skin is closed with a continuous simple or interlocking pattern of nonabsorbable material.

Points for consideration

- Abdominal ultrasonography should be used to verify that the fetus is still alive and, if possible, assess uterine integrity.
- Survival of the mare and/or foal may be influenced by degree of uterine torsion, ease of correction, elapsed time before correction, and stage of pregnancy.
- Regardless of the stage of gestation, if the cervix is open at the time of recognition of uterine torsion, delivery after standing intravaginal/intrauterine manipulation or ventral midline access for reposition and caesarian section is preferable to standing flank laparotomy or rolling of the mare.
- Occasionally a standing flank laparotomy will not have a successful surgical outcome due to the degree of rotation. If a more severe rotation (>360 degrees) is suspected or an excessively elevated heart rate (>70 beats/min) is identified, it may be better to perform a ventral midline laparotomy to better assess uterine health and gain the best physical advantage for uterine positioning. In addition, the fetus can be removed.

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Seasonal Movements and Home Ranges of Feral Horse Bands in Wyoming's Red Desert

RICHARD MILLER

Abstract

Feral horses have seasonal movement patterns which correspond to their use of water sources and areas near ridges. Home ranges of horse bands varied in size from 73 to 303 km². Some bands shared use of a common home range. Those bands having a common range followed similar movement patterns within their home ranges. A herd is defined as a structured social unit made up of bands following movement patterns within a common home range.

Movements of feral horses can be important to managers in determining the effects of the horses on the range and in setting boundaries of herd management units. Previously reported feral horse home ranges have varied from 0.9 km² on Sable Island (Welsh 1975) to 78 km² for a spring and summer home range in Stone Cabin Valley, Nev. (Green and Green 1977). As part of a general study of feral horses in Wyoming's Red Desert (Miller and Denniston 1979, Boyd 1980, Miller 1980, Miller 1981, Miller 1983), movements and home ranges of the horses were determined. The objectives of this portion of the study were to determine the size of band use areas and to determine if a pattern existed in time of use for any one area.

Methods

Research was begun in June 1976 and continued until September 1979. Bands were identified with a photo file and card file system keyed on color combinations and readily identifiable individual horses. When bands were identified their locations were plotted to quarter section of USGS to topographic maps. Bands were located from the ground using binoculars and a spotting scope. A number of locations were obtained while working on other portions of the study, such as while following breeding horses. When a band had not been located for 2 weeks, the known use area of that band and adjacent areas were searched. Between November 1977 and April 1979 bands were also located from an airplane during 16 flights made to study habitat use of feral horses and cattle (Miller 1983).

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In determining home ranges I modified Mohr's (1974) minimum home range method in that 2 outside points were not connected if the area which would be included was easily observed and regularly covered during field work. Any band using such an area should have been easily spotted. I made this modification to avoid inflating home range estimates.

Results

Only one of the home ranges I determined in the Red Desert fell within the range of previously reported sizes (0.9 km² to 78 km²) (Welsh 1975, Green and Green 1977). Most home ranges were larger than any previously reported (Table 1). Locations and identification of bands was much more difficult during winter, as bands were much more wary in winter. A band which we could regularly approach within 400 m in the summer often could not be approached within 1 km in winter. This wariness, combined with limited access to the area and fewer field days available during fall and winter, limited the number of fall and winter locations.

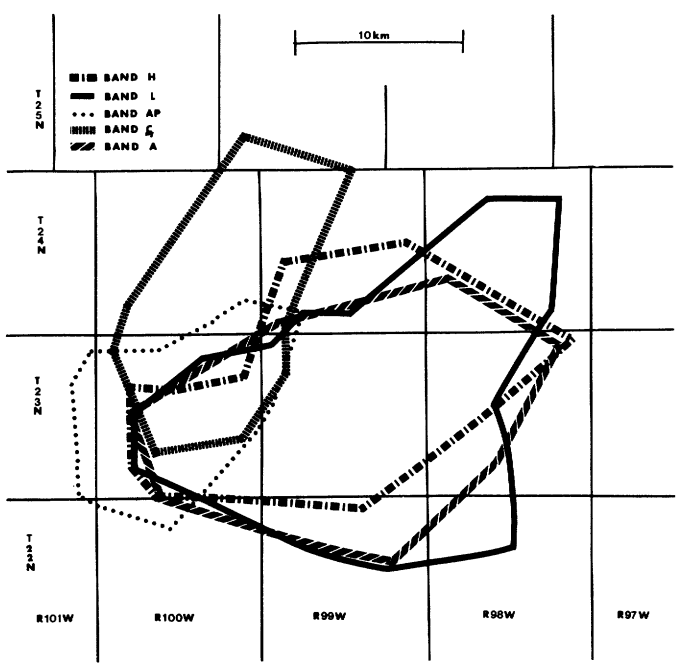

Fig. 1. *Home ranges of Bands A, H, and L from Herd #1 and Bands AP and CZ from other herds.*

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Table 1. Home ranges of feral horse bands in Wyoming's Red Desert.

Band	Home range size (km²)	Part of herd #1	Dates	Number of locations by season			
				Dec.–Mar.	Apr.–May	June–July	Aug.–Nov.
A	264	yes	8/76–2/79	5	8	26	19
B	197	yes	8/76–7/79	2	14	28	16
N	233	yes	8/76–9/79	6	12	52	22
L	303	yes	6/76–8/79	5	40	67	19
Y	73	no	8/76–10/79	1	6	51	16
AC	158	yes	4/77–10/79	1	2	58	6
AE	88	?	6/77–8/79	0	4	33	8
AP	101	no	6/77–9/79	0	10	57	9
CV	166	yes	10/77–10/79	0	7	29	9
CZ	145	no	5/77–7/79	1	7	30	15
AL	93	no	8/76–7/79	1	7	40	13

The home ranges of some horse bands in the Red Desert overlap almost completely (Fig. 1, Bands A, H and L), and I believe these bands can be said to have a common home range. Bands with a common home range follow similar movement patterns within that home range. An illustration of this is that bands with similar movement patterns were often first located and last located near a partcular well or foraging site with a few days of each other (Table 2).

Figure 1 illustrates home ranges for bands from more than one herd and shows that herd home ranges do overlap. Association of bands from different herds is not unusual at a water source or feeding site. All bands from one herd do not always move at the same time but usually follow similar patterns in their movements and usually move as groups of bands (Table 2).

Bands of horses followed very similar movement patterns each year of the study, arriving in a portion of their home range at approximately the same time each year. In effect, they followed something similar to a rotation grazing system in which the same pasture is used at the same season each year. A composite of the seasonal locations of 6 bands which had overlapping home ranges and similar movement patterns (Fig. 2) shows discrete winter and summer seasonal use areas, indicating the similarity in movements and habitat selection. The large sizes of the April–May and the August–November seasonal ranges (Fig. 2) reflect the variation in weather conditions during those 2 seasons and the responses of the

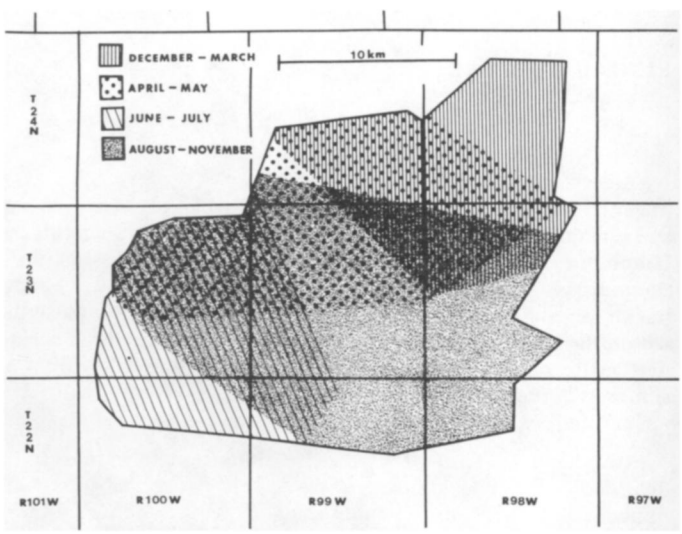

Fig. 2. *A composite of the home ranges and seasonal locations of Herd #1, Bands A, B, H, L, AC and CV.*

Table 2. Dates of locations of bands from Herd #1 at selected points in the Red Desert.

	Band					
	A	B	H	L	AC	CV
1976						
Last located at 4th of July well	8/8	9/26	8/15	—	—	
First located at well in S7,T23N,R99W	—	8/28	8/28	8/28	—	—
Last located at well in S7, T23N, R99W	—	9/10	10/16	9/25	—	—
1977						
First located at well in S7,T23N,R99W	4/24	7/7	7/6	4/30	5/28	—
First located at 4th of July well	6/10	5/25	6/10	6/10	6/10	-
Return to well S7,T23N,R99W	9/24	7/7	7/6	7/7	7/6	—
Last Located at 4th of July well	9/2	9/3	9/3	6/27	6/27	9/2
1978						
First located at well S7,T23N,R99W	4/23	—	—	4/23	—	—
First located at 4th of July well	6/7	6/7	6/7	6/8	6/15	6/17
Last located at 4th of July well	8/11	7/27	8/11	7/14	7/14	7/12
First located at El Paso well #1	9/9	9/9	9/9	7/27	7/27	7/27
Last located near Chandler-Simpson well	10/1	9/9	10/1	8/10	9/17	8/10
1979						
First location at well in S7,T23N,R99W	—	—	—	5/28	—	—
First location at 4th of July well	5/28	5/19	5/21	5/21	6/10	5/20
Last location at 4th of July well	disbanded	6/13	7/16	7/30	7/1	6/20

Dates given are those for locations and may not be the exact dates when bands arrived at or left any site, but they do serve to illustrate similarity and dissimilarity in the timing of movements for bands within one herd.

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horses to weather (Miller 1983).

Discussion

An interband dominance hierarchy has been described for the bands whose movements are reported here (Miller and Denniston 1979). Miller and Denniston (1979) suggested that a structured social unit made up of bands existed and defined that unit as a herd. The data presented here show that some of these same bands follow similar movement patterns within overlapping home horses. An expansion of the definition of a herd given in Miller and Denniston (1979) follows: a herd is a structured social unit made up of bands following similar movement patterns within a common home range. An examination of the home ranges presented in Green and Green (1977) and in Feist and McCullough (1976) suggests that herds may also be found in other feral horse populations.

Herds may be a deme or a breeding unit and could show significant morphological variation as found in demes of Uganda kob (Buechner and Roth 1974). Support for this idea is found in the variation in the proportion of certain coat colors seen in horses from different localities in the Red Desert. Pintos and greys, which are infrequently seen in the population as a whole, are common in certain areas.

Clutton-Brock and Harvey (1977 and 1978) suggested that large home ranges result where resources are strongly clumped. widely dispersed or unpredictable in abundance. Altmann (1974) and Crook et al. (1976) have predicted extensive home range overlap where several essential resources have very restricted distributions. Denham (1971) made a similar argument that clumping of populations should be high where predictability of resources is low. W ter sources and ridges which are used as cover in the Red Desert can be considered strongly clumped or as having restricted distributions and as being widely dispersed from one another. Marquiss (1957), studying vegetation composition and ground cover of relict areas in the sagebrush grassland type adjacent to my study area, found variations in ground cover and composition which were significant at the 0.01 level between sites and between years. From his data I believe vegetation and hence forage in this section of the Red Desert may be considered both patchy and unpredictable in availability from year to year. If these factors are producing the large home range sizes, extensive home range overlap, and strongly clumped population distributions found in the Red Desert horses, then changes in the distribution and/or availability of resources can be expected to change home range and movement patterns.

Herds of horses, because they follow similar seasonal movement patterns and thus have similar habitat using patterns within a common home range, are logical management units for future feral horse mangement in the Red Desert.

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The One-Migrant-per-Generation Rule in Conservation and Management

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Abstract: *In the face of continuing habitat fragmentation and isolation, the optimal level of connectivity between populations has become a central issue in conservation biology. A common rule of thumb holds that one migrant per generation into a subpopulation is sufficient to minimize the loss of polymorphism and heterozygosity within subpopulations while allowing for divergence in allele frequencies among subpopulations. The one-migrant-per-generation rule is based on numerous simplifying assumptions that may not hold in natural populations. We examine the conceptual and theoretical basis of the rule and consider both genetic and nongenetic factors that influence the desired level of connectivity among subpopulations. We conclude that one migrant per generation is a desirable minimum, but it may be inadequate for many natural populations. We suggest that a minimum of 1 and a maximum of 10 migrants per generation would be an appropriate general rule of thumb for genetic purposes, bearing in mind that factors other than genetics may further influence the ideal level of connectivity.*

La regla de Un-Migrante-Por-Generación en Conservación y Manejo

Resumen: *Debido a la continua fragmentación y aislamiento de habitats, el nivel óptimo de conectividad entre poblaciones se ha convertido en un tema central en la biología de la conservación. Una regla común sostiene que un amigrante por generación en una subpoblación es suficiente para minimizar la pérdida de polimorfismo y heterocigosidad en las subpoblaciones y al mismo tiempo permite la divergencia de frecuencias alélicas entre subpoblaciones. La regla de un-migrante-por-generación se basa en numerosas suposiciones simplifican que tal vez no sucedan en poblaciones naturales. En este trabajo examinamos las bases conceptuales y teóricas de la regla y consideramos factores genéticos y no genéticos que influyen en el nivel deseado de conectividad entre subpoblaciones. Concluimos que un migrante por generación es un mínimo deseable, pero puede ser inadecuado para muchas poblaciones naturales. Surgerimos que un mínimo de uno y un máximo de diez migrantes por generación seria una regla general apropiada para fines genéticos, sin olvidar que factores no genéticos pueden influir en el nivel ideal de conectividad.*

> . . . evolution depends on a certain balance among its factors. There must be gene mutation, but an excessive rate gives an array of freaks, not evolution; there must be selection, but too severe a process destroys the field of variability, and thus the basis for further advance; prevalence of local inbreeding within a species has extremely important evolutionary consequences, but too close inbreeding leads merely to extinction. A certain amount of crossbreeding is favorable but not too much. In this dependence on balance the species is like a living organism. At all levels of organization life depends on the maintenance of a certain balance among its factors.
>
> —S. Wright (1932:365)

Introduction

By the 1930s Sewall Wright had clearly established—based on experiments and historical breeding records—

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that inbreeding depression in small, isolated populations typically leads to adaptive decline and extinction (Provine 1986). In Wright's view small amounts of gene flow between partially isolated demes are necessary for long-term persistence. Prompted by Wright's work, the question of how many immigrants ("migrants" in population genetics literature) are needed to prevent population differentiation became the subject of a vast body of theoretical analysis (for review see Slatkin [1985, 1987]). From this analysis emerged a genetic rule of thumb that one migrant individual per local population per generation (OMPG) is sufficient to obscure any disruptive effects of drift (Spieth 1974; see also Kimura and Ohta 1971; Lewontin 1974).

Biologists concerned with population insularization caused by habitat fragmentation began advocating the application of this principle for conservation purposes. Several of the most cited papers advocating, in some sense, the movement of one individual per generation between isolated populations for conservation purposes include Franklin (1980), Frankel and Soulé (1981), and Allendorf (1983). More recently, the OMPG concept has been widely applied by scientists and managers charged with initiating connectivity between potentially isolated populations. For example, Gogan (1990) makes the statement for mammals in general that "Immigration to a subpopulation of only one individual once every five years reduces concerns about inbreeding depression to a negligible level." Triggs et al. (1989) suggest for the critically endangered kakapo (*Strigops habroptilus*) that "In future generations, exchanges of one young kakapo per generation between populations should be made to help maintain genetic variation and reduce inbreeding."

Mace and Lande (1991) have used the OMPG rule as a criterion for fragmentation in defining threatened species categories of the World Conservation Union. In the United States nearly every recovery plan that considers genetic issues and insularization applies the OMPG rule. For example, the black-footed ferret (*Mustela nigripes*) recovery plan states that "If the effective population size is to be maintained among many smaller populations, then exchange of genetic material at the rate of one individual/generation between local populations to ensure random and adaptive divergence among populations is necessary" (U.S. Fish and Wildlife Service 1988:18).

The simplicity of the OMPG—and its widespread adoption—belies confusion over the derivation of the rule, its real generality, and the specifics of implementing it (Varvio et al. 1986). Indeed, immediately after stating that subpopulations would not become fixed for different alleles if there were "an interchange of only one individual every other generation," Wright noted that violations of simplifying assumptions may mean that ". . . an interchange of the order of thousands of individuals per generation between neighboring subgroups of a widely distributed species might well be insufficient to prevent a considerable random drifting apart in their genetic compositions" (Wright 1931:128). Thus, from the outset Wright acknowledged the complicating role of the social, ecological, and genetic characteristics of immigrants; these real-world complexities are often neglected in contemporary reference to the OMPG rule.

Human-caused habitat modification is reducing many continuous populations to isolated or semi-isolated fragments (Soulé et al. 1988; Mills 1995). In light of these changes, concerns over the appropriate level of gene flow in fragments are well grounded, both because of the increased potential for demic extinction in isolated populations (Senner 1980; Soulé 1987; Lynch & Gabriel 1990; Mills & Smouse 1994) and because of potential consequences for adaptation to future disturbances (Soulé 1980; Frankel & Soulé 1981). The issue of actually prescribing gene flow in a management context is complicated, however, and it has received little empirical or theoretical consideration.

We describe the OMPG rule, present its theoretical underpinnings, and discuss its application and limitations from a genetics perspective. Finally, we make recommendations that apply both to highly manipulated systems, where managers physically move individuals, as well as to systems in which the movement rate is affected by the size and shape of available habitat or movement corridors.

The One-Migrant-per-Generation Rule

The one-migrant-per-generation rule originated in Wright's analysis of the interplay between genetic drift and gene flow in determining the expected patterns of genetic divergence among a series of subpopulations. Consider a large number of subpopulations in which genetic drift produces genetic divergence among the subpopulations at a rate inversely proportional to the local effective population size (N_e), which is assumed to equal the census size, N. Assume that in each generation a proportion m of the individuals in each subpopulation are migrants from outside that subpopulation and that the allele frequency in migrant individuals is the average allele frequency in all subpopulations. Such migration will limit the amount of divergence that will occur among subpopulations.

This model leads to the surprisingly simple result that at equilibrium the amount of divergence among subpopulations for reasonably small values of m is approximately

$$F_{ST} \approx \frac{1}{4mN + 1}, \quad (1)$$

where F_{ST} is the proportion of total gene diversity (heterozygosity) due to divergence among subpopulations. Note that because m is the proportion of migrants and N is the population size, mN is the actual number of migrants entering a subpopulation each generation.

The amount of divergence among subpopulations as measured by F_{ST} is independent of the total amount of gene diversity in a population (H_T). The F_{ST} can also be thought of as the reduction in heterozygosity within subpopulations (H_S) caused by random genetic drift and divergence among subpopulations. That is,

$$F_{ST} = 1 - \frac{H_S}{H_T}, \quad (2)$$

and F_{ST} is usually called the fixation index. In the case of more than two alleles, each allele may have a different fixation index. Nei (1973) introduced an analogous value (G_{ST}, the coefficient of genetic differentiation) that can be used to describe genetic differentiation for many loci with more than two alleles (Chakraborty & Leimar 1987).

This analysis has two notable results. First, the amount of divergence is dependent only on the number of migrants (mN) and is in a sense independent of population size. For example, we expect the same amount of divergence among subpopulations of size 1000 with 2.5% migration as we do among subpopulations of size 50 with 50% migration; in both cases there are 25 migrants per generation.

The dependence of divergence on the number of migrants, rather than the population size (N) or rate of migration (m), may at first seem counter-intuitive. Divergence, however, results from the opposing effects of migration and genetic drift. The larger the subpopulations, the slower they are diverging through drift; thus, proportionally fewer migrants are needed to counteract the effects of drift. Small subpopulations diverge rapidly though drift, and thus proportionally more migrants are needed to counteract drift.

The second notable aspect of this result is that remarkably small amounts of migration can have powerful genetic consequences on divergence among subpopulations at equilibrium; for example, OMPG is expected to yield an equilibrium F_{ST} of 0.2 (Fig. 1). If we examine the actual distribution of allele frequencies among subpopulations, it is clear that OMPG will also make it unlikely that polymorphism will be lost within subpopulations (Fig. 2; Wright 1931; Fig. 6). With at least one migrant per generation, subpopulations are unlikely to reach equilibrium gene frequencies where one allele or the other is lost (frequency of zero) or "fixed" (frequency of 1.0).

Nevertheless, substantial divergence in allele frequencies across populations is expected with one migrant per generation (Wright 1931, 1969; Allendorf & Phelps 1981). Thus, OMPG provides a desirable balance between drift and gene flow by preventing the loss of alleles and minimizing loss of heterozygosity within subpopulations but allowing genetic divergence to exist among subpopulations. Low amounts of exchange will maintain qualitative similarity among subpopulations and also allow substantial quantitative divergence in allele frequencies.

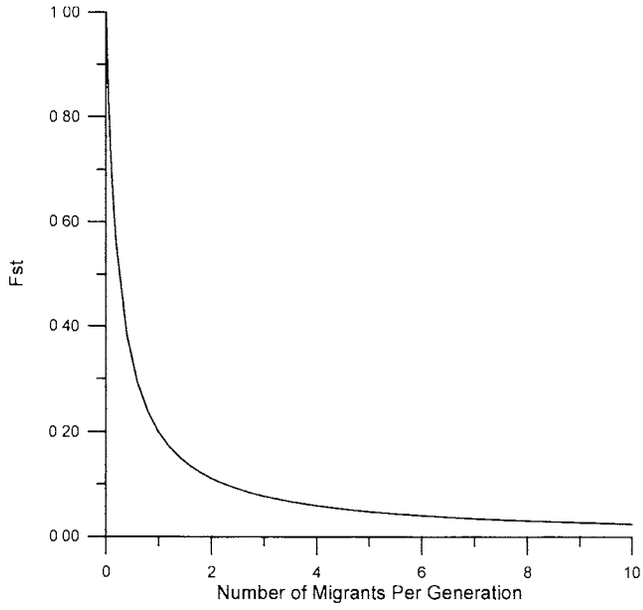

Figure 1. Relationship between number of migrants per generation (mN) *and the fixation index* (F_{ST}).

We want to emphasize that OMPG is not a discrete quantitative threshold and does not imply panmixia (random mating) among individuals of different populations. Rather, the basis of OMPG originates from a qualitative evaluation of tradeoffs between loss of genetic diversity within populations versus homogenization among populations.

Assumptions of the Rule

The OMPG rule results from a simple model of population structure based upon a host of unrealistic assumptions. We consider the primary assumptions that affect the applications of the results of this basic model to conservation.

(1) *Island model of migration*: A migrant is equally likely to come from any subpopulation so that there is no geographical pattern to gene flow. The assumption is also usually made that there are an infinite number of subpopulations of equal size.

(2) *Selective neutrality and no mutation*: There are no selective differences among genotypes, and no new mutations enter the population. That is, gene-frequency dynamics are determined entirely by the interaction between genetic drift and gene flow.

(3) *Ideal populations*: The subpopulations have the characteristics of an ideal genetic population, so that the census number of individuals equals the effective population size. For general purposes, the ideal population consists of a constant number of N diploid individuals ($N/2$ females and $N/2$ males) in which all parents have

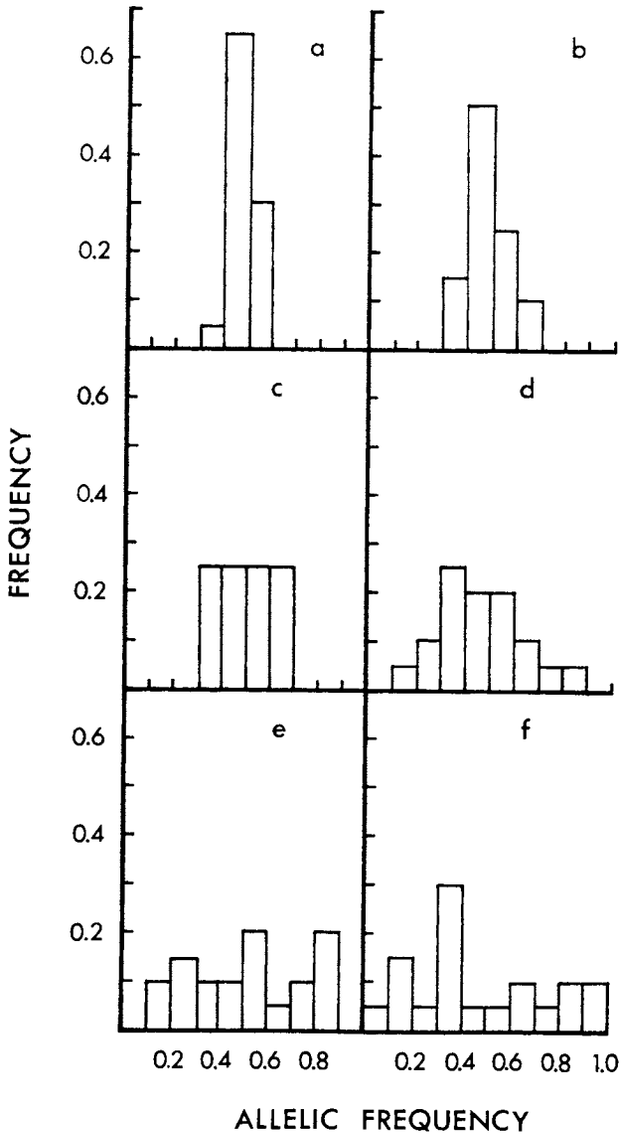

Figure 2. Distribution of allele frequencies in 20 subpopulations (N = Ne = *200 each) with different amounts of migration, based upon computer simulations of the island model for two alleles at a single locus (Allendorf & Phelps 1981). For panels a-e, number of migrants per generation decreases and* F_{ST} *increases, as follows:* mN = *25,* F_{ST} = *0.0080 (a);* mN = *10,* F_{ST} = *0.0175 (b);* mN = *5,* F_{ST} = *0.0509 (c);* mN = *2,* F_{ST} = *0.1104 (d);* mN = *1,* F_{ST} = *0.2513 (e); and* mN = *0.5,* F_{ST} = *0.3221 (f).*

equal probability of contributing an offspring to the next generation.

(4) *Demographic equality*: Immigrants have the same demographic attributes, in terms of survival and reproduction probabilities, as resident individuals.

(5) *Equilibrium*: The subpopulations persist long enough to reach steady-state or equilibrium conditions with respect to gene frequencies.

Limitations of the Rule

What are the consequences of violating the simplistic assumptions of the OMPG rule? We consider how population size may affect the predicted effects of OMPG, then evaluate each of its assumptions. In each case we determine whether each complicating factor is expected to increase or decrease the effectiveness of OMPG or whether "it depends."

Census Population Size

As noted in the derivation of the OMPG rule, one of its interesting and attractive features is that it is independent of population size, as long as gene dynamics are driven entirely by drift and gene flow (mutation and selection play no role). There are, however, at least two caveats to this generalization. First, mN migrants into a small population will represent a larger proportion of the population than if they had entered a larger population; any disruptive social or mating system effects of the immigration will therefore be magnified in smaller populations (Frankel & Soulé 1981; Frankel 1983).

Second, the usual formulation for the loss of genetic variation under drift-migration equilibrium (Eq. 1) is an approximation of the exact equation and holds true only when m (migration rate) is "small" (Wright 1969:291). For a fixed mN, such as one migrant, m becomes large with small population size. For example, when $N = 2$, $m = 0.5$, a large value that leads to less loss in variation (smaller F_{ST}), with the exact equation than expected with the approximation (Fig. 3). We note, however, that the overestimation of F_{ST} with the approximate equation is insignificant with subpopulation sizes large enough to be likely to persist in the face of demographic stochasticity for even relatively short periods of time. For example, for $N = 20$, the exact F_{ST} is 94% of the approximation.

Both of these issues imply that when populations become quite small less migration may be appropriate, both because of possible social disruption and because very small populations are not losing variation as fast as predicted by the F_{ST} approximation. Other factors, such as Allee effects and demographic or environmental stochasticity, lead to the opposite conclusion, with immigrants initiating an important "rescue effect" (Brown & Kodric-Brown 1977).

Island Model: Pattern of Migration

The immigrants in Wright's infinite island model are randomly chosen from the pool of an infinite number of subpopulations. If individuals in the real world come from nearby populations, their gene frequencies would differ little from the target population, so more immigrants would be necessary to prevent loss of variation

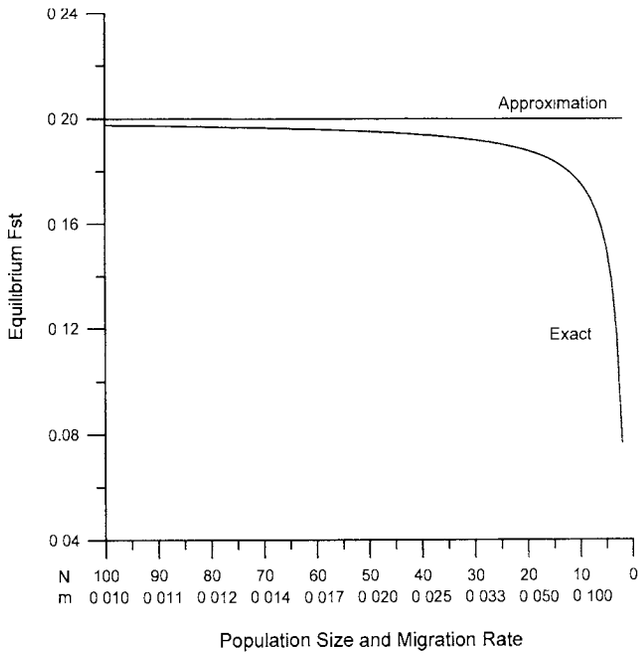

Figure 3. Expected F_{ST} *at equilibrium for one migrant per generation (*mN = *1) using the exact equation (from Eq. 12.2 in Wright 1969) and the commonly used approximation (Eq. 1). Unlike the approximation, which assumes small* m, *the exact equation changes as* m *changes.*

(Wright 1931; Levin 1988). Crow and Aoki (1984) conclude, however, that unless the number of populations is very large, this neighborhood effect is relatively small; in a finite population model, migrants from neighboring groups are about half as effective in their genetic effects. Kin-structured migration—in which individuals disperse in the company of relatives—will also have the effect of requiring more immigrants for the same effect on genetic drift (Levin 1988).

Directional movement patterns are an important and widespread exception to the assumption of the island migration model. In many situations migrants from a relatively large subpopulation may be moved into a smaller subpopulation that is isolated; this is referred to as a continent-island pattern of migration (Hedrick 1983:278). For example, grizzly bears (*Ursus arctos*) from the Yellowstone National Park region are isolated from other populations. The recovery plan for grizzly bears includes regularly moving bears from more northern populations in Montana that are contiguous with grizzly bear populations in Canada into the Yellowstone region (U.S. Fish and Wildlife Service 1993).

The genetic effects of this type of directional movement pattern are compatible with the OMPG rule. The isolated population can be considered one subpopulation of the island model in which migrants from the larger population represent the migrant pool of the island model. Thus, the predictions of the OMPG rule will be valid as long as the donor population is large so that genetic drift is minimal. Weishampel (1990) cautions that if the donor population has lower genetic variation than the receiving population, migration can actually decrease genetic variation in the population of interest.

Another characteristic of the pattern of migration is the interval between immigrants. In its simplest form the island model assumes that probability of migration per generation is constant. Will 1 migrant per generation have the same effect as 10 migrants every 10 generations? Lacy (1987:153) concludes that sporadic immigration should be just as effective as a steady supplementation, provided that the same long-term average rate is maintained. On the other hand, Levin (1988:645) concludes that "a variable migration rate homogenizes neutral allele frequencies less than a uniform migration rate with the same mean." This effect is quite small, however, based upon theory (Nagylaki 1979). Experimental work offers no insight into this question. In many cases the driving criteria for the timing of immigrants will probably not be genetic calculations but logistical concerns (transplanting one animal per generation will often be impractical) and behavioral and social considerations whereby groups of immigrants are more likely to experience successful reproduction.

In short, real-world deviations from the simple pattern of migration used to derive the OMPG rule mean that somewhat more than one migrant per generation may be necessary to achieve the desired genetic goals; the necessary increase will depend on particular circumstances, but it is probably small.

Island Model: Number of Subpopulations

The expected value of F_{ST} at equilibrium can be corrected as shown below to take into account a finite number (n) of subpopulations (Slatkin 1995):

$$F_{ST} \approx \frac{1}{4mNa + 1}, \tag{3}$$

where

$$a = \left(\frac{n}{n-1}\right)^2. \tag{4}$$

Total gene diversity (H_T) can be partitioned into within- and between-subpopulation components. Isolation causes a decrease in within-subpopulation variation (H_S), whereas fixation for different alleles maintains total gene diversity (H_T). As long as the subpopulations are connected by gene flow, an allele will eventually be lost from all subpopulations (and therefore the entire population) by genetic drift if there are a finite number of subpopulations. If the subpopulations are isolated, however, then different alleles will drift to fixation in differ-

ent populations, so more alleles will be maintained in the entire population.

There may be merit (Chesser 1983) in maintaining isolated populations so that more alleles can be retained in the entire population. Nevertheless, these benefits must be weighed against increased probability of extinction due to within-population inbreeding depression (Varvio et al. 1986; Mills & Smouse 1994). In short, the tradeoff between within- and among-population variation may dictate more or less migration than the OMPG rule does (Figs. 1 & 2).

Natural Selection and Mutation

The theoretical model upon which the OMPG rule is based assumes selective neutrality—that is, there are no differences in fitness among genotypes. Nevertheless, the OMPG rule itself does take into account natural selection. The OMPG rule was chosen because it is sufficient to avoid the loss of alleles in subpopulations caused by genetic drift but will allow the allele frequencies within subpopulations to respond to local selective pressures.

Even weak natural selection can have a major effect on the amount of genetic divergence among populations (Allendorf 1983). The effects of natural selection will be greater in larger subpopulations because of the reduced effects of drift in larger populations. In general, natural selection will not have a major effect on the distribution of allele frequencies if the selection coefficient (s) is less than the migration rate (m) (Wright 1940). Thus, natural selection will have less effect on allele frequency divergence among subpopulations with smaller subpopulations for the same amount of migration (mN) (Allendorf 1983).

The effect of selection upon the expected amount of divergence with a given amount of gene flow depends upon the pattern of selection. Similar selection in different subpopulations will tend to reduce divergence, whereas different selective conditions will increase divergence among subpopulations (Allendorf 1983). It is likely that both of these patterns of selection will occur at different loci within the same population. Thus, the OMPG rule seems appropriate when we consider the potential effects of natural selection.

Mutation is expected to have little effect on the application of the OMPG rule. The expected amount of divergence at equilibrium for G_{ST} (the multiple-allele form of F_{ST}) is independent of the mutation rate and the number of alleles, as long as the migration rate is much greater than the mutation rate (Hartl & Clark 1989). This condition will be met for all loci in which migration rates are on the order of one per generation ($mN = 1$) or greater.

Ideal Populations and Demographic Equality

The ratio of effective population size to census population size is expected to affect the consequences of varying migration rates. Wright (1931:128, 1969:292) noted that the smaller N_e is relative to the census N, the larger the number of migrants necessary to minimize differentiation. This is because as N_e decreases below N the population loses variation faster via drift, and a given migrant contributes less to the breeding population, than when N_e equals N. Therefore, populations with highly skewed N_e/N ratios, as is often the case in wild vertebrates (Frankham 1995), may require more than one migrant per generation. How many more are necessary is uncertain; presumably it will depend on why N_e is less than N (for example, because of skewed sex ratio or variance in family size) and on the reproductive characteristics of the immigrants.

Demographic equality also assumes that immigrants are equivalent to residents. To the extent that an immigrant is more or less likely to survive and breed relative to individuals in the target population, it may count as more or less than one migrant. It seems unlikely that immigrants will be demographically equivalent to resident individuals in most situations. In the case in which immigration is imposed by moving captured individuals, there are a variety of reasons to expect immigrants to be less likely to survive and reproduce than residents.

There are also a few situations in which immigrants may be more successful than residents. For example, individuals with rare genotypes have a reproductive advantage in plant species with self-incompatibility systems (Charlesworth 1985). Thus, immigrants from a genetically different subpopulation may have a reproductive advantage in this case.

Because deviations from ideal population structure will tend to compromise the effectiveness of migration relative to expectations, including social structure and taking into account the relative reproductive success of immigrants will mean that more than one migrant per generation may be necessary.

Equilibrium

The OMPG rule is based upon the assumption of equilibrium between the effects of migration and genetic drift. Equilibrium will be reached more quickly with smaller population sizes and higher migration rates (Varvio et al. 1986). Thus, greater migration may be initially desirable with larger subpopulations in order to reach equilibrium more quickly. Equilibrium between gene flow and drift is approached fairly quickly with small population sizes (Table 1; see also Allendorf & Phelps 1981; Table 2).

Before the population reaches equilibrium, the restorative effect of immigration is initially greater for small populations than for large because they have drifted to lower levels of variation (Lacy 1987). This implies that increases above OMPG may well be beneficial when migration is initiated for the first time.

Table 1. Observed and expected genetic divergence among five subpopulations of Leadbeater's opossum connected by gene flow in a simulation study (Lindenmayer & Lacy 1995).*

mN	H_s	*Expected* F_{ST}	*Observed* F_{ST} *20 yr*	*50 yr*	*100 yr*
0	0.28	1.000	0.170	0.309	0.509
0.7	0.60	0.186	0.105	0.128	0.130
1.5	0.70	0.096	0.074	0.068	0.079
7.4	0.80	0.021	0.021	0.022	0.024

**The* mN *values were calculated with the annual migration rate (*m*) used in the simulations, a generation interval of 3.71 years and an* N *of 40 based on carrying capacity (see text for discussion of using* N *versus* N_e*).* H_s *values represent observed mean heterozygosity within subpopulations at 100 years. Expected* F_{ST} *at equilibrium was calculated using Eq. 3 and the actual* mN *values used in the simulations. Observed* F_{ST} *values were calculated using Eq. 2.*

Implicit in the equilibrium assumption is that populations all persist long enough to reach equilibrium. McCauley (1993) reviews the consequences of relaxing this assumption: incorporating extinction and recolonization dynamics can either increase or decrease genetic differentiation, depending on how high population turnover rates are, where the migrants originate, how many migrants move together, and whether the migrants settle in unoccupied or occupied patches. Extinction and recolonization dynamics tend to accentuate population differentiation most when migrant individuals move into established populations more than colonizing new patches. Therefore, if conspecific attraction (Reed & Dobson 1993) tends to increase the likelihood of a dispenser settling in an already occupied area rather than an unoccupied area in a metapopulation, then those migrants will be less effective at minimizing differentiation in the metapopulation; if they settle more in empty patches, then extinction and recolonization dynamics predict that fewer migrants are needed to prevent differentiation (McCauley 1993).

In general, then, violation of the equilibrium assumption means that somewhat more than one migrant per generation may be beneficial. Extinction and recolonization dynamics could either increase or decrease the desirable number.

Empirical and Simulation Results

What insights can be gained from field studies, laboratory experiments, and comprehensive computer studies of natural populations that violate many of the above assumptions at the same time? Because it is not possible to test the predictions of the OMPG rule within a reasonable time frame for most species of interest, empirical tests will initially come from model genetic species with short generation times.

Little experimental work considers the issue of how many migrants are optimal in natural—or even captive—populations. The most direct experimental work is that of Spielman and Frankham (1992). They found strong and significant increases in reproductive fitness in 10 replicate inbred lines of *Drosophila melanogaster* that received only a single immigrant per generation. On average, OMPG restored nearly 50% of that fitness lost under isolation. Newman (1996) has found similar results with experimental populations of the plant *Brassica campestris*. One migrant per generation among 10 subpopulations had a significantly positive effect for five of six fitness components measured. In contrast to these results, Backus et al. (1995) found no increase in fitness associated with a single immigrant (although higher levels of gene flow did increase fitness) among five subpopulations of the housefly (*Musca domestica*).

Studies that use simulations or analytical approaches incorporating actual life-history information for long-lived species also provide insight into how effective OMPG is expected to be in the face of real-world deviations from the simplifying assumptions. For example, Hedrick (1995) used a variety of theoretical population genetic models to test the validity of the OMPG rule in the case of the Florida panther (*Felis concolor coryi*). The report of a workshop for genetic restoration of the Florida panther recommended an initial influx of 20% of the population, followed by gene flow of one animal per generation. Hedrick's models included various combinations of gene flow and genetic drift. He also examined several violations of the assumptions of the basic model: natural selection, directional gene flow, and nonequilibrium initial conditions. Despite these complicating factors, he concluded that this recommendation was sufficient to eliminate lowered fitness due to inbreeding depression and to retain adaptive alleles in the Florida population.

Allendorf et al. (1991) used simulations with the computer program GAPPS (Harris et al. 1986; Harris & Allendorf 1989) to examine the effects of immigration on effective population size and the rate of loss of genetic variation in an isolated population of grizzly bears in the Rocky Mountains. These simulations used a directional (continent-island) pattern of gene flow in a discrete-time, stochastic computer program that followed each individual bear from birth to death. The estimated effective population size of the isolated population was approximately 30% of the census population size. Their results are in general agreement with those expected with the basic model: the introduction of a few bears per generation greatly reduced the rate of loss of variation in the isolated population. The introduction of males had a greater effect than the introduction of females in the model, but this effect may be outweighed by the reduced likelihood of males being incorporated in the breeding population into which they are introduced be-

cause of the behavioral differences between males and females (Allendorf et al. 1991; U.S. Fish and Wildlife Service 1993).

Dobson et al. (1991) used GAPPS to simulate the viability of isolated populations of black rhinos (*Diceros bicornis*) in Kenya. They used an island-continent pattern of migration and demographic data obtained from black rhino sanctuaries. They also included the effects of inbreeding depression in some of their simulations. They found that one immigrant every 10 years had a major effect on the population size and persistence of isolated populations.

The most realistic test of the OMPG rule using simulations that we are aware of was done by Lindenmayer and Lacy (1995) with Leadbeater's possum (*Gymnobelideus leadbeateri*). They used the computer program VORTEX (Lacy 1993) to model the viability of metapopulations with subpopulations occupying a small number of habitat patches of varying sizes. They used values for life-history parameters drawn from extensive field studies, allowed local subpopulations to become extinct, and included the effects of inbreeding depression. Dramatic effects of migration were observed in their largest metapopulation, which consisted of five subpopulations of 40 individuals each (Table 1).

The results for Leadbeater's possum also can be used to quantitatively test the predictions in a system that violates many of the assumptions upon which the OMPG is based. The observed amount of genetic divergence among the five subpopulations estimated from the data of Lindenmayer and Lacy (1995) is similar to that predicted by the simple equilibrium model (Table 1). As expected, near-equilibrium values were obtained more rapidly with greater amounts of gene flow. Even with $mN = 1.5$, however, the observed F_{ST} value was nearly 75% of that expected at equilibrium after approximately five generations.

Recommendations for Implementing Gene Flow

When connectivity is implemented for genetic purposes, we are interested in finding the middle ground between loss of alleles in local populations (fixation, or loss in heterozygosity) and uniformity of allele frequencies across local populations. One migrant per generation is an appropriate lower limit to the amount of gene flow that is desirable. Nevertheless, sometimes more than an average of one migrant per generation will be necessary because of deviations in real populations from the ideal conditions assumed in developing the OMPG rule.

We hesitate to give "cookbook" prescriptions, but an increase in migration above the mean of one migrant per generation may be desirable under the following conditions: (1) inbreeding depression is thought to be a problem in the local population; (2) migrants are closely related to each other or to the local population; (3) social, behavioral, or logistical factors prevent single individuals from immigrating, so that movement is in pulses of several animals every several generations; (4) N_e is much less than total population size; (5) migrants are likely to be at a disadvantage in terms of survival and breeding success; (6) the receiving population has been isolated for many generations; and (7) demographic or environmental variation indicates a high danger of extinction without aggressive supplementation.

If one migrant per generation is not enough in many circumstances, then the next question is how much gene flow may be too much to maintain the balance we are seeking. This question is difficult to answer without extensive genetic and demographic information on the population under consideration. Concerns over local adaptation and outbreeding depression dictate that, under some conditions of phenotypic or genotypic dissimilarity, immigration into an isolated population should be minimized (for cogent discussions see Templeton [1986] and Leberg [1990]). Frankel and Soulé (1981) proposed an upper limit of 5 migrants per generation because of concerns with disruptive effects on social structure in small populations. We suggest that up to 10 migrants per generation is not likely to tip the balance too far by causing uniformity of allele frequencies across subpopulations (Fig. 2).

The characteristics of the migrants is as important as the number of migrants. Social, behavioral, and demographic characteristics that affect the likelihood of survival and reproduction of migrants may overwhelm genetic considerations in assessing the "ideal" candidates to recruit as migrants (Kleiman 1989; Stanley-Price 1989; Short et al. 1992). Likewise, considerations of mating system and social structure will affect the genetic impact of an immigrant. Greater numbers of randomly chosen migrants may be necessary to achieve the same genetic effect as fewer migrants chosen to maximize contribution to the effective population size.

Finally, we stress that criteria other than genetic variation will often play pivotal roles in determining the appropriate level of connectivity between semi-isolated fragments. For example, small populations subject to blinking out due to demographic or environmental stochasticity can experience a "rescue effect" (Brown & Kodric-Brown 1977), with immigrants increasing numbers away from the boundary of zero. In this case more migrants increase population persistence, regardless of genetic considerations. On the other hand, contagious diseases have been raised as a factor decreasing the benefits of connectivity (Wilson et al. 1994; Hess 1994).

Conclusions

The OMPG rule has provided much-needed guidance in management and has illuminated the need for genetic

connectivity among isolated populations. Like many theoretical applications in conservation, its utility is strongest when its complexity is appreciated (Doak & Mills 1994). We have seen several compelling real-world factors that suggest more than one migrant per generation may be necessary to achieve genetic goals, and some situations (albeit fewer) in which less connectivity is desirable. One key to practical decision making is to define clearly the desired genetic outcome, as well as other objectives and constraints of the supplementation program (Lacy 1987).

One migrant per generation is an acceptable minimum, but it may be inadequate for many natural populations. Different levels and schedules of supplementation are appropriate under different environmental conditions and with different species. Nevertheless, we suggest that a minimum of 1 and a maximum of 10 migrants per generation would be an appropriate general rule of thumb for genetic purposes.

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M. A. Sanjayan provided early enthusiasm and references on the topic to L. S. M., and reviewed the manuscript. F. W. A. was supported by a grant from the National Science Foundation (DEB-9300135) while preparing this paper. We appreciate reviews and comments on various drafts by D. Doak, D. Tallmon, and W. Morris (who also suggested the examination of how expected and exact F_{ST} values diverge at small numbers). L. S. M. would like to acknowledge Michael Soulé, a mentor and friend; F. W. A. would like to acknowledge Michael Soulé, a bodhisattva and friend. This paper is contribution No. 96-1 of the Montana Forest and Conservation Experiment Station, Missoula.

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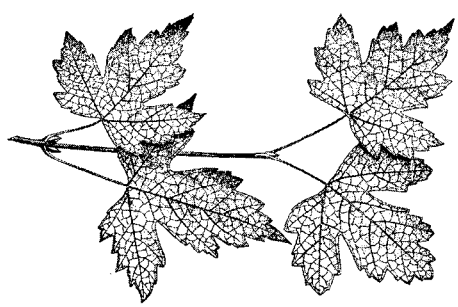

United States Department of the Interior
Bureau of Land Management
National Wild Horse and Burro Program
1340 Financial Boulevard
Reno, Nevada 89502-7147

In Reply Refer To:
4700 (WO261)

Memorandum

To: District Manager, Burns District Bureau of Land Management

From: Paul Griffin, Ph.D.
Wild Horse and Burro Program, Research Coordinator, Bureau of Land Management, 2150 Center Ave., Building C, Fort Collins, Colorado, 80526

Subject: National Research Council's 2015 report to BLM

Mr. Cain,
In reference to the proposed Mare Sterilization Research Environmental Assessment, I am providing you the following information in support of your analysis. This letter includes the internal deliberations provided to BLM by the National Research Council regarding the three proposed wild horse sterilization studies that would take place in Burns, Oregon.

On March 6, 2014, the BLM made public its request for applications to a research program titled "Wild horse and burro sterilization or contraception – development of techniques and protocols." The National Research Council provided scientific reviews of research proposals that BLM received in response. The National Research Council's report to BLM (NRC 2015) included a letter and two appendices. In order to ensure that the reviews of those proposals were rigorous, confidential, and unbiased, the specific critiques of proposals were sent to BLM as nonpublic attachments labeled Appendix A and Appendix B. I also note here that, for administrative reasons including available funding, the specific proposals that BLM chose to fund were a subset of the proposals recommended in NRC (2015).

In this letter, I am quoting, in their entirety, the paragraphs of text in Appendix A that pertain to the three Oregon studies under your consideration, other than personally identifiable information. These proposals were labeled proposals 8, 9, and 19 by the review committee of the National Research Council (2015). After that text I am also copying over complete page images from the specific reviews of those three proposals from Appendix B, except again with personally identifiable information removed.

Sincerely,

Paul Griffin

Literature Cited

National Research Council. 2015. Review of proposals to the Bureau of Land Management on Wild Horse and Burro sterilization or contraception; a letter report. Committee for the review of proposals to the Bureau of Land Management on Wild Horse or Burro Sterilization or Contraception. The National Academies Press.

Quoted text from Appendix A of NRC (2015), related to proposed Burns, Oregon studies

"Appendix A

Recommended Proposals

In its public session with the Bureau of Land Management (BLM), the committee understood that the agency was looking for research projects that would yield results quickly and ones that may take more time to come to fruition. With that in mind, it has grouped the recommended proposals into ones that can be completed and (if successful) have their results implemented in 2 years or less, ones that may yield practical results in the medium term (2–5 years), and ones that hold promise to yield results in the long term (5–10 years). A strategy for setting funding priorities among the projects and ways in which BLM could work with the investigators to strengthen their proposals are suggested in the last section of this appendix.

RESEARCH PROPOSALS ON STERILIZATION THAT COULD BE COMPLETED IN 2 YEARS OR LESS

The committee grouped Proposals 8, 9, 12, and 10 because they all focused on permanent sterilization through straightforward surgical procedures or, in the case of Proposal 10, a single injection. They all also had relatively low budgets and should be completed within 2 years. The committee believes that all four proposals feature methods that could be used safely in the near future (1–3 years from now) and therefore recommends them all for funding (Table A-1). When these research activities have concluded, BLM will be in a position to evaluate which approach or combination of approaches works best under what conditions, such as time of year, open or pregnant mares, stage of pregnancy, and the terrain and size of the population in the Herd Management Area where a gather, sterilize, and release program is planned. It can then proceed with the best options, having evaluated the merits of each approach. With regard to Proposal 10, the committee does not think that treatment of stallions will have an effect on the population growth rate of herds. However, BLM will capture stallions with mares and foals when it conducts gathers, and having an easy-to-apply, one-dose treatment option available for all mature animals would be optimal for the agency. It should fund Proposal 10 to verify whether such an injection can be safely developed for free-ranging stallions.

The committee also examined a proposal on conducting ovariectomies on free-ranging mares: Proposal 19, Functional assessment of ovariectomy (spaying) via colpotomy of wild mares as an acceptable method of contraception and wild horse population control; PI, [*personally identifiable information omitted here*]. Ovariectomy is regularly conducted on domestic mares, but some of the protocols used in domestic animals after surgery would not be applicable to free-ranging mares because they cannot be held still for 48 hours. The committee did not view this as a research proposal because the procedure is already done regularly in mares and therefore it contains no science or experimentation related to technique. For that reason, the committee did not group Proposal 19 with the research proposals listed in Table A-1. Nevertheless, it evaluated the usefulness of ovariectomy as a tool for population-growth suppression and the risks associated with it. Details of the committee's conclusions (including the effects of ovariectomy on fetuses before 90 days of gestation, from 90 to 120 days of gestation, and after 120 days of gestation) are in Appendix B. In short, the committee believes that this procedure could be operationalized immediately to sterilize free-ranging mares but that the techniques in Proposals 8, 9, and 12 would be less invasive. If the research projects of Proposals 8, 9, or 12 prove successful, their procedures should replace the procedure described in Proposal 19."

The following four pages are copies from NRC (2015) Appendix B, Proposal Review Comments. These are the reviews for the three proposed studies that would take place in Burns, Oregon, but with personally identifiable information redacted.

Proposal Number: 8

Proposal Title: Hysteroscopy and laser ablation of the oviduct papillas as a minimally invasive method of sterilization in standing, sedated wild horses and burros

Principal Investigator: [redacted] Oregon State University

Funding Recommendation: Fund.

COMMITTEE DISCUSSION SUMMARY

The objective of this proposal is to sterilize nonpregnant mares by hysteroscopic laser ablation of the oviductal papilla while the mares are under standing sedation. Fertility would be assessed by allowing treated mares to mate with a fertile stallion.

The committee finds the simplicity of this proposal to be its greatest strength. The procedure is minimally invasive and would require only restraint in the stocks with standing sedation. In practiced hands, the entire procedure should take 20–30 minutes per horse. With some training, many veterinarians could become proficient in performing this procedure. There is no question that the laser would damage the oviductal papilla. Whether the scar damage is sufficient to sterilize the mare permanently is the question at hand in this proposal.

The committee considered this proposal in conjunction with Proposals 9 and 12, which also aim to sterilize mares through minimally invasive procedures. The committee concludes that Proposals 8, 9, and 12 should all be funded. The budgets for these proposals are not large, and each should be completed in a short period (no more than 2 years). When these research activities have concluded, BLM would be in a position to evaluate which approach or combination of approaches works best under what conditions, such as time of year, open or pregnant mares, stage of pregnancy, and the terrain and size of the population in the Herd Management Area where a gather, sterilize, and release program is planned.

That being said, a number of improvements could be made in Proposal 8, and the committee is skeptical about one important factor. The committee is not confident that many mares gathered from an HMA would be eligible for this procedure because it is aimed at nonpregnant mares. Free-ranging mares are extremely sound reproductively, and many are in foal by the age of 2 years. Free-ranging mares that are not pregnant after the age of 2 years are probably subfertile. Therefore, the procedure would be applicable primarily to subfertile mares or yearlings that are 8 months old and/or weigh around 300 kg. The committee is not confident that many gathered horses will fit that description. It would not be practical to conduct a gather to perform only this procedure given the likelihood of a low number of candidate fillies and subfertile mares.

Regarding the low number of candidates for this procedure, the committee wonders whether inducing early embryonic death is acceptable. If it were acceptable, this procedure could be used in early spring in most mares. Abortion itself would not be induced because any embryos lost would simply be resorbed by the mare rather than expelled.

Another option would be to use this approach in tandem with the 1-year PZP vaccine. A gather could be conducted in year 1 to treat all captured mares with the PZP vaccine, after which they would be released. That would help to ensure a large number of nonpregnant mares when a gather occurred in the following year. That approach would add expense by requiring two

gathers in consecutive years, but infertility after the second year would be permanent (assuming that the procedure is successful), and the need for future gathers would be obviated for some time.

With regard to improving the proposal, many details are omitted or assumed by the investigators. The committee interprets the proposal to indicate that the investigators plan to use 50 mares from a BLM holding facility in Burns, Oregon. If that is the case, it could be stated more clearly. No control group of mares is mentioned. Similarly, it is not said how the fertility of the stallion given access to the mares would be determined. And, methods for capturing, sedating, and minimizing pain and stress in the animal are not described or addressed.

Another issue not addressed in the proposal is tubal pregnancies. The investigators report that if a mare is bred in the preceding 6–7 days, tubal pregnancy may result. In an animal with a 21-day cycle, 5% of open mares would be expected to be in estrus on any given day, so in 6–7 days as many as 30% of open mares could have been recently bred. Tubal pregnancy would be a painful, iatrogenic condition and should be considered as a welfare concern.

A proof-of-concept study could be carried out quickly with domestic mares before the technique is introduced to the horses in Burns or perhaps the step in Burns could be skipped entirely. Using domestic mares that could be easily handled would allow well-managed breeding (via artificial insemination) with semen of known fertility. Pregnancy status could then be diagnosed within 2 weeks of insemination. After proof of the concept, the technique could be moved into a free-ranging herd to test the difficulty of using it in true field conditions.

The committee was confused by the statement at the end of the proposal: “Beginning in the spring, assuming our experimental population has remained at Burns. . . .” It is not clear why the investigators cannot assume that the experimental population would still be available. If that population were not available, this would be another justification for conducting the experiment in domestic mares to which access could be guaranteed from the start to the finish of the experiment.

Finally, BLM would need to invest in many expensive endoscopes and have access to a number of trained veterinarians if the procedure is to have an effect on the population growth rate of a herd. The costs for the proposed project are appropriate, although the indirect costs included in the budget are higher than the 15% for the Wild Horse and Burro Program.

Proposal Number: 9

Proposal Title: Minimally invasive tubal ligation of wild horse and burro mares as a method of contraception and population control

Principal Investigator: [redacted] Oregon State University

Funding Recommendation: Fund.

COMMITTEE DISCUSSION SUMMARY

The procedure put forward in this proposal uses a flexible endoscope inserted through the anterior vagina to cauterize and transect the oviduct. Before treatment, the mare would be sedated, and then the abdomen would be distended with filtered air or carbon dioxide to aid in visualization of the ovaries and oviducts. The endoscope would pass through an incision in the anterior vagina and be used to visualize each oviduct, and the oviduct would be cauterized and transected. An epidural would be performed if necessary. The ligation of the oviduct would render the mare permanently sterile but still able to cycle normally. The investigators plan to perform this procedure on pregnant mares.

The committee is concerned that the ovaries may not be visible in late pregnancy because the mare's ovaries are pulled medially and anteriorly as the pregnant uterus moves over the pelvis and down to the floor of the abdomen. The collapse of the anterior vagina would also prevent passage of the endoscope in pregnant mares. Conducting this study would answer whether or not those concerns are warranted.

The committee considered this proposal in conjunction with Proposals 8 and 12, which also aim to sterilize mares through minimally invasive procedures. The committee concludes that Proposals 8, 9, and 12 should all be funded. The budgets for these proposals are not large, and each should be completed in a short period (no more than 2 years). When these research activities have concluded, BLM will be in a position to evaluate which approach or combination of approaches works best under what conditions, such as time of year, open or pregnant mares, stage of pregnancy, and the terrain of and size of the population in the Herd Management Area where a gather, sterilize, and release program is planned.

The committee offers some caveats regarding this proposal. The major risk to the mare would be abdominal pain in the 24 hours after the procedure. Injection of lidocaine or similar products into the ovary or oviduct during the procedure is not mentioned. Given the experience of some committee members, this additional step is recommended for pain management.

The proposal could be written more clearly and with more detail. It states that mares would be restrained in a hydraulic, padded chute that is specifically and successfully designed for routine processing of free-ranging horses. However, no further information is provided regarding animal-handling facilities. It is also unclear whether the investigators would have access to the number of animals (50) that are proposed for use in this study.

The committee is skeptical of the claim that four horses could be treated in an hour. Given the time needed to inflate the abdomen, two is more likely. There is also the issue of skilled practitioners: this approach would require more training than the form of sterilization discussed in Proposal 8.

The costs for the proposed project are appropriate, although the indirect costs included in the budget are higher than the 15% for the Wild Horse and Burro Program.

Proposal Number: 19

Proposal Title: Functional assessment of ovariectomy (spaying) via colpotomy of wild mares as an acceptable method of contraception and wild horse population control

Principal Investigator: [redacted] Oregon State University

Funding Recommendation: Fund with nonresearch money.

COMMITTEE DISCUSSION SUMMARY

This proposal focuses on demonstrating the value of ovariectomy in free-ranging horses. The investigator proposes to evaluate the feasibility of using surgery as a management tool in controlling the growth rate of free-ranging horse herds.

The proposal contains no science or experimentation related to technique. Colpotomy, as described in this proposal, is not a new technique; the only novelty in the proposal is that the procedure would be performed on free-ranging rather than domestic horses. The committee did not consider this difference to be a matter of research.

Nevertheless, the committee evaluated the proposal's usefulness as a tool for population growth suppression and the risks associated with it.

Ovariectomy via colpotomy is a relatively common procedure and a standard veterinary practice that is performed widely by practitioners in domestic horses. The death rate associated with the procedure is low. Domestic mares are typically cross-tied to keep them standing for 48 hours post-surgery to prevent evisceration through the unclosed incision in the anterior vagina. That protocol would not be possible with free-ranging mares because they cannot be held still for so long. Therefore, there is some concern that the investigator may see more fatalities after surgery than the 1% quoted in the protocol, which is based on domestic mares. The procedure should be 100% effective in sterilizing treated mares.

The proposal does not state at what early stage of pregnancy a mare would be excluded from the study. The mare's ovaries and their production of progesterone are required during the first 70 days of pregnancy to maintain the pregnancy. It is the opinion of the committee that if this procedure were performed in the first 90 days of pregnancy, the fetus would be resorbed or aborted by the mother. If performed after 120 days, the pregnancy should be maintained. The effect of ovary removal on a pregnancy at 90–120 days of gestation is unpredictable because it is during this stage of gestation that the transition from corpus luteum to placental support typically occurs. The proposal also does not state how long it would take to perform the procedure on a mare. That would be useful information for BLM to have in assessing its practicality.

The committee believes this procedure could be operationalized immediately to sterilize mares, with the caveat that fatalities may be higher than the 1% reported in the literature. The sterilization techniques put forward in Proposals 8, 9, and 12 would be safer—with less risk of hemorrhage and evisceration—and probably less painful. Therefore, if the techniques in Proposals 8, 9, and/or 12 prove to be successful after the research has been conducted, the committee thinks that these techniques should replace Proposal 19's method of ovariectomy via colpotomy as surgical approaches for permanent sterilization.

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December 1980

Sterilization of Dominant Males Will Not Limit Feral Horse Populations

Kurt J. Nelson, Wildlife Biologist
Carson National Forest, Questa, New Mexico[1]

Abstract

Sterilization of dominant stallions is impractical for controlling feral horse populations. Evidence does not support the premise that reproductive units are stable, with one male and many females. Individual horses, including reproductively active females, move between bands during the breeding season.

[1]*The author was a graduate student, New Mexico State University, Las Cruces. This research was supported by the Rocky Mountain Forest and Range Experiment Station with headquarters at Fort Collins in cooperation with Colorado State University and by the New Mexico State University Agricultural Experiment Station in Las Cruces. The manuscript was submitted as New Mexico State University Agricultural Experiment Station Journal Article 774. Supervision was provided by David R. Patton, project leader at the Station's research work unit at Tempe in cooperation with Arizona State University.*

Sterilization of Dominant Males Will Not Limit Feral Horse Populations

Kurt J. Nelson

Management Implications

Permanent or temporary sterilization of the dominant stallion in a feral horse herd has been suggested to control horse populations. This control method would work only if bands of horses were stable, if reproductive units consisted of one male and many females, and if females did not move between bands. A study of the behavior of bands of horses from the Jicarilla herd shows that female fidelity is weak or nonexistent and that some breeding in the bands is by subdominant males. The amount of sterilization that would have to be done to control horse numbers indicates that the technique is not practical. Another reason to avoid selective sterilization of dominant males is that these animals represent the best genetic material for the population.

Introduction

The Wild Free-Roaming Horses and Burros Act (PL 92-195) of 1971 provides federal protection and custody for feral equids, including feral horses (*Equus caballus*), on specified public lands. Prior to 1971, feral horse populations were limited through relatively unrestricted removal of animals. Since protection began in 1971, some of these populations may have increased at rates approaching 20-25% per year (Blaisdell 1977), resulting in overuse of available resources (Hall 1972). An acceptable method is needed to regulate feral horse numbers at levels compatible with environmental constraints while maintaining the populations at viable levels.

Population management has three distinct goals: (1) to keep a species on a "sustained yield basis" (harvest game management); (2) to increase the density of a species that is dangerously low (endangered species management); or (3) to decrease the density of a species (pest management) (Caughley 1976). With respect to feral horse populations on western rangelands, population management might be viewed as an amalgamation of goal 2, maintaining viable populations of feral horses, and goal 3, limiting growth of local populations that reach pest proportions.

One suggested technique for imposing population control artificially is temporary or permanent sterilization of the dominant stallions in a feral horse population to reduce the rate of reproduction (Baxter 1977, Frei 1977). The technique is appealing because it requires manipulation of only a few animals, and it is acceptable to various agencies and preservation groups (National Wild Horse Forum 1977).

The hypothesis that sterilization of dominant stallions would control population is based on the following assumptions:

1. Only certain males (harem stallions) participate in the breeding.
2. The number of breeding males is small relative to the number of reproductively active females, so that one male breeds many females.
3. A feral horse society is composed of groups that are stable over time.
4. Any changes in band affiliations are primarily among the immature, prereproductive individuals in the population.

If any of these seemingly plausible assumptions is untrue, sterilization is not practical as an artificial means of imposing density limitations.

This hypothesis thus raises questions on feral horse biology: What is the sex and age composition of the population? When do mating and parturition occur? At what age do both males and females reach sexual maturity? What type of social organization is expressed? Are the dominant stallions of feral horse bands doing all of the breeding within their bands? Do peripheral males participate in breeding receptive females? How stable are feral horse bands, and how much exchanging of members between bands occurs? How are bands formed and who forms them?

Only recently have there been attempts to obtain information on the population biology of feral horses (Feist 1971, Feist and McCullough 1976, Green and Green 1977, Hall 1972, Hall and Kirkpatrick[2]).

Various authors have described the social organization of both wild and feral equids. Generally, they have noted two distinct types: those equids in which the males are strictly territorial, such as in Grevys zebra (*Equus grevyii*) (Klingel 1969a, 1975a, 1975b), in some

[2]*Hall, R., and J. F. Kirkpatrick. 1975. Biology of the Pryor Mountain Wild Horse. Unpublished report, 21 p. Bureau of Land Management, Salt Lake City, Utah.*

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feral ass (*E. asinus*) populations in Death Valley (Moehlman 1974), and those equids in which the males are nonterritorial and form harem units, such as in plains zebra (*E. quagga*), mountain zebra (*E. zebra*) (Klingel 1966, 1967, 1968, 1969b, 1975b), and feral horse (*E. caballus*) (Fiest 1971, Feist and McCullough 1976, Green and Green 1977).

Feist (1971), Feist and McCullough (1976), and Green and Green (1977) stressed the highly stable nature of the harem social unit in their studies of feral horse populations. Band stability, it is hypothesized, is determined by the stallion's dominance over the other individuals of his band. Interchanges that did occur were primarily in the prereproductive segment of the population. Thus, a feral horse society is thought to be one of female fidelity to a particular band and not one of sporadic association with many different bands.

It can be concluded from computer simulations of population responses to changes in demographic parameters that sterilization of the dominant stallions is an impractical method of population control (Nelson 1978). It was shown that to initiate a population decline, the percent of successful breeding of females must be reduced to less than 10% (fig. 1).

Another method of control of these populations appears to be continuous cropping of 15% of the population per year. Cropping of entire band units would cause the least disruption to the social structure of the entire population. For a discussion of such a cropping procedure, see Fowler and Smith (1973). For a more thorough discussion of the computer simulations see Nelson (1978).

The research presented here is an evaluation of male sterilization techniques based on observations of population biology.

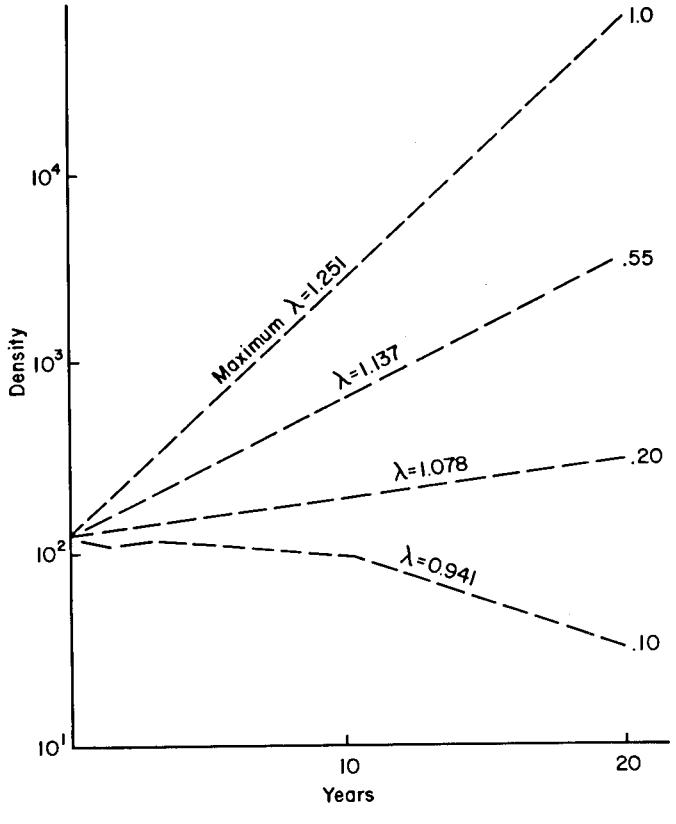

Figure 1.—Changes in the finite rate of increase in a feral horse population as a result of varying percent breeding. Proportion breeding shown at end of each line.

Study Area

The study area was on the Jicarilla District of the Carson National Forest, 90 km northeast of Farmington, N. Mex. The northern portion of this public land has been designated as the Jircarilla Wild Horse Territory and is approximately 29,000 ha. This study was restricted to 14,200 ha. The study area is heterogeneous, including four major canyons (10,000 ha) administered by the USDA Forest Service (FS) and 4,200 ha of land administered by the Bureau of Land Management (BLM). There is movement of feral horses across the FS-BLM boundary line.

Topographic features include wide canyon bottoms, steep-sided rocky slopes, and broad mesas. Elevations range from 1,800 to 2,275 m. The area lies within the San Juan Basin, with underlying rock of the Wasatch and Naciminento formations. The mesa soils are of sandstone origin and quite variable. The slopes and bottomlands are a mixture of soils derived from shale and sandstone and are easily eroded (Dane 1948, Simpson 1950). There are alluvial deposits 6 m or more in depth in the main canyons; canyon bottoms vary in width from a few hundred meters to 1.5 km or more. Deep gullies have been cut through the alluvia, and sheet erosion is common.

Precipitation occurs in the form of winter snow and summer rains. Water availability is limited during the dry months prior to the onset of summer rains. The development of stock tanks to hold runoff and the presence of intermittent springs along the main canyons provide water sources during the drier months. With the onset of the summer rainy season, water is readily available throughout the area.

Vegetative types include sagebrush-grass association, pinyon-juniper woodland association, pinyon-juniper/sagebrush-grass ecotone association, pine/Douglas-fir association, and revegetation association.

Approximately 800 ha of range revegetation has been done in the study area over the past 10 years. These areas begin vegetative growth 3 to 5 weeks earlier than the surrounding nonmanipulated land. A corresponding increase in use of these areas by feral horses, mule deer (*Odocoileus hemionus*), and elk (*Cervus elaphus*) was noted in response to early spring growth. Revegetation areas provide an important food source before spring growth begins on other parts of the range. A substantial road network exists on the study area enabling fairly complete coverage from a vehicle.

Methods

This study involved intensive observation of daily and seasonal activity of selected feral horse bands. The usual procedure was to observe a band for 4 to 12 hours. Most of the study area was visited at least every 2 or 3 days.

A 20X spotting scope, 8X binoculars, and a tape recorder were used. Observations were recorded in three formats, adapted from procedures outlined by Altmann (1974). Behavior-time matrices, including both social and nonsocial behaviors, were constructed for observations of bands and individuals within bands. The number of horses engaged in different activities was recorded at 5-minute intervals, and the relative position of the horses plotted. During each hour that a band was observed, activities of a randomly chosen individual were observed for 30 minutes and recorded separately. For individuals, observed activities were noted as occurring within any given minute during the 30-minute observation period (Lagerspetz 1964, Conley 1976). Band identification, date, time, weather conditions, vegetative association, and location were recorded for all observations. In addition, sex, age, identifying color and marks, and familial relationships (as discernible) were recorded on the individual's data sheets. A continuous account of all other behavior was recorded throughout an observation period in conjunction with the other two formats. From these three methods of recording information, construction of a complete behavior profile is possible for both individual and group activity patterns over the course of a year.

An aerial count of the feral horse population was conducted January 18-20, 1977, after sufficient snowfall to facilitate observation. This aerial survey and knowledge of the number of horses in the study area were used to estimate total population size.

Fieldwork began on September 13, 1976, and terminated August 17, 1977. Approximately 1,100 hours of behavior observations on individual horse bands and 400 hours of behavior observations on individuals within bands were recorded during the field study.

Results

The aerial census revealed 226 individuals in this population. The population segment that received intense study consisted of 116 individuals in 17 different bands. Each band included a mature stallion, one to six mature females and their offspring, and an assortment of immature females not necessarily of familial relation. Two bands contained more than one stallion.

Table 1 shows the age structure and composition of this population segment. Four age classes could be recognized: (1) foal, individuals up to 1 year of age; (2) yearling, individuals from 1 to 2 years of age; (3) immature, individuals from 2 to 3 years of age, and (4) mature, individuals 3-1/2 years and older. Some overlap exists in the immature and mature age classes because of difficulty in determining the age of certain females. If the three youngest age classes are grouped together, 52.6% of the population was born between 1975 and 1977.

Table 1.—Sex and age composition of the population segment that received intense study in summer 1977

	Foal	Yearling	Immature	Mature	Total	Percent
Male	12	7	10	17	46	39.7
Female	9	9	14	38	70	60.3
Total	21	16	24	55	116	100.0
Percent of total population	18.1	13.8	20.7	47.4	100.0	
Ratio of males to females	1.33	0.77	0.71	0.44	0.66	

Very little is known of reproduction in feral horses. Nishikawa et al. (1952), Hafez and Jainudeen (1974), and Nishikawa and Hafez (1974) gave a thorough summary of reproduction in domestic horses. A brief description of reproduction in domestic horses provides a reference to evaluate feral horse reproduction. In domestic stallions, sexual maturity may be attained before the age of 2 years. Testicular growth begins at 11 months with spermatogenesis occurring by 12 months. Males possess viable sperm year-round, although photoperiod appears to affect both the quantity and quality of semen. A domestic stallion can normally service 60 to 80 females during the breeding season. In female domestic horses, sexual maturity may be attained as early as 12 to 18 months. Estrous may be acyclic at first, then becomes cyclic. The length of the estrous cycle varies from 16 to 24 days, and duration of estrus itself is 3 to 11 days. Ovulation occurs 24 to 48 hours prior to the end of estrus. Diestrus lasts 14 to 19 days when ovulation occurs and 7 to 10 days when ovulation does not occur. The duration of gestation is 340 ± 30 days. A post-partum estrus may occur 5 to 15 days following birth. In domestic horses, reproductive efficiency is low, with a 60-65% conception rate and 50% foaling rate. Abortion frequency is 10% with mares 3 to 6 years old having the lowest abortion frequency. Prenatal mortality is common to lactating mares mated early in the season and to those which conceive during post-partum estrus.

Based on recent field observations of feral horse populations, it appears that reproduction in feral horses is similar to that in domestic horses. Feist (1971) and Feist and McCullough (1975) observed that 43.2% of mature females foaled in 1970. Two mares in the immature age class foaled, and two sets of twins also were noted. The breeding season of this population of feral horses was restricted to the spring and summer, with all foals born between April and June, and a peak in foaling in May. All mating occurred within the

harem unit with all copulations by the dominant male stallion. The behavioral sequences of mating have been described by Feist and McCullough (1976).

In the Jicarilla feral horse population, mating was observed throughout the year. Most observed copulations coincided with the spring and summer foaling season. Of 19 observed copulations, 17 were by dominant harem stallions. These stallions copulated with female members of their own bands and other receptive females. Nine of the observed copulations were with females from another band.

Evidence does not support the premise of stable reproductive units. Fidelity to a particular band is weak or nonexistent in many females of this population.

Twenty-one foals were born to the 38 mares in the mature class giving a 0.55:1 ratio. There were no twins. This rate of recruitment indicates most females do not foal in successive years, a finding that is further supported by field observations that 29% of the mares had foals in each of the past 2 years. The peak foaling period in this population occurred from April through May. By the end of May, 66% of all foals had been born, although foaling continued through the end of August.

There were differences in reproductive effort between females with access to revegetation areas and females without such access. Females with access to these areas began foaling on April 6 and completed foaling in May. Other females began foaling on May 3 and continued through August. Of the mature females, 64% had access to revegetation areas. These females contributed 73% of the foals (table 2).

Mortality was low in this feral horse population. During the study, 25 skulls were collected and age-at-death estimates were derived using tooth-eruption and tooth-wear criteria following procedures used by Ensminger (1969). Twenty-two of these horses were less than 12 years of age at the time of death. The presence of canine teeth indicated 15 of the skulls were from male horses (Ensminger 1969). There was no mortality in the foal class. Of 21 foals born in the spring and summer of 1977, all survived through August 1977 when this study ended. It is possible some mares gave birth to foals that died prior to being observed.

Table 2.—Differences in reproductive effort of females with access to revegetated areas and females without access

Activity area	Females	Foals	Foal to mare ratio	Standard error
	------- *percent* -------			
Revegetated areas	64	72	0.62	±0.08
Nonrevegetated areas	36	28	.43	± .10
Total	100	100	.55	± .06

Different mortality rates between sexes are evident in this population. The male to female ratio at birth approaches 1.0; however, the sex ratio approaches 0.4 in the mature age classes. This suggests that males are incurring higher mortality or that a disproportionate number of males left the study area. Of 10 males in the immature age class, only 4 were in harem units, while all 14 females in this immature class associated with one or more harem bands.

Mountain lion (*Felis concolor*) and coyote (*Canis latrans*), both present in the study area, are potential predators on feral horse populations (Dobie 1952, Young and Goldman 1946). However, no deaths within this population could be attributed to predation during the study.

Observations suggest that survival patterns are characterized by low mortality in the foal and yearling classes, and higher male mortality in the immature and mature age classes. Dispersal movements are included as a component of mortality estimates, thus making estimates conservative.

Social Structure

A band is a recognizable aggregate of individuals in a somewhat stable association. A band may be a harem group or it may be an all-male aggregation of immature, subdominant members of the population. Female aggregations were not classified as bands because they were in frequent association with one or more harem groups over the course of the year. An assumption in this study was that the stallion is the alpha individual, thus band identity was determined by his presence or absence. Most bands contain a core of individuals which usually remain together with the stallion. I have called such a group the primary band. If a female was recognized as a member of a particular band but was not with the stallion, she was classified as out of the band.

The dynamics of group formation and group structure are of particular importance in a social system. Feral horse bands were formed in four ways: (1) peripheral males acquiring females from existing bands (observed three times); (2) peripheral males forming a band with females which had previously been moving between bands (observed twice, although neither was permanent); (3) a peripheral stallion assuming control over a harem band following disappearance of the dominant stallion (observed once); and (4) bands splitting into two new bands as a result of competition between two mature males (observed once and appeared to be happening in another band at the close of field observations).

Size and composition of some bands were fairly stable while others were unstable. Changes in band size and composition occurred throughout the year, but there was a strong seasonal pattern in some bands. The monthly mean size of each band indicated an increase in general movements of individuals between bands and out of bands during the spring and summer months. Certain bands having access to revegetation areas exhibited greater interchange of members be-

tween bands (fig. 2). Prior to the onset of breeding and parturition in late March, these bands begin to break up, with females and their offspring moving between adjacent bands. By May, very few females were with their primary band, and were either with another band, by themselves, or with all-female factions of a previous band. Bands not having access to revegetation areas appeared to be relatively stable (fig. 3). Considerable movement occurred even among the more stable bands but was usually short-term, with individuals returning to their primary band (fig. 4).

Sixty-one percent of the females in the study area were either alone, or with other females and no stallion, or in association with another band at some time during the year. Females moved more during the time of breeding and parturition. This amount of movement would not necessarily negate the hypothesis that the population could be controlled by sterilizing dominant males, provided the animals involved in the movement were the nonreproductive and/or immature segments of the population. However, the most active segment of females were mature (78% of the observed movements). Immature females had a higher movement activity in the fall and winter periods. Yearling females generally stayed with their primary band, moving only with other females.

Discussion

These findings suggest that dominant males do most of the breeding and that there are few such males relative to the number of sexually active females. However, the majority of the observations were of bands which included a dominant stallion, resulting in a bias in favor of such observations. While copulations involving peripheral stallions were not observed, the fact that females associate with these males at various times, and spend long periods of time outside any harem band, suggests that some reproduction may be attributable to these males.

An explanation for the disproportionate sex ratio in the mature age class is not readily available. Two solitary stallions and one group of five immature and subdominant stallions were observed during the study. Dispersal of males to areas outside the study area or differential mortality directed toward the male segment of the population are two possible reasons for the observed imbalance; however, it seems possible that males from other areas would immigrate into this population segment, resulting in no net changes.

Most movements of individuals between bands or out of bands coincided with the breeding season. Not only was there movement between groups, but this movement included the reproductively active females. The high percentage of the female population involved in either long- or short-term movements outside the primary band negates the effectiveness of male-limited population control. Evidence that over 50% of the observed copulations were with females from other bands further indicates the ineffectiveness of sterilizing only certain harem stallions.

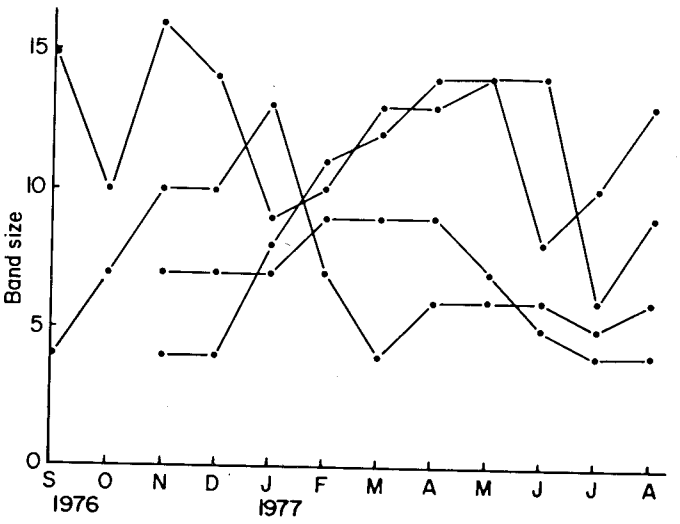

Figure 2.—Maximum monthly band size of four bands with access to revegetated areas.

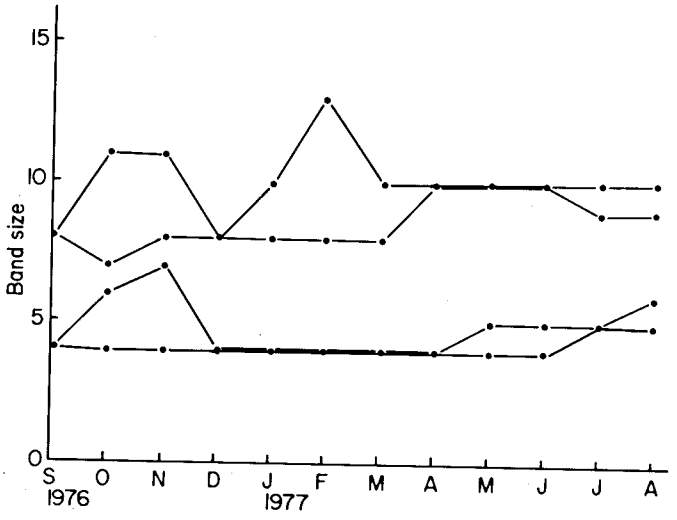

Figure 3.—Maximum monthly band size of four bands without access to revegetated areas.

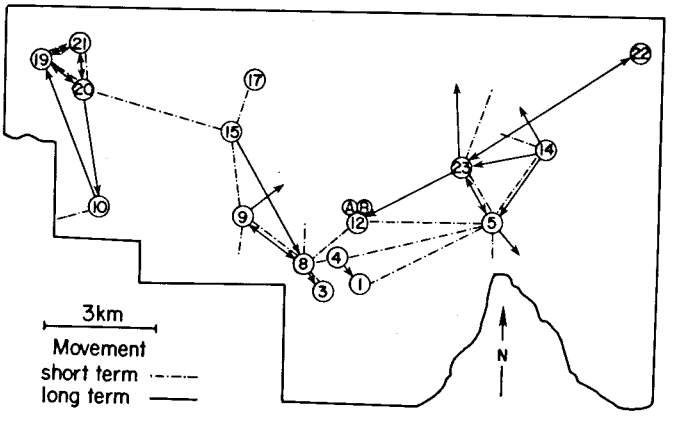

Figure 4.—Movement of individuals between bands and out of bands within the study area in the Jicarilla feral horse population.

Given the potential of feral horse populations to increase as evidenced in the past few years, it seems unlikely that sterilizing dominant males would reduce reproduction enough over the time necessary to cause a decline in numbers. Approximately 25% of the harem stallions were replaced each year. If these observations are representative of feral horse populations in general, male-sterilization techniques would have to be implemented on a yearly or every-other-year basis, an impractical management approach.

In addition to failing to limit the population, selective sterilization of alpha males would eliminate the major contributors of quality genetic material from the gene pool. Breeding by peripheral, subdominant males, might then introduce inferior genotypes, a result which runs counter to the management objective of maintaining viable feral horse populations.

Future Research

The biology of this population has been made more complex by the differential behavior of individuals with access to revegetation areas compared to those without access to such areas. The higher foal:mare ratio of females with access to revegetated areas implies increased reproductive value with increasing nutritional resources. Clegg and Ganong (1969) found that domestic mares would breed at any season if well fed. Hall (1972) relates reproductivity to nutrition in the Pryor Mountain feral horse population, since foaling coincided with the onset of spring growth of vegetation. That these females with access to revegetation areas are foaling earlier in the spring suggests that the timing of reproductive efforts are tied to differences in forage availability and are in this sense vegetation generated. While these differences do not directly relate to the questions of limiting population growth by sterilizing dominant males, a management alternative might be to concentrate on those groups of animals which exhibit higher production, if the goal is to limit growth of the population. Research completed by the University of Arizona will provide new information on the use of revegetated areas by wild horses.[3]

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Untersuchungen zu den Auswirkungen des Kastrationszeitpunktes von Lamahengsten (*Lama glama*) auf ihr Verhalten

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Schlüsselwörter:
Lama, Kastration, Verhalten, Androgen

Zusammenfassung:

Gegenstand und Ziel: In der Arbeit wurde der Effekt der Kastration auf das Verhalten sowie den Hormonstatus von Lamahengsten in Abhängigkeit vom Alter der Tiere untersucht. **Material und Methoden:** Bei je sechs adulten und juvenilen Hengsten wurden das Sozial- und Sexualverhalten vor und nach Kastration beobachtet sowie Plasmatestosteronwerte bestimmt. **Ergebnisse:** Bei den fertilen Althengsten war die durchschnittliche Zahl geringer sozialer Distanzen signifikant niedriger. Die Anzahl stieg nach Kastration in beiden Gruppen ebenso wie der Anteil direkter Körperkontakte. Die Zahl kurzer Auseinandersetzungen sank bei den Althengsten nach Kastration ebenso wie der Anteil der Kämpfe mit eindeutigem Ausgang. In beiden Untersuchungsgruppen veränderte sich die Rangordnung der Tiere durch die Kastration. Vor dem Eingriff führten alle Althengste Deckakte ohne Unterbrechung aus. Danach unterbrachen alle die Kopulationen mehrfach bei reduzierter Gesamtdeckzeit. Bei den Junghengsten fanden sich vor der Kastration nur kurze Deckzeiten mit vielen Unterbrechungen. Die Plasmatestosteronwerte betrugen bei den adulten Hengsten 4,1 ng/ml (± 2,8) und bei den Junghengsten 1,1 ng/ml (± 1,0). Die Testosteronwerte und Rangindices waren nicht korreliert. **Schlussfolgerung und klinische Relevanz:** Die Gruppen unterschieden sich signifikant in Verhalten und Hormonstatus. Die Kastration führte in beiden Gruppen relativ rasch zu einer besseren Verträglichkeit der Tiere. Die sexuelle Aktivität wurde durch die Kastration signifikant beeinflusst. Das grundsätzliche Interesse an Stuten blieb allerdings bei den Althengsten erhalten. Die Kastration ist für Hengste, die nicht zur Zucht eingesetzt werden sollen, anzuraten. Sie sollte möglichst vor der Geschlechtsreife durchgeführt werden, aber nicht vor einem Alter von 12 Monaten.

Key words:
Llama, castration, behaviour, androgen

Summary:

Objective: The aim of the study was to prove the effects of castration on behaviour and hormone levels in sexually mature and immature Llama males. **Material and methods:** The study comprised six sexually mature and six sexually immature llama males. Their social and sexual behaviour was observed before and after castration. Testosterone levels were analysed regularly. **Results:** The percentage of low social distances was significantly lower in mature males and increased in both groups after castration as well as the number of direct body contacts. Short agonistic interactions and the percentage of fights with clear result (one male was the winner or looser) decreased after castration in mature males. In both groups order of rank changed after castration. Before castration all mature males mated the female without breaks. After castration they interrupted copulations several times and total time of mating was decreased. Immature males showed short mating times with many interruptions before castration. The testosterone level changed between the months of observation in the mature male group. In the mature male group blood testosterone level was 4.1 ng/ml (± 2.8), and in the immature group it was 1.1 ng/ml (± 1.0). There were no correlations between testosterone level and order of rank. **Conclusion and clinical relevance:** Mature and immature males differed significantly in behaviour and hormone levels. Castration did improve social behaviour as the number and intensity of agonistic interactions decreased. Sexual behaviour was also significantly affected by castration in mature males. It can be concluded that keeping and handling of geldings is less problematic. Therefore, castration can be recommended as a management tool after 12 months of age, but before sexual maturity.

Effects of castration on the behaviour of male llamas (*Lama glama*)

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Einleitung

Die Lama- und Alpakahaltung erfreut sich in Mitteleuropa mittlerweile im Hobby- und Freizeitbereich einer großen Beliebtheit (10). Lamas werden unter anderem zum Trekking genutzt. Dazu eignen sich besonders männliche Tiere, die nicht bzw. nicht mehr züchterisch zum Einsatz kommen. Bei der Hengsthaltung können jedoch Probleme auftreten. Geschlechtsreife Hengste zeigen ein ausgeprägtes Aggressionsverhalten gegenüber anderen Hengsten, in dessen Folge mitunter lebensbedrohende Verletzungen entstehen (7). Die Haltung mehrerer geschlechtsreifer Hengste in Stutenherden ist deshalb nicht möglich. Bei den nicht domestizierten Neuweltkameliden (Guanako und Vikunja) vertreibt der Althengst die Junghengste aus dem Herdenverband, wenn diese die Geschlechtsreife erreichen (14). Aus diesem Grund sowie zur Kontrolle des Reproduktionsgeschehens in der Herde werden in der Praxis geschlechtsreife Hengste häufig einzeln gehalten. Die Einzelhaltung, ohne Kontakt zur Herde, muss als nicht tiergerecht bewertet werden. Häufig führt sie zur Entwicklung von Verhaltensanomalien. Die gemeinsame Haltung kastrierter männlicher Tiere scheint dagegen problemlos möglich (8). Männliche Kastraten gelten bei verschiedenen Tierarten als unproblematischer im Umgang (20). Auch bei Neuweltkameliden erlischt nach frühzeitiger Kastration das geschlechtsspezifische Aggressionsverhalten, sodass Wallache sowohl mit anderen Wallachen als auch in Stutenherden mit Fohlen und Junghengsten gehalten werden können (4). Dabei spielt möglicherweise der Zeitpunkt der Kastration eine wichtige Rolle. Er bestimmt mit, in welchem Maße das Verhaltensrepertoire ausgebildet bzw. tradiertes Verhaltensrepertoire noch verändert werden kann (1). Für Lamas wird entsprechend ein früher Kastrationszeitpunkt empfohlen. Nachteile der frühen Kastration zeigen sich jedoch in anatomischen Problemen, wie dem eunuchiden Riesenwuchs, weshalb die Tiere zum Kastrationszeitpunkt ein Mindestalter von 12 Monaten erreicht haben sollten (12).

Ziel der vorliegenden Arbeit war, den Effekt der Kastration auf die geschlechtliche Entwicklung und das Verhalten von Lamahengsten in Abhängigkeit vom Alter und der geschlechtlichen Reife der Tiere zum Zeitpunkt des Eingriffs zu untersuchen. Darüber hinaus wurden mögliche Effekte der Testosteronkonzentration im Hinblick auf die Rangordnung ermittelt.

Material und Methoden

Tiere und Versuchsbedingungen

Für die Untersuchungen standen 12 Lamahengste (*Lama glama*) zur Verfügung. Eine Gruppe umfasste sechs geschlechtsreife, fertile Hengste im Alter von 3–8 Jahren, die andere Gruppe sechs infertile Tiere im Alter von 9–18 Monaten (zu Beginn der Untersuchungen zeigten alle Tiere noch eine präputiale Verklebung).

Die Untersuchungen fanden in der Lehr- und Forschungsanstalt Oberer Hardthof der Justus-Liebig-Universität in Gießen über einen Zeitraum von 10 Monaten (Mai bis März) statt. Beide Herden wurden auf zwei unterschiedlich großen Weiden mit Unterstand gehalten (erste Weide: ca. 2500 m², zweite Weide ca. 5000 m² Fläche), wobei einmal wöchentlich ein Wechsel der Weiden erfolgte. Ein Sichtkontakt der Gruppen untereinander oder zu Stuten bestand nicht.

Die Lamahengste wurden mit Heu und Wasser ad libitum versorgt und erhielten jeden zweiten Tag Kraftfutter (Kraftfutter für Schafe bzw. Pferde, 100 g pro Tier und Tag). Außerdem stand ein Salz- und Mineralleckstein zur freien Verfügung. Alle Tiere hatten freien Zugang zu Futter und Wasser.

Versuchsdurchführung

Vier Monate nach Untersuchungsbeginn wurden die Hengste mittels skrotaler Methode kastriert (18), die Junghengste und die Althengste jeweils an einem Tag. Alle Tiere waren zu diesem Zeitpunkt älter als 12 Monate.

Im wöchentlichen Wechsel wurde jede Herde auf der größeren Weide von einer Beobachtungskanzel aus durch eine Person im Time-sampling-Verfahren (5-Minuten-Raster) von Montag bis Freitag für 8 Stunden täglich (2 × 4 Stunden) zu unterschiedlichen Tages- und Nachtzeiten beobachtet. Die wöchentliche Beobachtungszeit umfasste so fast zwei volle Tage. Zu jedem Beobachtungszeitpunkt wurden die geringen sozialen Distanzen zwischen den Tieren erfasst (direkter Körperkontakt, Abstand < 1 m ohne Kontakt, Abstand 1–5 m). Der bstand bezog sich dabei auf die am nächsten zueinander befindlichen Körperteile.

Die Tiere konnten aufgrund ihrer individuellen Färbung eindeutig identifiziert werden, sodass eine zusätzliche Kennzeichnung unterblieb. Die Focal-sampling-Methode (6) wurde verwendet, um verschiedene Parameter im Funktionskreis des Sozialverhaltens (agonistische Interaktionen mit Sieg oder Niederlage innerhalb der Dyade, Kampfelemente, soziale Distanzen, Verhaltensweisen der Kontaktaufnahme) zu erfassen. Die Berechnung der Rangordnung erfolgte nach folgender Formel: $R = V - D / V + D$ (15, 19). Dabei bedeutet R den Rangindex, V die Frequenz gewonnener und D die Frequenz verlorener Auseinandersetzungen. Die Indices können zwischen –1 und +1 liegen, wobei +1 absolute Dominanz und –1 absolute Subdominanz bedeuten (15, 19). Weiterhin wurden die Dauer der Auseinandersetzungen (in Sekunden) gemessen und agonistische Verhaltensweisen (z. B. Drohen, In-den-Hals-Beißen) dokumentiert. In die agonistischen Interaktionen (AI) waren alle Hengste involviert (zwischen 38,5% und 63,8% der AI wurden von den jeweiligen Hengsten als Akteur eingeleitet), sodass auf der Basis der von Lundberg (15) und Puppe (19) entwickelten Formel eine fundierte Aussage zur sozialen Rangordnung möglich wurde. Unentschiedene agonistische Interaktionen (beide Tiere in einer Dyade weichen einander aus) blieben bei der Berechnung der Rangindices unberücksichtigt.

Zur Bewertung des Sexualverhaltens wurden die Hengste im Untersuchungszeitraum einmal pro Woche einzeln immer derselben deckbereiten Stute zugeführt. Es wurde Wert darauf gelegt, dass die Reihenfolge der Hengste stets wechselte. Erfasst wurden das Interesse an der Stute, bei Kopulation die individuelle Deckzeit (die Maximalzeit wurde auf 5 Minuten begrenzt) sowie etwaige Unterbrechungen des Deckakts.

Einmal wöchentlich erfolgte bei allen Tieren eine Blutprobenentnahme aus der Vena jugularis zur Bestimmung der Testosteronkonzentration (11, 16).

Statistische Auswertung

Die statistische Auswertung der Daten erfolgte mit dem Programmpaket SPSS Version 8.0 für Windows, wobei folgende statistische Verfahren zur Anwendung kamen: Prozedur ANOVA (multipler Mittelwertvergleich nach Student-Newman-Keuls), Prozedur Kreuztabellen (Chi-Quadrat-Unabhängigkeitstests in Kontingenztafeln) sowie Korrelations- und Regressionsanalysen.

Ergebnisse und Diskussion

Die Individualdistanz zwischen Tieren ist ein wichtiger Indikator des Sozialverhaltens (5). Die Häufigkeit des Vorkommens geringer sozialer Distanzen gibt damit Auskunft über die Verträglichkeit der Tiere (2). Die Distanzen zwischen den Tieren wurden in

der vorliegenden Studie in Bereiche bis 1 m und 1–5 m eingeteilt. Außerdem wurde das Vorkommen direkter physischer Körperkontakte vermerkt. Bei den Althengsten war die durchschnittliche Zahl geringer sozialer Distanzen signifikant ($p < 0,01$) niedriger als bei den Junghengsten. Die Anzahl stieg nach Kastration rasch an ($p < 0,01$). In der Althengstgruppe fanden vor der Kastration 41,5% (n = 105) von allen engen sozialen Kontakten (n = 253) im Bereich bis zu 1 m statt, 58,5% (n = 148) im Bereich von 1–5 m. Nach Kastration stieg die Häufigkeit der engen sozialen Kontakte im Bereich bis 1 m auf 63,9% (n = 133). Der Anteil der sozialen Kontakte mit physischem Körperkontakt verdoppelte sich nahezu von 19,9% vor auf 39,1% nach Kastration ($p < 0,01$). Innerhalb der Junghengstgruppe waren ähnliche Entwicklungen feststellbar. Es wurden 514 Verhaltensweisen vor und 386 nach der Kastration beobachtet und ausgewertet. Der Anteil geringer sozialer Distanzen im Bereich bis 1 m war allerdings bereits vor Kastration signifikant ($p < 0,01$) höher (59,5%) als der Anteil sozialer Kontakte bis 5 m. Bei 24,1% der Beobachtungen hatten die Tiere vor der Kastration direkten Körperkontakt. Nach Kastration lagen bei 68,7% der Beobachtungen der geringen Distanzen die Abstände zwischen den Tieren unter einem Meter. Bei 33,7% der Beobachtungen hatten die Tiere direkten Körperkontakt. Die Unterschiede in der Junghengstgruppe waren zu den beiden Zeitpunkten signifikant ($p < 0,05$).

Die Kastration führte damit in beiden Gruppen relativ rasch nach dem Eingriff zu einer deutlich günstigen Beeinflussung des Sozialverhaltens, das heißt zu einer besseren Verträglichkeit der Tiere. Dies deckt sich mit Beobachtungen bei anderen Tierarten mit ähnlichen sozialen Strukturen wie z. B. dem Pferd (13).

Zur Bestimmung des Aggressionspotenzials und der Rangordnung wurden die Ergebnisse der beobachteten agonistischen Interaktionen sowie deren Dauer festgehalten. Die Dauer agonistischer Interaktionen kann genutzt werden, um die Intensität von Kämpfen und möglicherweise daraus resultierende Verletzungen zu bewerten (6). Allerdings traten im Untersuchungszeitraum keine klinisch relevanten Verletzungen auf, weder bei den Althengsten noch bei den Junghengsten. Es wurde auch kein Tier aus dem Gruppenverband ausgeschlossen. Auseinandersetzungen von weniger als einer Minute sind häufig gefährlicher bzw. ernsthafter als länger anhaltende Kämpfe. Bei Letzteren besteht häufig eine Vermischung mit spielerischen Elementen. Dies zeigte sich auch bei den eigenen Untersuchungen. Bei den Althengsten konnten 301 AI vor und 421 AI nach der Kastration dokumentiert werden. Bei den Junghengsten beliefen sich die entsprechenden Stichprobenumfänge auf 257 bzw. 218 AI. 77,4% aller Auseinandersetzungen dauerten in der Gruppe geschlechtsreifer Tiere vor Kastration kürzer als eine Minute. Nach der Kastration reduzierte sich der Anteil auf 64,6% ($p < 0,01$). Bei den jungen Hengsten lag das Verhältnis bei 59,5% vor und 58,3% nach Kastration ($p > 0,05$) (Abb. 1). Der Schwerpunkt der eigenen Untersuchungen richtete sich auf das agonistische Verhalten, wobei verschiedene Verhaltensweisen der Aktion (= des Angriffs) dokumentiert wurden, unter anderem „Halsbeißen", „Verfolgen", „Beinebeißen", „Halsdrücken", „Genitalienbeißen", „Ohrenbeißen", „Schreien". Submissive Verhaltensweisen, die generell methodisch schwieriger zu erfassen sind, wurden indirekt über die Einhaltung unterschiedlicher sozialer Distanzen berücksichtigt.

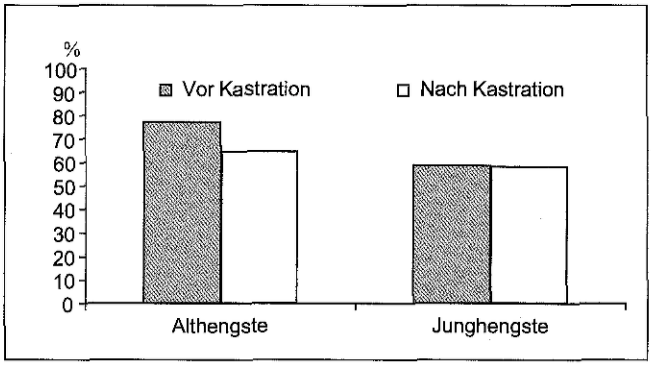

Abb. 1 Häufigkeit (%) kurzer agonistischer Interaktionen (bis 1 Minute) vor und nach Kastration

Während bei den Althengsten vor Kastration etwa 55% aller Kämpfe einen eindeutigen Ausgang hatten, nahm nach der Kastration der Anteil der unentschiedenen Kämpfe hochsignifikant ($p < 0,01$) zu. Die eindeutig gewonnenen und verlorenen Interaktionen sanken auf etwa 44% und die Intensität der Auseinandersetzungen verringerte sich. Dies entspricht den Ergebnissen anderer Untersuchungen bei Neuweltkameliden (17). Offensichtlich änderte sich auch die Motivation für Auseinandersetzungen, indem post castrationem 11% der AI spielerische Ursachen hatten und eine Unterschreitung der Individualdistanz nur noch in 10,2% der Fälle aggressives Verhalten auslöste.

Die ermittelten Rangindices lagen vor Kastration bei den Tieren der geschlechtsreifen Gruppe zwischen –0,5 und +0,7. Nach Kastration variierten sie zwischen –0,7 und +0,9 (Tab. 1). Bei den Hengsten der unreifen Gruppe lagen sie vor Kastration zwischen –0,6 und +0,9, danach zwischen –0,7 und +1,0 (Tab. 2). In beiden Untersuchungsgruppen veränderte sich die Rangordnung der Tiere über einen Zeitraum von 6 Monaten nach Kastration, blieb dann aber relativ stabil. Dies entspricht den Erfahrungen bei anderen Tierarten.

Tab. 1 Rangindices und Rangplätze vor und nach Kastration bei Althengsten

Hengst	Vor Kastration		Nach Kastration	
	Rangindex	Rangplatz	Rangindex	Rangplatz
1	+0,7	1	+0,9	1
2	+0,1	3	+0,5	2
3	–0,6	6	–0,7	5
4	–0,4	4	–0,5	3,5
5	–0,5	5	–0,5	3,5
6	+0,2	2	–*	–*

* Hengst verstorben

Tab. 2 Rangindices und Rangplätze vor und nach Kastration bei Junghengsten

Hengst	Vor Kastration		Nach Kastration	
	Rangindex	Rangplatz	Rangindex	Rangplatz
1	+0,9	1	+1,0	1
2	–0,5	4,5	–0,4	5
3	+0,1	2	–0,1	3,5
4	–0,6	6	–0,1	3,5
5	–0,5	4,5	–0,7	6
6	–0,1	3	+0,4	2

Abb. 2 Vor Kastration hatten alle adulten Hengste die Stute ohne Unterbrechung für 5 Minuten gedeckt. Danach wurde der Deckakt stets mehrfach unterbrochen.

Vor Kastration hatten alle Althengste die Stute ohne Unterbrechung für 5 Minuten gedeckt (Abb. 2). Nach Kastration unterbrachen alle Hengste die Deckakte vor Ablauf der maximal definierten Zeit. Die durchschnittliche Deckdauer reduzierte sich auf 280 Sekunden mit mehreren Unterbrechungen. Dabei fielen extreme Variationen zwischen den Hengsten auf (88–283 Sekunden). Einige Hengste reduzierten die Gesamtdeckzeit (Summe der einzelnen Deckintervalle) bis zum Ende der Untersuchungen nur unwesentlich. Alle Hengste unterbrachen die Deckakte allerdings immer wieder. Die Junghengste deckten vor der Kastration die Stute durchschnittlich 55 Sekunden lang. Nur in 18% der Fälle wurde die Kopulation nicht mehrfach abgebrochen. Nach Kastration veränderte sich die durchschnittliche Deckzeit nicht signifikant. Allerdings erfolgte nur noch in 6,4% der Fälle eine Kopulation ohne Unterbrechung. Kastrierte Tiere zeigen also immer noch ein mehr oder weniger entwickeltes, wenn auch reduziertes Interesse an den Stuten.

Unterschiede im Deckverhalten unter Bezug auf die Rangposition traten nur bei Althengsten und nur nach der Kastration auf. Während die beiden ranghöchsten Hengste noch bei 88,9% bzw. 77,8% aller Deckakte kopulierten, ergab sich bei den übrigen Hengsten ein Abfall auf 16,7 bis 5,6%.

Die für die Kopulation erforderliche Libido wird in starkem Maße durch den zentralnervalen Effekt der Androgene gesteuert. Da es nach Kastration zu einem unmittelbaren Abfall der Testosteronkonzentration kommt, erklärt sich damit das Auftreten reduzierter Deckzeiten bzw. das verringerte sexuelle Interesse (3). In der vorliegenden Untersuchung konnte für die Althengste keine Beziehung zur geschlechtlichen Aktivität (Erfahrungen) vor dem Versuch hergestellt werden, da diese unbekannt war. Die bis zum Zeitpunkt der Untersuchung gesammelten Erfahrungen (tradiertes Verhalten) mögen einen Einfluss auf das Sexualverhalten genommen haben und somit die Variationen zumindest zum Teil erklären (13).

Die Plasmatestosteronwerte betrugen bei den geschlechtsreifen Hengsten vor Kastration durchschnittlich 4,1 ng/ml (± 2,8), bei den unreifen Hengsten 1,1 ng/ml (± 1,0). Bei den geschlechtsreifen Tieren schwankten die individuellen Durchschnittswerte zwischen 1,8 und 7,9 ng/ml ($p < 0,01$). Die Werte lagen damit im Bereich der Literaturangaben (9). Die Testosteronwerte variierten bei den Althengsten zwischen den Untersuchungsmonaten vor dem Zeitpunkt der Kastration signifikant (Abb. 3). Saisonale Schwankungen wur-

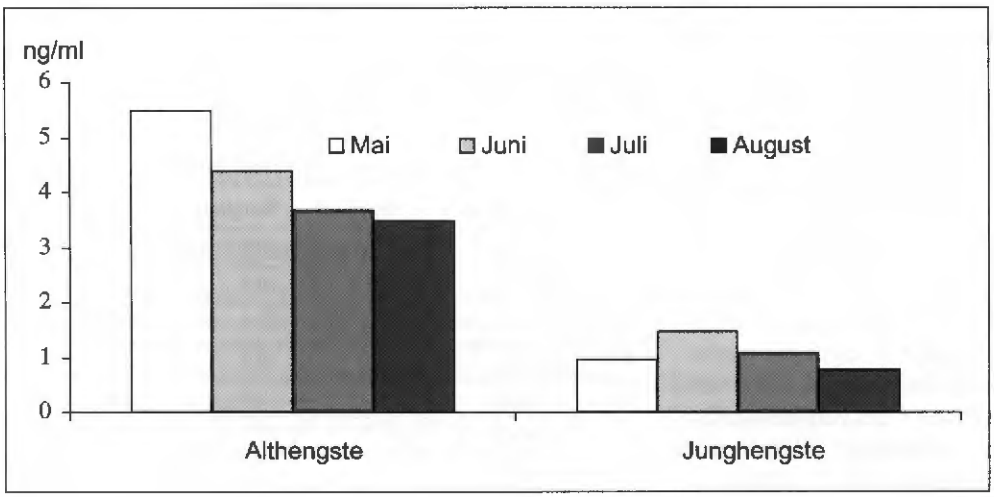

Abb. 3
Mittlere Testosteronwerte (ng/ml) beider Gruppen pro Monat

den in der Literatur bereits beschrieben (9). Zwischen Testosteronwert und Rangindex konnte keine signifikante Korrelation berechnet werden. Die verwendete Formel zur Berechnung des Rangindex führte zu keiner Überschätzung der sozialen Stellung einzelner Hengste, da es keine ungewöhnlich hohen Zahlen an agonistischen Interaktionen in einzelnen Dyaden gab.

Schlussfolgerungen

Die Gruppen der geschlechtsreifen und der juvenilen Hengste unterschieden sich signifikant in Verhalten und Hormonstatus. Die Unterschiede glichen sich nach Kastration an. Nach Kastration schwächte sich das Aggressionsverhalten der Althengste signifikant ab. Während vor der Kastration ausgeprägte Territoriumskämpfe auftraten, die meistens einen eindeutigen Ausgang hatten, und keine spielerischen Interaktionen zu beobachten waren, traten nach Kastration deutlich mehr unentschiedene Kämpfe und spielerische Auseinandersetzungen auf. Die Zahl der geringen sozialen Distanzen sowie die der direkten Körperkontakte stieg signifikant an, ebenso die Häufigkeit der spielerischen Interaktionen. In beiden Gruppen verschob sich nach Kastration die Rangordnung. Die Rangordnungen waren in den Beobachtungszeiten vor und nach Kastration sehr stabil. In der Junghengstgruppe wurden bereits vor Kastration agonistische Interaktionen auf einem zahlenmäßig niedrigeren Niveau beobachtet. Es kam häufiger zu direkten Körperkontakten.

Die sexuelle Aktivität wurde bei den adulten Hengsten signifikant durch die Kastration beeinflusst. Das grundsätzliche Interesse an Stuten blieb allerdings erhalten.

Grundsätzlich kann festgestellt werden, dass die Kastration das Sozialverhalten günstig beeinflusste, das heißt eine bessere Verträglichkeit vor allem der adulten Tiere zur Folge hatte. Die Kastration ist deshalb für Hengste, die nicht zur Zucht eingesetzt werden sollen, anzuraten. Der Eingriff sollte noch vor Eintritt der Geschlechtsreife erfolgen, wobei ein Alter von mehr als 12 Monaten noch als ausreichend früh angesehen werden kann. Auch Zuchthengste lassen sich tiergerecht in Gruppen halten, wenngleich nach Literaturangaben und den eigenen Untersuchungen mit relativ regelmäßig auftretenden agonistischen Interaktionen unterschiedlicher Schwere (z. B. unterschiedliche Dauer, Verletzungsgefahr) gerechnet werden muss.

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Gelding Is Likely To Cause Wild Horses Undo Suffering

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Nuclear receptor subfamily 3, group C, member 4, call it NR3C4, is an intracellular protein that is widely expressed in many types of cells and tissues throughout the body. It is a ligand-dependent transcription factor that is inactive until an androgen, either testosterone or its metabolite, 5α-dihydrotestosterone, forms a high-affinity ionic bond with it. The resulting NR3C4-androgen complex translocates from the cell cytosol to the cell nucleus where it binds to androgen-response elements in the regulatory regions of target genes. It then regulates the flow of genetic information from DNA to messenger RNA. The NR3C4-androgen complex plays key roles in an array of biological processes, including those integral to normal brain development, structure and function, and maintenance of musculoskeletal, reproductive, cardiovascular, immune and haemopoietic systems.

Gelding, i.e., castration, removes the primary source of androgen from the body. Gelded horses can live long and happy lives … in captivity aided by our management and the lack of challenges associated with life in the wild. However, a gelded horse lacks the capacity to regulate the transcription of significant genetic information. All of the physiological and structural processes mentioned above are compromised … not just his capacity to reproduce. He lacks androgen-dependent biochemical, physiological and anatomical advantages that evolved over millions of years to allow him to 1) survive without undue suffering, and 2) reproduce. Anyone who would ask a gelded horse to negotiate the challenges of a natural environment and lifestyle is either callus or has only a superficial knowledge of the impact of castration on an individual.

There is extensive scientific literature pertaining to the role of androgens in physiology and behavior. There is no justification for releasing gelded horses back into a natural environment. There is already sufficient evidence indicating it is likely to cause unwarranted suffering. If I were to propose such an experiment as part of a grant application, the federal government would require that I get institutional approval for the project based on the judgement of the *Institutional Animal Care and Use Committee* — a panel comprised of 14 full-time faculty investigators, a veterinarian, one layperson not affiliated with Washington University School of Medicine, and one non-scientific member. That committee is charged with assuring animals are humanely treated and, importantly, that experiments with animals provide significant new information that is not already available and cannot be acquired by means other than animal experimentation. When managing horses and proposing research projects (i.e., Antelope and Triple B Complexes Gather Plan, part 2.2.3.3.), the federal government should be held accountable to the same standards it rightfully imposes on others.

4H

Menopause ...and Wild Horse Management

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Menopause & Wild Horses

Menopause is a natural biological process. Even so, the physical and emotional consequences can be so severe some women seek hormone replacement therapy despite warnings of potential health risks. Sleep disruption, hot flashes, loss of energy, night sweats, insomnia, mood swings, headaches, dizziness, heart palpitations, sexual disinterest, joint stiffness, weight gain, depression, anxiety, irritability, urinary incontinence and so on are all listed as potential consequences of menopause. In addition, postmenopausal women are at increased risk for osteoporosis, heart disease, macular degeneration, glaucoma and colon cancer.

Menopause is generally defined as the permanent end of menstruation and fertility. However, it might be more accurately defined by the cessation of ovarian function. Most importantly for this discussion, it is associated with a reduction in the synthesis and release of estrogens, e.g., estradiol-17ß, and progestins, e.g., progesterone.

It is the decline in the levels of these ovarian hormones that cause trouble for so many women. And, of course, it is these hormones that hormone replacement therapy restores.

You might be wondering what this has to do with wild horse management, right? Let me explain. In 2010, the Bureau of Land Management (BLM), raised the possibility of gelding males (removing their testes) and spaying females (removing their ovaries) before returning them to the range.[1] Now, they have once again proposed spaying females as a method to control wild horse populations. Technically speaking, horses have estrus cycles, not menstrual cycles.[2] Nevertheless, spaying removes the primary source of estrogens and progestins from the body, just like menopause, and there is no reason to believe the consequences are less severe for mares than they are for woman. In fact, for woman the surgical removal of the ovaries results in more severe symptoms of menopause, because in natural menopause the ovaries continue to produce low levels of female hormones. It is hard to imagine that removal of these hormones from the body by spaying won't compromise a mare's ability to survive and thrive in the wild.

You see, the ovarian hormones are powerful steroid hormones. They have widespread effects on physiology and behavior. Estrogen, for example, is essential for

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[1] Working Toward Sustainable Management of America's Wild Horses and Burros: Draft Goals, Objectives and Possible Management Actions, Bureau of Land Management, Department of the Interior, June 2010.

[2] If conception does not occur during a cycle, animals that have menstrual cycles slough off the uterine lining during menstruation, whereas animals that have estrous cycles reabsorb the uterine lining. The function and components of the reproductive endocrine system, the hypothalamic-pituitary-gonadal axis, are essentially the same.

healthy bone. When estrogen production declines due to menopause, whether naturally occurring or surgically induced, or after exposure to radiation or chemotherapeutic drugs, bones become brittle and break easily. Spaying mares is likely to have similar effects on bones density and strength. I wonder, does the BLM really think returning mares to the open range in a condition associated with the increased risk of breaking bones is a good idea? Have they given it any thought at all? The image of a horse suffering with a broken leg on the open range due to compromised bone density isn't a pretty one.

Ovarian hormones also influence many neural circuits throughout the brain by binding to transcription factors which regulate the activity of certain genes.[3] In addition to the hypothalamus and other brain areas related to reproduction, they affect the activity of serotonin pathways, catecholamine neurons, and the basal forebrain cholinergic system. They influence the functioning of the hippocampus, a brain region involved in spatial and declarative memory. Ovarian steroids also have measurable effects on affective state, e.g., mood/emotion, and cognition … the mental processes involved in learning, memory, problem solving and decision making. They affect cardiovascular health and immune function. And, anyone who has watched mares in "heat" might guess, ovarian hormones have potent effects on social relations too … an

[3] My PhD thesis research at the Institute of Animal Behavior, Rutgers University, focused on the regulation of these transcription factors in brain by neurotransmitter systems: Nock, B.: Noradrenergic Regulation of Female Sexual Behavior and Hypothalamic Steroid Receptors, PhD Thesis, Rutgers University, 1980.

effect incompatible with the BLM's goal of creating a "thriving natural ecological balance" for the wild horses (see [4]).

But, taking out the ovaries doesn't just remove ovarian hormones from the body. There's more to it … a lot more. It affects other endocrine systems as well. Even forty years ago endocrinologists appreciated the subtle intricacies of endocrine system function and the complexities of hormone interactions. My endocrinology professor in graduate school, Dr. Alan Leshner PhD, drove home time and again the point that "No hormone works independently of other hormones." Take away one hormone and it affects the levels of other hormones too. Remove ovarian hormones and pituitary hormone releasing and inhibiting hormone activity changes, gonadotropic hormone levels go up, adrenocorticotrophic hormone levels go up, cortisol levels go up, thyroid hormone levels go down, and so on and so on. It's a fact. Moreover, the assessment of the long-term consequences of such hormonal imbalances requires in-depth, long-term comprehensive field studies by professional scientists … individuals trained and experienced in the art and science of science. Veterinarians don't currently know the consequences. BLM personnel don't currently know the consequences. The National Science Foundation doesn't currently know the consequences. But, it is reasonable to suggest based on what we do already know that spaying is likely to have serious consequences for a mare's ability to survive and thrive on the open range.

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[4] Nock, B.: Wild Horses and Ecological Balance. Liberated Horsemanship Press. Commissioned by The American Wild Horse Preservation Campaign.

So, the idea of spaying mares as a method of population control doesn't sit well with me when I think beyond how it impacts reproduction. I'm also skeptical about whether it can be done safely in the field. Taking out the ovaries which reside in the abdominal cavity is a much more serious procedure than removing the testes of a male. When we ovariectomize rats for research, we do it in a state-of-the-art animal facility. We're required, by federal law, to use sterile procedures and defined recovery and followup protocols. I'm wondering, can and will the BLM, a federal agency, adhere to the practices the federal government requires us to follow for rats when removing the ovaries of horses in the field? I seriously doubt it is possible.

Even if such precautions are effectively implemented, I doubt the wisdom of returning spayed horses to the open range when bone health, mental and emotional processes and social behaviors have been compromised. The endocrine system is one of the major signaling systems of the body and ovarian hormones are major components. They are powerful agents which function to coordinate behavior and physiology. Inducing what could be described as physiological chaos by removing ovarian hormones and disrupting the normal functioning of neural and other endocrine systems to boot is very likely, in my opinion, to compromise a mares ability to thrive as a fully integrated herd member. *Bruce - 314-740-5847 BruceNock@mac.com*

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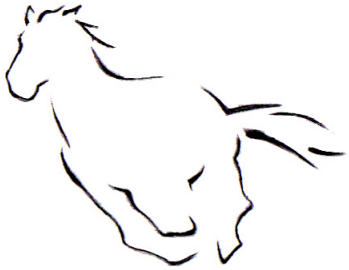

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Ovarian function following immunocontraceptive vaccination of mares using native porcine and recombinant zona pellucida vaccines formulated with a non-Freund's adjuvant and anti-GnRH vaccines

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ABSTRACT

An important determinant in the selection of any contraceptive agent is the impact on ovarian function, both in the short and longer term. In this study, ovarian activity was monitored in mares immunised with one of the following vaccine formulations; native porcine zona pellucida (pZP), recombinant zona pellucida proteins ZP3 and ZP4 (reZP), pZP and reZP combined or a commercially available anti-GnRH vaccine. The ZP antigens were prepared in an adjuvant formulation consisting of 6% polymeric adjuvant (Montanide™ PetGel A, Seppic, France) and 500 μg polyinosinic-polycytidylic acid - TLR3-agonist (Poly(I:C) HMW VacciGrade™, Invivogen, USA). A vehicle-only control group was administered the adjuvant formulation without antigen. Ovarian activity was monitored using clinical observations (transrectal palpation and ultrasonography of the reproductive tract) in addition to blood sampling for serum progesterone and anti-Müllerian hormone (AMH) concentrations while employing a low sampling frequency. Treatments and measurements were initiated in December (southern hemisphere summer) and subsequent data collection was performed in January, February, March and May. Both reZP and anti-GnRH vaccination were associated with clinically evident ovarian suppression in the short term. Ovarian activity in mares administered a reZP or anti-GnRH vaccine was significantly different to adjuvant control and pZP treated mares. Serum AMH concentrations were different between pZP and anti-GnRH treated mares 3.5 months after the final vaccination. Serum AMH concentrations were significantly correlated with mare age, serum progesterone and ovarian volume.

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1. Introduction

A number of antigens have been proposed as targets for fertility control *via* vaccination. These include peptide hormones, oocyte and sperm proteins and other molecules associated with fertilization and early embryonic development [1]. Two immunogens studied extensively in the horse and other species as potential contraceptive agents are gonadotrophin releasing hormone (GnRH) and native porcine zona pellucida (pZP) proteins [1].

An important determinant for the selection of a contraceptive agent is the effect on ovarian function, both in the short and long term [1]. The presumed immunocontraceptive mechanism of pZP in the horse involves antibody binding to the ZP sperm receptor sites and subsequent prevention of sperm-oocyte binding and fertilisation. Based on this supposition, pZP immunisation should not affect the hypothalamic-pituitary-gonadal axis, thereby permitting continuation of cyclical ovarian activity [2,3] and associated behaviours [1]. However, pZP-based immunocontraception causes irreversible ovarian damage in some species [4–6]. By contrast, anti-GnRH vaccines trigger production of antibodies that

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neutralise endogenous GnRH, which prevents receptor binding and activation of pituitary gonadotrophs. The suppression of gonadotrophin secretion causes reproductive quiescence characterised by cessation of cyclical ovarian activity [7,8]. Anti-GnRH vaccines therefore also suppress both physiological and behavioural oestrus in the immediate [7,9–11] and longer terms [8]. Ovarian suppression onsets within three months of treatment [8–12] and is associated with decreased ovarian weight [12], length [9], volume [11,12], and reductions in serum progesterone [9–12], oestradiol-17β [10], LH [12] and FSH [12] concentrations. Ovarian suppression has also been reported in mares subsequent to immunocontraception using pZP vaccine [13–15]. In this respect, apparent cessation of oestrous cyclicity and erratic cyclicity into the non-breeding season have been reported after long term treatment (>3 years) with pZP vaccines [1,16]. More recently, 93% of mares treated with a pZP vaccine ceased cyclical activity within four months of treatment [17]. Abrogated cyclicity was associated with both consistently low serum progesterone and minimal ovarian activity as determined by clinical, macroscopic and histological examination of the ovaries. Recombinant vaccines have been developed by the expression of porcine ZP3 and ZP4 in *Escherichia coli* (reZP) [18]. A recent report has described the intensively monitored ovarian function and fertility of pony mares subsequent to treatment with either a pZP or a reZP vaccine [19]. Control mares retained cyclical ovarian activity throughout the trial whereas six out of seven pZP treated mares and one reZP mare entered an extended (albeit reversible) anoestrus characterised by clinically apparent ovarian suppression and basal serum ovarian steroid concentrations. Pregnancy was established in 0%, 57% and 100% of pZP treated, reZP treated and control mares, respectively [19]. A recent vaccination trial in donkey jennies studied the effects of pZP and reZP on oestrous cyclicity and fertility [20]. The vaccines were similarly formulated to that of previous pZP and reZP vaccines administered in horses [19], using Freund's adjuvants. Seven of 9, 6/8 and 0/8 jennies entered anoestrus within three months after the final vaccination for the reZP, pZP and control jennies, respectively. No jennies in the two vaccinated groups became pregnant compared to 6/8 control jennies.

The advantages of a reZP (compared to pZP) vaccine include production efficiency and the avoidance of contamination with non-ZP proteins and heat-resistant microorganisms [19,21,22]. However, the efficacy of pZP or reZP as an immunocontraceptive agent relies on the inclusion of a strong adjuvant [23]. Freund's complete modified adjuvant (FCMA) is typically used for the primary inoculation followed by Freund's incomplete adjuvant (FIA) for booster inoculations. Freunds' adjuvants can cause undesirable side effects, which can be severe and persist for months [23]. The use of alternative adjuvants that produce a similar or better immune response with less severe side effects would be advantageous.

A more complete understanding of ovarian suppression subsequent to ZP-based immunocontraception will better define the mechanism of the contraceptive effect [19]. However, populations requiring contraception are typically managed under extensive conditions and, therefore, practical methods with limited intervention opportunities are commonly required for monitoring effects [21]. Anti-Müllerian hormone (AMH) has been proposed as a tool for assessing ovarian function during ZP-based immunocontraception [24] as it is reportedly a consistent [25] and useful biological marker of ovarian function [26].

The current study aimed to describe ovarian function in mares managed under extensive conditions following treatment with pZP or reZP vaccines formulated using non-Freund's adjuvants or a commercially available anti-GnRH vaccine. In addition, AMH concentrations were compared between treatment groups.

We hypothesised that immunocontraception using pZP proteins formulated with non-Freund's adjuvants would have similar ovarian effects as anti-GnRH vaccination. Furthermore, immunocontraception using reZP proteins formulated with non-Freund's adjuvants were expected to elicit similar ovarian responses as pZP vaccination. Additionally, we anticipated changes in AMH concentrations in ZP immunocontracepted mares.

2. Materials and methods

2.1. Mare selection, management and environment

A population of mixed breed mares (light body type: Arabian, Quarter Horse, Draught and Thoroughbred cross; age: 2–10 y) were studied from November 2016 to May 2017. Inclusion criteria were non-pregnant, normal oestrous cyclicity, good physical and reproductive health and no previous immunocontraceptive treatment [19]. Fifty barren or maiden mares were initially screened for inclusion during a 30-day monitoring period. In this group, regular oestrous cyclicity was confirmed in 26 mares on the basis of periodic changes in the serum progesterone concentration [27]. Lactating mares at the same site were recruited following re-establishment of oestrous cycle activity (assessed by transrectal palpation and ultrasonography of the reproductive tract). Thirty-nine mares were ultimately enrolled (26 maiden or barren and 13 lactating). Mares were maintained on a single extensive mountainous grassland site (3000ha) in pre-existing groups. The study site was located at 29° 51′ 30.8664″ S 29° 20′ 46.9068″ E. The study occurred during the physiological breeding season [28]. The natural day length and environmental temperature range at the beginning and end of the study period were 13 h 18 m, and 7–31 °C and 10 h 20 m, and −3 to 24 °C, respectively.

2.2. Study design

Horses (n = 39) were stratified by body condition scores (BCS: 1–9) [29], parity and age (Table 1) for random assignment to one of five treatment groups. Repeated measures data were gathered *via* clinical observation and venous blood collection.

2.3. Formulation of vaccines

The same adjuvant formulation was used for each of the control, pZP-only, reZP-only and combined pZP and reZP groups. Each vaccine dose (1 mL) was constituted by combining the antigen, 6% polymeric adjuvant (Montanide™ PetGel A, Seppic, France) and 500 μg polyinosinic-polycytidylic acid - TLR3-agonist (Poly(I:C) HMW VacciGrade™, Invivogen, USA). The amount of antigen *per* treatment was as follows: 100 μg pZP (Trumpeter Farms and Veterinary Service, Winters, California, USA) for pZP treatments; 250 μg recombinant ZP3 (containing tetanus toxoid epitope) and 250 μg ZP4 (containing bovine RNase epitope; reZP; supplied by BioSciences, CSIR, South Africa) for reZP treatments and no antigen for the control group. Multi-dose vials of each vaccine formulation were prepared, lyophilised and reconstituted with sterile water for injection.

2.4. Vaccine administration

The adjuvant control group (n = 8) was treated on d = 35 and again five weeks later (d = 70).

The pZP-only group (n = 7) received an initial vaccination at d = 35 followed by an identical booster vaccination after five weeks (d = 70).

The reZP-only group (n = 8) received an initial vaccination at

Table 1
Treatment groups sub-divided on the basis of mare distribution by: age, median (range); parity, median (range); and BCS (1–9), median (range). P > 0.05.

Mare information	Treatment					P value
	Control (n = 8)	pZP-only (n = 7)	reZP-only (n = 8)	pZP and reZP (n = 8)	GnRH (n = 8)	
Age (years)	4 (2, 9)	4 (2, 8)	4 (2, 10)	3 (2, 7)	4 (2, 7)	1.000
No. previous parities	1 (0, 3)	1 (0, 3)	1 (0, 5)	1 (0, 3)	1 (0, 4)	1.000
BCS (1–9)	5 (4, 7)	6 (4, 7)	5 (4, 7)	5 (3, 7)	6 (2, 8)	0.700

pZP, native porcine zona pellucida vaccine; reZP, recombinant porcine zona pellucida vaccine; GnRH, anti-GnRH vaccine (Improvac®).
Body condition score (BCS).

d = 0, followed by two identical boosters at five week intervals (d = 35 and d = 70).

The pZP and reZP group (n = 8) received an initial vaccination of pZP at d = 35 followed by a booster vaccination of reZP after five weeks (d = 70).

The anti-GnRH group (n = 8) received an initial 2 mL vaccination containing 400 μg GnRH-protein conjugate with an diethylaminoethyl (DEAE)-dextran adjuvant (Improvac®, Zoetis, South Africa) at d = 35 followed by an identical booster vaccination five weeks later (d = 70).

All vaccines were administered by deep intramuscular injection into the gluteal muscle mass; boosters were administered into the contralateral musculature.

2.5. Data collection

Animals were examined and samples collected in December (d = 0), January (d = 35), February (d = 70), March (d = 105) and May (d = 175). Transrectal palpation and ultrasonography of the reproductive tract was performed at d = 0, d = 35, d = 70, d = 105 and d = 175. During the examination, ovarian volume, presence of follicles ≥15 mm diameter, presence of a CL (confirmed retrospectively by serum progesterone >1 ng/mL), uterine and cervical tone and the presence of uterine oedema were recorded for each mare. Oestrous cyclicity or activity was defined as the presence of follicles ≥15 mm and ovarian volume ≥25 cm^3 (prolate ellipsoid formula) and the confirmation of an ovulation (if present) was confirmed by the presence of a previously unrecorded CL or corpus haemorrhagicum in conjunction with serum progesterone levels >1 ng/mL. In the absence of a CL, oestrous cyclicity was determined on the basis of observed tubular genital tract characteristics [30]. Ovarian inactivity was defined as bilaterally small ovaries (both <25 cm^3), the absence of a CL or any follicles ≥15 mm and basal (<1 ng/mL) serum progesterone [8,11,19,31].

Blood samples were collected by jugular venipuncture at d = 0, d = 35, d = 70, d = 105 and d = 175. Samples were centrifuged and serum stored at −20 °C until assayed.

2.6. Pasture breeding

Three mature, clinically-healthy and proven fertile stallions (6–8 years of age) were selected for pasture-based breeding. One stallion was randomly selected for each of the three breeding herds and introduced in March (d = 105). All stallions remained with the mares until July. Foaling outcome was assessed at the end of the subsequent physiological breeding season based on available records.

2.7. Hormone assays

Serum progesterone was measured using a chemoluminescence technique (Immulite® 1000, Siemens, Germany) [32]. Serum AMH concentrations were determined using a commercially available ELISA according to the manufacturer's instructions (AMH Gen II ELISA; Beckman Coulter, Brea, CA, USA). This assay has been validated for use in mares [33] and the detection limit of the assay was 0.08 ng/mL. Intra- and inter-assay coefficients of variation were 3.7% and 4.4% respectively, for a low AMH concentration (3.82 ng/mL), and 3.4% and 4.0%, for a high AMH concentration (16.45 ng/mL).

2.8. Statistical analyses

Data concerning the presence/absence of individual measures of normal oestrous cyclicity were compared among treatment groups using mixed effects logistic regression. Quantitative data were log transformed and analysed using mixed effect linear regression. Regression models included fixed effect terms for treatment group, sampling time (categorical with five levels), a group by time interaction (AMH only) and age to adjust for potential confounding. Mare was included as a random effect and a first-order autoregressive correlation structure was used to account for repeated sampling. Post-hoc tests in the mixed-effects models were adjusted using the least significant differences (LSD) or Bonferroni method. Serum AMH concentrations at each sampling time were compared among groups using one-way ANOVA with multiple *post-hoc* comparisons adjusted using Bonferroni correction of P values. Pairwise correlations were estimated using Spearman's rho or Pearson's correlation coefficient as appropriate. Statistical testing was performed using commercially available software (IBM SPSS Statistics Version 25) and significance was set at $P \leq 0.05$.

3. Results

3.1. Ovarian activity

Treatment groups were comparable in respect to age, parity, and BCS (Table 1) and all mares had evidence of cyclic ovarian activity prior to treatment (Table 2). Treatment (P = 0.001) and time (P < 0.001) both had a significant effect on the presence/absence of normal ovarian activity. Mares in the control and pZP treated groups expressed normal cyclical ovarian activity most commonly followed by mares within the combined pZP and reZP, reZP-only, and GnRH treated groups in descending order of frequency. When summarized for all observation times, control mares were more likely to be cycling compared to reZP (P = 0.005) and GnRH (P < 0.001) treated mares. Similarly, pZP treated mares were also more likely to be cycling compared to reZP (P = 0.002) and GnRH (P < 0.001) treated mares. Five weeks after the first treatment and first booster for reZP-only (d = 70), 8/8 control mares, 6/7 pZP-only mares, 5/8 pZP and reZP mares, 3/8 reZP-only mares and 2/8 anti-GnRH mares were demonstrating normal oestrus cyclicity. Five weeks after the final booster (d = 105), 5/8 control mares, 4/7 pZP-only mares, 3/8 pZP and reZP mares, 1/8 reZP-only mares and 0/8 anti-GnRH mares were demonstrating normal oestrus activity. By the end of the active monitoring period (d = 175), 3/8 control

Table 2
Number of mares displaying ovarian activity or inactivity at each time-point during anti-ZP or -GnRH vaccination or adjuvant-only (control) treatment.

Time-point	Treatment									
	Control (n = 8)		pZP-only (n = 7)		reZP-only (n = 8)		pZP and reZP (n = 8)		GnRH (n = 8)	
	Active	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Active	Inactive
d = 0	8	0	7	0	8	0	8	0	8	0
d = 35	8	0	7	0	8	0	8	0	8	0
d = 70	8	0	6	1	3	5	5	3	2	6
d = 105	5	3	4	3	1	7	3	5	0	8
d = 175	3	5	3	4	0	8	0	8	0	8

pZP, native porcine zona pellucida vaccine; reZP, recombinant porcine zona pellucida vaccine; GnRH, anti-GnRH vaccine (Improvac®).

mares, 3/7 pZP-only mares, 0/8 pZP and reZP mares, 0/8 reZP-only mares and 0/8 anti-GnRH mares had evidence of normal ovarian activity. At the end of the subsequent breeding season the records for seven mares were available. Three control mares foaled and one pZP-only mare experienced a late-gestation abortion.

3.2. AMH

Treatment group had a significant effect on the serum AMH concentrations collected over the entire study ($P = 0.030$). Furthermore, there were significant differences among treatment groups at d = 105 ($P = 0.037$) and d = 175 ($P = 0.019$). No post hoc pairwise comparisons were significant at d = 105 but at d = 175, mares treated with the anti-GnRH vaccine had higher concentrations compared to pZP-only treated mares ($P = 0.029$). The difference between pZP-only treated and control mares at this time-point was not significant ($P = 0.084$). Serum AMH concentrations changed over time in reZP-only mares with higher concentrations at d = 70 compared to d = 105 ($P = 0.047$) (Table 3). Serum AMH concentrations were positively correlated with ovarian volume ($r = 0.171$, $P = 0.035$) and mare age (ordinal categories; >3 y, 3–6 y, >6 y) ($\rho = 0.269$, $P < 0.001$) but negatively correlated with serum progesterone ($r = -0.373$, $P = 0.014$).

4. Discussion

The immunocontraceptive method of action of pZP in the horse, and other species, has been proposed to involve the prevention of sperm–zona binding, with oestrous cyclicity presumed to continue undisturbed [2,3]. However, the results of the present study demonstrate varying degrees of ovarian suppression across all treatment groups. Significantly more reZP-only and anti-GnRH mares stopped cycling sooner after vaccination than either the control or the pZP-only mares. A two-pronged treatment protocol utilising pZP as the primary inoculation and reZP for the booster had a protracted effect. Ovarian suppression following anti-GnRH vaccination has been reported and the mechanism (suppression of FSH and LH secretion) is well understood [7,8,11]. There is increasing evidence that, at least in some species, ovarian suppression is a contributory factor in the contraceptive efficacy of ZP vaccination [4,5,15–17,19,34]. However, more research is required to define the mechanism of ZP-associated ovarian suppression.

A previous study from our research group reported higher incidences of anoestrus and a superior contraceptive effect in mares treated with pZP compared to reZP [19]. However, the reZP vaccine [18] used in that study was manufactured in a different laboratory, was formulated with Freund's adjuvant and only a single booster treatment was administered. The current study is the first to report a non-Freund's adjuvant for ZP-based immunocontraception in the horse.

The reduction in ovarian activity in control mares was possibly an effect of season. A significant effect of time on ovarian activity was evident for all groups, and while this is expected in seasonally breeding animals, it was a limitation of the current study. Reproductive activity is determined by season, primarily photoperiod, and to a lesser extent nutrition and environmental temperature [35]. The physiological breeding season in the southern hemisphere is October to March but variations have also been reported [28,30]. Only 26 of the 50 mares initially assessed for the study were cyclic at the end of November and this delayed the initial administration of treatments and subsequent introduction of stallions for breeding. An additional limitation was the paucity of foaling records at the end of the following breeding season. Further investigations into the contraceptive efficacy, reversibility and safety of the novel formulation used in this study are warranted.

The AMH results of the present study were partially consistent with previous reports [24]. Mean AMH concentrations differed across groups at d = 105, but differences between pZP-only and

Table 3
Mean (95% CI) serum anti-Müllerian hormone concentrations (AMH; ng/mL) in mares over a 6 month period during anti-ZP or -GnRH vaccination or adjuvant-only (control) treatment.

Time-point	Treatment					P value†
	Control (n = 8)	pZP-only (n = 7)	reZP-only (n = 8)	pZP and reZP (n = 8)	GnRH (n = 8)	
d = 0	1.21 (0.58, 2.56)	0.80 (0.36, 1.78)	$0.88^{\Delta\square}$ (0.46, 1.70)	0.64 (0.29, 1.40)	0.89 (0.53, 1.50)	0.380
d = 35*			$0.70^{\Delta\square}$ (0.41, 1.19)			
d = 70	1.04 (0.59, 1.84)	0.68 (0.39, 1.20)	0.96^{Δ} (0.50, 1.83)	0.64 (0.37, 1.09)	0.93 (0.39, 2.19)	0.730
d = 105	0.80^{a} (0.33, 1.96)	0.47^{a} (0.38, 0.57)	$0.44^{a,\square}$ (0.18, 1.07)	0.67^{a} (0.39, 1.18)	1.02^{a} (0.60, 1.72)	0.037
d = 175	0.96^{ab} (0.48, 1.92)	0.45^{a} (0.21, 0.95)	$0.67^{ab,\Delta\square}$ (0.33, 1.35)	0.90^{ab} (0.43, 1.87)	1.16^{b} (0.69, 1.96)	0.019
P value¤	0.180	0.330	0.049	0.140	0.690	

pZP, porcine zona pellucida vaccine; reZP, recombinant porcine zona pellucida vaccine; GnRH, anti-GnRH vaccine (Improvac®).
* reZP-only treatment group additional measurement.
† Based on 1-way ANOVA comparing AMH among groups within each time-point. Means with different superscripts (letter) differ significantly after *post-hoc* testing incorporating Bonferroni correction.
¤ Based on mixed effects linear regression comparing AMH over time within each treatment group including a random effect for mare to account for the repeated sampling and fixed effects of age and time-point. Means with different superscripts (symbol) differ significantly after *post-hoc* testing incorporating Bonferroni correction.

anti-GnRH mares were only evident at d = 175. The reduced sampling frequency and relatively small group sizes in this study might have contributed to the absence of other significant differences. As a result, it is not clear whether AMH is a suitable indicator of the effect of ZP vaccination on ovarian follicular activity in mares managed under extensive conditions (i.e. sampled infrequently). However, AMH concentrations were correlated with ovarian volume and serum progesterone, suggesting that it can be useful under certain circumstances. Previous work by our research group noted the potential of serum AMH concentrations for monitoring ovarian function following immunocontraception in mares [24]. Samples were collected weekly from October to March in this previous work [19,24] but only analysed for five strategic time periods. The results of the previous study served to inform the frequency of sampling and study design for the current project. Among other hypotheses, the current study investigated the premise that less intensive sampling for serum AMH would still be useful for monitoring ovarian function. This is important because most populations of horses that require immunocontraception are feral or semi-feral, greatly limiting the ease and frequency of interventions such as blood sampling. Sample collection coinciding with other interventions, such as inoculations, would enhance practicality in such circumstances. Serum AMH has less cyclical variability in the horse [25] and may therefore be less influenced by season than clinical measures of cyclicity.

Serum AMH tended to be higher in older mares and there is a need to further investigate the dynamics of AMH concentrations in younger, albeit sexually mature, mares. This positive correlation between age and AMH has also been reported in Japanese Black cows [36].

In conclusion, a non-Freund's adjuvated reZP vaccine is a promising alternative for immunocontraception in the mare when ovarian suppression is an acceptable outcome. Serum AMH concentrations following ZP-based vaccination may be used to infer reductions in both ovarian volume and serum progesterone under extensive conditions.

Authorship

M.B. Nolan and M.L. Schulman contributed to the study design, data collection, data analysis and interpretation, preparation and final approval of the manuscript. H.J Bertschinger and G.T. Fosgate contributed to the study design, data analysis and interpretation, preparation and final approval of the manuscript. R. Roth and M. Crampton prepared the reZP and contributed to final approval of the manuscript. I.S. Martins contributed to the data collection and final approval of the manuscript. T.A.E. Stout contributed to data interpretation and preparation and final approval of the manuscript.

Authors' declaration of interests

No competing interests to declare.

Ethical animal research

The study was approved by the University of Pretoria Animal Ethics Committee (V124-16).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.theriogenology.2018.07.044.

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Immunocontraception in Wild Horses (*Equus caballus*) Extends Reproductive Cycling Beyond the Normal Breeding Season

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Abstract

Background: Although the physiological effects of immunocontraceptive treatment with porcine zona pellucida (PZP) have been well studied, little is known about PZP's effects on the scheduling of reproductive cycling. Recent behavioral research has suggested that recipients of PZP extend the receptive breeding period into what is normally the non-breeding season.

Methodology/Principal Findings: To determine if this is the case, we compiled foaling data from wild horses (*Equus caballus*) living on Shackleford Banks, North Carolina for 4 years pre- and 8 years post-contraception management with PZP (pre-contraception, n = 65 births from 45 mares; post-contraception, n = 97 births from 46 mares). Gestation lasts approximately 11–12 months in wild horses, placing conception at approximately 11.5 months prior to birth. Since the contraception program began in January 2000, foaling has occurred over a significantly broader range than it had before the contraception program. Foaling in PZP recipients (n = 45 births from 27 mares) has consistently occurred over a broader range than has foaling in non-recipients (n = 52 births from 19 mares). In addition, current recipients of PZP foaled later in the year than did prior recipient and non-recipient mares. Females receiving more consecutive PZP applications gave birth later in the season than did females receiving fewer applications. Finally, the efficacy of PZP declined with increasing consecutive applications before reaching 100% after five consecutive applications.

Conclusions/Significance: For a gregarious species such as the horse, the extension of reproductive cycling into the fall months has important social consequences, including decreased group stability and the extension of male reproductive behavior. In addition, reproductive cycling into the fall months could have long-term effects on foal survivorship. Managers should consider these factors before enacting immunocontraceptive programs in new populations. We suggest minor alterations to management strategies to help alleviate such unintended effects in new populations.

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Introduction

The extirpation of predator species has resulted in the expansion of free-ranging ungulate populations in North America [1], necessitating their regulation. Immunocontraceptive management has become increasingly popular as culling programs are seldom well-received by the general public. In females, the most common form of immunocontraception, porcine zona pellucida (PZP), stimulates the production of antibodies that bind sperm receptors on the egg's surface, thereby preventing sperm attachment and fertilization [2].

In recent decades, the wild horse (*Equus caballus*) has become a model for evaluating the effects of PZP, thanks chiefly to the seminal nine-year study of contracepted mares on Assateague Island National Seashore [3]. Subsequent research on this population has shown that PZP has little to no effect on recipient physiology or behavior [4,5,6]. For example, in Assateague horses, PZP has no effect on the duration of individual estrous cycles [6], and researchers have reported only minor ovulation failure and depressed urinary oestrogen concentrations with repeated applications [4]. However, behavioral research on the horses of Shackleford Banks, North Carolina, and other wild ungulate species suggests that PZP affects the reproductive physiology of recipient animals [7,8,9]. In each of these studies, females treated with PZP extended reproductive behaviors into the non-breeding season. While these results are consistent with an extension of ovulatory cycling into the post-breeding season when most females are normally anovulatory [10,11], this possibility has not yet been tested.

Mares are seasonally polyestrous and extended estrous periods have been documented [11]. Tropical species, for example, are less strictly seasonal, and in some cases, reproduce throughout the year [12,13]. In addition, variability in the cycling schedules and receptivity of individual mares [14], and the performance of estrous behavior and copulatory activities during the non-breeding season [15] have been documented in temperate species. Such

variation in ovulatory scheduling and receptivity suggest that the seasonality of reproductive behavior in *Equus caballus* females is characterized by a substantial degree of plasticity. As the physiological state of contracepted animals has been significantly altered, the possibility of prolonged extended cycling is even more feasible.

In wild horse societies, the harem is the core social group, consisting of usually one, but sometimes two or three harem male(s), one to several female(s), and their offspring [16,17,18,19,20,21]. Harem males will sometimes fight to acquire mares from other groups, but stallions almost always retain their mares [18,19,20,22]. In temperate environments, food availability is lower during the fall and winter months and free-ranging horses will alter their activity to maximize food intake and reduce energetic costs [23,24]. Mares are typically anovulatory at this time and sexual behavior in males is largely absent [11,14].

On Shackleford Banks, increased reproductive behavior in the post-breeding season by mares has resulted in increased male attentiveness [9]. Such behavior (by males) has been shown to restrict the movement of females, thus reducing their grazing efficiency [21,25]. The occurrence of this behavior during a time of year when animals typically increase group spread to acquire adequate forage [19,24], represents a change in behavior fundamental to the animals' survival [26,27]. Offspring conceived during the post-breeding season are likely subject to decreased resource availability as lower quality forage can affect mares' ability to produce sufficient milk [27]. Finally, regardless of the timing of titer decline post-treatment [9,28,58], when anti-PZP antibody titers decrease during the fall months [28,29], extended reproductive cycling among recipient mares will increase their chances of conception, thereby lowering the vaccine's overall efficacy. Determining whether PZP recipients are likely to extend reproductive cycling is therefore of great importance if managers are to limit animal numbers while still maintaining functional, healthy populations.

Here we use the birth dates of foals to estimate dates of conception for PZP recipient and non-recipient mares on Shackleford Banks, North Carolina. Gestation length in wild horses is 11–12 months [30]. Therefore, dates of conception can be reliably estimated as approximately 11.5 months prior to birth. Breeding normally occurs from March through August, with most births occurring in April and May [11]. Given the extension of reproductive behavior in PZP- treated mares [9], we hypothesize that PZP recipients will extend cycling into the non-breeding season more often than will non-recipients. Therefore, when PZP recipients conceive and give birth, they will do so later on average and over a wider range of months than will non-recipients.

Methods

Study area

This study was conducted on Shackleford Banks, a barrier island located approximately 3 km off the coast of North Carolina, USA. The island was 15 km in length, and varied between 0.5 and 3 km in width. The horse population on Shackleford Banks has been co-managed by the National Park Service and the Foundation for Shackleford Horses since 1996.

Study subjects

The reproductive units of Shackleford horses are typical of feral equids. They are coherent harem groups of one, or sometimes two or three stallion(s), one to several mare(s) and their offspring [18]. Predominantly, the harem groups are not territorial and animals move within overlapping home ranges, although this has not always been the case [18].

Management

PZP Contraception. In January 2000, the National Park Service began the application of PZP for the purposes of immunocontraception. The National Park Service administers PZP in the spring (late February through April) each year. Mares receive their initial treatment at 1.5–2 years of age. Each injection includes 100 micrograms of PZP with an adjuvant (combined at the darting site). Initial doses include Freund's Complete Adjuvant, Modified, Mycobacterium butyricum (Calbiochem #344289). All succeeding doses include Freund's Incomplete Adjuvant (Sigma #F5506). In a given year, an average of 63% of all reproductive mares are inoculated with the vaccine (range = 37–88%). The authors of the present study are not and have never been in charge of making management decisions regarding this herd.

Gathers and Removals. The National Park Service began their management of the Shackleford Banks population prior to the use of PZP, performing five gathers between November 1996 and January 2000. During these gathers the majority of the population was rounded up and individuals testing positive for equine infectious anemia were removed and either euthanized or quarantined. As such forms of management could conceivably influence reproductive cycling, we analyzed foaling dates before and after gathers (but before contraception management) using a linear mixed effects model (see Statistical Analyses).

From January 2000 – January 2008, 38 foals (conceived due to contraception failure or administration scheduling) were removed from the island for the purpose of population control. The majority of removals (92%) were conducted in the January following the foals' birth.

Foaling Data

We recorded foaling data before contraception management (1995–1997) during a study of mother-infant behavior [31]. We obtained foaling data for 2000 and post-contraception years (2001–2008) from the National Park Service at Cape Lookout National Seashore, North Carolina. Although PZP was first administered in 2000, foals born in this year were conceived before contraception management. As such, we considered 2000 a pre-contraception year.

We identified individual horses by color, sex, age, physical condition, and other distinguishing markings including freeze brands. We monitored pregnant mares 2–3 times per week to ensure accurate estimation of foaling date. Births are not commonly witnessed, as mares will generally give birth away from their harem group [31]. This does not significantly affect the determination of birth dates because typically, mares return to their groups within hours (depending on the length of the foaling process and the mares' and foals' condition afterward). In the event that we did not locate mares for more than one week, the birth dates of new foals were estimated by comparing the condition of the foals' coat, mane and tail hair, and locomotor ability to that of foals for which the exact age was known. Using these methods, we were able to estimate birthdays within a range of 1–2 days to 1 week. Focal animals in this study were observed from a minimum of 15 m away at all times. The observers did not obstruct or manipulate the animals' natural behavior in any way. As such, approval for this study by a review board or ethics committee was unnecessary.

We designated births as coming from current recipient, prior recipient, or non-recipient mares. Current recipients received PZP

treatment the year they conceived (the year previous to foaling). Prior recipients received PZP treatment at some point earlier in their lifetime, but not the year of conception. Non-recipients had never received PZP at the time of conception.

Physical Condition

Physical condition is an important factor to consider when monitoring reproductive behaviors. Animals in better condition will have more resources to allocate to mating behavior and physiology than will animals in poorer condition. We assessed mare condition via rump scoring. We determined rump scores examining the curvature of the line between the tailbone and the point of the hip. Scores were based on a scale from 1 to 5; a score of 1 being the poorest [32].

Weather Data

We collected all climatology data from the Morehead City WNW Station at 34° 44′N; 76° 44′W, approximately 8 km from the study site. We obtained all data from the National Climate Data Center (2009) [33].

Statistical Analyses

We analyzed data in R version 2.11.1 (R Core Group, Vienna Austria) and JMP, version 7 (SAS, Cary NC, USA). We used Fligner-Killeen tests to determine if variation in foaling dates differed before and after contraception management and between PZP recipients and non-recipients. This test is more conservative than Fisher's or Bartlett's tests and is less sensitive to outliers and non-normal data [34]. Results from these tests show Bonferroni corrected *P*-values.

We used linear mixed effects models to analyze monthly weather patterns. These statistical models included year as a random effect and the following fixed effects: linear through fourth order polynomial functions of month (to account for nonlinear fluctuations in temperature and rainfall), management regime (before or after contraception management), and two-way interactions with management regime. We selected best fit models using Akaike's Information Criterion adjusted for small sample size (AICc). Because temperature data were temporally auto-correlated, we included an autoregressive moving average correlation structure [35]. For each year of the study, we calculated the mean of the monthly residual values from both the rainfall and temperature models from July through November. We included these terms in the mixed effects models described below to determine if weather affected foaling date.

To test for differences in foaling date with PZP treatment, while controlling for pseudo-replication and unequal variances, we used a linear mixed effects model. The model included mare ID as a random effect and a function allowing different variances across groups (pre-contraception management, post-contraception management non-recipient, post-contraception management current recipient, and post-contraception management prior recipient) [35]. We included mare age as a covariate, as this has been suggested to influence female reproductive behaviors and physiology [36,37]. Additionally, a one-way ANOVA revealed no differences in mare age among treatment groups ($F_{2,84} = 2.03$, $P = 0.14$), suggesting that age did not confound differences among groups. From the above mixed effects model, differences in foaling dates among current, prior, and non-recipients were calculated using an F-test for linear combinations [35].

As gathers could have affected foaling date, we used a linear mixed effects model to test for differences between foaling dates before and after gathers (but before contraception management). After controlling for weather and mare age, mean foaling date did not differ before and after gathers ($P = 0.63$). Additionally, the range of birth dates did not differ significantly between these groups (SD before gathers = 1.04, SD after gathers = 0.77, $P = 0.36$). Finally, AICc from our analysis of foaling dates (see Results) suggested that combining data before and after gathers yielded a more parsimonious model than did separating those categories (AICc separating pre- and post-gathers = 551.02, AICc combining pre- and post-gathers = 550.60). Consequently these groups were combined as pre-contraception management animals for all analyses.

To determine whether the number of PZP applications (total or consecutive) or the number of years between pregnancies (total or consecutive) influenced foaling date, we ran separate linear mixed effects models using these as predictor variables. Each model used only data from PZP recipients and included mare ID as a random effect. To determine if results from this analysis could be influenced by year, we ran a linear mixed effects model on post-management, non-recipients predicting foaling date by year.

We analyzed PZP efficacy using a generalized linear mixed effects model with a binomial error distribution and mare ID as a random effect [38]. This analyzed the probability of conceiving using age at first PZP application and the number of consecutive PZP applications as fixed effects. All mixed effects models initially included two-way interactions, which were removed if their *P*-values were >0.10.

Results

Foaling

After contraception management, foaling occurred over a broader range of months than before contraception management (see Fig. 1, SD pre-contraception = 0.91 months, SD post-contraception = 2.04 months; Fligner-Killeen Test for Homogeneity of Variances: Median Chi Square = 28.10, $P<0.0001$; pre-contraception: n = 65 births from 45 mares over 4 years; post-contraception: n = 97 births from 46 mares, over 8 years). In the eight years following contraception management, mares receiving PZP foaled over a broader range of months than did non-recipient mares (see Fig. 1, SD recipients = 2.40 months, SD non-recipients = 1.54 months; Fligner-Killeen Test for Homogeneity of Variances: Median Chi Square = 11.76, $P = 0.001$; recipients: n = 45 births from 27 mares; non-recipients: n = 52 births from 19 mares).

On average, current PZP recipients gave birth 3.36 months later than did pre-contraception mares, according to our linear mixed effects model, which controlled for heterogeneity of variances, weather influences, and mare age (estimate = 3.36, SE = 0.51, t = 6.64, $P<0.001$; overall model: Likelihood ratio (compared to null model): 40.79, generalized $r^2 = 0.48$, $P<0.001$). Mares that had received PZP earlier in their lifetime, but not during the year of conception (prior recipients), gave birth 0.90 months later than pre-contraception mares on average (estimate = 0.90, SE = 0.40, t = 2.27, $P = 0.03$). Non-recipient mares that never received PZP themselves, but gave birth after the general population was managed with PZP, gave birth 1.01 months later than pre-contraception mares on average (estimate = 1.01, SE = 0.25, t = 4.04, $P = 0.001$). This translates into current PZP recipients giving birth 2.34 months later than non-recipient animals and 2.46 months later than prior recipients (F-tests for linear combinations: $F>16$, $P<0.001$ for each comparison). Furthermore, birth dates were about 0.38 months later for each degree centigrade above average in the latter half of the breeding season and 0.05 months later for each centimeter of rain above average (temperature residuals July-Nov: estimate = 0.38, SE = 0.13, t = 2.84, $P = 0.001$; rainfall residuals July-Nov: esti-

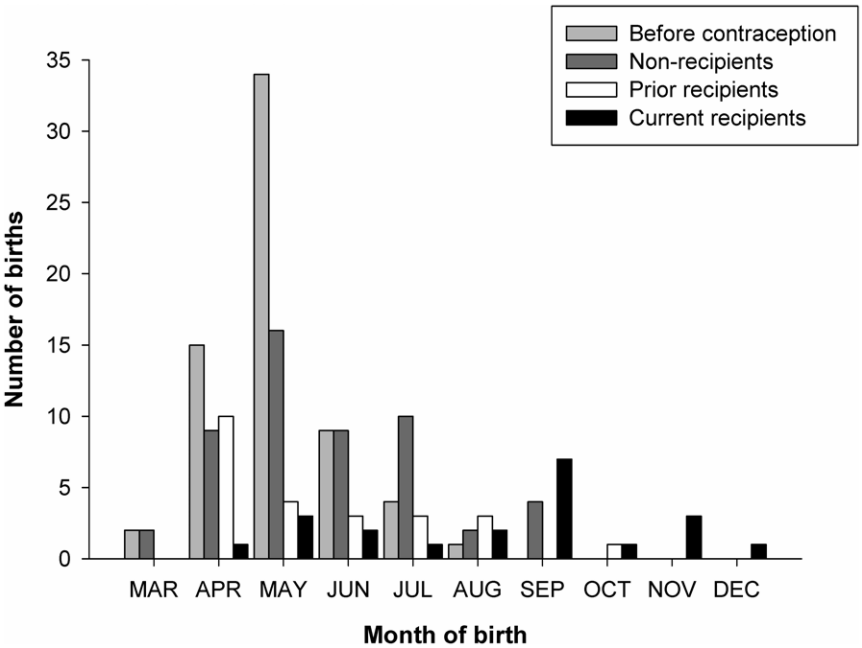

Figure 1. The distribution of births for mares on Shackleford Banks, NC, pre-contraception and post-contraception management. Mares gave birth over a wider range of months after the onset of contraception; this effect was more pronounced in PZP recipients than non-recipients.
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mate = 0.05, SE = 0.03, t = 1.83, $P = 0.07$). In general, mares gave birth 0.05 months earlier for every year of age (estimate = −0.05, SE = 0.02, t = −2.03, $P = 0.05$).

Among mares treated with PZP, those receiving a higher total number of applications foaled later in the season than did those receiving fewer applications, with each additional application associated with a 0.5 month delay in foaling date (Linear Mixed Effects Model: estimate = 0.55, SE = 0.21, t = 2.61, $r^2 = 0.65$, $P = 0.01$, see Fig. 2A). In addition, each consecutive PZP application was associated with a 0.8 month delay in foaling date, on average (Linear Mixed Effects Model: estimate = 0.83, SE = 0.23, t = 3.64, $r^2 = 0.65$, $P<0.0008$, see Fig. 2B). While the consecutive and total number of PZP applications were highly correlated, comparing the AICc between the prior two models suggested that the number of consecutive PZP treatments more accurately predicted month of birth than did the total number of PZP treatments (AICc total = 202.96, AICc consecutive = 196.83). The number of years that mares did not conceive (total or consecutive), however, did not correlate with foaling date (Linear Mixed Effects Model: total years: estimate = 0.31, SE = 0.26, t = 1.19, $r^2 = 0.57$, $P = 0.24$; consecutive years: estimate = 0.26, SE = 0.28, t = 0.92, $r^2 = 0.44$, $P = 0.36$). It is unlikely that these patterns resulted from a general trend across years, as birth dates among non-recipient animals did not change with calendar year (Linear Mixed Effects Model: $r^2 = 0.17$, estimate = 0.05, SE = 0.09, t = 0.57, $P = 0.57$).

Mare Condition

A generalized linear model, which controlled for mare age, showed that among post-contraception animals, recipient mares (both current and prior) were in better physical condition, with rump scores approximately 0.4 points higher than those of non-recipients (overall model (compared to null model): Chi Square = 7.74, $P = 0.05$; PZP treatment: estimate = 0.38, SE = 0.15, Chi Square = 6.13, $P = 0.01$; age: estimate = −0.005, SE = 0.06, Chi Square = 0.007, $P = 0.93$; PZP treatment × age: estimate = −0.02, SE = 0.06, Chi Square = 0.13, $P = 0.72$).

Weather

The best fit model of monthly mean temperature showed that overall temperatures were approximately 0.6°Cwarmer after the onset of contraception management (see Fig. 3A; overall model: Likelihood ratio (compared to null model) = 358.40, $P<0.0001$, generalized $r^2 = 0.88$; month: estimate = −8.49, SE = −.97, t = −8.78, $P<0.0001$; $month^2$: estimate = 3.88, SE = 0.29, t = 13.43, $P<0.0001$; $month^3$: estimate = −0.45, SE = 0.03, t = −13.70, $P<0.0001$; $month^4$: estimate = 0.015, SE = 0.001, t = 12.20, $P<0.0001$; contraception management (before): estimate = 0.58, SE = 0.27, t = −2.13, $P = 0.06$). The best fit model of monthly precipitation showed no overall differences between the periods before and after contraception management, but there were significant interactions between month of the year and the periods before and after contraception (see Fig. 3B; overall model: Likelihood ratio (compared to null model) = 38.43, $P<0.0001$, generalized $r^2 = 0.20$, $P<0.0001$; month: estimate = −11.19, SE = 4.96, t = −2.25, $P = 0.03$; $month^2$: estimate = 3.66, SE = 1.46, t = 2.51, $P = 0.01$; $month^3$: estimate = −0.38, SE = 0.17, t = −2.27, $P = 0.02$; $month^4$: estimate = −0.01, SE = 0.006, t = 1.88, $P = 0.06$; contraception management (before): estimate = 6.99, SE = 4.22, t = 1.66, $P = 0.12$; month × contraception management (before): estimate = −2.97, SE = 1.43, t = −2.07, $P = 0.04$; $month^2$ × contraception management (before): estimate = 0.22, SE = 0.11, t = −2.03, $P = 0.04$).

PZP Efficacy

We defined PZP efficacy during the year of administration as the number of vaccinated mares that did not became pregnant divided by the total number receiving the vaccine. Across the first four consecutive PZP applications, this efficacy declined from 97% to 87%, returning to 100% after five or more consecutive applications (see Fig. 4). A generalized mixed effects model shows that this pattern

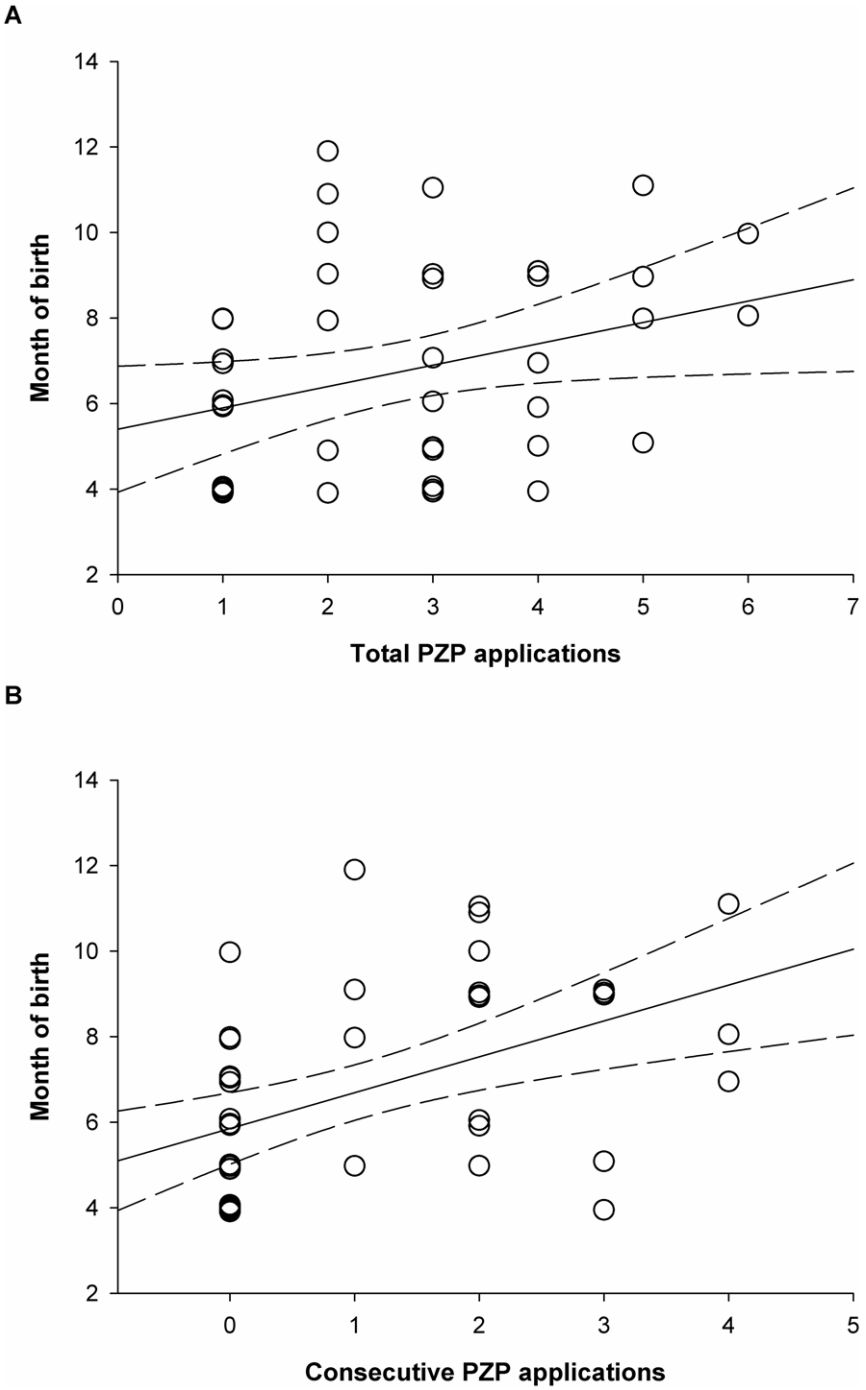

Figure 2. Birth month and number of A) total PZP applications, and B) consecutive PZP applications. In the events of ties, month of birth has been jittered by 0.2 years to allow clear visualization of every individual. Mares receiving more applications of PZP foaled later in the year on average than did mares receiving fewer applications. Although the number of total and consecutive applications is highly correlated, AICc suggests that the number of consecutive applications explains more of the variation in the data.
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is significant, even when controlling for mare age (overall model with binomial error distribution: Log Likelihood = −61.79, $P = 0.01$, generalized $r^2 = 0.17$; consecutive PZP applications: estimate = 2.98, SE = 1.20, z = 2.49, $P = 0.01$; $(\text{consecutive PZP applications})^2$: estimate = −0.51, SE = 0.22, z = −2.33, $P = 0.02$; age at first PZP application: estimate = 0.10, SE = 0.05, z = 1.84, $P = 0.07$). Prior research has shown that five to seven years of consecutive PZP treatment can be associated with ovulation failure [4]. The present dataset is consistent with this result, as no mare receiving the vaccine for five or more consecutive years became pregnant.

Discussion

Here we show that PZP recipients exhibited a change in their reproductive schedule: recipient mares gave birth over a broader time period than did non-recipients, with current recipients giving

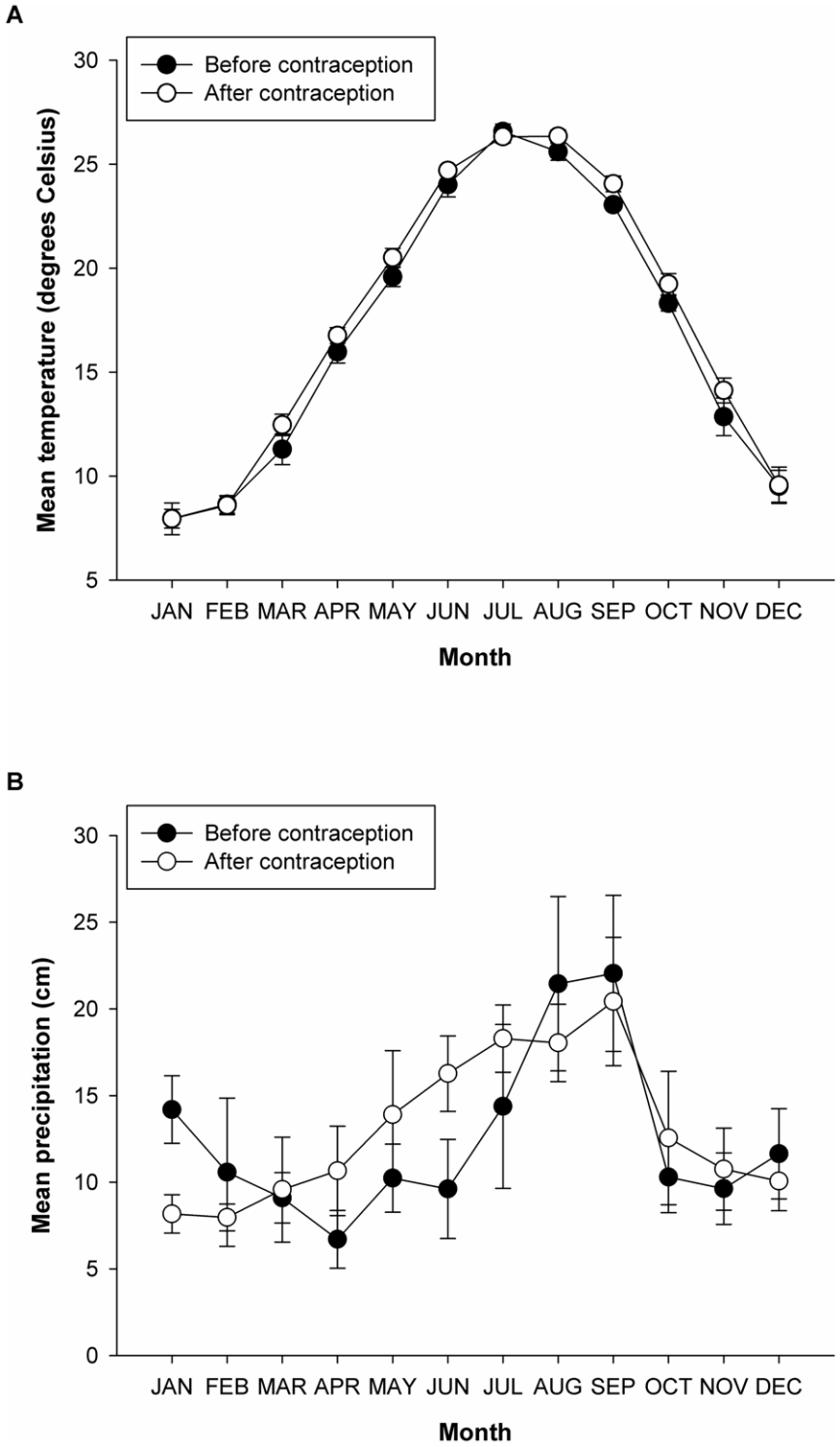

Figure 3. Weather data for six years pre-contraception (1995–2000) and eight years post-contraception (2001–2008) management. Data were collected from Morehead City, North Carolina, approximately 8 km from the study site (Shackleford Banks, North Carolina). Temperatures (A) were marginally warmer post-contraception than they had been pre-contraception. Overall rainfall (B) did not differ before and after contraception, though the seasonal patterns were marginally different pre- and post-contraception.
doi:10.1371/journal.pone.0013635.g003

birth later in the year than prior recipient and non-recipient mares. Given that gestation in wild horses lasts approximately 11 to 12 months [30], this change indicates a corresponding change in the schedule of ovulatory cycling. Contraception with porcine zona pellucida is popular amongst managers specifically because it effectively reduces the odds of conception without the application of exogenous steroids [2]. Long-term studies on Assateague Island have reported that PZP has little to no effect on reproductive

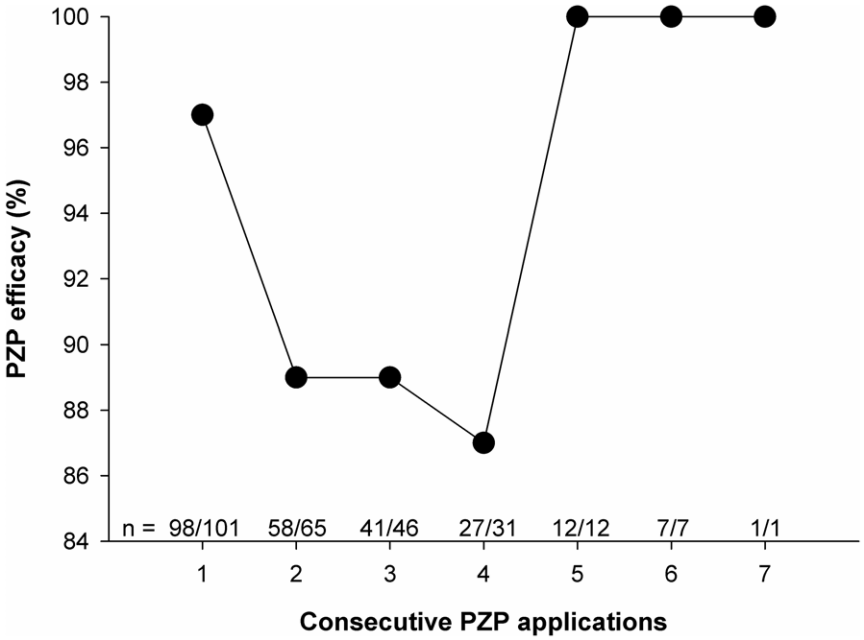

Figure 4. PZP efficacy and number of consecutive PZP applications. PZP efficacy was defined as the number of recipient mares that did not become pregnant divided by the total number of mares receiving the vaccine. Across the first four consecutive applications, PZP efficacy declined, returning to 100% after five or more consecutive applications (5–7 applications have been shown to result in ovulation failure and decreased oestrogen levels [5,40]).
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hormone levels, the schedule of reproductive cycling, or the social behaviors of recipient animals [4]. However, studies in other wild horse populations have shown that recipient mares both initiate and receive more instances of reproductive behavior during both the breeding [39] and non-breeding seasons [9]. This study provides the first evidence that mares treated with PZP can extend ovulatory cycling beyond the normal breeding season. This suggests that populations of wild ungulates can vary in their response to similar contraceptive treatment. Careful consideration of baseline population dynamics should be made prior to treatment in order to fully assess possible PZP effects.

Foaling Date

Mares receiving PZP at any point during their lifetime gave birth over a broader time period than did non-recipient animals. This larger variance among PZP mares is likely driven by the fact that current recipients gave birth later than did prior recipients (see Results, Fig. 1). Moreover, mares receiving more consecutive applications foaled later in the season than did mares receiving fewer applications. Increases in the average interbirth interval for recipient mares did not seem to be driving this result, as foaling date was not affected by the number of years (cumulative or consecutive) that mares failed to conceive. This discrepancy may be due to high variability in the conception and foaling dates of treated mares. First, it is less likely that an animal vaccinated with PZP will conceive at all, thus reducing sample size. Second, due to contraceptive failure, some treated mares will conceive during the normal breeding season, further increasing variability. Interestingly, prior to the application of PZP, the average month of birth did tend to increase with interbirth interval (Linear Mixed Effects Model: estimate = 0.30, SE = 0.17, t = 1.82, $r^2 = 0.06$, $P = 0.07$) [22], demonstrating at least some plasticity in the scheduling of reproductive cycling in Shackleford mares. On Assateague, PZP recipients experience normal reproductive cycling and mate at rates similar to non-recipients [40]. However, when such behavior fails to result in conception over several years, it follows that individuals extending reproductive cycling will be able to achieve conception later in the year if the contraceptive effects of PZP have decreased sufficiently [28,29].

Because feral horses are highly social, such changes can have cascading effects on other group members and throughout the population. Our research has shown that after contraception management, PZP recipients both attract and initiate more instances of reproductive behavior [9] and are more often the harem male's nearest neighbor during the fall/winter (Nuñez, unpublished data), indicating that group spreads are reduced. Such changes represent an increase in energy expenditure and a potential decrease in nutrient intake during a time of year when sufficient energy reserves are at a premium [27]. Moreover, early foal development in unmanaged populations typically occurs during the spring and summer when resources are plentiful [11,27]. Offspring born in the fall/winter months face nutritional and thermoregulatory challenges not experienced by their counterparts born during the normal foaling season, potentially making developmental benchmarks difficult to achieve [27].

Such predictions are not consistent with data from Assateague Island where mares show increased survival, only minimal physiological side effects, and no behavioral or demographic changes [4,5,6]. In addition, foal survival does not differ between foals born in or out of the normal foaling season [41]. However, on Shackleford Banks, recipient mares change groups more often, elicit and receive more instances of reproductive behavior, and receive more harassment from harem males [9,42]. Given these differences in mare response to PZP management in the two populations, it follows that predictions based on the data from one site are not necessarily applicable to the other.

These population differences may be due to the scheduling of PZP administration at the two sites. When the contraception

program on Assateague began in 1994, the priorities for treatment followed a hierarchical approach based on the previous breeding success of the population, ensuring that all mares were given an opportunity to reproduce [3]. Females for which there was a high priority for treatment included those that had produced at least one surviving offspring. Low priority females included those that were less than four years of age. Females greater than four years old that had not produced surviving offspring did not receive treatment. In addition, the plan stipulated that only mares that had produced at least three surviving offspring or two generations of offspring would receive more than three consecutive years of treatment. Foals were not to be removed as removal increases a mare's reproductive success in the subsequent year [43,44,45]. Finally, it was recognized that this plan was subject to change as the population numbers decreased [46]. In the present study, Shackleford mares were contracepted between 1.5 and 2 years of age and received an average of 3.4 ± 0.2 (mean $\pm$ standard error) consecutive years of contraception, regardless of their productivity. To further control population numbers, foals born to these mares (due to contraception failure or changes in the application schedule), were likely to be removed. This difference in PZP administration and subsequent discrepancy in early life experience may contribute to the behavioral differences between the populations, as the ability to conceive with a harem male is likely critical to establishing lasting harem fidelity [16] and the retention of foals (until at least two years of age) is important to maintaining normal reproductive function [43,44,45].

Possible Mechanisms

Although the effect was more pronounced in recipients of PZP, both recipients and non-recipients showed a wider range of foaling dates after contraception management (after 2001). While relatively rare, such extended periods of estrous have been documented in several equine species. Tropical species, for example, have been observed to reproduce throughout the year [12,13,47]. Similarly, studies of temperate species have shown that individuals can vary significantly in reproductive timing [14] and estrous behaviors during the non-breeding season [15]. Our data show that Shackleford mares exhibit at least some plasticity in their reproductive cycling. This plasticity enables mares to time their reproductive cycling according to ecological, sociological, and physiological cues.

For example, our results show that the reproductive changes exhibited by Shackleford mares correlate with warmer temperatures occurring later in the calendar year, after contraception management. Increases in rainfall late in the breeding season also correlate (albeit weakly) with later births. Both warmer temperatures and increased rainfall could result in higher resource availability [27] and afford females the additional reserves necessary to extend reproductive cycling into what is typically the non-breeding season.

The physical condition of mares may also play an important role in the extension of reproductive cycling. On Shackleford Banks, recipient mares are currently in better physical condition than are non-recipients. This is likely due to the fact that successfully contracepted mares are unconstrained by the costs of pregnancy and lactation [48]. Recipient mares will therefore have more resources to allocate to additional reproductive cycles. This effect of PZP, coupled with warmer temperatures occurring later in the year, may act to increase a mare's chances of conceiving later in the calendar year, if PZP antibody titers are sufficiently low [29].

Additionally, extended cycling in non-recipient mares could be influenced by the physiology and behavior of recipients. Shackleford males exhibit higher rates of sexual behavior towards recipient females during both breeding and non-breeding seasons [9,42]. These overt social stimuli may entrain some non-recipients to continue reproductive behaviors and cycling into the early fall. Such stimuli are commonly used to induce receptivity in several domestic species including horses [49], pigs [50], and cows [51]. In the wild, courtship signals from conspecifics advance gonadal cycles or maturation in several taxa, including mammals [52,53,54], birds [55], amphibians [56], and reptiles [57]. Given the importance of social cues in the timing of reproduction among such diverse species, this possibility warrants further investigation in Shackleford mares.

Finally, the declining efficacy of PZP with increased consecutive applications is likely a contributing factor to the later foaling dates of recipient mares. Lyda and colleagues' research with captive, wild mares has shown that antibody titers against PZP remain high for up to ten months after initial treatment [28]. In addition, research with both Shackleford and Assateague horses has shown that initial applications of PZP are often effective over multiple years [9,58], suggesting that antibody titers can remain high for longer. However, laboratory research has shown considerable variability in anti-PZP titers [29], as did Lyda and colleagues' work in which half the mares treated with PZP and Freund's Complete Adjuvant fell below contraceptive levels within the ten months of study [28]. Our data show that increasing the number of consecutive applications can reduce the single year efficacy of PZP by roughly 10%, indicating that either antibody titer or reactivity can decrease more rapidly with consecutive applications. Such patterns could result from the induction of immunological tolerance [59], which reduces responsiveness to self-tissues or repeatedly encountered, non-pathogenic antigens [60]. PZP is designed to mimic host tissue and induce an immune response against self tissue: the recipient's own zona pellucida [2]. As such, it seems reasonable that at least some animals would mount tolerance mechanisms to combat this autoimmunity. In addition, the repeated application of a specific antigen generates an antibody response that is increasingly more specific to that particular antigen [29]. The antibodies produced by mares against porcine zona pellucida should, therefore, become less cross reactive with horse zona pellucida over time. Of course, PZP efficacy will vary depending on mare age and timing of inoculation [61]. Regardless, if PZP recipients extend reproductive cycling and behavior into the non-breeding season, any decrease in efficacy that leaves them fertile in the fall/winter will help drive increases in late season conception.

Although the removal of offspring can induce estrous cycling in ungulate species [62], it is unlikely that the removal of foals has influenced foaling date among PZP-treated mares on Shackleford Banks. Thirty-nine foals (conceived due to contraception failure or administration scheduling) have been removed from the island. Approximately 55% of these foals were born to non-recipient animals. The majority of foal removals were conducted in the January following foal births. Given that non-recipient animals did not give birth later than September and most recipient animals gave birth before December, it is unlikely that foal removals in January induced late-season estrus in Shackleford mares. It is equally unlikely that increases in mare condition due to the alleviation of lactation costs resulted in early resumption of estrus the following spring [27]. If that were the case, during the early spring months we would expect to see an increase in the number of foals born to mares subjected to offspring removal. This is not borne out by the data. Still, the removal of foals is ill-advised as it increases mare fecundity the following year [43,44,45].

Management Implications

When the alternative (gather and removal) is considered, PZP is currently managers' most humane and effective option for population control. However, careful study of the animals' demography, physiology, and behavior is necessary prior to and during treatment to ensure that a) the potential effects of PZP can be assessed accurately, and b) within managerial constraints, unintended effects of PZP are ameliorated. Differences in habitat, resource availability, and demography among conspecific populations will undoubtedly affect their physiological and behavioral responses to PZP contraception, and need to be considered. For instance, while Assateague horses show no behavioral and only minor physiological responses to PZP, horses on Shackleford Banks [9,42] and in the western United States [39] alter social and reproductive behaviors in response to PZP. Our data suggest that mare condition and warming trends may present additional complications. Increases in physical condition and changes in average temperature may interact with management regimes, enabling mares to alter their reproductive physiology even further. Moreover, these data emphasize the importance of study during both the breeding and non-breeding seasons. Much of the research showing little to no effect of PZP on feral horse behavior or physiology has been performed exclusively during the breeding season [4,5,10], potentially missing important differences in recipient response.

If population numbers are managers' primary concern, our data show that giving five or more consecutive applications of PZP will result in 100% contraception efficacy. This is consistent with data from Assateague where mares receiving 5–7 consecutive PZP applications exhibited ovulation failure and decreased urinary oestrogen concentrations [5,40]. However, if managers are tasked with the maintenance of natural behaviors and foaling schedules, consecutive PZP applications should be avoided. Research has shown that one application of PZP is often effective over multiple years, exhibiting yearly efficacy declines similar to that of 2–4 consecutive treatments (on Shackleford) [9,58]. Our data show that current recipients gave birth later than both prior recipient and non-recipient animals. However, prior recipients of PZP gave birth on schedules similar to non-recipients, suggesting that breaks between treatments can ameliorate unintended behavioral and physiological changes in recipient animals. Contraception on such schedules will still maintain lower pregnancy rates, but will allow for the birth of a manageable number of offspring which are also important to the maintenance of normal behaviors [9]. These foals should be allowed to remain in the population for at least two years as earlier removal has been shown to increase a mare's reproductive success in the subsequent year [43,44,45]. Additionally, subadult, dispersing females should be allowed to settle into harems and have at least one foal before receiving contraception [16]. Management regimes such as this would of course necessitate a higher minimum population level. Additional research is needed to determine whether these larger, but still limited population sizes could achieve management goals. If so, this could prove a cost-effective means of controlling animal numbers while maintaining their natural physiology and behavior.

The broader implications of this research are considerable. As this study suggests, the physiological and behavioral effects of PZP are not fully understood. Still, the vaccine is currently administered to many different species including white-tailed deer, *Odocoileus virginianus* [7], elk, *Cervus elaphus* [8], black bears *Ursus americanus* [63], and African elephants, *Loxodonta Africana* [64]. As with conspecific equid populations, habitat, resource, and demographic differences among species will affect their responses to PZP contraception and need to be considered. For social species like the horse, a proper balance between managing population size and maintaining a more natural physiological and behavioral regime is particularly important.

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Author Contributions

Conceived and designed the experiments: CMVN. Performed the experiments: CMVN. Analyzed the data: CMVN JSA. Wrote the paper: CMVN JSA DIR.

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Research article

Lingering effects of contraception management on feral mare (*Equus caballus*) fertility and social behavior

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Due to the extirpation of their natural predators, feral horse populations have expanded across the United States, necessitating their management. Contraception of females (mares) with porcine zona pellucida (PZP) is a popular option; however, effects to physiology and behavior can be substantial. On Shackleford Banks, North Carolina, USA, treated mares have exhibited cycling during the non-breeding season and demonstrated decreased fidelity to the band stallion, but PZP's long-term effects on mare physiology and behavior remain largely unexplored. After the contraception program was suspended in this population, we examined how prior exposure to varying levels of PZP treatment impacted (1) foaling probability and foaling dates (a proxy for ovulatory cycling) from 2009 to 2014 and (2) mare fidelity to the band stallion and reproductive behavior during 2013 and 2015. Additionally, we evaluated the effects of time since the mares' last treatment on these factors. Mares receiving any level of prior PZP treatment were less likely to foal than were untreated mares. Among mares that received 1–3 PZP applications, foaling probability increased with time since last treatment before declining, at ~6 years post-treatment. Mares that received 4+ applications did not exhibit a significant increase in foaling probability with time since last treatment. Moreover, previously treated mares continued to conceive later than did untreated mares. Finally, mares previously receiving 4+ treatments changed groups more often than did untreated mares, though reproductive behavior did not differ with contraception history. Our results suggest that although PZP-induced subfertility and its associated behavioral effects can persist after the cessation of treatment, these effects can be ameliorated for some factors with less intense treatment. Careful consideration to the frequency of PZP treatment is important to maintaining more naturally functioning populations; the ability to manage populations adaptively may be compromised if females are kept subfertile for extended periods of time.

Key words: Contraception, feral horse, immunocontraception, management, prolonged subfertility

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Introduction

The extirpation of predator species in North America has precipitated the expansion of free-ranging ungulate populations (Eberhardt *et al.*, 1982; McCullough *et al.*, 1997), escalating human–wildlife conflict (Conover, 1995). Perhaps the most well-known example is that of increasing white-tailed deer (*Odocoileus virginianus*) populations across the United States.

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These deer damage crops, feast on suburban ornamentals, spread disease, and are involved in both vehicle and train collisions (Conover, 1997), resulting in human injury, the loss of human life and costs amounting to more than 1 billion USD of property damage per year (Mastro *et al.*, 2008). Elk (*Cervus canadensis*) populations have also increased in some areas, with severe ecological impacts. In the Yellowstone ecosystem, unfettered elk populations have been associated with an ecological cascade of diverse effects including a reduction in available forage (Ripple and Beschta, 2003) and subsequent decreases in various vertebrate species including grizzly bears (*Ursus ortcos*) (Ripple *et al.*, 2014) and various songbird species, including the Common Yellowthroat (*Geothlypis trichas*), Lincoln's sparrow (*Melospiza lincolnii*), and Warbling Vireo (*Vireo gilvus*) (Baril *et al.*, 2011). Feral horse populations have also greatly expanded across the United Sates. In the west, feral horses and livestock compete for forage and water (BLM, 2011), while in the east, feral horses can negatively impact barrier island plant (Furbish, 1980) and estuarine (Levin *et al.*, 2002) communities. Managers have few options with which to control these populations (e.g. culls, removals), and fewer still that are acceptable to the public (Hobbs *et al.*, 2000; Porton, 2005). Consequently, contraceptive management has become increasingly popular (Hobbs *et al.*, 2000; Porton, 2005).

A commonly used contraceptive in female ungulates, porcine zona pellucida (PZP), stimulates the production of antibodies that bind sperm receptors on the egg's surface, thereby blocking fertilization while still allowing ovulation and the associated cycles of sex hormones (Sacco, 1977). Because PZP does not affect hormone levels directly, it was previously suggested that PZP treatment did not significantly affect recipient behavior or physiology (Kirkpatrick *et al.*, 1996, 1997; Powell and Monfort, 2001; Turner *et al.*, 1997). However, research on white-tailed deer, elk and feral horses has shown that there can be unintended effects on reproductive behavior (McShea *et al.*, 1997; Heilmann *et al.*, 1998; Nuñez *et al.*, 2009; Madosky *et al.*, 2010; Ransom *et al.*, 2010) and physiology (Nuñez *et al.*, 2010; Ransom *et al.*, 2013); in feral horses, these effects can be exacerbated in mares receiving higher numbers of total and/or consecutive PZP applications (Madosky *et al.*, 2010; Nuñez *et al.*, 2010). Briefly, studies investigating the response of free-ranging elk and white-tailed deer to PZP treatment indicate that recipient females extend reproductive behaviors into the post-breeding season (McShea *et al.*, 1997; Heilmann *et al.*, 1998). Authors suggest that in response to repeated unsuccessful mating attempts, females continue cycling in an attempt to gain additional reproductive opportunities (McShea *et al.*, 1997; Heilmann *et al.*, 1998). In feral horses, PZP-induced subfertility was also correlated with reproductive cycling in the non-breeding season (Nuñez *et al.*, 2010; Ransom *et al.*, 2013), increases in the amount of reproductive behavior directed towards mares by band stallions (Ransom *et al.*, 2010), and an increase in group transfer and reproductive behavior by mares (Nuñez *et al.*, 2009; Madosky *et al.*, 2010). Few studies have documented how permanent such side-effects may be (Kirkpatrick, 1995; Madosky *et al.*, 2010; Ransom *et al.*, 2013) and to our knowledge, none have investigated whether the intensity of PZP management can influence female physiology and behavior after the suspension of treatment in managed populations.

Here, we examine the rate at which mares on Shackleford Banks, North Carolina, USA are able to return to their physiological and behavioral baselines after varying levels of contraception management and with increasing time since treatment. Specifically, we monitored per capita births and the effects of varying levels of PZP treatment on the likelihood of conception in individual mares, and foal birth dates from 2009 to 2014, during which time the contraception program was largely suspended. In addition, we examined mare group transfer and reproductive behavior in the summers of 2013 and 2015 to determine whether PZP-induced subfertility had any long-term behavioral effects on previously treated mares. This information is critical to understanding the full effects of long-term subfertility on individuals and populations, and will be integral to designing best-management practices for PZP contraception.

Methods

Study area

Shackleford Banks is a barrier island ~3 km off the coast of North Carolina, USA. The island measures ~15 km in length, and varies between 0.5 and 3 km in width. The Shackleford horses are of Spanish origin and were likely first introduced ~400 years ago (Conant *et al.*, 2012). Currently, the population is co-managed by the National Park Service and the Foundation for Shackleford Horses (National Park Service, 2004). In 1997, federal law was instituted mandating the maintenance of at least 100–110 horses on Shackleford Banks (United States Congress, 1997). The National Park Service and the Foundation for Shackelford Horses limit the population to ~120 individuals, to reduce damage to the ecosystem.

Study subjects

The reproductive units of Shackleford horses are typical of feral equids, consisting of coherent bands of one, or sometimes two or three stallion(s), with one to several mares and their offspring (Rubenstein, 1981). Double-male bands are present, though they make up a small portion of the Shackleford population (5–10% of bands during the course of this study). In addition, differences in the degree of band territoriality have been associated with the island's varying ecology, making the region in which animals reside a reliable proxy for social variation in this population (Rubenstein, 1981). Though the bands are predominantly non-territorial, individual bands are spatially distinct from one another (Feist and McCullough, 1976; Rubenstein, 1981, 1986). Interactions that occur between different bands typically involve younger individuals engaging in exploration and/or play, the dispersal of sub-adult individuals

(both male and female), male–male assessments and/or contests, and the transfer of mares from one band to another (personal observation). Historically, band membership has been long lasting with most changes involving the dispersal of immature individuals. Band stallions sometimes fought to acquire mares from other groups, but stallions almost always retained their mares (Feist and McCullough, 1976; Rubenstein, 1981). More recently, PZP-induced subfertility has been associated with decreases in mare fidelity to the band stallion, increases in mare reproductive behavior, and mare cycling in what is typically the non-breeding season for these mares (Nuñez *et al.*, 2009, 2010; Madosky *et al.*, 2010).

PZP contraception

The National Park Service began the application of PZP for the purposes of immunocontraception in January 2000. In 2009, the program was provisionally suspended because the population was deemed stable (Mason, 2017). The contraception of all but five previously treated mares was halted, and all previously untreated mares of reproductive age were given one dose and booster of PZP. All treatments were administered by the National Park Service from late February through April each year. Each injection contained 100 micrograms of PZP plus an adjuvant (mixed at the darting site). Initial doses contained Freund's Complete Adjuvant, Modified, *Mycobacterium butyricum* (Calbiochem #344 289). All subsequent boosters contained Freund's Incomplete Adjuvant (Sigma #F5506).

For the animals in this study, PZP deterred pregnancy in 99.91% of cases when administered during the same year (Stuska, 2014). These values are similar to those published for Assateague horses, in which PZP deterred pregnancy in 94% of cases (Turner *et al.*, 1997). Over the course of the contraception program, ~19% of pregnancies were due to contraception failures. The contraception histories for the mares considered in this study are displayed in Table S1.

Pregnancy and foaling

Fecal samples are collected by the National Park Service in January of each year. All pregnancy testing is completed by enzyme immunoassay of fecal material at the Science and Conservation Center at ZooMontana in Billings, Montana. Using the methods of Kirkpatrick *et al.* (1991), water extracts of fecal samples are assayed for estrone conjugates and nonspecific progesterone metabolites. In addition, the National Park Service has tracked the foaling records, including foal birth dates, for each mare since 2000 (Stuska, 2014). These data were used to supplement assay results.

Behavioral and demographic sampling

Behavioral and demographic sampling was conducted primarily by two observers (C. Alvarez in 2013 and H. Carr in 2015) and was supplemented with data from three additional observers (B. Streater, 2013; M.A.F. Kent and K.E. Monroe, 2015). All observers were trained by C.M.V. Nuñez. We observed behavior in the majority of reproductive mares (aged 4 years and older) present during the study. We recorded group changing and reproductive behavior for 73% (43/59) and 95% (55/58) of mares in 2013 and 2015, respectively. The number of bands we observed comprised 68% (13/19) and 95% (18/19) of bands on the island in 2013 and 2015. Comparable to previous studies (Nuñez *et al.*, 2009; Ransom *et al.*, 2010), we observed mares for 7 weeks each year from mid-June to late August, totaling 350.15 hours of observation, averaging 3.13 h per mare (range = 1–6.05, SE ± 0.12). Horses were identified individually by color, sex, age, physical condition and other distinguishing markings including freeze brands. Ages are known from long-term records for the identified horses of Shackleford Banks (Nuñez, 2000).

We located each band an average of 0.87 times each week (range = 0.28–1.83, SE ± 0.03). We recorded its GPS location and composition, paying particular attention to the presence or absence of females. Group transfers were recorded if a mare was (a) not in the band she had been seen with previously, or (b) in a different band than she had been seen with previously. Mares did not need to remain with the 'new' group for a minimum length of time for the behavior to be considered a group transfer since even short-lived changes can affect band stability (Madosky *et al.*, 2010) and mare stress physiology (Nunez *et al.*, 2014). We remained with each group for a minimum of 30 min to ensure that individuals recorded as absent were not actually nearby, but out of our sight; when compared to 60-min field observations, 30 min ensured accurate data collection and allowed for larger sample sizes (Nuñez *et al.* 2010). We rarely witnessed mare transfer activity directly ($n = 1$); therefore, mare absence from a band was an important metric with which we measured the number of mare transfers between groups. On average, transfer behavior was confirmed by the mares' presence in new bands within 12.32 days of mare absence (range = 2–31, SE ± 1.44).

All incidences of reproductive interest (including copulation, mounting, genital sniffing, and rump rubbing) directed to and initiated by mares were recorded *ad libitum* during scan sampling (Altmann, 1974) as in Nuñez *et al.* (2009).

Statistical analyses

We analyzed data in R version 3.1.1. We used a linear mixed effects model (nlme package; Pinheiro and Bates, 2000) to determine whether the number of births had increased after the suspension of the contraception program ($n = 157$ total births; 112 births during the program; 45 births after program suspension). All births, regardless of mare age and treatment status at the time of foaling, were included in this analysis. Models included year as a random effect and the following fixed effects: mare treatment (previously treated/untreated), timing (during/after the contraception program), the interaction between mare treatment regime and timing, and a function allowing different variances across groups. We analyzed our data in both raw and arcsine-square root

transformed formats as per Ahrens *et al.* (1990). The arcsine-square root transformation has been shown to correct deficiencies in the normality of error terms, homogeneity of variance, and additivity of percentage data (Ahrens *et al.*, 1990).

To determine whether a mare's contraception history (number of total treatments received) affected her ability to foal in the years following contraception suspension, we used a generalized linear mixed model with a penalized quasi-likelihood (PQL) function (package MASS (Venables and Ripley, 2002)). The GLMM accounts for intra-subject correlation and individual heterogeneity (Jang and Lim, 2006) while the PQL function has proven reliable for the analysis of independent counts (Dean *et al.*, 2004). Models included mare identity as a random effect and the following fixed effects: linear and second-order polynomial functions of mare age (to account for both linear and non-linear effects on the likelihood of foaling), mare contraception history (0 treatments/1–3 treatments/4+ treatments (Kirkpatrick and Turner, 2002), n = 68 mares, Table S1), and island region.

To test for differences in mares' average foaling date with contraception history, we used linear mixed effects models, including a function allowing different variances across groups, and the following fixed effects: linear and second-order polynomial functions of mare age, mare contraception history (0 treatments/1–3 treatments/4+ treatments), and island region. We considered 37 births from 24 mares for this analysis (see Table S1).

To examine mare group changing and reproductive behavior, we analyzed the number of group changes mares made and the number of instances of reproductive interest both initiated and received by mares (n = 59 mares) over two field seasons conducted in 2013 and 2015 (see Table S1). We used linear mixed effects models including mare as a random factor, and the following fixed effects: linear and second-order polynomial functions of mare age, mare contraception history (0 treatments/1–3 treatments/4+ treatments), and island region. All behavioral data were Poisson distributed and log-transformed for analysis.

Parameters were removed from models by backwards elimination if $P > 0.05$. For each analysis, final models were run including linear and second- order polynomial functions of time since last treatment; for this analysis, only mares receiving at least one PZP treatment were considered (see Table S1). Mares less than 4 years of age and the 5 individuals that received treatment between 2009 and 2014 were excluded from all analyses save our per capita birth analysis (see above). In addition, where applicable, models were run both with and without mares belonging to double-male bands. Excluding these mares did not significantly change our findings; therefore, the models presented here include mares belonging to both single- and double-male bands. Where appropriate, we selected best fit models using Akaike's Information Criterion adjusted for small sample size (AICc) (Burnham and Anderson, 2002).

Results

Births

Not surprisingly, yearly per capita births were considerably lower for treated mares than for untreated mares during the contraception program (Linear Mixed Effects Model: mare treatment regime (previously treated); estimate = −0.41, SE = 0.08, t = −5.30, P = 0.0003). This pattern did not change from 2009 to 2014 when the contraception program was largely suspended; previously contracepted mares continued to exhibit subfertility even after the cessation of treatment (Linear Mixed Effects Model: treatment group (previously treated) * timing (after contraception suspension); estimate = 0.07, SE = 0.13, t = 0.50, P = 0.62, Fig. 1). Results were similar with arcsine-square root transformed data (Ahrens *et al.*, 1990) (Linear Mixed Effects Model: mare treatment regime (previously treated); estimate = −0.46, SE = 0.10, t = −4.61, P = 0.0007; treatment group (previously treated) * timing (after contraception suspension); estimate = 0.13, SE = 0.17, t = 0.74, P = 0.50). AICc analysis suggested that models without a variance function were a better fit to the data (AICc without variance function = 5.97 (raw), 24.19 (arcsine transformed); AICc with variance function = 8.89 (raw), 28.20 (arcsine transformed)).

Contraception history and foaling after contraception suspension

Even while controlling for the effects of mare age (Generalized Linear Mixed Model, mare age: estimate = 0.59, SE = 0.27, t = 2.20, P = 0.03; mare age^2: estimate = −0.02, SE = 0.01, t = −2.18, P = 0.03), previously treated mares were less likely to give birth during the study period, regardless of the number of prior treatments received (Generalized Linear Mixed Model, number of treatments (1–3 treatments): estimate = −3.00, SE = 1.24, t = −2.40, P = 0.02; (4+ treatments): estimate = −4.19, SE = 1.27, t = −3.30 P = 0.001, Fig. 2). Island region had no effect on the probability of mare foaling (all $P > 0.20$). These results cannot be explained by inherent subfertility in mares slated for treatment; prior to contraceptive treatment, all mares exhibited equivalent fertility (Generalized Linear Mixed Model, treated vs. untreated: estimate = 0.36, SE = 0.43, t = 0.84, P = 0.41, Table S2).

In addition, we sought to determine whether time since last treatment impacted the probability of foaling in previously treated mares. We found that mares previously receiving 1–3 treatments exhibited an initial increase in foaling probability, followed by a subsequent decrease (Generalized Linear Mixed Model, time since last treatment: estimate = 2.03, SE = 0.43, t = 4.67, $P < 0.00001$; time since last treatment^2: estimate = −0.18, SE = 0.04, t = −4.07, P = 0.0001, Fig. 3A). Conversely, mares receiving 4+ treatments did not exhibit a discernable increase in foaling probability even after several post-treatment years (Generalized Linear Mixed Model, time since last

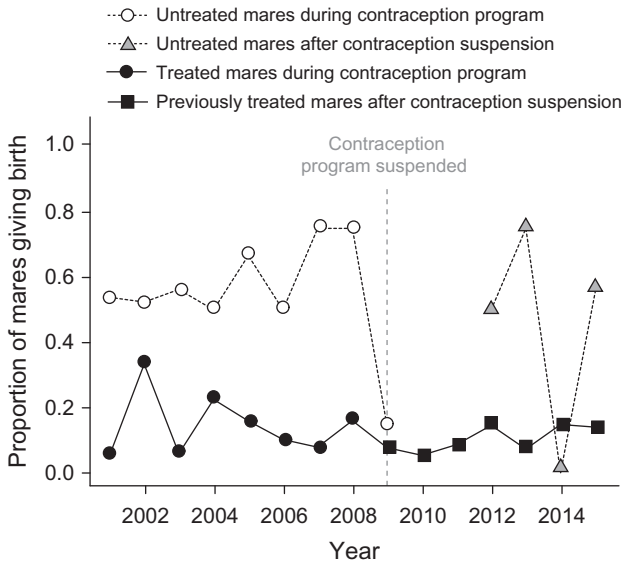

Figure 1: Proportion of mares giving birth in the years during and after contraception management. During the contraception program, treated mares (black circles) showed markedly lower birth rates than did untreated mares (open circles). In 2009, all untreated mares received PZP, leaving no untreated animals of reproductive age on the island in 2010 and 2011. Even after the suspension of treatment, previously treated mares (black squares) did not return to a level of fertility comparable to untreated mares before 2009 (open circles) or to younger, untreated animals that became reproductive between 2012 and 2015 (gray triangles).

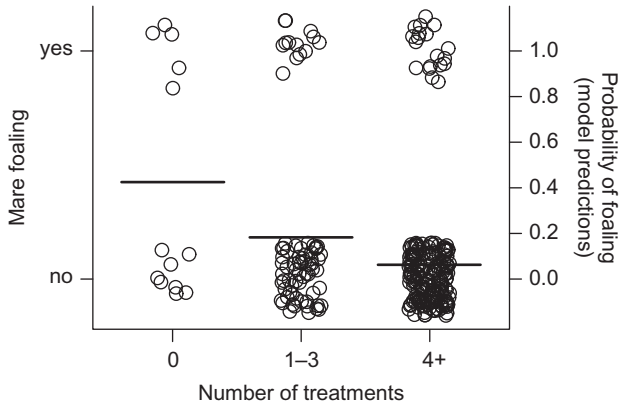

Figure 2: Probability of foaling and contraception treatment; circles represent individual births. Mares previously receiving any level of treatment were less likely to give birth than were untreated mares.

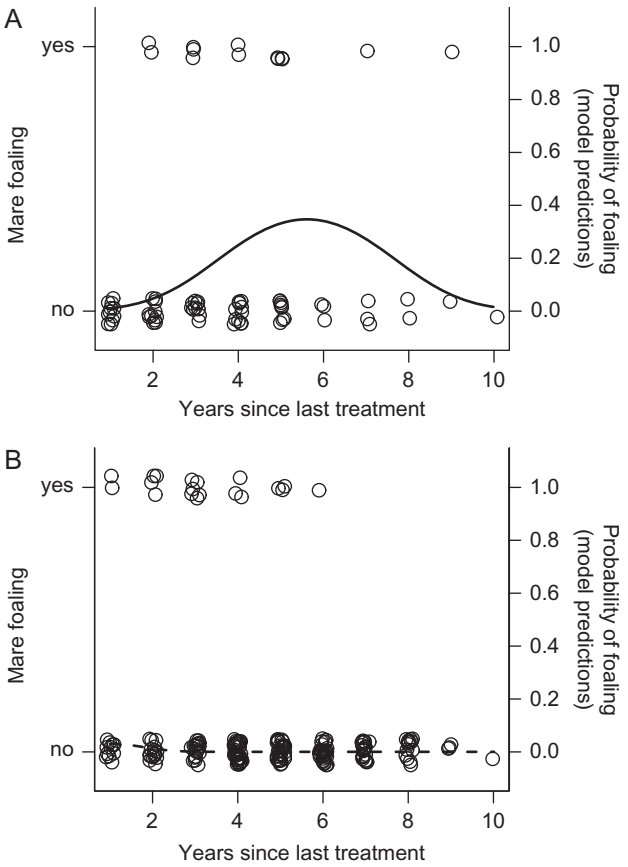

Figure 3: Probability of foaling after contraception suspension and mare treatment; circles represent individual births; solid and dashed lines indicate model predictions. Mares previously receiving 1–3 treatments (**A**) showed a gradual increase in foaling probability, peaking at ~6 years post-treatment, followed by a subsequent decrease. Mares previously receiving 4+ treatments (**B**) did not show a significant increase in foaling probability regardless of time since last treatment.

treatment * number of treatments (4+treatments): estimate = −0.79, SE = 0.25, t = −3.14, P = = 0.002, Fig. 3B).

Foaling date

Mares previously receiving any level of treatment gave birth later in the year than did untreated mares (Linear Mixed Effects Model, number of treatments (1–3 treatments): estimate = 2.29, SE = 0.78, t = 2.92, P < 0.00001; (4+ treatments): estimate = 3.50, SE = 0.90, t = 3.88, P < 0.0001, Fig. 4). Mares having received 1–3 treatments and 4+ treatments did not differ in their average foaling date (Tukey Honest Significant Difference Test: estimate = 1.21, SE = 0.87, z = 1.39, P = 0.34, Fig. 4). Island region and linear and second-order polynomial functions of both time since last treatment and mare age proved insignificant (all P-values > 0.08). Results suggested that models including a variance function were a better fit to the data (AICc with variance function = 155.56; AICc without variance function = 163.02).

Mare fidelity and reproductive behavior

Contraception history had a significant effect on mares' group changing behavior. Even after controlling for the effects of mare age (Linear Model: estimate = −0.04, SE = 0.01, t = −3.04, P = 0.004), mares that previously received 4+ contraceptive treatments changed groups more often than did

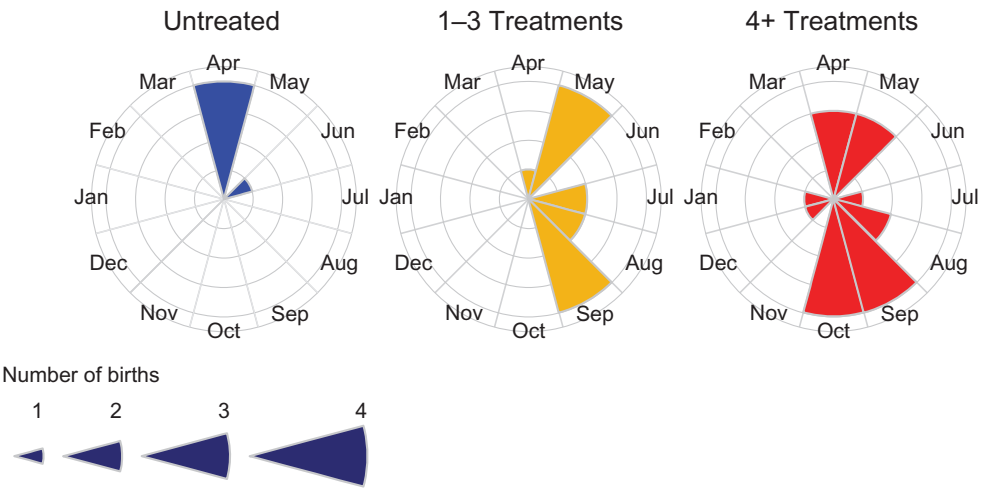

Figure 4: Average foaling date for untreated mares vs. previously treated mares. Mares previously receiving any level of treatment foaled later in the season than did untreated mares. Mares previously receiving 1–3 and 4+ treatments did not differ in their average foaling date.

untreated mares (Linear Model: estimate = 0.75, SE = 0.27, t = 2.80, P = 0.007, Fig. 5). Mares that previously received 1–3 treatments were equivalent to both untreated mares (Linear Model: estimate = 0.43, SE = 0.26, t = 1.66, P = 0.10, Fig. 5) and those receiving 4+ treatments (Tukey Honest Significant Difference Test: estimate = 0.32, SE = 0.15, z = 2.04, P = 0.10, Fig. 5). Island region, linear and second-order polynomial functions of time since last treatment, and second-order polynomial functions of mare age proved nonsignificant (all P-values > 0.08). Results suggested that models without variance functions were better fits to the data (AICc without variance function = 206.55; AICc with variance function = 207.62).

Contraception history did not affect mare reproductive behavior (Linear Mixed Effects Model: all P-values > 0.10). Results suggested that models without variance functions were better fits to the data (AICc without variance function = 131.49; AICc with variance function = 133.76). Island region, linear and second-order polynomial functions of both time since last treatment and mare age proved insignificant (all P-values > 0.07).

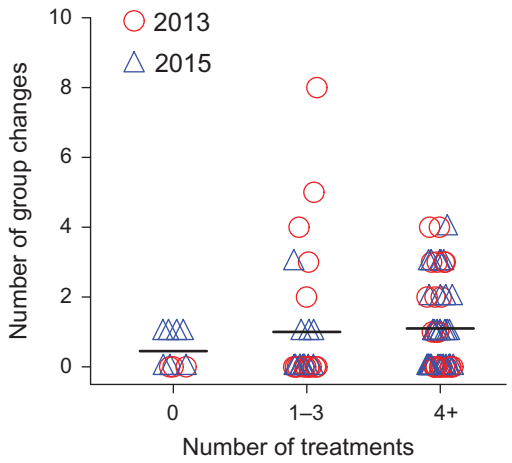

Figure 5: Number of group changes for untreated mares vs. previously treated mares in 2013 (red circles) and 2015 (blue triangles). Mares previously receiving 4+ contraceptive treatments made more group changes than did untreated mares. Mares previously receiving 1–3 treatments did not differ from untreated mares or those receiving 4+ treatments. Segmented lines indicate mean values.

Discussion

We investigated the likelihood that previously treated mares on Shackleford Banks, North Carolina USA, would return to baseline fertility, foaling dates and behavior after the cessation of contraceptive treatment with PZP. Mares received varying numbers of total PZP inoculations, allowing us to more closely investigate the effects of treatment intensity on mare fertility and subsequent effects to mare behavior. This contraceptive has been administered to several mammalian species including, white-tailed deer (*O. virginianus*) (Miller *et al.*, 1999), elk (*C. canadensis*) (Shideler *et al.*, 2001), black bears (*Ursus americanus*) (Lane *et al.*, 2007), bison (*Bison bison*) (Duncan *et al.*, 2013), and African elephants (*Loxodonta africana*) (Fayrer-Hosken *et al.*, 2000); these species have evolved an array of reproductive behaviors and social systems, which are vital to their survival and well-being. A more complete understanding of PZP contraception's potential long-term effects on recipient physiology and behavior is, therefore, of utmost importance if we are to manage and conserve species in the most effective and ethical manner possible. Feral horses have evolved a complex social structure, the maintenance of which is paramount to the overall health of group members

(Kaseda *et al.*, 1995; Linklater *et al.*, 1999). Careful study of mare physiological and behavioral responses to long-term contraception management can serve as a model for other social species for which the formation and maintenance of social bonds is necessary for survival.

Even when controlling for the effects of mare age on fertility, previously treated mares were less likely to give birth than were untreated mares during the study period. Moreover, the number of treatments that mares previously received affected the likelihood that mares would return to fertility after treatment suspension. Regardless of age, mares that previously received 1–3 treatments were more likely to return to fertility than were mares that received 4+ treatments. These results cannot be explained by inherent subfertility in mares slated for treatment: prior to contraceptive treatment, all mares exhibited equivalent fertility. In addition, time since last treatment was an important predictor of foaling probability for mares that received 1–3 treatments, but not 4+ treatments. Mares receiving 4+ treatments remained largely subfertile even after several post-treatment years. Conversely, mares that received fewer treatments exhibited a gradual increase in foaling probability until ~6 years post-treatment, after which, foaling probability decreased. This decrease was likely due to the typical age-specific reproduction in feral mares (Garrott *et al.*, 1991; Carnevale *et al.*, 1994). Feral mares more than 15 years of age exhibit decreased foaling rates (Garrott *et al.*, 1991) and pony mares at or older than 20 years of age are less likely to ovulate at regular intervals (Carnevale *et al.*, 1994). In this study, mares that previously received 1–3 treatments were, on average, 12.42 years of age in 2009, when they received their last inoculation. At 6 years post-treatment, these mares would have been old enough to exhibit the reproductive senescence typical of the species. However, the fact that mares receiving fewer treatments were able to return to fertility while mares receiving more treatments were not is telling; these results demonstrate that mares can retain more natural physiological functioning with less intense contraceptive treatment.

Foaling date was also was also affected by contraception history. Mares previously receiving any level of treatment exhibited later foaling dates than did untreated mares. The persistence of this effect after treatment suspension may be related to increases in mare condition commonly associated with contraception treatment (Turner and Kirkpatrick, 2002). In domestic mares, increased body condition or 'fatness' has been associated with higher circulating levels of leptin (Fitzgerald and Bates, 2000). Mares exhibiting higher circulating leptin were more likely to exhibit reproductive activity during the winter months, suggesting that leptin may modify mare reproductive response to inhibitory photoperiod (Fitzgerald and McManus, 2000). Treated Shackleford mares exhibit the increased physical condition typically demonstrated by contracepted mares (Nuñez *et al.*, 2010). It may be, therefore, that increases in body condition and concomitant increases in circulating leptin have contributed to prolonged effects on mare cycling even after contraception treatment suspension.

Potential effects of late birth dates to foal survival are difficult to assess on Shackleford Banks. In the past, animals perceived to be in poor condition have been removed from the island and adopted off, complicating the analysis of foal survival. As such, we cannot look to our data with confidence to determine if foal survival is compromised by later birth dates. Notably, foal survival is affected by birth date in western feral horses (Ransom *et al.*, 2013). In these populations, the probability of foal survival decreases as parturition date moves further away from the summer peak in resources (Ransom *et al.*, 2013), though these animals are undoubtedly subject to harsher environmental conditions than are Shackleford horses.

Contraception history also had an effect on mare fidelity to the band stallion. Mares that previously received 4+ treatments changed groups more frequently than did untreated mares. Mares that previously received 1–3 treatments did not differ from either untreated mares or those that previously received 4+ treatments. Combined with our findings on fertility, these results demonstrate that unintended side-effects of PZP treatment have the potential to become long term, i.e. they persist with continued subfertility, even if treatment, per se, has ended. However, our results also show that with less frequent treatment, some of these effects can be ameliorated with time, enabling more flexible population management.

Interestingly, our mare fidelity results are similar to those demonstrated in previous studies conducted during the contraception program (Nuñez *et al.*, 2009; Madosky *et al.*, 2010). In those studies, treated mares transferred groups more often than did untreated mares. These behaviors were linked to the presence of a foal (Nuñez *et al.*, 2009): mares with foals, regardless of treatment status, tended to change groups less often, indicating that mares, having successfully reproduced with a stallion, were more likely to remain with said stallion and/or, that mares with foals required a more stable environment in which to raise offspring. In the present study, we found that mares that had previously received 4+ treatments changed groups more often than did untreated mares. The lower foaling probability exhibited by these mares, even after several post-treatment years, is likely the proximate mechanism behind this behavior, rather than PZP treatment, per se. These results show that changes in mare behavior formerly associated with active contraception treatment have persisted with prolonged subfertility, lending additional support to the assertion that such unintended side-effects can persist, even after active contraceptive treatment has ended. Moreover, such behavior represents a significant decrease in mare fidelity by previously treated mares. Prior to the contraception program, Rubenstein (1981) reported that across the entire island, only 10.8% of mares transferred groups, indicating high band fidelity by females. This is in stark contrast to

what was found in our previous study, during which 53% of mares changed groups (Nuñez *et al.*, 2009), in the 2 years of the Madosky study during which 66% and 73% of mares changed groups in 2007 and 2008, respectively (Madosky *et al.*, 2010), and in our current study, during which 42% and 49% of mares changed groups, in 2013 and 2015, respectively.

Contrary to our results from an earlier study (Nuñez *et al.*, 2009), previously treated mares did not engage in more reproductive behavior than did untreated mares. The disparity in our results may be due to differences in the timing of the two studies: the current study was conducted during the breeding season, when breeding behavior is relatively frequent amongst reproductive individuals; our previous study was conducted during the non-breeding season when breeding behavior is more variable. It is worth noting that in the Madosky study (2011), also conducted during the breeding season, Shackleford mares treated with PZP received more harassment from band stallions than did untreated mares. Taken together, these results could suggest that the amount of reproductive behavior in previously treated mares has returned to baseline levels. However, it is not clear whether 'harassment' (Madosky, 2011) is indicative of reproductive behaviors as we have defined them here. As such, our ability to compare results across time is limited.

Our results demonstrate the potential for long-term physiological and behavioral effects to PZP-treated individuals and that these effects can persist with continued subfertility, even after the cessation of treatment. However, our results also suggest that with less frequent treatment, these effects can be ameliorated with time, enabling more flexible population management.

Our results also contrast with claims that PZP is 'completely reversible'; claims upon which feral horse management decisions have been defended (BLM, 2010). Similar long-term subfertility with intense PZP treatment has been found in feral horse populations living on Assateague Island National Seashore, MD (Kirkpatrick and Turner, 2002), in the closely related, but critically endangered Przewalski's horse (*E. ferus przewalskii*) (Feh, 2012), and in feral horses living in the western United States (CO and WY) (Ransom *et al.*, 2013), indicating that these effects are not limited to specific sites or populations, but are pervasive amongst equids. In the Assateague study, nearly 70% of mares receiving three consecutive PZP treatments failed to demonstrate fertility for 1–4 years post-treatment (Kirkpatrick and Turner, 2002). In the Przewalski's horse, only 45% of 20 mares that had been treated with PZP for three consecutive years demonstrated fertility; those that did foal, did not do so until 6.7 years after their last treatment, on average. Moreover, four of nine females receiving only one treatment (one primer injection and one booster injection) did not produce offspring for 4+ years post-treatment (Feh, 2012). Though the mechanism behind the prolonged subfertility in Przewalski's mares is unknown, some of the Assateague mares demonstrated ovulatory failure (Kirkpatrick *et al.*, 1992; Kirkpatrick and Turner, 2002). In western feral horse populations, previously treated mares were less likely to give birth and gave birth later into the calendar year for up to six post-treatment years, again demonstrating the potential for long-term side effects of prolonged, PZP-induced subfertility (Ransom *et al.*, 2013).

The present study demonstrates that the intensity of treatment is also important: mares previously receiving 4+ treatments are less likely to return to fertility and exhibit decreased fidelity to the band stallion. Mares receiving any level of treatment are more likely to give birth later into the calendar year. Conversely, mares receiving 1–3 treatments are more likely to return to fertility and demonstrate behavior more typical of the species (Feist and McCullough, 1976; Rubenstein, 1981; Berger, 1986). These results largely support our previous assertion that more natural physiology and behavior can be maintained in feral horses with less aggressive contraception schedules (Nuñez *et al.*, 2009).

Results similar to those demonstrated in this study have been documented in several captive species (Penfold *et al.*, 2014). A retrospective analysis, combined with published literature and reliable anecdotal reports, demonstrated that extended periods of infertility in females can induce reproductive changes that negatively impact future fertility. This was the case for individuals treated with a contraceptive agent and also for those left untreated, but separated from male counterparts (Penfold *et al.*, 2014). The mechanisms underlying these impacts are varied, but include endometrial hyperplasia [(Asian (*Elephas maximus*) and African elephants, Seba's bats (*Carollia perspicillata*), felids], uterine insufficiencies [wildebeest (*Connochaetes*)], asymmetric reproductive aging [white rhinoceros (*Ceratotherium simum*)], and enlarged ovaries and exuberant uterine responses [stingrays (*Myliobatoidei*)] (Penfold *et al.*, 2014).

The close association with PZP-induced subfertility and mare behavior demonstrated here points to an important link between animal physiology and behavior that should be considered when implementing any management program. Alterations to an animal's reproductive physiology—for whatever managerial gain—will likely be associated with concomitant changes in behavior (McShea *et al.*, 1997; Heilmann *et al.*, 1998; Nuñez *et al.*, 2009; Gilman *et al.*, 2010; Madosky *et al.*, 2010; Ransom *et al.*, 2010). The effects of these subsequent changes—both to management program efficacy and to animal welfare—ought to be weighed against the perceived advantages of our physiological manipulations.

The broader implications of this research are notable. Because other methods for controlling feral horse populations can have pronounced side effects and are often unpopular with the public, PZP is undoubtedly an important option in a manager's toolkit (Hobbs *et al.*, 2000; Kirkpatrick and Turner, 2003; Porton, 2005). However, our results suggest

that the best use of this tool will balance attaining target population sizes against over-treating individual animals. Although the prolonged effects of PZP contraception outlined here could actually assist managers in keeping population sizes low (in feral horses and other over-abundant wildlife), the use of PZP with small populations and/or those of conservation concern should be approached with caution (Ransom *et al.*, 2014). For instance, the fact that mares do not easily return to fertility after the cessation of treatment could limit the ability of populations to rebound in the face of stochastic declines. In the case of Shackleford Banks horses, prolonged PZP-induced subfertility has likely reduced the ability to adaptively manage the population; stopping treatment has not resulted in increased fertility, particularly for mares that previously received 4+ treatments. Given these results and the prolonged effects of PZP-induced subfertility on foaling schedules and behavior (and the potential for additional effects to non-recipient animals (Ransom *et al.*, 2010)), we suggest that monitoring individual treatment histories, or modeling the statistical probability of multiple treatments per individual, could prove critical in maintaining more natural physiological and social dynamics, all while allowing for maximal flexibility in attaining management objectives.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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Behavioural effects of ovariohysterectomy on bitches

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ABSTRACT

A questionnaire about their dogs' behaviour was administered to the owners of 150 spayed bitches at the time of spaying and again six months later. It was also administered twice with the same time interval to a control group of 150 unspayed bitches, group matched for breed and age. Principal component analysis of the questionnaire responses yielded 13 factors. On two of these factors, 'indiscriminate appetite' and 'dominance aggression towards family members', the scores of the spayed bitches showed a significant increase compared with their controls. The spayed bitches most likely to show an increase in dominance aggression were puppies under one year, already showing some aggression.

INTRODUCTION

In bitches, the most frequently performed surgical operation is that of ovariohysterectomy, or spaying. Although it is sometimes performed for medical reasons, its most usual purpose is the avoidance of pregnancy and the inconveniences of a bitch in oestrus. It is occasionally undertaken as a treatment for a behavioural problem. It is sometimes stated that spaying affects temperament adversely (for example that spayed bitches 'lack personality' or are 'frankly neurotic' (Jones and Joshua 1982)). It has been observed clinically (Voith and Borchelt 1982, O'Farrell 1986) that owners of bitches presenting with problems of aggression frequently report an exacerbation after spaying. However, there are no objective and controlled studies in this area. The aim of the present study was to fill this gap.

MATERIALS AND METHODS

The owners of 150 bitches were interviewed at the time that the bitch was spayed and again six months later. They were an unselected sample, being the first 150 owners contacted through veterinary practices who were available for interview. These formed the experimental group. The control group was composed of 150 unspayed bitches: their owners were also interviewed twice with an interval of six months. The controls were matched for breed with the bitches in the experimental group and the groups were matched for age. No dog was included in the study which was less than four months old at initial interview or which had been acquired less than one month previously.

Questionnaire

The interview took the form of a questionnaire with 60 items. Twenty of the items were concerned with basic information about the dog and its circumstances: age, age when acquired, number of dogs and people in the household, number of pregnancies, etc. The rest of the questionnaire was concerned with the dog's behaviour over the previous month. The following areas were covered: aggression (to owners, visitors, other dogs), fears, activity level, excitability, destructive chewing, reactions to separation from owner, behaviour in the car, roaming, feeding, urination and defecation and scavenging. Most of the questions were phrased in such a way that the replies were in the form of an estimated absolute or relative frequency of the behaviour.

RESULTS AND DISCUSSION

To simplify the results of the responses to the 40 behavioural items, the correlations between the items were subject to principal component analysis. The results are shown in Table 1.

Table 1. Principal component analysis of questionnaire items (Varimax rotation)

	Factor loadings
Factor 1. Protective aggression (9·8% of variance)	
Threatens people outside family	0·76
Barks, growls when owner opens door to someone	0·68
Gets excited when owner opens door to someone	0·60
Factor 2. Aggression towards other dogs (7·2% of variance)	
Growls at other dogs	0·85
Growls at dogs outside the household	0·83
Factor 3. Urination or defecation indoors (5·2% of variance)	
Urinates in house	0·83
Defecates in house	0·78
Factor 4. Destructive behaviour (4·8% of variance)	
Destructive when left alone	0·88
Destructive chewing generally	0·81
Factor 5. Phobias (4·4% of variance)	
Afraid of visitors	0·73
Afraid of specific kind of person (eg, men)	0·76
Afraid of things (eg, vacuum cleaner)	0·57
Factor 6. Dominance aggression towards family members (4·3% of variance)	
Threatens if disturbed when resting	0·78
Threatens family members	0·72
Threatens when groomed	0·50
Threatens when something taken away (eg, food)	0·35
Factor 7. Excitement in car (3·6% of variance)	
Sits quietly in car	−0·85
Jumps about or barks in car	0·81
Factor 8. Indiscriminate appetite (3·5% of variance)	
Eats meals quickly	0·83
Eats any commercial dog food	0·83
Eats rubbish, carrion, etc	0·36
Factor 9. Dislike of separation from owner (3·3% of variance)	
Follows owner to door on departure	0·74
Looks miserable when owner leaves	0·63
Takes no notice when owner leaves	−0·55
Factor 10. Territorial urination (3·1% of variance)	
Adopts non-squatting posture when urinating	0·74
Urinates frequently on walks	0·72
Factor 11. Activity level in the house (2·8% of variance)	
Moves around constantly	0·74
Reacts to noises	0·48
Follows owner around house	0·38
Factor 12. Perverse appetite (2·7% of variance)	
Eats dog faeces	0·87
Eats rubbish, carrion, etc	0·57
Factor 13. Reactivity (2·6% of variance)	
Reacts to noises	0·72
Threatens when groomed	0·45
Barks when left	0·43

Table 2. Differences between experimental and control groups at first interview

Spayed group	
1 Was acquired at older age	P<0·001
2 Had fewer administrations of synthetic progestogen	P<0·01
3 Had fewer dogs in household	P<0·0001
4 Reacted less to separation (factor 9)	P<0·01
5 Jumped about less in car	P<0·01
6 Roamed more from house	P<0·02

Table 3. Change in factor scores in unspayed group between first and second interview

Factor	Change
1 Protective aggression	+
2 Aggression towards other dogs	+
3 Urination or defecation in the house	− (P = 0·002)
4 Destructive behaviour	−
5 Phobias	−
6 Dominance aggression towards family members	−
7 Excitement in car	− (P < 0·05)
8 Indiscriminate appetite	− (P < 0·01)
9 Reaction to separation from owner	−
10 Territorial urination	+ (P < 0·01)
11 Activity level in the house	−
12 Perverse appetite	−
13 Reactivity to stimuli	−

Where changes are significant, significance level is shown (Wilcoxon test)

For each interview, a score on each factor was calculated by summing the score on the relevant items, weighted by their factor loadings. In analysing the results, for the most part, these factor scores were used, rather than the scores on the individual items.

Next, the results were examined to see if there were any significant differences between the spayed and unspayed groups at initial interview. Any significant differences between the groups would mean that groups were not matched on those variables. The mean age of the experimental group was 33·47 months (SD 33·95). The mean age of the control group was 33·29 months (SD 30·52). These ages were not significantly different: the groups, therefore, had been successfully matched for age. There were, however, significant differences between the groups on some variables: these are shown in Table 2. The relevance of these differences is discussed later.

The factor scores on first and second interview for the unspayed bitches were then compared (Wilcoxon test for matched pairs) to determine what changes in behaviour had occurred due only to the passage of time. The changes in the factor scores are shown in Table 3. It can be seen from this table that significant decreases occurred in urination and defecation in the house, excitement in the car and indiscriminate

Table 4. Change in factor scores in unspayed group between first and second interview by age

Factor	Change		
	11 months and under	12-24 months	25 months and over
1 Protective aggression	+	+	−
2 Aggression towards other dogs	+	+	−
3 Urination or defecation in the house	− (P = 0·003)	−	−
4 Destructive behaviour	−	−	+
5 Phobias	+	+	− (P = 0·002)
6 Dominance aggression towards family members	−	−	−
7 Excitement in car	− (P = 0·02)	−	−
8 Indiscriminate appetite	− (P = 0·01)	−	−
9 Reaction to separation from owner	+	−	−
10 Territorial urination	+ (P = 0·005)	+	+
11 Activity level in the house	−	+	−
12 Perverse appetite	−	+	−
13 Reactivity to stimuli	−	+	−

Where changes are significant, significance level is shown (Wilcoxon test)

appetite: a significant increase occurred in territorial aggression. The unspayed group was then divided into three age groups: 11 months and under (puppies), one to two years and over two years. The changes occurring in these groups over the six months were examined. These are shown in Table 4. It can be seen that where significant changes occurred in the group as a whole, these were due to changes in the puppy group. These changes are not surprising: they are the changes one would expect in puppies as they mature. The finding, however, is reassuring, as it confirms the validity of the questionnaire. It is interesting that fears do not follow the same pattern: they do not decrease until the bitch is over two years old.

The changes in the spayed group from first to second interview were then calculated and the changes compared with the changes in the unspayed group (Wilcoxon test). The comparison was also made with the puppies excluded, in case changes in the puppy group were masking changes due to spaying. The factors for which the changes in the spayed and unspayed group differed significantly are shown in Table 5.

The first question to be considered is whether these differences are due to spaying or whether they might be accounted for by any of the differences between the two groups at initial interview. It seems unlikely that the difference on factor 6 (dominance aggression) or on factor 8 (indiscriminate appetite) could be accounted for by any of the initial differences. However, in the case of factor 7 (excitement in the car) the items which loaded on this factor were 'does not sit quietly in the car' and 'jumps about in the car'. On examining the changes on these two items, it was found that changes in the 'jump about' item were solely responsible for the significant difference in the changes between the groups on factor 8. The initial scores of the two groups on the 'jump about' item were significantly different (Table 2): it cannot be concluded, therefore, that spaying caused an increase in excitement.

With regard to factor 8 (indiscriminate appetite), it seems safe to conclude that the relative increase in the experimental group when puppies were excluded was due to the effects of spaying. Presumably this behavioural change

Table 5. Change in factor scores between first and second interview: significant differences between experimental and control groups

Factor	
6 Dominance aggression towards family members	
Spayed group increase	
Unspayed group decrease	
	P<0·05
7 Excitement in car	
Spayed group increase	
Unspayed group decrease	
	P<0·05
8 Indiscriminate appetite (puppies excluded)	
Spayed group increase	
Unspayed group decrease	
	P<0·05

Table 6. Changes in dominance aggression by age

Age	Aggression increases	Aggression stays the same	Aggression decreases	Total
11 months or less	S = 13 U = 4	S = 28 U = 31	S = 4 U = 6	S = 45 U = 41
12-24 months	S = 6 U = 6	S = 31 U = 26	S = 5 U = 7	S = 42 U = 39
2 years +	S = 14 U = 11	S = 38 U = 43	S = 11 U = 16	S = 63 U = 70
Total	S = 33 U = 21	S = 97 U = 100	S = 20 U = 29	

S Number of bitches in spayed group
U Number of bitches in unspayed group

Table 7. Changes in dominance aggression by initial aggression score

Initial Aggression	Aggression increases	Aggression stays the same	Aggression decreases	Total
Some aggression	S = 16 U = 8	S = 7 U = 5	S = 20 U = 29	S = 43 U = 42
No aggression	S = 17 U = 13	S = 90 U = 95	—	S = 107 U = 108
Total	S = 33 U = 21	S = 97 U = 100	S = 20 U = 29	

S Number of bitches in spayed group
U Number of bitches in unspayed group

Table 8. Changes in dominance aggression by age and initial aggression

	Aggression increases	Aggression stays the same	Aggression decreases	Total
Bitch under 12 months showing some initial aggression	S = 6 U = 1	S = 2 U = 0	S = 4 U = 6	12 7
Bitch over 12 months showing no initial aggression	S = 10 U = 10	S = 64 U = 64	—	74 74

sometimes results in the weight gain which is often cited as a possible side-effect of spaying.

Similarly, it seems safe to attribute the change in factor 6 (dominance aggression towards family members) to spaying. The results were examined more closely in an attempt to discover which bitches are most at risk of developing increased aggression following spaying. Table 6 shows the changes occurring in bitches spayed at different ages. It can be seen that although there is no clear trend with increasing age, the greatest difference between experimental and control group occurs in the youngest age group. Table 7 shows a comparison between bitches showing some aggression at initial interview and those showing none. It can be seen that there is a greater risk of aggression increasing in those already showing some aggression. Combining the variables age and initial aggression, Table 8 compares puppies under one year old already showing some aggression with dogs over one year showing none. If a puppy showing some aggression is spayed, there is a 50/50 chance that her aggression will increase. If she is not spayed, the chances are 6 to 1 that her aggression will decrease. On the other hand, if an adult bitch showing no aggression is spayed, this does not increase at all the chances that she will subsequently show some aggression: these are 6 to 1 in either case.

CONCLUSIONS

There is no evidence that spaying has beneficial effects on behaviour (excluding, of course, behaviour directly connected with oestrus).

The operation should not be undertaken as a treatment for behavioural problems.

Spaying is accompanied by the risk of certain behavioural changes. There is a risk of increase in indiscriminate appetite. More importantly, there is a risk of increase in dominance aggression towards family members. This risk is greatest in puppies under one year already showing some aggression. For these, some alternative method of controlling oestrus (eg, a synthetic progestogen or a surgical procedure which does not remove all ovarian tissue) might be preferable. (Unfortunately, the figures do not, of course, indicate whether the risk diminishes for these dogs as they get older). On the other hand, there seems no risk of increased aggression resulting from spaying for older dogs not showing any aggression. In addition the study showed no adverse effects on other kinds of behaviour.

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BOOK RECEIVED

Handbook on Animal Diseases in the Tropics

Edited by M. M. Sewell and D. W. Brocklesby. 4th edn. Published by Bailliere Tindall, London. Price £14.99, 385 pages. 1990.

Comparison of Transvaginal Natural Orifice Transluminal Endoscopic Surgery (NOTES®) and Laparoscopy for Elective Bilateral Ovariectomy in Standing Mares

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Objective: To compare surgical trauma, perioperative pain, surgical time, and complication rate in mares undergoing standing bilateral ovariectomy by natural orifice transluminal endoscopic surgery (NOTES) or laparoscopy.
Study Design: Experimental study.
Animals: Healthy mares (n = 12).
Methods: Ovariectomy was performed with a vessel-sealing device by transvaginal NOTES (n = 6) using a flexible endoscope and specialized instruments or by bilateral flank laparoscopy (n = 6). Preoperative and postoperative complete blood count (CBC), plasma fibrinogen concentration, serum amyloid A concentration, peritoneal nucleated cell count, and total protein concentration were compared using repeated measures ANOVA. Surgical times were compared using an unpaired t-test. Mares were monitored for 2 weeks postoperatively and necropsy was performed at 3 weeks (n = 6) or 3 months (n = 6) to assess short- and medium-term effects of each procedure. Complication rate, necropsy, and bacteriology findings were compared using a Fishers exact test. Significance was set at $P < .05$.
Results: All surgical procedures were performed successfully and all but one laparoscopy mare were bright, alert, and maintained a good appetite until euthanasia. Mean ± SD surgical time was similar between groups (NOTES: 100 ± 40 minutes; laparoscopy: 107 ± 47 minutes). There were no significant temporal differences in vital parameters, CBC, plasma fibrinogen concentration, serum amyloid A concentration, peritoneal nucleated cell count, and total protein concentration between groups.
Conclusions: Bilateral ovariectomy by transvaginal NOTES or laparoscopy resulted in minimal inflammation and surgical trauma.

Minimally invasive techniques are continuing to expand in veterinary surgery, with decreased soft tissue trauma, reduced postoperative pain and morbidity, and faster return to function cited as key advantages.[1] Standing laparoscopy is currently the gold standard minimally invasive technique for elective bilateral ovariectomy in mares. This technique allows excellent viewing and hemostasis through small body wall incisions.[2]

Natural orifice transluminal endoscopic surgery (NOTES) consists of passing a flexible endoscope through a natural orifice (typically vagina or mouth), creating an opening to access the abdomen (typically transvaginal or transgastric routes) and perform intra-abdominal procedures. Since 2004,[3] flexible endoscopy for abdominal surgery has been used in laboratory studies[4,5] and is currently being performed for cholecystectomy in human clinical trials.[6] A recent retrospective case-control study compared transvaginal cholecystectomy to conventional laparoscopic cholecystectomy.[7] Whereas similar complication rates between groups were reported, the NOTES group had a lower need for postoperative analgesia, a faster recovery, and better cosmetic results compared to the laparoscopy group. Controlled studies comparing transgastric NOTES to laparoscopy in pigs suggest that viewing, cardiopulmonary and immunologic effects are similar.[8–12]

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NOTES techniques have recently been introduced to veterinary surgery,[4,13] and our group described transvaginal ovariectomy in 10 mares using a NOTES approach.[14] Transvaginal abdominal exploration using flexible and rigid endoscopes was also recently reported in horses.[15] Transvaginal access uses the principles of the colpotomy technique to access the abdominal cavity of mares. Ovariectomy by blind colpotomy using a chain écraseur has been used in standing mares because it is a quick and inexpensive procedure that avoids skin incisions.[16,17] However, disadvantages of the colpotomy technique include blind access to the abdominal cavity and pedicle transection by mechanical means that can potentially lead to major intra- and postoperative complications including fatal hemorrhage, peritonitis, intestinal puncture, and evisceration through the colpotomy site.[18] By providing direct viewing during the transvaginal abdominal approach, and of the surgical site during hemostasis and transection of the ovarian pedicle, the transvaginal NOTES approach has the potential to increase the safety of the colpotomy technique for bilateral ovariectomy in healthy mares.[15]

Because the NOTES approach eliminates the need for skin and body wall incisions, it may offer advantages over standard laparoscopy with respect to fewer incisional complications and faster return to function. A single small vaginal wall incision allows access to both sides of the abdominal cavity when performing a NOTES ovariectomy.[14] Compared to conventional laparoscopic ovariectomy, the transvaginal approach has fewer incisions through a thinner muscle layer, resulting in less overall tissue disruption for surgical access. This has the potential to decrease the risk for incisional complications such as seroma, infection, or dehiscence, as well as reduce the degree of postoperative incisional pain. A concern raised with the introduction of NOTES in the clinical setting has been the perceived increased risk of abdominal infection after access through a nonsterile orifice.[19] However, to date, an increased risk of abdominal infection after transvaginal NOTES compared to laparoscopy has not been documented. Clinically, perioperative antimicrobial administration and disinfection of the natural orifice have been used to limit contamination during surgery.

Surgical invasiveness, as measured by postoperative pain and recovery time, is more easily and accurately evaluated in people than in horses. Even though pain scoring systems have been described and validated in horses,[20] pain assessment is associated with a certain degree of subjectivity. Acute phase proteins, such as serum amyloid A (SAA) or fibrinogen, provide a quantitative assessment of inflammation and correlate well with the degree of surgical trauma in horses.[21] We are unaware of a controlled study comparing the outcome of NOTES to laparoscopic procedures in horses. Should NOTES ovariectomy be shown to have advantages over laparoscopy, the spectrum of minimally invasive abdominal surgeries in the horse could be expanded using natural orifice surgical techniques.

Our primary objective was to compare perioperative pain and surgically induced trauma in standing mares undergoing bilateral ovariectomy by NOTES or conventional laparoscopy. Further, we compared surgical time and complication rate of the 2 procedures. We hypothesized that transvaginal NOTES would improve the clinical outcome after bilateral ovariectomy in the mare by minimizing soft tissue trauma and reducing pain and inflammation when compared to a conventional laparoscopic approach.

MATERIALS AND METHODS

Horses

Twelve mares of various breeds (age 8–18 years; weighing 358–625 kg) were studied. The mares had no history of previous abdominal surgery and were determined to be healthy and free of reproductive abnormalities based upon physical, rectal, and ultrasound examinations. Mares were admitted 7 days preoperatively for an acclimation period and were monitored daily for 2 weeks postoperatively. Each mare was randomly assigned to either the transvaginal NOTES or laparoscopic ovariectomy group with 6 horses in each group. All surgeries were performed during the same month (July); however, no attempt was made to manipulate the reproductive cycle in the mares or to time the surgery with the stage of the reproductive cycle.

Preoperative Phase

Mares were housed in the same facility for the duration of the study. During this acclimation phase, preoperative behavior scores and activity levels were measured. Hay was withheld for 3 days and a complete pelleted feed was removed 24 hours before surgery, but mares had free access to water. Complete blood count (CBC), plasma fibrinogen concentration, and SAA concentration were determined preoperatively. A catheter was placed in the left jugular vein and potassium penicillin (22,000 U/kg, intravenously [IV]) and flunixin meglumine (1.1 mg/kg, IV) were administered within 1 hour of surgery. Mares were restrained in standing stocks and sedated with detomidine hydrochloride (0.01 mg/kg, IV) and butorphanol tartrate (0.01 mg/kg, IV). Caudal epidural anesthesia was performed with a combination of xylazine hydrochloride (0.2 mg/kg) and morphine (0.2 mg/kg), made up to a volume of 15 mL with sterile (0.9% NaCl) solution. Additional doses of detomidine hydrochloride and butorphanol tartrate were administered IV if the mare displayed signs of pain during surgery (kicking, shifting weight, and excessive body movement). In the NOTES group, the perineal region was clipped, the vulva and vaginal walls were cleaned and aseptically prepared using a surgical scrub brush of chlorhexidine gluconate 4% solution (Pre-scrub II™, BioMed Systems Inc, Norwalk, CT), as previously described.[14] A 30 F Foley catheter was introduced into the bladder and left in place for the duration of surgery to allow decompression of the bladder. The

pelvis and perineal region were draped, leaving a fenestration for the vulva. In the laparoscopy group, both paralumbar fossae were clipped, aseptically prepared, and draped for surgery.

Surgical Procedures

A surgical resident (KP) with similar proficiency in NOTES and laparoscopy techniques performed all surgical procedures assisted by 2 board certified surgeons (TBL, LJF).

NOTES Group. Transvaginal ovariectomy was performed as described.[14] The technique previously described was modified by using a 15-mm optical trocar (instead of a 12-mm trocar) in order to accommodate the endoscope for viewing entry into the abdominal cavity. Briefly, the cervix and vaginal fornix were identified using a 1-m-long single working channel flexible endoscope (Olympus® GIF P-140, Olympus America, Inc., Center Valley, PA). The transvaginal portal was made through the vaginal wall at the 11 o'clock position, 4 cm away from the cervix by introducing a 15-mm controlled access cannula (Endopath® Xcel™ Bladeless, Ethicon Endo-Surgery, Inc., Cincinnati, OH) under endoscopic guidance followed by placement of a 33-mm diameter Endopath® Large-Port assembly (LTK33, Ethicon Endo-Surgery, Inc.). Once the 33-mm diameter cannula was inserted through the vaginal wall, the endoscope was introduced in the abdominal cavity and the ovaries were identified. On-demand room air introduced by pressing the air ingress button on the endoscope was used to improve observation. No positive-pressure insufflation was used and intra-abdominal pressures were not recorded. Local anesthesia was achieved by infiltrating 35-mL 2% lidocaine hydrochloride in the mesovarium of each ovary using a 17-g endoscopic injection needle (17-Gauge Lance-A-Lot, Mila International, Inc., Erlanger, KY). Claw-jaw laparoscopic grasping forceps (ARO910136 BM, Arrow Medical Supply, Inc., Libertyville, IL) were used to grasp and elevate the ovary during hemostasis and transection of each ovarian pedicle using a 60-cm long, 10-mm diameter customized vessel sealing device (LigaSure™, Valley Lab, Boulder, CO).[14] All instruments and endoscope were positioned side by side within the 33-mm cannula and therefore, no extra portals were made throughout the procedure. After complete transection of the ovarian pedicle, the ovary was removed through the vaginal opening after removal of the 33-mm cannula. The 33-mm cannula was reinserted and the procedure was repeated for the second ovary, which was removed through the same vaginal opening after removal of the cannula. Each ovarian pedicle was examined to ensure complete hemostasis. The transvaginal portal site was closed primarily by placing a single cruciate suture of 1 polydioxanone through the mucosa and muscularis layers of the cranial vaginal wall. Culture swabs of the cranial vaginal fornix (after sterile preparation and before placement of the transvaginal portal) and the edges of the incision (before closure) were obtained using a guarded uterine swab (Culture swab 25 in., Continental Plastics, Delavan, WI) and submitted for microbial culture to determine bacterial contamination during surgery.

Laparoscopy Group. Each proposed portal site was infiltrated with 20-mL 2% lidocaine hydrochloride. The 1st portal was made in the left paralumbar fossa, midway between the last rib and tuber coxae and just dorsal to the crus of the internal oblique muscle. A 1.5-cm stab incision was made and an 11-mm diameter, 20-cm-long laparoscopic cannula (30103 H3, Karl Storz Endoscopy, Goleta, CA) with a blunt obturator (62103 AL, Karl Storz Endoscopy) was introduced into the abdomen perpendicular to the skin. A 10-mm diameter, 33-cm long 0°-angled laparoscope (62033 APA, Karl Storz Endoscopy) was placed in the 1st portal and intra-abdominal location was confirmed. The abdomen was insufflated with CO_2 (620-040-600, Stryker Endoscopy, San Jose, CA) at a flow rate of 6–8 L/min to an intra-abdominal pressure of 12–15 mmHg. Two additional portals were made in a similar fashion, 1 between the 17th and 18th ribs ~3 cm ventral to the ventral border of the tuber coxae and the other about 10 cm ventral to the initial portal. The laparoscope was transferred to the cranial most portal and the left ovary was identified. The mesovarium was infiltrated with 35-mL 2% lidocaine hydrochloride using a laparoscopic injection needle (250-080-703, Stryker Endoscopy) inserted through the dorsal instrument portal. The injection needle was removed and the left ovary was grasped using 10-mm claw-jaw grasping forceps (60510 FSL, Karl Storz Endoscopy) placed through the ventral instrument portal. Hemostasis and transection of the ovarian pedicle was achieved with a 10-mm vessel-sealing device (LigaSure™) inserted through the dorsal instrument portal.[22] After the dissection was complete, the left ovary was kept in the jaws of the grasping forceps and passed underneath the rectum to the right side of the abdomen.

The procedure was repeated in a similar manner on the right side. The right ovary was removed first, by extending the ventral instrument portal site ~4 cm dorsally. The skin and external oblique muscle were incised using sharp dissection while a grid technique was used for deeper muscle layers. The left ovary was transferred to the right-sided grasping forceps and removed similarly through the right body wall incision. The incision used for ovary removal was closed in 3 layers. The body wall, incorporating internal and external oblique muscles, was sutured with 1 polydioxanone in a cruciate pattern. The subcutaneous tissue was closed with 0 polydioxanone in a simple continuous pattern. The skin was closed with 2-0 polypropylene in a simple continuous pattern. The other laparoscopic portal sites were closed in 1 layer with 2-0 polypropylene in a simple interrupted pattern in the skin. Culture swabs (Culturette, Becton Dickinson Microbiology Systems Inc., Sparks, MD) of the skin before incision (after sterile preparation) and swabs of the body wall musculature before closure, were obtained and submitted for microbial culture.

Postoperative Phase

Time 0 (t0) was identified as the time the horse returned to the stall after surgery. Mares were administered a single dose of potassium penicillin (22,000 U/kg, IV) immediately after surgery upon return to their stall. Flunixin meglumine (1.1 mg/kg, IV) was administered every 12 hours for 2 days after surgery. In the laparoscopy group, skin sutures were removed 14 days postoperatively.

Monitoring

Surgical Variables. Total surgery time was recorded for each surgical procedure and was defined as the time from completion of the caudal epidural to final closure of the portal sites, including the time necessary for sterile preparation of the surgical site and setup of the operating room. Intraoperative complications such as hemorrhage of the ovarian pedicle after transection, poor viewing, instrument failure, retroperitoneal insufflation, or drop of the ovary were recorded. At procedure completion, each ovary was measured and in the laparoscopy group, the length of the incision used to retrieve the ovaries was recorded. Postoperative monitoring of mares for surgical complications included observing for abdominal discomfort, fever, diarrhea, peritonitis, laminitis, incisional complications, vaginal edema, and discharge.

Physiologic Variables. Body weight was recorded 3 days preoperatively and at day 14. Complete physical examination was performed during the acclimation phase, before surgery (baseline examination), at time = 0, 6, 12, 18, 24, 36, 48, 60, and 72 hours, and then daily until study completion. CBC, plasma fibrinogen concentration, and SAA concentration were determined before surgery and at days 3 and 10 postoperatively. Blood for SAA measurement was collected into a plain tube, allowed to clot, centrifuged at $2500 \times g$ for 15 minutes, and the serum harvested and stored at –70°C. All samples were assayed after study completion by using a commercially available solid phase sandwich ELISA method (Phase Serum Amyloid A ELISA Kit TP 802, Tridelta Development Ltd, Bray, Ireland).[23] Peritoneal fluid was collected percutaneously in an aseptic manner at days 0, 3, 14, and at 3 months (long-term group) and the abdominal nucleated cell count determined using an automatic cell counter (Cell-Dyn® 3700, Abbott Laboratories, Abbott Park, IL). Abdominal total protein concentration was measured using a refractometer (Vet 360, Reichert®, Depew, NY). For total protein concentrations <1 g/dL, specific gravity was recorded and total protein concentrations were determined using published conversion tables.[24] Microbial culture of the peritoneal fluid was performed on day 14.

Pain Assessment. Heart rate was recorded before surgery, 5 minutes after initial sedation, and every 10 minutes during surgery. Postoperative behavior scores were recorded by one nonblinded observer at times 0, 6, 12, 18, 24, 48, and 72 hours after surgery and then daily for 11 days using a previously described numerical rating scale (NRS).[20,25] Briefly, the NRS of behavior was calculated as a sum of scores assigned for 9 observations and was subdivided into posture and socialization scores. Each observation was scored from 1 (absence of gross pain, head above withers, ears forward, alert, moves freely, freely lift feet when asked, responds to door opening, approach, and reaches for grain) to 4 (continuous signs of gross pain, head below withers, ears back, does not move, located in the middle or facing the back of the stall, unwilling to lift feet, no response to door opening, observer approaching, or offer of grain).

Activity level was recorded using a 3-dimensional accelerometer (AAM, Respironics Mini Mitter Division, Bend, OR) placed around the right front pastern beginning 2 days before surgery and continued for 6 days postoperatively. The device uses an accelerometer to measure the occurrence and intensity of motion and includes a sensor that produces a variable electrical current when the device is subjected to acceleration changes. Increased activity results in an increase in voltage and this information is stored as activity counts. This device has been used to continuously record the intensity, frequency, and duration of physical activity in dogs[26]; however, it has not been validated in horses.

Necropsy Examination. Mares were euthanized at 3 weeks (n = 6; 3/group) and 3 months (n = 6; 3/group) after surgery to evaluate short- and medium-term effects of the procedures. A complete necropsy was performed and the abdominal cavity was examined specifically for signs of peritonitis or adhesions. Surgical sites, adjacent organs, and the gastrointestinal tract were evaluated for gross pathology or local peritonitis. In the NOTES group, the vaginal vault was examined for hemorrhage/hematoma, adhesions, or gross evidence of infection (purulent exudate, abscess formation). The surgical sites with ~2 cm of surrounding connective tissue and the incision sites were removed en bloc and fixed in 10% neutral buffered formalin in preparation for histologic evaluation. Tissue samples were embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin and microscopic evaluation was performed. Swabs of the surgical sites obtained at necropsy were submitted for microbial culture.

Statistical Analysis

Measured variables (physiologic and pain assessment variables) were compared to baseline values determined before surgery. Data were expressed as mean ± SD or median and interquartile range and $P < .05$ was considered significant. The main effects of treatment (2 levels), time (2, 3, 4, or 24 levels), and the interaction between treatment and time were evaluated using repeated measures ANOVA (Proc mixed, SAS 9.2, SAS Institute Inc., Cary, NC) and a compound symmetry covariance matrix. Non-normally distributed data were ranked before repeated measures ANOVA was performed to approximate a normal distribution

and homogeneity of variance. When indicated by a significant F-test for the interaction term, between treatment comparisons at each time interval, and within treatment comparisons to postoperative time = 0 minutes, were conducted using Bonferroni-corrected *P* values. Surgical time and amount of sedatives were compared between groups using an unpaired t-test. Proportional comparisons (complication rate, postmortem adhesions, abscess formation, and positive bacterial cultures) were made using a Fisher's exact test. Because the NRS of behavior was calculated as a sum of scores assigned for 9 observations and a valid statistical test for comparing combined scores is not available, statistical analysis was not performed and descriptive statistics are reported. A Spearman rank correlation test was used to determine whether the pain assessment variables of heart rate, NRS of behavior, and activity level were correlated. Sample size calculations performed before the study were based on the primary hypothesis using SAA concentration, heart rate, and mean surgical time as the primary outcome variables.

RESULTS

Mares were healthy and CBC, plasma fibrinogen concentration, and SAA concentration were similar between groups before surgery (Table 1). All surgical procedures were performed successfully and all mares survived until study completion.

Surgical Variables

Overall mean surgical times were 100 ± 40 minutes and 107 ± 47 minutes for transvaginal NOTES and laparoscopy, respectively ($P = .80$). There were no differences in the incidence of intraoperative and postoperative complications between groups. Intraoperative complications included difficult abdominal access and retroperitoneal insufflation (LAP, n = 1), suboptimal view of the surgical site (NOTES, n = 3), and mild hemorrhage of the ovarian pedicle during hemostasis/transection (NOTES, n = 3; LAP, n = 1). During hemostasis and transection of the right ovarian pedicle in 1 mare of the NOTES group, the tip of the vessel-sealing device was applied to the duodenum located immediately cranial to the ovarian pedicle. Energy was applied for ~1 second before it was recognized. The vessel-sealing device was immediately released and a small-blanched area was present on the serosal surface of the duodenum. Because the damage appeared to be confined to the serosa, no further action was taken and the procedure was completed. In the NOTES group, 1 ovary was dropped during removal through the vaginal opening. The ovary was identified immediately adjacent to the transvaginal portal and successfully retrieved. Ovaries were of similar size in both groups (NOTES, 6.2 ± 0.7 cm × 4.0 ± 0.5 cm × 4.5 ± 0.9 cm; LAP, 6.4 ± 1.2 cm × 4.1 ± 0.9 cm × 4.7 ± 1.3 cm). Mean length of the skin incision used to retrieve the ovaries in the laparoscopy group was 6.2 ± 1.8 cm.

Except for 1 mare in the laparoscopy group, all mares were bright, alert, and maintained a good appetite until euthanasia at 3 weeks or 3 months. In the laparoscopy group, 1 mare developed diarrhea in the immediate postoperative phase and signs of colic on day 3. The diarrhea resolved within 3 days and abdominal pain responded to administration of flunixin meglumine (1.1 mg/kg, IV) and butylscopolamine (0.2 mg/kg, IV). On day 7, this mare also developed incisional infection of all laparoscopic portal sites. The sutures were removed, the wounds flushed daily, and the mare was administered trimethoprim-sulfamethoxazole (30 mg/kg orally twice daily for 5 days). The surgery sites healed by second intention. *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli*, *Klebsiella oxytoca*, and *Myroides* sp. were cultured from the incision sites.

All NOTES mares had vulvar edema with clear vaginal discharge, which resolved within 24 hours after surgery. In 1 mare, however, serosanguineous vulvar discharge developed on day 12. Palpation of the vagina led to retrieval of a blood clot but no ongoing bleeding was evident. The vagina was cleansed with warm water and the serosanguineous discharge stopped. The mare with the duodenal injury did not show signs of abdominal pain and kept a good appetite

Table 1 White Blood Cell Count, Plasma Fibrinogen Concentration, Serum Amyloid A Concentration, and Abdominal Nucleated Cell Count and Total Protein Concentration in 12 Mares Immediately Before Surgery (Day 0) and After Bilateral Ovariectomy via NOTES (n = 6) or Laparoscopy (LAP, n = 6). Data are Presented as Median and Interquartile Range. (*) Significant Difference from Baseline Within Each Group. Serum Amyloid A Concentration and Abdominal Total Protein Concentration Increased Significantly in Response to Surgery. ND = Not Determined

	Group	Day 0 (Baseline)	Day 3	Day 10	Day 14
White blood cells ($10^3/\mu$L)	NOTES	8.5 (7.4–9.7)	6.0 (4.9–7.5)	10.2 (9.8–11.6)	ND
	LAP	7.1 (6.5–9.0)	5.7 (5.1–6.1)	10.2 (6.0–13.5)	ND
Fibrinogen (mg/dL)	NOTES	227 (210–245)	293 (283–301)	269 (240–275)	ND
	LAP	259 (251–271)	290 (255–361)	302 (261–403)	ND
Serum amyloid A (μg/mL)	NOTES	1.7 (0.3–19.1)	47.6 (30.9–48.5)*	2.6 (0.9–11.3)	ND
	LAP	5.7 (0.3–16.9)	48.6 (47.8–48.7)*	2.3 (1.4–7.2)	ND
Abdominal nucleated cell count (/μL)	NOTES	1400 (1390–2600)	10,150 (7800–17,100)	ND	31,950 (15,400–38,300)*
	LAP	2000 (963–2300)	23,050 (19,500–44,400)*		9030 (1570–86,500)*
Abdominal total protein (g/dL)	NOTES	1.1 (1.0–1.2)	3.1 (2.5–3.5)*	ND	2.8 (2.7–3.0)*
	LAP	1.0 (1.0–1.4)	2.7 (2.1–3.2)*		1.7 (1.0–2.5)

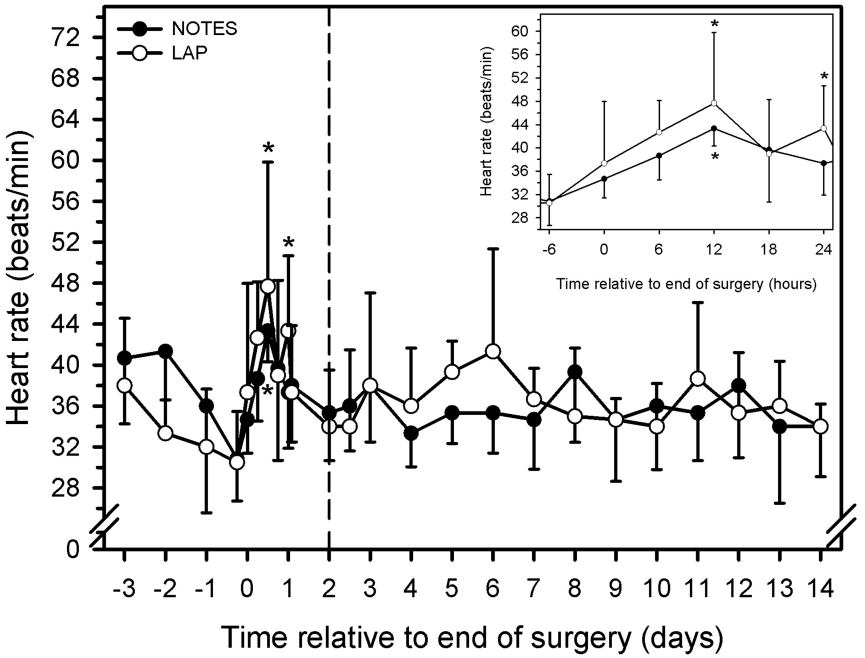

Figure 1 Mean ± SD heart rate in mares undergoing bilateral ovariectomy with NOTES or laparoscopy (6/group). Baseline evaluation was made 6 hours before surgery. Flunixin meglumine discontinued on day 2 (round dash line). The perioperative period (from t–6 to 24 hours postoperatively) is shown as an inset in the upper right corner of the graph. No significant difference between groups was found at any time point. (*) Significant difference from baseline (t–6) within groups. Return to baseline heart rate values after surgery was slower in the laparoscopy group (36 hours) than in the NOTES group (18 hours).

throughout the study. Other postoperative complications included transient fever (NOTES, n = 1; LAP, n = 3), incisional seroma (LAP, n = 3), persistent incisional swelling (LAP, n = 1), and subcutaneous emphysema (LAP, n = 6).

Physiological Variables

Body weight was similar between groups and remained stable throughout the study. There was no significant main effect of group or interaction effect between group and time in temperature, heart rate or respiratory rate at any time point of the study.

As expected, both groups had a significant elevation in heart rate after surgery (Fig 1). Heart rate returned to baseline values within 18 hours after surgery in the NOTES group and within 36 hours in the laparoscopy group. White blood cell count, plasma fibrinogen concentration, SAA concentration and abdominal nucleated cell count, and total protein concentrations over time are reported in Table 1. No significant differences in vital parameters, CBC, plasma fibrinogen concentration ($P = .10$), SAA ($P = .49$), peritoneal nucleated cell count ($P = .39$), and total protein concentration ($P = .59$) were found between groups across time. SAA concentration and abdominal total protein concentration increased significantly in response to surgery. Cytologic evaluation of the peritoneal fluid at days 3 and 14 revealed evidence of postoperative inflammatory response in all cases; however, no evidence of toxic or degenerative changes in neutrophils or intra/extracellular bacteria were observed. In the long-term group, peritoneal fluid cytology at 3 months revealed a transudate in all horses with normal differential counts.

Pain Assessment

There was no difference in the intraoperative respiratory and heart rates between groups. There was no significant difference in the total amount of detomidine hydrochloride ($P = .85$) or butorphanol tartrate ($P = .96$) administered during the procedure.

All mares appeared comfortable after surgery; however, there was a significant decrease in measured activity levels from 2–6 days postoperatively compared with baseline levels in both groups (Fig 2). Mean NRS of behavior, including posture and socialization scores, was numerically similar between groups preoperatively (NOTES, 11.5 ± 1.8; LAP, 10.8 ± 1.2) and at all time points of the study. Mean NRS of behavior reached a maximal value immediately after surgery in both groups (t0: NOTES, 15.8 ± 2.8; LAP, 12.0 ± 1.6), whereas the scores returned to baseline values within 18 hours after surgery in both groups (t18: NOTES, 11.5 ± 3.4; LAP, 10.4 ± 1.7). There was a weak but significant correlation between the NRS of behavior and activity levels (Spearman's rho = 0.22; $P = .005$), the NRS of behavior and heart rate (Spearman's rho = 0.24; $P = .001$),

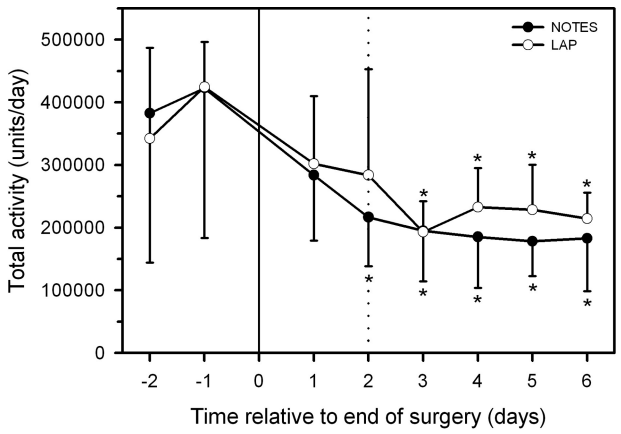

Figure 2 Mean ± SD activity levels in mares undergoing bilateral ovariectomy with NOTES or laparoscopy (6 per group). Day 0 is surgery day. Flunixin meglumine discontinued on day 2 (round dash line). No significant differences between groups were found at any time point. (*) Significant difference from baseline (day -1).

and the activity levels and heart rate (Spearman's rho = 0.23; $P = .003$).

Morphologic Findings

At 3 weeks postoperatively, there was no evidence of infection or adhesions in the abdominal cavity. Fibrosis of the small colon mesentery was found in 1 laparoscopy and 1 NOTES mare. A line of whitened tissue was present at all sites of transection of the ovarian pedicles. An incision line abscess was found in 2 mares from the laparoscopy group. One of these mares had secondary healing of all portal sites after suture dehiscence, whereas 1 mare had incisional swelling present for the duration of the study without wound drainage or suture dehiscence. A hemorrhagic lesion was found within the vaginal wall of the mare that had serosanguineous vaginal discharge at day 12, but no adhesion was present within the vaginal vault. The transvaginal portal site was completely closed and healed well in all 3 mares, without evidence of vaginal adhesions. The duodenum that was accidentally grasped with the vessel-sealing device was intact with a small area of serosal fibrosis at the injured site.

At 3 months postoperatively, there was no evidence of infection or adhesions within the abdominal cavity in any of the mares. All ovarian pedicle transection sites had fully healed. In 3 laparoscopy mares, the incision sites appeared completely healed. The transvaginal portal site was difficult to identify in all 3 NOTES mares and had healed completely.

Histologic evaluation of the ovarian pedicle transection sites revealed satisfactory healing of all surgery sites at 3 weeks and 3 months. Histological evaluation of the transvaginal portal sites revealed the presence of scar tissue consistent with complete tissue healing in all NOTES mares.

Microbiology Findings

There was no growth from pre- and postoperative swabs submitted from either group. A single isolate grew from peritoneal fluid obtained on day 14 in 1 (16%) NOTES mare. Of samples submitted from the ovarian pedicle transection sites at necropsy, there was 1 (8%) positive culture in the laparoscopy group and 3 (25%) in the NOTES group. Details regarding microbial findings and corresponding peritoneal nucleated cell counts, total protein concentrations, and cytologic findings are reported in Table 2.

DISCUSSION

Our aim was to determine whether transvaginal NOTES ovariectomy in the mare is associated with less-surgical trauma compared to the current gold standard minimally invasive technique, laparoscopy. Our data showed no difference between the 2 techniques in terms of pain and inflammation resulting from surgery, as measured by physiologic variables (heart rate, respiratory rate, rectal temperature), plasma or serum concentration of positive acute phase proteins (fibrinogen, SAA), peritoneal total nucleated cell count and total protein concentration, or pain assessment. Laparoscopy and transvaginal NOTES resulted in minimal inflammation and both techniques were performed successfully. Surgical time and complication rates associated with laparoscopy and transvaginal NOTES were similar. Thus, each method was found to provide a satisfactory minimally invasive technique for bilateral ovariectomy in standing mares.

Documentation of the response to surgical trauma resulting from these minimally invasive techniques was performed in an effort to quantify their invasiveness. SAA was used as the primary marker of tissue trauma because it is the only major acute phase protein with low concentrations in healthy horses that demonstrates a 10- to 1000-fold increase in acute phase states.[27] Concentrations of fibrinogen or SAA correlate well with the intensity of surgical trauma in horses.[21] As expected, SAA concentrations increased above the preoperative values at day 3 in response to the surgical trauma, and returned to baseline levels at day 10; however, there was no difference between groups at any time point of the study. Our results corroborate previous studies that demonstrated that SAA concentrations increase rapidly during the acute phase of inflammation and decrease rapidly after recovery because of its short half-life.[21,23] Three days after uncomplicated, standing castration, mean SAA concentration increased several 100-fold[23] whereas we demonstrated a relatively smaller increase after either transvaginal NOTES or laparoscopy. Our findings suggest that transvaginal NOTES and laparoscopy are both minimally invasive techniques that result in minimal tissue damage and a mild inflammatory response.

Longer surgical times and a steep learning curve are to be expected when introducing a new surgical technique.

Table 2 Description of Microbiological and Corresponding Cytological Findings of Peritoneal Fluid in Mares with Positive Culture at 14 Days, 21 Days, and/or 3 Months (92 Days) Postoperatively

Mare	Group	Day	Source	Bacteria	Peritoneal Nucleated Cell Count (/μL)	Peritoneal Total Protein (g/dL)	Cytological Findings
1	LAP	92	Right mesovarium	*Micrococcus* sp. *Pseudomonas vesicularis*	928	1.0	Normal peritoneal fluid
2	NOTES	92	Right mesovarium	*Bacillus Pumilus Corynebacterium* sp.	1000	1.0	Normal peritoneal fluid
4	NOTES	14	Peritoneal fluid	*Bacillus* sp.	45,600	3.2	Moderate neutrophilic inflammation, 84% nondegenerate neutrophils, no evidence of infectious agents
		92	Left mesovarium	*Staphylococcus epidermidis*	683	1.0	Normal peritoneal fluid
8	NOTES	21	Right mesovarium	*Gram negative rod*	38,300	3.0	Exudate with rare evidence of acute hemorrhage, 85% nondegenerate neutrophils, no evidence of infectious agents.

In contrast to our pilot study,[14] where operative times were extended during technique and instrument development, the surgical times for transvaginal NOTES ovariectomy were considered clinically acceptable. Accordingly, surgical times were similar for performing bilateral ovariectomy using either transvaginal NOTES or laparoscopy. This is in contrast to most published controlled studies comparing NOTES to laparoscopy in which longer operative times are consistently reported for NOTES procedures.[4,13] We found that mesovarial infiltration of local anesthetic was faster and more readily achieved laparoscopically using the laparoscopic injection needle when compared with using the flexible endoscopic injection needle passed through the working channel of the endoscope during the transvaginal NOTES procedures. The ovaries were more easily removed through the vaginal opening than through the body wall, especially in larger mares. Transvaginal NOTES may therefore be advantageous in mares with a thick body wall or narrow paralumbar fossa when ovary removal by laparoscopy may be challenging. However, laparoscopy would be preferred in cases of enlarged ovaries or other pathology of the reproductive tract when access or surgical field of view could be impaired with the transvaginal NOTES approach.

Intra-abdominal observation was considered excellent in all laparoscopic mares whereas in 3/6 NOTES procedures, viewing of the caudal abdomen was hindered by the presence of small intestine loops. The field of view with the endoscope was narrower than with the laparoscope and loops of bowel occasionally obscured the endoscopic view. Viewing of the ovarian structures was more consistent than in our preliminary study,[14] presumably because of a longer preoperative fasting regimen. Controlled pneumoperitoneum during transvaginal NOTES procedures could improve the intra-abdominal endoscopic view further. Most human studies describe the use of a hybrid NOTES technique using a laparoscopic portal at the umbilicus to create and maintain CO_2 pneumoperitoneum and thus facilitate the endoscopic procedure.[6,28] Recently, methods for monitoring,[29] regulating, and insufflating the abdomen through a flexible endoscope with either air or CO_2 were developed and assessed.[12] A well-sealed portal site is necessary for successful abdominal insufflation, whether it is performed with laparoscopic or NOTES techniques. During the transvaginal NOTES procedures, the flexible endoscope and 2 long-handled rigid instruments were introduced through the single 33-mm transvaginal portal resulting in an inability to create a sealed portal. A multi-instrument portal for transvaginal access similar to those used in single incision laparoscopic surgery could maintain a seal and permit abdominal insufflation for NOTES techniques.

Clinically, all but 1 mare recovered well after surgery. Heart rate and respiratory rates returned faster to baseline values in the NOTES group (18 hours postoperatively) than in the laparoscopy group (36 hours postoperatively). These results may indicate that transvaginal NOTES produced less pain postoperatively; however, pain assessment revealed that NRS of behavior scores returned to baseline values within 18 hours after surgery in both groups. Activity monitoring was used postoperatively to complement physiologic and behavioral pain assessments. We found that there was a weak but significant correlation between pain scores, activity levels, and heart rate. Accelerometers have been validated in people[30] and dogs,[26] and to our knowledge our study is the first report of using accelerometers in horses to quantify activity pre- and postoperatively. Although we did not validate the accelerometers by correlating our measurements to continuous video recording, each horse was used as its own control during the acclimation phase.

Activity levels followed the same pattern in both groups with an overall activity decrease in the immediate postoperative period with stabilization to levels lower than baseline from days 2 to 6. Lack of activity after surgery has been previously associated with pain in horses[20]; however, that study only assessed activity for the first 24 hours postoperatively. In the present study, the activity pattern observed postoperatively may reflect adaptation of the mares to the housing environment and the daily husbandry routine after surgery.

Alternatively, since activity levels were only significantly decreased after flunixin meglumine was discontinued, the reduction in activity could be as a result of pain. Interestingly, if this were the case, activity monitoring would be a sensitive indicator of postoperative pain in horses since pain assessments were unremarkable from days 2 to 6 and physiologic variables had all returned to baseline preoperative values. Continuous activity monitoring for a longer period (such as 21 days) may have helped elucidate whether decreased activity was pain or housing/husbandry related. Activity monitoring as an assessment of postoperative pain may warrant more detailed investigation.

We found a high incidence of incisional infection (33%) in the laparoscopy group. Our results might have been affected by the small sample size as they are in contrast to previous studies in which infection after laparoscopic ovariectomy occurred in only 6/129 mares (4%).[2,22,31–39] There was no evidence of incisional infection in the NOTES group during necropsy examination at 3 weeks or 3 months. Our results are consistent with a recent, large human retrospective study, in which incisional infection after transvaginal NOTES cholecystectomy was reported in 0.8% of patients.[40] Other complications reported included vaginal bleeding, urinary tract and vaginal infection, and rectovaginal abscessation, with a combined incidence of 1%. Except for the mare with postoperative serosanguinous discharge, vaginal examination was not performed during the course of the study because results from our preliminary study did not show evidence of infection of the transvaginal portal site in any mare at 7 or 14 days postoperatively.[14]

The elevation of peritoneal nucleated cell count and total protein concentration after surgery were similar between groups and likely reflected the mild inflammatory response associated with laparoscopic or transvaginal NOTES ovariectomy. Previous laparoscopic studies reported similar results and showed elevation of peritoneal nucleated cell counts 24 hours after CO_2 pneumoperitoneum.[41,42] The time required for the peritoneal nucleated cell count to return to baseline values after abdominal surgery is unknown and therefore, further studies are warranted to follow peritoneal fluid values over time after laparotomy, laparoscopy, or NOTES techniques.

Necropsy and histologic examination revealed no evidence of infection or adhesion with satisfactory healing of all surgery sites. The risk for intra-abdominal contamination and infection in transluminal procedures has been one of the major obstacles to the widespread clinical use of NOTES in people.[19] Intraoperative contamination was not detected through pre- and postsurgical incisional cultures in either group, and thus we found that vaginal preparation with 4% chlorhexidine gluconate solution was effective for transvaginal access. Microbiologic evaluation of the peritoneal fluid at 14 days, or the mesovaria at necropsy, was positive in 4 mares. Two mares from the NOTES group had a positive microbiologic culture within 21 days postoperatively, along with elevated peritoneal nucleated cell count (38,300–45,600 μL). Postoperative septic peritonitis has been previously defined as a combination of clinical signs (depression, fever, endotoxemic shock, and abdominal pain) and abnormal peritoneal fluid (peritoneal nucleated cell count $>100{,}000/\mu$L with cytologic evidence of free or phagocytosed bacteria).[43] Neither of these mares had clinical signs consistent with septic peritonitis and postoperative peritoneal nucleated cell counts were $<100{,}000/\mu$L, even in light of the positive bacterial cultures. There was no evidence of sepsis present on cytology and the peritoneal fluid in these 2 mares was consistent with a postoperative inflammatory response, as expected after abdominal surgery. In addition, the cytologic findings and peritoneal differential counts were similar to those obtained from mares without positive cultures at the corresponding time points.

At 3 months, 4 mares (NOTES, n = 3; LAP, n = 1) had positive microbial cultures upon sampling of the mesovaria at necropsy, while normal cytological findings and differential counts of the peritoneal fluid were present. Microbiologic identification and quantification of all plates with positive growth showed either 1 or 2 colonies in each instance with organisms interpreted as contaminants by the microbiologist in each case. This was consistent with cytological findings of the peritoneal fluid and the concern of contamination during transvaginal NOTES could not be substantiated in this study. It is possible that mares with positive microbiological cultures had recovered from a low-grade peritoneal infection, although there is a lack of clinical, histological, or cytological evidence to support this speculation. Further studies may be warranted to further characterize the potential for contamination during/after transvaginal NOTES in mares. Sterile preparation of the vagina and use of systemic perioperative antibiotics is recommended for transvaginal NOTES in mares based on our current results.

The remaining open question is whether or not transvaginal NOTES should be used instead of laparoscopy for ovariectomy in mares or in which cases one technique should be preferred over the other. Both techniques have disadvantages in the cost of the specialized equipment, the learning curve associated with the procedures and the need for a hospital setting. Furthermore, customized instruments are currently required for the transvaginal NOTES technique because of the insufficient shaft length of conventional laparoscopic instruments.[14] Although in the present study exclusion criteria were applied to history of previous surgery, we believe that transvaginal NOTES may offer an advantage in cases where adhesions are suspected along the parietal peritoneum. Because there is no need for skin or body wall incisions, transvaginal NOTES eliminates risks for body wall incisional complications (seroma, infection, dehiscence, or subcutaneous emphysema) and thus mares may be able to resume exercise within a few days after transvaginal NOTES ovariectomy.

One limitation of the present study may be considered the small number of mares used for comparison of the techniques and their outcome. Detecting no difference between groups for the outcomes evaluated warrants examination of the statistical power of these findings. With a sample size of 6 mares/group, power calculation showed that we had 80%

power to detect a difference of 13 μg/mL in SAA concentrations at 3 days. When designing the study, we anticipated that a SAA difference of 100 μg/mL between groups would be of clinical significance, based upon a previous study in horses when SAA concentrations reached ~500 μg/mL.[21] Based on our SAA data, a total of 42 horses/group would have been necessary to detect the small difference found, with a power of 80% and 95% confidence level (ie, P-value < .05). However, it is very unlikely that such small differences are of clinical significance. Similarly for our other primary outcome variables of heart rate and surgical time, we believe the findings of no difference between groups are clinically applicable.

Summarily, we found that transvaginal NOTES could be performed safely as an alternative to conventional laparoscopy for standing bilateral ovariectomy in healthy mares. These 2 techniques induced similar levels of surgical trauma and inflammation and both were shown to be acceptable in terms of clinical outcome. Like many new surgical methods, transvaginal NOTES requires specific instrumentation and experience and thus appropriate training is necessary before routine clinical use of the technique. Further evaluation in clinical cases is warranted.

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The effect of spaying on the racing performance of female greyhounds

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ABSTRACT

The effect of spaying (ovariohysterectomy) on racing greyhound performance was analysed. Using data from a longitudinal observational study design, random effects models were used to compare the racing performance before and after spaying. Greyhounds were matched by distance and date of racing, and then both spayed and entire bitches were compared for each stadium which had spayed greyhounds on the racing strength. The racing performance of entire females was selected from the anoestral period (from 90 days post-oestrus to the next oestrus).

Spaying had no real effect on performance, with a mean difference of +0.066 seconds (90% CI: −0.004, +0.135 s) over 480 metres. The effect of time since spaying had no effect (90% CI: −0.0002 to +0.0001 s/day). Spaying of racing greyhound bitches produced no overall change in performance, and had no apparent long term effect on performance. These results are discussed within the context of the current information on the advantages and disadvantages of spaying at a young age.

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Introduction

The Greyhound Board of Great Britain (GBGB) wishes to determine the effect of spaying on the performance of female racing greyhounds in order to be able to advise trainers about an alternative to the use of oestrus suppressants, such as norethisterone. Recent analyses by the author (Payne, 2013) have shown that female greyhounds lose performance in the dioestral period. Thus there are performance penalties in allowing bitches to cycle normally. The aim of the present study was to determine whether spaying affects performance and to advise on the suitability of spaying (ovariohysterectomy or ovariectomy) as an alternative to the medical suppression of oestrus and dioestrus. Spaying would also help to reduce the rehoming costs on retirement, and eliminate the on-going veterinary costs associated with repeat examinations, and the prescription and supply of oestrus suppressants.

There are health benefits to spaying. In particular there is a reduced incidence of mammary neoplasia, which is time-dependent and greatest when the spaying is performed before the first season (odds ratio [OR] = 0.05), decreasing slightly if spayed before the second season (OR = 0.08), and decreasing further if spayed after the second season but before 2.5 years old (OR = 0.26); spaying after 2.5 years old has no protective effect again mammary neoplasms (Romagnoli, 2008). Given that 94.3% of actively racing female greyhounds are <5 years old, and 40.3% are <2.5 years old, there is a high proportion (54%) of racing bitches which are >2.5 years, yet are not spayed until retirement, and so are not protected against mammary neoplasia (unpublished data). This is sufficient justification to investigate whether spaying during a bitch's racing career has any effect on performance whilst still gaining an important health benefit.

Recent investigations (Payne, 2013) have shown that the dioestral performance of female greyhounds changes within the same time frame as the changes in serum progesterone concentrations. On the basis of this evidence it was suggested that serum progesterone had a significant effect on performance as a direct consequence of its biochemical effects. Thus it would seem logical that spaying would remove these effects and produce a female greyhound which has a stable performance pattern throughout the year, and thus does not require any lay-off time post-oestrus under the GBGB Rules of Racing.[1]

In order to further investigate the effect of spaying on the performance of racing greyhounds, a comparison of the performances of entire versus spayed racing bitches was conducted to determine both the immediate and long-term effects of spaying.

Materials and methods

Study design

A longitudinal observational study was used. The entry point for each greyhound was the start of its racing career provided that it had never had its oestrous cycles suppressed. Only greyhounds attached to tracks were included in the study in order to produce a nested data set. Open race greyhounds can race at any track, and thus prevent nesting of the data (i.e. open race form creates a multi-membership model).

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[1] GBGB Rules of Racing 1 January 2013; see: http://www.gbgb.org.uk/files/GBGB%20Rules%20of%20Racing%20January%202013.pdf.

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Participants

Greyhounds were selected into three groups: (1) spayed, then began racing, (2) spayed during their racing career, and a control group (3) of entire bitches that ran over the same distances and dates as groups (1) and (2). Previous work by the author (Payne, 2013) showed that performance is variable between oestrus and 80–90 days post-oestrus, after which the performance does not change with time. In order to compare with the performance prior to spaying, only the race performance for the entire bitches in the period more than 90 days post-oestrus to the next oestrus was used. Greyhounds left the study on the last date of their race form.

Data sources and management

A list of greyhounds reported to have been spayed between May 2004 and March 2012 was generated by the GBGB/PA Sport, along with their race form, together with a corresponding dataset representing entire bitches that had raced at the same stadia. Six stadia were included in the study, and only races of 480 m were used in the analysis. Since spaying is not a common procedure for greyhounds of racing age, the datasets for both the spayed and entire groups were matched for race date and stadium. This produced a dataset covering the six stadia comprised 347 spayed and 136 entire females ($n = 483$), together with 11,592 lines of race form. The dataset comprised the fields as shown in Table 1.

The data were held in a relational database management system (SQL Server Express 2012, Microsoft[2]) with a separate front-end in Access 2010 (Microsoft Office 2010[3]) which contained the structured query language (SQL) queries. Outputs from the queries were exported into Excel 2010 (Microsoft Office 2010[4]) for further calculations and thence for subsequent export into the statistical packages.

Variables and data calculations

The dependent variable was unadjusted race time (IndTime, in seconds to two decimal places) and was used as a relatively unbiased measure of performance. The effect of differing running conditions could not be reliably assessed, and was accepted as an unexplainable error. The outcome variable was the greyhound's actual race time for each race or trial (IndTime, seconds), as this provided the best unbiased estimate of performance. Adjusted times were not used as these are modified by a human judgement of the going allowance, and thus were considered to be biased.

The time since spaying was simply calculated as the interval between the date of spaying and date of the race, reported as an integer. This value was named days since spaying (DSS) and could be positive where a race was after spaying or negative where a race was before spaying. Thus day zero was the reported day of spaying.

When comparing performance between pre- and post-spay, the fixed binary variable spayed was used in the models. The entire or spayed state was thus indicated by the values of 0 for entire and 1 for spayed.

Race distance was described by the stadium categorical variable and represented a random effect. Age was not included, since there would be a high correlation between age and DSS.

DSS and spayed variables were calculated by SQL code, whilst repeat measure codes were produced in Excel 2010 by sorting the race form by race date for each greyhound and then generating an ordinal measure. Data checking and removal of illogical values were also performed in Excel 2010.

Study size and power analysis

Alpha and beta were calculated to be 10% and 6%, respectively, to balance and minimize the summed probability of committing Type I and Type II errors (Verrill and Durst, 2005; Nakagawa and Cuthill, 2007; Mudge et al., 2012). The effective sample size in the multilevel models was calculated to be $n = 164$, and could detect an effect size equivalent to 0.08 s for a race of approximately 29.8 s over 480 m, whilst maintaining alpha at 10% and beta at 6%. Results are expressed as the mean together with the 90% confidence interval (CI).

Statistical methods

The initial output from the SQL queries was exported into Excel 2010 for further calculations and analysed in Analyse-It v.3.15 (Analyse-it[5]) and StatsDirect v.2.7.9 (Stats Direct[6]) in order to generate the descriptive statistics. The data were then imported into MLwiN v.2.27 (University of Bristol[7]) in order to generate the multilevel models. The models were built by manual forwards addition, and random effects modelled by greyhound ID. The fixed effect of stadium ID provided a measure of the effect of the differences in track design despite the same race distances of 480 m. Thus the two levels were greyhound ID and repeat measures for the races. Two models were used to independently describe the effects of DSS and spaying. Once the models had been built in MLwiN and run as iterative generalized least squares (IGLS) models, the parameters were then used to generate Bayesian models using a Markov Chain Monte Carlo (MCMC) method via Gibbs sampling. Model checking was performed in MLwiN.

Table 1
Data fields extracted from the GBGB/PA Sport race form and signalment information.

Fields	Signalment table	Raceform table
Primary keys*	Greyhound ID*	Greyhound ID* Race date*
Data	Date of birth Date of spaying	Stadium ID Race distance Race time

Results

Descriptive statistics

Analysis of IndTime showed it to be normally spread, and a split of the race times by entire versus spayed showed similar times (Fig. 1), with a random scatter of race time against DSS (Fig. 2).

Multi-level models

The two multilevel models (MLM) described the effect of the time before and after spaying and the effect of spaying. The effect of time (DSS) on performance was negligible (90% CI: −0.0002, +0.0001 s/day). The analysis assumed a linear regression, which was reasonable considering previous findings (Payne, 2013) and by inspection of the scatter plot seen in Fig. 2.

The effect of spaying was marginal at +0.066 s. The 90% CI was −0.004 to +0.135 s, which includes zero, i.e. the null hypothesis of no effect could not be rejected. In more general terms, the results indicate that there was no evidence in this dataset for an effect of spaying. Additionally the residual error in the analyses was ±0.394 s, indicating that other unmodelled factors have a greater effect on race time. Model checking showed a normal distribution of the respective standardized residuals against DSS in the time model, and by group in the spayed model, together with a linear plot of standardized residuals against normal scores.

Discussion

The models show that spaying is unlikely detract from prior performance, and that post-spay the performance appears to be stable. Additionally, there were no differences over time between entire and spayed greyhounds. Both these results indicate that there are no significant short or long-term effects of spaying on performance ($P < 0.10$).

The comparison was made between race performances over 90 days since oestrus and after spaying. The finding that there was no difference adds support to previous findings that progesterone is likely to be involved in the depression of performance post-oestrus (Payne, 2013), since in both the over-90 day post-oestrus periods and the post-spay periods used in the current analysis, the progesterone levels were essentially zero due to either inactivity in, or absence of, the ovaries (Cox, 1970; Wheeler, 2011).

A specific analysis of the effect of spaying on athletic performance has not been undertaken before, so comparisons with previous studies are not possible. Likewise, this paper does not address any possible effects of reproductive hormones (or lack of) on musculoskeletal strength, in particular bone strength.

[2] Microsoft SQL Server, http://www.microsoft.com/en-us/sqlserver/editions/2012-editions/express.aspx.
[3] Microsoft Office Access 2010, http://office.microsoft.com/en-gb/access/.
[4] Microsoft Office Excel 2010, http://office.microsoft.com/en-gb/excel/.
[5] Analyse-it for Microsoft Excel, Analyse-it, http://analyse-it.com/.
[6] Stats Direct Statistical Software, www.statsdirect.com.
[7] Centre for Multilevel Modelling, University of Bristol, http://www.bristol.ac.uk/cmm/.

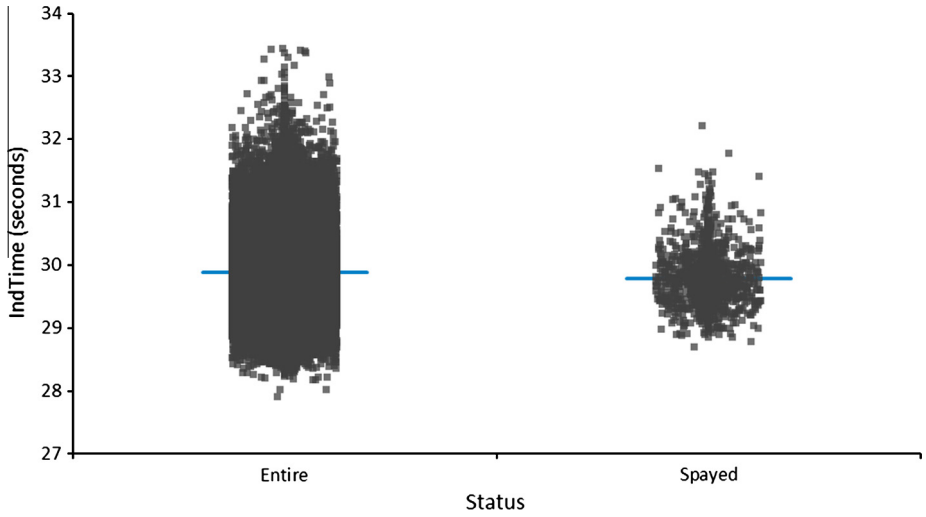

Fig. 1. Descriptive statistics for the entire dataset, split by entire versus spayed race times (IndTime). Mean race time for entire bitches = 29.880 s (SD = 0.596); mean race time for spayed bitches = 29.793 s (SD = 0.442). *N* = 11,592 races, 483 bitches. Distance = 480 m.

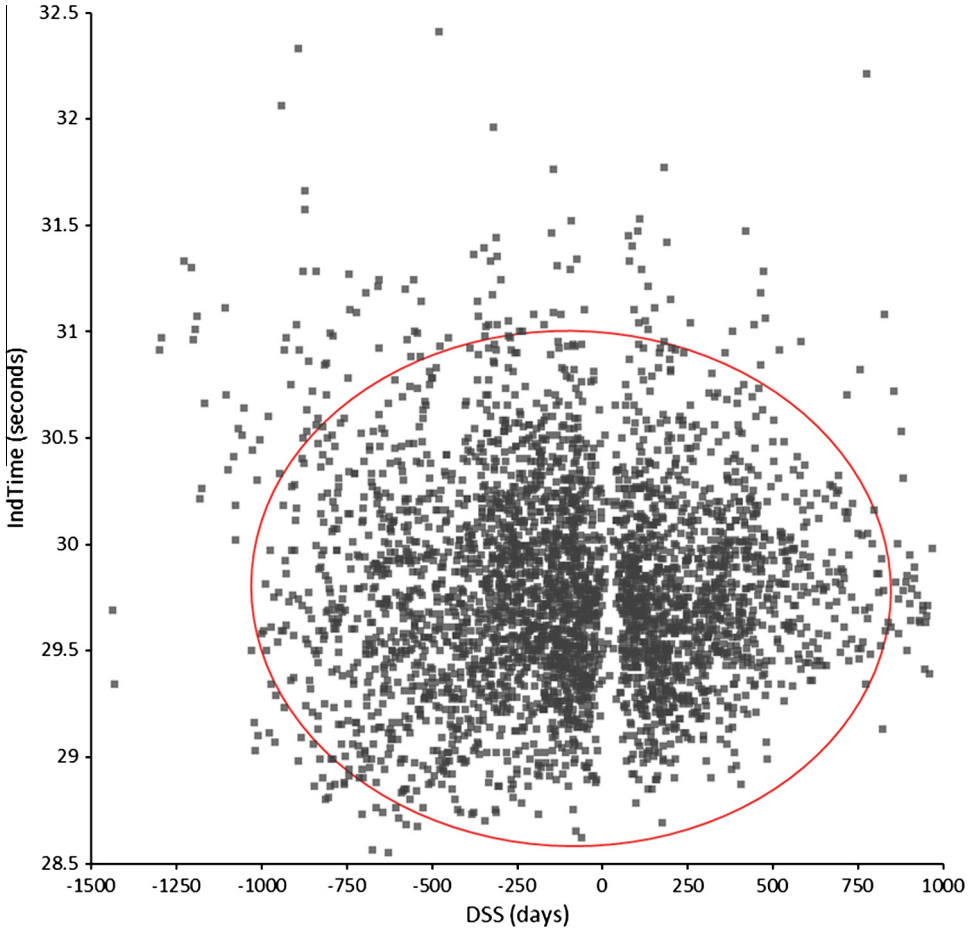

Fig. 2. Scatter plot of race time (IndTime, s) against days since spaying (DSS, days). Distance = 480 m. A negative DSS is the period before being spayed.

Against the benefits of spaying, the reported disadvantage of urinary incontinence has been found to be variable (de Bleser et al., 2011) and more likely to be related to tail docking and obesity (a possible consequence of spaying) than to the hormonal effects of ovariohysterectomy (Gregory, 1994), although Romagnoli (2008) does suggest spaying under 3 months old can increase the risks of incontinence. Neither tail docking nor obesity are likely to occur in racing greyhounds.

Conclusions

Spaying does not materially affect the individual racing performance of greyhound bitches, either as a shift in performance or as a gradual loss of performance ($P < 0.10$). Spaying during their racing careers can be considered for bitches that are unlikely to be bred from, and can secure recognized health benefits without a performance penalty as well as providing an alternative to the use of oestrus suppressant/postponement medications.

Conflict of interest statement

The author of this paper has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

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of part of the normal bacterial flora leading to an acute oesophagitis and death in some from inspiration of regurgitated food and liquid.

No incidents of this type have been encountered among other broiler breeders within the same company. Managemental changes have been subsequently introduced as part of a husbandry development programme that are designed to give more even distribution of food and greater uniformity of birds within pens during rearing, which should help to avoid such incidents in the future.

C. J. RANDALL
Ministry of Agriculture
Fisheries and Food,
Veterinary Laboratory,
Eskgrove,
Lasswade,
Midlothian
C. P. J. MILLS
D. B. Marshall (Newbridge)
Ltd,
Newbridge,
Midlothian

Meat hygiene

From Mr F. Jagger

SIR,—Henry Carter's characteristic but not unjustified comments (VR, February 16, p158) prompt me to join in this correspondence.

As one, until lately, closely associated with meat hygiene politics I share his disappointment but not his despondency. I believe the BVA proposals as a step towards centralisation had much to recommend them and the Ministry's argument that "hybrid" control would be unworkable, unconvincing. The proposed dual accountability of the official veterinary surgeon to both the local authority and Ministry of Agriculture would seem to be but another example of "hybrid" control.

Nevertheless many of us have felt that veterinary and environmental health officers' interests must be harmonised having regard to the contribution each can most advantageously make in the realm of food hygiene. The proposed regulations in my view bring this position nearer.

If one applies the yardstick of "what is in it for us?" to the proposals, one learns:

(1) That the EHO shall be allowed to certify meat products for export in plants divorced from abattoirs, the meat used however bearing veterinary certification from the export plant of origin, and in this capacity he shall be accountable to the Ministry of Agriculture. (2) That the meat inspector status will not change. (3) That an OVS shall be in hygienic control of all export approved abattoirs where existing dual standards shall cease—not an insignificant step forward when viewed in the context of the continuing trend towards concentration of slaughter. (Two per cent of abattoirs are responsible for some 80 per cent of all meat produced in this country —a fair proportion of these abattoirs having export approval.)

What of the future? In the short term a considerable responsibility rests on those of us engaged in meat hygiene to demonstrate that the veterinarian has something positive and purposeful to contribute to the business of meat production and in the longer term the schools must persevere in their efforts to create a force dedicated to establishing the profession in its rightful place in the wide field of food hygiene.

F. JAGGER
Murivance,
Shrewsbury

Libidinous behaviour in a gelding

From Mr O. D. Pearce

SIR,—The letter from S. Borsberry (VR, January 26, p89) referring to libidinous behaviour in a pony gelding reminded me of a similar case brought to my attention last year.

The owners of a trekking centre bought in extra ponies for the summer and ran them together with their own horses, mares and geldings mixed.

They stated that they would often see the geldings covering the mares and had decided to keep a record of the offending ponies. During the course of the summer, seven out of 12 geldings had been seen to cover mares.

It would appear that the nearer the pony was to the native breed, the more active it was.

I had personally gelded one of the newly bought in ponies at least six years previously. It had never shown such behaviour until last summer, even though it had run with mares after it was gelded.

I wonder whether such change in behaviour can be explained by the pony feeling threatened by the changes in environment and pony population. The native pony would presumably have stronger herding instincts in this respect.

OWEN D. PEARCE
Penmellyn,
St Columb,
Cornwall

Penicillin: Suspected adverse reaction

From Mr A. B. Marshall

SIR,—The recent paper by Nurmio and Schulman (VR, February 2, p97) is a welcome addition to the few papers giving detailed observations of possible drug reactions, but their conclusion that "the pathogenesis of these reactions is most likely to be the hypersensitivity to penicillin" is not justified.

A literature search covering the period 1950-1978 reveals only about a dozen papers reporting possible penicillin allergy in the common domestic species. Some of these are mainly review papers and thus contribute little in the way of new observations.

Strikingly, only one paper makes any attempt to prove the involvement of the penicillin component. Leemann and others (1969) report anaphylactic shock or generalised urticaria in cows after penicillin injections, the incidence increasing markedly after a foot-and-mouth disease vaccination programme. Penicillin was used in the preparation of some FMD vaccines and Leemann and others initially postulated that this was the sensitising agent. However they could not detect anti-penicillin antibodies in the cattle sera. In skin tests of cattle that had experienced anaphylactic reactions they obtained negative results to fresh solutions of bulk benzyl penicillin and to penicillin degradation products but the commercial preparation elicited strong urticarial reactions. Thus the reactions were due to some component other than the penicillin. Further work demonstrated that carboxymethyl cellulose, a stabilising component in some penicillin suspensions, was the real cause of the allergy in these cows.

The majority of suspected penicillin allergies involve the procaine salt, see for example Anon (1978) (quoting FDA); Ndiritu and Enos (1977) and several other anecdotal reports. The procaine moeity has been established as causing allergy in pigs (Clarke and Clarke 1975), and Goodman and Gilman (1975) in discussing procaine benzyl penicillin preparations make the following statement, "The possibility of allergy to procaine must be considered when hypersensitivity to such preparations occurs." Direct procaine toxicity should also be considered.

The paper by Brisbane (1963 — quoted by Nurmio and Schulman) is based on a retrospective survey of 22 veterinarians and indicated that 1083 animals showed anaphylaxis or urticaria associated with pencillin or penicillin / streptomycin administration. However there is no data to show that the penicillin component caused the reaction and the particular penicillin salts involved are not stated. Ndiritu and Enos (1977) attribute adverse reactions in a foal and a cat given potassium penicillin to the potassium ion. The foal was subsequently treated uneventfully with procaine penicillin.

Three aspects of the paper by Nurmio and Schulman indicate that the reactions were not due to penicillin hypersensitivity. First, they saw no problems in the ampicillin treated group, although there is generally considered to be cross sensitivity between the members of the penicillin family (Dewdney 1977). Secondly, the very high incidence (17 of 17, 3 of 3 and 4 of 4) of adverse reactions to the particular preparation rules out allergy. Thirdly, procaine benzyl penicillin

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Original Article

How to perform ovariectomy through a colpotomy

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Keywords: horse; ovariectomy; colpotomy

Summary

Bilateral ovariectomy of mares is performed most commonly to eliminate or diminish unwanted behaviour, create a teaser mare, sterilise a mare so that it can be registered in its breed association, or produce a recipient mare for embryo transfer. A practical technique of bilateral ovariectomy that can be easily performed without the mare sedated and without a surgical facility is ovariectomy through a colpotomy. Ovariectomy through a colpotomy is less expensive than ovariectomy using other approaches because it is performed with the mare standing, can be performed quickly and the only specialised instrument required is a chain écraseur. Complications of ovariectomy performed through a colpotomy are uncommon when the mare is correctly prepared for the procedure and when proper precautions are taken during and after the procedure.

Both ovaries are sometimes removed from mares to eliminate the regular oestrous cycle, create a teaser mare, or produce a recipient mare for embryo transfer (Hooper *et al.* 1993). Surgical approaches described for bilateral ovariectomy of mares include celiotomy through one or both flanks, or through the vagina (Hooper *et al.* 1993; Palmer 1993; Pader *et al.* 2011). A practical technique of bilateral ovariectomy that can be performed easily without anaesthetising the mare and without a surgical facility is ovariectomy through a colpotomy, with the use of an écraseur. Ovariectomy performed with an écraseur through a colpotomy, although once performed commonly (Nichols 1988; Embertson 2009), is now performed rarely because of perceived dangers associated with the procedure and the increasingly widespread use of laparoscopy to remove ovaries. Ovariectomy through a colpotomy is relatively inexpensive because it is performed with the mare standing, can be performed quickly and the only specialised instrument required is an écraseur. Removing one or both ovaries through a colpotomy avoids scarring, which is common when ovariectomy is performed using other approaches.

Ovariectomy through a colpotomy is usually performed to spay mares because most neoplastic ovaries are too large to be removed through a vaginal celiotomy. A neoplastic ovary is usually removed using a ventral midline, or oblique paramedian celiotomy (Moll *et al.* 1987; Westermann *et al.* 2003). Neoplastic ovaries less than about 10 cm in diameter, however, can be removed through a colpotomy (Colbern 1993; Moll and Slone 1998). Complications of ovariectomy performed through a colpotomy are uncommon when the mare is prepared properly for the procedure and when the correct precautions are taken during and after the procedure (Nichols 1988; Moll and Slone 1998; Embertson 2009; Pader *et al.* 2011).

Preparation of the mare for the surgery

Feed should be withheld for 24 h if the surgeon is inexperienced to reduce the number of faecal balls in the small colon, in order that a faecal ball is not confused with an ovary during the procedure. Administering 4 L of liquid paraffin 12–24 h in advance of surgery decreases the likelihood of faecal balls being present in the small colon. The mare is restrained in an equine stock and sedated with detomidine HCl (0.002–0.022 mg/kg bwt i.v.) and butorphanol (0.002–0.022 mg/kg bwt i.v.) (Pleasant and McGrath 1998). Administration of detomidine HCl should precede administration of butorphanol to avoid opioid-induced excitement. Sedation can be maintained by administering sedatives i.v. by constant rate infusion. Drugs commonly administered by constant rate infusion include detomidine alone (0.02 mg/kg bwt/h, i.v.), or in combination with butorphanol (0.012 mg/kg bwt/h, i.v.), or morphine (0.05 mg/kg bwt/h, i.v.) (Doherty and Valverde 2006). To avoid inducing excitement when using butorphanol in combination with detomidine, a loading dose of detomidine (0.008 mg/kg bwt i.v.) should be administered initially before a loading dose of butorphanol (0.022 mg/kg bwt i.v.) is administered (Doherty and Valverde 2006).

The mare is administered antimicrobial therapy and an anti-inflammatory, analgesic drug such as phenylbutazone (2.2 mg/kg bwt i.v.) and the perineum, vestibule and vagina desensitised by infusing 2% mepivacaine HCL (1.8 mg/kg bwt) alone, or in combination with, 2 or 10% xylazine HCl (0.18 mg/kg bwt), into the epidural space. A mare can be ovariectomised safely through a colpotomy without receiving epidural anaesthesia (authors' observation), but epidural anaesthesia, in addition to desensitising the vestibule and at least a portion of the vagina, prevents the mare from defaecating during surgery.

The tail is bandaged with rolled gauze, elevated and secured to the overhead cross-bar of the stock. The faeces are removed manually and the mare's ovaries and uterus evaluated by palpation per rectum. Hair surrounding the perineum is clipped and the perineum scrubbed with an antiseptic soap. Irrigating the vagina with 1 L isotonic saline solution not only cleans the vagina and vestibule, it also induces pneumovagina, easing the introduction of a hand and arm into the vagina.

Preparation of the surgeon

Although the procedure can be performed with the surgeon and assistant wearing only sterile obstetrical sleeves, we prefer to wear sterile surgical gowns, the arms of which are

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covered with a sterile obstetrical sleeve to decrease friction between the arm and vagina. Sterile surgical gloves are donned over the hands of the obstetrical sleeves. The glove and sleeve on the surgeon's dominant arm are lubricated with sterile KY jelly to ease introducing the hand and arm into the vagina and into the abdomen after colpotomy.

Surgical procedure

A small pack of sterile gauze swabs, tethered to a sterile suture (e.g. umbilical tape) and saturated with local anaesthetic solution, such as mepivacaine HCl or lidocaine HCl, is introduced into the vagina with the surgeon's dominant arm (**Fig 1**). Pressing the pack to the fornix of the vagina for several minutes ensures that the mucosa at the fornix is desensitised. A stab incision is made with a No.10 or 15 scalpel blade, tethered to sterile strand of suture (e.g. umbilical tape), at the dorsolateral aspect of the fornix of the vagina, at the 10.30 or 13.30 h position, about 2 cm dorsolateral to the base of the cervix. This incision is cranial and dorsal to the vaginal branch of the internal pudendal artery (i.e. the vaginal artery) (Embertson 2009), which can usually be easily palpated when the vagina is distended with air (**Figs 2** and **3**). A right-handed surgeon can perform bilateral ovariectomy more easily through a colpotomy created on the right aspect of the vaginal fornix (i.e. the 13.30 h position), whereas a left-handed surgeon can perform bilateral ovariectomy more easily through a colpotomy created on the left aspect of the vaginal fornix (i.e. the 10.30 h position).

The blade is inserted through vaginal mucosa and submucosa and the stab incision is spread, first with the jaws of a haemostat and then with fingers, until an opening in the mucosa and submucosa is created that can accommodate the entire hand into the retroperitoneal space. Fascia and peritoneum are torn with a finger to create a hole into the abdominal cavity large enough to accommodate the hand and forearm of the surgeon. Trying to thrust a finger through the peritoneum, rather than tearing the peritoneum with a finger, is ineffective because this manoeuvre pushes the peritoneum away from the abdominal wall.

Fig 1: A pack of sterile gauze, tethered to a sterile suture and saturated with local anaesthetic solution, is introduced into the vagina.

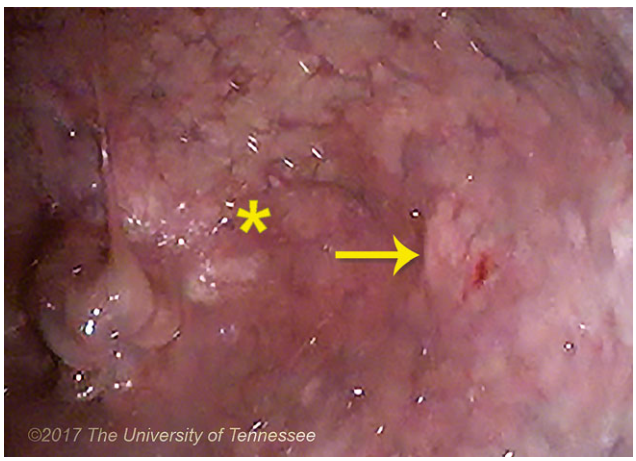

Fig 2: A stab incision is made with a No. 10 or 15 scalpel blade at the dorsolateral aspect of the fornix of the vagina, at the 10.30 or 13.30 h position, about 2 cm away from the base of the cervix and cranial and dorsal to the vaginal branch of the internal pudendal artery. *, site of incision; arrow, points to the vaginal branch of the internal pudendal artery.

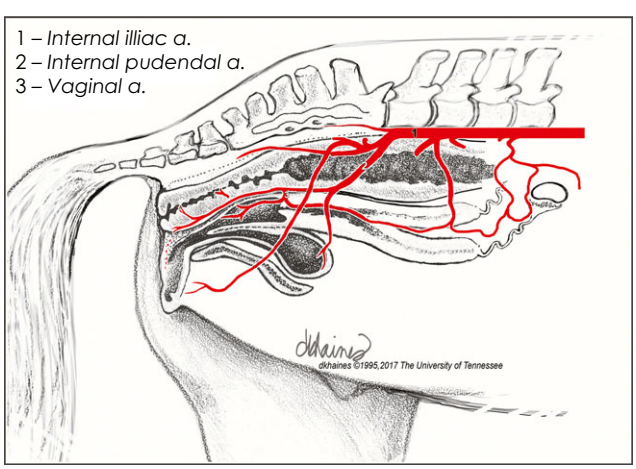

Fig 3: Schematic diagram showing the vaginal branch of the internal pudendal artery. 1, internal iliac artery; 2, internal pudendal artery; 3, vaginal artery.

The ovaries are identified and the pedicle of each ovary desensitised by pressing sterile gauze swabs, saturated with local anaesthetic solution, to each ovarian pedicle for several minutes (**Fig 4**). The gauze is tethered to a long, sterile suture (e.g. umbilical tape) to ensure that the gauze can be retrieved from the abdomen, if the gauze swabs are accidently dropped. The chain of the écraseur is secured over the dominant hand of the surgeon by placing four fingers through the loop of the chain of the écraseur and inserting the hand, the chain and the end of the écraseur through the colpotomy into the abdomen. The chain of the écraseur is positioned, with tension, against the surgeon's proximal row of phalanges as the end of the écraseur is introduced into the abdomen. The écraseur preferred by the authors is the Chassaignac écraseur[1] (**Fig 5**).

The left ovary should be removed first, if the colpotomy was created on the right side of the vagina, so that when

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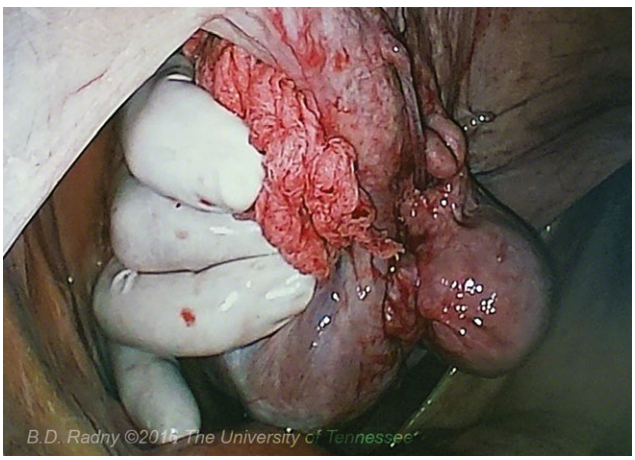

Fig 4: Ovaries are identified and the pedicle of each ovary is desensitised by pressing sterile gauze, saturated with local anaesthetic solution, to each ovarian pedicle.

Fig 5: The écraseur preferred by the authors is the Chassaignac écraseur[1].

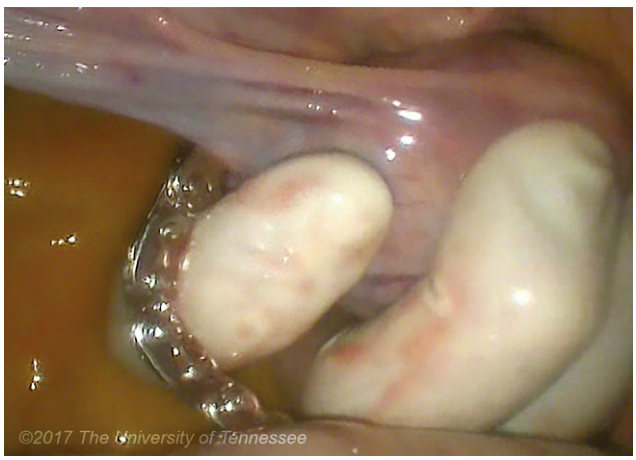

Fig 6: The chain of the écraseur is secured against the proximal row of the surgeon's phalanges, the ovary grasped, and the chain slipped over the hand to encircle the ovarian pedicle. This photograph was obtained through a laparoscope introduced into the abdominal cavity at the mare's flank, for the purpose of demonstrating ovariectomy through a colpotomy.

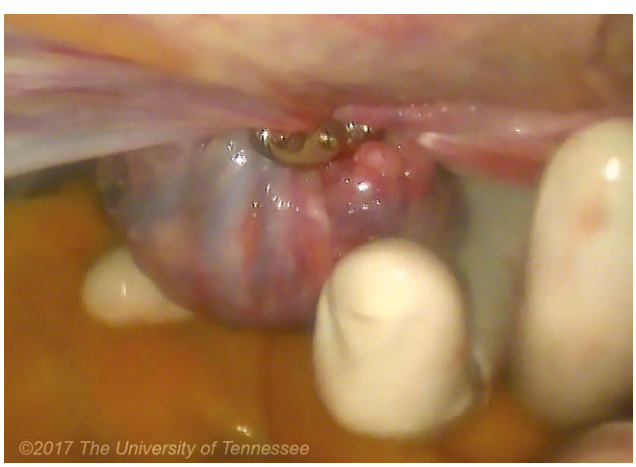

Fig 7: The chain is tightened, using the ratchet, until the pedicle is severed. This photograph was obtained through a laparoscope introduced into the abdominal cavity at the mare's flank, for the purpose of demonstrating ovariectomy through a colpotomy.

the right ovary is removed, the pedicle of the left ovary is not disturbed by the arm of the surgeon. When removing the ovary on the side contralateral to the colpotomy, the surgeon should ensure that his, or her, hand and the écraseur have passed beneath the small colon to access the ovary, so that the ovary is not grasped through the mesocolon. Removing an ovary encased by mesocolon risks severing a colonic vein and artery and creates a hole in the mesocolon through which intestine can become entrapped.

With the chain of the écraseur secured against the proximal row of the surgeon's phalanges, the ovary is grasped and the chain is slipped over the hand to encircle the ovarian pedicle (**Fig 6**). The chain is tightened around the pedicle, being careful that the chain encircles no other structure, such as a loop of intestine. The surgeon, or preferably an assistant surgeon, slowly tightens the chain by using the ratchet on the end of the écraseur while the surgeon holds the ovary and ensures that the ovarian pedicle is not stretched during this part of the procedure. The chain is tightened, using the ratchet, until the pedicle is severed (**Fig 7**). Stretching the pedicle while the chain is tightened may cause the pedicle to recoil when the pedicle is severed, which, in turn, may result in excessive haemorrhage from the ovarian artery. The ovary is extracted through the colpotomy and the contralateral ovary is removed in a similar manner.

The palm of a hand is held beneath each pedicle at the end of the procedure to feel for bleeding from the pedicle (**Fig 8**). If, on rare occasion, the amount of blood emanating from a pedicle is alarming, a long forceps, such as a Knowles cervical forceps, can be inserted vaginally, through the colpotomy, applied to the pedicle and left in situ for 1 h or more to induce haemostasis. The colpotomy is usually left unsutured to heal by second intention because suturing the colpotomy is difficult and may induce vaginitis, causing the mare to strain.

The mare should receive a Caslick's vulvoplasty if its perineal conformation is poor, to prevent pneumovagina and subsequent contamination of the vestibule, vagina and abdomen. Antimicrobial and analgesic therapy is continued for 3–5 days after surgery. The mare should be cross-tied for 2–3 days to prevent it from becoming recumbent because rising from recumbency increases abdominal pressure which, in turn, increases the likelihood of evisceration (Embertson 2009). Within about 3 days, the colpotomy contracts to a diameter that accommodates only one finger (**Fig 9**). The hole is usually completely sealed from the abdomen within

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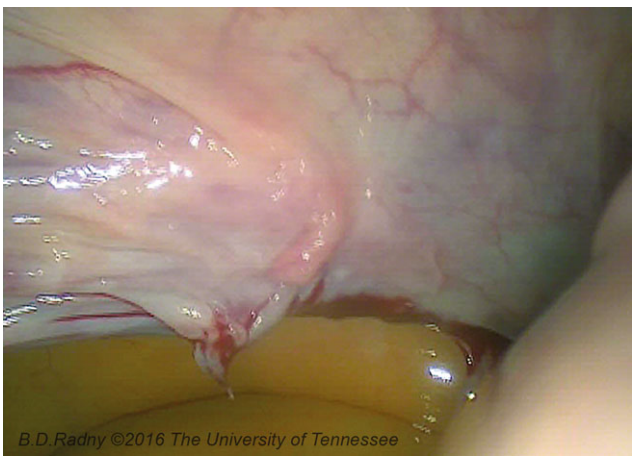

Fig 8: The palm of a hand is held beneath each pedicle at the end of the procedure to detect bleeding from the pedicle. This photograph was obtained through a laparoscope introduced into the abdominal cavity at the mare's flank, for the purpose of demonstrating ovariectomy through a colpotomy.

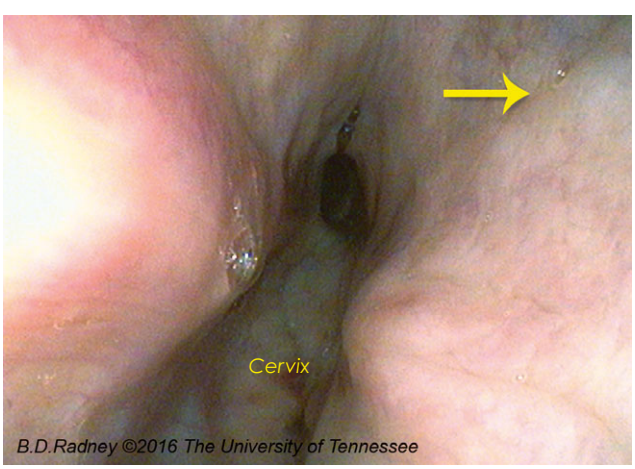

Fig 9: Endoscopic view of the fornix of the vagina 3 days after colpotomy. The colpotomy has contracted to a diameter smaller than that of one finger. The bulge in the vaginal wall (arrow) is the vaginal artery.

3 weeks (Moll and Slone 1998). The mare can be allowed unrestricted exercise after about 5 days (Nichols 1988; Colbern 1993; Moll and Slone 1998; Embertson 2009; Pader *et al.* 2011).

Discussion

The primary disadvantage of ovariectomy by colpotomy is that the ovarian pedicle is crushed and transected blindly, making detection of excessive intra-abdominal haemorrhage difficult. Because of this difficulty, the mare's haematocrit and serumal total protein should be determined before surgery and periodically after surgery to ensure that haemorrhage from the severed ovarian pedicles is not severe. Determining the magnitude of blood loss by monitoring the horse's haematocrit and total serumal solids during the first 6–24 h after ovariectomy, however, is difficult, because with severe blood loss, a decrease in total serumal protein is often not evident for 6 h and a decrease in haematocrit may not be evident for 12–24 h (Getman 2009).

Clinical signs associated with substantial blood loss include tachycardia, tachypnoea, a weak pulse, pale mucous membranes, prolonged capillary refill, cold extremities and weakness (Mudge 2014). If severe haemorrhage is suspected, the horse should receive fluid therapy and transfusion of blood from an acceptable donor should be considered. A complication of colpotomy itself is fatal haemorrhage caused by inadvertent perforation of the vaginal artery with a scalpel blade, but this artery is avoided if it is located by palpation before the fornix of the vagina is incised and if the incision is created at the proper location (Embertson 2009).

One of the authors (J.S.) has observed only two surgical complications after performing over 100 ovariectomies by colpotomy. One mare experienced severe haemorrhage, requiring multiple blood transfusions, but survived. The cause of severe haemorrhage was thought to result from transecting the ovarian pedicle while the pedicle was under tension. Another mare strained after surgery, presumably because of vaginitis induced by an unsuccessful attempt to suture the colpotomy. Straining gradually diminished over several days.

Persistence of unwanted behaviour is a complication of bilateral ovariectomy performed to eliminate or ameliorate that behaviour (Hooper *et al.* 1993; Kamm and Hendrickson 2007; Crabtree 2016). Success of ovariectomy in eliminating or ameliorating unwanted behaviour is likely if that behaviour occurs primarily during oestrus and if hormonal therapy has been shown to improve the mare's behaviour (Kamm and Hendrickson 2007). Ovariectomy is unlikely to resolve unwanted behaviour, if that behaviour is sexual behaviour that occurs during oestrus, because ovariectomy commonly results in continued display of sexual receptiveness with loss of normal cyclic activity (Hedberg *et al.* 2007).

Bilateral ovariectomy eliminates the production of oestrogen by eliminating the theca and granulosa cells of the ovarian follicles (Christensen 2011). Continued signs of oestrus displayed by ovariectomised mares is most likely due to absence of the corpora lutea, the primary source of progesterone, because progesterone is responsible for inhibiting the behavioural signs of oestrus (Watson and Hinrichs 1989).

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Not applicable.

Source of funding

None.

Authorship

The manuscript was written by both authors who also obtained the photographs.

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Population dynamics of brushtail possums subject to fertility control

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Summary

1. Reducing the fertility of pest species using immunocontraceptive vaccines holds promise for the humane and effective control of vertebrate pest populations. However, despite much research into the development of contraceptive vaccines, there are few data illustrating the effectiveness of fertility control for managing wild populations of vertebrate pests.
2. In New Zealand, introduced brushtail possums *Trichosurus vulpecula* have been the focus for the development of contraceptive vaccines to provide a possible alternative to lethal control techniques.
3. The effect of fertility control on the population dynamics of brushtail possums was investigated experimentally using surgical sterilization to suppress breeding on six 12-ha study sites between January 1996 and October 2000. Sterilization treatments, consisting of 0%, 50% and 80% of resident females sterilized, were applied at a particular site, with adjustments made annually on recruits to maintain levels of sterility. Mark–recapture trapping was used to estimate age-specific demographic rates and their relative contribution to age-specific population growth rates (λ).
4. Sterility treatments resulted in reductions to *per capita* rates of local recruitment (surviving young per adult female) of 60% and 74% on average for 50% and 80% levels of sterility, respectively. However, immigration by yearling-aged possums compensated for reductions in local recruitment because of sterility treatments, leading to the stable ($\lambda = 1$) population trajectories on sterility treatment sites.
5. There was some evidence that sterilized females had higher annual survival rates than fertile females, but otherwise there was no evidence of any other compensatory responses to imposed sterility. However, additional studies are needed to clarify possible density-dependent responses that could compensate for the effects of fertility control.
6. *Synthesis and applications.* Demographic compensation by pest populations can negate the effects of fertility control. Results from this study suggest that immigration can easily compensate for fertility control in pest species where adult survival is high and fecundity is low. This suggests that fertility control will need to be applied at large spatial scales to reduce this effect. Where adult survival makes a relatively high contribution to the population growth rate, the benefits of fertility control would be enhanced if integrated with conventional control to provide rapid initial population reduction.

Key-words: demographic contributions to lambda, immunocontraception, lambda, population growth rate, reverse-time mark–recapture, *Trichosurus vulpecula*

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Introduction

Australian brushtail possums *Trichosurus vulpecula* Kerr, introduced into New Zealand in the 1850s to establish a fur trade, are now a serious pest (Clout & Sarre 1997; Clout & Erickson 2000) and the main wildlife vector for bovine tuberculosis (Tb), which they transmit to livestock (Caley *et al*. 1999). Current methods of managing possums in the wild usually involve the use of poison bait, spread over large areas. While this method is effective, it is often costly and needs continual maintenance to give long-lasting results. There

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are increasing concerns about possible effects on non-target populations and public health, as well as ethical considerations (Morgan & Hickling 2000). For these reasons, research is currently aimed at developing fertility control using contraceptive vaccines for possums, to provide a possible alternative long-term, cost-effective solution. Current research is mainly directed at reducing fertility by developing immunological blocks to fertilization or embryonic development (Cowan 1996; Cowan 2000), or manipulating hormonal regulation and gonadal development (Eckery *et al*. 1998).

While fertility control is seen as a possible solution to public concerns and animal welfare issues surrounding conventional lethal control, there are few data illustrating its effectiveness as a management tool. Models of fertility control in mammals suggest it will be relatively more effective on species with high fecundity and shorter lifespan (Hone 1992), and hence is predicted to be less effective on marsupials compared with eutherians, as marsupials generally have lower birth rates and higher survivorship than eutherians (Sinclair 1997). Models of fertility control for brushtail possums suggest the expected level of population suppression for a given sterilization rate is dependent on assumptions about the strength of density-dependent compensation, with fertility control being less effective if density-dependent compensatory responses operate through recruitment rather than adult survival (Barlow 1994; Barlow, Kean & Briggs 1997).

The mating system and social structure of the population may also influence the effectiveness of fertility control if they affect the birth and/or death rates (Caughley, Pech & Grice 1992; Barlow 2000). Ji, Clout & Sarre (2000) examined the responses of male possums in populations where 50% of the adult females were surgically sterilized. They found that surgically sterilized females continued to have oestrous cycles outside the normal breeding season. This resulted in an increase in the local density of males, which, it was hypothesized, were being attracted to the area because of the prolonged breeding activity. However, the potential effects of these behavioural and other compensatory responses to fertility control on the population dynamics of possums remain unknown. As compensatory increases in either adult or juvenile survival or immigration rates may compromise the efficacy of fertility control (Sinclair 1997), empirical evidence is needed on the magnitude of these responses (if any) before fertility control can be considered a viable management technique for brushtail possums.

To investigate the efficacy of artificially inhibiting the birth rate as an alternative control technique for brushtail possums, a field experiment was undertaken over 5 years to determine the effect of various levels of infertility (sterilized females) on population dynamics. Specifically, age-specific local recruitment, immigration and survival were estimated together with their relative contribution to the population growth rate (λ) for populations where either 50% or 80% of adult females had been made permanently infertile by surgical sterilization. Comparisons were made between these parameters and those on control sites where no sterility treatments were applied.

Materials and methods

STUDY AREAS

The experiment was undertaken on six 12-ha live trapping grids at two different locations. Three sites were established in the Orongorongo Valley, east of Wellington (174°58′E, 41°21′S), and three in the Turitea catchment, east of Palmerston North (175°41′E, 40°26′S), New Zealand. Sites were established in October–November 1995 in native podocarp/hardwood forest. Vegetation in the Orongorongo Valley was dominated by emergent species *Metrosideros robusta* A. Cunn., *Dacrydium cupressinum* Lamb., *Prumnopitys ferruginea* D. Don. and *Prumnopitys taxifolia* D. Don. (Fitzgerald 1976; Campbell 1990; Ramsey *et al*. 2002). The forest canopy was between 6 and 20 m and comprised mainly *Elaeocarpus dentatus* J. R. Forst. & G. Forst., *Laurelia novae-zelandiae* A. Cunn., *Melicytus ramiflorus* J. R. Forst. & G. Forst., *Hedycarya arborea* J. R. Forst & G. Forst., *Knightia excelsa* R. Br., *Weinmannia racemosa* L. f., *Schefflera digitata* J. R. Forst. & G. Forst., *Pseudowintera axillaris* J. R. Forst. & G. Forst. and five species of tree fern. The area also included stands of kanuka *Kunzea ericoides* A. Rich. and hard beech *Nothofagus truncata* Col. Ckn. The Turitea catchment site was situated in the Tararua ranges near Palmerston North and had vegetation comprising remnant podocarp/kamahi forest now dominated by a canopy of *Beilschmiedia tawa* A. Cunn. with associated *Melicytus ramiflorus*, *Hedycarya arborea*, *Knightia excelsa* and scattered *Dacrydium cupressinum* and *Prumnopitys ferruginea* (Esler 1969). At each site, between 132 and 150 cage traps were placed on a marked grid at 30 m spacing. Populations in the Orongorongo Valley typically have one birth pulse per year in autumn (March–July), while populations in the Turitea catchment have been observed to have an occasional second, smaller pulse of breeding in spring (September–December) (D. Ramsey, unpublished data).

POPULATION MONITORING

Trapping followed the robust design (Pollock 1982; Nichols & Pollock 1990), with each population trapped for a minimum of three primary sessions a year with each primary session consisting of secondary sessions of four or five consecutive nights trapping. Trapping started on all sites during October–November 1995 and continued until October 2000. The exception was the control site in the Orongorongo Valley, which has been continuously trapped since 1966 (Crawley 1973; Efford 1998). Newly caught possums were anaesthetized with ether (1995–99) or 50–100 mg ketamine hydrochloride (2000) (Parnell Laboratories New Zealand

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Ltd, Auckland, New Zealand) by intramuscular injection and given a unique tattoo and ear tag. Before the animal was released at the point of capture, measurements were taken that included head, body and total lengths; testis length and width for males; weight and tooth wear. For females, an estimate of breeding success was made in late June by examining the pouch for young. Head lengths of pouch young were measured to estimate date of birth (Efford 1998) by assuming a head length at birth of 7 mm and a linear growth rate of 0·27 mm day^{-1} (Orongorongo Valley) or 0·35 mm day^{-1} (Turitea) (D. Ramsey, unpublished data). Pouch young caught with their mothers in September–October were usually large enough to tag. Recaptured animals were identified and weighed before being released. Animals not first caught with their mother were assigned a minimum age using a logistic regression algorithm discriminating on body-size measurements. Separate regressions were constructed for Orongorongo Valley and Turitea using possums whose birth cohorts were known. Independent possums were classified as either yearlings (= 20 months old) or adults (> 20 months old) (Efford 1998).

STERILIZATION TREATMENTS

Fertility control was imposed by surgically sterilizing females by ligating the oviducts. This method ensured ovarian function remained intact and mimicked the action of the most likely candidate fertility control vaccines. Three levels of sterilization treatment were applied, with 0%, 50% or 80% of adult females at a particular site sterilized. As the effect of surgery on survival of animals was unknown, sham operations (no sterilization) were undertaken to balance the level of surgical manipulation across treatments. However, no sham operations were undertaken on the Orongorongo Valley control (0% sterility) site, so that consistency could be maintained with the previous long-term monitoring data collected at this site. Two replicates of each treatment were undertaken, with one complete replicate at sites in the Orongorongo Valley and one replicate in the Turitea catchment. Treatments were assigned to sites within each area at random, with the exception of the control site in the Orongorongo Valley. Sterilization treatments were applied during January–April 1996, with adjustments to maintain sterility levels made annually on recruits. All trapping and surgical procedures were approved by the Landcare Research Animal Ethics Committee, Lincoln, New Zealand.

DECOMPOSITION OF THE ANNUAL POPULATION GROWTH RATE

A retrospective analysis (Caswell 2000) was undertaken to determine the relative contribution of demographic parameters (survival, recruitment) to the annual population growth rate (λ). Trapping undertaken in September–October was chosen as the annual census date because pouch young were marked with their mothers during this period. Capture data for the intervening periods were not used. Here λ is the realized annual growth rate rather than the asymptotic growth rate derived from population projection matrices (Caswell 2001), and is defined as:

$$\lambda_i = \frac{N_{i+1}}{N_i} \qquad \text{eqn 1}$$

where λ_i is the population growth rate during year i, and N_i and N_{i+1} are the population abundance estimates in year i and $i + 1$, respectively. Following Nichols *et al.* (2000), λ can be decomposed into contributions from survivors and new recruits as:

$$\lambda_i = \frac{\gamma_{i+1} N_{i+1} + (1 - \gamma_{i+1}) N_{i+1}}{N_i} \qquad \text{eqn 2}$$

where γ_{i+1} is the probability that a member of the population at time $i + 1$ (N_{i+1}) was a member of the population at time i (N_i) (e.g. survivors from time i), and hence $(1 - \gamma_{i+1})$ is the probability that a member of the N_{i+1} was recruited between time i and $i + 1$. Thus, the γ_{i+1} can be regarded as a measure of the relative contribution of survivors and recruits to population growth between years i and $i + 1$ (Nichols *et al.* 2000; Nichols & Hines 2002).

Recruitment into a population can potentially come either from reproduction from resident females (local recruitment) or from animals arriving from outside the study population (immigration). In order to decompose λ into contributions from local recruitment, immigration and survival, it is necessary to condition on age structure, as recruitment into the next age class that cannot be explained by survival from the previous age class must be a result of immigration (Stokes 1984; Nichols & Pollock 1990). Three age classes were considered in the analysis: pouch young, dependent young first caught with their mothers and tagged at approximately 8 months old; yearlings, possums first caught in the year following their birth at approximately 20 months old; and adults, all possums approximately ≥ 32 months old.

The relative contribution (γ) of demographic parameters (survival, fecundity/local recruitment and immigration) of each age class to the annual population growth rate λ was estimated using methods detailed in Nichols *et al.* (2000). This retrospective analysis uses reverse-time capture–recapture analysis to estimate γ. By reversing the time order of capture history data, inference can be made on the recruitment process, which is statistically equivalent to inference on the survival process using forward time (Pollock, Solomon & Robson 1974; Pradel 1996). However, the analysis of the contribution of different demographic components to λ for age-structured data is not straightforward (Nichols *et al.* 2000). For example, using reverse-time capture–recapture, it was desirable to condition on animals caught as yearlings at time $i + 1$ and estimate the probabilities that those animals were either pouch young of resident females at time i (γ_{i+1}) or new immigrants

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arriving between time i and $i + 1$ $(1 - \gamma_{i+1})$. As there were three age classes, the population growth rate given in equation 1 can be partitioned into two components. The population growth rate of adults is given as:

$$\lambda_i^2 = \frac{N_{i+1}^2}{N_i^2} \qquad \text{eqn 3}$$

where N_i^2 and N_{i+1}^2 are the population abundance of adults in year i and $i + 1$, respectively. The population growth rate of the yearling component is given by:

$$\lambda_i^1 = \frac{N_{i+1}^1}{N_i^1} \qquad \text{eqn 4}$$

where N_i^1 and N_{i+1}^1 are the population abundance of yearlings in year i and $i + 1$, respectively. The N_i^a $(a = 2, 1)$ was estimated using the jack-knife closed population estimator (Burnham & Overton 1978), which is robust to individual heterogeneity in capture probabilities. Estimates of λ_i^2 and λ_i^1 and their sampling variance in each year were obtained using a bootstrap procedure. For each age class within each primary trapping session, a random sample of the capture histories over the secondary capture periods was selected (with replacement), conditional on the total number of individuals captured. The ratio of 50 bootstrap jack-knife estimates of abundance in year $i + 1$ and year i was used to generate 2500 estimates of the population growth rate. The mean and standard error of the population growth rate was estimated as the mean and standard deviation of these 2500 bootstrap estimates. An estimate of the overall average population growth rate over the study period was made by fitting a linear regression to the natural log of the jack-knife abundance estimates of the total population in September–October each year. The average growth rate ($\bar{\lambda}$) was estimated as e^b, where b was the slope of the regression line (Caughley 1977).

The decomposition of λ_i^2 is given by the following expression (Nichols *et al*. 2000):

$$\lambda_i^2 = \frac{\gamma_{i+1}^{22} N_{i+1}^2 + \gamma_{i+1}^{21} N_{i+1}^2 + (1 - \gamma_{i+1}^{22} - \gamma_{i+1}^{21}) N_{i+1}^2}{N_i^2} \qquad \text{eqn 5}$$

where λ_i^2 is the population growth of adult possums during the ith year; γ_{i+1}^{22} is the probability that an adult possum alive at time $i + 1$ was in the adult population at time i; γ_{i+1}^{21} is the probability that an adult possum alive at time $i + 1$ was in the yearling population at time i; and $1 - \gamma_{i+1}^{22} - \gamma_{i+1}^{21}$ is the probability that an adult possum alive at time $i + 1$ immigrated between time i and $i + 1$. Similarly, the decomposition of the population growth rate of the yearling subpopulation can be expressed as:

$$\lambda_i^1 = \frac{\gamma_{i+1}^{10} N_{i+1}^1 + (1 - \gamma_{i+1}^{10}) N_{i+1}^1}{N_i^1} \qquad \text{eqn 6}$$

where λ_i^1 is the annual population growth rate of yearling-aged possums during the ith year; γ_{i+1}^{10} is the probability that a yearling possum alive at time $i + 1$ was a dependent pouch young at time i; and $1 - \gamma_{i+1}^{10}$ is the probability that a yearling possum alive at time $i + 1$ immigrated between time i and $i + 1$. These γ_i^{rs} [rs = 22, 21 or 10, referring to either age classes 2 (adults), 1 (yearlings) or 0 (pouch young)] were estimated using the closed-form maximum likelihood estimators (MLE) detailed in Nichols *et al*. (2000), for example:

$$\hat{\gamma}_i^{22} = \frac{\hat{M}_{i,i-1}^{22}}{n_i^2}; \; \hat{\gamma}_i^{21} = \frac{\hat{M}_{i,i-1}^{21}}{n_i^2}; \; \hat{\gamma}_i^{10} = \frac{\hat{M}_{i,i-1}^{10}}{n_i^1}$$

Where:

$$\hat{M}_{i,i-1}^{rs} = \frac{m_{i,i-1}^{rs}}{\hat{p}_{i-1}^{s*}} \qquad \text{eqn 7}$$

and $m_{i,i-1}^{rs}$ are the members of the n_i^r that were caught as members of the n_{i-1}^s where n is the total number of animals caught in periods i or i^{-1} for either age class r or s. The capture probability parameter ($\hat{p}_{i-1}^{s*}$) in equation 7 is specific to reverse-time analysis and is calculated as:

$$\hat{p}_i^{s*} = \frac{\hat{p}_i^s \hat{\eta}_i^s}{1 - \hat{p}_i^s (1 - \hat{\eta}_i^s)} \qquad \text{eqn 8}$$

where $\hat{p}_i^s$ is the capture probability for age class s at period i using conventional forward time modelling, and $\hat{\eta}_i^s$ is the probability that an animal of age class s captured at period i survives trapping and handling and is released (Nichols & Hines 2002). Equations 7 and 8 were used to produce unbiased estimates of γ_i^{rs} in the presence of losses on capture. During 1998 and 1999 an attempt was made to limit the influx of immigrant yearling possums on sterility grids by removing a proportion of unmarked yearlings during the October trapping session. This attempt was largely unsuccessful. However, not accounting for losses on capture could produce biased estimates of γ_i^{21} as the yearling possums that were removed could not be seen again as adults. When there are no losses on capture (i.e. $\eta_i^s = 1$) the forward-time and reverse-time estimates of capture probability are equal (Nichols & Hines 2002).

Estimation of $\hat{\gamma}_i^{22}$ and $\hat{\gamma}_i^{21}$ was relatively straightforward, with the forward-time capture probability for adults ($\hat{p}_i^2$) and yearlings ($\hat{p}_i^1$) estimated by fitting Cormack–Jolly Seber (CJS) models, conditioning on age at first capture using the program MARK (White & Burnham 1999). Models were fitted that allowed for both time-dependent and time-independent age-specific capture probability, noting that the capture probability of pouch young cannot be estimated (e.g. a possum released as a pouch young that survived over the next year interval is caught as a yearling). Only models with time-dependent age-specific survival rates were considered as part of the candidate set of models. The set of models was ranked using methods detailed in Burnham & Anderson (1998) using Akaike's information criterion adjusted for sample size (AIC_c). The most general model considered (time-varying age-specific survival and capture probability) was tested for goodness-of-fit using the parametric bootstrap test in program

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MARK. The overdispersion parameter c was estimated by dividing the observed deviance of the general model by the mean of 100 bootstrap samples. Models were corrected for overdispersion using this estimate of c, and the fit of all the models considered was compared using Akaike's information criterion corrected for overdispersion ($QAIC_c$) by taking the difference between all models and the model with the lowest $QAIC_c$ (Burnham & Anderson 1998; White & Burnham 1999). The relative support for each of the candidate models was assessed by calculating normalized Akaike weights (w_i) (Burnham & Anderson 1998). Once estimates of $\hat{p}_i^s$ were obtained, $\hat{p}_i^{s*}$ was estimated using data on losses-on-capture (see Appendix 2) to estimate $\hat{\eta}_i^s$.

In order to estimate $\hat{\gamma}_i^{10}$, it is necessary to have information on capture probability of pouch young $\hat{p}_{i-1}^0$ that is not directly available from standard age-structured models. Capture probability of dependent pouch young can be estimated from an approximation of the total size of the pouch young cohort:

$$\hat{p}_i^0 = \frac{n_i^0}{\hat{N}_i^0} \qquad \text{eqn 9}$$

where n_i^0 is the number of pouch young tagged with their mother, and $\hat{N}_i^0$ is an estimate of the total size of the pouch young cohort. This was obtained using:

$$\hat{N}_i^0 = \frac{n_i^{1f}}{\hat{p}_i^{1f}} BR_i^1 + \frac{n_i^{2f}}{\hat{p}_i^{2f}} BR_i^2 \qquad \text{eqn 10}$$

where n_i^{1f} and n_i^{2f} are the total number of yearling and adult female possums caught at time i, respectively; BR_i^1 and BR_i^2 are the breeding rates of yearling and adult female possums, respectively; and $\hat{p}_i^{1f}$ and $\hat{p}_i^{2f}$ are the capture probabilities of female yearling and adult possums, respectively, obtained from the conventional age-structured CJS analysis applied to capture histories for female possums.

An estimate of sampling variance for each of the MLE of the γ_i^{rs} was obtained by a bootstrapping procedure. Conditioning on each of the n_i^r ($r = 2, 1$) releases at time i, capture histories were simulated in reverse time using the point estimates of the γ_i^{rs} and age-specific capture probabilities. For each individual in each n_i^r, a random uniform number between 0 and 1 was drawn and compared with the appropriate γ_i^{rs}. If the random number was less than the estimate the animal was deemed to have been present at time $(i - 1)$, if otherwise it was not. If the animal was present at time interval $(i - 1)$ another random number was drawn and compared with the appropriate capture probability estimates from the forward-time age-structured model (i.e. p_{i-1}^s). If the random number was less than the estimated capture probability, the animal was assumed to have been caught at time interval i^{-1} and thus was entered as a member of the $n_i^s (r = 2, 1, 0)$. The n_i^r and simulated n_{i-1}^s were used to calculate the $m_{i,i-1}^{rs}$ and, hence, an estimate of the γ_i^{rs} using equation 7. This bootstrapping process was repeated 1000 times for each γ_i^{rs}, with the standard error estimated as the standard deviation of the 1000 bootstrap estimates.

Once estimated, the values of γ_i^{22}, γ_i^{21} and γ_i^{10} were compared between sterility treatments using generalized linear models. A binomial error structure was assumed by using a logit link function, with the number of n_{i-1}^s ($s = 2, 1, 0$) releases for each time period used as weights for each value of γ_i^{rs}. The rate of increase (λ_i^2 or λ_i^1) was included in the analysis as a covariate, to determine whether year-to-year variation in γ_i^{rs} was dependent on the age-specific rate of increase. Area (Orongorongo or Turitea) was also included as a fixed effect in the analysis.

Finally, estimates of λ_{i+1}^{10} were used to make an estimate of *per capita* local recruitment, defined as the number of pouch young born in year i surviving to the yearling age class in year $i + 1$ per adult female in year i, thus:

$$\hat{B}_{i+1} = \frac{\hat{N}_{i+1}^1 \hat{\gamma}_{i+1}^{10}}{\hat{N}_i^{2f}} \qquad \text{eqn 11}$$

where $\hat{B}_{i+1}$ is the *per capita* rate of local recruitment, $\hat{N}_{i+1}^1$ is the jack-knife estimate of abundance of yearling possums in year $i + 1$, and $\hat{N}_i^{2f}$ is the jack-knife estimate of abundance of adult female possums in year i. The sampling variance of $\hat{B}_i$ was calculated using the delta method (Seber 1982) as:

$$\mathrm{Var}(\hat{B}_i) = \frac{\mathrm{Var}(\hat{N}_{i+1}^1 \hat{\gamma}_{i+1}^{10})}{(\hat{N}_i^{2f})^2} + \frac{(\hat{N}_{i+1}^1 \hat{\gamma}_{i+1}^{10})^2 \mathrm{Var}(\hat{N}_i^{2f})}{(\hat{N}_i^{2f})^4} \qquad \text{eqn 12}$$

Where:

$$\mathrm{Var}(\hat{N}_{i+1}^1 \hat{\gamma}_{i+1}^{10}) = (\hat{N}_{i+1}^1)^2 \mathrm{Var}(\hat{\gamma}_{i+1}^{10}) + (\hat{\gamma}_{i+1}^{10})^2 \mathrm{Var}(\hat{N}_{i+1}^1) + \mathrm{Var}(\hat{N}_{i+1}^1)\mathrm{Var}(\hat{\gamma}_{i+1}^{10})$$

This assumes that each of the random variables is independent.

OTHER ANALYSES

The effect of the sterilization procedure on individual females was examined by comparing the annual survival of sterilized adult females with fertile adult females by fitting CJS models in the program MARK. Sterile and fertile individuals were categorized by site, which was included as a fixed effect in the analysis. The goodness-of-fit of the 'global' model was assessed using the pooled chi-squared goodness-of-fit tests from tests 2 and 3 using program RELEASE (Burnham *et al*. 1987). Models for capture probability were fitted first, and included the interactive effects of site, sterility and time. The 'best' model of capture probability was then used to model survival and included the same effects. Models were ranked as previously using AIC_c and Akaike weights (Burnham & Anderson 1998; Burnham & Anderson 2001).

The breeding rate of fertile females was compared between sterility treatments using logistic regression (Collett 1991). Area (Orongorongo/Turitea) was fitted

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as a fixed effect in the model. The median birth date for pouch young born to resident females was similarly compared using ANOVA. All analyses were performed using Splus 6·1 (Insightful Corporation 2001).

Results

BREEDING STATISTICS

Low breeding rates occurred on all sites during 1996, with only 24% and 45% of adult females breeding on control sites in the Orongorongo Valley and Turitea, respectively (Fig. 1a). However, in subsequent years treatment levels of sterility successfully suppressed breeding, with median levels of 69%, 30% and 14% of adult females breeding on control, 50% and 80% sterility sites, respectively (Fig. 1a). Accounting for the fixed effect of area (Orongorongo or Turitea), the rate of breeding in fertile females was similar among sterility treatments (logistic regression $\chi^2_2 = 0{\cdot}79$, $P = 0{\cdot}67$; Fig. 1b) and, similarly, there were no significant differences in the median dates of birth (ANOVA $F_{2,25} = 0{\cdot}03$, $P = 0{\cdot}97$). The rate of spring breeding also did not appear to increase among fertile females at sites with imposed sterility ($\chi^2_2 = 1{\cdot}7$, $P = 0{\cdot}43$). Thus, sterility treatments did not significantly influence either the rate or the timing of breeding in unsterilized females.

POPULATION TRAJECTORIES

Between 1996 and 2000, population abundance exhibited no strong upward or downward trend on any site, with the exception of the Turitea control (0% sterility) site, which averaged a 20% annual increase (Fig. 2a,b and Table 1). Overall, average population growth rates ($\bar{\lambda}$) were close to unity for all sterility treatment sites (Table 1).

DECOMPOSITION OF THE ANNUAL POPULATION GROWTH RATE

Forward-time CJS models fitted to mark–recapture data from the September–October trapping sessions

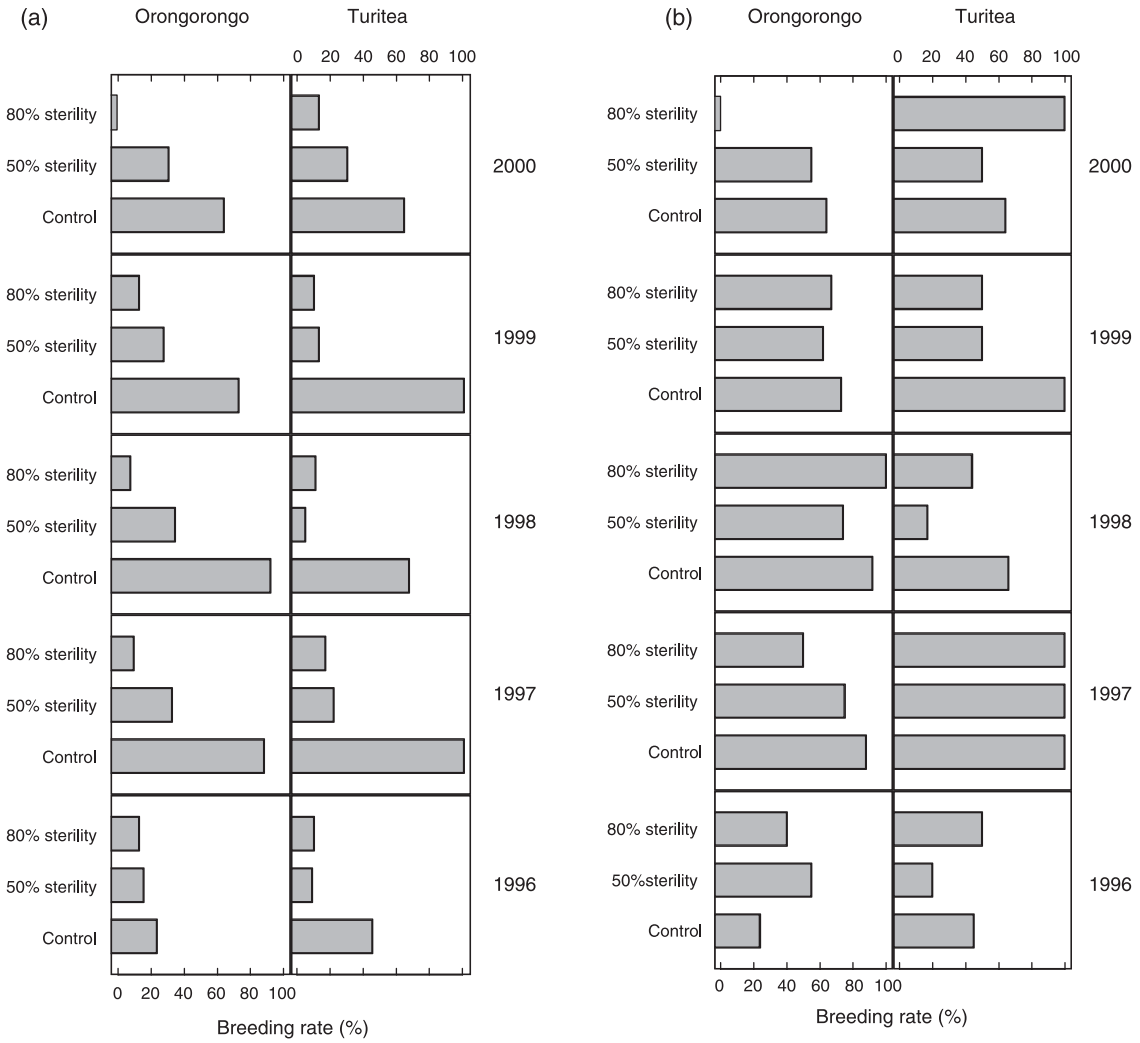

Fig. 1. Breeding parameter estimates for sterility treatment sites in the Orongorongo Valley and Turitea from 1996 to 2000. (a) The percentage of all adult females with dependent young in September–October of each year; (b) the percentage of adult fertile females with dependent young in September–October of each year.

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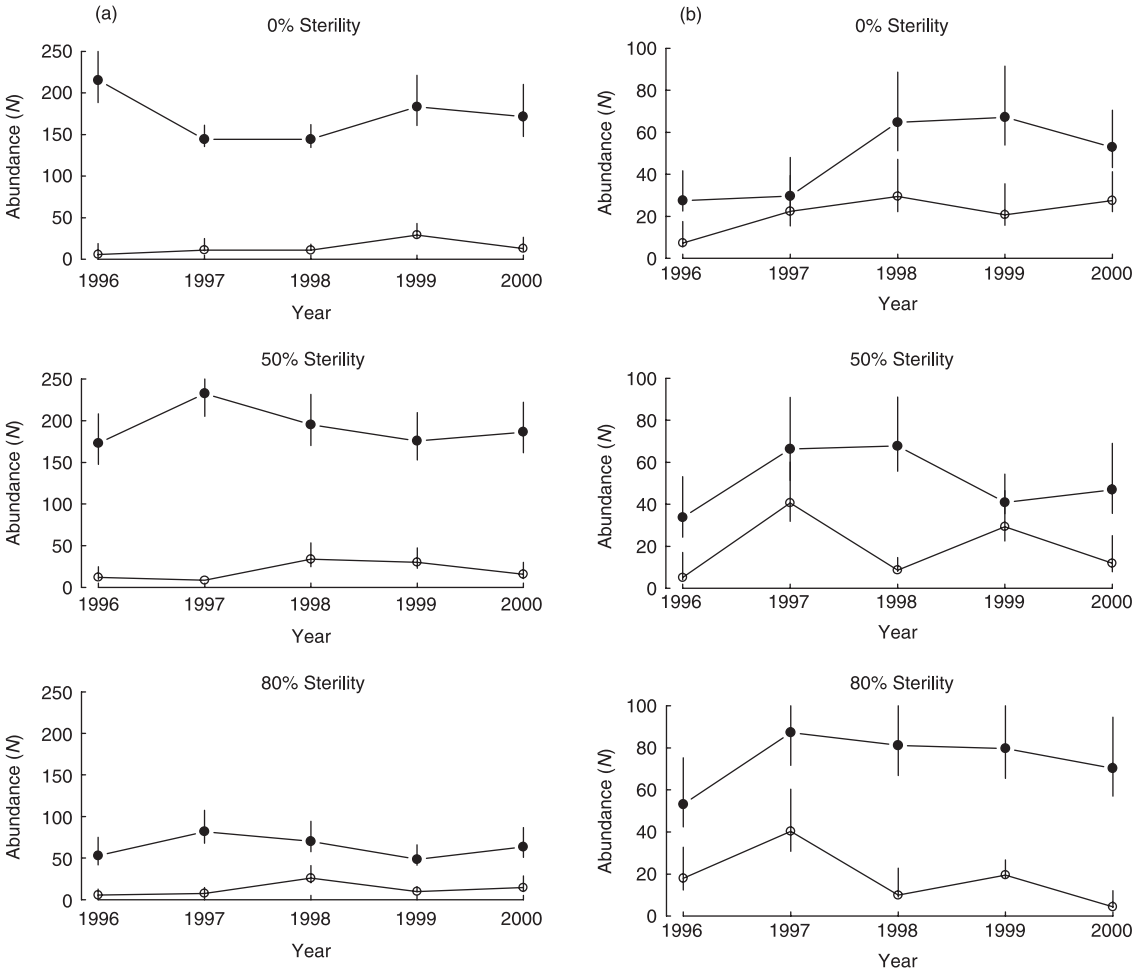

Fig. 2. Jack-knife estimates of population abundance (N) in September–October of each year between 1996 and 2000 for (a) sterility treatment sites in the Orongorongo Valley, and (b) sterility treatment sites at Turitea. Open circles are the yearling subpopulation size and closed circles are the adult subpopulation size. Error bars are the 95% confidence limits.

Table 1. The average population growth rate ($\bar{\lambda}$) between September and October each year from 1996 to 2000 for each sterility treatment site in each of the Orongorongo Valley and Turitea

	Turitea		Orongorongo	
	$\bar{\lambda}$	(95% CI)	$\bar{\lambda}$	(95% CI)
Control	1·21	(0·977–1·49)	1·01	(0·851–1·20)
50%	1·04	(0·709–1·54)	1·01	(0·900–1·14)
80%	1·01	(0·794–1·30)	1·06	(0·847–1·318)

Table 2. Results from logistic regression analysis of the effects of area [Orongorongo Valley (OV) and Turitea (TU)], adult population growth rate (λ_i^2) and sterility treatment on the proportional contribution of adult survivors to the adult population growth rate (γ_{i+1}^{22})

Term	D.f.	Deviance	Residual d.f.	Residual deviance	P
Null			23	163·5	
Area (OV or TU)	1	8·6	22	154·9	< 0·01
λ_i^2	1	72·7	21	82·2	< 0·01
Sterility treatment	2	3·4	19	78·8	0·19

each year to estimate annual age-specific capture probabilities indicated that models with capture probability constant across time and age classes, but differing for each site, were the most preferred based on $\Delta QAIC_c$ and Akaike weights.

The relative contributions to the adult population growth rate of adult survivors (γ_i^{22}), yearling recruits (γ_i^{21}) and immigrants ($1 - \gamma_i^{22} - \gamma_i^{21}$) and their bootstrap standard errors in each year are given in Appendix 1. Logistic regression analysis of γ_i^{22} revealed no significant differences between sterility treatments. However, there were significant differences between areas and a significant effect because of λ_i^2 (Table 2). Similar analysis for γ_i^{21} revealed no significant differences as a result of sterility treatment, area or λ_i^2 (Table 3). Survival of adults from the previous year (γ_i^{22}) made the dominant contribution to the adult population growth rate, contributing around 65% to the adult population growth rate when λ_i^2 was high ($1{\cdot}3\ \text{year}^{-1}$) and around 79% when λ_i^2 was low ($0{\cdot}9\ \text{year}^{-1}$) (Fig. 3). Recruitment of resident yearling-aged animals (γ_i^{21}) made a minor contribution

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Table 3. Results from logistic regression analysis of the effects of Area [Orongorongo Valley (OV) or Turitea (TU)], adult population growth rate (λ_i^2) and sterility treatment on the proportional contribution of yearling recruits to the adult population growth rate (γ_{i+1}^{21})

Term	D.f.	Deviance	Residual d.f.	Residual deviance	*P*
Null			23	14·7	
Area (OV or TU)	1	3·1	22	11·7	0·08
λ_i^2	1	0·4	21	11·3	0·53
Sterility treatment	2	2·3	19	8·9	0·31

Table 4. Results from logistic regression analysis of the effects of area [Orongorongo Valley (OV) or Turitea (TU)], yearling population growth rate (λ_i^1) and sterility treatment on the proportional contribution of locally born young to the yearling population growth rate (γ_{i+1}^{10})

Term	D.f.	Deviance	Residual d.f.	Residual deviance	*P*
Null			23	91·7	
Area (OV or TU)	1	22·2	22	69·5	< 0·01
λ_i^1	1	6·8	21	62·7	< 0·01
Sterility treatment	2	29·8	19	32·8	< 0·01

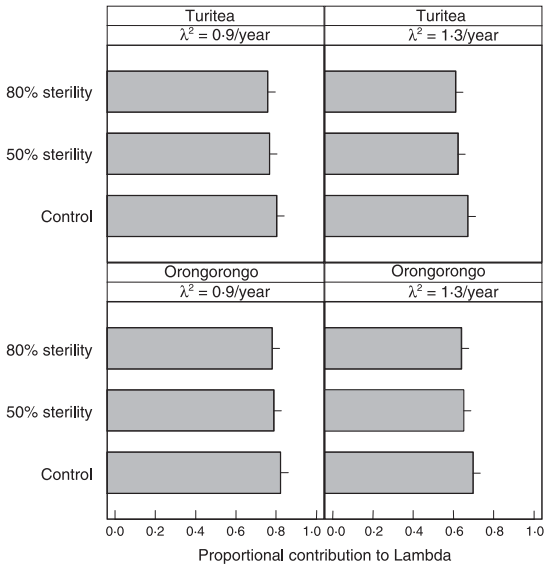

Fig. 3. Predicted values from a logistic regression model of the effects of area (Orongorongo and Turitea), adult population growth rate (λ_i^2) and sterility treatment on the proportional contribution of adult survivors to the adult population growth rate (γ_{i+1}^{22}). Predicted values are for each level for each categorical variable (area, sterility treatment) and for the upper and lower quartile of the observed values for λ_i^2. Error bars are the standard error.

to the growth rate of adults, especially on sites in the Orongorongo Valley (Fig. 4).

The relative contributions to the yearling population growth rate of recruitment of locally born young (γ_i^{10}) and yearling immigrants ($1 - \gamma_i^{10}$) and their bootstrap standard errors in each year are given in Appendix 1. Logistic regression analysis of the contribution of local recruits to the yearling population growth rate revealed that γ_i^{10} differed significantly because of the additive effects of area, λ_i^1 and sterility treatments (Table 4). Local recruitment made the dominant contribution to the yearling growth rate on control sites, predicted to contribute 51% to the growth rate of yearlings when λ_i^1 was low (0·5 year^{-1}) and 41% when λ_i^1 was high (2·5 year^{-1}) (Fig. 5). In comparison, local recruitment on 50% and 80% sterility sites was predicted to contribute only 21% and 19%, respectively, when λ_i^1 was low, and 14% and 13%, respectively, when λ_i^1 was high (Fig. 5).

Because of the significant reductions in γ_i^{10} as a result of sterility treatments, estimates of *per capita* local recruitment $\hat{B}_i$ were also reduced by sterility treatments. The number of offspring surviving until yearling age per adult female averaged 0·13 and 0·17 on control (0% sterility) sites in the Orongorongo Valley and Turitea, respectively, but only 0·03 and 0·05, respectively, on 80% sterility sites (Fig. 6). Estimates of $\hat{B}_i$ and their standard errors in each year are given in Appendix 1.

Model selection results for CJS models fitted to estimate survival rates of sterilized and fertile females revealed that models of capture probability that included the interactive effects of site, sterility treatment and time (year) received the most support, as judged by ΔAIC_c values. Using this best model of capture probability, four survival rate models that included either the additive or interactive effects of site and sterility, and an additive effect of time, received the most support as judged by ΔAIC_c values (Table 5). Although average

Table 5. Model selection results for CJS models fitted to estimate apparent annual survival (ϕ) for adult fertile and sterile female possums. *S*, site effect; *St*, sterility effect; *t*, time (year) effect. Models are ranked according to differences between the model AIC_c and the model with the lowest AIC*c* (ΔAIC_c). w_i indicates the relative support (Akaike weights) for each model. The capture probability component of all models includes the interactive effects of all factors (e.g. $S \times St \times t$)

Model	Deviance	*K*	AIC_c	ΔAIC_c	w_i
$\phi(S + St)$	215·4	38	1303·8	0	0·33
$\phi(S \times St)$	213·9	39	1304·5	0·70	0·23
$\phi(S)$	218·3	37	1304·6	0·74	0·23
$\phi(S + St + t)$	212·3	40	1305·2	1·35	0·17
$\phi(.)$	225·5	36	1309·5	5·69	0·02
$\phi(S \times t)$	199·5	48	1310·5	6·64	0·01
$\phi(S + t)$	216·1	41	1311·3	7·43	0·01
$\phi(St)$	225·5	37	1311·7	7·88	0
$\phi(S \times St + t)$	211·1	44	1313·0	9·20	0
$\phi(St \times t)$	218·4	41	1313·5	9·71	0
$\phi(t)$	223·8	39	1314·5	10·63	0
$\phi(St + t)$	223·6	40	1316·5	12·71	0
$\phi(S \times St \times t)$	189·5	55	1316·7	12·88	0

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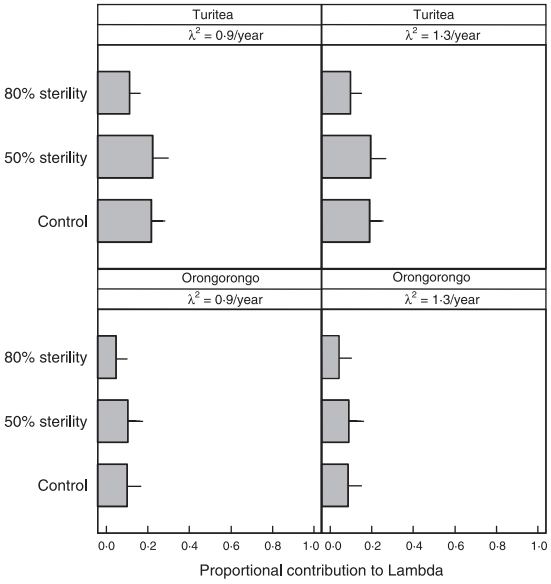

Fig. 4. Predicted values from a logistic regression model of the effects of area (Orongorongo and Turitea), adult population growth rate (λ_i^2) and sterility treatment on the proportional contribution of local yearling recruits to the adult population growth rate (γ_{i+1}^{21}). Predicted values are for each level for each categorical variable (area, sterility treatment) and for the upper and lower quartile of the observed values for λ_i^2. Error bars are the standard error.

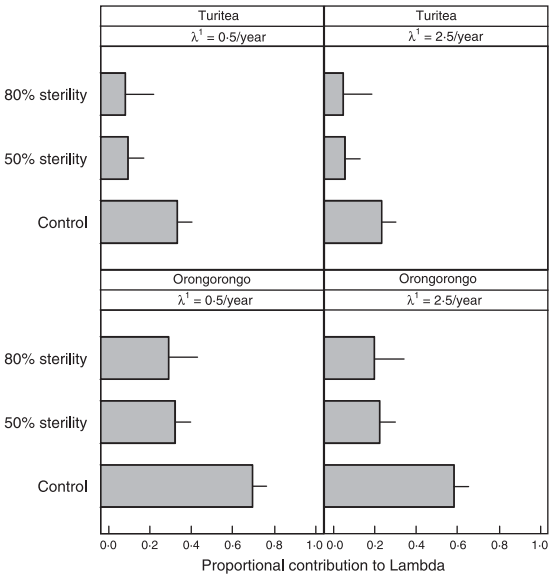

Fig. 5. Predicted values from a logistic regression model of the effects of area (Orongorongo or Turitea), yearling population growth rate (λ_i^1) and sterility treatment on the proportional contribution of local recruitment to the yearling population growth rate (γ_{i+1}^{10}). Predicted values are for each level for each categorical variable (area, sterility treatment) and for the upper and lower quartile of the observed values for λ_i^1. Error bars are the standard error.

survival rates of sterile females were higher than fertile females at the same site (Fig. 7), the 95% confidence intervals (CI) for the sterility term in the model with the most support ($S + St$) overlapped 0 ($\hat{\beta}_{sterility} = 0{\cdot}65$; 95% CI = −0·03, 1·32 on a logit scale). A likelihood ratio test between this model and the more restricted model without the sterility term indicated a marginal level of significance ($P = 0{\cdot}09$).

Discussion

The methods presented here for decomposing contributions to λ from different age classes differ slightly from the robust design approach given in Nichols *et al.* (2000). They advocate calculating age-specific capture probabilities using the ratio of the number caught to an estimate of population size using a closed capture estimator, for example:

$$\hat{p}_i^a = \frac{n_i^a}{\hat{N}_i^a}$$

where $\hat{p}_i^a$, n_i^a and $\hat{N}_i^a$ are the capture probability estimate, total number caught and population size estimate, respectively, for age class a in primary capture session i. Unfortunately, this estimator did not prove to be very efficient when applied to the possum capture data, with imprecision in estimates of $\hat{N}_i^a$ leading to many estimates of γ greater than 1 (and estimates of 1 − γ less than 0). Applying conventional age-structured CJS models in forward time for the estimation of the p_i^a greatly ameliorated this problem, as model selection procedures could be employed. This analysis suggested that capture probabilities for yearling- and adult-aged possums could be adequately modelled using estimates of p_i^a that were constant across time and age classes. An alternative approach using joint likelihoods and multistate models under the robust design also allows the construction of reduced parameter models (Nichols *et al.* 2000). However, because of the complications of estimating capture probability and population size of pouch young (which was dependent on the capture probability, population size and breeding rate of their mothers), this was not attempted for the possum data set.

Permanent sterilization of a proportion of females successfully suppressed breeding rates, regardless of whether the population was a notionally spring breeding population or not. In both the Orongorongo Valley (no spring breeding) and Turitea (spring breeding), fertile females on sterility sites did not breed earlier and did not have an increased breeding rate compared with females on control sites. Levels of spring breeding were also similar between sterility treatments. Hence, there was no evidence that fertile females on sterility sites attempted to compensate for reduced productivity caused by sterilization treatments.

Population abundance exhibited no significant upward or downward trend over the duration of the study, with the exception of the control site at Turitea, which exhibited an average 20% annual increase. Thus, reductions in the birth rate because of sterility treatments did not result in population declines over the course of the study. Examining the contribution of population

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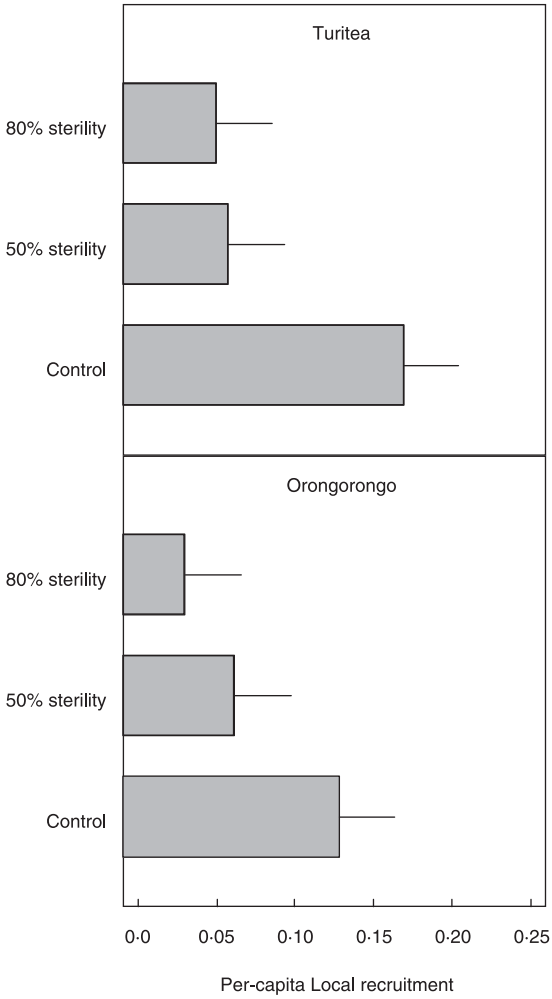

Fig. 6. Mean *per capita* local recruitment rates ($\hat{B}$) for each sterility treatment site in the Orongorongo Valley and Turitea. Error bars are the standard error of the mean ($n = 4$).

vital rates (births, survival and immigration) to annual population growth at each site revealed the contribution of underlying demographic processes driving annual population change.

Overall, sterility treatments suppressed *per capita* rates of local recruitment. In this context, local recruitment refers to both male and female young born *in situ* that survived until the late yearling stage (i.e. 20 months old) per adult female. Averaged over areas, the reduction in the *per capita* rate of local recruitment was 60% and 74% on 50% and 80% sterility treatments, respectively. The contribution of local recruitment to the population growth rate of yearlings was similarly reduced, but was dependent on the magnitude of the yearling population growth rate and differed among areas. The population growth rate of yearlings in year i (λ_i^1) that would have occurred through local recruitment only can be calculated as $\lambda_i^1 \gamma_{i+1}^{10}$. These calculations show that local recruitment only would be inadequate to replace the disappearance of yearling-aged possums through death, emigration or recruitment to the adult age class on any site (Table 6). However, average population growth rates revealed that the yearling subpopulation was increasing, on average, over the course of the study on all sites (Table 6). On sterility treatment sites, immigration made the dominant contribution to the population growth rate of yearlings, compared with that made by local recruitment. On control sites, local recruitment made the dominant contribution to the population growth rate of yearlings on one site only (Orongorongo Valley). At Turitea, the contribution to the yearling population growth rate was dominated by immigration on all sites. However, the contribution by local recruitment to the yearling population growth rate on sterility treatment sites was reduced compared

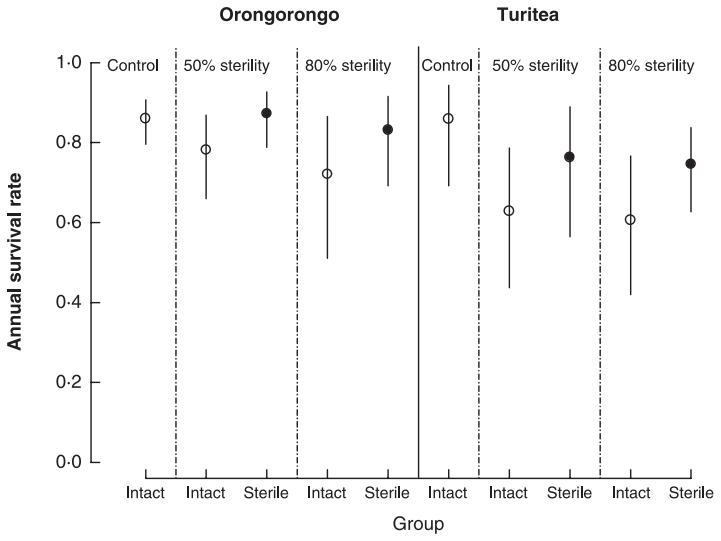

Fig. 7. Estimates of the mean annual apparent survival rates of intact (fertile) adult females (open circles) and sterile adult females (closed circles) for each sterility treatment site in the Orongorongo Valley and Turitea. Estimates are from model including the additive effects of site and sterility [$\phi(S + St)p(S \times St \times t)$] (see text for details).

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Table 6. The average population growth rate of yearlings on control and sterility treatment sites predicted from local recruitment only ($\lambda_i^1\gamma_{i+1}^{10}$) compared with the average population growth rate because of both local recruitment and immigration (λ_i^1)

	Orongorongo		Turitea	
	Local recruitment only	Including immigration	Local recruitment only	Including immigration
Control	0·94	1·51	0·25	1·60
50% sterility	0·39	1·63	0·13	3·11
80% sterility	0·09	1·96	0·12	1·20

with that on the control site, because of the reduction in *per capita* rates of local recruitment by sterility treatments.

This suppression of local recruitment indicates that the apparent survival rate of pouch young did not compensate for reductions in the birth rate because of sterility treatments. Hence, it is expected that the percentage suppression of local recruitment would be approximately linear with the percentage suppression of the birth rate as a result of sterility. The greater than expected suppression on 50% sterility sites, and the Turitea 50% sterility site in particular, was mainly because of the sterility rate on this site being higher than the nominal 50% over most of the study period (Fig. 1a). In addition, there were no compensatory increases in either the yearling recruitment to the adult population or the annual apparent survival rates of adults in terms of their contribution to the adult population growth rate. Hence, the only demographic response to artificial reductions in fertility has been compensatory increases in the immigration rate of yearling-aged possums, resulting in stable population trajectories ($\lambda = 1$). These results are in contrast to the studies of Twigg & Williams (1999) and Twigg *et al*. (2000) on the effect of sterility on wild rabbit populations in Australia, who found that local recruitment rates compensated for reductions in the birth rate as a result of sterility. One caveat to our conclusion, however, is that densities of possums on most sites have been relatively constant, because of the stabilizing effect of immigration. Hence, density-dependent compensation in local recruitment or adult apparent survival was unlikely to be observed.

The compensatory yearling immigration observed in this study suggests that yearling dispersers may be tuned to the local density of resident yearlings. Hence, a decision to 'settle' in a particular area may be partially based on an assessment of the number of resident yearlings, as an indication of the number of potential 'vacancies'. This makes sense for a species with high adult survival, where existing resident yearlings could rapidly fill any vacancies caused by turnover in the adult subpopulation. However, the rules governing settlement decisions by dispersing possums remain speculative (Efford 1996; Efford 1998). However, if true, this mechanism governing settlement by dispersing yearlings could have direct implications for the operational success of fertility control, as spatial variation in fecundity (i.e. spatial variation in the proportion sterilized) is likely to be inversely related to the settlement patterns of dispersing yearling possums that will limit the overall effectiveness of fertility control. Spatial variation in the proportion sterilized is likely where vectored immunocontraception is used (Barlow 1994) and there is spatial aggregation of the vector. Likewise, fertility control applied at too small a spatial scale would be nullified by immigration.

Comparisons of the survival rates of fertile and sterile females suggested that sterile females had higher survival rates. Such a finding is expected if there are significant costs to reproduction, and is similar to the results observed for the effect of sterility on female rabbit survival (Twigg *et al*. 2000). Theoretical studies of fertility control have explored the potential for changes in a population's vital rates to compensate for reductions in the birth rate (Bomford 1990; Caughley, Pech & Grice 1992; Barlow, Kean & Briggs 1997; Sinclair 1997; Hobbs, Bowden & Baker 2000; Hood, Chesson & Pech 2000; Davis & Pech 2002). Generic stage-structured modelling undertaken by Davis & Pech (2002) suggested the overall response of populations to fertility control was critically dependent on the role of sterilized females in density-dependent processes. If sterile females are not involved in any density-dependent process then they accumulate in the population without (negatively) influencing the population growth rate. Such a scenario can lead to fertility control, resulting in higher populations than uncontrolled populations (Davis & Pech 2002). However, such a scenario is unlikely for brushtail possums as the existing evidence suggests possums are regulated primarily by extrinsic rather than intrinsic mechanisms (i.e. competition for food resources) (Thomas *et al*. 1993; Ramsey *et al*. 2002). Thus, sterile females are expected to play a role in any exploitative competition for food and therefore should contribute to any density-dependent response.

Presently, there are no data on the probable magnitude of compensatory responses to fertility control in brushtail possums at reduced population density. As the most likely scenario for practical application of fertility control is following an initial population reduction using conventional control (Cowan 1996; Bayliss & Choquenot 1999), additional studies will need to be undertaken to determine the operational nature and magnitude of any density-dependent compensatory responses. This will be necessary so that the potential of fertility control for inhibiting population recovery

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can be predicted. Future studies will need to reduce population density experimentally on sites with imposed sterility to determine the effect of reduced density on both recruitment and mortality, and attempt to determine the strength and nature of any compensatory responses.

In summary, the results to date suggest sterility rates of 50% or greater can result in at least a 50% reduction in *per capita* recruitment when populations are close to carrying capacity (K). However, moderate levels of immigration can ameliorate this reduction. It is not known to what extent density-dependent compensation in recruitment rates will impair the effectiveness of fertility control. The high contribution of adult survival to the annual population growth rate of adults, and the higher survivorship of sterile females compared with fertile females, suggests the benefits of fertility control would be enhanced if integrated with conventional control to provide rapid initial population reduction. However, population models will need data on the nature and strength of compensatory responses at reduced density to improve predictions on the rate of recovery of possum populations following application of integrated conventional and fertility control strategies.

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Supplementary material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JPE/JPE1006/JPE1006sm.htm.

Appendix 1. The proportional contribution to the annual population growth rate by age and treatment, and the *per capita* local recruitment rates

Appendix 2. Summary statistics used in the mark–recapture analysis

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Research Article

Effects of Fertility Control on Behavior and Disease Transmission in Brushtail Possums

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ABSTRACT Fertility control is currently under development for the control of brushtail possums (*Trichosurus vulpecula*), one of New Zealand's most serious vertebrate pests. Despite intensive research into various methods for achieving infertility, including immunocontraception and disrupting endocrine control of reproduction, researchers know little about the potential effects of these methods on the behavior of wild possums. We assessed the effects of surgically imposed sterility, either to block fertilization (tubal ligation) or to disrupt endocrine control of fertility (gonadectomy), by using radiotelemetry on the movement patterns and site fidelity of wild brushtail possums. In addition, we assessed the effect of gonadectomy on the transmission rate of a commonly occurring, directly transmitted pathogen in possums, *Leptospira interrogans* serovar *balcanica* (hereafter *L. balcanica*), to determine the effect of any behavioral changes on possum contact rates. Both tubal ligation and gonadectomy of females did not appear to have any appreciable effect on behavior, with sterilized females having space-use patterns and fidelity to seasonal breeding ranges similar to those of fertile females. However, gonadectomy of male possums resulted in a significant reduction of 42% and 47% in the 95% and 70% isopleth seasonal breeding ranges, respectively. Furthermore, the transmission rate of *L. balcanica* in gonadectomized male and female possums was reduced by 88% and 63%, respectively, compared with that in fertile male and female possums. Overall, these results suggest that fertility control, either by blocking fertilization (e.g., immunocontraception) or by disrupting endocrine control of reproduction (e.g., gonadotropin-releasing hormone vaccines), is unlikely to have an impact on social organization and behavior of brushtail possums in ways that may compromise the efficacy of fertility control for reducing population density. However, the reduction in the transmission rate of *L. balcanica* indicates that fertility control that interferes with endocrine control of reproduction is likely to reduce the contact rate between possums. This could have implications for the control of other wildlife diseases requiring direct contact for transmission. (JOURNAL OF WILDLIFE MANAGEMENT 71(1):109–116; 2007)

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KEY WORDS disease transmission, fertility control, home range, *Leptospira interrogans* serovar *balcanica,* leptospirosis, *Trichosurus vulpecula*.

Brushtail possums (*Trichosurus vulpecula*) are a serious vertebrate pest in New Zealand, and research into methods of fertility control of brushtail possums was initiated to develop techniques for environmentally acceptable, humane, and cost-effective population control (Cowan 1996, Roger 1997). Current research has focused on developing immunological blocks to fertilization by immunizing possums against proteins specific to the zona pellucida or sperm (i.e., immunocontraception; Roger 1997, Cowan 2000) or by interfering with central endocrine control of reproduction, such as immunization against gonadotropin-releasing hormone (GnRH) or using GnRH-toxin conjugates that disrupt pituitary gonadotropes (Herbert and Trigg 2005). Recent work has documented the effect of induced sterility on the population dynamics of brushtail possums (Ramsey 2005), but an important issue remains about the potential effects of fertility control on possum behavior. Methods of fertility control that interfere with endocrine control of reproduction may have adverse behavioral repercussions, such as effects on dominance hierarchies that may lead to higher fertility rates in subordinate individuals (Caughley et al. 1992). In contrast, methods of fertility control that block fertilization, such as immunocontraception, should leave the individual hormonally intact and should not significantly interfere with any established social structure.

Brushtail possums are essentially solitary and spend less than 1% of their time directly interacting, except during the breeding season (Day et al. 2000). In New Zealand populations, possums have widely overlapping ranges and do not appear to defend a territory, but at low density they may have an exclusive area that is defended (Day et al. 2000). Male possums generally have a larger range size than females, and male ranges are larger in summer and autumn during the breeding season than in other months, apparently to maximize their access to reproductive females (Cowan and Clout 2000, Ramsey et al. 2002). Although no studies have directly examined the effects of fertility control on the social organization of wild possums, disruption of sex steroid hormone levels in dominant male and female possums in captive populations did not appear to alter the existing dominance status of individuals (Jolly and Spurr 1996, Jolly et al. 1996). However, if the larger range size in male possums is dictated largely by the need to gain access to reproductive females, then it is predicted that hormonally compromised male possums should have a reduced range size compared with that of intact males.

The indirect effects of fertility control on behavior in wild populations have received some attention; the transmission rate of a common, directly transmitted pathogen of possums, *Leptospira interrogans* serovar *balcanica* (hereafter *L. balcanica*), increased by 28% in populations in which approximately 80% of adult females were surgically sterilized by tubal ligation compared with untreated populations. Although the overall effect was statistically weak ($P = 0.16$), the observed increase in the transmission rate was hypothesized to be due to sterile but hormonally intact females having a higher contact rate due to an increased frequency of estrous cycles during the breeding season

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(Caley and Ramsey 2001). An increase in estrous activity due to tubal ligation has been demonstrated in both wild (Ji et al. 2000) and captive individuals (Rekha 1997). Caley and Ramsey (2001) also postulated that methods of fertility control that disrupt endocrine control of reproduction could have the reverse effect, with a reduction in the transmission rate of directly transmitted pathogens due to a decrease in the contact rate associated with mating.

We assessed the effects of both disrupting endocrine control of fertility (gonadectomy of M and F) and blocking fertilization (tubal ligation of females) on patterns of space use and site fidelity. In addition, we assessed the effect of disrupting endocrine control of fertility on contact rates between possums by comparing the transmission rate of *L. balcanica* between gonadectomized and intact possums.

STUDY AREA

We conducted the study on a population inhabiting a remnant patch of native forest on farmland near Porewa, Manawatu, New Zealand (40°01′N, 175°29′E). The site encompassed 3 closely spaced patches of native forest totaling about 14 ha, was surrounded by dairy pasture, and contained a population of approximately 100 possums. The forest canopy was dominated by tawa (*Beilschmiedia tawa*) and northern rata (*Metrosideros robusta*) as emergents, with mahoe (*Melicytus ramiflorus*), titoki (*Alectryon excelsus*), and kaikomako (*Pennantia corymbosa*) as subdominants. The understory was dominated by *Coprosma areolata* and ongaonga (*Urtica ferox*), with a sparse ground layer of ferns (predominantly *Blechnum* spp.), grasses, and forbs. Cattle periodically grazed in the forest.

METHODS

We placed 60 cage traps on the ground throughout the forest on a grid at 30-m spacing and we trapped for 4 consecutive nights, 3 times per year, in January, June, and September from September 1996 until January 2000. Thereafter, trapping occurred bimonthly until August 2001. We anaesthetized all captured possums with ether and gave each a unique tattoo and ear tag for identification. Capture rates of possums were high (60–80% over a 4-night session), ensuring that we recaptured individuals relatively frequently. We judged maturity in females from the presence of a fully formed pouch and evidence of past or existing lactation, and in males from the presence of testes >13 mm in diameter. We scored age from tooth wear following Cowan and White (1989).

Sterility Treatments

We artificially imposed 2 alternative methods of fertility control on individual possums to determine their potential effects on behavior. The first method was surgical ligation of female oviducts as a model for methods of fertility control that would potentially not interfere with hormonal regulation of fertility (e.g., immunocontraception targeting the zona pellucida). The second method was surgical removal of the gonads in both male (testes) and female possums (ovaries) as a model for methods of fertility control that block endocrine control of reproduction (e.g., immunization against GnRH).

We transported possums selected for sterilization to the laboratory in burlap sacks, where we performed surgery under aseptic conditions. We gonadectomized males transscrotally and we performed ovariectomy or tubal ligation in females through ventral midline incision. We did not perform any sham operations on control animals, as previous research in possums has shown that similar surgical procedures had no detectable effect on either possum body condition (Ji et al. 2000) or survival (Ramsey 2005). We placed possums in sacks in a quiet, warm room overnight to recover before being released at their point of capture. There was no evidence, based on trapping records postsurgery, that we displaced treated possums from their previous range. We performed all surgical procedures with approval from Landcare Research's Animal Ethics Committee (AEC No. 00/5/3).

Effect of Fertility Control on Seasonal Breeding Ranges and Movements

We examined the effects of fertility control treatments on the seasonal breeding range and movements of possums using radiotelemetry. Between January 1997 and January 2001, we fitted possums with radiocollars (Sirtrack Ltd., Havelock North, New Zealand) and tracked their movements from 2 fixed stations using twin-element Yagi null-peak antennas operated manually. We determined locations by triangulation of the bearings obtained from each station. Operators maintained radio contact, ensuring that we obtained bearings for each animal nearly simultaneously. We located possums 6 times per night over a 6-hour period beginning from sunset for 2 nights per week during the main breeding period (e.g., between Mar and May). The nightly schedule covered the period of greatest activity by possums (Ward 1978, Efford et al. 2000). We applied surgical sterilization to a random selection of male and female possums in January each year from 1998 to 2001.

We estimated the accuracy (bias and precision) of the radiotelemetry system by triangulating locations of radiocollars placed at known locations within the forest. During January 1997, we estimated the locations of a sample of trap sites in the forest using a differential Global Positioning System (GPS) using a mobile receiver unit placed on a 10-m pole to gain access above the canopy. We estimated locations recorded with this GPS system to within ±1 m. Those trap sites that could not have their location estimated using GPS (due to canopy ht and cover) had their location estimated using distance and bearing measurements taken from the nearest known trap location (i.e., within 30–60 m). During May, July, August, and December 1997, we estimated between 28 and 56 trap site locations within the forest by triangulation by placing radiocollars near ground level (<30 cm above ground), with operators asked to obtain bearings simultaneously. Operators had no knowledge about the whereabouts of each collar, only that it was somewhere in the forest. We estimated the bias of each receiving station by obtaining the mean difference between the estimated

bearing and true bearing for each known location, with precision given by the standard deviation of the bearing errors. We obtained an additional estimate of telemetry error by calculating the mean linear distance between the estimated and true locations. The mean linear distance between estimated and true locations is regarded as the best estimate of telemetry error as it takes into account both angular error and distance between the receiving stations and the transmitter (Zimmerman and Powell 1995).

We analyzed the distribution of the difference between estimated and true bearings between each receiving station and known locations for normality. Quantile plots revealed significant deviation from a normal distribution due to the presence of a few outlying values in both bearing errors and linear distances. Therefore, summary statistics used the median and median absolute deviation (MAD) to describe the location and scale of these distributions. We then used the average of the bearing error scale estimates to calculate error polygons for each location estimate (White and Garrott 1990).

We estimated seasonal breeding ranges for individual possums using a fixed bivariate normal kernel density surface (Worton 1989) estimated on a 10-m grid. Kernel density surfaces using fixed bandwidths are considered the most reliable method for estimating range size from radiotelemetry data (Powell 2000, Hemson et al. 2005). We estimated the bandwidth of the Gaussian kernel separately in the X (easting) and Y (northing) directions using the direct plug-in method (Sheather and Jones 1991, Wand and Jones 1995). This method of selecting the bandwidth from data is considered more optimal than older methods such as least-squares cross validation (LSCV; Wand and Jones 1995). In addition, recent studies have cast doubt on the reliability of LSCV for estimating the bandwidth from radiotelemetry data (Hemson et al. 2005). Once we fitted a density surface, we calculated the home range as the area that encompassed 95% of the probability density surface using bilinear interpolation. In addition, we calculated a core area as the area that encompassed 70% of the probability density surface. We considered core area ranges a more stable estimate of range size as a greater number of observations are used to fit the contour (i.e., the contour is less influenced by 1 or 2 extreme locations). We calculated all home range estimation procedures using the R statistical package (R Development Core Team 2005) using the KernSmooth package.

Disease Transmission

Leptospira balcanica (Kmety and Dikken 1993) is a commonly occurring pathogen in possums that is spread largely by possum-to-possum contact during affiliative or sexual interactions (Day et al. 1997*b*, 2000). Clinical disease due to *L. balcanica* infection in possums is rare (Day et al. 1997*a*) and point prevalence of the disease in wild populations is high, between 40% and 70% (Caley and Ramsey 2001). *Leptospira balcanica* appears to be transmitted primarily through sexual contact, and it was considered to be a useful model for determining the effect of fertility control on disease transmission.

From January 2000, we trapped at bimonthly intervals until August 2001. At each bimonthly trapping occasion, captured possums had approximately 5 ml of blood drawn from a tail vein. We determined the presence of *L. balcanica* infection in each sample using a serological microagglutination test (MAT), using doubling dilutions of serum beginning at 1:50, to determine the presence of leptospiral antigens (Horner et al. 1996). If we saw no reaction in the 1:50 dilution, we scored the result as negative (e.g., Caley and Ramsey 2001). Between January and September 2000, we surgically removed the gonads of a random sample of adult possums that were sero-negative for *L. balcanica*. A random sample of non-treated adult possums, also sero-negative for *L. balcanica,* served as controls.

Analysis

Seasonal breeding ranges and movements.—The number of years (breeding seasons) that we tracked a particular possum varied among individuals, as some individuals provided range estimates as both control and treatment individuals in consecutive years, while others provided home range estimates as just control or treatment in a single year. However, range estimates for individuals in multiple years were likely to be serially correlated. To account for this nonindependence of observations, we analyzed range estimates using linear mixed-effect models (Pinheiro et al. 2005). We fitted possum identification number as a random effect with the added effects of year and sterility treatment fitted as fixed effects. We modeled serial correlation among range estimates for individual possums by assuming the correlation structure followed a first-order autoregressive process. Models, fitted separately for males and females, included the fixed effects sterility treatment and year and their interaction. We assessed the significance of terms in the model using F-ratio tests calculated using marginal sums of squares. We natural log–transformed all estimates of range size before analysis to stabilize the variance.

As a further test of the effect of sterility treatment on seasonal breeding range size, we selected possums that had both pre- and posttreatment range estimates in consecutive years. We used paired t-tests to test for differences in range size pre- and posttreatment for each sex. We performed similar paired t-tests on possum range size in consecutive years for possums that did not receive a sterilization treatment. In addition to changes in range size, we compared the distance between centers of activity, defined as the point of highest probability density, derived from the kernel density surface, pre- and posttreatment to determine the extent of range fidelity between years among both intact and sterility-treatment individuals.

Transmission of L. balcanica.—The statistic of interest was the force of infection or instantaneous incidence, defined as the per capita rate that susceptible individuals become infected (McCallum et al. 2001). We obtained estimates of the force of infection (λ) by fitting exponential (constant force of infection) models to the disease incidence

data or failure times, recorded in discrete 2-month intervals, using maximum likelihood. We defined the failure time as the interval between the possum entering the study and the time of infection or censoring. We used right censoring to indicate the possum was either lost to follow-up (disappeared) or survived (and was seen) at the end of the study without record of infection. Hence, a right-censored time indicated that a possum was at risk of infection between entering the study and the time of censoring. For *L. balcanica*, we recorded a positive MAT for a possum at any point following its entry in the study as an infection event. We compared estimates of the force of infection between sterility treatments (gonadectomy/intact) and sex. We fitted main effects and the 2-way interaction sequentially and estimated their statistical significance by analysis of deviance (Collett 1991). We estimated mean times to infection events using the Kaplan–Meier product-limit estimator. We undertook all statistical analyses in R (R Development Core Team 2005).

RESULTS

The median difference between estimated and true bearings revealed that bearing errors were approximately unbiased for receiving station A (mean angular error of +0.2°). However, estimates for receiving station B revealed a negative bias of approximately 4° during the May, July, and August samples, indicating the presence of a misaligned compass rose. We corrected this, and the estimates we obtained during the December sample were approximately unbiased (mean angular error of +1.4°). We adjusted all bearings from the B receiving station taken during the 1997 breeding season by +3.9° to remove this bias.

Estimates of the median linear distance between estimated and true locations revealed that the effect of bearing errors was highest during the May, July, and August samples when the bias in the B receiving station was present (mean linear error of 44 m) and lowest in the December sample, following the correction of the bias (mean linear error of 28 m). Hence, following correction we expected that the median linear error would be in the order of 30 m. The mean of the MAD estimates of scale for receiving stations A and B were 2.3° and 2.1°, respectively. Therefore, we used 2.2° as the estimate of bearing precision to calculate error polygons for each location. We used only locations with error polygons <2 ha in area in further analyses.

Effect of Fertility Control on Seasonal Breeding Ranges and Movements

The numbers of possums in each treatment group in each year varied throughout the study (Table 1). For males, there were 81 estimates of seasonal breeding range size for 41 individual radiocollared possums, whereas for females there were 74 estimates of seasonal breeding range size for 41 individually collared females. The number of locations having error polygons ≤2 ha in area, used to calculate each seasonal range, ranged from a minimum of 15 to a maximum of 153, with the average number of locations per range being 73. The average size of the error polygons for this reduced data set was 0.5 ha.

Table 1. Numbers of radiocollared brushtail possums in each treatment group present in each year of the study, between 1997 and 2001 at Porewa, Manawatu, New Zealand.

		Yr				
Sex	**Group[a]**	**1997**	**1998**	**1999**	**2000**	**2001**
F	Intact	12	5	7	11	2
	Tubal	0	7	4	0	0
	Ovx	0	0	4	5	8
M	Intact	16	21	7	13	3
	Castrate	0	0	4	5	8

[a] Intact = non-treated possums, tubal = female possums sterilized by ligation of the oviducts, ovx = females sterilized by surgical removal of the ovaries, castrate = males sterilized by surgical removal of the testes.

The linear mixed modeling undertaken for both males and females indicated no significant interaction between year and sterility treatment for both 95% and 70% isopleth ranges (likelihood ratio tests $P > 0.29$). Therefore, we removed the interaction term from these models. For males, *F*-statistics calculated using marginal sums of squares indicated no significant effect due to year (95% isopleth: $F_{1,38} = 1.83$, $P = 0.18$; 70% isopleth: $F_{1,38} = 2.62$, $P = 0.11$) and a marginal level of significance for sterility treatment (95% isopleth: $F_{1,38} = 3.14$, $P = 0.08$; 70% isopleth: $F_{1,38} = 2.94$, $P = 0.09$; Figure 1). However, 2 outlying observations had a large influence on this result (1 intact M and 1 gonadectomized M; Fig. 1), and when removed, the effect of sterility treatment was highly significant (95% isopleth: $F_{1,36} = 6.88$, $P = 0.01$; 70% isopleth: $F_{1,36} = 7.09$, $P = 0.01$). For females, the equivalent *F*-statistics indicated no significant effect due to year (95% isopleth: $F_{1,30} = 2.1$, $P = 0.16$; 70% isopleth: $F_{1,30} = 0.34$, $P = 0.56$) or sterility treatments (95% isopleth: $F_{2,30} = 1.36$, $P = 0.27$; 70% isopleth: $F_{2,30} = 1.66$, $P = 0.21$) on seasonal breeding range size (Fig. 2). Removal of 2 outlying observations (1 intact F and 1 ovariectomized F; Fig. 2) made no difference to this analysis (sterility treatment effect: 95% isopleth: $P = 0.27$; 70% isopleth: $P = 0.21$).

We further tested the effect of sterility treatment using the subset of possums that had both pre- and posttreatment seasonal breeding range estimates in consecutive years. Paired *t*-tests indicated that males that were gonadectomized had a significant reduction in both their 95% and 70% isopleth ranges in the year following their treatment application, with a mean reduction in range size of 42% in the 95% isopleth ($t = 2.5$, df = 6, $P = 0.04$) and a 47% reduction in the 70% isopleth ($t = 3.1$, df = 6, $P = 0.02$). This compares with the sample of intact males, whose range size did not significantly change in the following year when we applied no treatment, with a 10% reduction recorded in the 95% isopleth ($t = 0.51$, df = 8, $P = 0.62$) and an 18% reduction in the 70% isopleth ($t = 1.05$, df = 8, $P = 0.32$). Comparisons of distance between centers of activity indicated that intact males had a mean shift in their centers of activity of 53 m between years. This compares with a

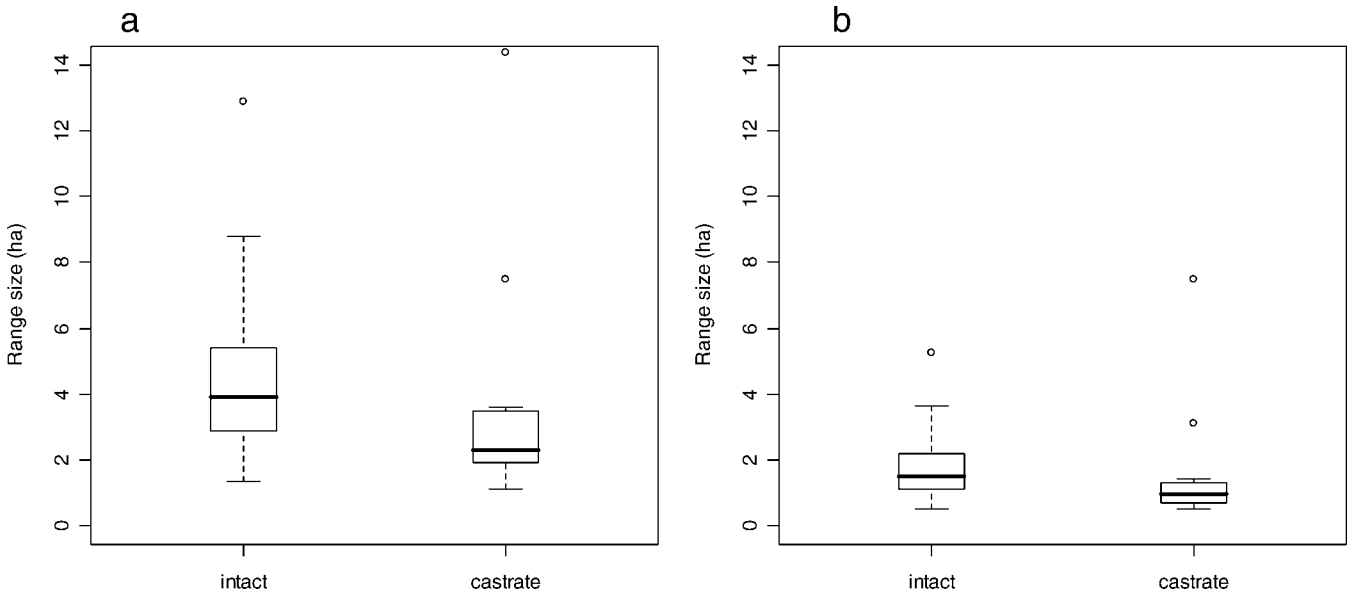

Figure 1. Box and whisker plots of the (a) 95% isopleth and (b) 70% isopleth seasonal breeding range size of intact and castrated male brushtail possums between 1997 and 2001 at Porewa, Manawatu, New Zealand. The length of the box represents the inter-quartile range; the thick horizontal line represents the median, and the whiskers reach to the most extreme data points within 1.5× the inter-quartile range from ends of the box. Circles represent data points outside this range (outliers).

mean range shift of 63 m for castrated males. These were not significantly different ($t = 0.54$, df = 13.5, $P = 0.6$ using Welch's approximation).

For females, paired *t*-tests indicated that females that were ovariectomized had a significant reduction in their 95%, but not in their 70% isopleth range size in the year following the application of the sterility treatment, with a mean reduction of 53% in the 95% isopleth ($t = 2.6$, df = 5, $P = 0.05$) and 44% in the 70% isopleth ($t = 1.8$, df = 5, $P = 0.13$). However, analysis of a similar sample of untreated females indicated that their range size also reduced in the same consecutive years with a mean reduction of 56% in the 95% isopleth ($t = 2.5$, df = 3, $P = 0.09$) and 55% in the 70% isopleth ($t = 2.2$, df = 3, $P = 0.11$), casting doubt about whether there was a true treatment effect. We did not conduct similar analysis on females that were given tubal ligations as there were insufficient numbers of non-treated female possums tracked in the same consecutive years for valid comparisons. Comparison of distance between centers of activity indicated that intact females had a mean shift in their centers of activity of 47 m between years. This compares with a mean range shift of 41 m for ovariectom-

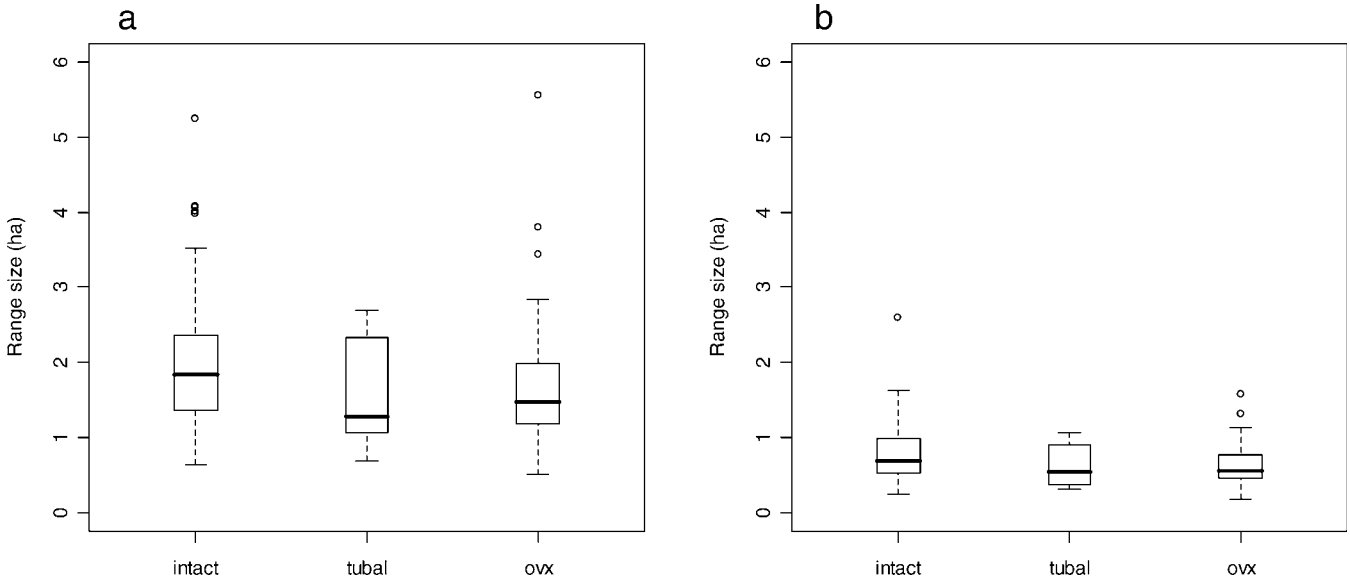

Figure 2. Box and whisker plots of the (a) 95% isopleth and (b) 70% isopleth seasonal breeding range size of intact female, tubally ligated female (tubal), and ovariectomized female (ovx) brushtail possums between 1997 and 2001 at Porewa, Manawatu, New Zealand. The length of the box represents the inter-quartile range; the thick horizontal line represents the median, and the whiskers reach to the most extreme data points within 1.5× the inter-quartile range from ends of the box. Circles represent data points outside this range (outliers).

ized females. As for males, these were not significantly different ($t = 0.21$, df = 8.3, $P = 0.84$ using Welch's approximation).

Disease Transmission

We randomly selected 26 possums to receive gonadectomies (12 M and 14 F) and 33 sexually intact possums served as controls (17 M and 16 F). Of the gonadectomized possums, 3 had sero-converted by the end of the study (2 M and 1 F). This compares with 10 control possums that had sero-converted over the same period (5 M and 5 F). The mean times to infection were 13.2 months (SE = 1.18) for intact possums and 18.7 months (SE = 0.72) for sterilized possums. Comparisons of the force of infection estimates between treatments and sex revealed that only the effect of treatment was significant ($P = 0.01$; Fig. 3). The estimated force of infection in gonadectomized possums was reduced by 88% in males and by 63% in females, compared with that in intact control possums.

DISCUSSION

Results from this study suggest that methods of fertility control that interfere with endocrine control of reproduction may significantly alter the behavior of brushtail possums. Although there is no convincing evidence of a direct effect of tubal ligation or ovariectomy on the behavior of females, castration of males significantly reduced their seasonal breeding range size compared with that of intact males in terms of their space-use patterns or range fidelity. Previous research has investigated the effect of surgical sterilization (tubal ligation) and hormonal sterilization of rats (*Rattus argentiventer*) on home range size and fidelity (Jacob et al. 2004). In contrast to this study, female rats receiving tubal ligations had significantly larger ranges than either hormonally sterilized or fertile rats. Studies in territorial species, such as foxes, have shown that surgically sterilized (but hormonally competent) females maintained similar-sized territories as intact females (Saunders et al. 2002). In pen trials, sterilized females did not appear to lose their dominance following sterilization (Newsome 1995). However, the effect of sterilization by endocrine disruption on the behavior of wild foxes is unknown.

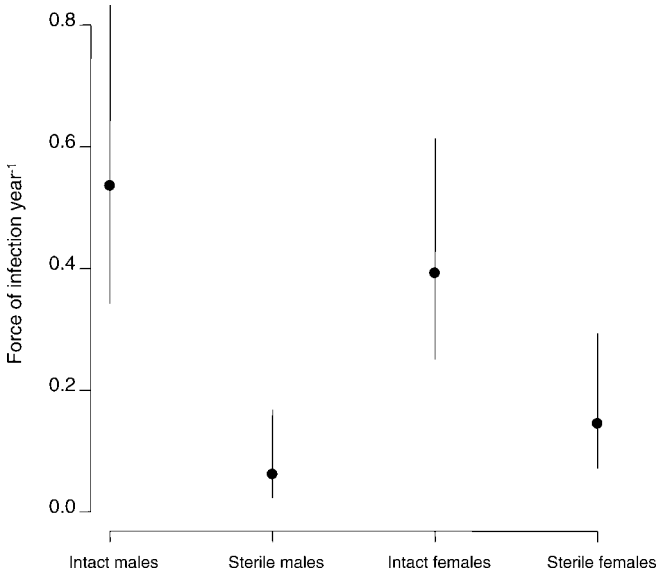

Figure 3. Estimates of the force of infection (λ ± SE) of *Leptospira interrogans* serovar *balcanica* for intact and sterilized (by gonadectomy) male and female brushtail possums between 2000 and 2001 at Porewa, Manawatu, New Zealand.

One potential negative outcome of sterilization by endocrine disruption of fertility of females in wild populations is that animals will lose their status and be forced to vacate their current territory. This could lead to compensatory immigration of fertile females into the area previously occupied by sterilized females, potentially negating the effect of fertility control (Jacob et al. 2004). Studies in captive possum populations have indicated that ovariectomy of the dominant female did not change her dominance status (Jolly and Spurr 1996, Jolly et al. 1996). In the present study, both 95% and 70% isopleth seasonal breeding ranges of ovariectomized females were similar to both intact and tubally ligated females. In addition, ovariectomized females were no more likely to shift their ranges than intact females. This suggests that female possums in wild populations that are sterilized by endocrine disruption of fertility would not lose their dominance status and hence are likely to maintain the same fidelity to their ranges following sterilization as fertile females.

In addition, gonadectomy of both males and females significantly reduced the incidence of *L. balcanica* infection compared with that in intact possums. These results support the hypothesis of Caley and Ramsey (2001), who suggested that methods of fertility control that interfere with hormonal control of reproduction would result in a reduction in the horizontal transmission rate of directly transmitted pathogens, due to a reduction in the contact rate. A study of contacts between male and female brushtail possums in the wild using proximity data loggers revealed that contacts were primarily sexual in nature, occurring more frequently in the breeding season with individual females interacting with up to 4 males (Ji et al. 2005). In addition, a study by Ramsey et al. (2002) found that male, but not female, home range size increased during the breeding season when population density was artificially reduced. The result of this range enlargement was that the male contact rate with females did not reduce in proportion to the reduction in density. This suggested that males were attempting to maintain their contact rate with females following the density reduction (Ramsey et al. 2002). Hence, the reduction in the transmission rate of *L. balcanica* in gonadectomized possums observed in this study was probably due primarily to a reduction in the contact rate associated with mating. Numerous studies have illustrated the effects of male castration on reduction in sexual behavior (Larsson 1978, McGill 1978, Lisk and Heimann 1980, Knol and Egberlink-Alink 1989*b*). In general, castration is followed by a decline or absence in copulatory behavior, declines in agonistic interactions with other males, as well as reductions in movements or roaming behavior (Knol and

Egberlink-Alink 1989*a*,*b*). For males, the reduction in the range size of castrated males would result in a reduction in the number and the extent of overlap with female ranges, reducing their contact rate. In addition, agonistic interactions with other males would also be reduced. For females, we assume that ovariectomized female possums would not be undergoing estrous behavior (Rekha 1997) and would not be sexually receptive. All non-primate mammals studied (with the exception of the rabbit) copulate only when the ovaries are present and undergoing follicle maturation (Lisk 1978). This suggests that the lower transmission rate of *L. balcanica* observed in ovariectomized female possums is driven largely by a significant reduction in their rate of contact with breeding males, without needing to invoke any changes in their movement patterns.

MANAGEMENT IMPLICATIONS

Overall, these results suggest that fertility control, either by immunocontraception or disruption of endocrine control of reproduction, are unlikely to have an impact on social organization and behavior of brushtail possums in ways that may compromise the efficacy of fertility control for reducing population density. Indeed, the results from this study suggest that disrupting endocrine control of reproduction is likely to have an additional benefit of reducing the transmission rate of directly transmitted pathogens. This finding may have important implications for the use of fertility control for other wildlife diseases, providing an additional benefit over and above that achieved through reductions in population density by also reducing contact rates between individuals.

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Quantifying Equid Behavior—A Research Ethogram for Free-Roaming Feral Horses

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Introduction

Feral horses (*Equus caballus*) are globally distributed in free-roaming populations on all continents except Antarctica and occupy a wide range of habitats including forest, grassland, desert, and montane environments. The largest populations occur in Australia and North America and have been the subject of scientific study for decades, yet guidelines and ethograms for feral horse behavioral research are largely absent in the scientific literature. The U.S. Geological Survey (USGS) Fort Collins Science Center conducted research on the influences of the immunocontraceptive porcine zona pellucida (PZP) on feral horse behavior from 2003–2006 in three discrete populations in the American west (see Ransom and others, 2007; Ransom, 2009). These populations were the Little Book Cliffs Wild Horse Range in Colorado, McCullough Peaks Herd Management Area in Wyoming, and Pryor Mountain Wild Horse Range in Montana; the research effort included over 1,800 hours of behavioral observations of 317 adult free-roaming feral horses. The following ethogram was developed during the course of this study to facilitate accurate scientific data collection on feral horse behavior, which is often challenging to quantify. By developing this set of discrete behavioral definitions and a set of strict research protocols, scientists were better able to address both applied questions, such as behavioral changes related to fertility control, and theoretical questions, such as understanding social networks and dominance hierarchies within social groups of equids.

Background

Feral horses in North America are descendents of animals that escaped domesticity or were intentionally released by settlers, explorers, and military personnel, and because of their highly adaptive nature were able to thrive in a multitude of environments. In the United States most feral horses are federally protected by the Wild Free-Roaming Horses and Burros Act of 1971, which refers to them as "living symbols of the historic and pioneer spirit of the West," stating that "they contribute to the diversity of life forms within the Nation and enrich the lives of the American people" (Public Law 92-195, 85 Stat. 649, as amended). Roughly 30,000 feral horses are distributed across 10 western States in the United States on 21.4 million ha of federally designated horse range. With annual population growth rates reported at 15–25 percent (Eberhardt and others, 1982; Wolfe and others, 1989; Garrott and Taylor, 1990; Garrott and others, 1991) and very limited natural depredation, the influence of feral horses on native flora, fauna, and ecosystem processes varies greatly (see Smith, 1986; Fahnestock and Detling, 1999; Coughenour, 2002; Levin and others, 2002; Beever and Herrick, 2006; Beever and others, 2008). Understanding animal behavior can be an important tool in addressing a species' influences and roles in an ecosystem and can lead to better management and conservation practices (Sutherland, 1998; Buchholz, 2007). Additionally, when management practices such as fertility control alter natural biological processes, it is important to consider the ethical implications, such as influences on animal behavior and social structure (Nettles, 1997; Asa and others, 2005; Porton, 2005).

Social Organization

Feral horses arrange themselves in family groups known as bands, which range in size from 2 to 20 or more animals. Band size is variable and may be a response to both the environment the horses inhabit and population density. Animals can reduce risk of predation by living in groups (Pusey and Packer, 1997), and in open habitats without cover in which to hide, animals may form larger groups (Molvar and Bower, 1994). The latter was found to be true in the USGS study,

where mean band size in the sage steppe habitat found at McCullough Peaks was 8.01 ± 0.38 (SE) horses, while habitats with trees and dense vegetation had smaller mean band sizes—4.45 ± 0.16 horses at Little Book Cliffs and 4.16 ± 0.17 horses at Pryor Mountain.

Feral horse bands typically consist of a single polygynous stallion, adult females, foals of the year, and yearling males and females. Bands with multiple stallions do occur and were first reported by Denniston (1979) and Miller (1979). Miller (1979) found that multiple stallions were present only in bands consisting of greater than 9.3 horses. This finding was supported by the USGS study, during which multiple-stallion bands were recorded only at McCullough Peaks; multiple stallions occurred in five bands of horses consisting of 9–18 animals each. At 2 years of age, subordinate males typically disperse the band into bachelor groups of two to several horses, but occasionally 2- and 3-year-old males remain with the band if the stallion does not drive them away. Rutberg and Keiper (1993) found that the age at which male horse dispersal occurs is strongly correlated with the number of peers in the natal group.

Feral horse bands are not geographically territorial and home ranges overlap with those of other bands in the population. This geographically non-territorial, harem-type social structure is known as Type I organization and also is used by the Przewalski horse (*Equus ferus przewalskii*), Plains zebra (*Equus burchellii*), and Mountain zebra (*Equus zebra*) (Klingel, 1975; Rubenstein, 1989). The other organizational structure found in equids, Type II organization, involves adult males establishing geographic territories and females moving between them. Type II organization is used by the Grevy's zebra (*Equus grevyi*), African wild ass (*Equus africanus*), and Asiatic wild ass (*Equus hemionus*). While feral horses do not exhibit geographic territoriality, the USGS study did document resource-oriented territoriality whereby stallions (with the entire band present) defended mineral sources repeatedly and often with elevated agonistic interactions when other bands approached.

Ethological organization within horse bands is characterized by a dominance hierarchy, but the literature has conflicting functional explanations for hierarchical structures in equids. Houpt and others (1978) found that body weight affected social rank but age did not; however, other researchers (Keiper and Sambraus, 1986; Rho and others, 2004; Heitor and others, 2006) found age to be linearly correlated with social rank. Reproductive status of females has been shown to influence their social dominance in a band, as well as their rate of interchange between bands (van Dierendonck and others, 2004). Most studies, however, indicate that agonism is strongly correlated with dominance rank among females (Houpt and others, 1978; Waring, 1983; Keiper and Receveur, 1992; Weeks and others, 2000). The USGS study found that the most dominant members of feral horse bands were female, and stallion rank varied by band. This ethological structure is reported in much of the existing literature (Houpt and Keiper, 1982; McCort, 1984; Keiper and Sambraus, 1986; Klimov, 1988; and Keiper and Receveur, 1992). Lack of stallion dominance has been attributed to the amount of time stallions spend away from the band while recruiting new females or engaging in agonistic interactions with other stallions; females in a band, however, can establish stronger hierarchical relationships because they continuously remain in close proximity to one another (Keiper and Receveur, 1992). Social rank in bachelor groups, which are often unstable, has only been attributed to individual temperament (Tilson and others, 1988).

Ethological Data Collection and Analyses

It is possible to quantify behavior through the systematic enumeration of discretely defined categorical behaviors in terms of counts of occurrence or proportions of time exhibited. Collection of ethological data may be accomplished through several techniques, and the ultimate decision rests on the precisely defined research objectives. The reader is referred to Altmann (1974) for a definitive overview of sampling methodology for observational data. In the last 30 years, many insights have contributed to refining these methods, such as how to determine the appropriate time period between sampling when using the instantaneous scan sampling method and a better research design to capture infrequent behavioral expressions. In sampling feral horse behavior, the USGS study found that instantaneous scan sampling (the method of recording the categorical behavior of each focal animal at precise moments in time) at 1-min intervals was ideal for quantifying time budget data. Continuous sampling is an alternative to this technique and involves recording a focal animal's behavior continuously and noting the point in time that a new behavior begins. Continuous sampling was impractical for our study due to the number of animals and observers involved. Mitlohner and others (2001) found no significant difference between behavioral data recorded with instantaneous scan sampling versus continuous sampling when intervals were 15 minutes or less, and found a high correlation between the techniques when using 1-min intervals.

Collecting data on time budgets using instantaneous scan sampling is fairly straightforward, but behaviors that are infrequent and expressed briefly are not likely to be captured adequately (Houpt, 1991; Doran, 1992). Two relatively good options exist for collection of such data: the all-occurrence method and focal sampling. All-occurrence data collection records every behavior every time it is observed (Altmann, 1974) and can be a good technique when the number of animals in the focal band is not too large and the behaviors of interest are easy to detect. Although less efficient in terms of data collection, the focal sample method may be more reliable since the observer's focus is directed at only one animal (Altmann, 1974; Houpt, 1991). This method involves randomly selecting one individual in the band and continuously observing and recording all behavioral expressions. In feral

horse research, capturing briefly occurring expressions is most important for reproductive, social, and agonistic behaviors. Feeding, resting, and locomotion typically occur in periods long enough to be captured by most techniques.

Ethological protocols must be established prior to data collection and should be defined by research objectives. The discrete behavioral categories presented in this ethogram do not preclude the exhibition of simultaneous behaviors. For example, it is typical in feral horses for a comfort behavior such as masturbation to occur while the animal is feeding, so a protocol should be explicitly defined stating which behavior takes precedence in the data record. If the objective of your research is to determine the amount of time horses are feeding in relation to some covariate, then feeding is the appropriate category, and if expression of comfort behavior is the focus, then it should be recorded as such (or in this case, one might wish to subdivide comfort into several behaviors, since comfort is generally a short-duration behavior with many specific expressions). Regardless of the approach, notations of simultaneous occurrences are invaluable in retrospect.

Simultaneous behavioral expressions are not the only observational data collection pitfall. Some behaviors, such as olfactory investigation, can be ascribed to several categories. This behavior may fall under comfort, reproduction, or harem social behavior, but the correct categorization of such expressions is not subjective: each expression does have an absolute definition. In such cases, the context of the behavior will define its true characterization. When such potentially confusing behaviors are prevalent, they are addressed specifically in the ethogram.

Considerably more in-depth analyses of behavior are possible when covariates associated with individual focal subjects are collected reliably. Many factors may influence behavior, such as age, body condition, dominance rank, presence of dependent foal, climate, and habitat. Fortunately, many feral horses have unique natural markings, making identification of individual animals possible without the use of radio-collars or numbered tags. Pelage color; facial patterns such as snips, strips, blazes, and stars; and leg markings such as coronet bands, socks, and stockings collectively make cataloging and referencing study animals feasible (Sponenberg and Beaver, 1992; Gower, 2000; Green 2001). Properly cataloging and identifying individual horses in a research effort can provide the foundation from which to build in-depth covariate data and thus lead to a better understanding of animal behavior.

Analyses of behavioral data may incorporate many traditional statistical methods, but time budget data presents a particularly challenging dilemma. These analyses must consider the compositional nature of a time budget: models exhibiting strong support for one behavioral category must also be considered for each other category. Proportions of time spent in behavioral categories are compositional dependent variables because the sum of the proportions in all behavioral categories sum to 1. In other words, there is a unit sum constraint (Aitchison, 1986) by which more time spent in one or more behavioral categories must be associated with less time spent in one or more other behavioral categories. These compensatory shifts in estimates are absolute, leading to a dependent relationship among behaviors constituting the time budget being analyzed (Elston and others, 1996). Many compositional data-analysis procedures have been developed (for example, log-ratio procedures; Aitchison, 1986, 1992), but few are appropriate for data that exhibit real values of 0 or 1. It may be important that the amount of time an animal exhibits a given behavior is truly 0 percent or 100 percent. Many methods, such as log-ratio analysis, require data transformations that eliminate values of 0 and 1 by assigning very small or very large values (greater than 0 or less than 1) (Bakeman and others, 1992; Clark and Messina, 1998). This process ultimately alters the distribution of data and may adversely affect conclusions drawn about individual behaviors in the composition (Bingham and Brennan, 2004). Once transformed, the common method of log-ratio analysis also produces only quantitative statements about the overall distribution of the composition in comparison to another and the sampling distribution of the difference; it does not specify explicit contributions of each piece of the composition to those differences (Aitchison, 1986). If your data are not composed of real values of 0 and 1, log-ratio analyses may be appropriate. It is best suited for compositions with only two elements since conclusions about the change in allocation may be drawn easily (Elston and others, 1996), but most behavioral studies aim to address multiple behaviors. Some extensions of the algebra of log-ratio compositional measures have been made by Billheimer and others (2001), but currently these procedures are limited to three-part compositions and still suffer the restriction of not allowing zero proportions.

One solution to the problem of comparing time-budget compositions with several behaviors and true values of 0 and 1 is the use of a multi-response permutation procedure (MRPP; Mielke and Berry, 2007). This method does not require data-transformation and analyzes compositional differences by approximating probabilities of a test statistic based on average Euclidean distances for detecting distributional differences. In other words, the test is focused on differences in multivariate cumulative distributions rather than means. This approach may yield useful results for drawing conclusions about how different animals or treatment groups allocate their time, but it still does not provide insightful conclusions about specific behaviors within the composition of interest. No standardized way to approach this problem currently exists, but one solution is simply to model each behavior to query further covariates and relationships of interest: analyze each part of the behavioral composition as a univariate dependent variable. For such results to be interpretable, however, the same models must be applied and presented for all individual behaviors in the composition.

In simple compositions with few behaviors, the trade-off in time allocation may be evident. It is likely, however, that such clear trade-offs will not always be evident and multiple models must be presented for each behavior. For example, consider a simple composition of three horse behaviors

(Y_f = feeding, Y_r = resting, and Y_l = locomotion, where $Y_f + Y_r + Y_l = 1.0$). If Y_f has a strong linear increase with the predictor variable X_1 (for example, 0/1 indicator variable of sex) and a weak linear increase with predictor variable X_2 (for example, herd size), then Y_r, Y_l, or both must decrease with X_1, but the actual magnitude of that decrease will depend on both behaviors and the degree to which they also are influenced by X_2 (model 1 is $Y = f(X_1 + X_2)$). It could easily be that the strongest model for Y_r might include X_2 and another predictor (say X_3, and model 2 is $Y = f(X_1 + X_3)$). Thus, it is possible for model 1 to be strongly selected for analyzing the Y_f part of the Y compositional vector of behaviors but model 2 to be strongly selected for the Y_r part of the composition. Likewise, one model may be strongly supported for several behaviors and a complement model may be strongly supported for the remaining behaviors in the composition (see Ransom, 2009); however, because the behavioral composition really induces a relationship among the parts of the composition, statistical model results will be uninterpretable if a selected model is not considered to apply to all parts of the behavioral composition (for example, Y_f, Y_r, and Y_l).

The increasing use of maximum-likelihood and Bayesian approaches for solving complex problems may ultimately lead to the development of more lucid guidelines for many problems indicative of behavioral data. These methods can provide powerful tools for analyzing scientific data and can lead to a deeper understanding of the processes and errors involved (Hobbs and Hilborn, 2006). Regardless of the statistical approach, the path toward proper model selection for individual dependent behaviors in a composition has not been described in the literature. The use of model weights and model averaging may assist in drawing coherent conclusions about parameters of interest (Burnham and Anderson, 2002), but all supported models must be considered simultaneously for all dependent compositional variables to avoid violating the inherent statistical assumptions. A reasonable approach to assessing strength of evidence for compositional models, without violating assumptions of dependence, is to sum the minimum Akaike's Information Criteria (AIC: Burnham and Anderson, 2002) scores for the individually-modeled constituent behaviors in a composition; subsequently, each sum then forms an aggregate AIC score for each compositional model. The strength of evidence for the overall composition may then be assessed using AIC weights as Burnham and Anderson (2002) describe for individual models.

Ethogram

This ethogram was developed to investigate the influences of fertility control on feral horse behavior; thus, the behavioral groupings are broad in some contexts, such as feeding behavior, and much more detailed in areas of specific interest to the research objectives, such as agonism. Behavioral researchers often consolidate an array of behavioral expressions into broad categories since data collection on every specific behavior expressed is usually impractical and makes analyses excessively difficult. Therefore, this ethogram divides behavioral expressions into 13 discrete categories that cover the range of expressions a feral horse may exhibit in free-roaming conditions. Two additional categories (human awareness and out-of-sight) are used to account for biased observations. These 13 categories can easily be further aggregated *a posteriori* if desired, but categorizing these behaviors separately *a priori* allows for a wider range of possible analyses while still being practical for data collection. An example of a broader aggregation of these categories is the consolidation of grooming, comfort, standing attentive, and elimination into *maintenance*; herding, reproduction, harem tending, harem social, agonism, and submission into *social*; and human awareness and out-of-sight (unavailable to the observer) into *unknown* (fig. 1). At any given time during the course of observation, a focal animal will be exhibiting behavior that can be described by one of these categories. Developing a research ethogram from the several hundred behavioral expressions documented in equids can be a daunting task; therefore, this basic ethogram for feral horses is presented as a starting point for behavioral studies. For an excellent ethogram resource detailing complete equid behavior expressions, the reader is referred to McDonnell (2003). For a comprehensive work on equine behavior, the reader should also see Waring (1983).

Feeding

Feeding behavior occupies roughly half of the daily time budget of feral horses (fig. 1) and usually entails grazing. Grazing occurs as a horse bites off and ingests grasses and forbs close to the ground (fig. 2). The feeding category also includes browsing on woody plants and trees, eating snow, drinking, mineral licking, coprophagy (eating feces), and pawing at food resources. The latter is critical in defining feeding as a mutually exclusive category in that a horse may be pawing at soil, plants, or snow, but if the action is directly related to acquiring and ingesting a food resource, then it should be considered as feeding behavior. Also, horses move as they graze; therefore, as long as the horse is feeding while it is moving, it should be considered as feeding rather than locomotion.

Resting

The second largest amount of time in the daily time budget of feral horses is allocated to resting, which may occur during 25–35 percent of the daylight hours (fig. 1). Resting behavior is characterized by a general lack of attention and relaxed state and may occur in a standing position or in recumbency (fig. 3). This includes both relaxation and sleeping. Horses are able to sleep standing up due to the stay apparatus that allows their body to be supported without active muscular control (Dallaire, 1986). This behavior is typically observed

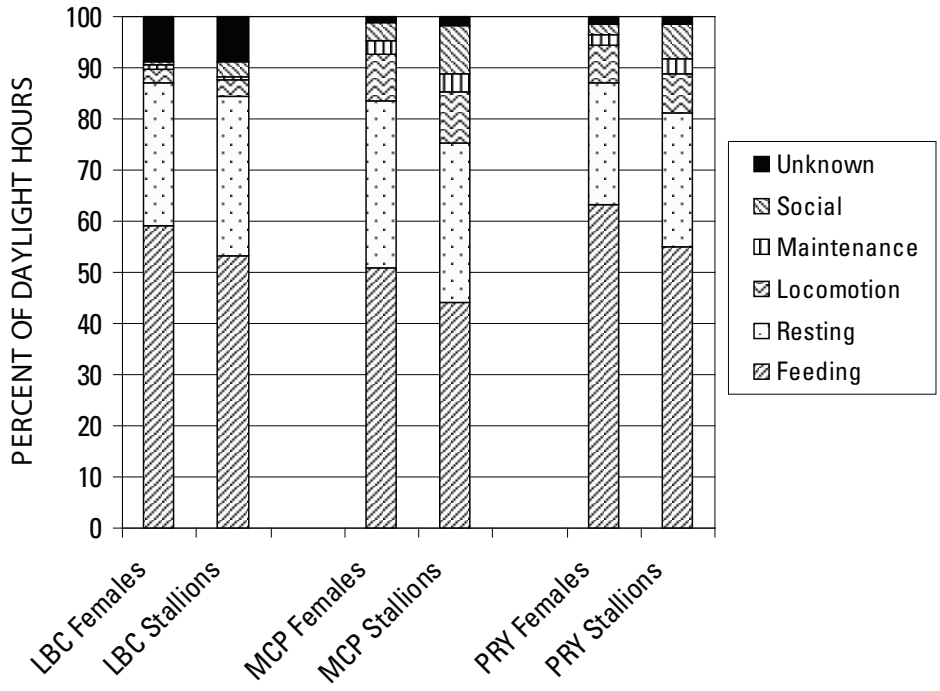

Figure 1. Observed daytime activity budgets of adult control female feral horses (n = 207, mean age = 7.79 yrs ± 0.33 SE, range = 2–21 yrs) and harem stallions (n = 110, mean age = 11.63 yrs ± 0.43 SE, range = 5–26 yrs) at Little Book Cliffs Wild Horse Range in Colorado (LBC), McCullough Peaks Herd Management Area in Wyoming (MCP), and Pryor Mountain Wild Horse Range in Montana (PRY), 2003–2006.

Figure 2. Feral horses grazing, the most typical *feeding* behavior.

Figure 3. *Resting* behavior in a band of feral horses is often characterized by huddling. Aside from the physical protection received by this behavior, the head-to-tail orientation of band members in this type of resting behavior also facilitates grooming. This allows for insect control around the head by the tail-swishing effect of neighbors.

as a body position with the head lowered, eyes closed, and one rear foot slightly elevated. Resting behavior in a band is often characterized by huddling, which facilitates insect control by the tail-swishing of close neighbors. Recumbent rest can be sternal or lateral. The comfort behavior of sun-basking is expressed in a laterally recumbent state, so a protocol must be defined *a priori* characterizing this state as rest or comfort, based on research objectives.

Locomotion

The third largest amount of the daily time budget is spent on locomotion. This behavior includes walking, trotting, cantering, galloping, jumping, and swimming and in feral horses is typically used for moving from one resource to another (fig. 4). Since most feral horse populations in North America occupy arid or semi-arid environments, movement to the few and scattered water sources is often the impetus for daily occurrences of extended locomotion (typically every 12 to 24 hrs). Otherwise, feral horses do not expend vast amounts of energy in locomotion, with the exceptions of brief social interactions between bands, stallion agonistic expressions, and female recruitment efforts by stallions. In this ethogram, locomotion integral to reproductive, harem tending, or comfort behaviors is considered part of its respective behavioral expression and not recorded as locomotion.

Maintenance

Grooming

Grooming behavior occupies a relatively small but important part of the daily time budget of feral horses and is often observed as rolling. Rolling occurs both on land and in water and is thought to assist with pelage health and insect control (fig. 5) (Waring, 1983). Other grooming behaviors include shaking, nibbling or licking on self, tail-swishing, rubbing, and periodic stomping to displace flies and biting insects. Allogrooming (also known as mutual grooming) in this ethogram is not considered categorically as a grooming behavior since it is also a social interaction that involves more than one animal. Depending on the nature of the research, it may be appropriate to consider allogrooming as part of the grooming category.

Figure 4. *Locomotion* is most typically expressed as walking from one place to another, as seen in this feral horse stallion. The higher energy-expense gaits of trotting, cantering, and galloping are observed far less frequently.

Figure 5. *Rolling* by this feral horse mare is a grooming behavior associated with pelage hygiene and insect control.

Comfort

Comfort behavior takes on a wide variety of forms in feral horses and includes any type of self-enjoyment expression, such as play, investigation, and stimulation (fig. 6). Some examples of comfort behavior are sun-basking, shelter seeking, masturbation, sexual play, object play, locomotor play, play fighting, and some olfactory investigations. Olfactory investigations also may be indicative of reproductive behavior or social behavior and should be included in the appropriate category for data collection based on specific research objectives. This ethogram also includes yawning and stretching as comfort behaviors, though it would not be inappropriate to include those expressions as a form of resting behavior.

Standing Attentive

When horses receive a stimulus that causes alertness, they react by exhibiting a rigid body posture with head upright, ears pointed forward, and eyes open and alert (fig. 7). This may be momentary in the case of a sound or smell causing alertness, but such standing attentive behavior also occurs for extended periods of time when a female is standing guard over a sleeping foal or a predator is nearby. In this ethogram, standing attentive does not include instances when the behavior is a result of human presence (fig. 8). In those cases, human awareness is considered its own category so that the biased data may be properly addressed during analyses.

Elimination

Elimination in horses is expressed as urination or defecation (fig. 9), though depending on research objectives, it should be determined whether any such expression will be included in this category or whether elimination with social implications is considered elsewhere. Socially, feral horse stallions create fecal middens known as stud piles and repeatedly defecate on them. These middens are thought to facilitate communication or ownership status of certain resources (Feist and McCullough, 1976; Rubenstein and Hack, 1992). When female feral horses urinate or defecate, it is common for the harem stallion to cover it with his urine for similar reasons. It has been reported that female horses do not cover the urine of other females in this fashion (McDonnell, 2003), though this was documented periodically during our study and may have implications in the female dominance hierarchy.

Figure 6. A typical *comfort* behavior for feral horse males is masturbation, which is expressed when a horse flexes his erect penis against his abdomen. This behavior is not expressed in conjunction with a nearby female and should not be confused with the penis drop observed when a male is reproductively tending a female.

Figure 7. Feral horse mares are focused on an external stimulus (in this case, a foal distress vocalization in the distance) and are exhibiting *standing attentive* behavior. Note the directionally pointed ears, rigid body position, and focused eyes characteristic of this expression. This behavior should not be confused with *human awareness*.

Figure 8. *Human awareness* is an important behavior to record when conducting any behavioral research so that biased data can be properly omitted from analyses of naturally occurring behaviors. Here, an entire band has paused because they noticed the photographer in the distance.

Figure 9. A female feral horse is exhibiting *elimination* by urination. Urination behavior in horses is sexually dimorphic: female horses urinate in a posterior direction and male horses urinate in an anterior direction.

In this ethogram, random urination and defecation are considered elimination, whereas systematic covering of urine or feces from the female by a harem stallion is considered as a harem tending behavior. The systematic covering of urine from a female by a female in the same harem is considered harem social behavior.

Social

Harem Social

Harem social behavior is considered to be social interaction among band members that is not specifically indicative of reproductive, harem tending, or agonistic behavior. Allogrooming is considered a harem social behavior in this ethogram (fig. 10), as is olfactory investigation (from one horse to another, fig. 11, or the systematic smelling of urine deposits among band members) and pair-bonding among juveniles (fig. 12).

Herding

When a feral horse stallion actively drives females of his harem together, it is considered herding behavior. This involves the stallion moving systematically behind the females, posturing with his head held low and ears laid back, and controlling the direction and movement of the band members (fig. 13). Waring (1983) termed this motion with the stallion's head moving side-to-side as snaking. Some researchers may choose to consider this as an agonistic behavior, but it is considered here as its own category since the function of the behavior is maintaining the harem rather than aggression toward specific females.

Harem Tending

Harem tending consists of stallion behaviors that are directed at maintaining the harem and include the defense and recruitment of females. The defense of females is expressed as a stallion positioning himself between his harem females and a perceived threat (typically another stallion) (fig. 14). It is also expressed by covering a female's urine or feces with his own, or by depositing feces on a stud pile (fig. 15). Recruitment, or stealing, of females by a harem stallion from another stallion is also considered harem tending behavior in this ethogram.

Reproduction

Reproductive behavior in feral horses is characterized by a series of stallion-initiated behaviors and a series of mare-initiated behaviors. The stallion reproductive sequence often

Figure 10. One of the most common *harem social* behaviors is allogrooming (also known as mutual grooming). It is expressed by the lateral parallel body position of two horses that allows for nibbling along the back or withers of each horse. While this behavior can be considered grooming, it is also thought to facilitate pair-bonding and dominance structure between band mates (Waring, 1983).

Figure 11. Olfactory investigation in the *harem social* behavior context is often observed as prolonged olfactory engagement of a female and her foal in a bonding regimen. This also may include licking and nudging.

Figure 12. Pair-bonding among juveniles within a band is also a common *harem social* behavior. In this example, female foals are closely engaging each other, which is thought to facilitate development of the female-female ethological relationships critical to band structure and stability later experienced as reproductive members of a family group (Crowell-Davis and others, 1986).

Figure 13. A feral horse stallion (leftmost horse) is *herding* females in his band by posturing and actively driving his females back into a cohesive group. Herding is the most common social behavior expressed by the dominant male toward females in his harem.

Figure 14. The blue roan stallion (foremost) is *harem tending* by expressing a posturing behavior and positioning himself between his harem females (several additional females out of the photo to the left) and an intruding male (right). Depending on the persistence and signals of the intruder, this behavior may develop into *agonism*.

Figure 15. Male feral horses also express *harem tending* by maintaining fecal middens, or stud piles, in which feces are aggregated at strategically located positions within the band's home range.

(but not always) begins with herding or reproductive tending behavior. Reproductive tending is the close following of a female by the male, without the directional driving observed in herding behavior. Following this tending behavior, the stallion often vocalizes and exhibits the olfactory response known as flehmen (fig. 16) (Stahlbaum and Houpt, 1989). He then proceeds by rubbing his head on the female's flanks and (or) resting his chin on her back, and extends his penis out of the prepuce (fig. 17). Some of these behaviors out of sequence are not necessarily associated with a stallion reproductive sequence; therefore, protocols to determine how to categorize the observed behavior are specified in figure 18.

Female reproductive behavior is signified by estrous, which can sometimes be challenging to detect from typical field observation distances. A mare initiates her reproductive sequence by presenting herself facing away from the stallion, lifting her tail, and vocalizing (though sometimes the female faces the stallion first). These behaviors alone do not necessarily signify estrous, since the female could simply be vocalizing and preparing to defecate. If in estrous, it is typically accompanied by posturing her body with hind legs slightly apart (an apparent squat) and often turning her head toward her posterior. The mare also will frequently emit small streams of urine, a behaviour that is often concurrent with rhythmic 'winking' of her vulva: winking can be observed by the periodic flashes of pink from the inner coloration of her vaginal membranes (McDonnell, 2003). These contrasting pigments can be seen in the rolling female in figure 5, simply because of her posture. The urine emitted from female horses in estrous consists of specific variations of urinary volatile compounds that may facilitate chemical communication during reproductive behavior (Ma and Klemm, 1997). Female horses may express estrous behavior during the anovulatory period (typically winter) as well as during pregnancy (Crowell-Davis, 2007). Such anomalies to our conventional understanding may lead to misinterpretation of estrous behavior in the field and care should be taken to correctly and systematically identify this behavior.

Once both male and female are exhibiting reproductive behaviors, copulation may occur with the stallion mounting the female from behind, neck arched over her back and forelegs resting on her sides (fig. 19). Several events could occur in this situation and should be categorized separately if reproductive behavior is a focus of the research. These include successful copulation, unsuccessful copulation (the stallion cannot adequately mount the female for some reason), mare acceptance, mare rejection (the mare kicks and moves away from the stallion), and forced copulation (the mare is not presenting or accepting the stallion's advance, but he relentlessly mounts her anyway).

Figure 16. Flehmen response in this feral horse stallion is displayed by the elevated head, and raised, inverted upper lip. This posture is a response to particularly exciting chemicals and olfactory signals that are detected by the main olfactory epithelium and concentrated directly into the vomeronasal organ, which in part controls sexual activity (Estes, 1972; Mills and Nankervis, 1999).

Figure 17. A feral horse stallion is exhibiting penis extension and rubbing of the female's flanks as part of the male reproductive behavior sequence.

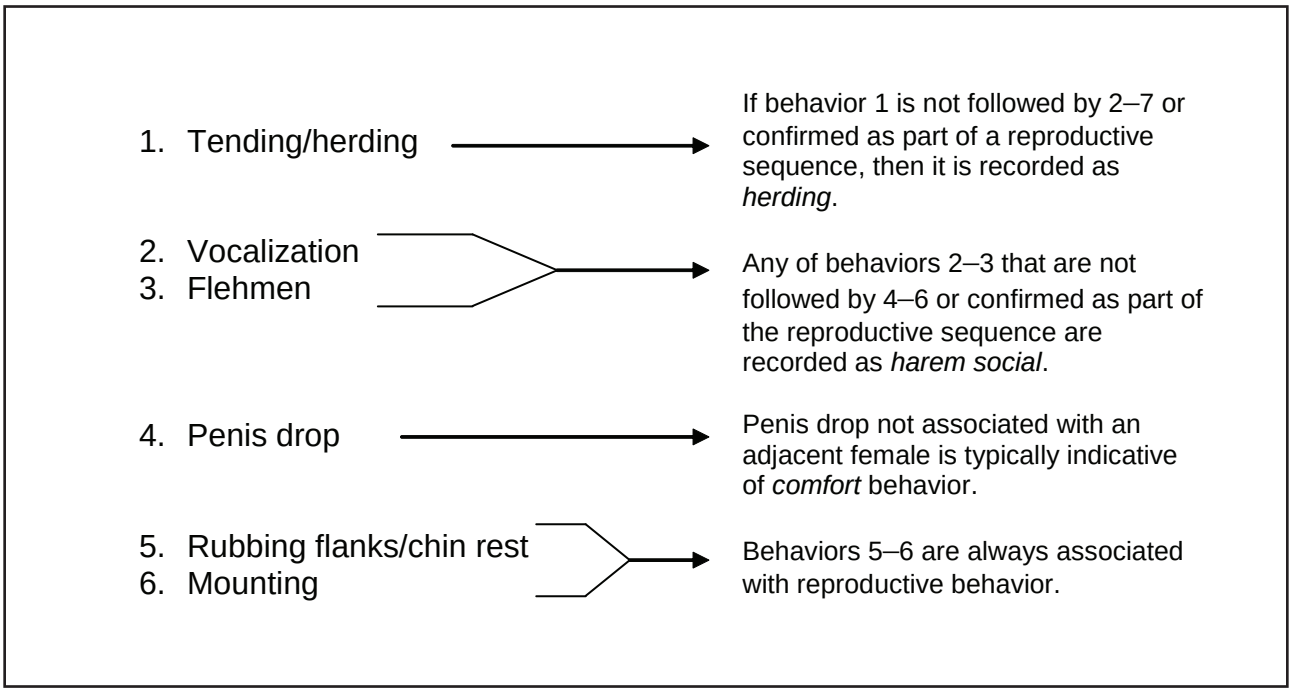

Figure 18. Basic guidelines for distinguishing reproductive and non-reproductive behaviors in stallions.

Figure 19. *Successful copulation* is occurring between this feral horse male and female. This reproductive behavior is characterized by a maintained mounting position on the female for the male and a relatively stationary, relaxed position for the female. *Unsuccessful copulation* is often a result of *mare rejection*, with the female expressing *agonistic* behaviors such as kicking toward the male and a brief mounting position for the male.

Parturition may also be included in the reproductive category or designated as its own behavioral category, depending on research focus.

Agonism

Agonism in feral horses is exhibited by a wide range of behaviors and in this ethogram is put into seven categories arranged by increasing intensity of the interaction. Note that any or all of the following behaviors may be exhibited in play by young animals, in which case they should be considered comfort behavior rather than agonism. The first four categories of agonism may occur between males only, females only, or between males and females, but the remaining levels of agonism are typically observed only between males.

1. The most common and least intense form of agonism is the threat. Threats are characterized by laterally pinned back ears, arched neck, and (or) a movement of the head toward the opposing horse, but with no physical contact (fig. 20). Most conflicts and dominance interactions among feral horses are resolved by these gestures alone.

2. The next agonistic expression is the bump or push, which is expressed by the aggressor making forceful contact with another horse using its head, neck, or shoulder (fig. 21).

3. As agonism escalates, the aggressor may chase its adversary at a gallop to displace the animal from the immediate area or with the intent of engaging the animal in more agonistic behavior (fig. 22). Chasing behavior is typically brief (seconds) or may last several minutes; however, the USGS study documented this behavior occasionally persisting over long time periods (greater than 1 hour) and great distances (greater than 3 km).

4. The fourth level of agonism involves biting an opponent or kicking with the hind legs (fig. 23). This level involves physical contact, though serious injury is rare.

5. Rearing occurs when the horse lifts its forelegs off the ground and elevates its body into a more vertical position, thus looking larger to the opponent (fig. 24). While technically only a threat, this body position typically signifies a more intense agonistic interaction and provides the initial position for commencement of stomping, striking, boxing, and dancing behaviors.

Figure 20. *Agonism* is expressed by both of these feral horses in the form of a *threat*. This is the most common form of agonism between feral horses and is most easily recognized by the laterally positioned, posterior pointing direction of the ears (shown in both animals here).

Figure 21. *Pushing* is an agonistic expression shown here by the foremost horse pushing toward the more distant horse.

Figure 22. *Chasing* is a moderately intense form of agonism and is shown here along with an expression of imminent biting behavior.

Figure 23. *Biting* is a common expression of agonism in equids and is expressed here between feral horse females to assert dominance.

Figure 24. *Rearing* is an expression often signifying a potentially intense agonistic interaction, typically between feral horse stallions.

Figure 25. *Striking* or *stomping* is being expressed by the feral horse stallion on the left. This behavior is typically indicative of highly agonistic expression and is expressed by the directed extension and downward motion of one or both front feet.

6. Stomping and striking occur when a horse uses one or both front feet to attack an opponent by extending the legs out and downward. This behavior has the potential to seriously injure the recipient (fig. 25).

7. The most intense form of agonism between feral horse stallions is boxing and dancing. These behaviors involve the engagement of the agonists in vertical body positions and the use of the front legs in striking (boxing) or closer engagement that results in head and neck biting (dancing) (fig. 26). On very rare occasions, feral horse stallions have been observed engaging in prolonged fights resulting in considerable injury and sometimes fatal outcomes.

Submission

Submission is exhibited by the loser of the agonistic encounter. This may be expressed by simply running away, but also may be expressed with laid down ears (distally or posteriorly), lowered head posture, lowering of the hindquarters, and sometimes jaw snapping (especially in juveniles) (fig. 27).

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Figure 26. *Boxing* and *dancing* between these two stallions is illustrative of the highest level of agonism that feral horses typically express.

Figure 27. *Submission* is being expressed by a young male horse (grullo [dark grey] horse in center) and directed toward the larger, dominant male (foremost), who is displaying *threat* behavior associated with dominance.

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Behavior of feral horses in response to culling and GnRH immunocontraception

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ABSTRACT

Wildlife management actions can alter fundamental behaviors of individuals and groups, which may directly impact their life history parameters in unforeseen ways. This is especially true for highly social animals because changes in one individual's behavior can cascade throughout its social network. When resources to support populations of social animals are limited and populations become locally overabundant, managers are faced with the daunting challenge of decreasing population size without disrupting core behavioral processes. Increasingly, managers are turning to fertility control technologies to supplement culling in efforts to suppress population growth, but little is quantitatively known about how either of these management tools affects behavior. Gonadotropin releasing hormone (GnRH) is a small neuropeptide that performs an obligatory role in mammalian reproduction and has been formulated into the immunocontraceptive GonaCon-B™. We investigated the influences of this vaccine on behavior of feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota, USA, for a year preceding and a year following nonlethal culling and GnRH-vaccine treatment. We observed horses during the breeding season and found only minimal differences in time budget behaviors of free-ranging female feral horses treated with GnRH and those treated with saline. The differences observed were consistent with the metabolic demands of pregnancy and lactation. We observed similar social behaviors between treatment groups, reflecting limited reproductive behavior among control females due to high rates of pregnancy and suppressed reproductive behavior among treated females due to GnRH-inhibited ovarian activity. In the treatment year, band stallion age was the only supported factor influencing herding behavior ($P < 0.001$), harem-tending behavior ($P < 0.001$), and agonistic behavior ($P = 0.02$). There was no difference between the mean body condition of control females (4.9 (95% CI = 4.7–5.1)) and treated females (4.8 (95% CI = 4.7–4.9)). Band fidelity among all females increased 25.7% in the year following vaccination and culling, despite the social perturbation associated with removal of conspecifics. Herding behavior by stallions decreased 50.7% following treatment and culling ($P < 0.001$), while harem-tending behavior increased 195.0% ($P < 0.001$). The amount of available forage influenced harem-tending, reproductive, and agonistic behavior in the year following culling and treatment ($P < 0.04$). These changes reflected the expected nexus

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between a species with polygynous social structure and strong group fidelity and the large instantaneous change in population density and demography coincident with culling. Behavioral responses to such perturbation may be synergistic in reducing grazing pressure by decreasing energetically expensive competitive behaviors, but further investigation is needed to explicitly test this hypothesis.

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1. Introduction

Gregarious relationships can impart individual benefits such as decreased depredation, increased foraging efficiency, and increased fecundity (Pusey and Packer, 1997). When resources to support populations of social animals are limited and populations become locally overabundant, managers are faced with the daunting challenge of decreasing population size without disrupting core behavioral processes. For example, the limited contiguous habitat and finite resources found on public wildlands cannot sustain overabundant populations of large ungulates, and managers continuously seek innovative and publically-acceptable tools for managing these species (Powers et al., 2011). Historically, such populations have been reduced by culling, using either lethal methods such as hunting, or non-lethal methods such as capture and translocation. Limiting fecundity through the use of immunocontraceptives is becoming a more commonly considered tool for controlling wildlife abundance. However, these tools can be accompanied by physiological changes that may alter behavior and ultimately influence population dynamics in unforeseen ways (Ransom et al., 2014). The paucity of quantitative data on natural behavior of wild free-roaming fauna, especially pertaining to those treated with fertility control agents, impairs our ability to understand species' influences and roles in ecosystems and thus our ability to effectively manage populations.

Elk (*Cervus canadensis*) and feral horses (*Equus caballus*) are increasingly targeted for fertility control management in the United States and both species exhibit complex social behavior and polygynous mating systems. Whereas elk congregate seasonally into mating groups (Geist, 1982), feral horses form bands that persist year-round with distinct associations of individuals sometimes lasting a decade or more (Klingel, 1982). These bands are largely maintained by reproductive, herding, agonistic, and defensive behaviors initiated by the polygynous male in the band, as well as hierarchical relationships among females (Ransom and Cade, 2009). Culling can result in discrete changes to population density and demography, which could perturb social organization. Likewise, effective fertility control agents lead to discrete changes in presence of offspring in the population, and thus may influence social behavior.

Approximately 33,000 feral horses roam 13 million ha of public lands in the western United States and managers have historically relied on nonlethal culling through a process of capture, removal, and public adoption to regulate animal abundance (Garrott and Oli, 2013). This process is unsustainable because demand in the U.S. for feral horses as domestic stock is less than half the number of animals removed, which has left as many horses in federally-subsidized pastures as persist in the wild. Several fertility control techniques have been investigated for use in feral horses, but most of these efforts resulted in tools that were publicly unacceptable, expensive to use, or impractical on a large scale. Among the most promising techniques are immunocontraceptive vaccines such as porcine zona pellucida (PZP) and gonadotropin-releasing hormone (GnRH). These agents are relatively easy to apply, are reasonably efficacious, and are not expected to be accompanied by serious contraindications (Kirkpatrick et al., 2011).

Gonadotropin releasing hormone is a small neuropeptide that performs an obligatory role in mammalian reproduction. When combined with a potent adjuvant, the GnRH vaccine stimulates a persistent immune response resulting in prolonged antibody production against GnRH, the carrier protein, and the adjuvant (Miller et al., 2008). The most compelling hypothesis of vaccine effectiveness suggests that antibodies to GnRH likely induce transient infertility by binding to endogenous GnRH, thus preventing attachment to receptors on gonadotropes and suppression of pulsatile luteinizing hormone (LH) secretion (Molenaar et al., 1993). Gonadotropin releasing hormone antibody titers are correlated with suppression of the reproductive system and infertility in a variety of species (Fagerstone et al., 2010). As anti-GnRH antibodies decline over time, concentrations of available endogenous GnRH increase and treated animals usually regain fertility (Powers et al., 2011).

This immunocontraceptive vaccine, known by the trade name as GonaCon-B^{TM}, has been shown to provide multiple years of infertility in several wild ungulate species including horses (Killian et al., 2008; Gray et al., 2010), bison (*Bison bison*) (Miller et al., 2004), elk (Powers et al., 2011), and white-tailed deer (*Odocoileus virginianus*) (Gionfriddo et al., 2009). Given the physiological mechanism of action, GnRH immunocontraception has the potential to reduce fertility and suppress the reproductive behaviors typically associated with copulation; however, in GnRH-vaccinated elk, such behaviors were not entirely diminished likely due to incomplete suppression of the hypothalamic pituitary gonadal (HPG) axis (Powers et al., 2011). Similar to elk, feral horses are polyestrous and fertility control agents that do not suppress these social behaviors can lead to females exhibiting frequent reproductive behaviors that can cascade into altered behaviors from associated band members and nearby males (Nuñez et al., 2009; Ransom et al., 2010).

Rigorous quantitative investigation into the potential effects of GnRH treatment on feral horse behavior is missing from the assessment of this immunocontraceptive as a potential management tool. Such data are

Table 1
Population metrics for feral horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota, USA, 2009 and 2010. Normalized Difference Vegetation Index (NDVI) is shown for only the behavior observation period in each year.

	2009	2010
Number of males	64	26
Number of females	74	59
Number of foals	27	31
Mean male age ± SE (range)	5.0 ± 0.45 (1–15)	7.7 ± 0.68 (1–16)
Mean female age ± SE (range)	5.3 ± 0.50 (1–20)	6.4 ± 0.45 (1–18)
Number of bands	19	16
Mean band size ± SE (range)	8.3 ± 0.07 (3–14)	8.7 ± 0.07 (3–19)
Total population size	165	116
Mean NDVI (95% CI)	0.44 (0.324–0.548)	0.43 (0.346–0.504)
Density (number of horses/ha)	0.009	0.006

also absent in regard to culling, which is arguably the most common feral horse management practice worldwide. We investigated the influences of GnRH treatment on behavior of feral horses at Theodore Roosevelt National Park, North Dakota, for a year proceeding and a year following GnRH vaccination and culling. Our objectives were to quantify effects of these two management tools on time budgets of individuals and social interactions between individuals. Based on the assumed mechanism of action of GnRH vaccine, we posited that inoculation would suppress reproductive behaviors in treated females compared to controls and that the sudden decrease in density and social perturbation caused by population reduction would measurably influence social behaviors of all horses.

2. Materials and methods

2.1. Study area

Theodore Roosevelt National Park is located in southwestern North Dakota, USA (45°55′N/103°31′W). The South Unit of the park, where this study was conducted, comprised approximately 19,000 ha and consisted of eroded badlands with gullies and ravines separated by large upland plateaus, and small buttes. The mixed-grass prairie vegetation was predominantly comprised of needle and thread (*Hesperostipa comata*), threadleaf sedge (*Carex filifolia*), western wheatgrass (*Pascopyrum smithia*), blue grama (*Bouteloua gracilis*) and little bluestem (*Andropogen scoparius*) (Butler et al., 1986). Sympatric ungulates included feral horses, pronghorn (*Antilocapra americana*), bison, mule deer (*Odocoileus hemionus*) and elk. Feral horses were confined to the South Unit by a 1.8–2.4 m high woven wire boundary fence and no other feral horse populations were nearby. All individual horses in this population were previously identified and assigned individual identity numbers by managers, and age and reproductive data for each animal have been maintained since 1993 (National Park Service, unpublished data).

2.2. Treatments and study subjects

In spring/summer 2009, when pre-treatment data were collected, 165 horses occupied the park and were organized into 19 bands (Table 1). All bands contained a single adult stallion and adult females and their offspring, with the exception of one band that also contained a second stallion. No females persisted outside of bands; whereas, bachelor males were distributed across the landscape individually or sometimes in small ephemeral bands.

Horses were gathered into permanent corrals and handling facilities using a helicopter during a scheduled management capture in October 2009. Managers attempted to gather all horses in the population at that time, and removed 78 horses based on the agency's specific management needs. Eighty-one percent of the culled animals were foals, yearlings, and 2- and 3-year olds, and 14% were bachelor males not associated with any band. The remaining individuals removed included three females age ≥16 years, and one 6-year old female; none of which were part of our experimental group. These removals inherently skewed the sex ratio of the population between years because so many bachelor males were removed; however, paternity attributed to bachelor males is thought to be low (Bowling and Touchberry, 1990) and all study bands were subjected to competition pressure equally in each year at the population level. Post-treatment data were collected in spring/summer 2010, when 116 horses (including those born in 2010) occupied the park (Table 1).

We selected 58 adult females (2–17 years of age) using a randomized complete block design consisting of a GnRH-vaccinated treatment group and a saline control group. The resulting experimental female population consisted of 29 GnRH-vaccinated females (treatment) and 28 saline-treated (control) females, with one mature female remaining in the population that could not be captured. Females that were not in either treatment group were excluded from behavior analyses. The GnRH vaccine ultimately did not prevent conception in all inoculated horses (Baker et al., 2013); therefore, to more clearly separate the effects of infertility on behavior, we also excluded from analysis observations of 14 treatment females that became pregnant in 2010 and produced offspring in 2011. Females of both treatment groups were dispersed randomly across 16 bands with no difference in proportion of treated and control females between bands (paired t-test for unequal means: $t = 0.116$, $P = 0.909$). The same adults and bands observed in 2009 were observed in 2010, with none of the research cohort or band stallions removed during the culling operation.

Veterinarians blinded to treatment status assessed the general health, pregnancy, and body condition of each

animal at the time of capture. Pregnancy status and approximate stage of gestation were determined using rectal palpation of the reproductive tract and transrectal ultrasound imaging (Bucca et al., 2005). We visually assessed body condition of all females and scored condition using a numerical index that increased 1–9 with increasing fatness (Henneke et al., 1983). Females in the treatment group received an intramuscular injection in the gluteus muscle, by hand-syringe, containing 2000 μg of GnRH vaccine (2 mL, GonaCon-B™ (National Wildlife Research Center, Fort Collins, CO, USA)). Females in the control group were injected in a similar manner with an equal volume of saline solution. All data for this study were collected in accordance with Colorado State University Animal Care and Use Committee standards (protocol 12-3197A).

2.3. Behavioral sampling

Behavioral observations were conducted during three daylight time periods (08:00–12:00 h, 12:01–16:00 h, and 16:01–20:00 h) from April 18, 2009–July 16, 2009 and April 8, 2010–August 19, 2010. Each observation session included collection of a 20 min instantaneous scan sample of time budgets at 1 min intervals for each adult band member (≥1 years old), and all-occurrence data collection for social interactions, exactly as implemented by a synonymous study of PZP in horses (Ransom et al., 2010). We used a feral horse ethogram that categorized specific behaviors into 15 discrete time budget categories; agonism, comfort, elimination, feeding, grooming, harem-social (e.g., allogrooming, pair-bonding, female-female urine marking), harem-tending (e.g. stallion defense of a band female or recruitment of a new female into the band), herding (e.g., driving or snaking behavior by the stallion), interaction-with-humans, locomotion, out-of-sight, reproduction, resting, standing attentive, and submission (Ransom and Cade, 2009). The infrequency and similar nature of several behaviors in the instantaneous scan samples led us to aggregate them into broader categories for time budget analyses. Grooming, comfort, standing attentive, and elimination were combined into *maintenance*; reproductive, harem-tending, harem-social, agonism, submission, and herding were combined into *social*; and interaction-with-humans and out-of-sight were combined into *unknown*. The time spent in the category *unknown* ultimately did not differ between treatment groups or between years.

We also collected social behavior data at every occurrence throughout each observation session because instantaneous scan sampling has the potential to underestimate rare events (Ransom and Cade, 2009). These all-occurrence behaviors included intraband social behaviors associated with herding, reproduction, agonism, and harem-tending and were retained as discrete categories for social behavior analyses. We observed all horses from the nearest distance that did not elicit attention of horses to the presence of the observer, typically 50–300 m. All observations were conducted using a 15–45 × 600 mm spotting scope or 10 × 42 mm binoculars when the distance between horses and observers was too far to allow unassisted detailed observation.

2.4. Statistical analyses

We modeled the frequency of each behavior using mixed-effects linear regression, where individual female identity and sampling time period (time of day) were included as random effects on the intercept term of each model. This accounted for variation that may have been present among individuals who were sampled repeatedly, though not always equally over time, and for temporal variation in behavior when samples were not equally collected across all times of day. We used the lme4 package of R version 2.14.1 (The R Foundation for Statistical Computing 2011) and SYSTAT 12.02.00 (SYSTAT Software, Inc. 2007) to calculate descriptive statistics and obtain mixed-effects model estimates using restricted maximum likelihood (Harville, 1977).

We fitted separate models for each year to assess the fixed effects of treatment (x_1, pre-treatment or pre-control in 2009, treatment or control in 2010), foal presence (x_2, dependent foal < 1 year of age present with the female, or no foal present with the female), female age (x_3), band size (x_4, total number of animals in the group), and band fidelity (x_5, number of times a female changed bands within the sampling year) on time budget behaviors. In the notation of Gelman and Hill (2007), models took the form of $y_{jk[i]} = \alpha_{0j[i]} + \alpha_{1k[i]} + \beta_0 + \beta_{x_1}x_{1[i]} + \beta_{x_2}x_{2[i]} + \beta_{x_3}x_{3[i]} + \beta_{x_4}x_{4[i]} + \beta_{x_5}x_{5[i]} + \varepsilon_{jk[i]}$, where the random effect intercept of $\alpha_{0j[i]} \sim N(\mu = 0, \sigma_j^2)$ represents time period j (08:00–12:00 h, 12:01–16:00 h, 16:01–20:00 h), for $i = 1,\ldots,n$ observations, and the random effect intercept $\alpha_{1k[i]} \sim N(\mu = 0, \sigma_k^2)$ represents $k = 1,\ldots,n$ individual horses for $i = 1,\ldots,n$ observations. This equation identifies the fixed effect intercept as β_0, x = independent variable with parameter β_x, ε = statistical error, and y = dependent variable. All-occurrence social behavior was modeled in the same framework, but because those data involved a given stallion directing a behavior toward a given female, we also included the additional variable of stallion age as a fixed effect.

In order to gain insight into the effects of removal versus fertility control, we also modeled time budget and all occurrence behavior across both years, replacing treatment status with observation year as a fixed effect. This allowed us to look at the broad paradigm change, independent of trying to separate the inherently related aspects of changing sex ratio, density, and competition. We included Normalized Difference Vegetation Index (NDVI) as a fixed effect in this model in order to estimate the temporal influence of forage availability on behavior that could have varied between years. The NDVI data were obtained from the National Aeronautics and Space Administration (http://modis.gsfc.nasa.gov) and reconciled using ArcGIS software (ESRI, Redlands, California). We also included sex as a fixed effect in this model to gain insight into both male and female time budgets in concert with the culling perturbation.

3. Results

The 2009 pregnancy assessment indicated that 89% of the control group and 86% of the treatment group were pregnant at the time of saline- or GnRH vaccine-injection. In the following year, 71% (95% CI = 53.9–89.3%) of control females and 72% (95% CI = 55.1–89.7) of treated females were observed with a dependent offspring. Mean body condition of pre-control females was 5.1 (95% CI = 4.8–5.4) and of pre-treatment females was 4.9 (95% CI = 4.7–5.2) in 2009. Likewise, mean body condition of control females was 4.9 (95% CI = 4.7–5.1) and of treated females was 4.8 (95% CI = 4.7–4.9) in 2010.

We collected behavioral data on 93 feral horses (23 males, 29 pre-treatment females, 27 pre-control females, 14 other females) for 582.4 h in 2009. The median age of observed stallions was 7 years (range = 3–14 years), median age of observed females was 5 years (range = 2–17), and median band size was 8 horses (range = 3–17). In 2010, approximately 5 months after treatment and culling, we observed 77 of the same feral horses (18 males, 29 treated females, 28 control females, two other females) for 1263.90 h. The median age of observed stallions was 9 years old (range 5–15), median age of observed females was 5 years old (range = 3–18), and median band size was eight horses (range = 3–19). The final data used for GnRH treatment analyses arose from 1331 observations of 29 pre-treated and 29-pre-control females in 2009 and 2299 observations of 15 treated and 28 control females in 2010. The final data used for culling analyses arose from 2115 observations of 23 males, 27 pre-control females and 29 pre-treatment females in 2009, and 3274 observations of 18 males, 28 control and 29 treatment females in 2010.

3.1. Time budgets

Band size and foal presence were influential in how females allocated their time during 2009, but there were no differences in behaviors between pre-treatment and pre-control females (Table 2). However, treated and control females spent their time differently in the year following inoculation. Treated females fed more and moved more than control females, and reciprocated by reducing time in resting and maintenance behaviors (Fig. 1). There were no changes in time spent engaging in social behaviors between treated or control females and their conspecifics (Table 2). Band size influenced feeding time, increasing 0.9 ± 0.40 (SE)% with each additional horse in the band in 2009. This effect was not evident in 2010 after the population was reduced. Time of day influenced feeding behavior in both years. Horses fed an estimated 7.3 ± 4.69% (2009) and 7.5 ± 5.01% (2010) less in the morning, as compared to 7.4 ± 5.00% (2009) and 9.0 ± 5.04% (2010) more in the afternoon.

Time spent feeding was largely reciprocated by time resting among both treated and control groups (Fig. 1). There were no differences between pre-treatment and pre-control females in time spent resting ($t_{1270} = 0.459$, $P = 0.646$), but like feeding, this relationship changed after inoculation, and treated females rested less than their control counterparts (Table 2). Foal presence influenced

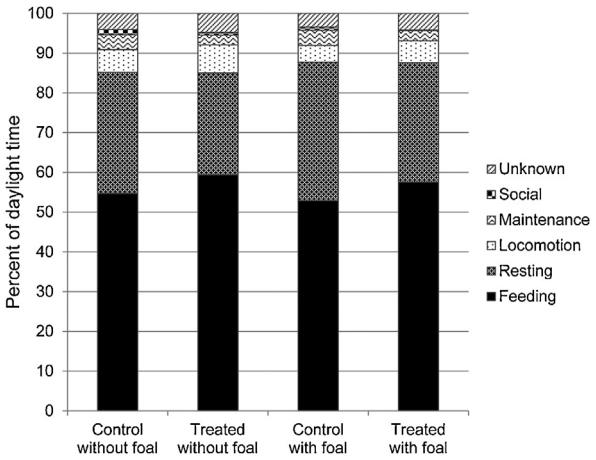

Fig. 1. Mean allocations of daylight time budget behaviors for feral horse (*Equus caballus*) females at median age (5 years old) and median band size (eight horses). Estimates are shown from mixed-effects linear regression considering individual identity and time of day as random effects. Data consisted of 2299 observations of 28 control and 15 treatment females in 2010 at Theodore Roosevelt National Park, USA. Foal presence was defined as a dependent born during the observation year.

resting in both years with independent females resting 4.4 ± 2.27% (2009) and 4.4 ± 1.83% (2010) less than females with a dependent foal (Table 2). Resting occurred temporally opposite of feeding activity, with 7.0 ± 4.43% (2009) and 6.5 ± 4.18% (2010) more resting in the morning and 6.8 ± 4.72% (2009) and 7.2 ± 4.22% (2010) less in the afternoon.

The remainder of horses' time was occupied by locomotion (about 5% of their time), maintenance (about 3% of their time), and social behavior (about 1% of their time) (Fig. 1). Treated females spent less time moving than controls and reciprocated with increased maintenance behavior (Table 2). Age was a statistically significant effect in modeling locomotion in 2010, but because time in locomotion only differed by 0.12% per year of age, this is unlikely to be biologically significant. Foal presence, however, was a strongly supported effect and females with a dependent were on the move 1.5 ± 0.35% more than independent females (Table 2). Maintenance behavior was influenced by band fidelity in 2010 and time spent exhibiting this behavior increased 1.6 ± 0.44% per band change. Six of 43 females (14%) were observed changing bands one to three times during 2010. In contrast, 23 of 58 females (40%) changed bands one to four times in 2009. The decreased band size in 2010 appears to have influenced time spent performing maintenance behavior (Table 2). Like age and locomotion, this effect was statistically significant but with an increase of only 0.1 ± 0.06% per additional band member it is unlikely to have biological meaning. Locomotion, maintenance, and social time budget behaviors were influenced minimally by time of day or individual variation (Table 2).

3.2. Social behaviors

All-occurrence social behaviors associated with herding, harem-tending, reproduction, and agonism from stallions toward females were observed 57% of the time

Table 2
Supported effects in a mixed-effects linear regression of feral horse (*Equus caballus*) time budget behaviors at Theodore Roosevelt National Park, USA. Variance for the random effects of time of day (j) and individual horse identity (k) are shown as σ_j^2 and σ_k^2.

Behavior	Supported effect	t	P	Difference	95% lower confidence limit	95% upper confidence limit	σ_j^2	σ_k^2
2009								
Feeding	Band size	2.217	0.027	0.878	0.101	1.654	0.589	0.163
Resting	Foal presence	−1.923	0.054	−4.370	−8.828	0.089	0.524	0.156
Locomotion	Band size	−2.369	0.018	−0.186	−0.340	−0.032	<0.001	0.009
Maintenance	None	–	–	–	–	–	0.007	0.100
Social	Foal presence	1.936	0.053	0.183	−0.002	0.369	<0.001	<0.001
2010								
Feeding	Treatment	−2.064	0.039	−4.685	−9.136	−0.234	0.716	0.188
Resting	Treatment	2.239	0.020	4.826	0.762	8.890	0.490	0.125
	Foal presence	−2.420	0.016	−4.425	−8.010	−0.840		
Locomotion	Treatment	−3.511	<0.001	−1.319	−2.056	−0.582	0.002	<0.001
	Age	2.059	0.040	0.117	0.005	0.229		
	Foal presence	4.304	<0.001	1.522	0.829	2.217		
Maintenance	Treatment	2.655	0.008	1.266	0.330	2.200	0.006	<0.001
	Band size	2.449	0.014	0.136	0.027	0.245		
	Band fidelity	3.617	<0.001	1.574	0.721	2.430		
Social	None	–	–	–	–	–	0.002	0.013

($n = 331$) during 2009 and 51% of the time ($n = 650$) during 2010. Herding by stallions was the most frequently observed social behavior. Band size influenced this behavior in 2009, with herding increasing 0.16 ± 0.56 times per hour (tph) per additional horse in the band (Table 3). In 2010, however, age of the stallion was the only important fixed effect on this behavior and herding increased 0.18 ± 0.038 tph per year of age. At median stallion age, median female age, and median band size, pre-treatment females received herding behavior 2.24 ± 0.743 tph and pre-control females received this behavior 1.98 ± 0.694 tph ($t_{274} = -0.892$, $P = 0.373$); likewise, treated females received herding behavior 1.06 ± 0.415 tph and control females received this behavior 0.99 ± 0.454 tph ($t_{602} = -0.271$, $P = 0.787$). Time of day was not an important influence on herding in either year and variance of this random effect was similar in both years (Table 3).

Harem-tending occurred less frequently than herding in 2009, but at a similar rate as herding in 2010. The supported fixed-effects in models of harem-tending were band size in 2009 and age of stallion in 2010 (Table 3). As band size increased per horse, harem-tending decreased 0.05 ± 0.020 tph in 2009, and as stallion age increased per year, harem-tending decreased 0.11 ± 0.026 tph in 2010. As with herding behavior, time of day did not influence harem-tending occurrence in either year and there was little individual variance in 2009.

Contrary to our prediction that reproductive behaviors would be reduced in GnRH-treated mares compared to controls, we observed almost exactly the same frequency in both years and between treatment groups. At median stallion age, median female age, and median band size pre-treatment females received reproductive behaviors 0.57 ± 0.374 tph and pre-control females received reproductive behaviors 0.40 ± 0.349 tph ($t_{274} = -1.054$, $P = 0.293$); likewise, treatment females received reproductive behaviors 0.59 ± 0.270 tph and control females received reproductive behaviors 0.56 ± 0.297 tph ($t_{602} = -0.177$, $P = 0.860$). The only supported fixed-effect in either year was female age in 2009, which estimated a 0.05 ± 0.024 tph decrease in reproductive behavior per year of age.

Agonism occurred at similar rates as reproductive behaviors in both years and was influenced by stallion age in both years (Table 3). Agonism from males toward females increased 0.03 ± 0.019 tph per year of male age in 2009 and 0.04 ± 0.017 tph per year of male age in 2010. At

Table 3
Supported effects in a mixed-effects linear regression of feral horse (*Equus caballus*) social behavior at Theodore Roosevelt National Park, USA. Variance for the random effects of time of day (j) and individual horse identity (k) are shown as σ_j^2 and σ_k^2.

Behavior	Supported effect	t	P	Difference	95% lower confidence limit	95% upper confidence limit	σ_j^2	σ_k^2
2009								
Herding	Band size	2.871	0.004	0.159	0.050	0.267	0.072	<0.001
Harem-tending	Band size	−2.731	0.007	0.054	−0.093	−0.015	<0.001	<0.001
Reproduction	Female age	−2.094	0.037	0.051	−0.099	−0.003	<0.001	0.073
Agonism	Stallion age	1.990	0.048	0.038	0.000	0.076	<0.001	<0.001
2010								
Herding	Stallion age	4.906	<0.001	0.184	0.110	0.258	0.047	0.153
Harem-tending	Stallion age	−4.234	<0.001	0.110	−0.162	−0.059	0.007	0.163
Reproduction	None	–	–	–	–	–	<0.001	0.096
Agonism	Stallion age	2.436	0.015	0.042	0.008	0.076	0.026	<0.001
	Band fidelity	2.411	0.016	0.201	0.037	0.365		

median stallion age, median female age, and median band size pre-treatment females received agonistic behaviors 0.26 ± 0.251 tph and pre-control female received agonistic behaviors 0.44 ± 0.234 tph ($t_{274} = 1.744$, $P = 0.082$); likewise, treatment females received agonistic behaviors 0.19 ± 0.184 tph and control females received agonistic behaviors 0.36 ± 0.203 tph ($t_{602} = 1.627$, $P = 0.104$). Band fidelity also influenced the 2010 estimate and agonism increased with number of band changes (Table 3). Neither time of day nor individual variance influenced agonism occurrence in either year.

3.3. Population reduction

Horses allocated time differently by sex and by year (Table 4). Males fed less, rested more, moved more and engaged in social interactions more often than females, but all horses fed less, rested more, moved more, and performed less maintenance behavior and engaged in more social behavior in 2010 as compared to 2009 (Fig. 2). Normalized Difference Vegetation Index was highly correlated between years ($R^2 = 0.90$). The finer scale all-occurrence behavioral data revealed that herding by stallions decreased 50.71% from 2009 to 2010, and appeared to increase in frequency concurrent with the seasonal green-up period (and concurrent reproductive season) (Fig. 3A). Harem-tending behavior increased 195.0% from 2009 to 2010, when both were estimated at median stallion age, median female age, median band size, and mean NDVI. Harem-tending also occurred in concert with forage production, but this relationship was more pronounced in 2010, when the frequency of occurrence was markedly escalated (Fig. 3B). Time of day and individual identity contributed little to variance in these models (Table 4).

4. Discussion

There were no differences in any behaviors between treatment groups before GnRH vaccination and culling. Females vaccinated against GnRH managed their time differently than controls. They fed less, rested more, traveled less, and performed more maintenance behaviors than their control counterparts, but engaged in the same amount of social behavior. The estimates across years, however, indicated that pronounced behavioral changes occurred in how stallions engaged with females in social behaviors. Stallions herded all females less and harem-tended more following the changes in population density, demography, and social perturbation that resulted from culling. The date of observation in relation to forage availability explained some differences between years, while the increased mean age of stallions as well as the decreased band size in 2010 also explained some of the differences. None of the models indicated that herding or harem-tending varied due to the treatment status of females, but rather implied that the paradigm shift in competition influenced how band stallions managed their family groups. Because the same stallions were observed in both years, the effect of age potentially reflected the additional year of experience each male possessed, in the context of competition pressure.

The similarity in female social behavior between treatment groups likely related to estrous behavior, a catalyst of other social behavior. Estrous behavior is influenced by the concentration and timing of progesterone and estradiol, the steroid hormones that fluctuate with occurrence of pregnancy (Katz, 2007). During pregnancy, high concentrations of progesterone usually inhibit estrous behavior (Crowell-Davis, 2007; Fabre-Nys and Gelez, 2007). The GnRH-antibodies stimulated by GnRH vaccination should have interrupted the hormonal cascade in females and ultimately suppressed pulsatile luteinizing hormone (LH) secretion, and thus ovulation (Powers et al., 2011). Consequently, we might expect that similar social behavioral expressions (albeit for different reasons) between pregnant females and GnRH-treated females would be observed. However, a similar study in elk concluded that GnRH was not completely suppressed in vaccinated females and thus some LH stimulation occurred and likely allowed limited production of estradiol (Powers et al., 2011). This permitted reproductive behaviors, at a reduced level, in GnRH-treated individuals. It should also be noted that estrous behavior has been observed with low frequency among both pregnant female and anovulatory female horses (Asa et al., 1983; Crowell-Davis, 2007). This is thought to be a bonding mechanism that assists in the maintenance of stable social groups of horses year-round, rather than ephemeral reproductive groups, such as those observed in elk. Also, the complex social behaviors among feral horses do not entirely center on reproductive receptivity, and other behaviors associated with dominance hierarchies, leadership, and gregarious lifestyles may or may not be as directly influenced by fluctuations in progesterone and estradiol.

Regardless of the similarity in social behaviors between treatment groups, treated females still allocated their time budgets differently than controls in 2010. Most GnRH-vaccinated females in the final dataset were likely not pregnant in 2010 because we categorically omitted the treated females that demonstrated unsuccessful response to vaccination by producing offspring in 2011. Pregnancy influences time spent feeding and resting simply due to the metabolic needs of barren females as compared to pregnant females (National Research Council, 2007). The composite nature of time budget behaviors requires that if any one behavior changes, then other behaviors such as locomotion or maintenance must also change; thus, the small differences between treatment groups were unsurprising.

The estimates of time spent per behavior for all horses in our study fell well within the ranges previously reported for daylight time budgets of horses (Mayes and Duncan, 1986; Boyd, 1998; Berger et al., 1999; Ransom et al., 2010). Ultimately, body condition scores of control and treated females were at parity in 2010. This may indicate that the metabolic demands of pregnant and non-pregnant females were compensated for behaviorally, that the body condition scoring system was not sensitive enough to detect finer-scale differences, or that forage abundance was high enough to obfuscate the effects of behavior on body condition. Foal presence influenced resting in both years with independent females resting less than females with a

Table 4
Between year differences in time budget and social behaviors of feral horses (*Equus caballus*) Theodore Roosevelt National Park, USA, as estimated by mixed-effects linear regression. Variance for the random effects of time of day (j) and individual horse identity (k) are shown as σ_j^2 and σ_k^2.

Behavior	Supported effect	t	P	Difference	95% lower confidence limit	95% upper confidence limit	σ_j^2	σ_k^2
Time budget								
Feeding	Year	2.141	0.032	2.141	1.838	4.178	0.743	0.098
	Sex	5.413	<0.001	6.725	4.289	9.160		
	Band size	3.699	<0.001	0.497	0.234	0.761		
	Age	−3.040	0.002	−0.477	−0.784	−0.169		
	NDVI	−22.407	<0.001	−115.698	−125.820	−105.576		
Resting	Age	2.844	0.004	0.445	0.138	0.752	0.515	0.104
	NDVI	19.903	<0.001	100.605	90.695	110.514		
Locomotion	Year	−5.726	<0.001	−1.280	−1.719	−0.842	0.001	<0.001
	Sex	−4.014	<0.001	−0.917	−1.366	−0.469		
	Band size	−3.720	<0.001	−0.098	−0.150	−0.046		
	NDVI	−5.433	<0.001	−6.307	−8.582	−4.031		
Maintenance	Year	5.122	<0.001	1.754	1.083	2.425	0.014	0.014
	Sex	−2.957	0.003	−1.279	−2.127	−0.431		
	NDVI	15.987	<0.001	27.654	24.263	31.045		
Social	Year	−3.862	<0.001	−0.714	−1.077	−0.352	0.001	0.014
	Sex	−3.095	0.002	−0.991	−1.618	−0.363		
	Band size	2.013	0.044	0.057	0.002	0.113		
	NDVI	6.798	<0.001	0.062	0.044	0.079		
All occurrence								
Herding	Year	7.581	<0.001	1.148	0.851	1.445	<0.001	0.063
	Stallion age	5.226	<0.001	0.026	0.088	0.193		
Harem-tending	Year	−9.483	<0.001	−0.838	−1.012	−0.665	0.004	0.970
	Stallion age	−4.578	<0.001	−0.078	−0.111	−0.044		
	Band size	−4.793	<0.001	−0.057	−0.080	−0.033		
	NDVI	4.918	<0.001	2.385	1.433	3.336		
Reproduction	Band size	−2.242	0.025	−0.025	−0.047	−0.003	<0.001	0.044
	NDVI	2.095	0.037	1.019	0.065	1.973		
Agonism	Stallion age	2.938	0.003	0.035	0.012	0.058	0.009	0.008
	NDVI	−2.736	0.006	−1.032	−1.772	−0.292		

dependent foal. Band size was also an important effect on some behaviors; most notably, herding and harem-tending behavior in 2009 were significantly related to band size, but this was not the case in 2010. This effect on social behaviors was replaced with stallion age in 2010. These contradictions may be in part due to the change in population size that occurred between years or due to changes in band composition. Even though band size did not change notably between years, some young animals were removed from bands.

Density of bachelor males in the population was likely influential in the large changes in herding and harem-tending behaviors observed between years: the number of bands and band size did not differ greatly between

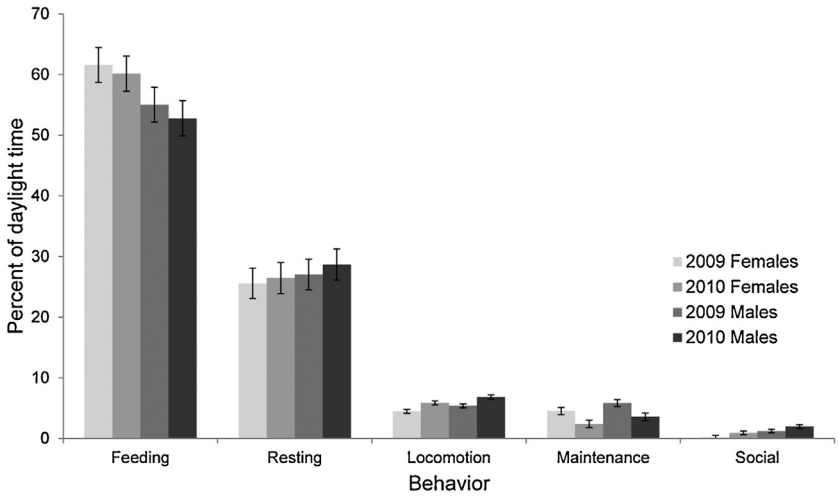

Fig. 2. Comparison of mean ± SE daylight time budget behaviors of feral horses (*Equus caballus*) before (2009) and after (2010) culling and GnRH treatment at Theodore Roosevelt National Park, USA. Estimates are shown at median age, median band size, and mean Normalized Difference Vegetation Index (NDVI) and arose from mixed-effects linear regressions considering individual identity and time of day as random effects.

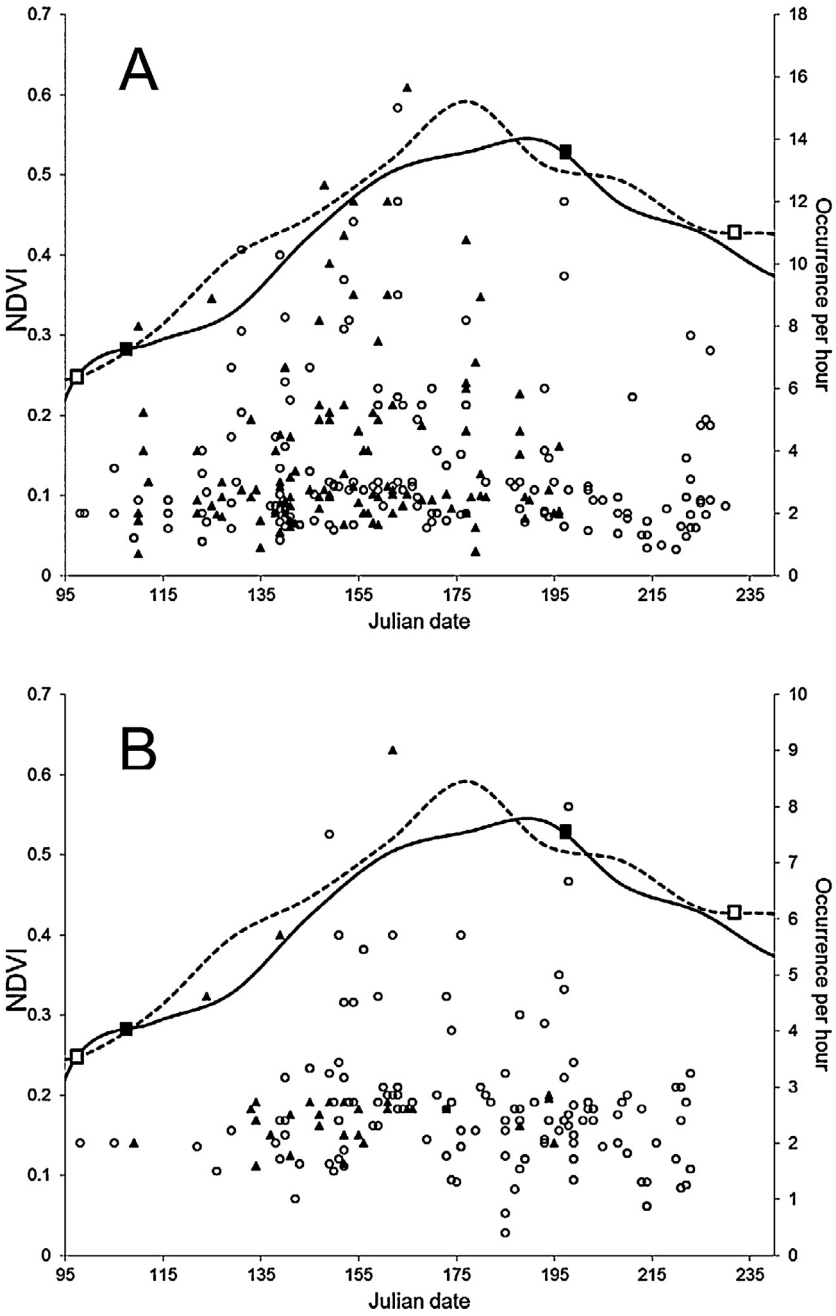

Fig. 3. Temporal occurrence and rate of feral horse (*Equus caballus*) herding (A) and harem-tending (B) behaviors in relation to seasonal trend in forage availability using Normalized Difference Vegetation Index (NDVI) as an index of greenness. NDVI for the pre-treatment year of 2009 is shown as a solid line with behaviors as solid triangles and NDVI for the post-treatment year of 2010 is shown as a dashed line with behaviors as hollow circles. Boxes on the NDVI trend lines designate the beginning and end of each behavior observation season.

years, but the number of available males in the population decreased 41% between years and the mean age of males in the population increased by nearly 3 years or 36%. This combination of effects presents compelling evidence that quickly and non-randomly reducing population density has behavioral implications for individuals and how bands function in the social dynamics of the population.

Herding and harem-tending behavior are important behaviors used by band stallions to maintain bands, but also represent an energetic cost for both the male and female(s) involved. Such costs may have biological meaning in terms of body condition and fidelity of females to the band (Ransom et al., 2010). The decline in herding behavior detected between years is not surprising given the decreased presence of competing males in the

population, which is often what prompts a stallion to actively herd females together (Waring, 2003). This decrease in potential energetic expense lends support for the practice of culling because while it decreases grazing impact by removing animals, it also leads to the remaining animals expending less energy (that would have to be replaced by grazing) by decreasing frequency of behaviors that arise from competition. Consequently, 2.2% less feeding behavior was estimated for stallions in 2010 as compared to 2009 in our study. This is supported by the observed increase in harem-tending behaviors in our study, most of which are likely driven by instinctual behavioral mechanisms more than active presence of a competitor. Such behaviors include stallions marking urine deposits of band members and constructing fecal middens that relate to reproductive status, communication, and band identification (Feist and McCullough, 1976; Rubenstein and Hack, 1992).

Little has been studied on the effects of culling in feral horse populations, and what has been presented in the literature focuses on stress and its effects on reproduction (Hansen and Mosley, 2000; Ashley and Holcombe, 2001). A short term study did collect data on percent of time horses spent feeding, resting, vigilant, traveling, and engaged in agonistic encounters and found no difference between control (horses not gathered or pursued) and simulated (horses that were pursued but evaded capture) groups (Hansen and Mosley, 2000). That study did not investigate the broader effects relating to density and sex ratio changes. We could find no other studies that investigated behavior before and after population removals of feral horses. There are some comparable studies of feral horse behavior in relation to immunocontraception however.

Porcine zona pellucida is the most extensively investigated immunocontraceptive for feral horses, and recent studies have found PZP-treated females exhibit more frequent reproductive behaviors and seasonally prolonged reproductive behaviors than their control counterparts. In a comparable study of three feral horse populations, 54.5% more reproductive behaviors were received by PZP-treated females than by control animals during the breeding season (Ransom et al., 2010). Likewise, in another feral horse population, more frequent reproductive behaviors among PZP-treated females were observed in the non-breeding season (Nuñez et al., 2010). Elevated rates of estrus have been reported in PZP-treated females of other species as compared to control females, which likely explains this increase in observed reproductive behavior (Mahi-Brown et al., 1985; Shumake and Wilhelm, 1995; Heilmann et al., 1998; Curtis et al., 2002). Seasonality of estrus in horses may not be as constrained by abiotic factors as previously thought, so it is possible that any contraceptive that does not completely suppress estrus could be associated with seasonally atypical expressions of reproductive behavior (Ransom et al., 2013). Decreased band fidelity has also been reported among PZP-treated female horses (Nuñez et al., 2009; Madosky et al., 2010), and in our GnRH study the per capita female interchange rate decreased 25.7% from 2009 to 2010. Given that some social reorganization was expected following the culling perturbation, the observed decrease in interchange provides additional evidence that GnRH-vaccinated females were not expressing the frequent reproductive behavior that appears to be coincident with decreased band fidelity.

Studies of the effects of GnRH immunocontraceptive vaccines on behavior of female horses are limited; nevertheless, a few small studies do illustrate some general patterns in responsiveness. In the only other feral horse study reported in the literature, no differences were observed between treatment groups in time spent feeding, resting, or traveling in either the breeding or non-breeding season (Gray et al., 2010). Of the 25 copulations observed in that study, only one was a GnRH-vaccinated female and it occurred during the non-breeding season. Both treated and control females demonstrated reproductive behavior roughly once every 2 h in our study. Our results concur with observations of reproductive behaviors in controlled experiments where captive mares were treated with GnRH vaccine. In those studies, treated mares showed suppressed ovarian activity and ovulation after immunization but continued to show evidence of estrous behavior at irregular intervals and duration (Dalin et al., 2002; Imboden et al., 2006). Similar behavioral responses to contraception induced by GnRH vaccine and GnRH agonist have been reported in other ungulate species as well (Baker et al., 2002, 2004; Conner et al., 2007; Powers et al., 2011). The parity we observed between treatment groups therefore simply reflects the limited reproductive behavior among control females due to high rates of pregnancy and suppressed reproductive behavior among treated females due to GnRH-inhibited ovarian activity. Since GnRH is highly conserved across mammalian species, fertility control technology founded on this hormone may suppress reproduction but does not always prevent the occurrence of sexual behaviors.

Our study was limited to only a pre-treatment and treatment year, and as such inferences should only be extended to the individual level in the short term. Both pregnancy and birth rates between treatment groups in our study were the same, but variation in birth interval among individuals can make longer term trends less certain in terms of births, dependent foals, physiology, and associated behaviors. While some studies have demonstrated females may be less likely to foal in consecutive years, as opposed to alternate years, others have found no differences (Roelle et al., 2010). Probability of foaling can also be strongly related to band size (Roelle et al., 2010), which was equitable between years in our study but may trend downward with effective fertility control over long time periods. For these reasons, the short duration of our study provides some relatively clear inference toward short-term effects of GnRH and culling on individual behaviors; however, long term effects of fertility control on behavior may manifest to all individuals as a population paradigm shifts to lower fecundity, and variable and unknown effects on social structure and dynamics emerge.

5. Conclusion

Our study found only minimal differences in behavior of free-ranging female feral horses treated with GnRH

and those treated with saline. Those differences could be physiologically explained and body condition between the two treatment groups was at parity. Following the same methodological protocols as a similar study of PZP-treatment on behavior of feral horses (Ransom et al., 2010), GnRH appears to lead to fewer behavioral modifications than PZP in this species in the short term. Long term influences of GnRH remain uncertain. The ultimate assessment of any fertility control agent must include the scope and magnitude of all potential contraindications, vaccine efficacy, and weighing the known effects against the benefit of reducing population growth. The differences in how band stallions socially interacted with all females between years provided strong evidence related to the effects of culling. These behavioral responses shed light on the effects of reducing competition and density in a social polygynous species with strong group fidelity. Such perturbation may be synergistic in reducing grazing pressure by decreasing energetically expensive competitive behaviors, but further investigation is needed to explicitly test this hypothesis.

Conflict of interest

We declare no conflict of interest among financial, personal, or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, our work.

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REVIEW

Ecological feedbacks can reduce population-level efficacy of wildlife fertility control

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Summary

1. Anthropogenic stress on natural systems, particularly the fragmentation of landscapes and the extirpation of predators from food webs, has intensified the need to regulate abundance of wildlife populations with management. Controlling population growth using fertility control has been considered for almost four decades, but nearly all research has focused on understanding effects of fertility control agents on individual animals. Questions about the efficacy of fertility control as a way to control populations remain largely unanswered.

2. Collateral consequences of contraception can produce unexpected changes in birth rates, survival, immigration and emigration that may reduce the effectiveness of regulating animal abundance. The magnitude and frequency of such effects vary with species-specific social and reproductive systems, as well as connectivity of populations. Developing models that incorporate static demographic parameters from populations not controlled by contraception may bias predictions of fertility control efficacy.

3. Many population-level studies demonstrate that changes in survival and immigration induced by fertility control can compensate for the reduction in births caused by contraception. The most successful cases of regulating populations using fertility control come from applications of contraceptives to small, closed populations of gregarious and easily accessed species.

4. Fertility control can result in artificial selection pressures on the population and may lead to long-term unintentional genetic consequences. The magnitude of such selection is dependent on individual heritability and behavioural traits, as well as environmental variation.

5. *Synthesis and applications*. Understanding species' life-history strategies, biology, behavioural ecology and ecological context is critical to developing realistic expectations of regulating populations using fertility control. Before time, effort and funding are invested in wildlife contraception, managers may need to consider the possibility that many species and populations can compensate for reduction in fecundity, and this could minimize any reduction in population growth rate.

Key-words: behaviour, demography, ecological process, fertility control, fitness, immunocontraception, population dynamics, population ecology, wildlife contraception, wildlife management

Controlling reproductive capacity of wildlife

Humans have been attempting to control the abundance of animals for over 13,000 years (Diamond 2002). Although regulating the herd size of domestic animals has been a feature of human economies for millennia, people have also sought to regulate populations of wild animals using hunting and culling techniques. Recent societal trends have motivated wildlife managers to seek nonlethal strategies of regulating births using fertility control (Hobbs, Bowden & Baker 2000; Porton 2005). This trend may be attributed to an increasingly mutualistic societal view arising from abundance of wildlife in suburban and urban areas (Teel & Manfredo 2009). In such circumstances, perception of wildlife species may focus less on

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their role as a natural resource and more on how they are part of our social environment (Leong 2010). This may lead humans to be more aware of, and concerned about, the welfare of these species. The balance of human perception and wildlife abundance is precarious because as highly adaptive species increase in density, wildlife–human conflicts increase, and society can then be confounded with the dichotomy of desire for wildlife protection and relief from wildlife conflict (Knuth *et al.* 2001; Lauber & Knuth 2004; Lauber *et al.* 2007; Hadidian 2009).

The practice of using fertility control to manage reproduction in wildlife emerged over 40 years ago (Asa & Porton 2005). Wildlife managers have been attracted to fertility control as a means to regulate overabundant wildlife when animals threaten people's lives, livelihoods or property; when they cause declines in more desirable species; and when their densities are high enough to increase disease transmission or disrupt ecosystem function (Caughley 1981; Hone 2007). The vast majority of empirical knowledge about wildlife fertility control comes from individual-level studies of drug safety and efficacy (Garrott 1995; Kirkpatrick, Lyda & Frank 2011). As a consequence, we know relatively little about how fertility control influences population ecology. It remains uncertain whether findings of research on the efficacy of fertility control at the individual level can allow inferences to populations. There may be compensation in vital rates that allow individuals to maintain fitness, thus offsetting the effects of fertility control agents. If such compensation occurs, then it may be infeasible to control some wildlife populations using fertility control.

Types of fertility control applied to wildlife include products that disturb normal reproductive hormone cascades or interfere with conception, such as immunocontraceptive vaccines, pharmaceuticals, hormone derivatives, agonists, antagonists, mechanical devices and surgical techniques (Asa 2005). All of these methods may prevent births, but they also may induce unintended changes to behaviour and physiology (Nettles 1997; Gray & Cameron 2010). It is not surprising that hormonal derivatives such as melengestrol and levonorgestrel affect behaviour (Gray & Cameron 2010), but it is surprisingly uncertain how variable such behavioural changes can be, even within similar taxa. Stump-tailed macaque *Macaca arctoides* females, for example, can become markedly more aggressive towards conspecifics when treated with a synthetic progestin (Linn & Steklis 1990), but hamadryas baboon *Papio hamadryas* females can become more passive when treated with a similar progestin (Portugal & Asa 1995).

Any fertility control application that changes the reproductive capacity of an individual has the potential to induce individual behavioural changes that can alter family group structure, influence interspecific and intraspecific interactions and ultimately shape population dynamics in unforeseen ways. Fertility control in free-roaming wildlife populations has been associated with changes in immigration (Ramsey 2005; Merrill, Cooch & Curtis 2006), decreased group fidelity (Nuñez *et al.* 2009; Madosky *et al.* 2010), increased survival (Caughley, Pech & Grice 1992; Kirkpatrick & Turner 2007; Williams *et al.* 2007), altered reproductive behaviour (Nuñez, Adelman & Rubenstein 2010; Ransom, Cade & Hobbs 2010) and shifted phenology (Ransom, Hobbs & Bruemmer 2013). Understanding and predicting these sometimes subtle and incremental shifts is difficult, and the challenge of separating the influences of multiple population growth controls can be daunting (Sibly & Hone 2002). To exacerbate these problems, the longitudinal studies needed to detect and quantify long-term population-level effects of fertility control treatments are expensive and time-consuming.

Insights into population-level effects of fertility control have largely been accomplished through efforts that simulate population dynamics (Caughley, Pech & Grice 1992; Hone 1992; Hobbs, Bowden & Baker 2000; Davis & Pech 2002; Merrill, Cooch & Curtis 2006). Simulation models can be useful tools for screening management alternatives; however, widespread behavioural and demographic changes induced by fertility control may subvert the underlying assumptions of demographic parameters in models that fail to consider these changes. The assumptions of many fertility control population models could be wrong if the vital rates informing them are based on the wealth of *a priori* ecological knowledge about species and systems before fertility control was applied.

Here, we synthesize findings from studies on population-level effects of wildlife fertility control and compare some of those outcomes with those from individual-level studies. We specifically investigate the demographic and behavioural components from wildlife fertility control studies, seeking to understand feedbacks that might be influencing population-level outcomes of fertility control strategies. We attempt to identify some of the underlying processes to help inform modelling efforts and future design for empirical population-level fertility control studies, and ultimately try to determine whether, in fact, nature can overcome control efforts at a scale that makes fertility control ineffective as a wildlife management tool.

The feedback dilemma

Considering animal behaviour in broad ecological and conservation contexts is critical to understanding population ecology (Sutherland 1998; Buchholz 2007); yet, a lack of rigorously quantified behaviour data often inhibits our ability to assess the magnitude of its importance. At the individual level, wildlife contraceptive studies have illuminated a wide array of behavioural changes attributed to fertility control applications (Nettles 1997; Gray & Cameron 2010). Studies on captive animals incorporate more experimental control than is usually possible with free-ranging wildlife, but the artificial environments inherent in captive studies often preclude the insights towards ecological feedbacks that studies of free-ranging wildlife

contribute. Consideration of results from both types of studies is needed to understand the potential population-level effects of fertility control acting through birth, survival, immigration and emigration. Feedbacks on population growth may be negative or positive, and the nature of feedback direction may vary with the ecology of the species in question.

Fertility control is designed to reduce the probability that an individual gives birth, so it should be expected that fecundity of treated animals will be less than that of untreated animals; however, indirect effects make the broader influence of treatment on birth rates less clear. Unintentionally protracted contraception has been attributed to physiological changes in ovarian function after fertility control treatments (Kirkpatrick *et al*. 1995; McShea *et al*. 1997; Nettles 1997; Powell & Monfort 2001; Stoops *et al*. 2006). Such residual infertility was observed in feral horses *Equus caballus* and Przewalski horses *E. ferus przewalskii* repeatedly treated with porcine zona pellucida (PZP) and was further complicated by shifts in the seasonal birth pulse when fertility resumed (Nuñez 2009; Feh 2012; Ransom, Hobbs & Bruemmer 2013). Increased frequency of reproductive behaviour has been observed in females treated with PZP vaccines and has the potential to focus attention of breeding males away from fertile females and towards the attractive, but sterile, females (Shumake & Wilhelm 1995; McShea *et al*. 1997; Heilmann *et al*. 1998; Nuñez, Adelman & Rubenstein 2010; Ransom, Cade & Hobbs 2010). Similarly, both elk *Cervus elaphus* and mule deer *Odocoileus hemionus* males demonstrated increased reproductive behaviour towards females treated with gonadotropin-releasing hormone vaccine (GnRH) or leuprolide (a GnRH agonist) after the untreated cohort became pregnant (Baker *et al*. 2002, 2004; Conner *et al*. 2007; Powers *et al*. 2011). All of these studies represent a potential mechanism for decreased fitness in males and could result in a higher net contraceptive effect at the population level (Ransom 2012).

In contrast, fecundity of untreated female mice *Mus domesticus* increased when fertility-controlled females were present, attenuating the contraceptive effect (Chambers, Singleton & Hinds 1999). Many species have been reported exhibiting a compensatory reproduction response when a subset of the population has been perturbed, such as might be the case with large-scale fertility control application (Swenson 1985; Kirkpatrick & Turner 1991; Boyce, Sinclair & White 1999; Williams 1999). How such effects originate through changes in social networks is unknown. Likewise, the effects of fertility control on biochemical and olfactory communication are virtually unknown, but have been implicated in disrupting intraspecific interactions in a primate (Crawford, Boulet & Drea 2010) and could lead to greater uncertainty about fecundity at the population level.

Fidelity to family groups has decreased in conjunction with fertility control applications (Fayrer-Hosken *et al*. 2000; Nuñez *et al*. 2009; Madosky *et al*. 2010), and time spent with conspecifics or potential mates has also changed (Bertrand *et al*. 1996; Poiani *et al*. 2002; Harrenstien *et al*. 2004). Territoriality increased for pikas *Ochotona curzoniae*, and the increase was related to decreased litter size (Liu *et al*. 2012). These types of changes can affect time budgets such that basic maintenance behaviours of feeding and resting decrease, resulting in lower body condition in animals receiving contraception (see Ji, Clout & Sarre 2000; for example). Similarly, female elk treated with leuprolide had comparable maintenance behaviours as untreated elk, but retained less body fat than untreated, pregnant females, possibly due to decreased anabolic hormone exposure during their infertile year (Conner *et al*. 2007).

Increased or decreased agonism between conspecifics has also been attributed to some contraceptive applications (Linn & Steklis 1990; Portugal & Asa 1995; Penfold, Patton & Jochle 2005; Snape, Hinds & Miller 2011). These behaviours can alter dominance hierarchies and thus access to resources (Dublin 1983; Schulte *et al*. 2000; Creel 2001). More directly, some deer treated with hormonal applications lost body condition or stopped eating altogether (Bell & Peterle 1975; White, Warren & Fayrer-Hosken 1994). Low body condition arising from any of these processes can enhance the fertility control effect by changing the ability of individuals to meet the nutritional requirements needed to support pregnancy and lactation (Cook *et al*. 2004; Rhind 2004).

Apart from the many potential direct and indirect effects of fertility control on births, some applications may also influence survival. Body condition and longevity can increase when the costs associated with reproduction decrease. For example, feral horses treated with a PZP vaccine had higher survival probability than untreated controls (Turner & Kirkpatrick 2002; Ransom 2012). Similarly, survival was higher among fertility-controlled rabbits *Oryctolagus cuniculus* (Twigg *et al*. 2000; Williams *et al*. 2007), coyotes *Canis latrans* (Bromley & Gese 2001) and foxes *Vulpes vulpes* (Saunders *et al*. 2002). These results show that reductions in recruitment caused by fertility control can be offset at least in part by enhanced survival of females, thereby attenuating the effect of fertility control on population growth rate.

Feedbacks amplifying the effect of fertility control can also occur. Disease transmission was 28% higher in populations of possums *Trichosurus vulpecula* with sterile females, illustrating the potential for decreased survival in fertility-controlled populations (Caley & Ramsey 2001). Phenological shifts in birth patterns among females that formerly received contraception may also have implications for survival of both females and their neonates if births occur asynchronously with forage resources (Ransom, Hobbs & Bruemmer 2013).

Perhaps, the most challenging impediment to predicting population-level effects of fertility control occurs when populations are open to immigration and emigration, processes that can be accelerated by feedbacks from the

application of fertility control. Male possums, for example, increased in frequency at sites where females received contraception (Ji, Clout & Sarre 2000), and in one study, the rate of immigration equalled the rate of reduction in births attributed to fertility control (Ramsey 2005). A similar feedback was also observed in deer (Merrill, Cooch & Curtis 2006). Home ranges of fertility-controlled female rats *Rattus argentiventer*, koalas *Phascolarctos cinereus* and white-tailed deer were larger than those of fertile females (Jacob, Matulessy & Sudarmaji, 2004; Gilman *et al*. 2010; Hynes *et al*. 2011). This was explained by an absence of range-limiting dependent young, and in deer, this larger home range was positively correlated with increased mortality rates (Gilman *et al*. 2010).

For nearly all of the collateral effects discussed here, there are almost as many cases where no collateral effects were detected when a different fertility control method was used or a different species was studied (Gray & Cameron 2010: Fig. 2). Such variation in individual and species responses emphasizes the need for careful consideration of species biology, reproductive system, physiology, behavioural ecology, population biology and ecological context when considering fertility control management for a population.

Population-level effects of fertility control

Scientific progress towards understanding population-level effects of fertility control has been slow despite an articulate call for population-level research of wildlife contraception nearly two decades ago (Garrott 1995). We reviewed 479 papers in the scientific literature on wildlife fertility control from 1980 to 2011 and found that 90% focused on individual-level effects (Fig. 1). Half of the papers were published in the last decade, reflecting an increasing interest in wildlife fertility control. There was an increasing trend in population-level studies, but still only 10% of the papers reviewed addressed population-level effects.

Only 6·5% of all papers we reviewed considered behavioural or indirect demographic effects in population-level assessment of fertility control (Table 1), and only half of those quantitatively considered such effects in modelling population dynamics with fertility control (Table 2). This is surprising given that such a large number of individual-level studies describe the potential for indirect effects to influence fertility control efficacy at the population level (Nettles 1997; Gray & Cameron 2010). The number of studies that empirically tested fertility control management for achieving long-term reduction in population size was too small for us to conduct a meaningful quantitative meta-analysis. It is worth noting, however, that the studies evaluating fertility control in open populations of promiscuous breeders did not sufficiently reduce population size for long time horizons (Twigg *et al*. 2000; Ramsey 2005; Jacob, Singleton & Hinds 2008), and a study of an open population of deer had mixed results (Merrill, Cooch & Curtis 2006). Efforts to manage relatively small, closed populations of feral horses, deer, elephants and feral cats using fertility control were much more successful (Rudolph, Porter & Underwood 2000; Delsink *et al*. 2006; Rutberg & Naugle 2007; Kirkpatrick & Turner 2008; Mendes-de-Almeida *et al*. 2011; Ransom 2012).

A broad consensus among both empirical and simulated studies is that in most free-roaming wildlife populations, a majority of the individuals in one or both sexes must be infertile to realize meaningful reduction in population growth. Reduction in abundance can require aggressively maintained treatment for long periods of time (Hobbs, Bowden & Baker 2000). This can mean that >50% of females must be treated in closed populations of long-lived, low-fecundity species to achieve moderate reduction in growth (Hone 1992; Hobbs, Bowden &

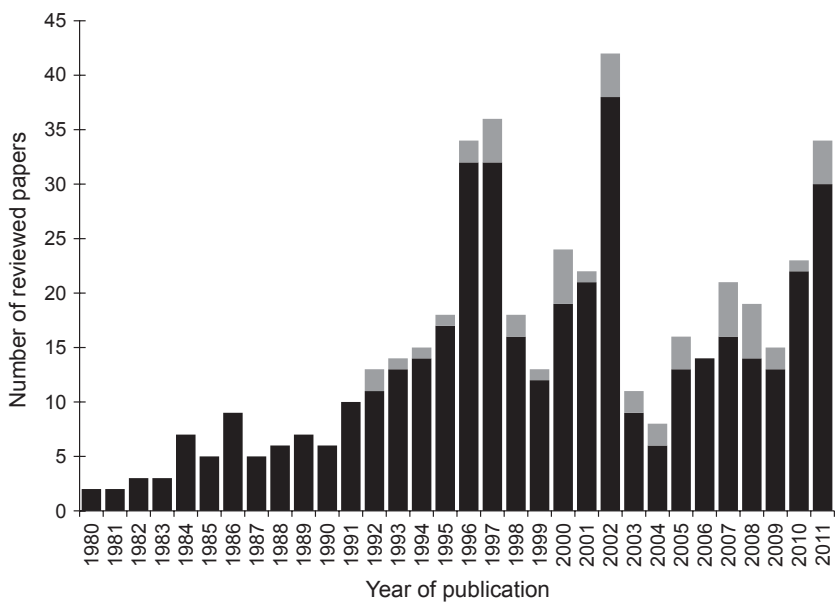

Fig. 1. Trends in publication (n = 479 papers) for research on fertility control in wild and feral fauna from 1980 to 2011. Proportion of each bar in grey depicts investigations that included empirical or simulated population-level effects.

Table 1. Number of reviewed scientific publications from 1980 to 2011 that included population-level effects of fertility control on wild or feral animals in theoretical, simulation or empirical contexts and the number of those publications that considered behavioural or indirect demographic feedbacks on population-level effectiveness of the application

	Type of study			Feedback on fertility control efficacy		
Fauna type	Theoretical	Simulation	Empirical	Negative	Positive	Not considered
Ungulate	1	10	6	4	0	13
Rodent	2	2	6	6	1	3
Marsupial	0	1	3	4	0	0
Pachyderm	2	2	1	2	0	3
Carnivore	0	3	1	0	0	4
Fish	1	0	0	1	0	0
Non-specific	4	3	0	4	0	3
Total	10	21	17	21	1	26

Baker 2000), and perhaps, >90% of females must be treated in open populations where immigration can compensate for birth reductions (Merrill, Cooch & Curtis 2006). Controlling populations of short-lived, high-fecundity animals is even more daunting because the high recruitment rates require fertility control treatments to be applied frequently (Singleton *et al*. 2002; Williams *et al*. 2007; Jacob, Singleton & Hinds 2008). In some applications, the lag time between treatment and effect on birth rates can result in too many new fertile females being recruited into the population for treatment strategies to be efficiently applied, even in long-lived, low-fecundity species (Nielsen, Porter & Underwood 1997; Whyte, van Aarde & Pimm 1998). Achieving population goals using fertility control for highly promiscuous breeders may not be feasible (Caughley, Pech & Grice 1992).

Some simulations have concluded that the response of a population to fertility control is nonlinear, and if survival increases as fecundity declines, then the survival is more influential on population growth than births (Hone 1992; Sinclair 1997; Hobbs, Bowden & Baker 2000; Merrill, Cooch & Curtis 2003). The logical conclusion to this is that fertility control is generally more effective at maintaining populations than actually reducing them, and thus, effective use of fertility control to maintain a population at reduced abundance may require culling to get the population to the desired level within a manageable time frame (Hone 1992; Barlow, Kean & Briggs 1997; Hobbs, Bowden & Baker 2000). Perhaps, most alarming is that many of these simulations assume 100% sterility, which is generally only achieved using surgical applications. Physically treating such large numbers of animals may be prohibitive due to the labour, time and expense necessary for capture, handling and application.

In contrast to these discouraging results, there is some evidence that fertility control can reduce and maintain populations at desired targets for abundance. Female feral horses have a long lifespan and can potentially produce offspring annually for up to 20 years of their life. A fertility control study at Assateague Island, the USA, reported zero population growth in only 2 years, with 42–76% of adult female feral horses treated annually with PZP (Kirkpatrick & Turner 2008). Population decline began 8 years after treatment began, and by year 11, the population had decreased nearly 23%. This sharply contrasts most simulation predictions, but may be explained by the indirect effects of treatment, the population parameters, life-history strategies of the species and the continual accessibility to individuals for treatment. This example reflects a relatively small, closed population of a polygynous species with strong social hierarchies. Thus, the lack of immigration and potentially reduced fecundity in subordinate females may act to supplement the effects of fertility control at the population level. Residual effects of treatment and decreased birth rate among untreated females contributed to a 33% difference in expected and realized births in another study of PZP in feral horses (Ransom 2012). Survival, however, appears to have increased in older females in both studies, which partially offsets the effects of fertility control. This complex system of feedbacks demands a deeper understanding of the influences acting on vital rates in populations proposed for control, and before we categorically dismiss the use of fertility control to functionally reduce populations, we must carefully consider how those feedbacks are operating.

Implications towards fitness

The genetic consequences of controlling any individual's fertility could have long-term effects on population fitness. Such artificial selection can theoretically occur in two ways. First, by purposeful or inadvertent non-random selection of animals targeted for contraception, managers may prevent reproduction in wildlife in ways that change gene flow in a population. This would have genetic effects similar to those that may arise from traditional means of wildlife management, such as non-random lethal removal of individuals from the gene pool. The second and far more uncertain mechanism is that inferred by the animal itself. Behavioural decisions unique to individuals shape their fitness, especially among males (Smith & Blumstein 2008). Such may be the case when polygynous males divert reproductive behaviour or when agonism increases or decreases in response to fertility control in the

Table 2. Population-level effects of fertility control in simulated (S) and empirical (E) studies that explicitly incorporated demographic feedbacks into analyses

Source	Taxa	Data	Individual treatment efficacy	Percent of population treated	Population effect	Feedback	Mechanism
Caughley, Pech & Grice (1992)	Gregarious, stratified by dominance structure	S	Non-specific: 100%	0–100% in social groups of up to 5 females	If female loses dominance at treatment, productivity increases	Negative	Increased births from subordinate females
Hone (1992)	a: long-lived, low fecundity b: short-lived, high fecundity	S	Non-specific: 100%	20–100% of original fecundity of females	Curvilinear response: for b, 80% fertility reduction ≈ 50% abundance reduction	Negative	Increased survival
Seagle & Close (1996)	White-tailed deer (*Odocoileus virginianus*)	S	Non-specific: 100%	0–100% of females	No reduction at <50% of females treated in open population	Negative	Increased immigration
Barlow, Kean & Briggs (1997)	a: monogamous and, b: polygamous	S	Non-specific: 100%	0–100% of females and/or 0–100% of males	Greatest effect in (a) with both sexes treated; less effect in (b)	Negative	Increased survival; increased births in b
Twigg *et al.* (2000); Williams *et al.* (2007)	European rabbit (*Oryctolagus cuniculus*)	E	Tubal ligation: 100%	0%, 40%, 60%, 80% of females	After 4 years, no difference in population size between treatments	Negative	Increased survival, increased immigration, decreased emigration
Singleton *et al.* (2002)	House mouse (*Mus domesticus*)	E, S	Tubal ligation & ovariectomy: 100%	67% of females	75% density reduction after 11 weeks, but compensation by 20–24 weeks	Negative	Increased births per female
Davis & Pech (2002)	Based on red fox (*Vulpes vulpes*)	S	Non-specific: 100%	0–100% of females	Absent density dependence, density increased with up to ~50% treatment	Negative	Increased survival
Merrill, Cooch & Curtis (2006)	White-tailed deer (*Odocoileus virginianus*)	S	Non-specific: 100%	30%, 45%, 60% of females	No population reduction if movement rates were >25% of observed and treatment was <45%	Negative	Increased immigration

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Table 2. (continued)

Source	Taxa	Data	Individual treatment efficacy	Percent of population treated	Population effect	Feedback	Mechanism
Ramsey (2005)	Brushtail possum (*Trichosurus vulpecula*)	E	Tubal ligation: 100%	0%, 50%, 80% of females	60% recruitment for 50% treated/74% for 80% treated: no population effect	Negative	Increased immigration, increased survival
Kirkpatrick & Turner (2008)	Feral horse (*Equus caballus*)	E	PZP: 0–8% birth rate in females	42–76% of females	Population decreased 22·8% in 11 years	Negative	Increased survival
Jacob, Singleton & Hinds (2008)	Ricefield rat (*Rattus argentiventer*)	E	Tubal ligation, progesterone: 100%	Up to 76% of females	No population effect in open population	Negative	Increased births, increased survival
Budke & Slater (2009)	Feral cat (*Felis catus*), closed population	S	Surgical or contraception: 100%	0–30% of females	91% of adult females need to be sterile to stop population growth	Negative	Increased survival

population. The age of individuals also interacts with demographic parameters of the population, as well as environmental variation, to influence fitness (Gaillard *et al*. 2000).

Individual physiology introduces a special consideration in the case of immunocontraceptives, and some authors have questioned the soundness of using a wild animal's immune system to select for the ability to reproduce (Nettles 1997; Cooper & Herbert 2001; Cooper 2004; Cooper & Larsen 2006). Immunocontraceptives are typically <100% effective at preventing pregnancy because they impose their effects through stimulation of the immune system. Depending on the magnitude of heritability, it is possible to select for decreased immune function and likely decreased fitness in relatively few generations. For example, if 10% of females in a population fail to mount a significant antibody response to an immunocontraceptive vaccine, and that phenotype has 80% heritability, then approximately 20% of female progeny will likewise not respond (Cooper & Larsen 2006). Thus, the trait has doubled in a single generation, assuming all females within the population were vaccinated. This has the potential to lead to immune incompetence and resistance to immunocontraception in the population. If immune responses responsible for contraception are mediated through the same or similar genetic pathways as those responsible for responding to pathogens and disease states, it is possible and even likely to select for decreased population fitness.

Alternatively, if a large proportion of the variation in contraceptive response is attributable to the environment, then even intense selection will have little effect on the phenotype of future generations (Magiafoglou *et al*. 2003). Difficulties in estimating heritability and finding reliable, accurate and sensitive indicators of changes in phenotype of immune function, given multigene effects on the system, pose significant challenges to resolve this question. However, there is evidence from studies with mice, chickens and pigs that strongly suggest that antibody production, delayed type hypersensitivity and phagocytic activity are heritable traits (Mouton, Sant'Anna & Biozzi 1988; Sarker *et al*. 1999; Wilkie & Mallard 1999; Pinard-van der Laan 2002). Significant changes in both humoral and cell-mediated immune responses can be achieved in as few as three generations in controlled environments (Sarker *et al*. 1999). Genetic variability in phenotype can change in response to environmental conditions (Hoffman 1999). This emphasizes the need for both laboratory and field studies of immunocontraceptives when investigating variance in immune response.

The proportion of the population targeted for fertility control will also influence selection pressure applied by non-response. From a practical standpoint, managers are likely to treat as many animals as possible when beginning a fertility control programme, particularly in extensively managed populations that are above management objectives. This will maximize non-response selection

pressure (Garrott 1995). Targeting for contraception may be more discriminating in intensively managed feral horse herds by selecting only a proportion of the population and ensuring that each female contributes an offspring to the population prior to contraception (Nuñez, Adelman & Rubenstein 2010). This strategy would result in decreased selection for non-response. Additionally, immigration and emigration will affect gene flow in the population and dilute the selection pressure. Treatment application intensity, non-response rate, change in fitness and migration will all influence the strength of artificial selection. This is one aspect of fertility control that wildlife managers have little, if any, valid data with which to make an informed decision. A cross-disciplinary approach involving immunologists, reproductive scientists, population or conservation geneticists and wildlife biologists may begin to answer these questions.

CONCLUSION

Study of individual effects of fertility control applications is an important step towards technology development of potential tools for managing wildlife, but understanding their ecological effects is a critical prerequisite for using them. Natural systems and processes are strained under anthropogenic pressures and ever-fragmenting landscapes, and resources to address these challenges are often lacking. Actions such as population-level fertility control demand that practitioners weigh not only the biological and ecological costs, but also the economic, political and regulatory costs involved for implementation.

Definitive generalizations about the biological and ecological conclusions of fertility control across taxa and biomes remain elusive. Primary empirical studies are still needed to evaluate population-level effects of fertility control on large wildlife populations over long time periods. Given the resources necessary to conduct such studies, it is likely that most future insight will arise from adaptive management applications. As such, fertility control as a management tool must be applied in thoughtful, calculated ways to achieve the greatest likelihood of success at reducing or maintaining population numbers. Current evidence suggests that population-level management of wildlife using fertility control is more likely to be successful if it is applied to populations (i) that are relatively small, (ii) that have little or no opportunity for an influx of new animals, (iii) that are comprised of a species that exhibits a relatively long gestation period and (iv) where a large proportion of individual animals can be accessed and aggressively treated. Fertility control management of non-native species might be more successful than management of native species because non-natives have not evolved in the ecosystem of concern, and the system is not dependent upon the species for natural function. For these reasons, alterations to life history, natural selection or behaviour may be also more publically acceptable, as long as they do not affect the conservation of native wildlife species. This hypothesis has not been tested to date. Likewise, applications to wildlife species occupying highly disturbed ecosystems may be effective because there may be disproportionately large influences from anthropogenic sources (i.e. where ecosystem drivers are more dependent on human actions than on natural processes), and the effects of fertility control on natural processes may be relatively minor in comparison.

If the necessary biological and ecological conditions can be met for effective use of fertility control to regulate wildlife populations, it is critical to address regulatory, political and economic concerns through public discourse and debate between agency, academic, political and public stakeholders who value their common wildlife resources for a wide variety of reasons. This includes transparent consideration of laws and regulations governing label and off-label use of drugs and vaccines; the welfare of animals subjected to capture, handling and treatment; and public acknowledgement that the targeted population effect and the ability of a management tool to reach such goals are not absolute. Rigorous economic analysis of the cost-effectiveness of fertility control relative to other management options is necessary to economically justify investing in the control actions (sensu Sebastián-González *et al.* 2011). This requires quantitative evaluation of the population-level and ecosystem-level benefits of the proposed actions.

Robust quantitative tools are necessary for modelling outcomes due to the disparate responses observed across species, populations and control options. Feedbacks from fertility control application vary widely and can produce quite unexpected results. In some cases, unintended changes to births, survival, immigration or emigration could provide a formidable barrier to effective management of populations using fertility control. Scientists, managers and practitioners are thus faced with understanding the often confounding nature of the actions implemented to achieve goals for populations. Fertility control can lead to complex population-level effects on the target species as well as cascading impacts on ecosystems. Both population-level and ecosystem-level impacts can and should be explored using a range of tools including empirical studies and simulation modelling. Expanding the prevailing birth-centric research theme to more thoughtful data collection and modelling that considers births, deaths, immigration, emigration, gene flow and ecological interactions will improve the quality and utility of fertility control studies.

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Spaying-induced coat changes: the role of gonadotropins, GnRH and GnRH treatment on the hair cycle of female dogs

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What is known about the topic of this paper

- It is well known that neutering of bitches may lead to a change in coat quality, although the nature of the change has never been described in detail.
- The gonadotropin concentrations of spayed untreated bitches were several times higher than concentrations found in intact or spayed female dogs treated with gonadotropin-releasing hormone (GnRH).
- Side effects of neutering of bitches, for example, urinary incontinence, may be treated with GnRH analogues.

What this paper adds to the field of veterinary dermatology

- After spaying, more follicles were in anagen and fewer in telogen compared with intact bitches.
- Spaying resulted in coat changes in 20% of the bitches.
- GnRH treatment resulted in a temporary improvement of coat changes in 79% of patients; however, the anagen : telogen ratio did not change after treatment.

Abstract

Although spaying can result in qualitative hair coat changes in dogs, the influence of spaying on the hair growth cycle has never been described. The study aims were to examine the effect of spaying and gonadotropin-releasing hormone (GnRH) treatment on canine hair coat, cycle stages of hair follicles, plasma gonadotropin concentrations and mRNA transcription of luteinizing hormone (LH) and GnRH receptors in hair follicles. Fifteen female dogs were examined before and 1 year after spaying and 24 spayed dogs before and after GnRH treatment. Spaying resulted in increased plasma gonadotropin concentrations and increased anagen : telogen ratio of hair follicles, but only 20% of the dogs developed coat changes. No differences were found in mRNA transcription of LH and GnRH receptors. GnRH treatment resulted in reduced plasma gonadotropin concentrations and improvement of coat changes in 79% of patients. This was associated with an increase in catagen hair follicles without changes in the anagen : telogen ratio. The present study demonstrated that spaying had an effect on the anagen : telogen ratio of hair follicles. Spaying-induced coat changes did not correlate with the anagen : telogen ratio. GnRH treatment reduced gonadotropin concentrations and reversed coat changes in some dogs, but had no effect on the hair growth cycle other than increasing the number of catagen hair follicles. A weak positive correlation between the plasma LH concentration and the anagen : telogen ratio was noted; however, our data did not suggest a direct receptor-mediated hormonal effect on the hair follicle. The present study did not identify the pathomechanism of spaying-induced coat changes.

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Introduction

The factors influencing hair growth and quality are diverse. Apart from genetic causes, environment and diet, cytokines and hormones influence hair growth.[1,2] It is generally accepted that in addition to many others hormones, androgens, oestrogens and prolactin are involved in the control of the normal hair cycle.[2–8]

Certain breeds, such as Irish setter, cocker spaniel, long-haired dachshund, Eurasier, chow-chow, golden retriever, Newfoundland and Landseer, have a predisposition for coat changes after spaying.[9] Affected dogs have a significant increase of wool hair and decrease in hair colour intensity. The coat resembles that of young dogs and is called 'puppy coat'.[9,10] These may be so severe that breeders advise against spaying. However, it is unknown to what extent reproductive hormones influence hair growth.

Until recently, side effects of spaying – namely, changes in the hair coat and urinary incontinence – were believed to be largely the result of oestrogen deficiency after ovariectomy. Short-acting oestrogens, administered daily to weekly, can successfully treat the 'puppy coat' condition observed after spaying.[10] Some reports, however, do not support oestrogen deficiency as the underlying cause.[11]

Iris Margaret Reichler and Monika Welle contributed equally to this work.

Dogs are treated with depot-gestagens for the long-term postponement of oestrus which results in ovarian quiescence.[12] These gestagen-treated dogs have similar basal oestrogen values as spayed dogs; however, the type of incontinence seen in spayed dogs has not been reported in gestagen-treated dogs and coat changes rarely occur. Furthermore, although oestrogen receptors have been demonstrated immunohistochemically in various locations of the hair follicle, no difference was detected between intact and spayed dogs.[3]

After ovariectomy, not only the gonadal secretion of oestrogen and progesterone is lacking, but also the feedback of these hormones to the hypothalamus and pituitary gland is interrupted. As a result, plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in spayed dogs are several fold higher than that of sexually intact dogs during anoestrus.[11,13–15] Besides the reproductive integrity, body weight, breed and time point of ovariectomy influence plasma gonadotropin concentrations.[11] The gonadotropin secretion is under the control of gonadotropin-releasing hormone (GnRH), a decapeptide from the hypothalamus. Many forms of GnRH analogues with varying potencies and persistence of action have been synthesized.[16] Repeated administrations or continuous release of GnRH analogues suppresses the secretion of FSH and LH by desensitizing the pituitary gland and thus the gonadal function. In dogs, they were used for hormonal ablation of the gonads,[17–21] for the treatment of mammary tumours,[18] prostatic hyperplasia[22] and urinary incontinence after spaying.[23,24] After treatment of incontinent dogs with depot preparations of GnRH analogues owners reported an improved coat quality.[23,24] Furthermore, receptors for FSH, LH and GnRH have been demonstrated in various epithelial structures in the canine skin.[25] The authors therefore hypothesized that the increased secretion of gonadotropins after ovariectomy may play a role in the development of the so-called 'puppy coat' observed in certain breeds of dog after spaying.

The main goals of this study were to examine the effect of spaying and GnRH treatment on the hair coat, cycle stages of the hair follicle, plasma gonadotropin concentrations and mRNA transcription of LH and GnRH receptors in canine hair follicles. Female dogs before and 1 year after spaying and spayed dogs before and after GnRH treatment were examined.

Materials and methods

Dogs

All procedures dealing with animal care, handling and treatment in this study were reviewed, approved and monitored by the Canton of Zurich Veterinary Office.

All dogs included in the study were examined at the Veterinary Teaching Hospital in Zurich. Fifteen dogs were presented for spaying (ovariectomy or ovariohysterectomy) and 1 year thereafter. Four of these dogs were spayed in spring, two in summer, five in autumn and four in winter. These 15 dogs were evaluated exactly 1 year after spaying, eliminating any potential impact of season in the parameters investigated.

Twenty-four spayed dogs were presented for GnRH treatment and were re-examined 8–16 weeks after a single subcutaneous injection of GnRH analogue (Deslorelin acetate, Peptech Animal Health Australia, North Ryde, NSW, Australia). Dogs weighing less than 30 kg received 4.7 mg of GnRH analogue and dogs over 30 kg received 9.4 mg.[21] The dogs treated with GnRH were presented because they showed side effects of spaying. Seven dogs had urinary incontinence due to urethral sphincter incompetence and two had increased aggressiveness towards other bitches, which occurred after spaying. One of these dogs as well as 13 other dogs showed coat changes after spaying. Furthermore, two dogs that did not show side effects of spaying (including coat or behavioural changes) participated in the study. Three of these 24 dogs were presented for treatment in spring, 11 in summer, four in autumn and six in winter.

Clinical examination

At each visit, the dogs had a complete physical examination performed. The hair coat was evaluated empirically and categorized as 'unchanged' or 'changed' after spaying or before GnRH treatment. The coat was classified as 'unchanged' if it had the breed-specific features. The coat was assessed as 'changed' if loss of colour intensity, excessive wool hair, loss of guard hair, or increased curling were observed. The hair coat assessment after GnRH treatment was performed only in dogs with at least one of the above changes before treatment. The dogs whose coat quality improved after GnRH treatment were placed in the category of 'clinical effect'. The dogs with no improvement were classified as 'no clinical effect'.

The sexual cycle phase of the intact dogs was determined by the interval since the last oestrus, inspection of the external genitalia and the plasma progesterone value. Anoestrus was diagnosed if the external genitalia were inconspicuous and the progesterone concentration was below 2 ng mL^{-1}. Prooestrus was defined as the period from onset of vulvar swelling and sanguineous vaginal discharge to the first signs of accepting the male dog (standing behaviour). Metoestrus was diagnosed if oestrus behaviour was not present and the progesterone values were above 2 ng mL^{-1}.

Hormone analysis

At the initial presentation, 5 mL of blood was collected from the jugular vein for hormone analysis, either during clinical examination of the dogs presented for GnRH treatment ($n = 24$) or induction of anaesthesia in the dogs presented for spaying ($n = 15$). To determine the effects of spaying and GnRH treatment on FSH and LH concentrations, all dogs had plasma concentrations of FSH and LH measured before and after spaying or GnRH treatment. In addition, the 15 dogs presented for spaying had plasma progesterone concentrations measured before spaying to determine the phase of the oestrus cycle. For hormone analysis, blood samples were transferred immediately after collection into NH4-heparinized tubes and centrifuged within 15 min for 10 min at 1500 RCF (relative centrifugation forces). The plasma was transferred into polysterol tubes and stored at –20 °C until analysis. Canine LH was determined using a specific canine LH radioimmunoassay (Biocode canine LH kit, No. AH C002, DispoLab AG, Dielsdorf, Switzerland) with the lowest limit of detection of 0.2 ng mL^{-1}. According to the manufacturer, the assay is species specific although LH from other species can partially cross-react with canine LH. Weak cross-reactivity with other canine pituitary hormones was noted and was due to contamination of the hormonal preparation as verified by a receptor assay (data not shown). Reproducibility data for this assay were as follows: intra-assay coefficient of variation (CV) of 5.35%, 4.67% and 7.21% (three samples, 18 replicates; 4.39, 23.33 and 62.10 ng mL^{-1}) and interassay CV of 5.13%, 6.47% and 18.9% (same sample, eight separate assays).

Canine FSH was determined using a canine-specific FSH immunoradiometric assay (Biocode canine FSH kit, No. AH C001; DispoLab AG) with the lowest limit of detection of 0.2 ng mL^{-1}. The specificity of this test was the same as that of the LH test. The intra-assay reproducibility (18 replicates) for this test was as follows: using 2.24, 9.18 and 34.46 ng mL^{-1}, the CV was 4.07%, 1.81% and 1.69%. The interassay (same samples, eight separate assays) CV was 9.91%, 5.46% and 4.03%. For assay purposes the reported limit was set at the lowest supplied standard concentration. For LH and FSH, this was 1.0 and 1.5 ng mL^{-1}, respectively. Plasma progesterone concentrations were measured by radioimmunoassay using a commercial kit (Coat-A-Count® Progesterone, Bühlmann Laboratories AG; Schönenbuch,

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Switzerland) previously validated for dogs.[26] The intra- and interassay CV were 5.1% and 8.8%, respectively. For assay purposes the reported limit was set at the lowest and highest supplied standard concentration, this was 0.32 and 127.2 nmol L^{-1}, respectively.

Skin biopsies

To evaluate the effect of spaying and GnRH treatment on hair follicle cycle stages and mRNA transcription of LH and GnRH receptors, skin biopsies were performed before and after spaying or GnRH treatment. Skin biopsies were taken from the flank under full anaesthesia in the 15 dogs that presented for spaying. In 10 of these dogs an additional skin biopsy was taken from the abdomen to determine if body site influenced the anagen : telogen ratio. One year after spaying as well as before and after GnRH treatment, skin biopsies were taken under local anaesthesia using 2% lidocaine. In all 24 dogs treated with GnRH, biopsies were taken from the flank at the cranial-ventral border of the wing of the ilium. The first biopsy sample was taken from the left flank and the second from the right flank to avoid potential scar tissue from the first biopsy. Eight millimetre punch biopsy instruments were used to collect all samples and the biopsy sites were closed with single sutures (Supramid 4/0). One-half of each biopsy was fixed in 4% buffered formalin at room temperature for 24 h for evaluation of the hair follicle cycle stages. The other half of the biopsy was placed into 1 mL RNAlater (RNAlater™, Qiagen AG, Hombrechtikon, Switzerland) and stored at 4 °C until polymerase chain reaction (PCR) analysis of mRNA transcription of LH and GnRH receptors.

Histological examination for determination of hair follicle cycle stages

After embedding in paraffin wax, 4-μm sections were cut from each tissue block and stained with haematoxylin and eosin. A blinded histological examination was performed by one of the authors. The cycle stages of all hair follicles present in the vertically embedded biopsies were assessed. The follicles were defined to be in anagen if either a bulb or an inner sheath was visible. The follicles were defined to be in telogen if the inner sheath cornification was completely replaced by trichilemmal cornification. Hairless telogen follicles were telogen follicles where no hair shaft was present.[27] Catagen was characterized by having no bulb, increased numbers of apoptotic cells and partial replacement of the inner sheath by trichilemmal cornification. Hair follicle cycle stages were evaluated before and after spaying (n = 15 dogs) and before and after GnRH treatment (n = 24 dogs).

Real-time RT-PCR for mRNA transcription of LH and GnRH receptors

Skin biopsy specimens preserved for mRNA extraction were further processed as previously described.[25] Real-time RT-PCR for mRNA transcription of LH and GnRH receptors was performed using TaqMan Universal PCR Master Mix (Invitrogen AG, Basel, Switzerland). Mean values (± SE) of triplicate determinations of LH and GnRH mRNA were assessed and expression levels of the genes were given as values in arbitrary units relative to the amount of the constitutively expressed 'house-keeping' gene r18S.

Real-time RT-PCR was not performed in the present study to quantify the mRNA transcription of FSH receptors, as the results of preliminary examinations gave very low values and a high mean variation in biopsy duplicates.

Data analysis

Microsoft Excel 97 was used to record data and statistical analyses were performed using StatView 5.01 (SAS Institute Inc., Cary, NC, USA). Plasma FSH and LH concentrations and mRNA transcription of LH and GnRH receptors were analysed with histograms in order to evaluate the distribution of the values. Transcription values for LH and GnRH receptors were transformed for statistical analysis because the distribution was skewed. The effects of spaying (n = 15) or GnRH treatment (n = 24) on mRNA transcription of LH and GnRH receptors and on hair follicle cycle stages were analysed by paired Student's t-test and paired sign test. These tests were also used to analyse if the body location had an impact on the above-mentioned parameters for the 10 dogs in which skin biopsies from the flank and the abdominal wall were taken before spaying.

The coat categories 'changed' or 'unchanged' after spaying, and whether the GnRH treatment had 'clinical effect' or 'no clinical effect' on coat quality were summarized by proportions and the corresponding 95% confidence intervals (CI). For the evaluation of differences between the groups ('changed'/'unchanged'; 'clinical effect'/'no clinical effect'), the Mann–Whitney U-test for two independent spot checks was used. Additionally, a simple analysis of variance was used for the transformed data and a post-hoc analysis, according to Bonferroni, was performed to confirm differences between single groups.

In order to demonstrate possible relationships between the different parameters (body weight, age, season, plasma gonadotropin concentrations, percentage distribution of the follicular cycle stage, mRNA values of LH and GnRH receptors), Pearson correlation coefficients (r) on the log transformed values and the corresponding 95% CI were calculated. Linear regressions were also performed to show the possible influence of single parameters on the percentage distribution of the follicular cycle stage and on the mRNA values of LH and GnRH receptors.

Throughout the study, statistical tests with P-values less than 0.05 were considered significant.

Results

The effects of spaying and GnRH treatment on hair coat were evaluated in 15 and 24 dogs, respectively, by examining each dog twice. Compared to the dogs presented for GnRH treatment (n = 24) the dogs presented for spaying (n = 15) were significantly younger and had a lower body weight at the time of spaying and 1 year later (mean age at the time of GnRH treatment 6 years vs. 2 years at the time of spaying (P = 0.0020) and 3 years 1 year later (P = 0.0098); mean body weight at the time of GnRH treatment 24 kg vs. 14 kg both at the time of spaying and 1 year later (P = 0.0074 and P = 0.0110, respectively).

Effects of spaying on coat change, cycle stages of hair follicles, plasma FSH and LH concentrations and mRNA transcription of LH and GnRH receptors

The effect of spaying on coat appearance, cycle stages of the hair follicles, plasma FSH and LH concentrations, and mRNA transcription of LH and GnRH receptors was examined in 15 female dogs before and 1 year after spaying. At the time of spaying, 12 dogs were in anoestrus, two in prooestrus and one in metoestrus (Table 1).

Coat changes 1 year after spaying were observed in 2 of 12 dogs (16.7%) spayed during anoestrus. A Hovawart dog had increased wool hair at the caudal aspect of the thighs and a beagle dog had a lighter coat colour. Additionally, a cocker spaniel spayed during metoestrus had a lighter coat colour and increased wool hair on the trunk and caudal aspects of the thighs. No coat changes were noted in the two dogs in prooestrus. Therefore, 3 of the 15 dogs (20%) developed coat changes 1 year after spaying.

Skin biopsies from the flank of nine anoestrous dogs and of the dogs spayed in prooestrus (two) and metoestrus (one) were analysed histologically before and after spaying. The histological sections of the remaining three dogs were of insufficient quality. Statistical analysis was only performed for the dogs spayed in anoestrus (n = 9). Before spaying, 67% of the hair follicles were in telogen and only 29.2% in anagen (median anagen : telogen ratio = 0.4). One year after spaying, significantly fewer follicles were in telogen (40.5%) and significantly more in

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Table 1. Reproduction cycle, clinical assessment of the hair coat and anagen : telogen ratio of hair follicles in 15 female dogs before and 1 year after spaying

	Immediately before spaying			One year after spaying	
Breed	Reproduction cycle	Clinical assessment of hair coat	Ratio*	Clinical assessment of hair coat	Ratio*
Hunting dog-mixed breed	Anoestrus	Breed-specific	0.3	Breed-specific	1.6
Labrador-mixed breed	Anoestrus	Breed-specific	0.4	Breed-specific	3.3
Ariegois	Anoestrus	Breed-specific	0.5	Breed-specific	0.6
Shetland sheepdog	Anoestrus	Breed-specific	0.1	Breed-specific	1.5
West Highland white terrier	Anoestrus	Breed-specific	0.5	Breed-specific	1.1
Pug-mixed breed	Anoestrus	Breed-specific	0.0	Breed-specific	0.7
Golden retriever	Anoestrus	Breed-specific	1.0	Breed-specific	0.5
Hovawart	Anoestrus	Breed-specific	0.1	Increased wool hair at the caudal aspects of the thighs	1.8
Beagle	Anoestrus	Breed-specific	0.7	Lighter coloured hair	1.2
Bernese mountain dog	Anoestrus	Breed-specific	1.4	Breed-specific	ND
Beagle-mixed breed	Anoestrus	Breed-specific	0.1	Breed-specific	ND
Jack Russel-mixed breed	Anoestrus	Breed-specific	1.0	Breed-specific	ND
Border terrier	Prooestrus	Breed-specific	1.7	Breed-specific	0.4
Welsh corgi	Prooestrus	Breed-specific	3.2	Breed-specific	0.2
Cocker spaniel	Metoestrus	Breed-specific	0.6	Lighter coloured coat, increased wool hair on the trunk and the caudal aspects of the thighs	0.7

*Anagen : telogen ratio of hair follicles; ND, not determined.

Table 2. Plasma gonadotropin concentrations and cycle stages of hair follicles and mRNA transcription of LH and GnRH receptors in skin biopsies of the flank taken from female dogs before and 1 year after spaying

	Anoestrus	One year after spaying	*P*-value
Age (years) (n = 12)	2.0 (1.3, 3.1)	3.0 (2.4, 4.1)	< 0.0001
Body weight (kg) (n = 12)	8.9 (7.4, 22.8)	9.7 (7.6, 23.0)	0.0003
Gonadotropins (n = 12)			
LH (ng mL^{-1})	1.0 (0.75, 1.18)	4.6 (3.3, 6.4)	0.0104
FSH (ng mL^{-1})	3.9 (1.9, 6.8)	80.3 (32.4, 95.1)	0.0010
Percentage of hair follicles in each cycle stage* (n = 9)			
Anagen	28.0 (8.3, 34.1)	50.8 (35.3, 58.0)	0.0126
Catagen	5.5 (0.6, 8.0)	5.8 (4.0, 7.4)	0.5
Telogen†	68.0 (59.6, 79.6)	40.5 (34.7, 54.7)	0.0196
Hairless telogen	25.5 (9.0, 34.9)	17.1 (10.3, 22.0)	0.8
Anagen : telogen ratio	0.4 (0.09,0.6)	1.2 (0.6, .7)	0.0215
Receptor mRNA (n = 12)			
LH flank skin	61.0 (11.3, 168.4)	29.8 (11.2, 167.9)	0.8
GnRH flank skin	1.2 (0.5, 5.2)	3.5 (1.4, 7.1)	0.1242

Results are presented as median values with the 1st and 3rd quartile (Q1, Q3) in parentheses. *Unclear follicle cycle stages were taken into account in regards to the total number, but were not assigned to a cycle phase. †All telogen follicles including hairless telogen.

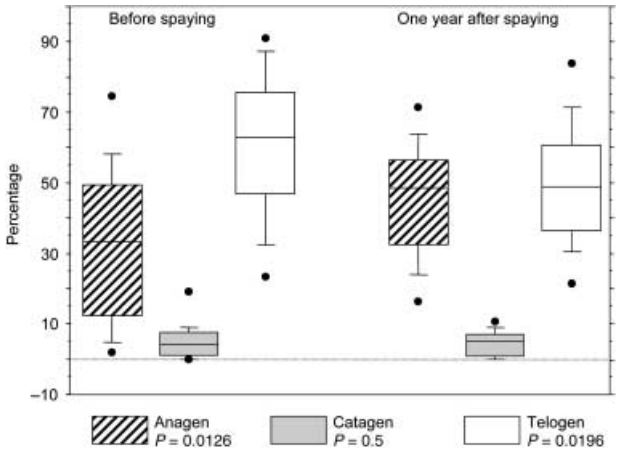

Figure 1. Percentage of anagen, catagen and telogen hair follicles in skin biopsies from the flank of nine dogs in anoestrus before spaying and 1 year after spaying. Values in the box plots reflect the 25th, 50th (median) and 75th percentiles. The whiskers represent approximately 90–95% of the values. Outliers are represented by dots.

anagen (50.8%, median anagen : telogen ratio = 1.2) (Fig. 1; Table 2). This increase of the anagen : telogen ratio was shown in eight of nine dogs (P = 0.0391).

No difference in the anagen : telogen ratio between dogs with and without coat changes after spaying could be observed (anagen : telogen ratio: 0.9 vs. 1.1, respectively; P = 0.9).

One year after spaying, the body weight as well as plasma LH and FSH concentrations of the 12 female dogs spayed during anoestrus were significantly increased. Although the median mRNA value of LH receptors was twice as low in spayed dogs and of GnRH receptor was nearly three times higher, these findings were not statistically significant. The data are shown in Table 2.

Effects of GnRH treatment on coat change, cycle stages of hair follicles, plasma FSH and LH concentrations and mRNA transcription of LH and GnRH receptors

The effect of GnRH treatment on coat quality as well as on the cycle stages of the hair follicles, plasma FSH and LH

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concentrations and mRNA transcription of LH and GnRH receptors was examined in 24 spayed dogs before and 8–16 weeks after treatment. Fourteen spayed dogs were presented for GnRH treatment because they had developed coat changes after spaying (Table 3). According to the owners, most dogs (n = 13) had significantly more wool hair especially on the trunk, caudal aspect of the thighs and the abdomen. In three dogs the coat was curlier after spaying. At re-examination 8–16 weeks after GnRH treatment, the coats of three dogs remained unchanged whereas in 11 dogs the coat quality improved. Two dogs had a 10- to 15-cm-wide stripe of a shinier, smoother and intensively coloured hair on the dorsum from the shoulder down to the sacral region (Fig. 2). The effect of duration of treatment varied considerably. In one dog, the improvement of coat quality lasted only 4 months, whereas in the majority of dogs, it lasted at least 6 months. Consistent with previous reports, all 24 female dogs tolerated the GnRH treatment and no systemic or local side effects were observed.[19,21,23,24,28,29] The body weight of the dogs before and after treatment was similar (Table 4).

The analysis of the follicular stages before and after treatment was performed only in 21 dogs because the histological sections of three dogs were of insufficient quality. Before GnRH treatment, spayed dogs with coat changes (n = 14) had a higher anagen : telogen ratio than spayed dogs with unchanged coats (n = 7) (median = 0.6 (1st and 3rd quartiles = 0.2, 1.2) vs. 0.2 (0.1, 05); P = 0.0560). Furthermore, higher plasma FSH concentrations before treatment were observed in dogs presented with coat changes compared to dogs with breed-specific coats, but this difference was not significant (median = 66.2 ng mL^{-1} (1st and 3rd quartiles = 54.4, 83.9) vs. 36.6 ng mL^{-1} (28.4, 70.0); P = 0.0862). Plasma LH concentrations and mRNA transcriptions of LH and GnRH receptors were similar in both groups before treatment. After GnRH treatment, the anagen : telogen ratio did not change significantly; however, a significant increase in the percentage of catagen follicles was observed. Plasma FSH and LH concentrations of all 24 dogs treated with GnRH decreased after treatment. The mRNA values for LH and GnRH receptors were similar before and after GnRH treatment. Data are shown in Table 4.

After GnRH treatment, no differences in mRNA transcriptions of LH and GnRH receptors, percentage of follicles in different cycle stages, and plasma FSH and LH concentrations were observed between dogs showing clinically improved coat quality (n = 11) and nonresponders (n = 3) (data not shown).

Influence of body weight, age, season, and plasma gonadotropin concentrations on the percentage distribution of the follicular cycle stages and on the mRNA transcription of LH and GnRH receptors

A weak relationship between the anagen : telogen ratio and the plasma LH concentrations in spayed dogs could be demonstrated (r = 0.324, P = 0.0705). Spayed dogs with high plasma concentrations of LH had higher percentages of anagen follicles than dogs with low LH plasma concentrations. A relationship between hair cycle stages and gonadotropin concentrations was not found in dogs in anoestrus or in spayed GnRH-treated dogs. No associations

Figure 2. Picture of a male dog not evaluated in the present study demonstrating the effect of neutering and gonadotropin-releasing hormone (GnRH) treatment on the hair coat. This dog had coat changes similar to those observed in the Malinois dog and cocker spaniel in the present study. (a) Cocker spaniel with the breed-specific coat before neutering. (b) The same dog 6 months after neutering. Note the marked wool hair, the dull coat colour and the increased hair curvature. (c) The same dog 12 weeks after GnRH treatment: the coat is darker, shinier and smoother on a 10-cm stripe at the dorsal midline from the shoulder to the sacral region. (d) The same dog 16 weeks after GnRH treatment: the hair on the dorsal midline is woollier and has a dull appearance.

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Table 3. Clinical assessment of the hair coat and anagen : telogen ratio of hair follicles in spayed dogs before and after GnRH treatment

	Before GnRH treatment		After GnRH treatment		Category after
Breed of dog	Clinical assessment of hair coat	Ratio*	Clinical assessment of hair coat	Ratio*	GnRH treatment
Newfoundland	Marked wool hair on the trunk	0.8	Coat easier to comb out	0.4	Clinical effect
Retriever-mixed breed	Marked wool hair, coat with increased curvature	5.0	Coat easier to comb out, less curvature of the coat	1.0	Clinical effect
Retriever/Irish setter	Marked wool hair, dun colour at tips of hair	0.5	Coat easier to comb out, more intensive colour	1.0	Clinical effect
Border-mixed breed	Marked wool hair, coat looks like fabric fur	0.2	More shiny, less wool hair	0.5	Clinical effect
Old German shepherd	Moderate wool hair, particularly on the caudal aspect of the caudal thigh	0.2	Coat easier to comb out	0.4	Clinical effect
Malinois	Marked wool hair, coat look dull	0.3	Marked stripe on the midline of the back	0.4	Clinical effect
Samoyed-mixed breed	Wool hair on the caudal thigh and ventral aspect of the trunk	1.3	Coat easier to comb out, dog looks slimmer	0.9	Clinical effect
Eurasian	Extensive wool hair on the neck, trunk and legs, combing out impossible	0.5	Coat more shiny, coat easier to comb out	0.8	Clinical effect
Eurasian	Marked wool hair, browning of the hair tips	1.1	Less wool hair	0.7	Clinical effect
Beagle	Pale coat colour	1.2	More intensive coat colour	1.7	Clinical effect
Cocker spaniel	Marked wool hair on the neck, head and trunk, coat is dull	0.7	Shiny stripe on the midline of the back with little wool hair, coat shiny	0.5	Clinical effect
Cocker spaniel	Marked wool hair, coat with significant curvature	1.1	No change	1.0	No clinical effect
Golden retriever	Marked wool hair, dun colour, slightly increased curvature	0.2	No change (possibly slightly darker)	0.1	No clinical effect
Wäller	Marked wool hair, dun colour	0.1	No change	1.0	No clinical effect
Dachshound	Breed-specific coat	1	Breed-specific coat	1.4	ND
Labrador	Breed-specific coat	0.5	Breed-specific coat	0.4	ND
Labrador	Breed-specific coat	0.1	Breed-specific coat	0.3	ND
Pit bull terrier	Breed-specific coat	0.2	Breed-specific coat	0.2	ND
Whippet	Breed-specific coat	0	Breed-specific coat	0.1	ND
Boxer	Breed-specific coat	0.3	Breed-specific coat	1.0	ND
Boxer	Breed-specific coat	0.1	Breed-specific coat	1.9	ND
Bernese mountain dog	Breed-specific coat	ND	Breed-specific coat	1.3	ND
Border terrier	Breed-specific coat	0.4	Breed-specific coat	ND	ND
Dalmatian	Breed-specific coat	0.3	Breed-specific coat	ND	ND

*Hair follicle anagen: telogen ratio. ND, not determined.

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Table 4. Plasma gonadotropin concentration, cycle stage of hair follicles and mRNA values of LH and GnRH receptors in skin biopsies from the flank of spayed dogs before and 8–16 weeks after GnRH treatment

	Before GnRH treatment	After GnRH treatment	*P*-value
Age (years)	5.7 (3.4, 9.7)	6.2 (3.9, 10.0)	< 0.0001
Bodyweight (kg)	23.0 (19.8, 31.7)	23.5 (20.3, 31.3)	0.3
Gonadotropins (n = 24)			
LH (ng mL^{-1})	5.7 (3.4, 7.0)	1.0 (0.75, 1.0)	< 0.0001
FSH (ng mL^{-1})	59.1 (39.8, 81.4)	1.0 (0.6, 2.2)	< 0.0001
Percentage of follicles in cycle stage* (n = 21)			
Anagen	30.1 (17.2, 43.4)	40.1 (24.8, 48.7)	0.2
Catagen	0.6 (0.0, 3.7)	4.0 (1.7, 7.0)	0.0302
Telogen†	67.9 (45.6, 81.2)	55.9 (49.6, 71.8)	0.1191
Hairless telogen	24.6 (18.8, 34.6)	26.6 (15.9, 32.7)	> 0.9
Anagen : telogen ratio	0.4 (0.2, 1.1)	0.7 (0.4,1.0)	> 0.9
Receptors (n = 24)			
LH flank skin	12.0 (8.0, 27.9)	20.6 (7.8, 45.2)	0.3
GnRH flank skin	0.7 (0.2, 1.9)	0.8 (0.3, 1.6)	0.3

Results are presented as median values with the 1st and 3rd quartile (Q1, Q3) in parenthesis. *Unclear follicle cycle stages were taken into account in regards to the total number, but were not assigned to a cycle phase. †All telogen follicles including hairless telogen.

were observed between the anagen : telogen ratio and body weight, age and season, respectively, in dogs that are in anoestrus, spayed, or spayed and GnRH treated (data not shown).

Plasma gonadotropin concentrations were significantly lower in spayed dogs with a higher body weight (LH r = –0.421, P = 0.0147, FSH r = –0.473, P = 0.0038). Age and season at the time of examination had no effect on plasma LH or FSH concentrations (data not shown).

A significant influence of body weight on mRNA values of LH receptors in skin biopsies of the flank of intact and spayed untreated dogs was demonstrated. The higher the body weight, the lower the mRNA transcription of LH receptors (intact: r = –0.530, P = 0.0062; and spayed: r = –0.373, P = 0.0350). This effect was not observed on mRNA values of GnRH receptors. After GnRH treatment, no associations were found between mRNA values of LH and GnRH receptors and body weight. There was no relationship between mRNA transcriptions of LH or GnRH receptors and plasma gonadotropin concentrations, age or season at the time biopsies were taken, respectively (data not shown).

Influence of biopsy site

Biopsies from the flank and from the abdominal wall could be evaluated in 10 of the 15 intact dogs. A higher anagen : telogen ratio in the biopsies of the flank compared to the abdominal wall was noted in samples from the same individual; however, the difference was not significant (P = 0.0673). This was due to a higher percentage of hairless telogen follicles in the abdominal wall (flank: median 22.3 (1st and 3rd quartiles = 11.5, 31.0) vs. abdominal wall: 35.6 (25.0, 44.0), P = 0.0135). The mRNA transcription of LH and GnRH receptors was similar in biopsies of the flank and abdominal wall in intact anoestrous dogs (median 50 (1st and 3rd quartiles = 9198) vs. 249 (17, 1130) P = 0.2; and median = 1.1 (0.4, 4.9) vs. 1.6 (1.3, 4.8) P = 0.3, respectively).

Discussion

Although it is frequently observed that in dogs of certain breeds spaying results in changes of the hair coat quality, to the authors' knowledge, the influence of spaying on the hair cycle and mRNA transcription levels of gonadotropin receptors has never been described.

In this study, 3 of 15 (20%) female dogs exhibited coat changes 1 year after spaying. The changes occurred in a Hovawart and a beagle dog in anoestrus and a cocker spaniel in metoestrous.

The anagen : telogen ratio was significantly increased 1 year after spaying independent of coat changes. A similar effect has also been demonstrated in rats where ovariectomy caused a profound and rapid telogen to anagen transition, with accompanying full hair growth.[30] The percentage of the different follicle stages may be influenced by body location, breed and season.[31–34] However, in the present study, it was possible to exclude these influences since the same dogs were reinvestigated at the same time of the year and biopsies from the same body location were taken. The only influence on the hair follicle cycle phases that could not be excluded entirely was age and body weight, although no significant relationship between these parameters could be demonstrated in this study.

Spayed dogs that were presented with coat changes for GnRH treatment had a higher anagen : telogen ratio compared to dogs with unchanged coats. The abnormal coat quality noted after spaying is called 'puppy coat'. After birth all hair follicles in dogs are in late stage of morphogenesis, similar to anagen, and the hair is of fairly constant diameter and curvature. At the age of 3–6 months, dogs 'lose' their puppy coat and develop the breed-specific adult coat. It is justifiable therefore to relate the changes in hair follicle cycle and coat quality observed in spayed dogs with the coat of puppies. The result of the present study may also explain the successful empirical oestrogen therapy in dogs with 'puppy coat'. Estrogens delay the initiation of the anagen hair follicle phase[35] and, thus, counteract the described spaying effect.

Subjectively, GnRH treatment was highly effective on the hair coat quality. At re-examination 8–16 weeks after treatment, nearly 80% of the dogs had less wool hair and a shinier and more intensively coloured coat. GnRH analogues are also given successfully to human patients with excessive hair growth.[36] It remains unclear why in the

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present study the response to GnRH therapy ranged from ineffective to long lasting. In dogs and other species, the required dose and duration effect of GnRH therapy are individually variable.[21,23,24]

Before and after GnRH treatment a large variability in the percentage distribution of hair cycle stages was observed. Although the same dog was examined before and after treatment, biopsies were taken from different breeds at various times of the year and these factors could also have affected the hair follicle stages. After GnRH treatment no significant difference in the anagen : telogen ratio was found. However, a significantly higher percentage of catagen follicles after treatment was observed, which may indicate that GnRH terminates the anagen phase and initiates the catagen phase, and thus has a similar effect to androgens. However, these data have to be considered with precaution since dogs were re-evaluated 8–16 weeks after GnRH treatment; therefore, at a different time of the year.

No difference in the percentages of follicular cycle stages was observed between dogs that had clinically improved coat quality after GnRH treatment and non-responders. This was unexpected as the owner's observation that the wool hair was easier to comb after GnRH treatment suggested that more telogen hair follicles was present. These findings could be the result of seasonal influences, since the repeated skin biopsies were taken 2 to 3 months apart; therefore, at a different season of the year. It has been shown in intact male dogs with same hair type but of different breeds that the time of the year influences hair growth individually.[33] A study including a greater number of treated dogs evaluated at the same season before and after treatment would be necessary to obtain more reliable data.

After spaying, plasma gonadotropin concentrations increased significantly. A weak positive relationship between plasma LH concentrations and the percentage of anagen follicles in spayed dogs could be demonstrated. A relationship between LH concentrations and hair growth is also known in humans. Women with polycystic ovarian syndrome have increased hair growth and abnormally high LH concentrations.[37] This finding supports a role for LH on the hair cycle.

In spayed dogs with coat changes, a tendency towards higher plasma FSH concentrations was observed compared to dogs without coat changes. This finding is supported, to some extent, by a study in postmenopausal women who complained about hair and skin changes.[38] Increased FSH values were seen in those women mostly bothered by such postmenopausal problems.[38] However, the possible role of FSH in the pathophysiology of spay-induced coat changes is unclear.

The plasma gonadotropin concentrations of all dogs treated with GnRH decreased significantly as previously reported.[23,24] Although most treated dogs showed an improvement of the hair coat quality, no relationship with lower plasma gonadotropin concentrations could be shown.

The influence of the sexual cycle on hair growth has been described in a large number of studies.[34,39–43] A direct receptor-mediated effect of oestrogens and androgens on the hair follicle is unlikely because the removal of the gonads does not seem to have an effect on these receptors.[3] Based on the present results, the same seems to be true for LH and GnRH receptors, since no significant changes could be shown after spaying or GnRH treatment.

Body weight seems to influence the mRNA transcription of LH receptors with lower levels noted in heavier dogs. It can be suggested that there is an association between the lower plasma gonadotropin concentrations in heavy dogs observed in another study,[11] and the lower receptor expression found in this study. However, in this study we could not find a significant correlation between body weight and gonadotropin concentrations. It is possible that receptor expression is not only influenced by body weight but also by other factors such as breed and season. However, studies need to be conducted to test this hypothesis.

Although FSH receptors were demonstrated in the canine skin by immunohistochemistry, and indirectly through the presence of low levels of FSH receptors mRNA by PCR,[25] a quantitative assessment of mRNA transcription of FSH receptors in the skin was not performed in the present study as preliminary results gave very low values and a high mean variation in biopsy duplicates.

The mRNA transcription of LH and GnRH receptors in the flank and abdominal skin was similar, although the percentage of the different follicle phases was significantly different. Therefore, a direct role of gonadotropins and GnRH influencing the hair cycle is not very likely. However, receptor protein is responsible for hormone binding in a target tissue and thus necessary for hormone action. A quantitative demonstration of receptor protein, and simultaneous evaluation of the plasma gonadotropin concentrations and the anagen : telogen ratio in dogs of the same breed before and after spaying would lead to more conclusive results about the role of FSH, LH and GnRH on the hair cycle.

It is likely that there is an indirect relationship between LH plasma concentrations and the percentage of anagen follicles. The LH secretion in intact animals is not only influenced by the feedback mechanism of oestradiol, but also by prolactin which suppresses the LH secretion. After gonadectomy, the prolactin concentration in rats significantly decreases, but can again increase with oestrogen treatment.[44,45] If the same endocrinological mechanisms occur in the dog, a causal role of prolactin would be conceivable. In addition, this might also explain the positive effect of oestrogen treatment seen in spayed dogs with a 'puppy coat'. Furthermore, after treatment with prolactin-suppressing substances a change of the coat colour was observed,[46] which would also be in agreement with a key role of prolactin. The absolute deficiency of prolactin in prolactin knockout mice results in slightly longer and coarser hair than in wild-type mice.[5] Because prolactin induces the catagen stage,[5,47,48] a deficiency of prolactin in spayed dogs could explain the shift in favour of anagen follicles as was observed in the present study. Prolactin and its receptor are expressed in hair follicle epithelium and show hair cycle-dependent expression in mice and mares.[47,48] Future studies are needed to clarify if prolactin plays a causative role in the pathomechanism of spaying-induced coat changes.

In summary, the present study clearly shows an increased anagen : telogen ratio in dogs after spaying independent

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of coat changes. Additionally, coat changes after spaying could be successfully treated with GnRH. However, GnRH treatment did not influence the anagen : telogen ratio and no changes in mRNA transcription of LH or GnRH receptors were found after spaying or after GnRH treatment. Thus, a direct receptor-mediated hormonal effect of the pituitary and hypothalamic hormones on the follicle is unlikely and the results of this study cannot clarify the underlying pathomechanisms of coat changes after spaying.

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Résumé Bien que la stérilisation puisse être responsible de modifications de la qualité du pelage chez le chien, son influence sur la pousse pilaire n'a pas été rapportée. Cette étude s'est intéressée à examiner les effets de la stérilisation et d'un traitement à la GnRH sur le pelage, le cycle folliculaire, les concentrations en gonadotropines plasmatiques et la transcription de l'ARNm des récepteurs à la LH et la GnRH dans les follicules pileux. Quinze chiennes ont été examinées avant et un an après une stérilisation et 24 chiens mâles castrés avant et après traitement à la GnRH. La stérilisation a provoqué une augmentation des concentrations plasmatiques en gonadotropines et une augmentation du rapport anagène/télogène, mais seulement 20% des chiens ont présenté des modifications du pelage. Aucune différence n'a été observée pour la transcription d'ARNm des récepteurs LH et GnRH. Le traitement à la GnRH a provoqué une diminution des concentrations en gonadotropine plasmatique, et une amélioration du pelage chez 79% des chiens. Ceci était associé avec une augmentation des follicules en phase catagène sans modification du rapport anagène:télogène. Cette étude démontre que la stérilisation a un effet sur le rapport anagène:télogène des follicules pileux. Les modifications pilaires ne corrèlent pas avec le ration anagène:télogène. Le traitement à la GnRH a diminué les concentrations en gonadotropines et a permis d'améliorer le pelage chez certains chiens, mais n'a pas eu d'effet sur le cycle pilaire, hormis une augmentation du nombre de follicules en catagène. Une corrélation faible entre les concentrations plasmatiques en LH et le rapport anagène:télogène a été observée, cependant nos données n'ont pas suggéré un effet direct médié par les hormones sur le follicule pileux. Cette étude n'a pas permis d'identifier le mécanisme pathogénique des modifications du pelages observées après stérilisation.

Resumen Aunque la esterilización puede resultar en cambios cualitativos en el pelaje de los perros, las influencia de la esterilización en el ciclo de crecimiento del pelo nunca ha sido descrita. Este estudio trata de examinar el efecto de la esterilización y del tratamiento con GnRH en el pelaje canino, los estadíos del ciclo de los folículos pilosos, las concentraciones de gonadotropina en el plasma y la transcripción del mRNA de los receptores LH y GnRH. Se examinarion quince hembras antes y después de un año del tratamiento de esterilización y 24 perros esterilizados antes y después del tratamiento con GnRH. La esterilización resultó en un aumento de la concentración de gonadotropina y un aumento de la relación anágena:telógena de los folículos pilosos, pero sólo un 20% de los perros presentaron cambios en el pelaje. No se observaron diferencias en el nivel de transcripción de los receptores de LH y GnRH. El tratamiento con GnRH resultó en una reducción de la concentración de gonadotropina y en una mejora de la calidad del pelaje en un 79% de los pacientes. Esto se relacionó con un aumento de los folículos en fase catágena sin cambios en la relación anágena:telógena. Este estudio demuestra que la esterilización tuvo un efecto en la relación anágena:catágena de los folículos pilosos. Los cambios en el pelaje inducidos por la esterilización no se asociaron con la relación anágena:telógena de los folículos pilosos. El tratamiento con GnRH redujo las concentrationes de gonadotropina e invirtió los cambios en el pelaje en algunos perros, pero no tuvo efecto el el ciclo de crecimiento del pelo, excepto por un incremento del número de folículos en fase catágena. Se observó una pequeña correlación entre la concentración de LH en plasma y la relación anágena:telógena; aunque nuestros datos no sugerían un efecto directo hormonal mediado por receptores en los folículos pilosos. Este estudio no identificó el mecanismo patogénico de los cambios en el pelaje inducidos por la esterilización.

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Zusammenfassung Obwohl die Ovariohysterektomie bei Hunden eine qualitative Veränderung des Haarkleides bewirken kann, wurde der Einfluss der Ovariohysterektomie auf den Haarwachstumszyklus nie beschrieben. Die Ziele dieser Studie bestanden darin, die Wirkung der Kastration und der GnRH-Behandlung auf das Haarkleid des Hundes, auf die Zyklusstadien der Haarfollikel, auf die Gonadotropinkonzentrationen im Plasma und auf die mRNA Transkription der LH und GnRH Rezeptoren in Haarfollikeln zu untersuchen. Es wurden fünfzehn Hündinnen vor und ein Jahr nach der Kastration, sowie 24 kastrierte Hündinnen vor und nach Behandlung mit GnRH untersucht. Die Kastration resultierte in erhöhten Gonadotropinkonzentrationen im Plasma und einem erhöhten Anagen-Telogen-Verhältnis der Haarfollikel, obwohl nur 20% der Hunde eine Veränderung des Haarkleides entwickelten. Bei der mRNA Transkription der LH und GnRH Rezeptoren wurden keine Unterschiede festgestellt. Die Behandlung mit GnRH ergab niedrigere Gonadotropinkonzentrationen im Plasma und eine Verbesserung der Fellveränderungen bei 79% der Patienten. Dies wurde im Zusammenhang mit einer Zunahme der katagenen Haarfollikel ohne Veränderungen des Anagen-Telogen-Verhältnisses gesehen. Die vorliegende Studie zeigte, dass die Kastration eine Auswirkung auf das Anagen-Telogen-Verhältnis der Haarfollikel hatte. Die durch die Kastration bedingten Veränderungen des Haarkleides korrelierten nicht mit dem Anagen-Telogen-Verhältnis. Die Behandlung mit GnRH reduzierte die Gonadotropinkonzentrationen und machte die Haarkleidveränderungen bei manchen Hunden rückgängig, hatte aber außer einer Zunahme an katagenen Haarfollikeln keinen Einfluss auf den Haarwachstumszyklus. Eine schwach positive Korrelation zwischen der LH Konzentration im Plasma und dem Anagen-Telogen-Verhältnis wurde beobachtet; unsere Daten wiesen jedoch nicht auf eine direkte, durch den Rezeptor vermittelte, hormonale Wirkung auf den Haarfollikel hin. Die gegenwärtige Studie konnte den Pathomechanismus der durch die Ovariohysterektomie bedingten Veränderungen des Haarkleides nicht identifizieren.

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Sexual behavior in geldings

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Abstract

In response to a request published in *Equus*, a magazine for those interested in horses, 85 owners of older geldings exhibiting sexual behavior completed history forms. The mean age of geldings was 16 ± 5 years. Only 39 of the owners had had the gelding for at least a year before the behavior was noted. These cases could be used to determine the true age of onset of the problem. When log survivorship was used to determine whether there were one or two different populations, a break or change in the slope at age 16 indicated that there are two populations. One population shows the behavior from the time of castration and the other first exhibits the behavior in old age, possibly in response to an ACTH secreting pituitary adenoma. A total of 40% of the horses were Quarterhorses, the most numerous breed in the US; 78% of the horses were purebreds. Fewer than half the owners knew the age at which their horse had been castrated because they did not own the horse at the time. The mean age at castration, when known, was 3.3 ± 2.5 years. The reason for contacting us was sexual behavior (70%), aggression (24%), or some other problem (1%). Whether or not aggression was the presenting problem, most of the horses showed aggression (95%), particularly towards other geldings (88%), but also towards people (31%). Copulatory behavior (mounting) was shown by 69% of the geldings and half of those were able to intromit. These findings indicate that the sexual behavior of geldings is a problem for owners and that aggression usually accompanies sexual behavior. The owners were encouraged to send serum samples taken before and after human chorionic gonadotropin (HCG) administration for testosterone and estrone sulfate analysis to determine whether residual testicular tissue was responsible for the horse's behavior. Of the 14 horses tested, only one had elevated levels of testosterone indicating that there was residual testicular tissue. A total of six of the owners agreed to treat their horses with cyproheptadine at a dose of 8 mg day^{-1} gradually increased to 88 mg day^{-1} per horse. A total of three of the horses showed a decline in sexual and aggressive behavior, one got worse and two had side effects and treatment was withdrawn.

Unilateral and Bilateral Laparoscopic Ovariectomy in 157 Mares: A Retrospective Multicenter Study

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Objective: To evaluate surgical techniques, perioperative complications and outcome of laparoscopic surgery for unilateral and bilateral ovariectomy in mares.
Study Design: Retrospective multicenter case series.
Animals: Mares (n = 157).
Methods: Indications for surgery were neutering, fertility problems, elimination of hormone-related abnormal behavior, ovarian tumors as well as nonregressive ovarian hematomas or cysts. Ovariectomy (n = 206) were performed by a standard laparoscopic procedure in 157 standing sedated mares. Dissection and hemostasis were achieved by using either (1) a linear stapling device, (2) bipolar electrosurgical instruments, with or without a modified Roeder knot, (3) a vessel-sealing system, or (4) a diode-laser with ligation. For some larger ovarian masses (>20 cm diameter), a 2-step surgical procedure was used with standing flank laparoscopic ovariectomy followed by ovarian retrieval through a median celiotomy. Surgical techniques, outcome, and perioperative complications of each method were recorded and analyzed.
Results: Laparoscopic dissection of the ovary was accomplished in all mares. Because of the size of the ovarian tumor (n = 7) or in 1 mare with behavioral problems (n = 1), the dissected ovary was removed through a median celiotomy under general anesthesia. Seventeen mares (10.8%) developed postoperative complications (eg, incisional drainage, incisional infection, seroma formation, dehiscence, transient fever and mild abdominal discomfort). All mares with flank incisional problems had ovarian size >12 cm and in 15 (88%) of these mares electrosurgical instruments were used for mesovarial dissection.
Conclusions: Laparoscopic ovariectomy in standing sedated mares provides good surgical access and is associated with low morbidity.

INTRODUCTION

Different conventional surgical procedures[1–5] and minimally invasive techniques[6–11] have been reported for unilateral and bilateral ovariectomy in mares. Ovariectomy seems to be associated with a higher frequency of perioperative complications than other elective surgical procedures.[12,13] Ovariectomy by laparotomy or colpotomy is associated with serious perioperative complications, including hemorrhage, myopathy and neuropathy, wound infection and dehiscence, eventration, peritonitis, adhesions, postoperative colic and ileus.[14,15] Minimal invasive surgery is considered to result in fewer complications and has become more popular in the last few years. In comparison to colpotomy and laparotomy, laparoscopic procedures offer the advantages of good intraoperative visibility, secure hemostasis, less invasiveness, reduced surgical morbidity, and shortened hospitalization.[8,16] According to individual surgeon preference, laparoscopic intervention can be in the standing sedated horse[9,17–25] or in dorsal recumbency.[10,11,26–28] Most authors favor surgery in standing sedated mares because of the normal anatomic orientation, increased operative visibility, and avoidance of the risks and complications associated with general anesthesia.

We report findings from a retrospective multicenter clinical study to evaluate the surgical techniques, intra- and perioperative complications, and outcome of laparoscopic surgery for unilateral and bilateral ovariectomy in mares.

MATERIALS AND METHODS

Horses

Medical records (1996–2009) of 157 mares that had either unilateral or bilateral ovariectomy at the Veterinary Clinic Starnberg, Germany, the Department of Equine Surgery, School of Veterinary Medicine, Justus-Liebig University, Giessen, Germany, and the Faculty of Veterinary Medicine, Department of Equine Sciences, Utrecht University, Netherlands, were selected. Clinical indications were neutering, fertility problems, elimination of hormone-related abnormal behavior, ovarian tumors as well as non-regressive ovarian hematomas or cysts.

A complete physical examination, a hematologic and serum biochemical profile and a gynecologic examination, including abdominal palpation per rectum with ultrasonographic evaluation of the reproductive tract were performed in all mares presented for ovariectomy, regardless of the reason.

Depending on the clinical indication, the behavior of the mare, the size of the ovary, and the surgeon's preference, ovariectomy was performed as a standing laparoscopic surgical procedure or a 2-step surgical procedure. For the 2-step procedure, in the 1st step, transection of the ovarian pedicle was carried out laparoscopically in the standing sedated mare and in the 2nd step, the dissected ovarian tumor was extracted through a median celiotomy in dorsal recumbency under general anesthesia.

Mares were provided free choice of water but fed only mash and pellets for 36–48 hours before surgery or had food withheld for 48 hours. Cefquinomsulfate (1.0 mg/kg intramuscularly [IM]), procaine penicillin G (22,000 U/kg, IM) or sodium benzylpenicilline (30,000 U/kg, IM) in combination with gentamicin (6.6 mg/kg intravenously [IV]), and flunixin meglumine (1.1 mg/kg, IV) or meloxicam (0.6 mg/kg, IV) were administered preoperatively. In 119 mares, butylscopolamine (0.2 mg/kg, IV) was administered preoperatively to achieve spasmoanalgesia.

Surgical Technique

Sedation and analgesia was achieved with detomidine (0.01 mg/kg, IV) and butorphanol (0.01 mg/kg, IV). Throughout the surgical procedure, sedation was adjusted as needed by administration of detomidine and butorphanol (each in a dosage of 0.005–0.01 mg/kg, IV).

According to the diagnosis, one or both paralumbar fossae were shaved and aseptically prepared. Local analgesia was achieved by a paravertebral anesthesia and or an infiltration anesthesia subcutaneously, intramuscularly and subperitoneally with 20 mL 2% lidocaine (with or without 2% adrenalin) each at the surgical sites: the paralumbar fossa and the 17th intercostal space at the level of the tuber coxae.

Laparoscopic ovariectomy was achieved through 2 ipsilateral instrument portals in the flank and 1 optic portal in the 17th intercostal space or just caudal to the last rib. After exposure of the ovary, infiltration anesthesia of the ovarian pedicle was performed using 10–20 mL 2% lidocaine (with or without 2% adrenalin). Before transection, the size of the ovary was reduced by suction of fluid, when possible. Dissection and secure hemostasis of the mesovarium was achieved using either (1) a linear stapling device (35 mm or 45 mm, Endo-GIA, Covidien, Service Center S.A., Elancourt, France); (2) bipolar electrosurgical instruments, with or without ligation; (3) a vessel-sealing system (LigaSure® Force Triad™, Tyco Healthcare Deutschland GmbH, Soltau, Germany); or (4) a diode-laser (Dornier MediLas D®, Dornier Medizinlaser GmbH, Wessling, Germany) with ligation. In 13 mares, the ovarian tumor was contained within in an intraabdominal sterile plastic bag, cut into pieces and then retrieved within the bag. In the mares, the ovaries were removed intact, when necessary by enlarging 1 of the instrument portals or by connecting the 2 instrument portals up to a length of 15 cm using a modified grid technique. After removal of the ovary, portals and flank incisions were sutured. In bilateral interventions, the same procedure was performed through the opposite paralumbar fossa to remove the contralateral ovary. In 1 mare, the dissected left ovary was moved from the left to the right side, and both ovaries were exteriorized through the same right-sided incision at the end of the procedure.

For ovarian tumors with a diameter >20 cm or if the mare displayed aggressive behavior, ovariectomy was accomplished in a 2-step procedure.

Surgical techniques for dissection and hemostasis, the diameter of the tumor (measured with a ruler after removal) and intra- and perioperative complications (bleeding of the mesovarium, wound healing problems, fever and abdominal discomfort) were evaluated. In 30 mares, surgical time was measured to evaluate the influence of the dissection technique (linear stapling, bipolar electrosurgery and vessel-sealing); these procedures were performed by 1 surgeon in horses with an ovarian tumor <12 cm diameter.

Postoperative Care and Follow-up

Cefquinomsulfate (1.0 mg/kg intramuscularly, IM), procaine penicillin G (22,000 U/kg, IM every 12 hours), or sodium benzylpenicillin (30,000 U/kg, IM) in combination with gentamicin (6.6 mg/kg, IV) and flunixin meglumine (1.1 mg/kg, IV) or meloxicam (0.6 mg/kg, IV) were administered according to the initial administration for 2–6 days. During hospitalization, a clinical examination and white blood cell count were performed the first 3 days postoperatively. Horses were discharged with a recommendation of stall rest for 10–14 days after laparoscopic intervention and for 6 weeks after a 2-step surgical procedure with hand-walking twice daily before resuming normal use. Short-term results (6–8 weeks) were determined by telephone questionnaire of owners, trainers or referring veterinarians in 72 mares.

RESULTS

Ovariectomy (n = 206) was performed in 157 mares. Laparoscopic ovariectomy was bilateral in 49 mares and unilateral in 108 mares. Clinical indications for bilateral ovariectomy were neutering (24 mares), nymphomania, hormone-induced abnormal behavior as well as estrus-related reduced athletic performance (24 mares), and bilateral ovarian tumor (1 mare). Unilateral ovariectomy was performed for granulosa-theca-cell tumor (GCT) in 103 mares, an ovarian cyst in 1 mare, and nonregressive ovarian hematoma in 4 mares. The diameter of the GCT in 68 mares was <12 cm (66%), in 23, between 12 – 20 cm (22%) and in 12, > 20 cm (12%).

Complete laparoscopic ovariectomy was achieved in 149 standing sedated mares. In 7 mares, laparoscopic dissection was followed by removal of the ovary through median celiotomy in dorsal recumbency because the ovarian tissue was > 20 cm diameter. In only 1 mare was conversion to celiotomy necessary after laparoscopic dissection of the ovarian pedicle because of inability to control behavioral problems in the standing mare.

Hemostasis and dissection of the ovarian pedicle was performed using either a 35 mm or 45 mm linear stapling device (n = 66), by vessel-sealing system LigaSure® (n = 49) or by using bipolar electrosurgical instruments as a single technique (n = 49) or in combination with a circular ligature around the main vessels (n = 38), knotted extracorporeally using a modified Roeder knot. In 2 mares, hemostasis was initially performed with a diode-laser and in 2 mares with a single modified Roeder knot. In these 4 mares, hemostasis was incomplete and bipolar electrocoagulation was necessary to occlude the bleeding vessels.

Use of linear stapling devices (Endo-GIA) was technically easy and created very precise cut edges. Mean ± SD surgical time was 40.2 ± 10.2 minutes. Two cartridges were necessary to completely divide the mesovarium of a normal ovary, whereas 3–4 cartridge units were needed for ovarian tumors.

Using the 10-mm bipolar vessel-sealing system, (LigaSure®) the ovarian pedicle was progressively coagulated and cut in a single-step process. The main vessels were sealed twice at a distance of 1 cm before cutting. The device allowed very efficient and safe sectioning of the mesovarium and created a smooth coagulated wound surface at the resection site. By using this system, smoke evacuation was minimal and hemostasis was secure. Mean surgical time was 46.2 ± 8.2 minutes.

Electrocoagulation was performed using a bipolar forceps with a mechanical cutting blade, which allowed tissue coagulation and division over a length of 5 mm. Because the cranial and caudal part of the mesovarium is less vascularized, a single coagulation was sufficient to achieve secure hemostasis. In the middle part, including the main vessels, multistage tissue coagulation was performed before dissection. At the resection site, a coagulated and carbonized wound surface was created. This technique turned out to be a time-consuming procedure before a secure hemostasis was achieved. Mean surgical time was 60 ± 15.4 minutes.

In 13 mares, the ovarian tumor was intraabdominally placed in a sterile plastic bag, cut into pieces with a scalpel blade (# 11 or 22) and then extracted in pieces. In the other mares, the ovaries were removed intact, when necessary by enlarging 1 instrument portal or by connecting 2 instrument portals.

No major intraoperative complications occurred. Seventeen mares (10.8%) developed postoperative complications, such as postoperative dehydration (n = 1), extensive subcutaneous emphysema (n = 1), wound swelling, and seroma formation followed by infection and dehiscence of the flank incision (n = 15). In 4 of these 15 mares, transient fever up to 39.2° C was observed over 3–5 days, starting on the 2nd postoperative day. Two of these mares had mild postoperative depression and colic signs on the 1st and 2nd postoperative day. In 1 of these mares, a fist-sized hematoma at the resection site was diagnosed by rectal palpation and ultrasonography. In the other mare, an adhesion between the resected ovarian pedicle and the base of the cecum was detected, which was easily released by transrectal manipulation. All postoperative complications occurred in mares with an ovarian tumor size >12 cm (in 15 of 27 mares where the tumor was removed by flank incision). No incisional problems occurred in the celiotomy wounds.

Transection of the mesovarium was carried out with the bipolar electrosurgical forceps in 10 of 15 mares with incisional problems, in 2 mares with a combination of bipolar electrocoagulation and circular ligature, in 1 mare where a linear stapling device was used and in 2 mares where the vessel-sealing system was used. The hematoma as well as the intraabdominal adhesion occurred in mares in which hemostasis and dissection of the ovarian pedicle was carried out with the bipolar electrosurgical forceps.

All postoperative complications responded to conservative treatment and all 157 mares were discharged from the clinic. Mares that had laparoscopic surgery had an overall postoperative morbidity of 10.8% and mortality of 0%.

In the follow-up questionnaire, a slight depression of the musculature was reported for 8 mares at the site of the enlarged flank-incision. No further complications were reported.

DISCUSSION

We report laparoscopic ovariectomy in 157 mares (46 bilateral, 97 unilateral) for removal of normal (48 mares) and pathologic (109 mares) ovaries, using different techniques for mesovarial dissection and transection, with no mortality and 10.8% morbidity that is substantially lower than is reported for conventional surgical ovariectomy techniques (mortality, 4.5–7.7%[14]; morbidity, 24.4–26.6%[29]).

Incisional problems were the predominant complications (15 mares) and only occurred in mares with ovarian

diameter > 12 cm. In 12 of these mares, mesovarial dissection was by electrosurgery (10 electrosurgery alone, 2 electrosurgery in combination with ligation). Others have reported incisional complications being statistically associated with ovarian tumor size[30,31], particularly when ovarian diameter is > 18 cm. Six to 8 weeks after surgery, 8 mares in our study had a depression at the flank incision site. We did not suture the internal abdominal oblique and transverse abdominal muscles in the mares in this study. It is possible that closure of the muscle layers may have prevented this cosmetic defect; however, suturing these layers is technically difficult because of the small wound size. Nevertheless avoiding cosmetic defects is important in mares used for competition or those intended for sale.

Different techniques were used for removal of the transected ovaries. After mesovarial dissection, complete ovaries < 15 cm diameter were removed by enlarging the instrument portals exclusively, using forceps and wound-retractors. Hand-assisted procedures[32], were not used. In 13 mares, we used an intraabdominal retrieval bag and fragmented the ovary to minimize the size of the mass being exteriorized. Other reported techniques include placing the mobilized ovary in a sterile plastic disposable surgical drape and, after intraabdominal transection into pieces with a scalpel or a morcellator[33], removal of the contained ovarian tissue through a smaller incision.[32,34,35] This technique offers also the advantages of decreasing the risks of losing the ovary and avoiding wound contamination by the tumor during removal. In an effort to prevent incisional complications and to optimize cosmetic outcome in 7 mares with a tumor diameter >15 cm, even after size-reduction by suction, we used a 2-step procedure with ovariectomy performed standing and ovarian exteriorization performed under general anesthesia through a median celiotomy.

We used different primary methods to achieve hemostasis of the mesovarium before or during transection: linear stapling (66 ovaries), vessel sealing (49 ovaries), electrosurgical dissection (49 ovaries), electrosurgery in combination with ligation (38 ovaries); diode laser and ligation (2 ovaries) and ligation followed by electrocoagulation (2 ovaries). Measured in 30 mares with the same surgeon, time to accomplish mesovarial separation of an ovary (<12 cm diameter), was comparable (stapling, 40.2 ± 10.2 minutes: vessel sealing, 46.2 ± 8.2 minutes) although slightly longer with the electrosurgical method (60 ± 15.4 minutes). Other reported techniques include use of a modified Roeder knot[32] or by diode-laser.[20] Use of a modified Roeder knot or endoloop ligature are reportedly more economical[36]; however, a disadvantage of these techniques is the potential for ligature slippage and bleeding during dissection especially with enlarged ovaries,[30] and which we also experienced in 2 mares. A combination of bipolar electrosurgical dissection of the cranial and caudal part of the mesovarium with a circular ligature around the main vessels was reliable in our hands for removal of normal and enlarged ovaries. Surgical lasers (eg, diode- or Nd:YAG) should only be used in addition to one of the techniques already described.[20] The coagulative abilities of these lasers are insufficient to achieve reliable hemostasis as the sole method. We had this experience in 2 mares and had to use electrocoagulation to achieve hemostasis. During laser-dissection, there is also risk of thermal damage to adjacent structures.[37]

A safe, effective, but expensive, technique for laparoscopic ovariectomy is use of a vessel-sealing system (LigaSure®).[35] We found this to be a quick, safe method for hemostasis of the mesovarial vasculature. Although vessel size may limit effective use of the LigaSure® especially in pathologic ovaries,[38] we did not encounter any difficulty in its effective use. Perhaps this is because we sealed the main vessels twice before cutting. Compared with monopolar or bipolar electrosurgical instruments,[39,40] surgical time is less with the Ligasure and thermal damage is confined to the target tissue.[41] The main disadvantage of electrosurgical instruments for ovariectomy in mares is the small-sized jaws (5 mm) of the bipolar forceps. Multistage tissue coagulation was needed in our mares to achieve reliable hemostasis causing a circumscribed thermal soft tissue necrosis with carbonization of the target tissue. As a consequence, in 1 mare a fist-sized hematoma occurred at the resection site and in another mare, an adhesion occurred between the resected ovarian pedicle and the base of the cecum. It seems that, the risk of postoperative adhesions might be higher when using electrosurgical instruments for coagulation of the ovarian vasculature.

Laparoscopic ovariectomy in the standing sedated mare has many advantages compared with conventional surgical ovariectomy through a median celiotomy. These include superior viewing, normal anatomic orientation, easier intraabdominal manipulation, and avoidance of the risks of general anesthesia.[9,42] In dorsal recumbency using conventional surgical technique, tension-free and secure ligation of the mesovarium under visual control cannot be guaranteed.[14,15,29] Excessive traction on the ovarian pedicle can result in intraoperative hypotension, which is believed to contribute to myopathy and neuropathy.[14,15,43] Using stapling devices for dissection of the mesovarium makes this surgical step technically easier. But even then, application of staples and ligatures tightened under tension may result in inadequate hemostasis.[44,45]

In our study, except for 8 mares, laparoscopic ovariectomy was completed in the standing position. Based on our favorable experiences with standing laparoscopic ovariectomy, recumbent ovariectomy should be limited to horses that exhibit behavioral problems during standing surgery. Laparoscopic ovariectomy in dorsal recumbency and 30° head down position has, from the technical point of view, no distinct advantages. Indeed, there is the potential disadvantage of cardiopulmonary derangement during laparoscopic surgery in the Trendelenburg position.[9,11,25,46]

Each surgical technique for ovariectomy in the mare has offered distinct advantages and disadvantages. Comparison of the different surgical techniques should be interpreted cautiously because treatments in this multicenter case series study were not conducted in a blind or random manner. Nevertheless, in our clinical experience laparoscopic ovariectomy in standing sedated mares has

been proven to be the preferred method for ovariectomy and avoids the risks of general anesthesia. It can be performed safely and reliably for removal of normal and enlarged ovaries and, compared with conventional surgical techniques, is less invasive[47,48] and is associated with substantially lower perioperative morbidity and mortality.

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Potential Demographic and Genetic Effects of a Sterilant Applied to Wild Horse Mares

Open-File Report 2015–1045

U.S. Department of the Interior
U.S. Geological Survey

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U.S. Department of the Interior
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U.S. Department of the Interior
SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2015

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Abbreviations Used in This Report

≥	Greater than or equal to
<	Less than
λ	lambda; annual population growth rate multiplier for a herd
BLM	Bureau of Land Management
K	carrying capacity
N	population size
U.S.	United States

Potential Demographic and Genetic Effects of a Sterilant Applied to Wild Horse Mares

By James E. Roelle and Sara J. Oyler-McCance

Abstract

Wild horse populations on western ranges can increase rapidly, resulting in the need for the Bureau of Land Management (BLM) to remove animals in order to protect the habitat that horses share with numerous other species. As an alternative to removals, BLM has sought to develop a long-term, perhaps even permanent, contraceptive to aid in reducing population growth rates. With long-term (perhaps even permanent) efficacy of contraception, however, comes increased concern about the genetic health of populations and about the potential for local extirpation. We used simulation modeling to examine the potential demographic and genetic consequences of applying a mare sterilant to wild horse populations. Using the VORTEX software package, we modeled the potential effects of a sterilant on 70 simulated populations having different initial sizes (7 values), growth rates (5 values), and genetic diversity (2 values). For each population, we varied the treatment rate of mares from 0 to 100 percent in increments of 10 percent. For each combination of these treatment levels, we ran 100 stochastic simulations, and we present the results in the form of tables and graphs showing mean population size after 20 years, mean number of removals after 20 years, mean probability of extirpation after 50 years, and mean heterozygosity after 50 years. By choosing one or two combinations of initial population size, population growth rate, and genetic diversity that best represent a herd of interest, a manager can assess the likely effects of a contraceptive program by examining the output tables and graphs representing the selected conditions.

Introduction

As the primary agency responsible for management of wild horses (*Equus caballus*) on public lands in the United States (U.S.), the Bureau of Land Management (BLM) has a need for a long-lasting, non-lethal method to control population growth rates. Given the protection afforded by The Wild Free-Roaming Horses and Burros Act of 1971 (P.L. 92–195 as amended) and general lack of natural predators, wild horse populations commonly increase at rates of 15–25 percent per year (Eberhardt and others, 1982; Garrott and others, 1991a) and quickly exceed the carrying capacity of their ranges. In the past, BLM has managed excess horses through a gather, removal, and adoption program, with unadopted horses held in short- and long-term holding facilities until the end of their natural lives. The sustainability of this strategy has recently been called into question, however (U.S. Government Accountability Office, 2008; Garrott and Oli, 2013; National Research Council, 2013). Therefore, BLM has increased efforts to develop a contraceptive that will reduce population growth rates in wild horse herds and thus reduce the number of excess animals that must be removed and cared for.

The contraceptive SpayVac has recently demonstrated the potential for long-lasting efficacy (at least 4 years) in wild horses (Killian and others, 2008). Maximizing the duration of contraceptive effectiveness is especially important in managing wild horses, which in most cases must be captured in order to successfully administer the drug. With extended efficacy (or perhaps even permanent sterility), however, comes increased concern for both the genetic health of a horse herd and the possibility of extirpation. Simulation modeling can be a useful tool in understanding the likely consequences of alternative management actions. We used the VORTEX software package (Lacy and others, 2014; Lacy and Pollak, 2014) to simulate the effects of treating various proportions of mares with a permanent sterilant. Response variables included population size, number of animals removed, average heterozygosity, and probability of local extirpation. (The VORTEX manual (Lacy and others, 2014) uses the term "extinction" rather than "extirpation." We believe that extirpation is more appropriate in this case, as we are simulating local populations, not the entire species.) We chose the VORTEX software because it can be used to simulate both demographic and genetic effects, and because it has been useful in simulating management of the horse population on Assateague Island (Ballou and others, 2008).

VORTEX is an individual-based model in which events happen or fail to happen on the basis of probabilities for each modeled individual. For example, if 100 females are of breeding age, each of which can produce at most one offspring, and fecundity is set to 80 percent, there is no guarantee that exactly 80 offspring will be born. Rather, for each female of breeding age, a random number chosen from a uniform distribution between 0 and 1 is compared to the probability of producing an offspring (in this example, 0.8). If the random number is less than 0.8, then the female is tagged in the model to give birth but otherwise does not. Thus, no two simulations are identical even when they use the same demographic parameters, and multiple iterations must be run in order to examine the possible range of outcomes. In general, we present results in the form of mean values for the multiple simulations; however, it is important to remember that extreme values can also be of interest. In addition, it is important to note that these simulations represent the results of management prescriptions applied consistently through time, without adaptation based on monitoring of results. In the real world, there would be many opportunities to adjust management strategies based on previous results.

Model Parameters

Parameter values and VORTEX options used in our simulations are summarized in table 1 and described in some detail in the sections that follow. Our knowledge of the biology and demography of horse populations and the efficacy of SpayVac and other forms of contraception is incomplete; what is applicable to one herd may not be applicable to all herds. In light of that fact, in cases where specific parameter values were in question, we tried to select the value or option that would have the largest effect on one or more of the response variables. For example, we don't know that SpayVac is a sterilant, but it may be. Furthermore, BLM is considering the use of other techniques (for instance, ovariectomy) that definitely will be permanent. So, for modeling purposes, we assume a permanent sterilant; once a mare is treated and the treatment works, she is infertile for life. This assumption is likely to minimize the number of animals tagged for removal during a simulation, while at the same time maximizing any decline in heterozygosity. We were similarly conservative in our choice of other parameter values and options, always choosing those that were likely to have the most detrimental effect on population demography and genetics.

Table 1. Parameter values and options used in simulations of the effects of a mare sterilant applied to wild horses.

[H_o, observed heterozygosity; ≥, equal to or greater than; BLM, Bureau of Land Management]

Parameter or option	Value selected for simulations
Mating system	Long-term polygyny
Age of first reproduction	Females = 3 years, males = 5 years
Maximum age (life span)	25 years
Maximum age of reproduction	20 years
Basic time cycle	1 year
Maximum litters per year	1
Maximum progeny per litter	1
Density-dependent reproduction	Not used
Reproductive rates	See tables 2 and 3
Mortality or survival rates	See tables 2 and 3
Sex ratio at birth	50 percent males
Mate monopolization	Mean of 3.0 mates per successful sire
Initial population size	100, 250, 500, 750, 1,000, 1,250, or 1,500
Initial age distribution	Stable age distribution calculated from age-specific schedules in table 2 and 3
Carrying capacity	Arbitrarily set at 5× the initial population size, but the contraception and removal programs prevent carrying capacity from having any effect
Initial heterozygosity	Actual allele frequencies at 13 microsatellite loci from the Lost Creek herd in Wyoming (high diversity, $H_o = 0.78$) or the Cibola-Trigo herd in Arizona (low diversity, $H_o = 0.49$)
Removals	When the simulated population reaches ≥150 percent of the initial population size, try to remove enough animals to get back to the initial population size. BLM's age-class removal criteria are used, but are constrained such that no more than 90 percent of any age and sex class are removed.
Efficacy of treatment	Treatment is efficacious in 90 percent of all newly treated mares
Duration of efficacy	If treatment is efficacious, female remains contracepted for life
Proportion of females treated	0 percent–100 percent, in 10 percent increments
Standard deviation due to environmental variability	$S_{EV} = 0.12$ for fecundity; $S_{EV} = 0.06$ for mortality
Concordance	Yes. Breeding and mortality are affected simultaneously.
Probability of catastrophe	10 percent annually
Impact of catastrophe	Reduce reproduction to 50 percent of normal; reduce survival to 75 percent of normal
Distribution of litter sizes	Not used; litter size in horses is virtually always 1
Number of iterations per simulation	100
Number of years per iteration	20 years for population size and removals; 50 years for heterozygosity and probability of extirpation
Number of populations in each simulation	1
Definition of extirpation	The population is considered to be extirpated when it no longer has individuals of both sexes.

Demographic Parameters

Mating System

VORTEX models breeding systems as monogamous or polygamous and short-term or long-term. With monogamous breeding, a male is needed for every breeding female; males may therefore become a limiting factor restricting breeding. In polygamous models, only one male is needed for all females to have an opportunity to breed. However, another parameter (see the section Mate Monopolization) can specify that only a subset of males has an opportunity to breed. For example, a polygynous system can be created in which successful males control harems of, say, five females on average, while the remaining males are excluded from breeding.

If a short-term option is chosen, then VORTEX assumes that mates are randomly reshuffled each year and that all available individuals have an equal probability of breeding. If a long-term option is chosen, then once harem relationships between stallions and mares are formed, those relationships remain intact across years of the simulation until a member dies or disperses to a different population. Demographically, it does not matter whether long-term relationships or rearrangement of harems each year is chosen. Genetically, there may be a small effect on the rate of loss of genetic diversity from the population, with greater loss of diversity expected in populations with higher harem fidelity.

We realize that wild horses do not necessarily mate for life (mares do move between harems) but, of the two choices available in VORTEX, long-term polygyny comes closer to representing the mating system of wild horses. We therefore used long-term polygyny as the mating system in all of our simulations. This is a conservative assumption, in the sense that it represents the greatest opportunity for loss of genetic diversity. Any additional interchange that does occur in the real world will tend to ameliorate effects and result in higher genetic diversity than predicted by our models.

Age of First Reproduction

VORTEX describes reproduction in terms of the age when the first offspring are born, not the age at onset of sexual maturity or the age at first conception. The program also assumes that breeding (and, for that matter, all other events) takes place at discrete intervals, usually years. Thus, age at first reproduction is entered as an integer value. The value used should represent something like the median age of first reproduction, not the earliest age ever observed, because the earliest observed age may not be typical of the population. We therefore used 3 years as the age of first reproduction in females and 5 years as the age of first reproduction in males (Garrott and Taylor, 1990; Garrott and others, 1991b; Roelle and others, 2010).

Maximum Age and Maximum Age of Reproduction

If the species being modeled does not reproduce throughout its entire adult life, the age when the species ceases to reproduce must be specified. For example, humans typically reach reproductive senescence before they die. If the typical life expectancy is 70 years but reproduction ceases at 50 years, then 50 years would be the maximum age of reproduction. VORTEX requires that the maximum age be the same for both sexes, but allows the maximum age of reproduction to vary by sex. Establishing the maximum age of reproduction is difficult because old animals are hard to age (unless they were marked or identified in the year of birth) and because sample sizes of old animals are typically small. Garrott and others (1991b) present information showing that some mares can reproduce at greater than 20 years

of age, whereas Roelle and others (2010) found no evidence of reproduction by females older than 16 years. Roelle and others (2010) also found that very few harems are held by stallions older than age 20. On the basis of available evidence, we used 20 years as the maximum age of reproduction and 25 years as the maximum age.

Maximum Number of Progeny per Year

VORTEX provides a great deal of flexibility in specifying the maximum number of progeny that can be born to a given female in a year (or other time interval), thus allowing species that produce multiple clutches or litters to be modeled. However, most of this flexibility is not needed for modeling a horse population. Given that horses produce only a single litter per year (the average gestation period is 335–342 days; Card and Hillman, 1993) and that twins are virtually unknown, we used 1 for the maximum number of litters per year, 1 for the maximum number of progeny per litter, and a year (365 days) as the basic time cycle.

Density-Dependent Reproduction

VORTEX allows the reproductive rate of the population being modeled to change with changing population size. For example, reproduction could be low when the population is small owing to a difficulty in finding mates (Allee effect) or, conversely, reproduction could drop off when the population is large (more specifically, at high density) owing to limited resources or territories, intraspecific competition, crowding, or stress.

Density-dependent reproduction, also known as compensatory reproduction, has been reported for wild horses (Kirkpatrick and Turner, 1991; Jenkins, 2000; Roelle and others, 2010), but quantifying the magnitude of the effect is difficult, and the information available is insufficient to allow generalization or calculation of some average function. In addition, many of our simulated populations differed only about 50 percent (that is, between N and 1.5 × N) because of the way we implemented the removal function (see section Removal and Contraception Programs). In our estimation, reproductive rates are not likely to change substantially owing to density in that relatively small range. We therefore did not use density-dependent reproduction in our simulations.

Reproductive and Mortality Rates

VORTEX models reproductive rate as the proportion of adult females giving birth each year (or other time interval), where an adult female is defined by the age of first reproduction (see previous discussion). Similarly, mortality rates are modeled as the proportion of animals dying in each age and sex class. From age 0 to the age of first reproduction, each age and sex class can have a different mortality rate; after the age of first reproduction, all animals are considered adults, and the mortality rate can vary only by sex.

While these parameters are fundamental to modeling population dynamics, values are well known for perhaps only a handful of wild horse populations. In our estimation, however, wild horse managers are more likely to have at least some idea of the annual population growth rate multiplier for a herd (λ, lambda), which is just a different way of expressing the growth rate itself. We therefore modeled a set of scenarios in which $\lambda = 1.05$, 1.10, 1.15, 1.20, or 1.25 (that is, annual growth rates of 5 percent, 10 percent, 15 percent, 20 percent, or 25 percent in the absence of contraception). To produce these growth rates, we used reproductive and survival rates (table 2) derived largely from Garrott

(1991a). We arrived at the values for λ = 1.25 by experimenting with parameters in the model. Table 3 provides an example of how we translated these parameters into VORTEX.

Table 2. Annual survival and reproductive rates used to simulate various annual population growth rates.

Population growth rate (percent)	Juvenile survival rate (percent)	Adult survival rate (percent)	Mares foaling (percent)
5	80	91	50
10	80	92	65
15	80	97	65
20	80	99	80
25	90	99	92

Table 3. Translation of the annual rates shown in table 1 into VORTEX parameter values for an annual population growth rate of 20 percent (λ = 1.20).

[λ, annual population growth rate multiplier for a herd; <, less than; ≥, equal to or greater than]

Reproduction		Female Mortality		Male Mortality	
Mare age (years)	Mares foaling (percent)	Age (years)	Rate (percent)	Age (years)	Rate (percent)
1	0	<1	20	<1	20
2	0	1 to <2	1	1 to <2	1
≥3	80	≥2	1	2 to <3	1
				3 to <4	1
				4 to <5	1
				≥5	1

We believe that, in the absence of contraception, growth rates of most wild horse herds will fall within the range shown in table 2. Rather than having to specify reproductive and mortality rates, a manager will need only to choose one or perhaps two values for the annual population growth rate to use the output from the model.

Sex Ratio at Birth

While foal sex ratio can differ from 50:50 in small herds in a given year (Roelle and others, 2010), over time the ratio tends to be fairly even in most herds. For example, Garrott (1991b) found that for a 12-year period 65 of 74 (88 percent) herds sampled in Nevada, Oregon, and Wyoming had a foal sex ratio that did not differ from 50:50. We therefore used 50 percent males in the foal crop in our simulations.

Mate Monopolization

As noted in the previous section, we ran our simulations using a polygynous breeding system. In order to do so, VORTEX requires that one of the following three parameters be specified: percentage of males in the breeding pool, percentage of males successfully siring offspring, or mean number of mates per successful sire. The remaining two parameter values are calculated from the one that is supplied based on the assumptions that some males are excluded from the breeding pool and that breeding

success among males in the pool of available breeders is described by a Poisson distribution. Only the first parameter (percentage of males in the breeding pool) is used by VORTEX.

Of the three ways in which mate monopolization can be described in VORTEX, mean number of mates per successful sire is the one most commonly discussed in the literature on feral horses, but comparisons among studies are hampered by inconsistencies in defining what constitutes a potential mate (that is, the age classes of mares). Roelle and others (2010) defined potential mates as all mares 2 years of age and older, and found a mean harem size of 2.2 mares (range 1.9 to 2.7) during 12 years. Using the same age criteria, Kaseda and Khalil (1996) found average harem sizes of 1.8 to 5.3 mares in many years.

Potential effects of using different values for the mean number of mates per successful sire are not intuitive; to a first approximation, however, one might expect that varying this parameter would affect genetics more than demography. We explored sensitivity of model results to mean number of mates per successful sire in a series of simulations with initial population size = 500, proportion of mares treated = 0.5, lambda = 1.05 (in the absence of contraception and removals), and initial heterozygosity = 0.77. We varied mean number of mates per successful sire systematically between 1.5 and 5.0. The fairly low growth rate and fairly high proportion of mares treated consistently kept the simulated population after 50 years at 200–250 animals; however, final heterozygosity ranged only from 0.74 to 0.75. Given the apparent lack of sensitivity of the model, we decided to set the value of mean number of mates per successful sire to 3.0 for all simulations.

Initial Population Size

Horse herds managed by BLM vary considerably in size, and the size of the herd is potentially very important relative to demographic and genetic results of contraception. We approached this problem by simulating herds with initial populations of 100, 250, 500, 750, 1,000, 1,250, and 1,500 animals. These initial populations should encompass the appropriate management level (AML; population goal) for many herds. We recommend a more detailed, site-specific analysis before a sterilant is applied to any herd with fewer than 100 animals. In addition, we implicitly assume that a herd is at its appropriate size before the results of these simulations can be used to evaluate a fertility control program. For example, the results of the simulations having an initial population and appropriate management level of 500 animals cannot be used if the population in fact contains, say, 1,000 animals.

Initial Age Distribution

VORTEX allows two options for specifying the initial age distribution used in a simulation. A specific age distribution (the number of individuals in each sex and age class) can be entered, or VORTEX can calculate the stable age distribution (proportion of animals) that would result from the age-specific schedules of reproduction and mortality described previously. We used the latter option, and the proportions were applied to the initial population size to determine the number of animals initially in each sex and age class.

Carrying Capacity

Carrying capacity (K) describes a theoretical upper limit to the size of a population. In VORTEX, if the actual population, N, exceeds K at the end of a particular year, then additional mortality is applied equally across all age and sex classes to reduce the population such that N = K. While carrying capacity is an important theoretical construct, values for K are difficult to estimate. We

somewhat arbitrarily set the value for K at 5 times the initial population size. With the contraception and removal programs specified as described in subsequent sections, simulated populations never reach carrying capacity.

Genetic Parameters

VORTEX allows inbreeding depression to be incorporated into the population model as a reduction in first-year survival among inbred individuals. Although some diploid species that have been studied show depressed fitness when inbred (O'Brien and Evermann, 1988; Quattro and Vrijenhoek, 1989), the rate at which inbreeding affects survival and reproduction is not well understood for most species. Therefore, we elected not to use this function in the wild horse model. A better approach for investigating the potential genetic consequences of contraception and gathers (round-ups) is to track the degree of genetic diversity, which can be loosely linked to fitness and an ability to adapt to environmental change.

VORTEX tracks changes in genetic diversity using heterozygosity (the frequency of two different alleles at a given gene) as a metric. Heterozygosity ranges from 0 (all individuals are homozygous and the same at all genes) to 1 (all individuals are heterozygous with different alleles at all genes). It is generally believed that higher levels of heterozygosity are associated with more genetic variation and lower probability of inbreeding depression.

VORTEX can track heterozygosity in two ways. Because VORTEX includes a detailed simulation of genetic change in the populations, one way to measure changes in heterozygosity is to start with maximum heterozygosity (1) by assigning each founder individual two unique alleles at a number of loci (genes). Each offspring is randomly assigned one of the two alleles from each parent at each locus. This method tracks relative loss of heterozygosity through time. Perhaps a more realistic way to examine loss of heterozygosity is to mimic real populations by starting with individuals that have a known level of heterozygosity. VORTEX allows this option by allowing real allele frequencies to be used as a starting point for model simulations. Thus, if we know the level of heterozygosity for a population, we can enter real allele frequencies such that the starting point of the simulations will have exactly that level of heterozygosity. We used the latter of these two approaches and ran the simulations for two different initial levels of heterozygosity (high and low) as reported for two real-world herds (Gus Cothran, Texas A&M University, unpublished reports prepared for BLM). Actual allele frequencies at 13 microsatellite loci were taken from the Lost Creek herd in Wyoming (high genetic diversity, $H_o = 0.78$; report prepared in 2010) and the Cibola-Trigo herd in Arizona (low genetic diversity, $H_o = 0.49$; report prepared in 2008). Absent site-specific information, we typically expect larger herds to have higher genetic diversity.

Removal and Contraception Programs

Removals

Given the limitations of available contraceptive methods, and facing population sizes that greatly exceed the desired number of animals in many herd management areas, BLM currently views contraception as a means of reducing the number of animals that must be removed, and perhaps increasing the interval between gathers, rather than as a replacement for gathers. We therefore simulated a gather-and-removal program that is as similar as possible to the program that BLM uses in the field. We modeled a gather-and-removal event in any year in which the simulated population was 150 percent or more of the initial population, and we attempted to remove sufficient animals to return the population

to its initial size. We removed animals from age classes according to BLM's removal criteria, which specify the following order: ages 4 and under, followed by ages 11–19, followed by ages 5–10. We constrained the number of animals removed, however, such that no more than 90 percent of any group was taken (because it would rarely be possible or desirable to remove every animal from a given age class). Our response variable is the total number of animals removed in 20 years averaged for 100 iterations.

Contraception

We implemented a simulated contraception program using capabilities in VORTEX called "population state" variables and "individual state" variables. Briefly, we used these variables to define the proportion of previously untreated females that are treated in each application and the probability that the treatment works if it is given (efficacy). In most cases, animals in BLM herds need to be gathered in order to apply a fertility control agent; we therefore treated mares following every gather and removal. Killian and others (2008) found that 17 percent of mares treated with SpayVac were pregnant in years 2 through 4 of their study, which implies efficacy of 83 percent. The most aggressive assumption would be that the treatment is 100 percent efficacious. However, it is probably also fair to assume that some mares will not respond to an immunocontraceptive such as SpayVac. We therefore assumed efficacy of 90 percent for all simulations. We also assumed that once a mare is successfully contracepted, she remains contracepted for life without further treatment (we assumed permanent sterility). We varied the proportion of mares treated from 0 to 100 percent in increments of 10 percent to illustrate the range of possible contraceptive effects. For example, when the proportion of mares treated is set at 20 percent, each mare not successfully treated in the past has a 20 percent chance of being caught and treated, and there is a 90 percent chance that the treatment will be effective. Note that high treatment rates (90–100 percent, for example) would likely be very difficult to achieve in the real world because some animals are difficult or impossible to gather.

Random Variation

Environmental Variation in Breeding and Mortality

In addition to the variability inherent in an individual-based model, VORTEX allows specification of variability (standard deviation) in the proportion of females breeding and in the proportion of both sexes dying due to factors external to the population (such as weather, predation, parasite loads). Estimates of variability in these parameters are generally not available for wild horse herds, but sufficient information was available to calculate standard deviations in fecundity and mortality for the Pryor Mountain herd (Roelle and others, 2010).

The standard deviation due to environmental variability, s_{ev}, for either fecundity or mortality, can be expressed as $s_{ev} = \sqrt{s_{tot}^2 - s_{ds}^2}$, where s_{tot}^2 is the total variance in the parameter for all years and s_{ds}^2 is the sampling (binomial) variance for all individual rates. From the Pryor Mountain data set (Roelle and others, 2010), we calculated $s_{ev} = 0.12$ for fecundity and $s_{ev} = 0.06$ for mortality. We combined ages and sexes for both of these calculations and used the resulting values in all simulations.

Concordance of Environmental Variation in Breeding and Mortality

Factors such as those already described (weather, predation, parasite loads) can affect reproduction and survival independently or simultaneously. Simultaneously means that good years for

reproduction are also good years for survival. We know of no information that can be brought to bear on this subject in regard to wild horse demography, but it seems logical that demographic effects would be most pronounced if these parameters vary simultaneously. We therefore opted for concordance in environmental variation.

Number and Types of Catastrophes

The VORTEX software allows specification of catastrophes (floods, forest fires, epidemic diseases) through particular effects on reproduction and survival. Perhaps the most common such event on western ranges is drought, and although droughts occur fairly frequently, we know of no specific information relating drought to reproduction and survival in wild horses. Nevertheless, we felt that it was important to include some form of catastrophe in our simulations. We therefore specified that drought would have a 10 percent probability in any year and that it would reduce reproduction to 50 percent of the normal level and survival to 75 percent of the normal level.

Distribution of Litter Sizes

VORTEX allows for one additional form of random variation if females can produce litters of different sizes. Because wild horses almost never produce more than one offspring, we did not use this parameter.

Simulation Control

Number of Iterations

As noted in the previous section, VORTEX uses a random number generator to simulate random events in an organism's life cycle. Therefore, no two runs or iterations of the model are identical even with a single set of input data. To obtain a more complete picture of demographic behavior, multiple iterations are required. We explored changes in the standard deviation of both mean population size and heterozygosity after 50 years while varying the number of iterations from 25 to 1,000. Because of the complexity of the removal and contraception functions, no consistent pattern emerged in the standard deviations of these parameters. More particularly, increasing the number of iterations to more than 100 did not consistently lead to lower variance about the mean. We therefore chose 100 iterations for all of our simulations.

Number of Years per Iteration

Initial simulations with some basic data indicated that significant changes in heterozygosity are rare in less than 50 years. On the other hand, significant demographic changes can occur in as little as 20 years. We therefore evaluated mean population size and removals at the end of 20 years of simulation, but we used 50 years to evaluate heterozygosity and probability of extirpation.

Number of Populations

VORTEX can be used to model a single, isolated population or a complex metapopulation composed of as many as 50 populations with interchange of animals between them. Although it is clear that some animals move between certain populations of wild horses, we know of no information on

rates of such movement. We therefore modeled only a single population without introduction of animals from outside, which is the worst case from the perspective of genetic diversity.

Extirpation Definition

VORTEX offers several methods for defining extirpation of a population. We used the definition that says a population is extirpated when it no longer contains individuals of both sexes.

Interpreting Model Output

Appendix A contains output from 70 sets of simulations representing two levels of genetic diversity (low or high), seven initial population sizes (100, 250, 500, 750, 1,000, 1,250, or 1,500 animals), and five population growth multipliers (1.05, 1.10, 1.15, 1.20, or 1.25). Each set of simulations is represented by a figure, and each figure contains five graphs and a table of supporting data. The order of the figures varies first by annual growth rate, then by population size, and then by genetic diversity. Thus, figure 1 has low genetic diversity, an initial population size of 100, and a growth multiplier of 1.05, and is followed by figure 2 with low genetic diversity, an initial population size of 100, and a growth multiplier of 1.10, and so on. In each figure, the first four (black and white) graphs and the table represent the same set of simulations, and the graphs and table have identical values. Due to a nuance in the way VORTEX operates, however, the final (color) graph represents a different set of simulations having the same parameter values but differing slightly due to random variation as already discussed. Thus, the value for average population size at 20 years on the color graph will not in general be identical to that in the table, but the two values will usually be very similar.

The black and white graphs present mean values (that is, averaged for 100 iterations) of four response variables on the vertical axes: mean population size after 20 years, mean number of removals after 20 years, mean probability of extirpation after 50 years, and mean heterozygosity in the herd after 50 years. In all cases, the horizontal axis is the percentage of previously untreated mares that are treated following each gather, varying from 0 percent to 100 percent. The colored graph represents the mean trajectory of the population size for a subset of the treatment levels (0 percent, 20 percent, 40 percent, and so on) depicted as lines of different color. It is very important to understand that there is some information that cannot be conveyed by mean values. For example, there are many cases where the table and black and white graphs show that animals were removed, but the colored graph lines never plot higher than 1.5 × the initial population. The appropriate interpretation is that on average the population stayed below the threshold (1.5 × the initial population), but in some individual iterations the population grew sufficiently to trigger a removal.

As an example application of model results, suppose a manager wants to know what proportion of mares can be treated with a sterilant in a herd having low genetic diversity, an initial population size of about 750, and a population growth multiplier of 1.15. This set of conditions is represented by figure 18 in appendix A. The first thing to notice about figure 18 is that no treatment level has any real effect on either mean probability of extirpation after 50 years or mean heterozygosity after 50 years. The graph of mean population size after 20 years, however, shows that treatment levels greater than 50 percent of the mares result in populations somewhat less than 750. We also see that if 50 percent of the mares are treated, the mean number of removals after 20 years is around 900, or about 500 fewer than in the scenario where no mares are treated. The number of removals can be further reduced by treating a higher proportion of mares, but only if average population levels below 750 are acceptable. The average trajectory of the population size through time can be examined by looking at the last (color) graph in the

figure 18, which also shows that treatment rates more than about 50 percent will result in populations that are commonly below the starting point of 750 animals.

Finally, uncertainties about level of genetic diversity, population size, or growth rate can be addressed by examining figures representing nearby values in the parameter space. Thus, in the example in the preceding paragraph, a manager might also wish to look at the figures representing 500 and 1,000 animals in the population and growth multipliers of 1.10 and 1.20 to gain some understanding of how sensitive model results are to these assumptions.

Conclusions

We believe that simulation results presented herein represent a useful starting point for evaluating a management program involving a contraceptive technique that is permanent (or nearly so). In particular, the results show that only in the most extreme circumstances (such as low initial genetic diversity, low population growth rate, high proportion of mares treated, no change in management for 50 years) would there likely be any noticeable effect on genetic diversity or a significant probability of extirpation of a herd. Monitoring and adaptive management would reduce the probability of unacceptable results even further. For example, a simple exchange program in which an animal or two were moved between herds periodically would help to maintain genetic diversity, and the contraceptive program could always be modified or discontinued in the event that the reduction of population was greater than anticipated. In short, we see nothing in our results that should steer wild horse managers away from permanent contraceptive techniques, as long as results are monitored and adjustments are made if necessary.

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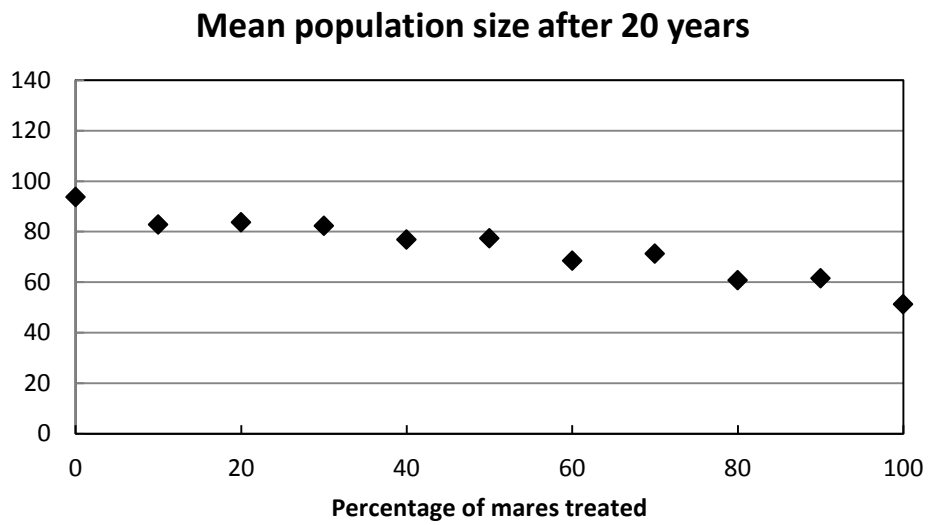
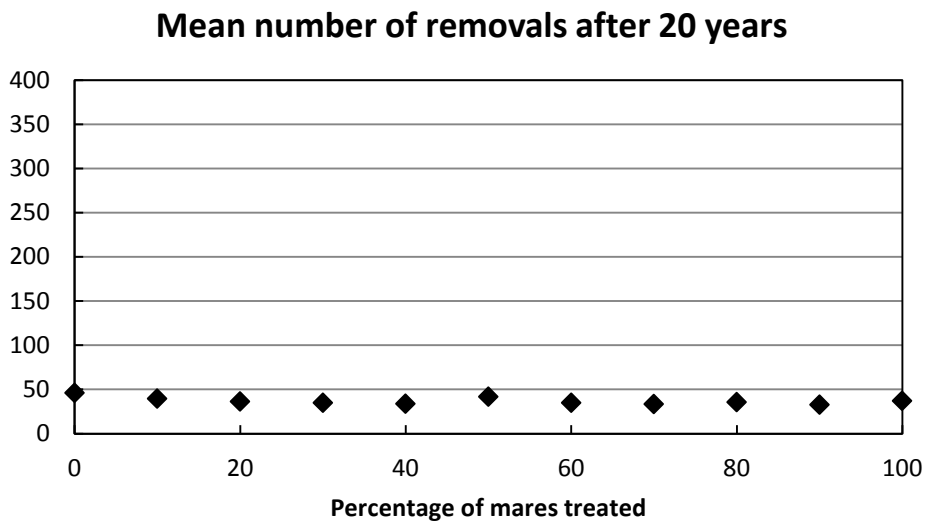
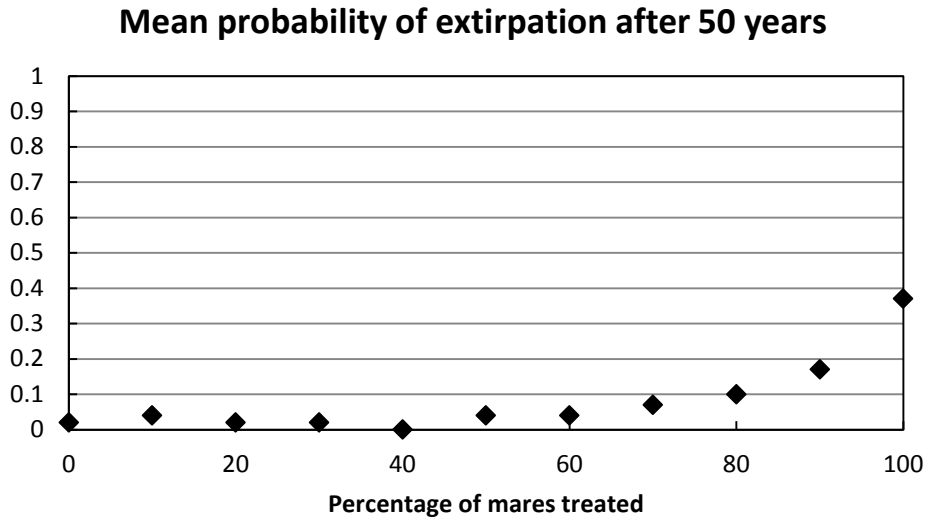
Appendix A. Simulation Results







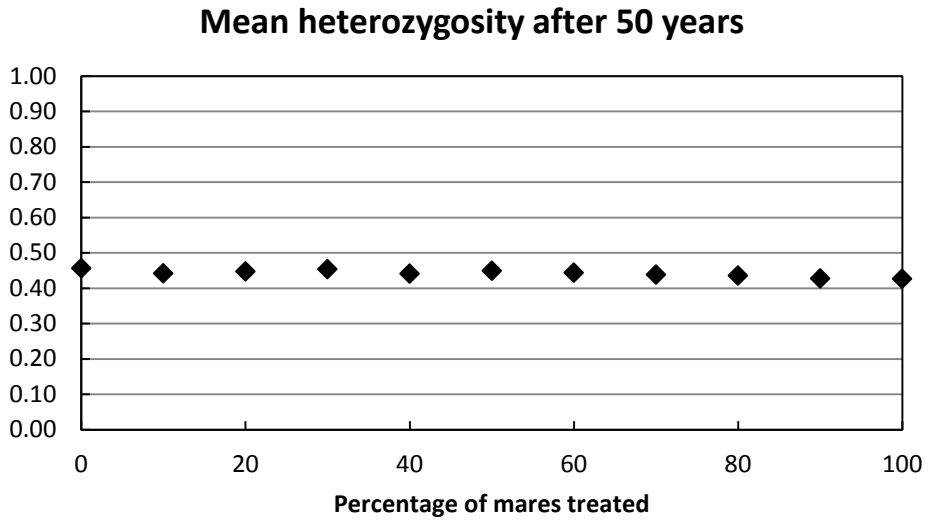


Figure 1. Initial population size = 100, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	93.71	82.73	83.68	82.28	76.81	77.32	68.49	71.19	60.66	61.53	51.22
Mean number of removals after 20 years	45.83	39.52	36.34	34.74	33.65	41.69	34.88	33.26	35.4	32.7	36.94
Mean probability of extirpation after 50 years	0.02	0.04	0.02	0.02	0	0.04	0.04	0.07	0.1	0.17	0.37
Mean heterozygosity after 50 years	0.46	0.44	0.45	0.45	0.44	0.45	0.44	0.44	0.44	0.43	0.43

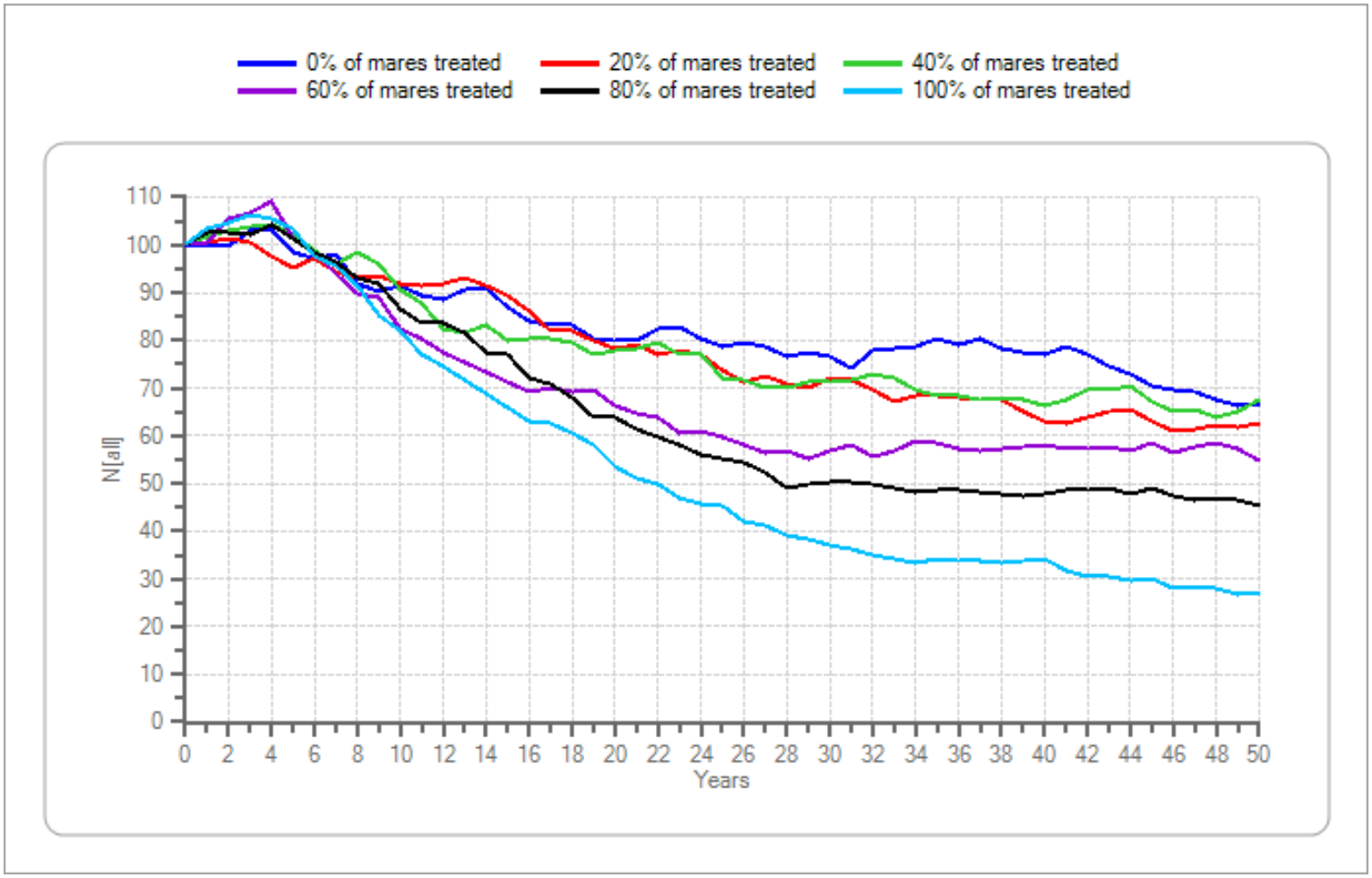


Figure 1. Initial population size = 100, lambda = 1.05, genetic diversity = low.—Continued

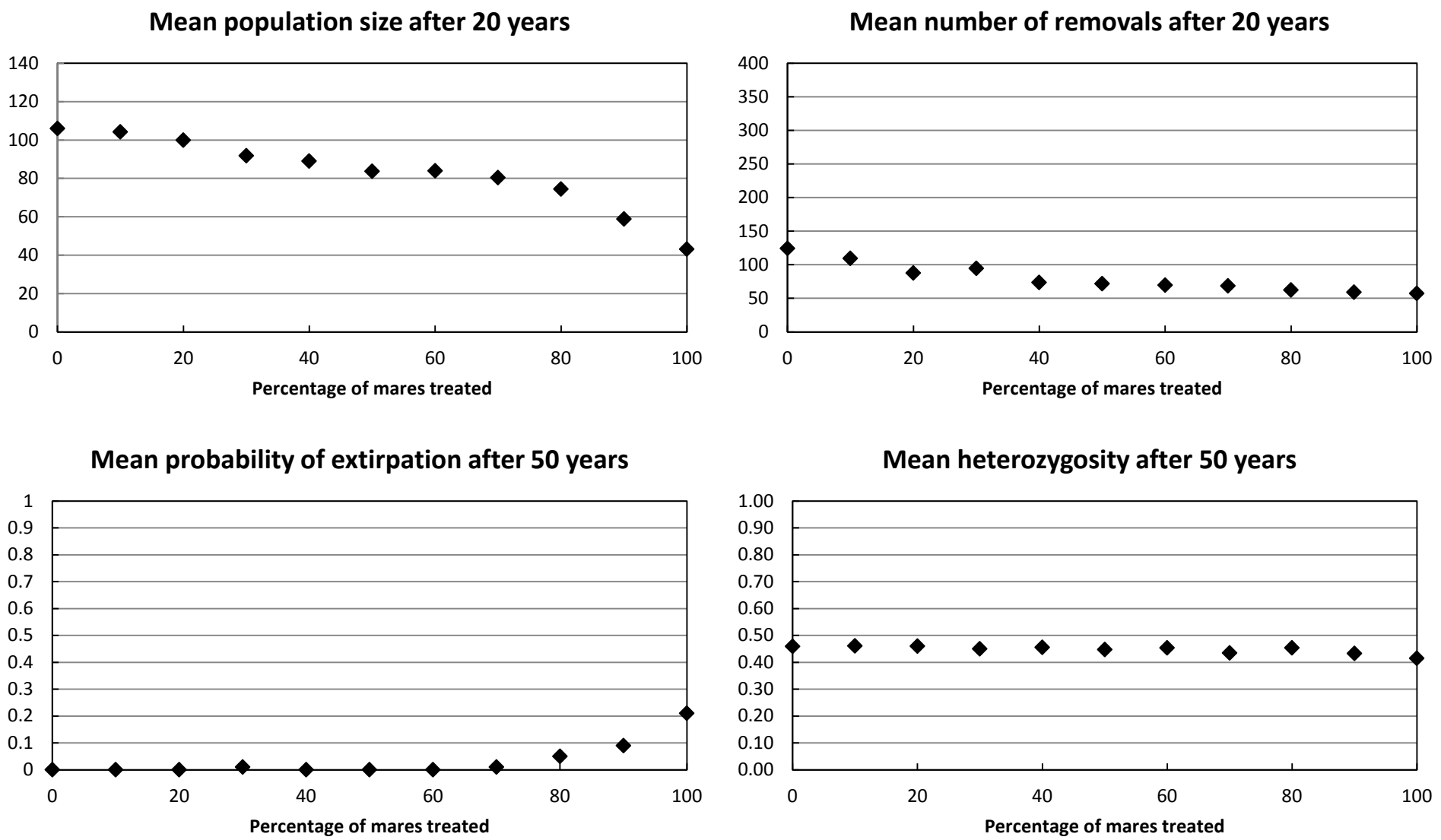


Figure 2. Initial population size = 100, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	105.96	104.13	99.93	91.74	89	83.71	83.91	80.41	74.37	58.79	43.07
Mean number of removals after 20 years	124.34	109.22	87.59	94.47	73.54	71.53	69.56	68.28	62.33	58.98	57.08
Mean probability of extirpation after 50 years	0	0	0	0.01	0	0	0	0.01	0.05	0.09	0.21
Mean heterozygosity after 50 years	0.46	0.46	0.46	0.45	0.46	0.45	0.45	0.43	0.45	0.43	0.41

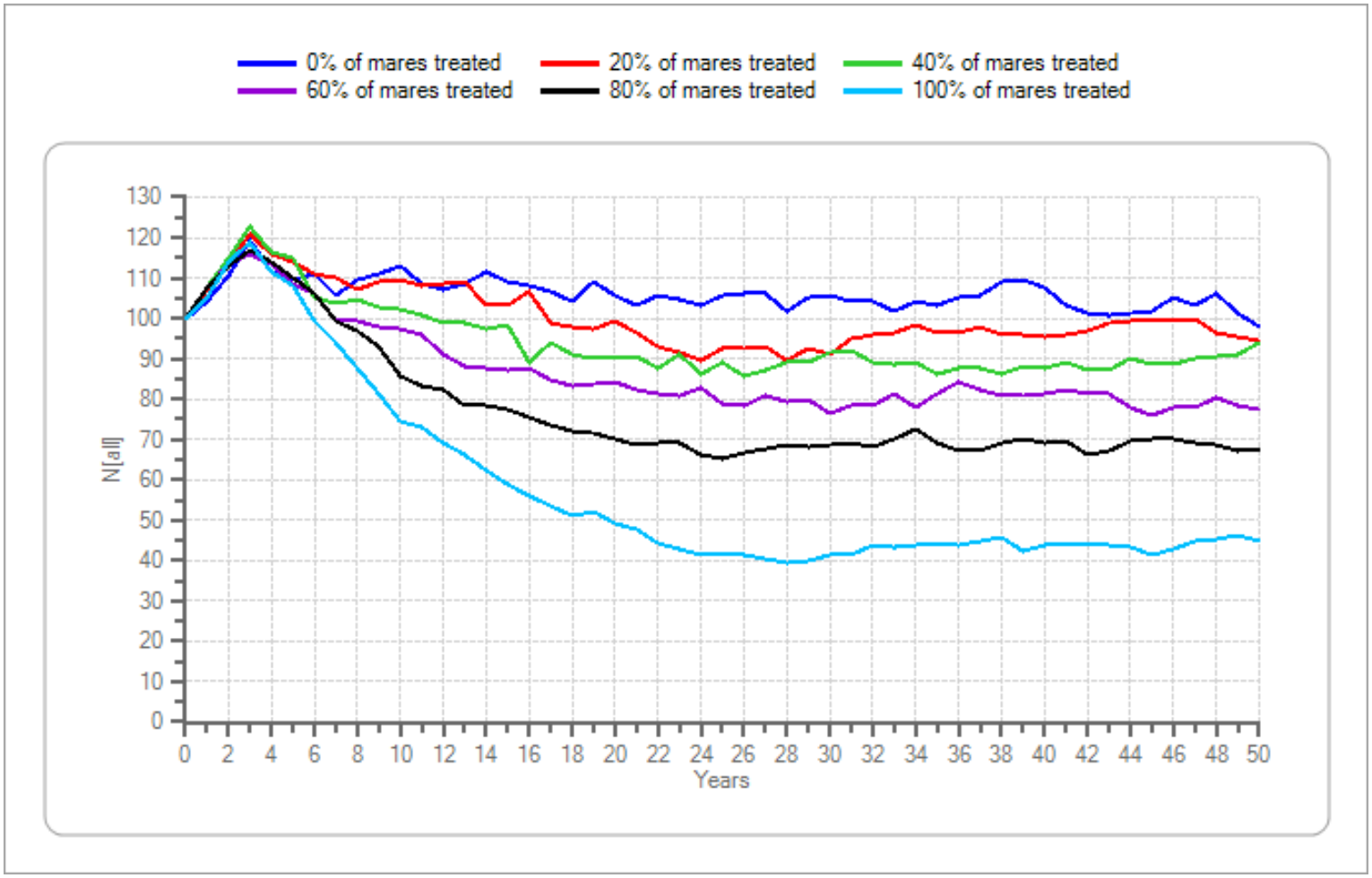


Figure 2. Initial population size = 100, lambda = 1.10, genetic diversity = low.—Continued

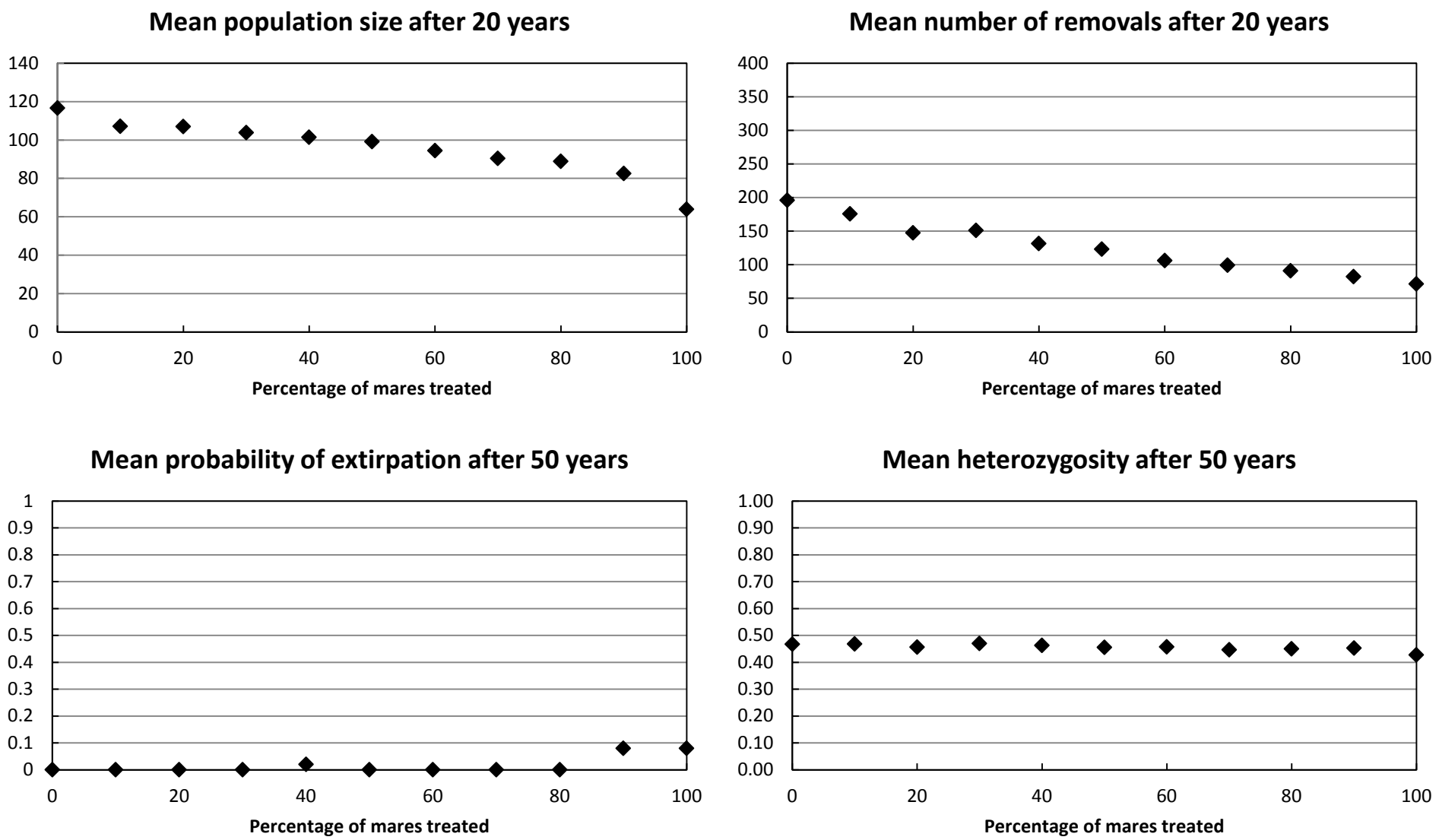


Figure 3. Initial population size = 100, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	116.67	107.04	106.93	103.8	101.36	99.18	94.47	90.36	88.82	82.5	63.9
Mean number of removals after 20 years	196.1	175.66	147.22	150.85	131.53	123.08	106.05	99.21	90.8	82.28	71.23
Mean probability of extirpation after 50 years	0	0	0	0	0.02	0	0	0	0	0.08	0.08
Mean heterozygosity after 50 years	0.47	0.47	0.46	0.47	0.46	0.46	0.46	0.45	0.45	0.45	0.43

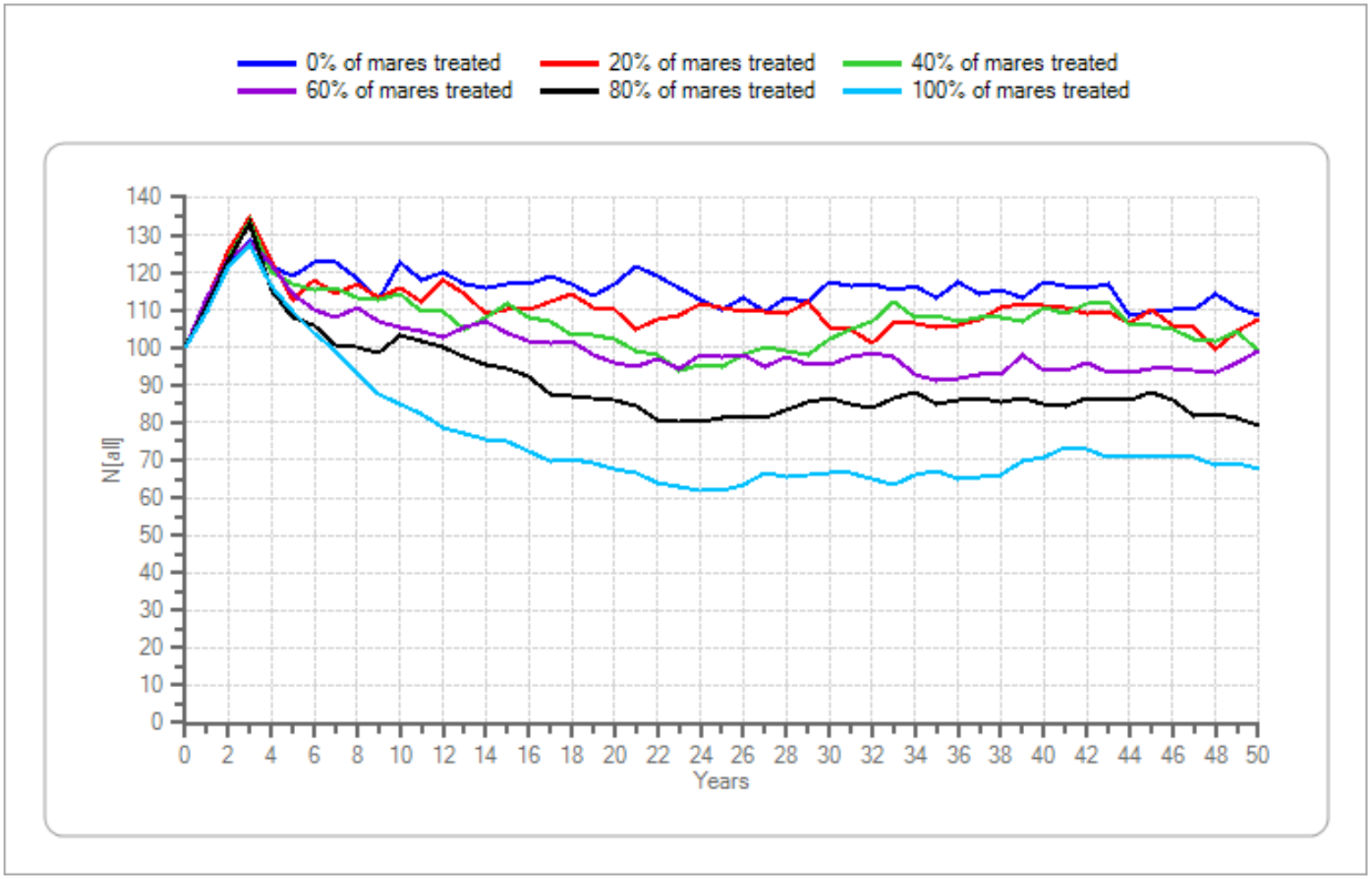
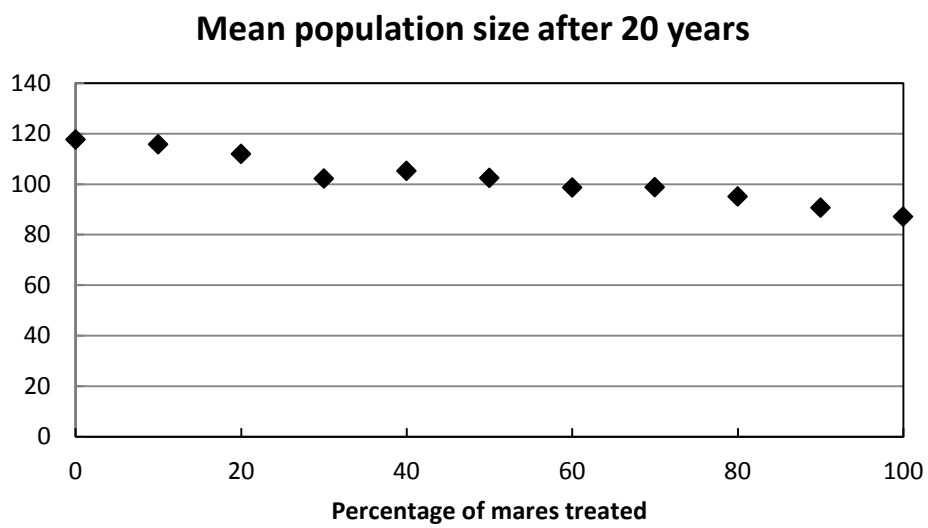
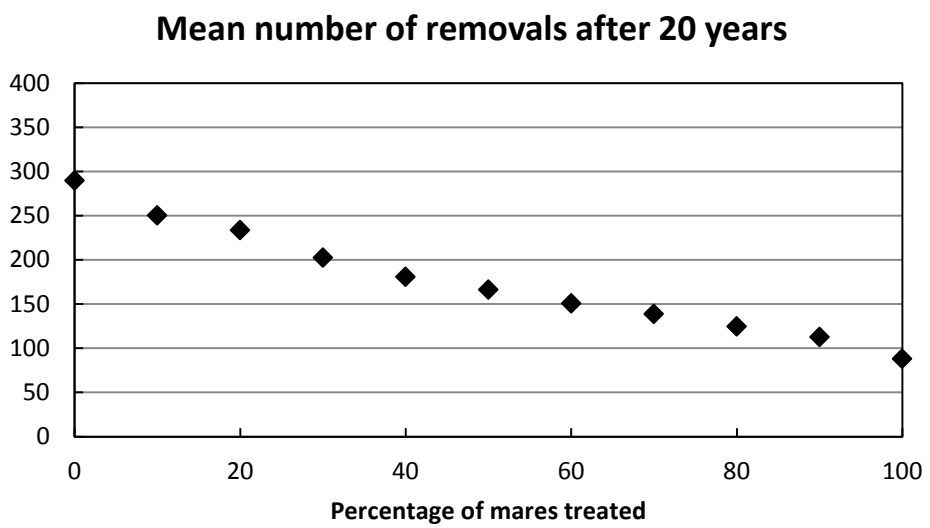
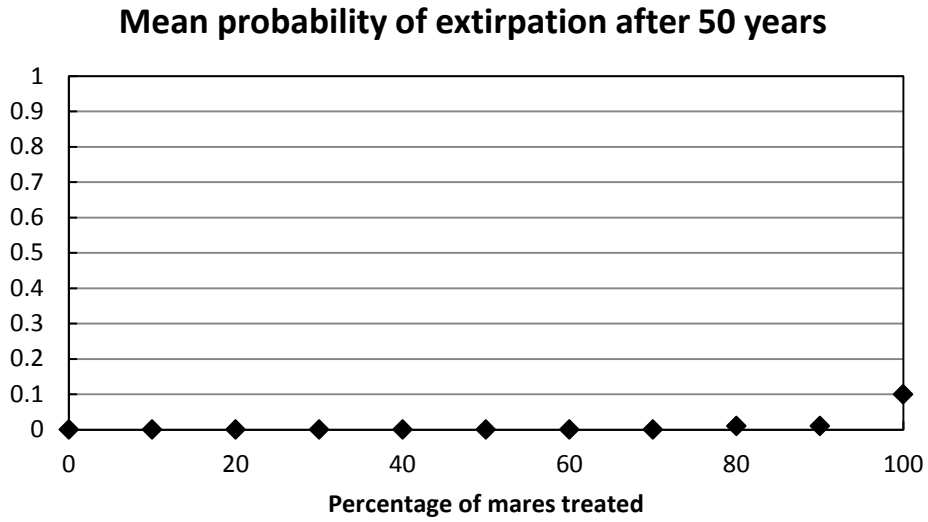


Figure 3. Initial population size = 100, lambda = 1.15, genetic diversity = low.—Continued







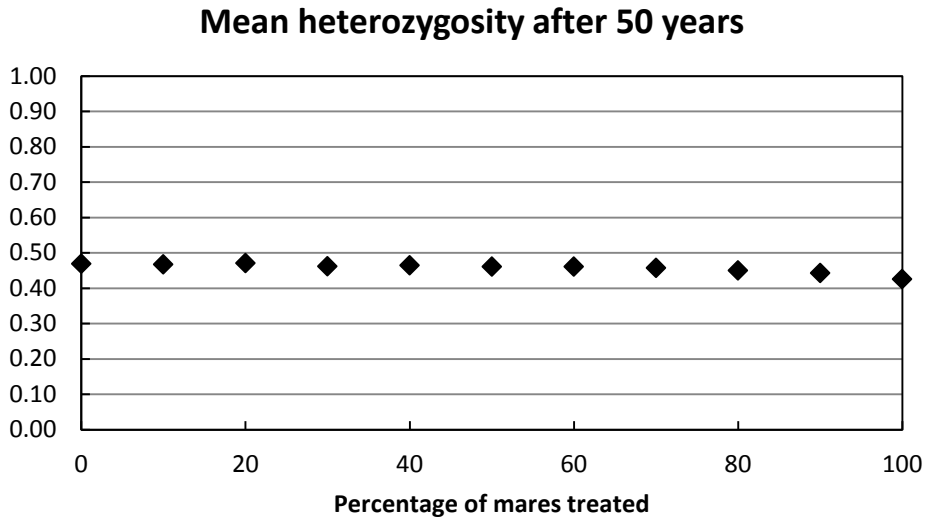


Figure 4. Initial population size = 100, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	117.66	115.72	111.98	102.21	105.14	102.37	98.63	98.73	95.01	90.61	87.04
Mean number of removals after 20 years	289.73	250.34	233.51	202.32	180.67	166.39	150.74	138.75	124.57	112.71	88.13
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0.01	0.01	0.1
Mean heterozygosity after 50 years	0.47	0.47	0.47	0.46	0.46	0.46	0.46	0.46	0.45	0.44	0.43

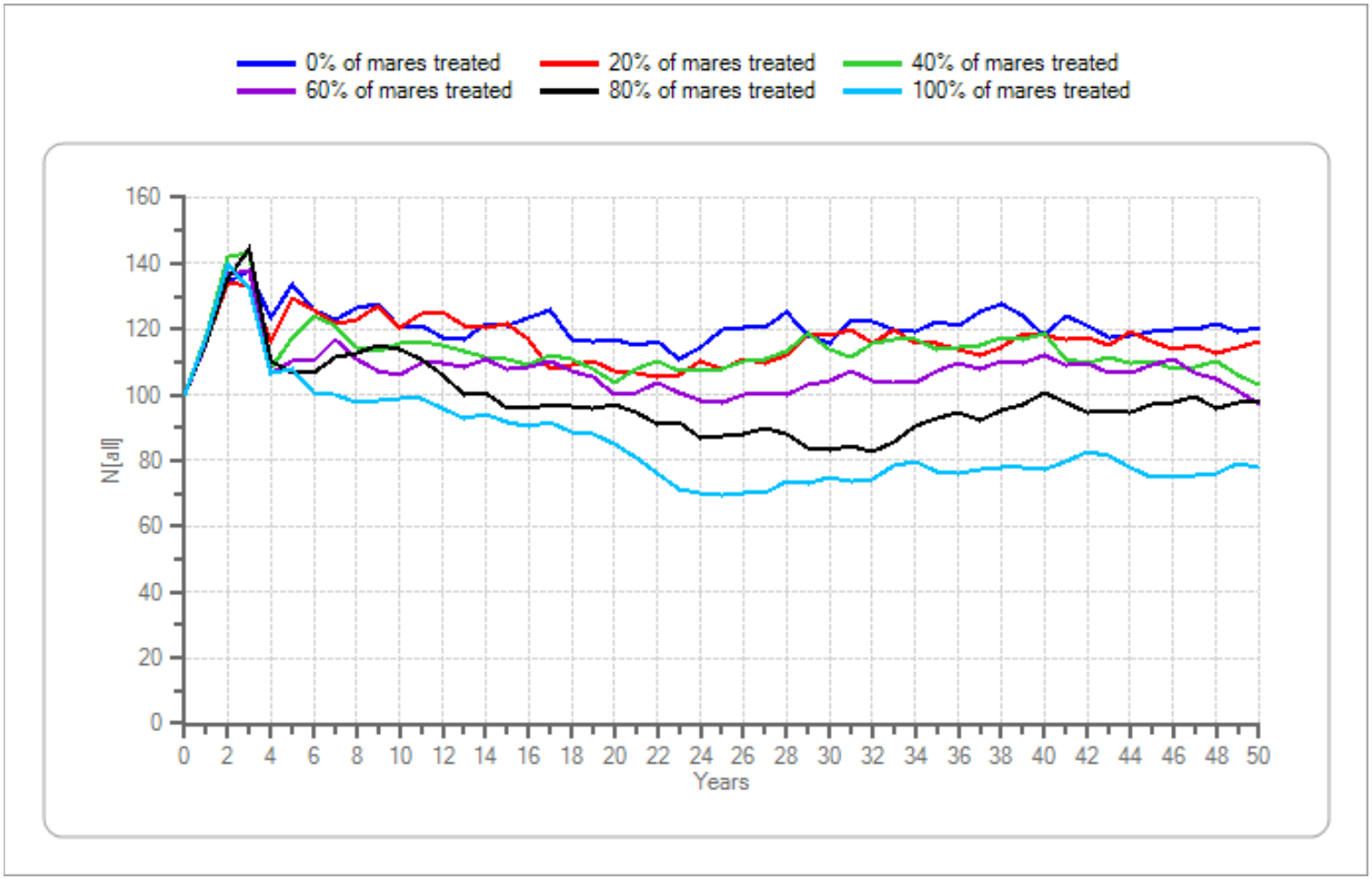


Figure 4. Initial population size = 100, lambda = 1.20, genetic diversity = low.—Continued

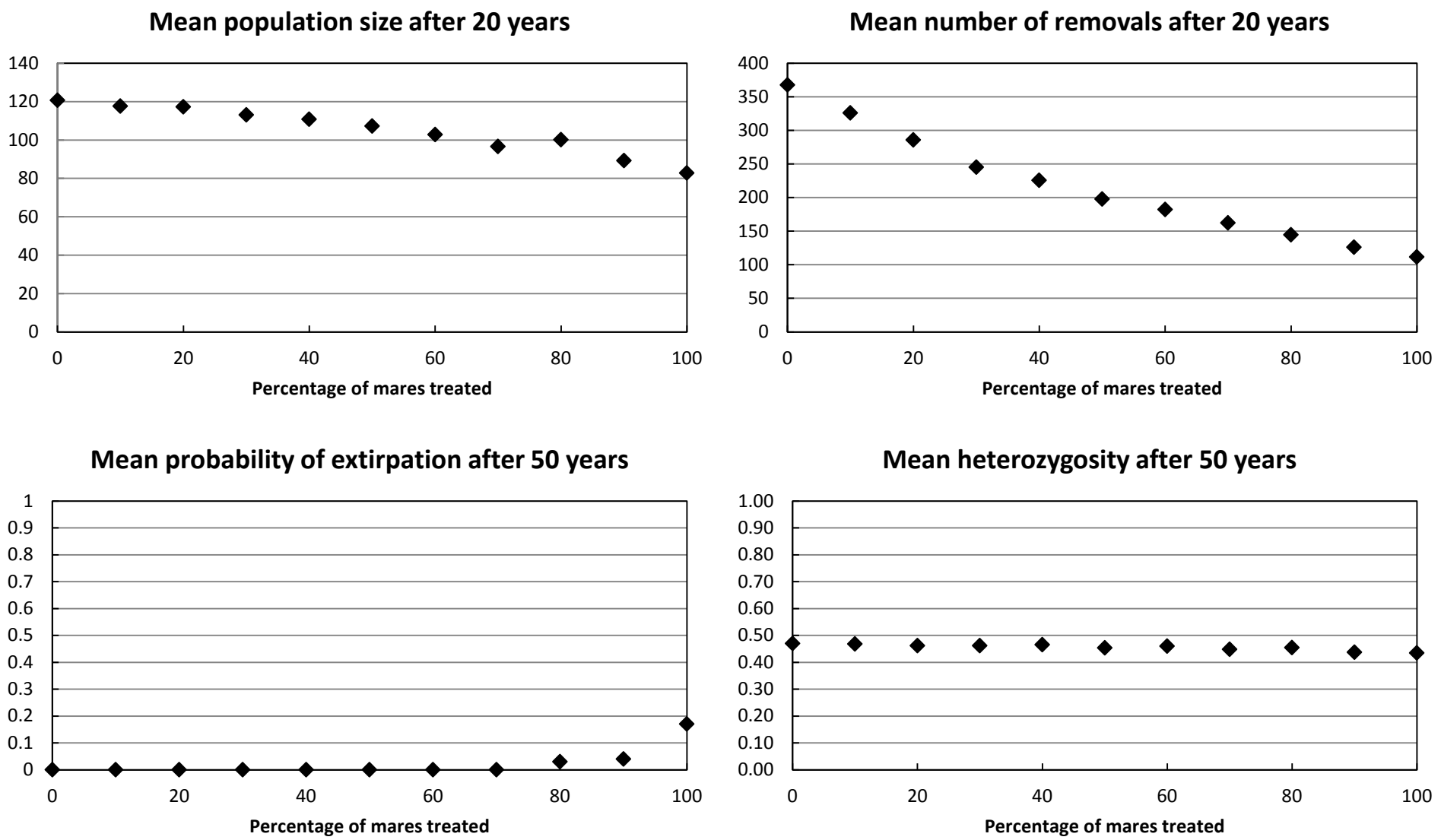


Figure 5. Initial population size = 100, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	120.71	117.59	117.22	113.11	110.75	107.17	102.75	96.64	100.1	89.18	82.72
Mean number of removals after 20 years	367.4	325.91	285.86	245.19	225.62	197.56	182.24	162.3	144.4	126.13	111.72
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0.03	0.04	0.17
Mean heterozygosity after 50 years	0.47	0.47	0.46	0.46	0.47	0.45	0.46	0.45	0.45	0.44	0.43

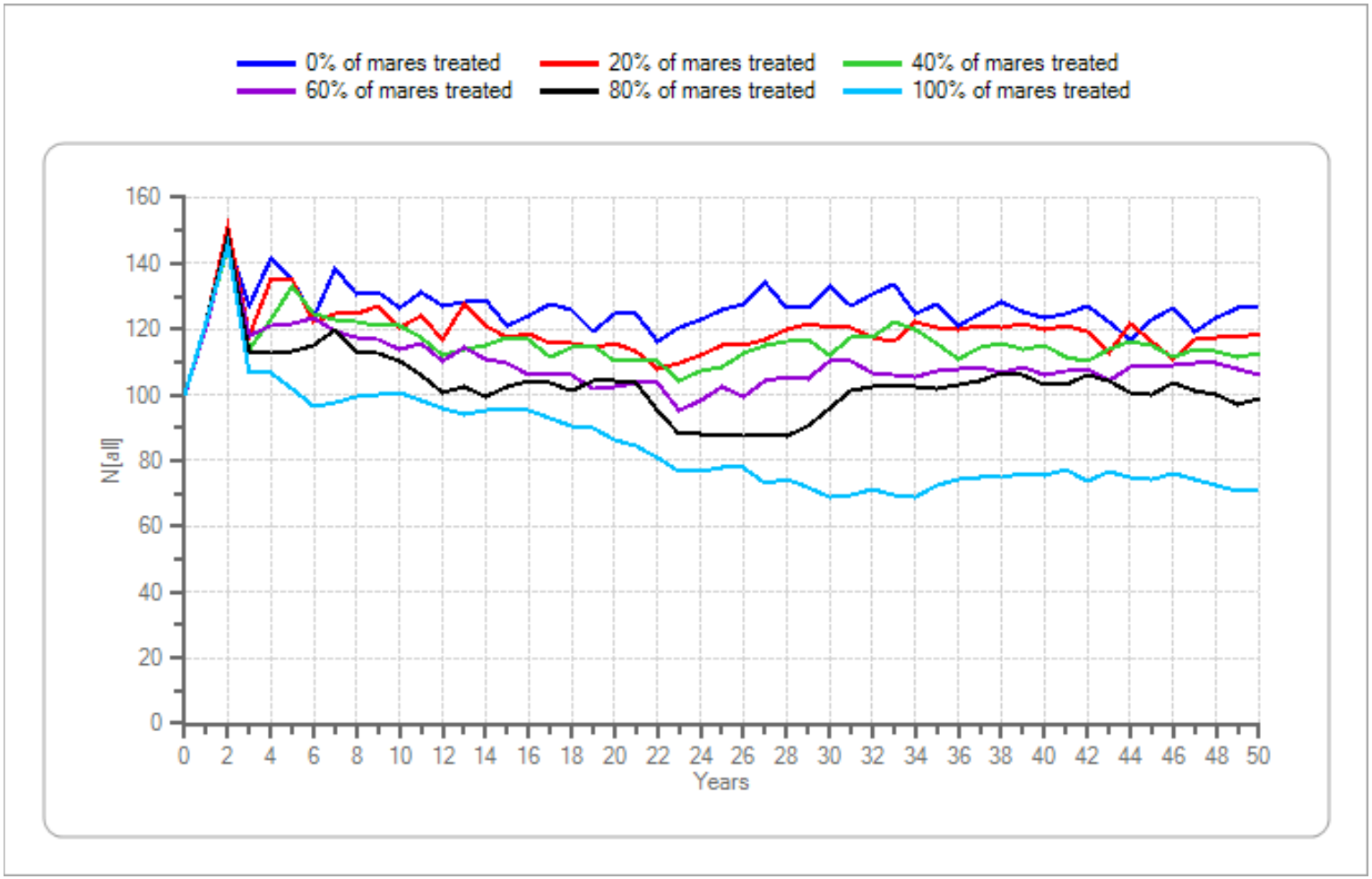
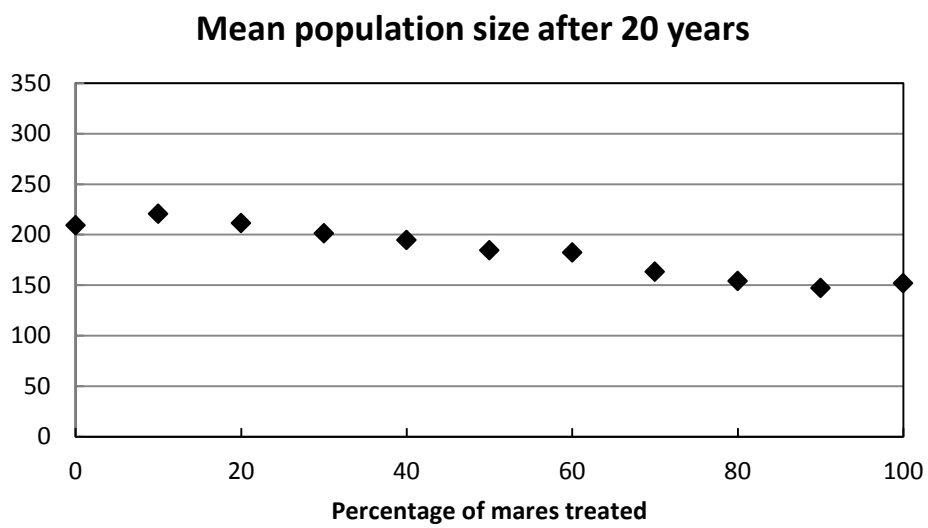
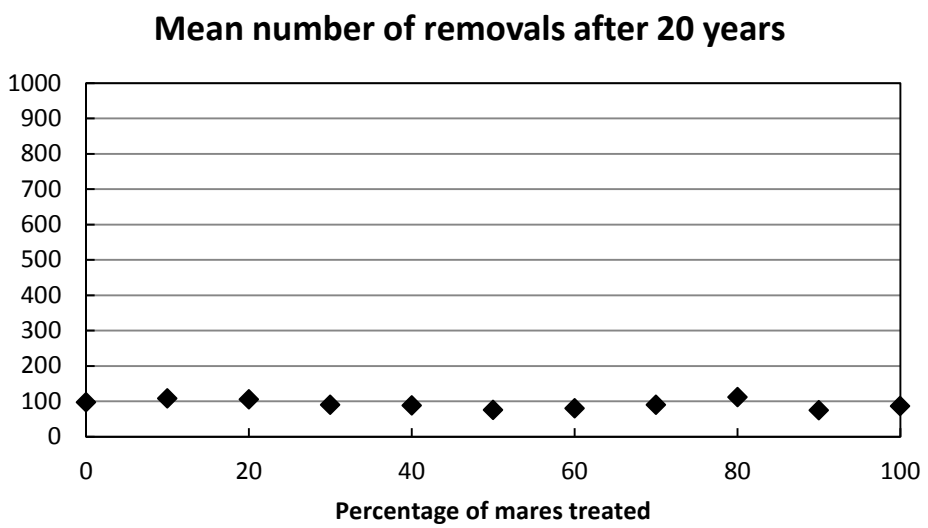
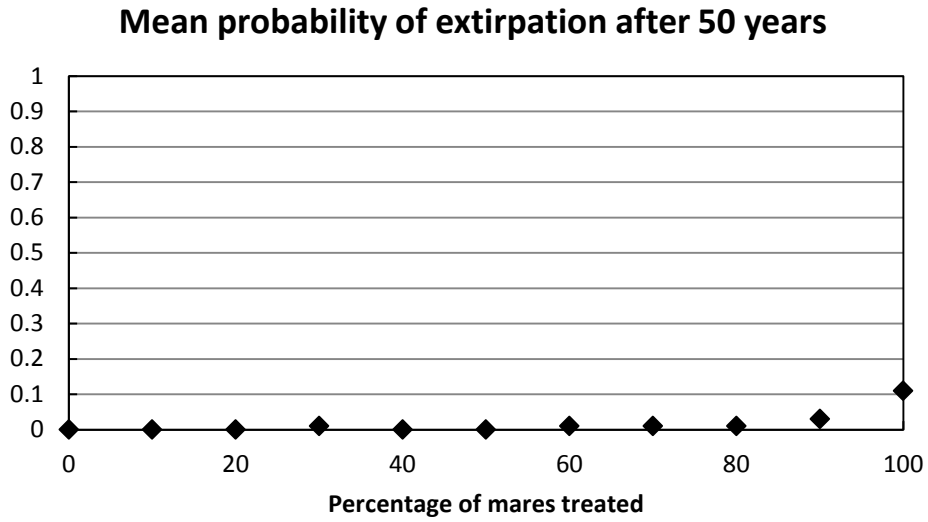


Figure 5. Initial population size = 100, lambda = 1.25, genetic diversity = low.—Continued







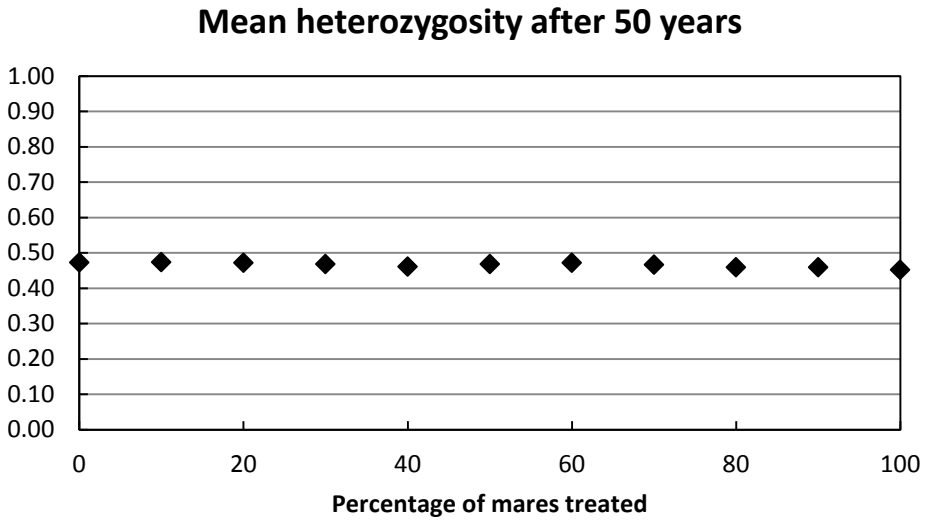


Figure 6. Initial population size = 250, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	209.16	220.46	211.25	201.33	194.71	184.37	182.2	163.33	153.91	147.12	151.88
Mean number of removals after 20 years	96.65	107.54	105.05	89.47	87.49	75.07	79.56	89.29	111.56	74	85.79
Mean probability of extirpation after 50 years	0	0	0	0.01	0	0	0.01	0.01	0.01	0.03	0.11
Mean heterozygosity after 50 years	0.47	0.47	0.47	0.47	0.46	0.47	0.47	0.47	0.46	0.46	0.45

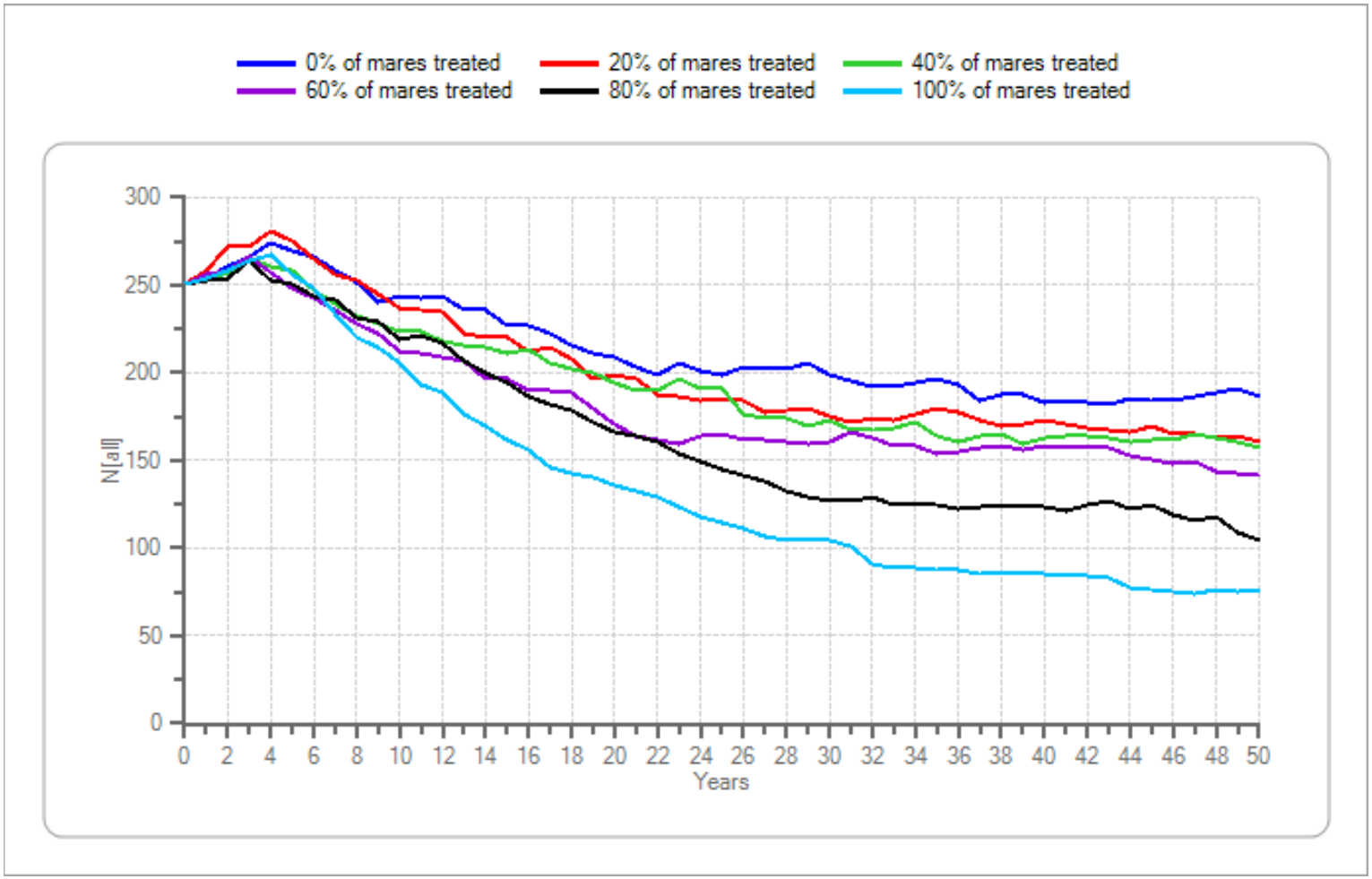
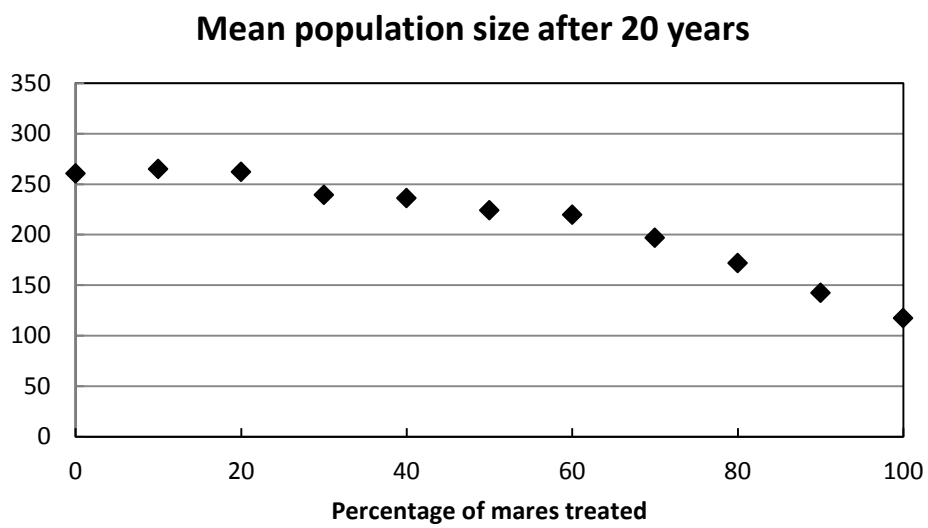
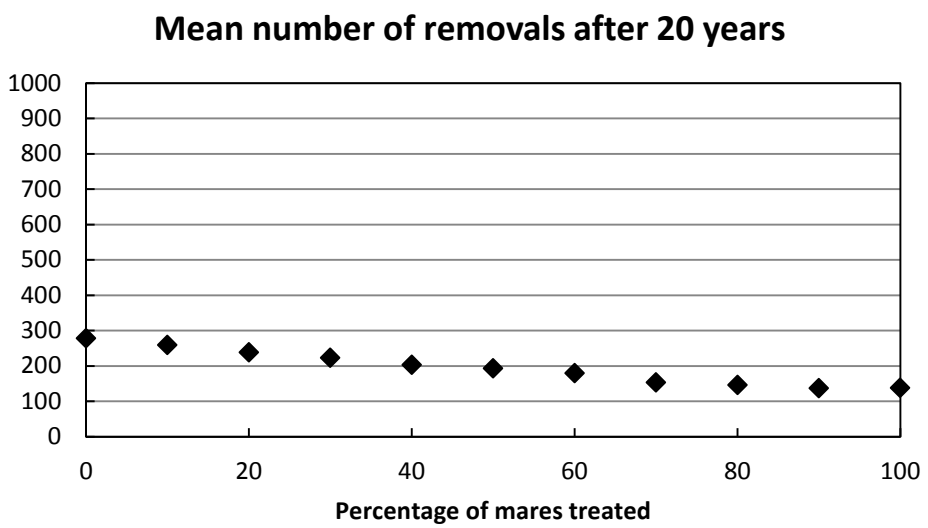
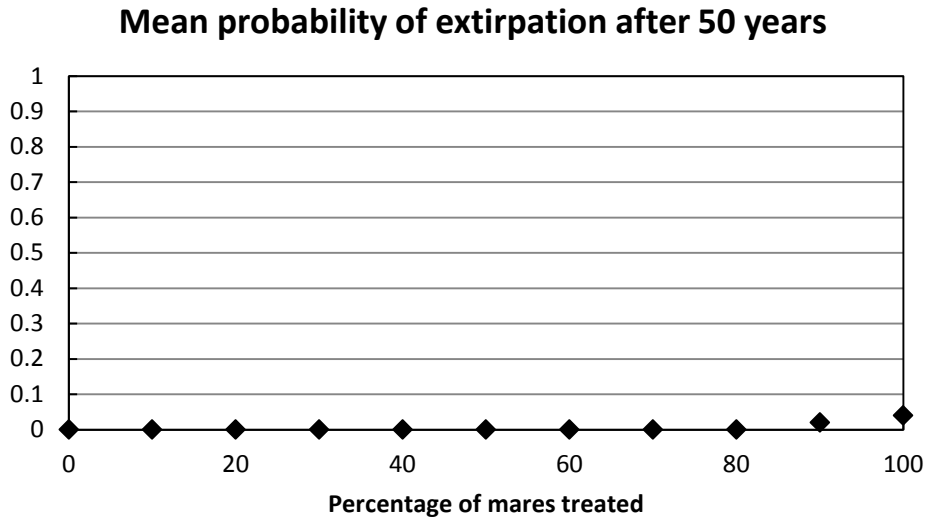


Figure 6. Initial population size = 250, lambda = 1.05, genetic diversity = low.—Continued







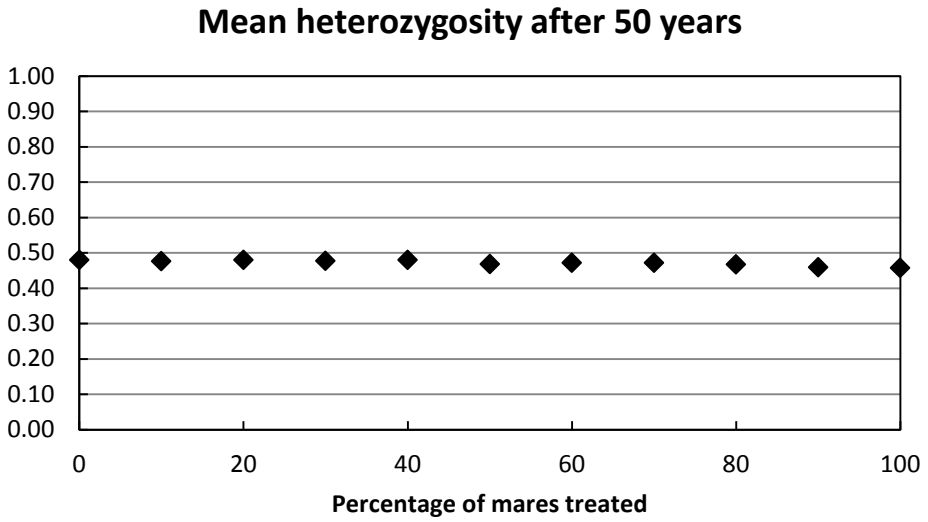


Figure 7. Initial population size = 250, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	260.38	264.77	262.05	239.17	236	224.01	219.74	196.91	171.78	142.36	117.4
Mean number of removals after 20 years	278.3	259.21	237.83	223.07	202.93	192.93	179.33	153.23	145.94	137.16	137.79
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0.02	0.04
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.47	0.47	0.46	0.46

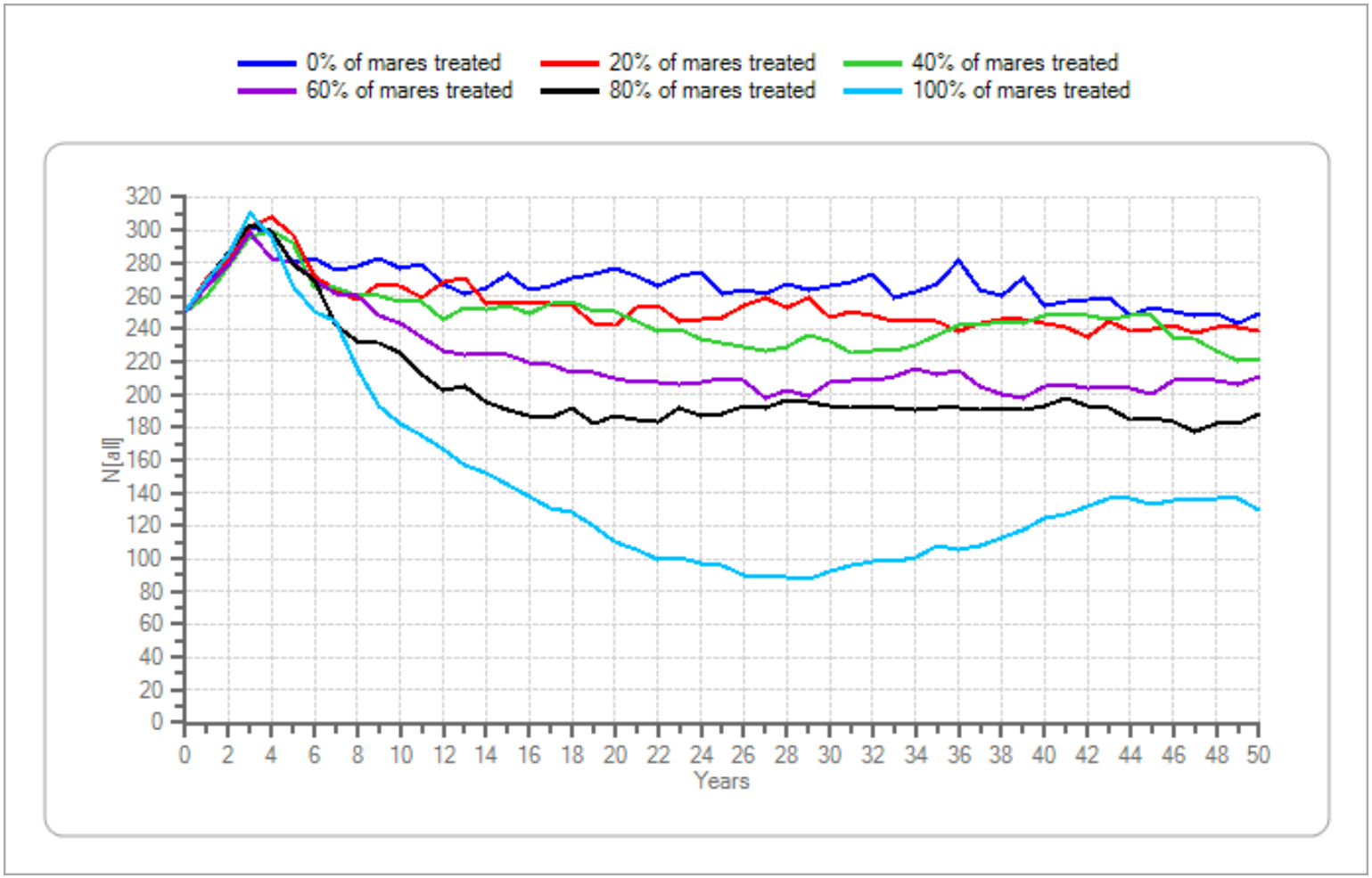
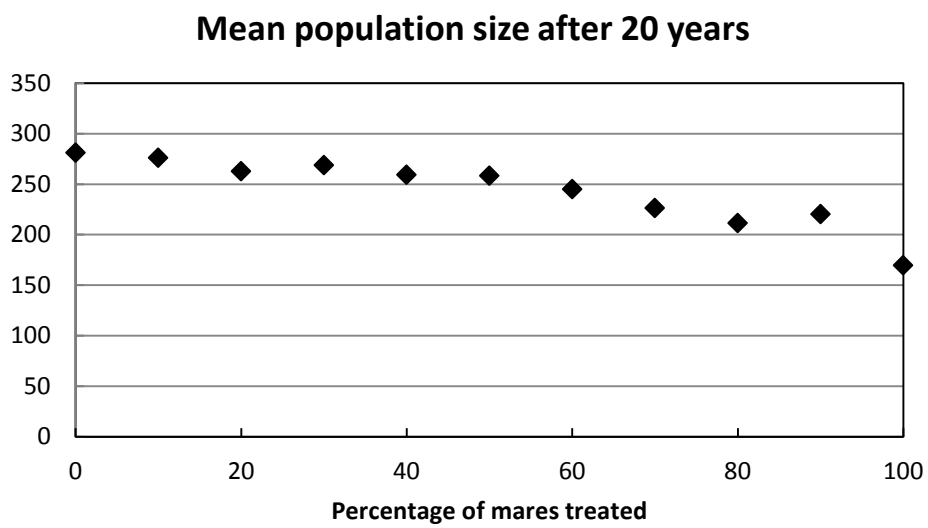
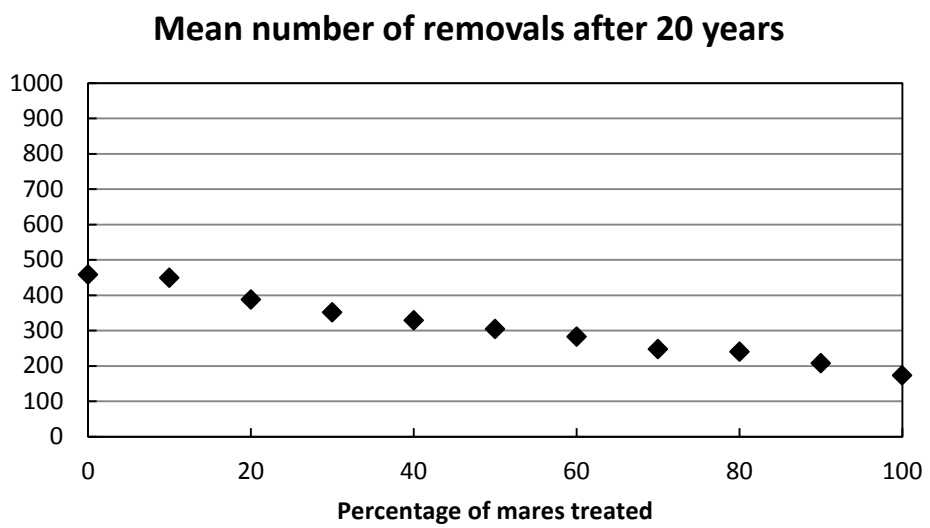
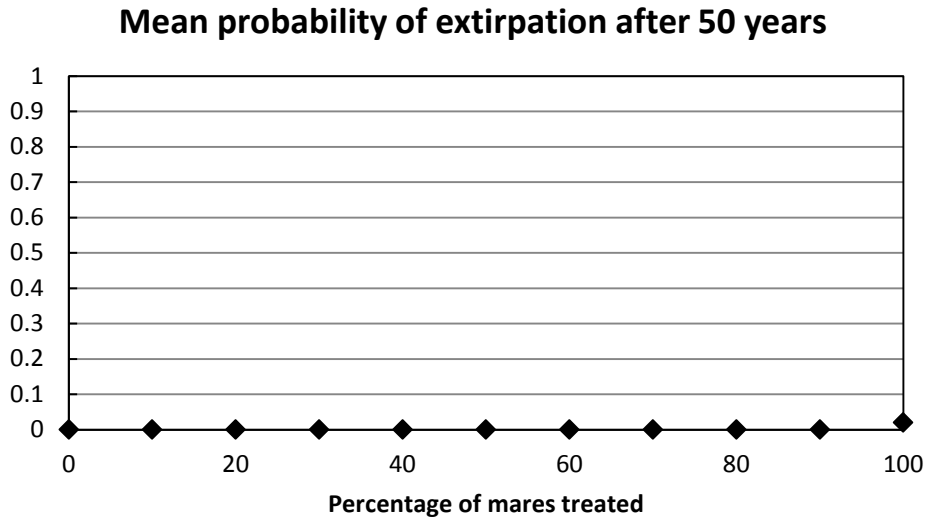


Figure 7. Initial population size = 250, lambda = 1.10, genetic diversity = low.—Continued







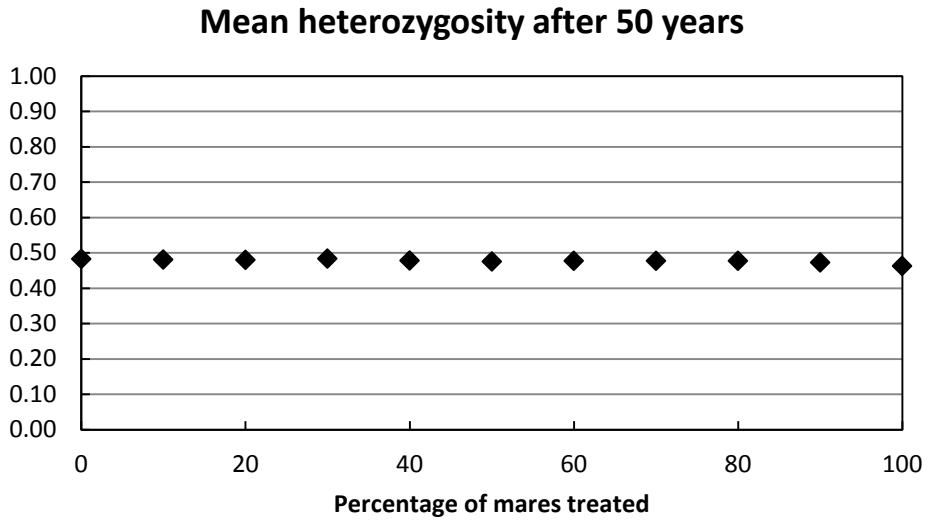


Figure 8. Initial population size = 250, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	281.13	275.97	262.64	268.65	259.12	258.31	244.81	226.37	211.37	220.14	169.63
Mean number of removals after 20 years	458.22	448.92	387.51	351.03	328.21	304.61	282.41	247.07	239.97	207.47	173.26
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.02
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47	0.46

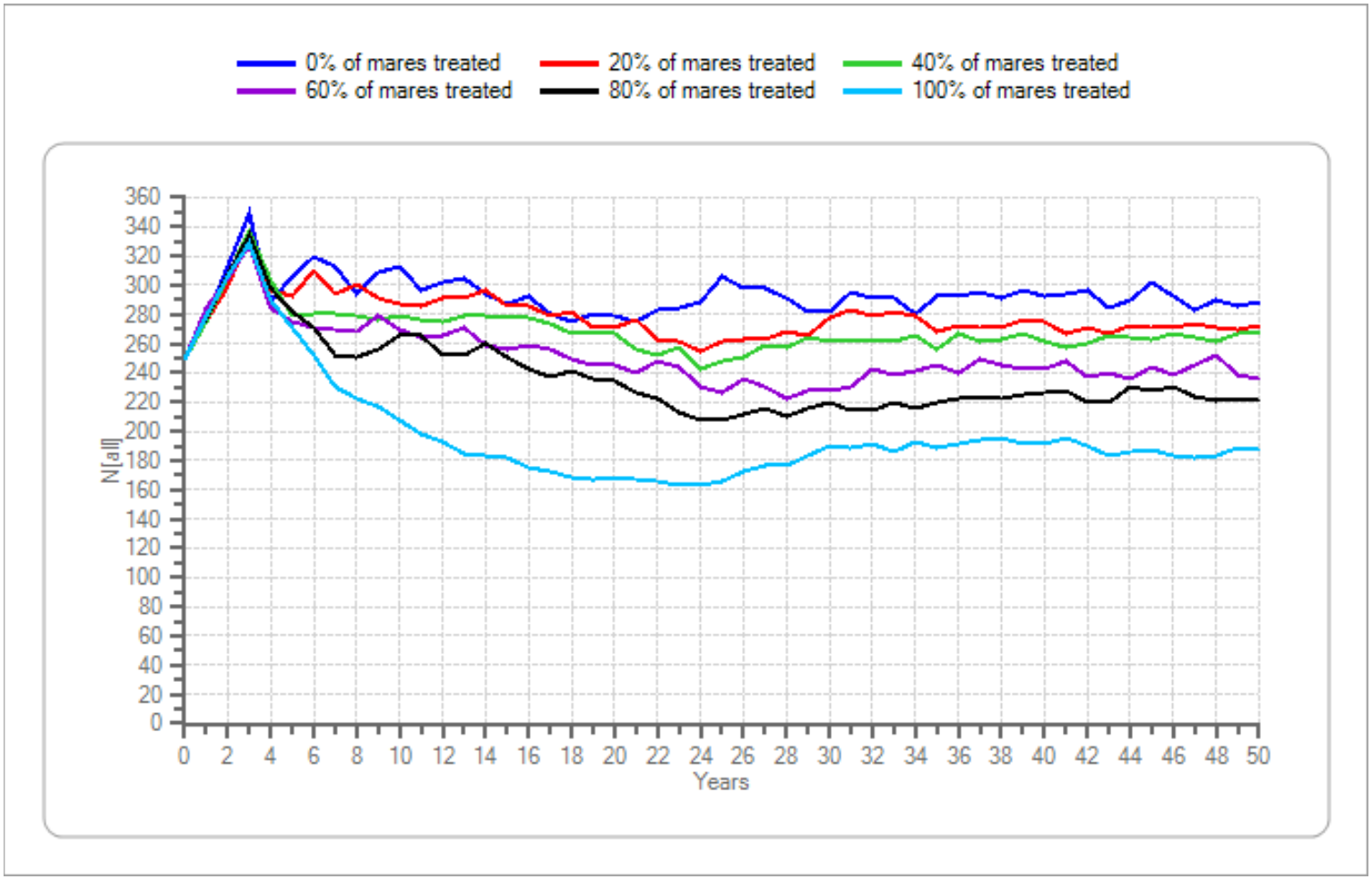
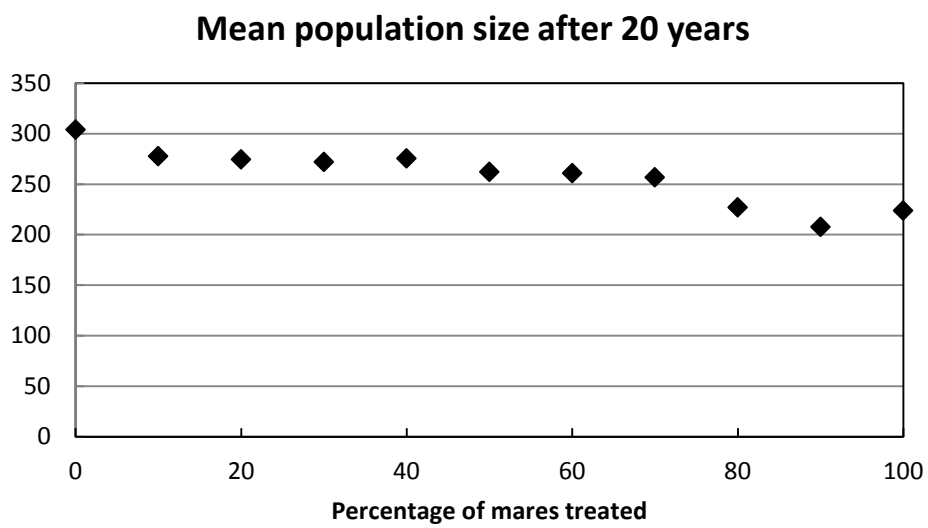
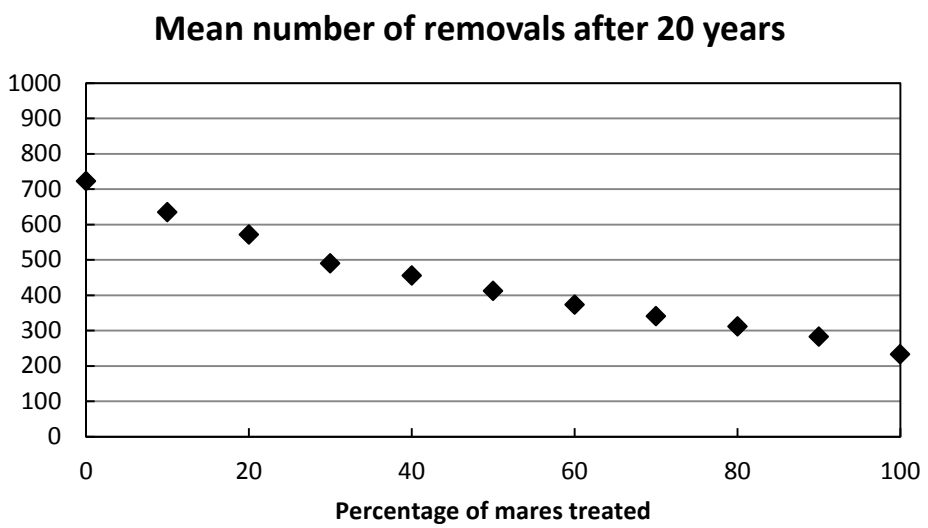
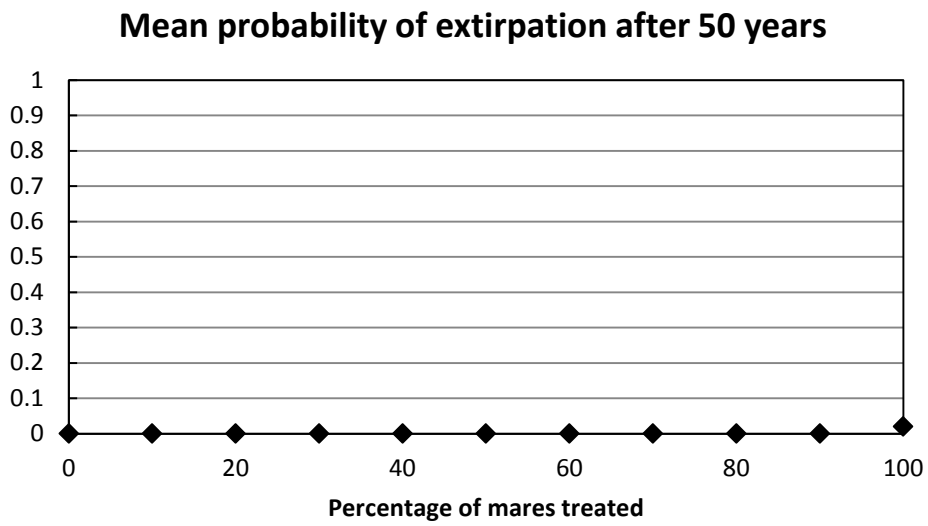


Figure 8. Initial population size = 250, lambda = 1.15, genetic diversity = low.—Continued







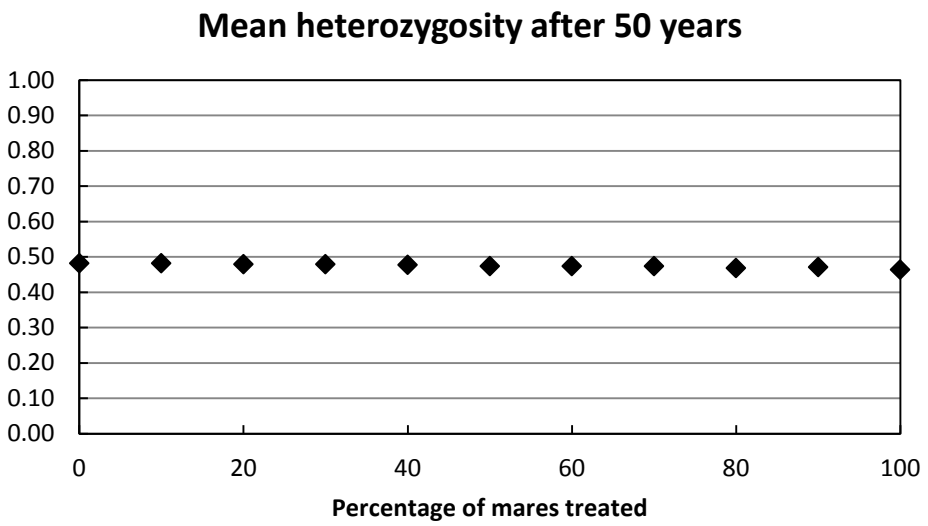


Figure 9. Initial population size = 250, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	303.72	277.47	274.27	271.98	275.36	262.1	260.81	256.62	226.89	207.59	223.76
Mean number of removals after 20 years	722.53	634.9	570.99	490.14	455.85	412	373.4	340.01	311.73	282.63	232.31
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.02
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.47	0.47	0.47	0.46

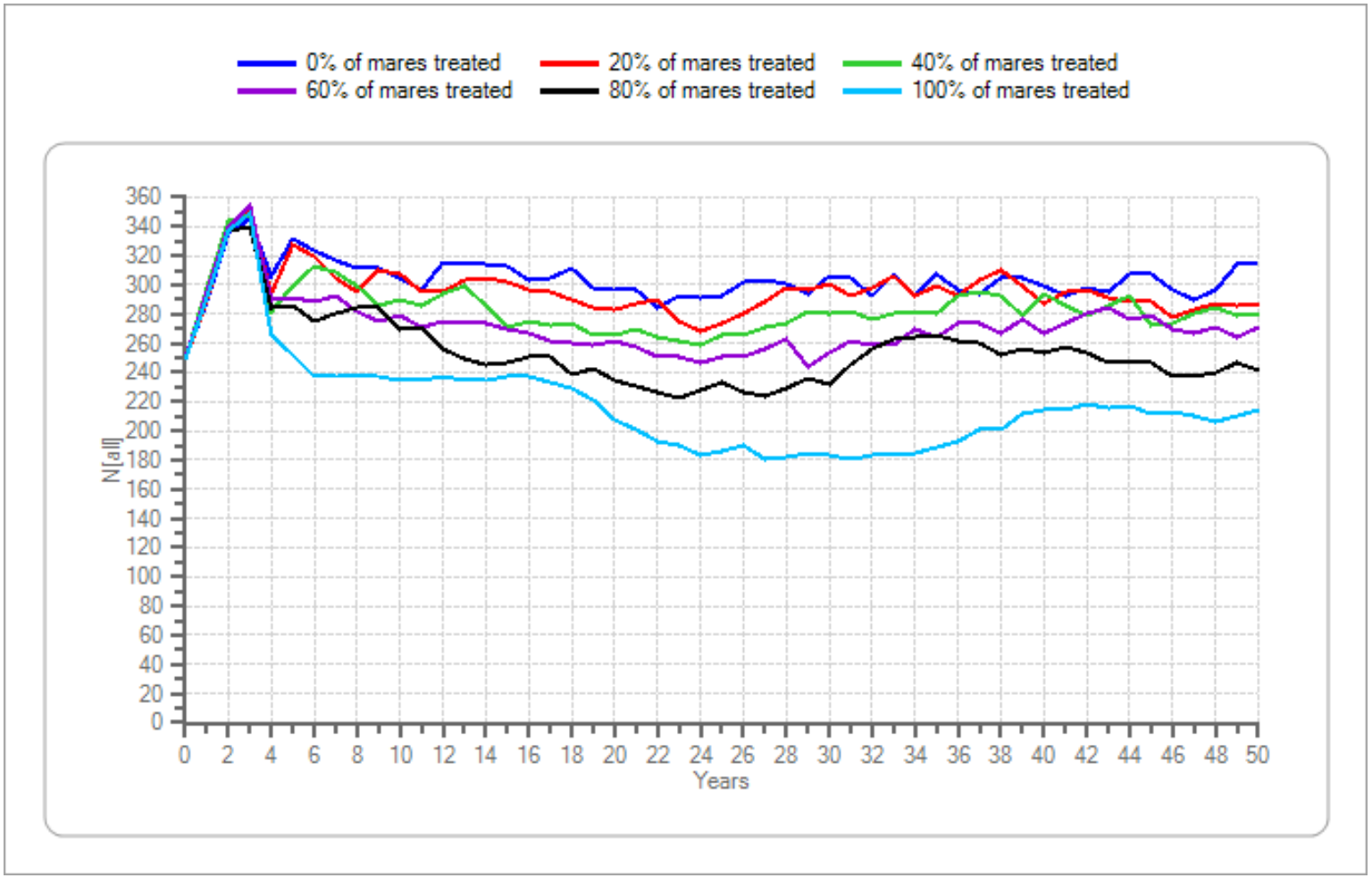
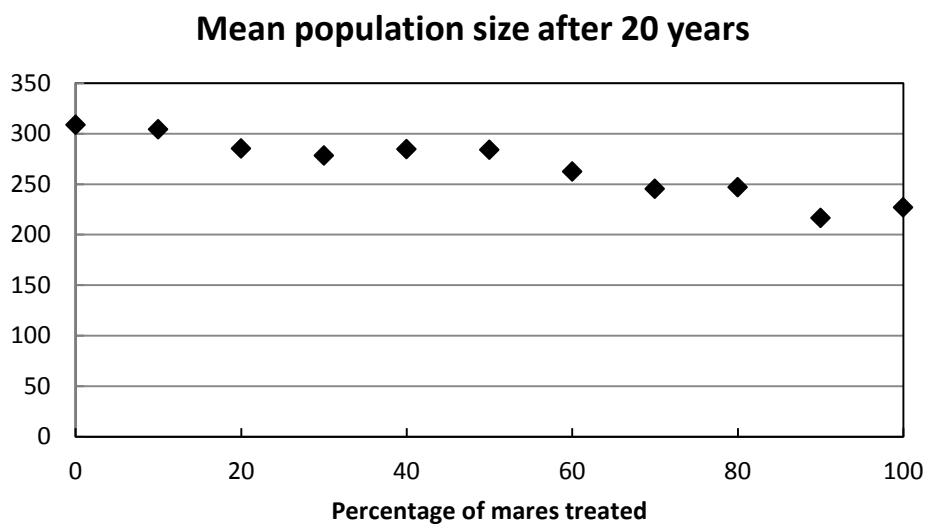
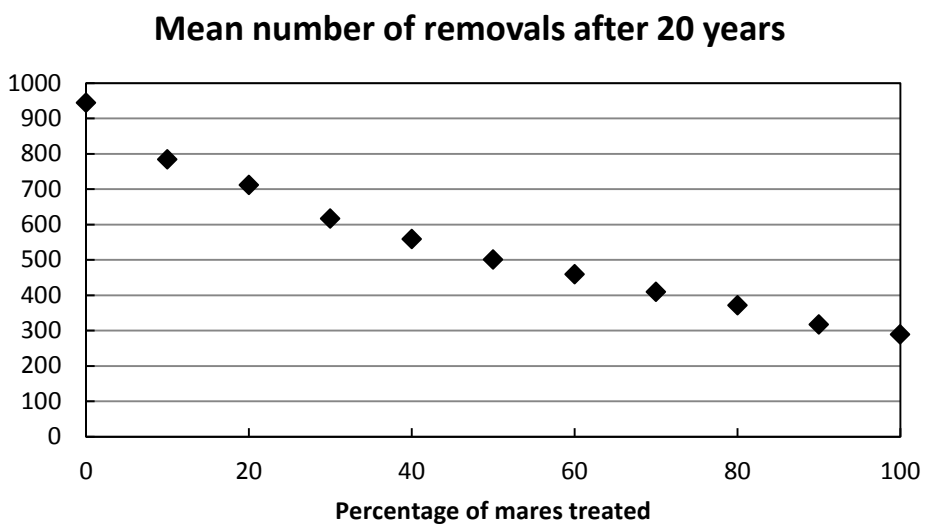
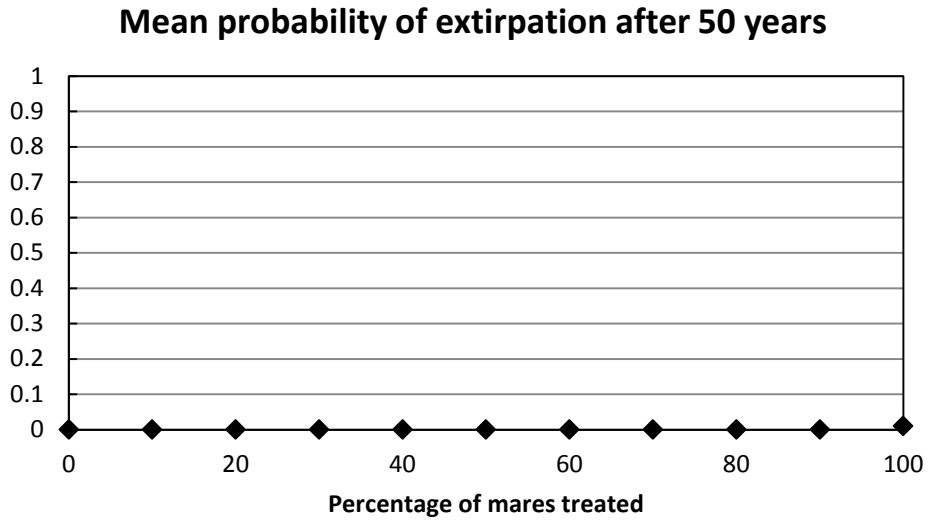


Figure 9. Initial population size = 250, lambda = 1.20, genetic diversity = low.—Continued







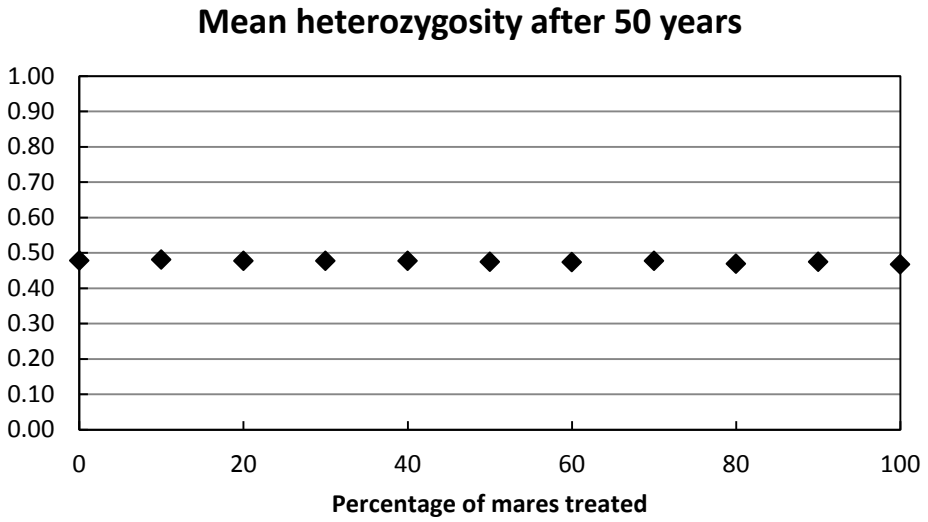


Figure 10. Initial population size = 250, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	308.52	304.15	285.11	278.16	284.69	283.85	262.37	245.28	246.89	216.51	226.76
Mean number of removals after 20 years	944.59	783.74	711.97	616.4	558.71	501.11	459.28	409.28	370.9	316.51	288.81
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.01
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.48	0.47	0.47	0.47

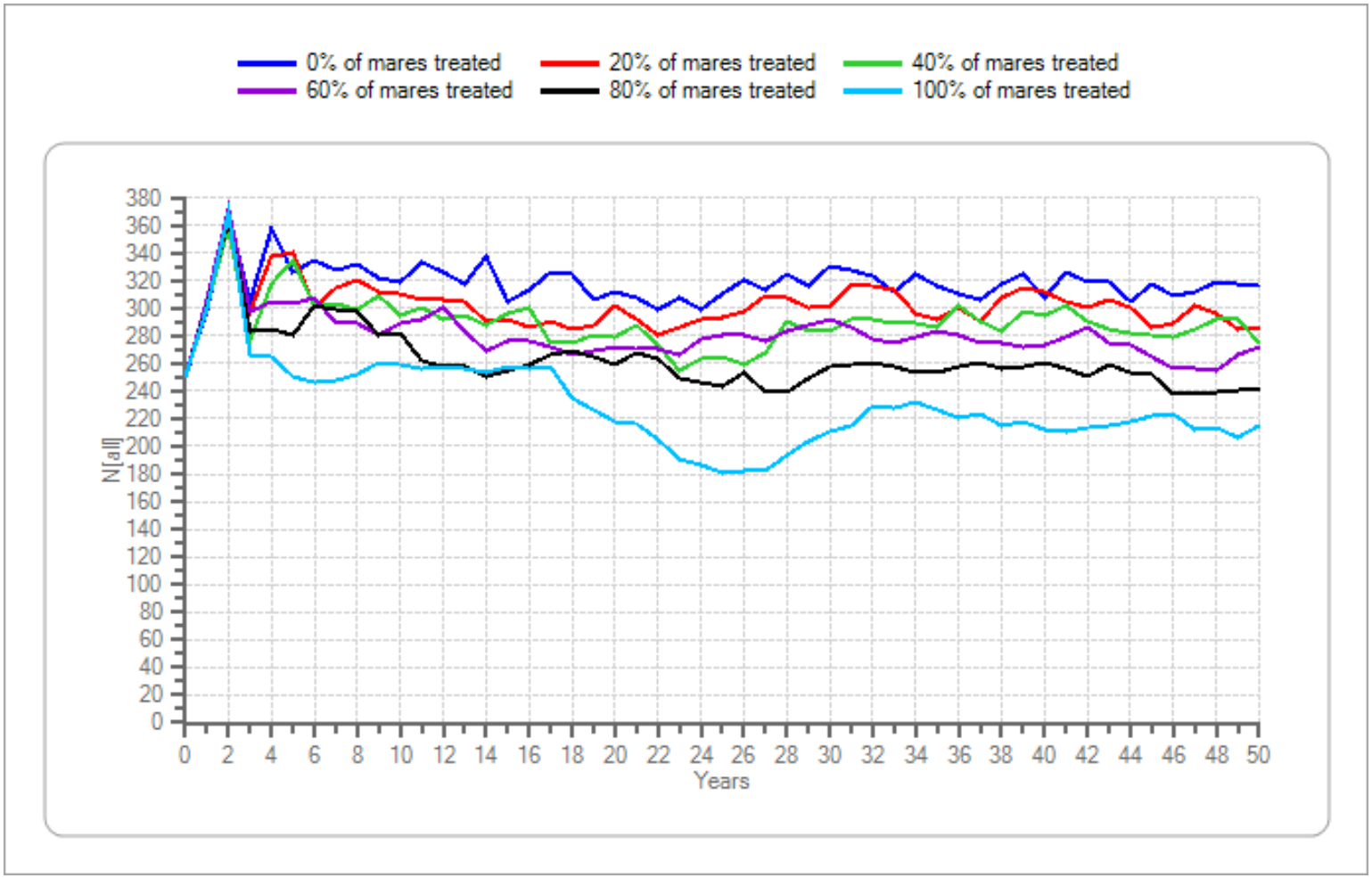
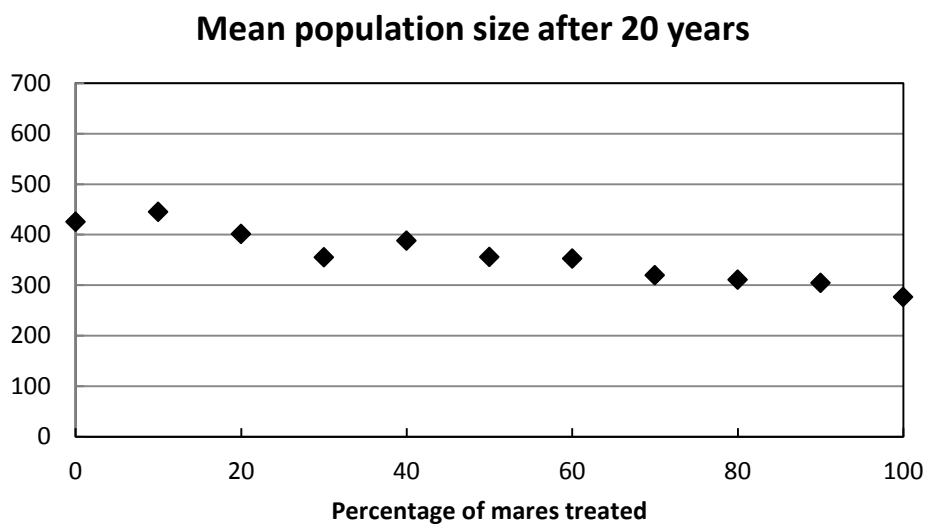
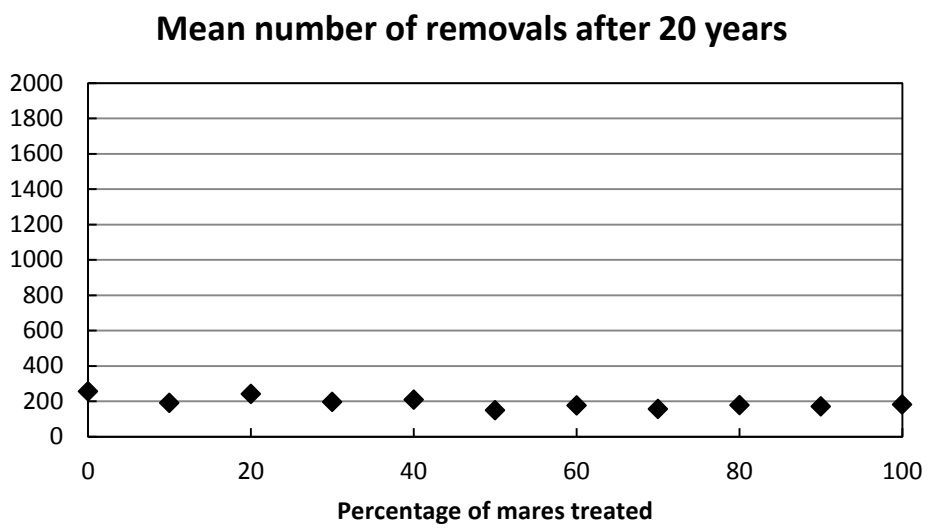
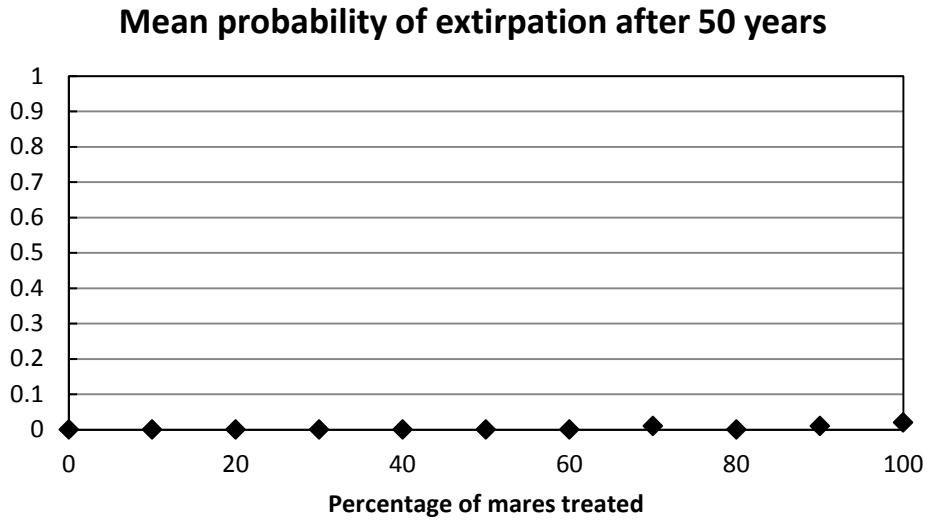


Figure 10. Initial population size = 250, lambda = 1.25, genetic diversity = low.—Continued







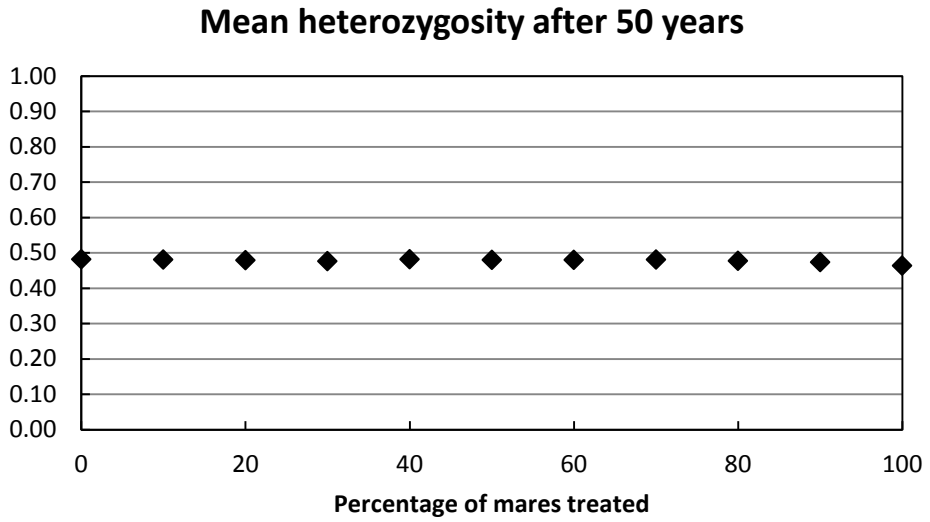


Figure 11. Initial population size = 500, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	425.35	444.78	401.43	354.61	387.64	355.61	352.23	319.32	310.27	304.15	276.61
Mean number of removals after 20 years	255.36	190.95	241.46	196.02	208.87	147.97	176.38	156.08	177.73	169.95	181.87
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0.01	0	0.01	0.02
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47	0.46

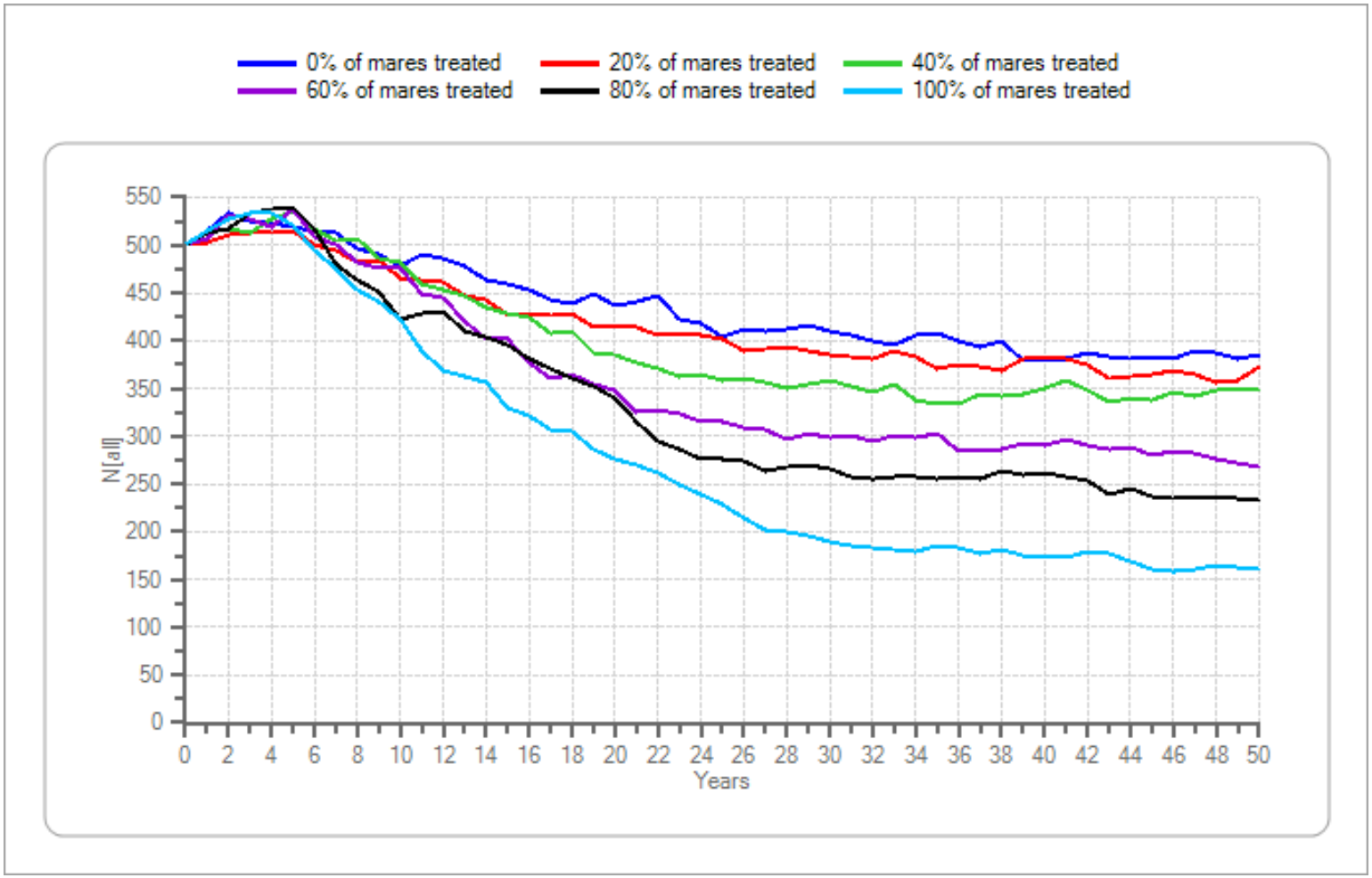
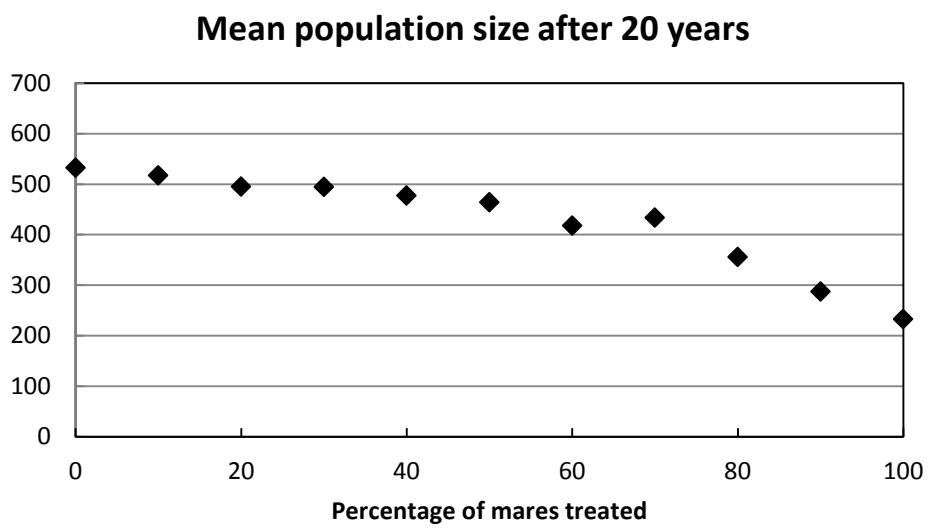
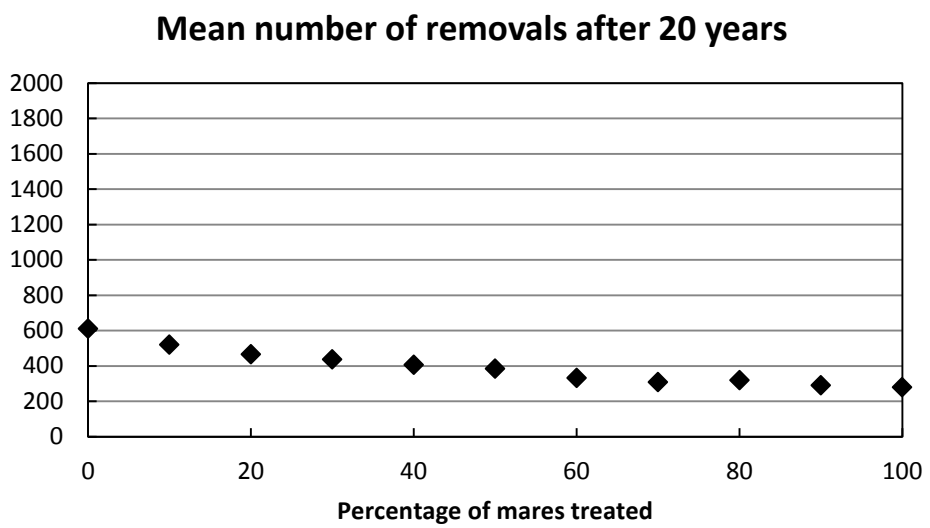
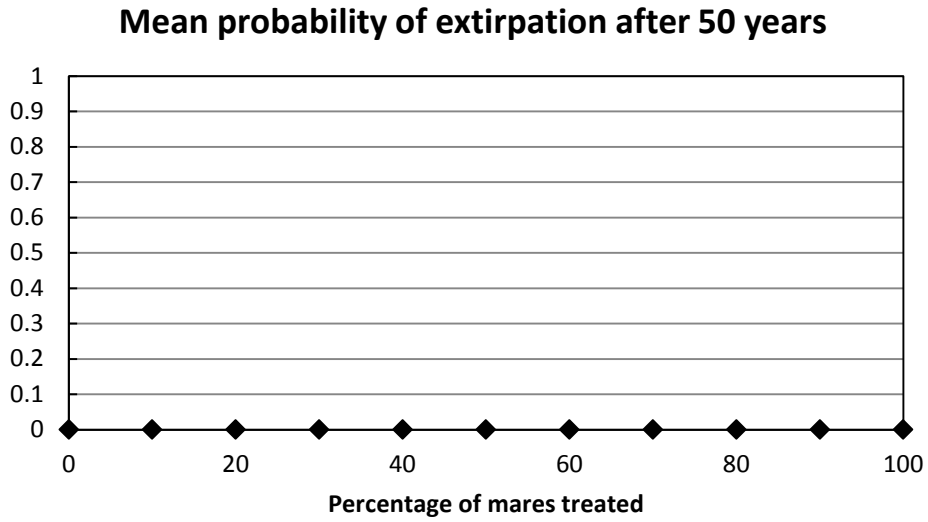


Figure 11. Initial population size = 500, lambda = 1.05, genetic diversity = low.—Continued







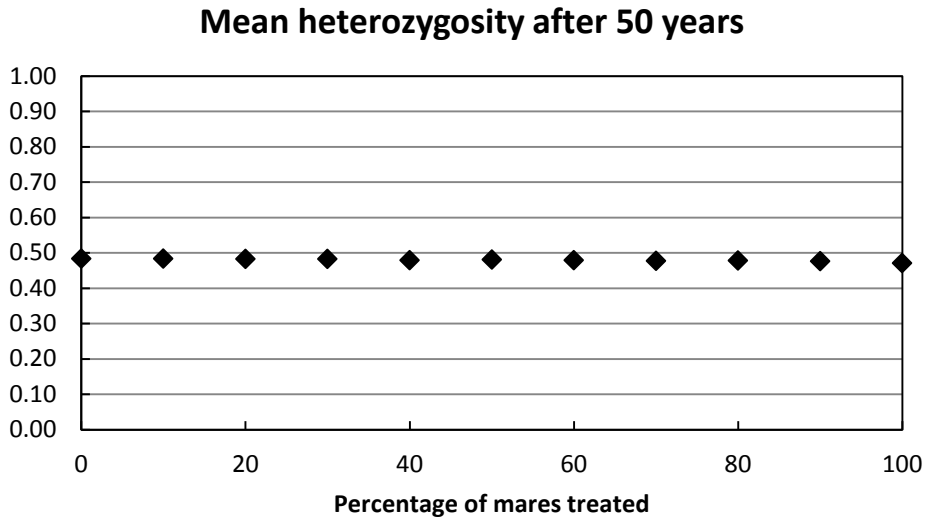


Figure 12. Initial population size = 500, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	532.14	517.32	494.85	494.04	476.94	463.89	417.36	433.64	355.55	286.94	232.49
Mean number of removals after 20 years	610.21	520.05	465.8	436.08	406.01	384.76	332.11	307.18	318.48	289.02	279.16
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47

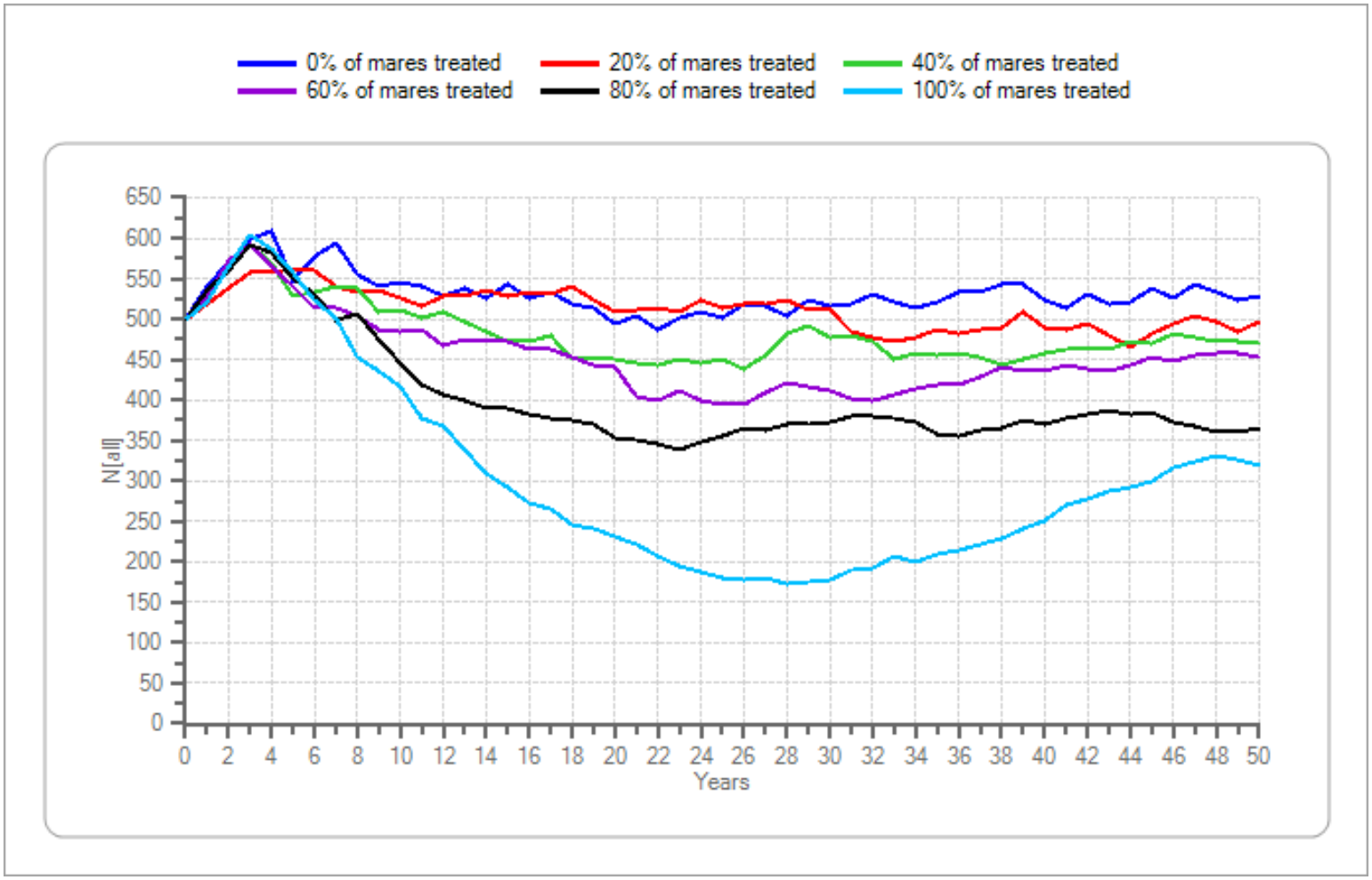
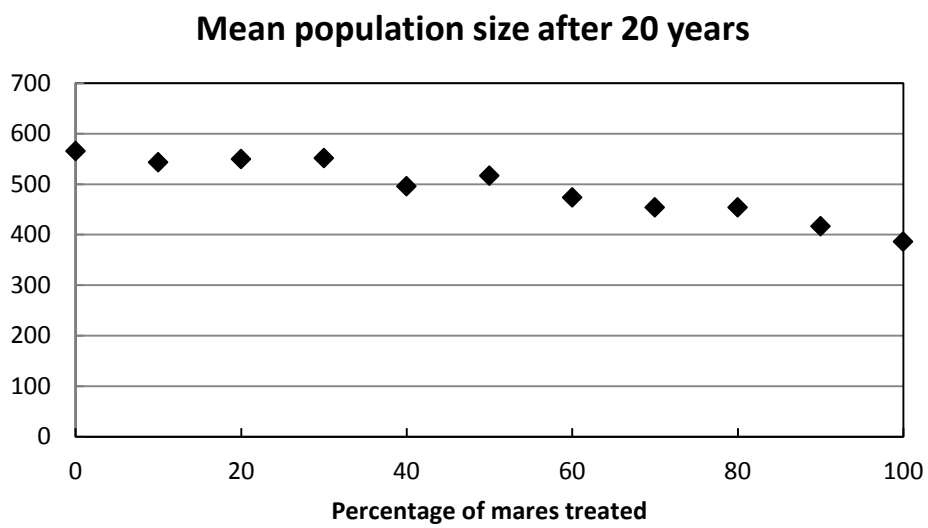
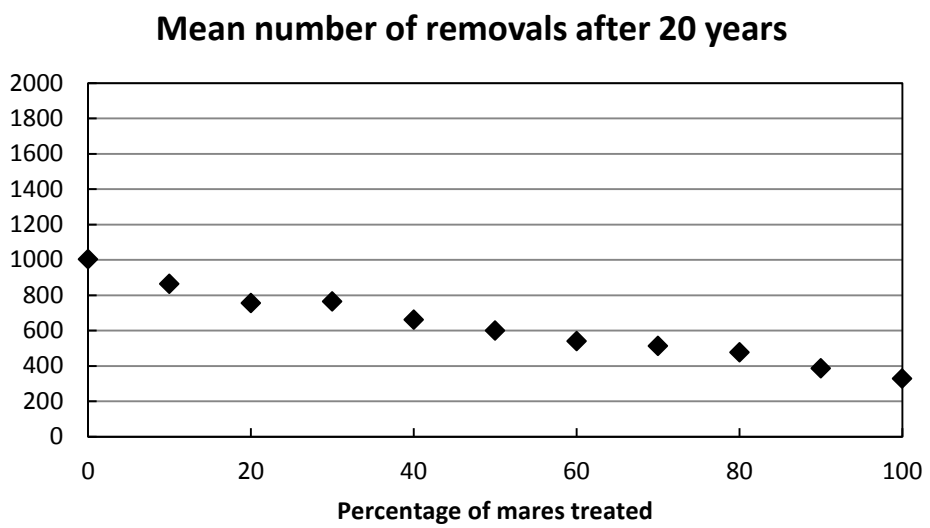
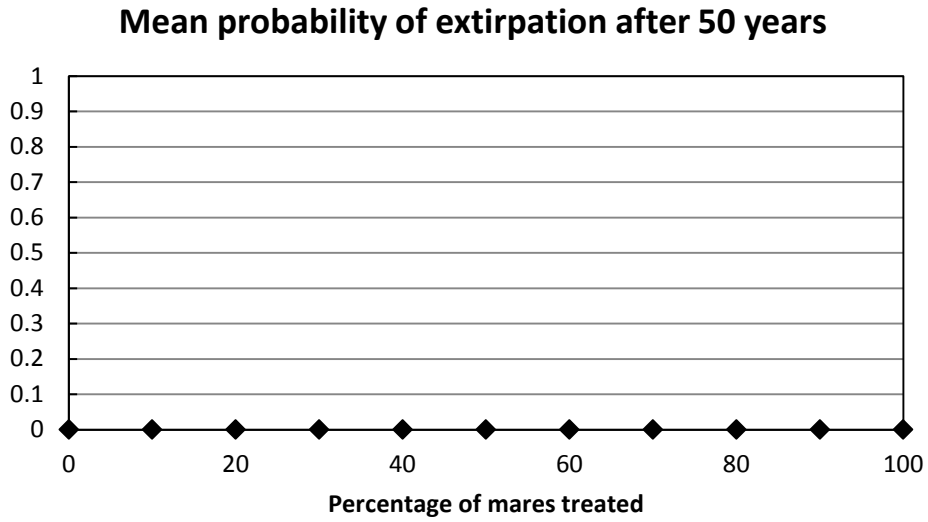


Figure 12. Initial population size = 500, lambda = 1.10, genetic diversity = low.—Continued







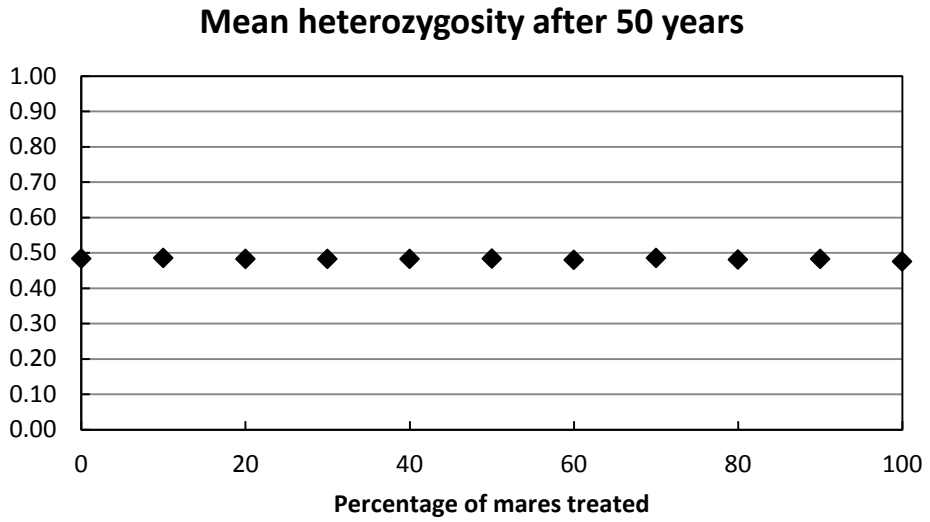


Figure 13. Initial population size = 500, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	565.34	543.12	549.14	551.57	495.37	516.42	473.26	453.73	453.48	416.08	385.94
Mean number of removals after 20 years	1002.92	863.83	755.3	764.15	660.85	598.87	539.63	512.76	475.42	384.89	328.2
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.49	0.48	0.48	0.48

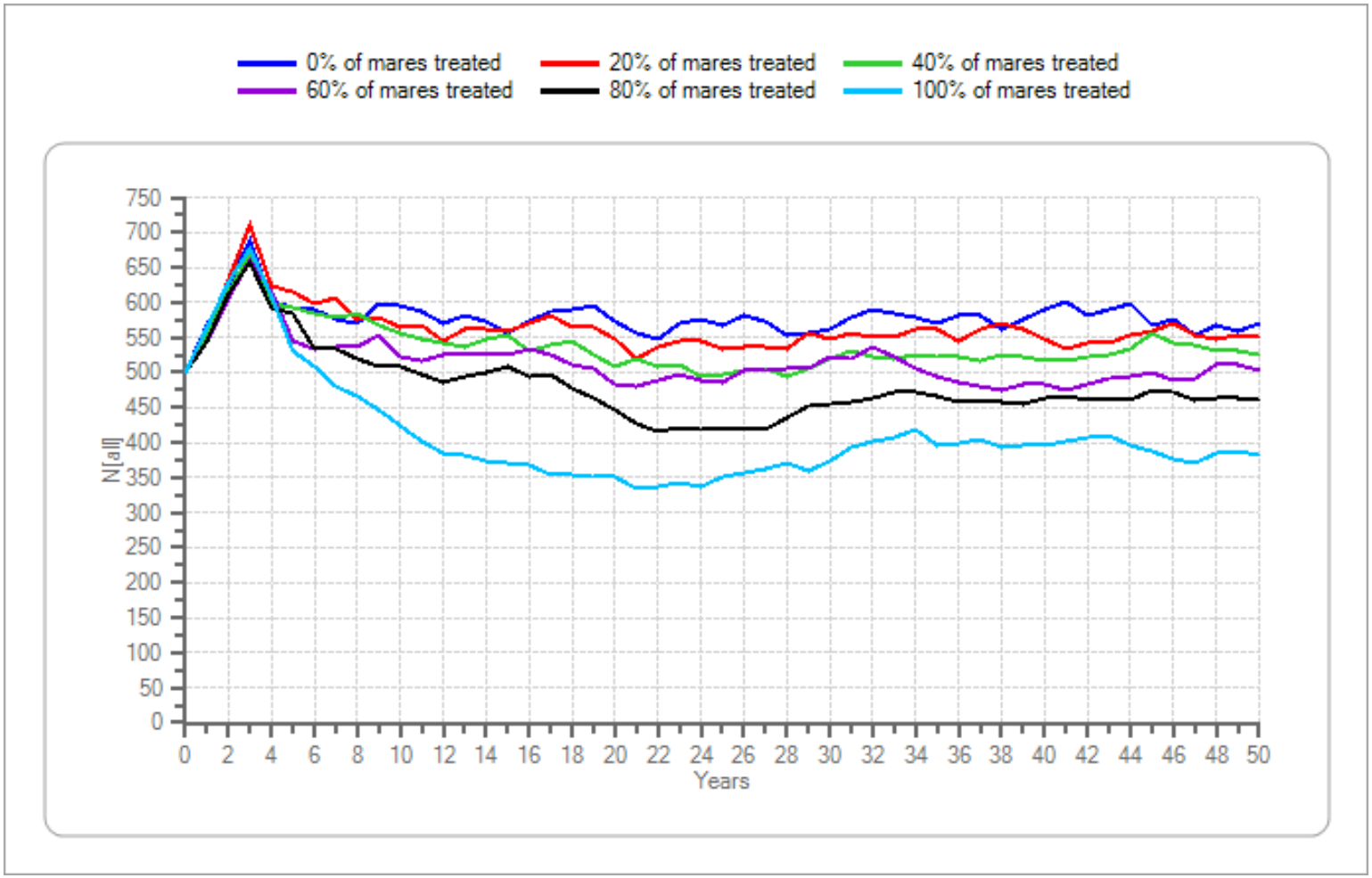
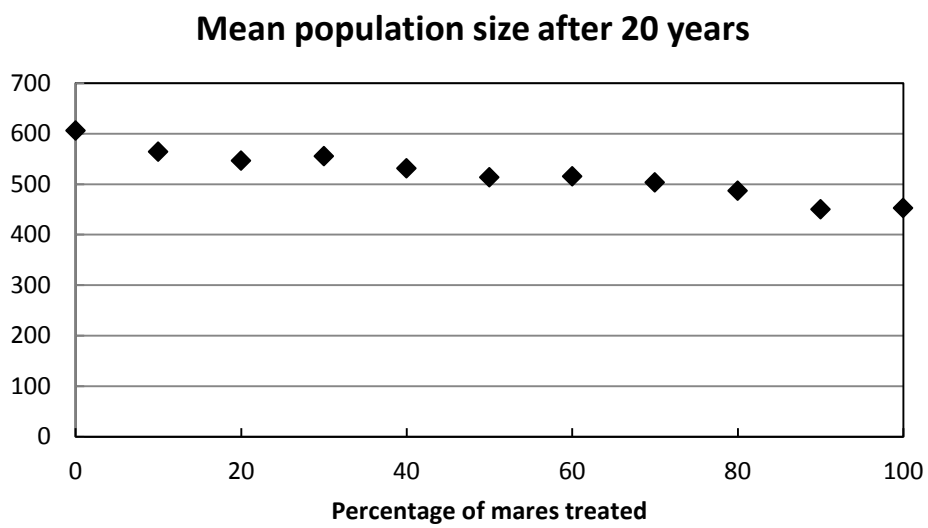
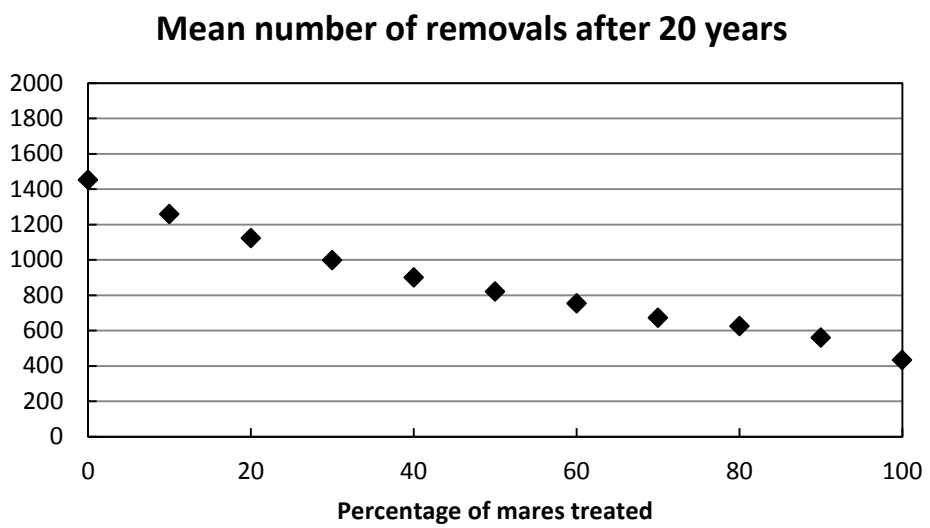
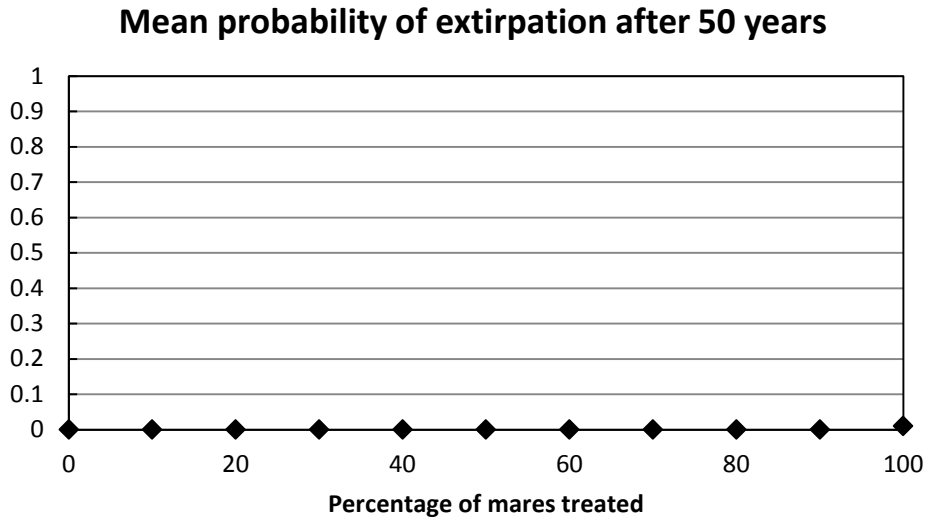


Figure 13. Initial population size = 500, lambda = 1.15, genetic diversity = low.—Continued







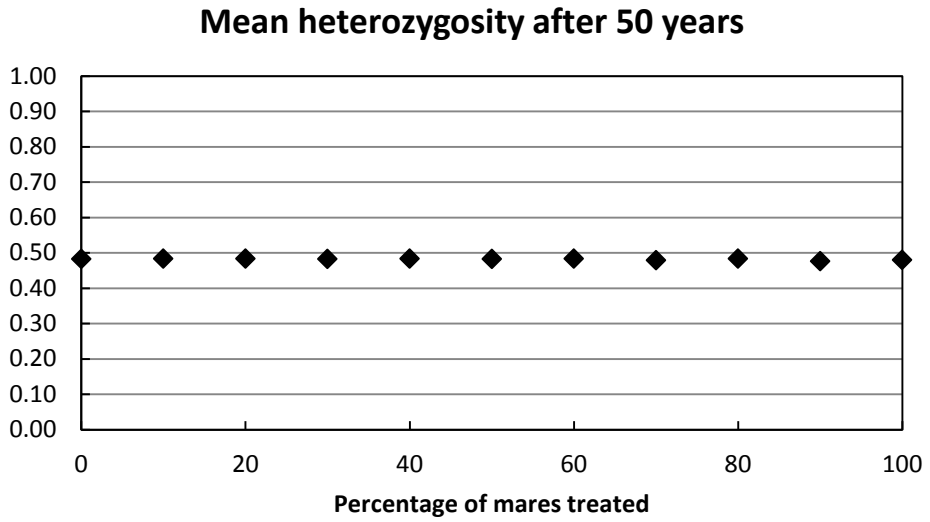


Figure 14. Initial population size = 500, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	606.16	564.12	546.4	555.28	531.1	513.41	515.42	502.94	486.58	450.15	452.21
Mean number of removals after 20 years	1451.75	1258.73	1122.91	997.36	900.23	820.29	752.55	671.84	624.39	559.54	432.37
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.01
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

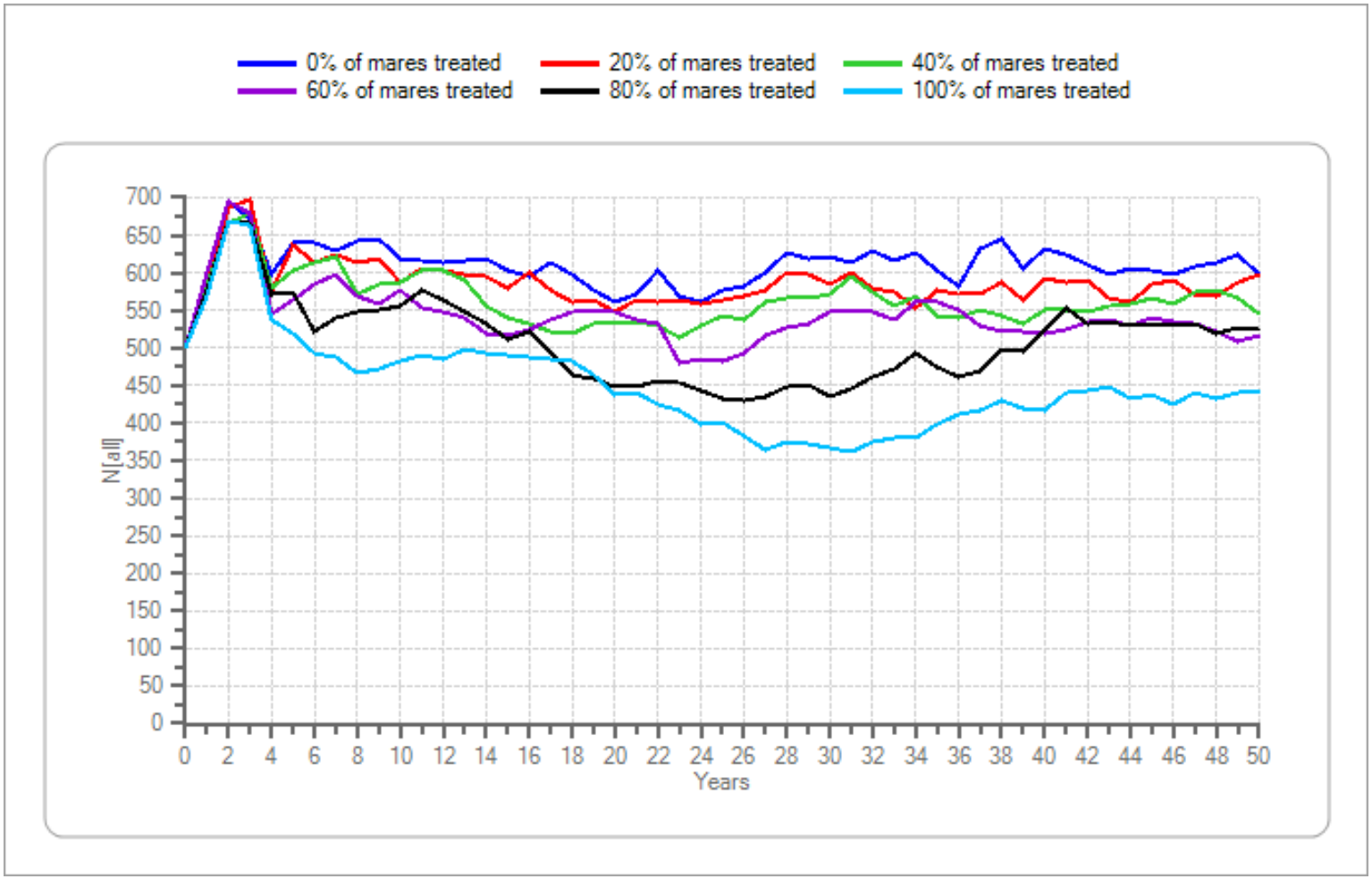
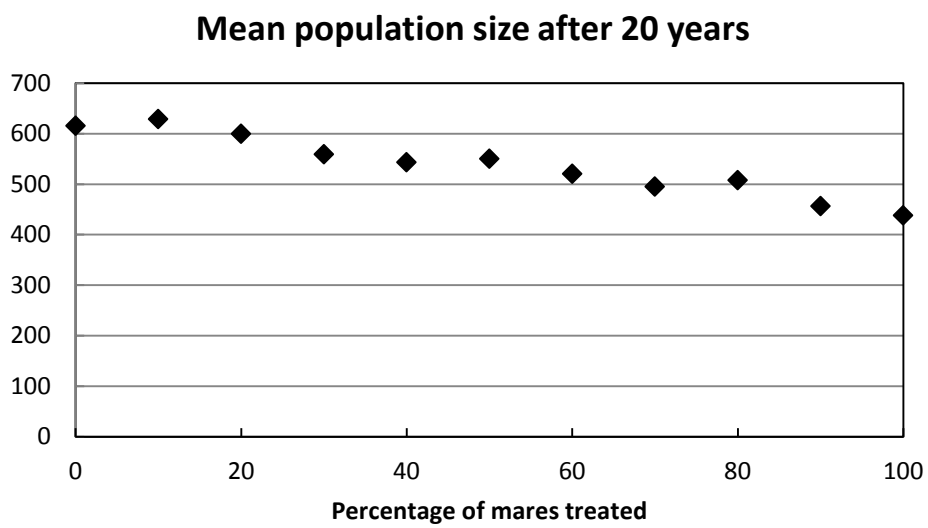
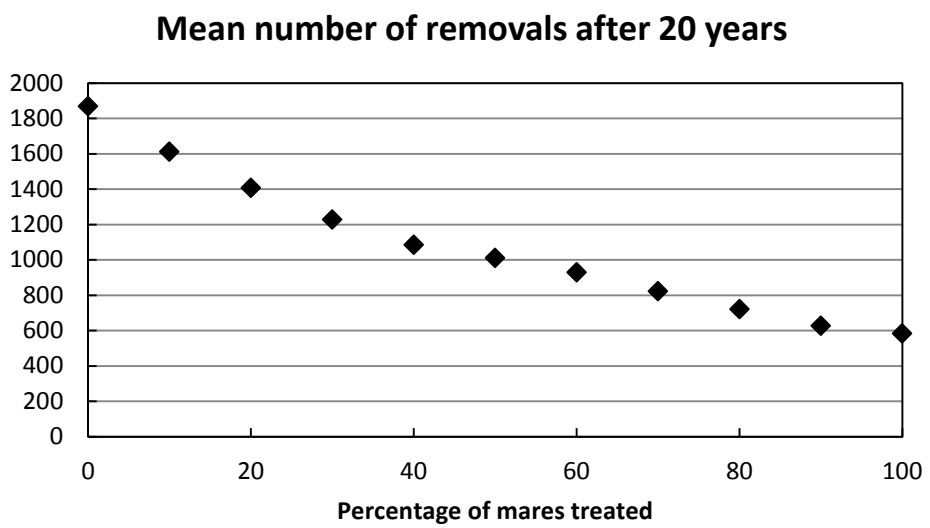
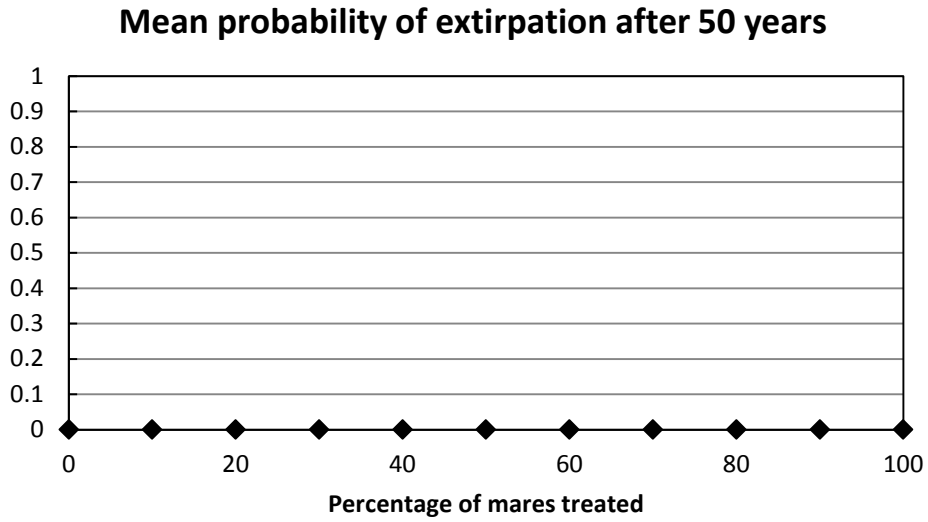


Figure 14. Initial population size = 500, lambda = 1.20, genetic diversity = low.—Continued







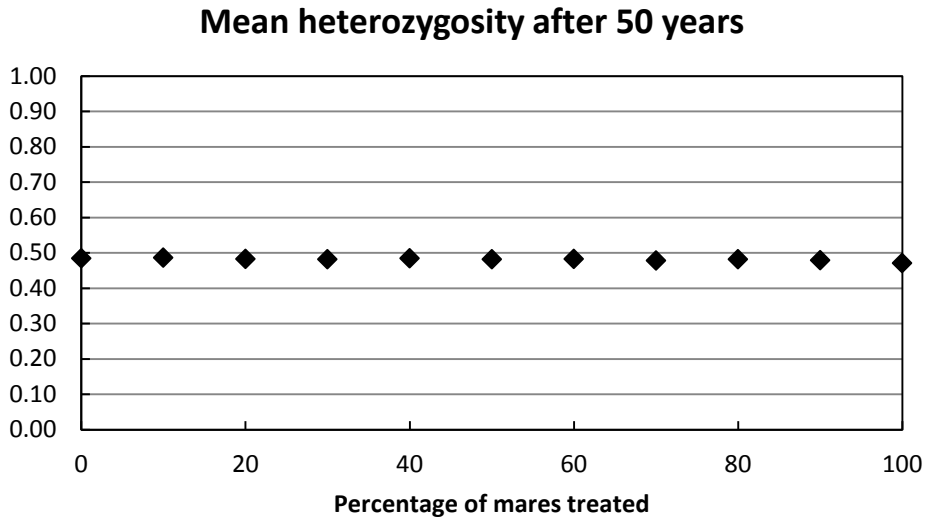


Figure 15. Initial population size = 500, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	615.29	628.52	599.27	559.22	543.09	550.12	520.04	495.03	507.73	456.42	437.97
Mean number of removals after 20 years	1868.95	1612.35	1406.33	1227.35	1083.85	1009.6	928.44	822.54	721.17	626.66	583.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47

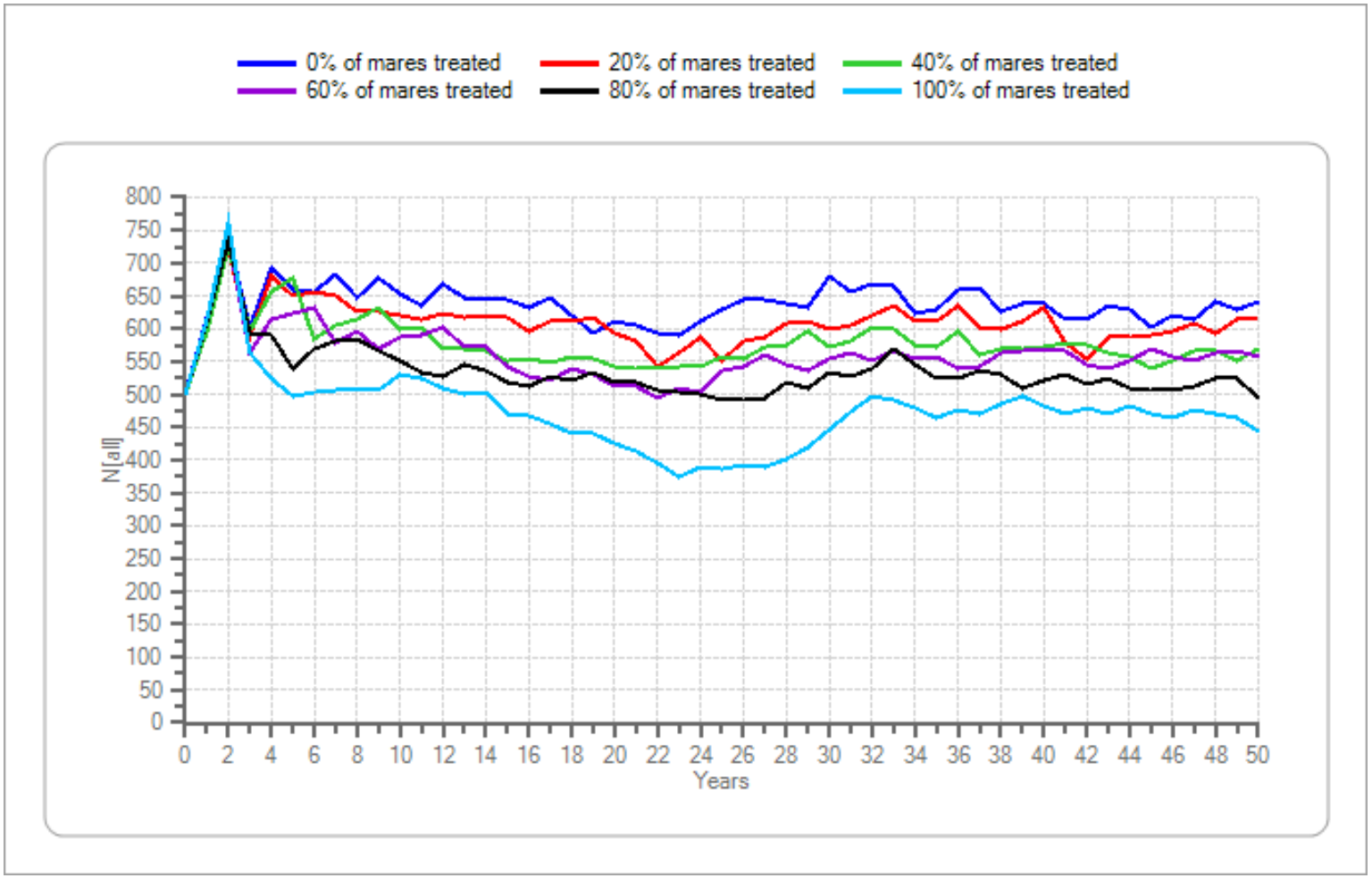
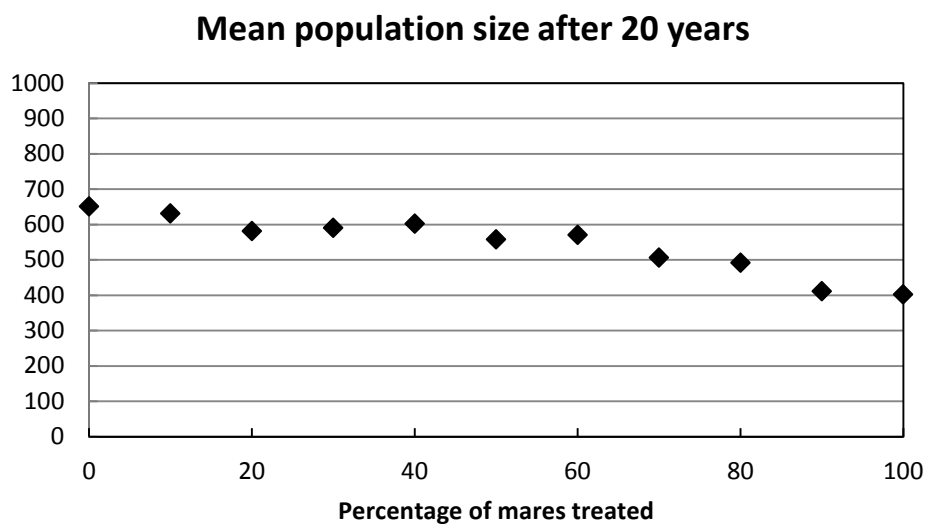
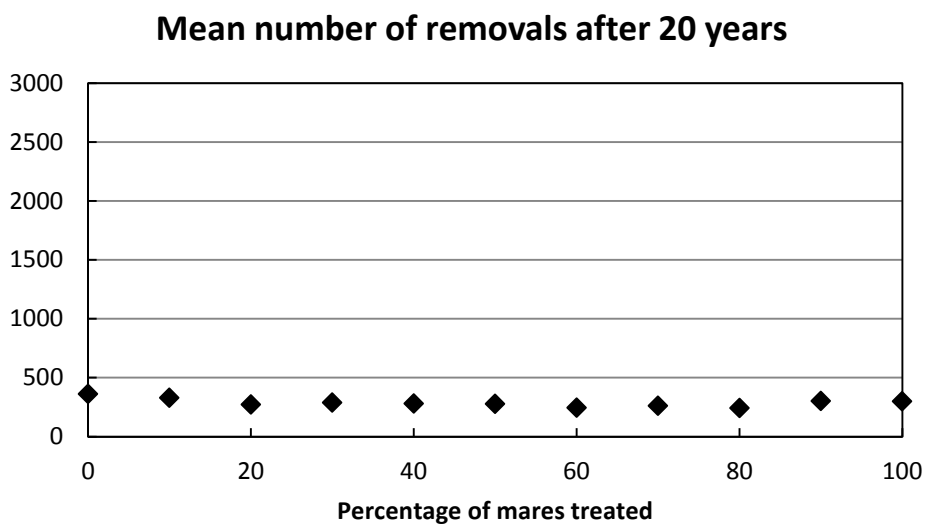
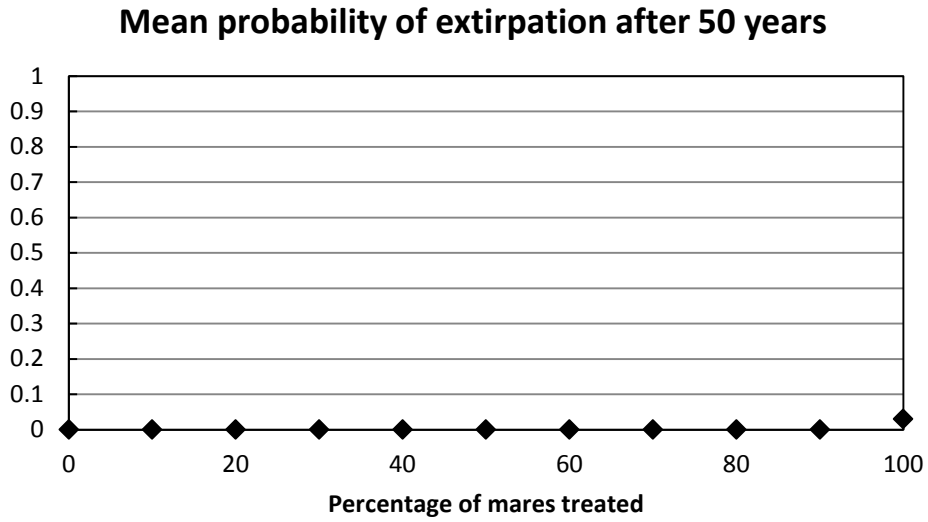


Figure 15. Initial population size = 500, lambda = 1.25, genetic diversity = low.—Continued







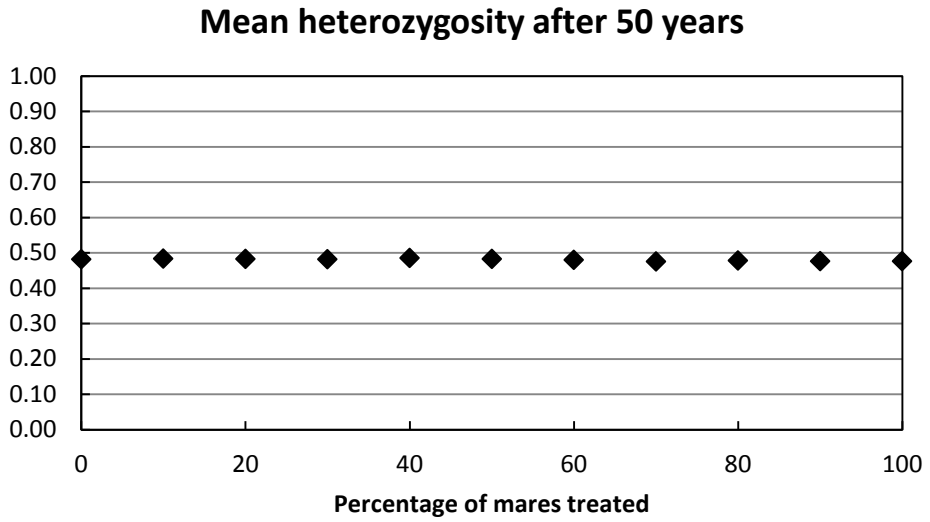


Figure 16. Initial population size = 750, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	650.88	631.43	581.13	590.12	602.15	557.46	570.81	506.51	491.17	411.2	401.89
Mean number of removals after 20 years	361.95	328.24	272.67	288.49	279.12	276.66	243.67	261.64	241.29	302.35	297.91
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.03
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.48

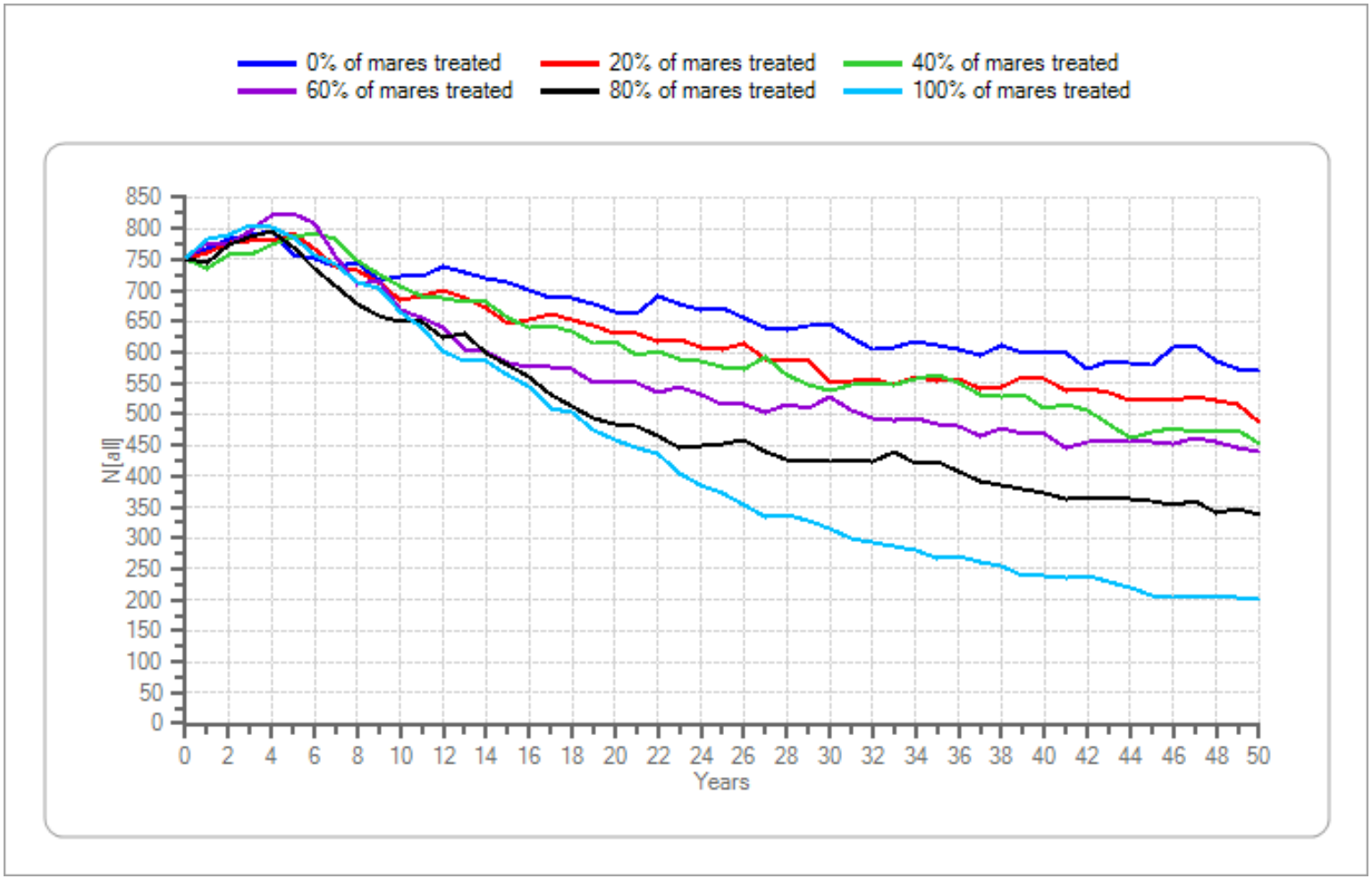
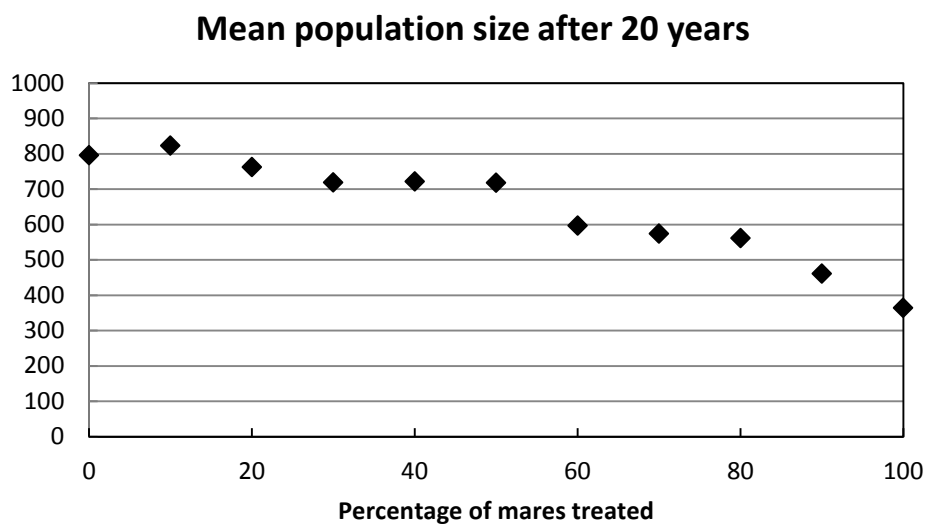
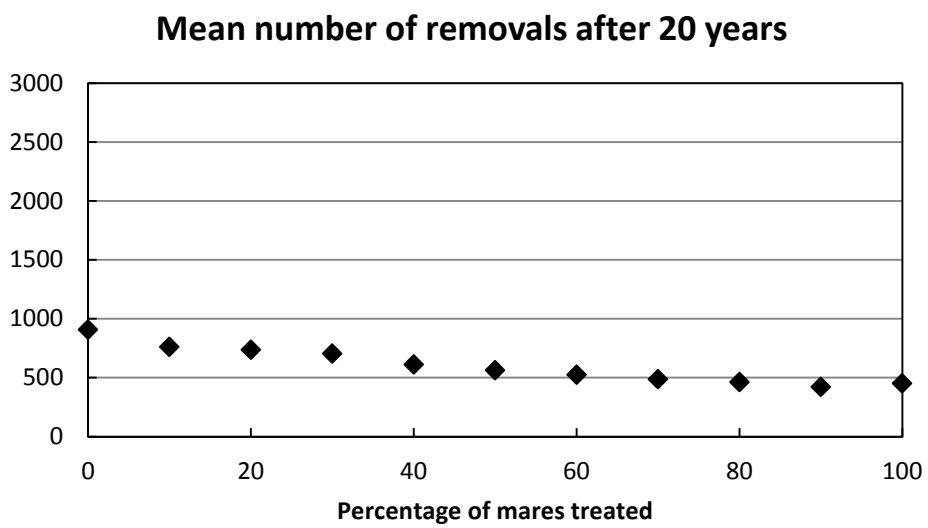
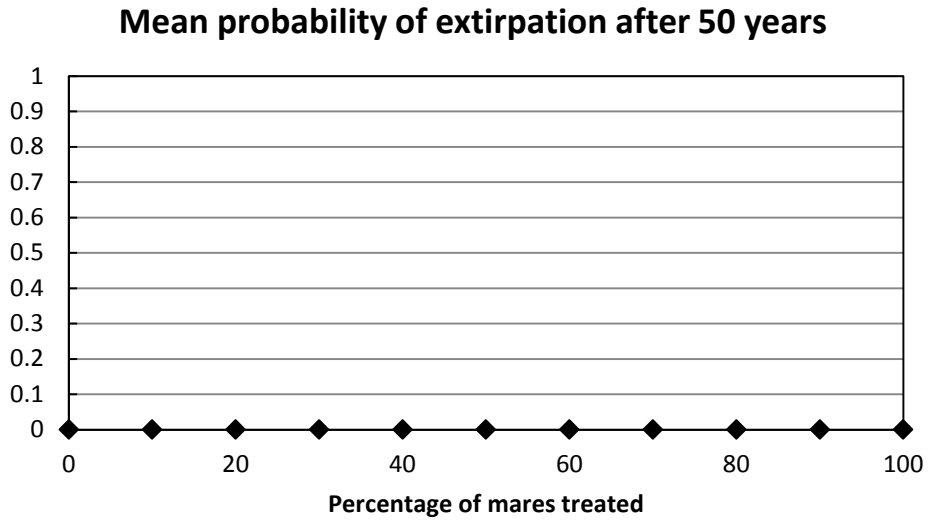


Figure 16. Initial population size = 750, lambda = 1.05, genetic diversity = low.—Continued







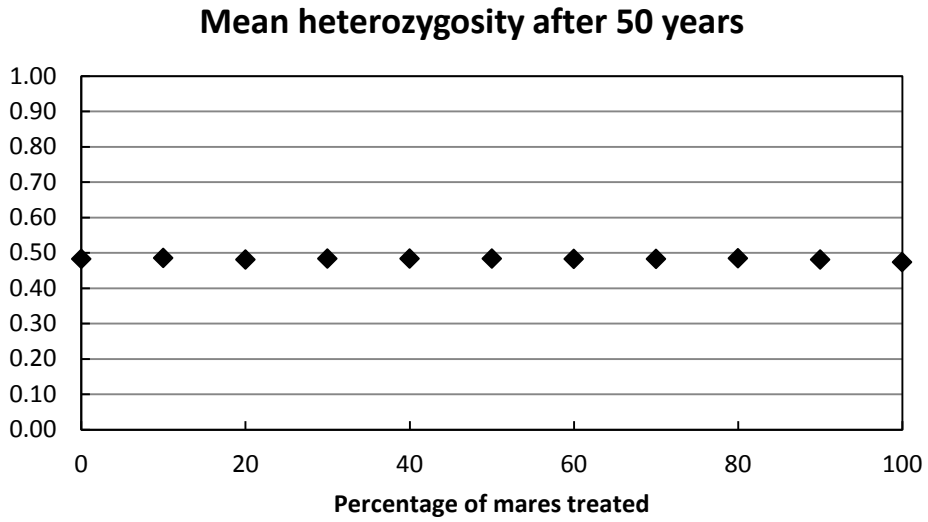


Figure 17. Initial population size = 750, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	795.56	823.24	762.24	718.7	721.89	717.77	597.06	573.89	561.16	460.9	363.9
Mean number of removals after 20 years	908.52	760.43	736.27	703.39	611.56	562.49	523.38	486.72	462.27	420.49	450.7
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47

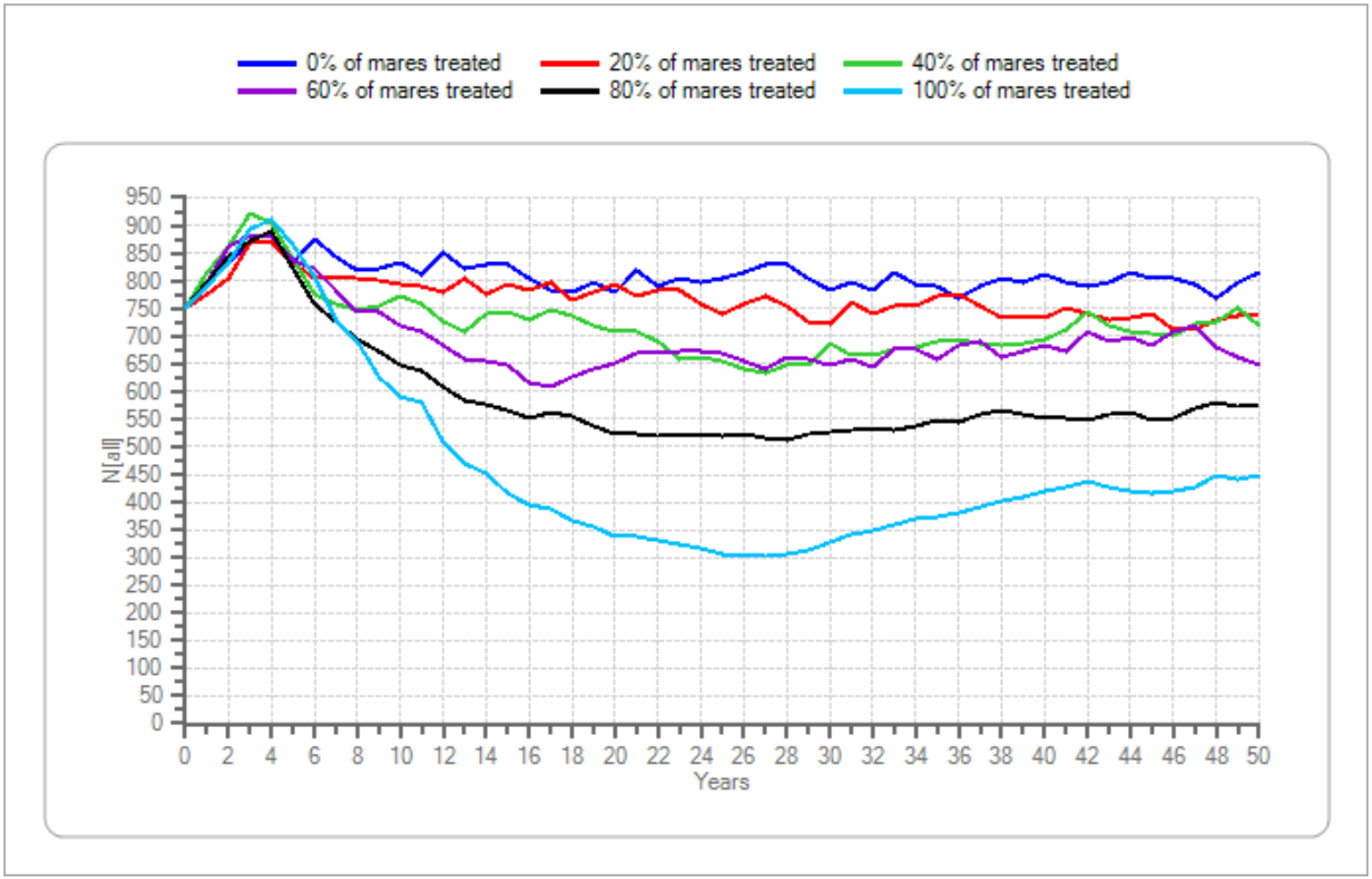
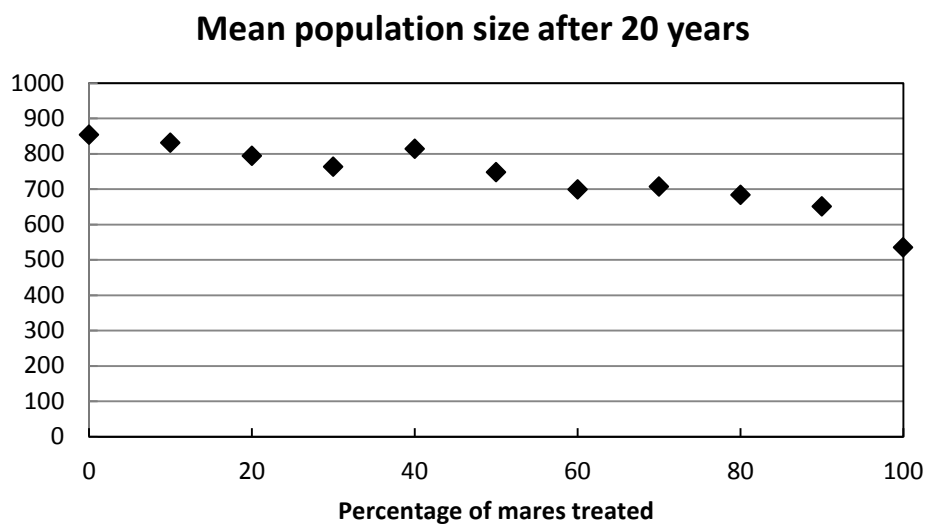
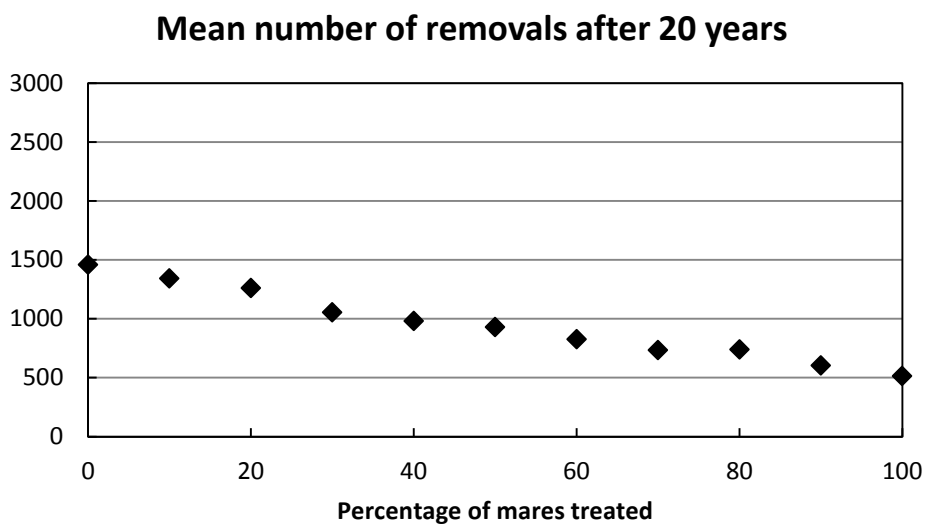
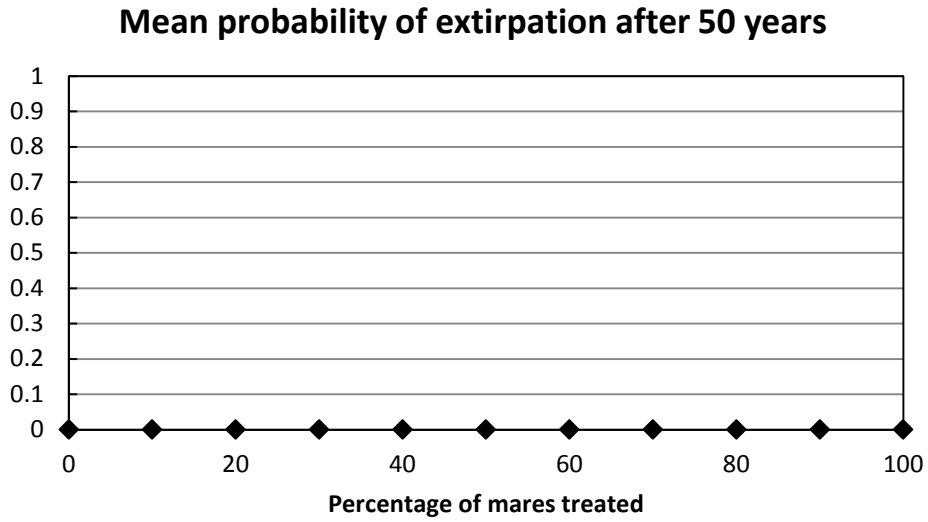


Figure 17. Initial population size = 750, lambda = 1.10, genetic diversity = low.—Continued







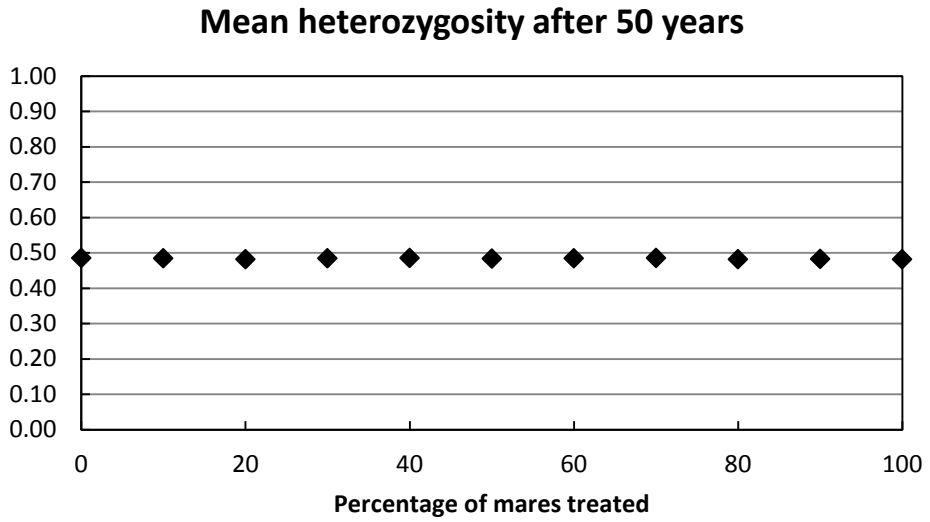


Figure 18. Initial population size = 750, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	853.53	831.08	794.36	763.4	813.88	747.49	698.91	707.4	683.74	650.95	535.33
Mean number of removals after 20 years	1458.62	1341.8	1260.79	1054.92	979.41	928.62	825.18	733.54	738.13	602.52	512.15
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.48	0.48	0.48	0.49	0.48	0.48	0.49	0.48	0.48	0.48

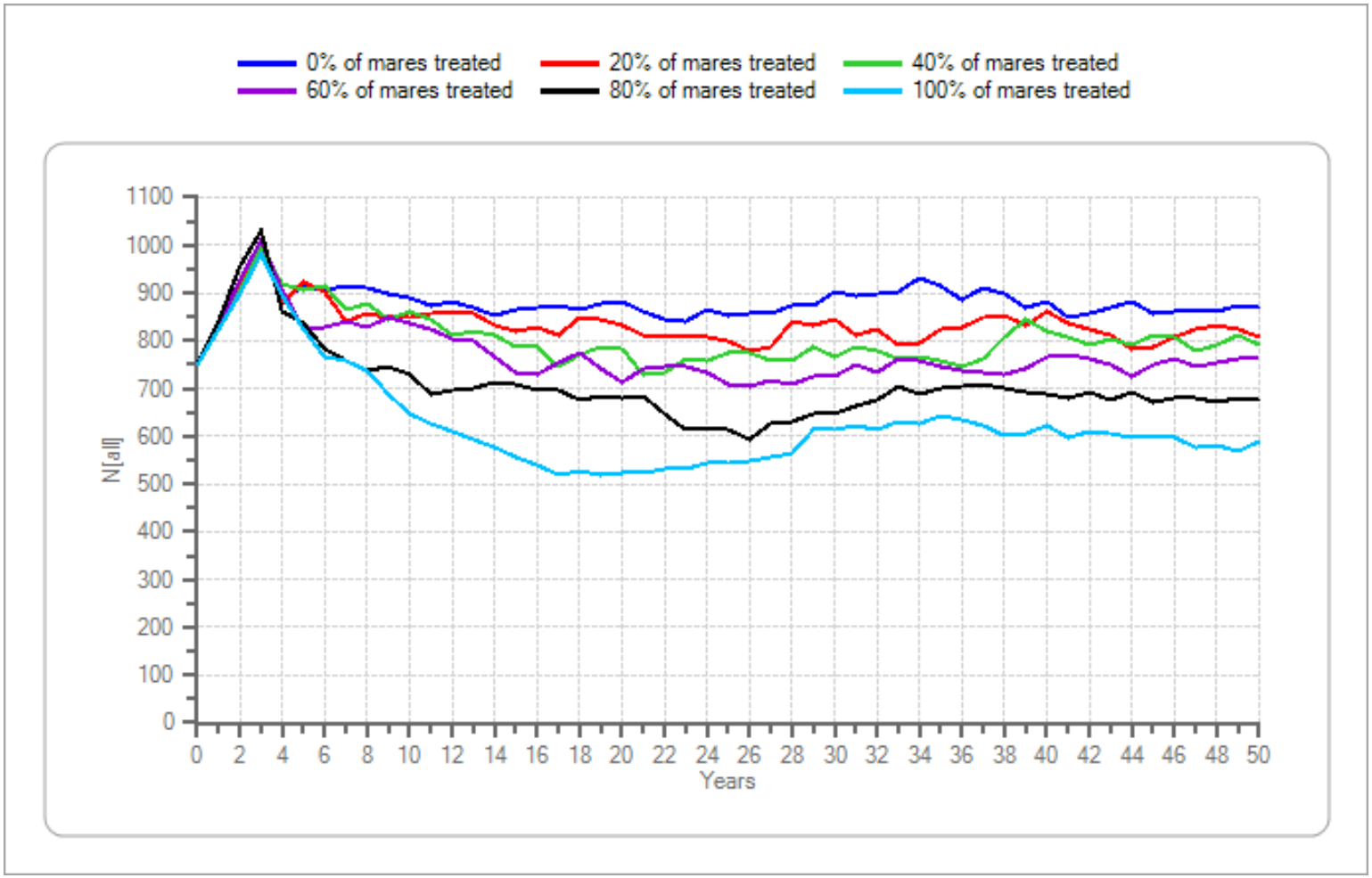
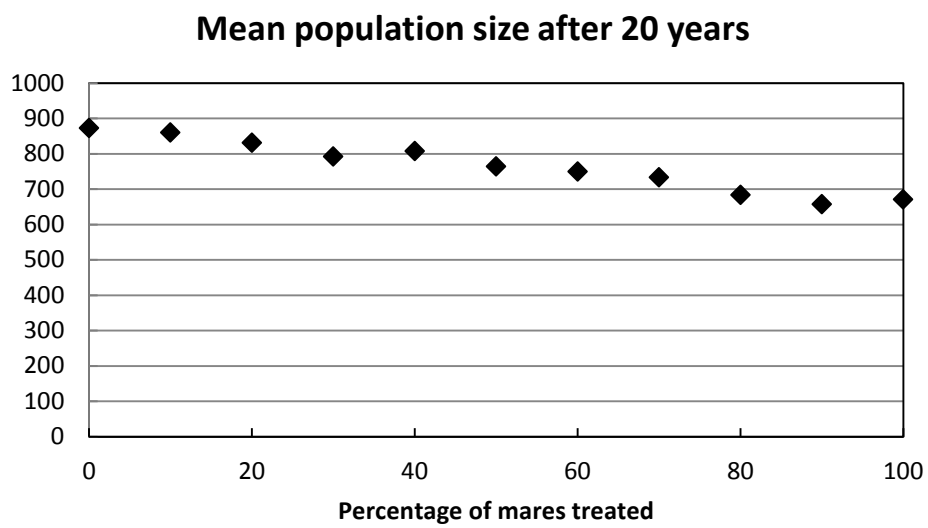
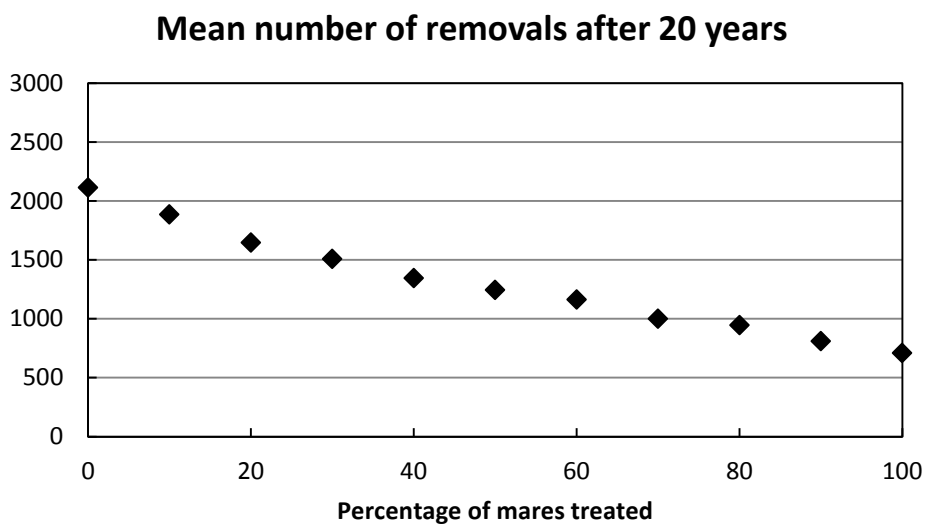
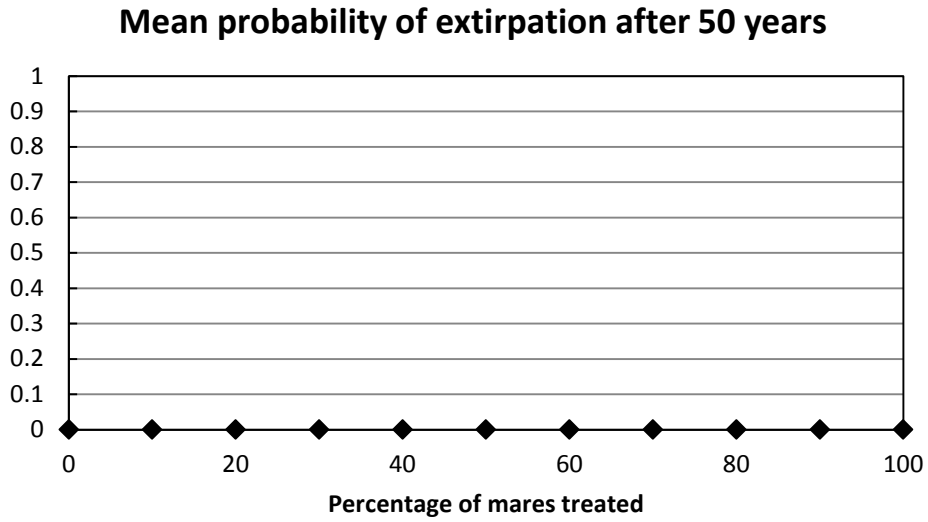


Figure 18. Initial population size = 750, lambda = 1.15, genetic diversity = low.—Continued







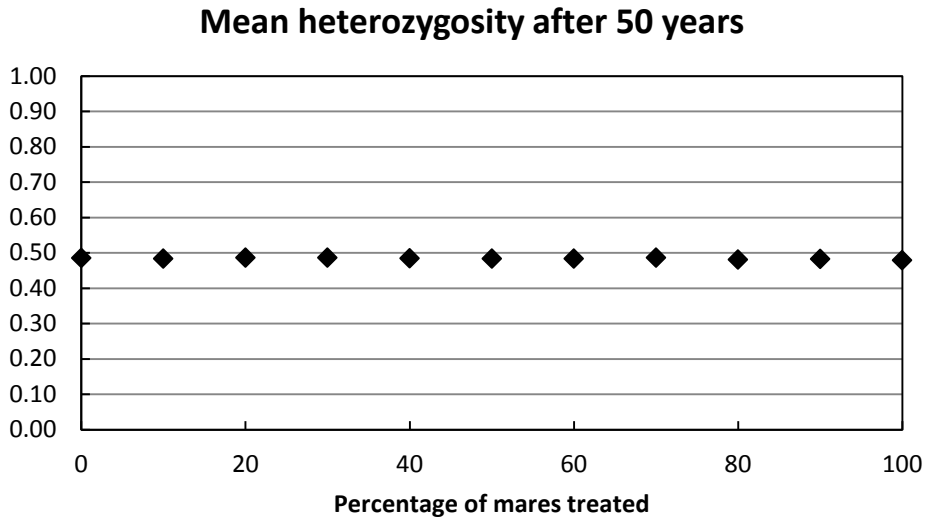


Figure 19. Initial population size = 750, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	872.42	860.25	830.74	792.41	807.75	763.95	749.48	733.6	683.58	657.07	670.54
Mean number of removals after 20 years	2112.42	1885.73	1646.6	1507.2	1345.72	1244.22	1162.02	999.18	944.68	809.02	709.93
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.48	0.49	0.49	0.48	0.48	0.48	0.49	0.48	0.48	0.48

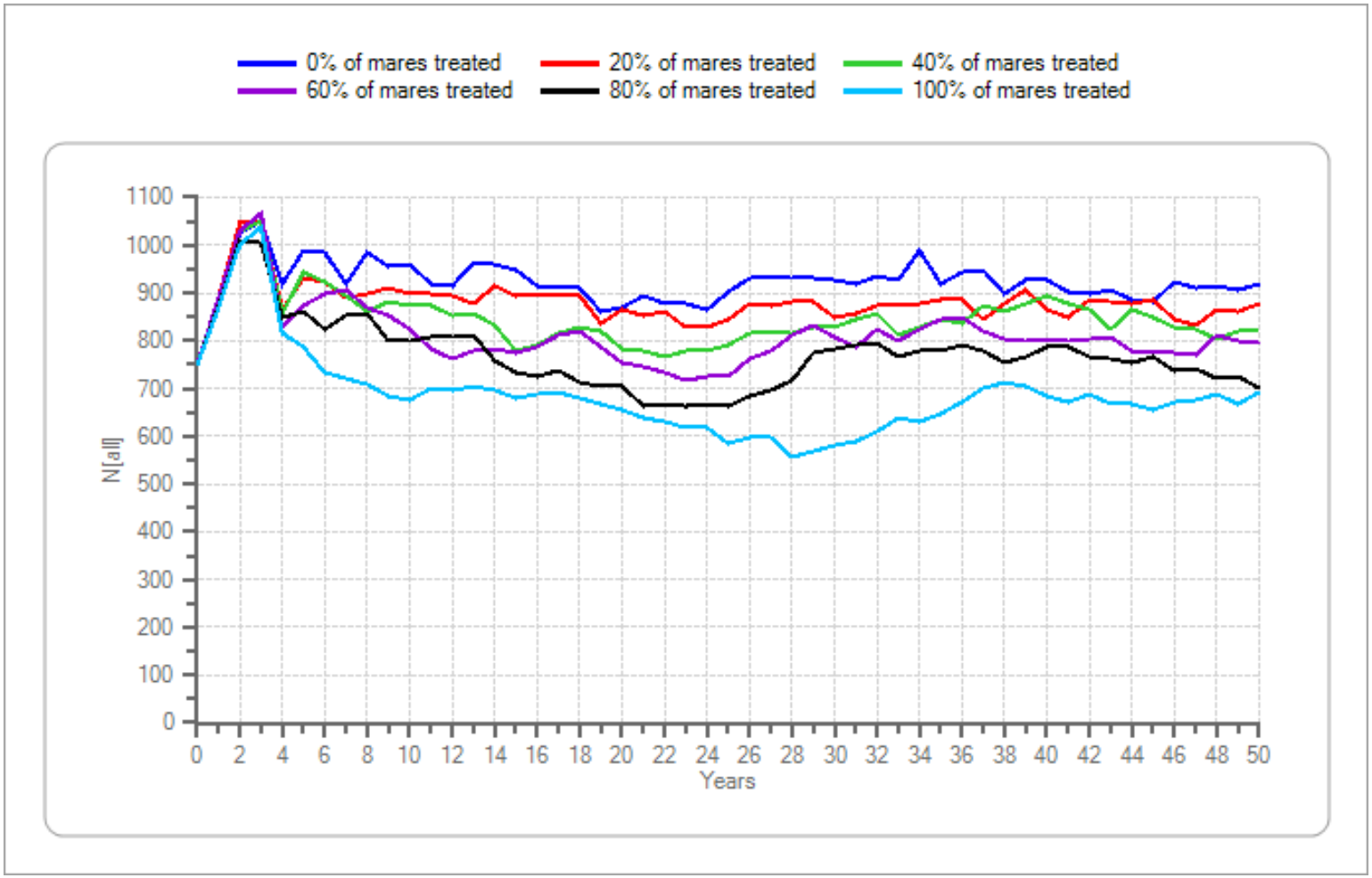
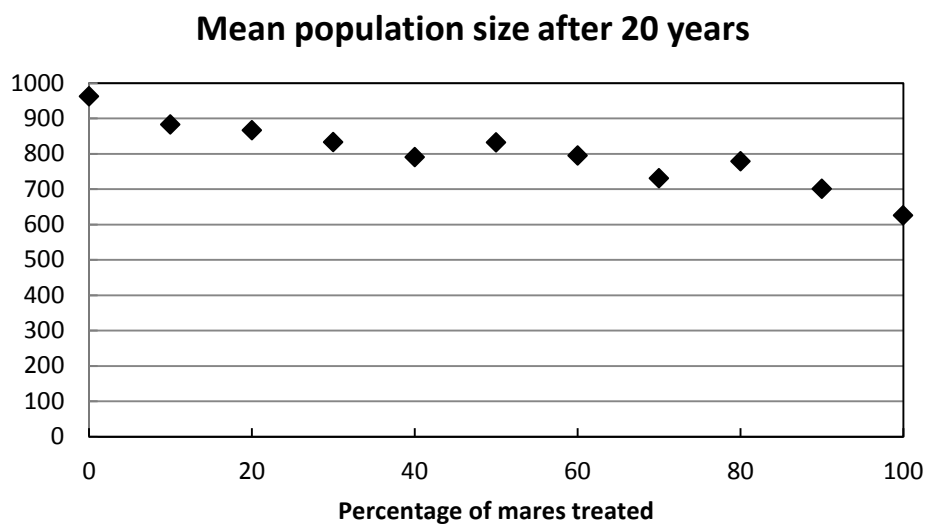
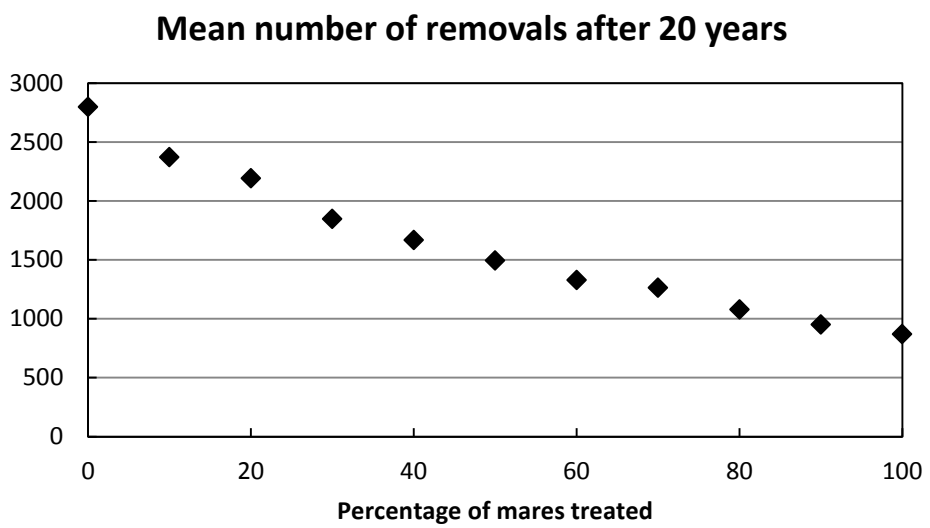
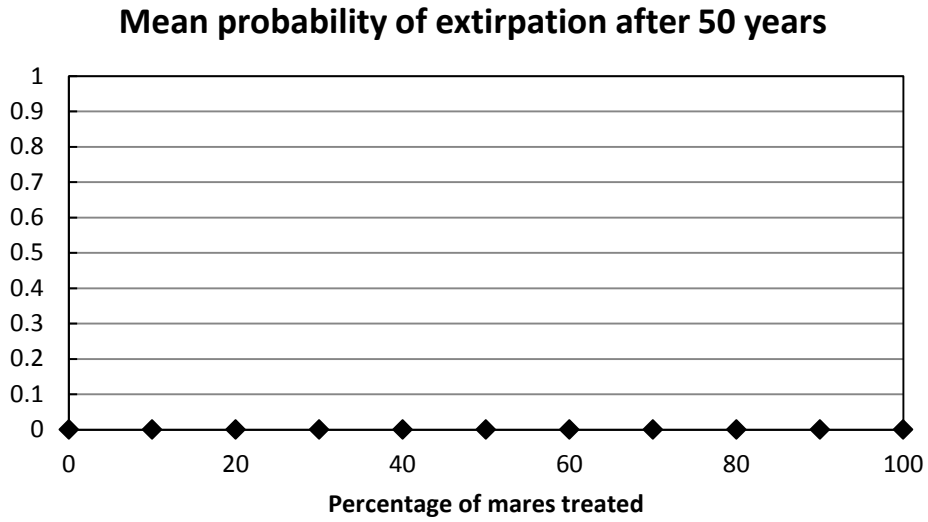


Figure 19. Initial population size = 750, lambda = 1.20, genetic diversity = low.—Continued







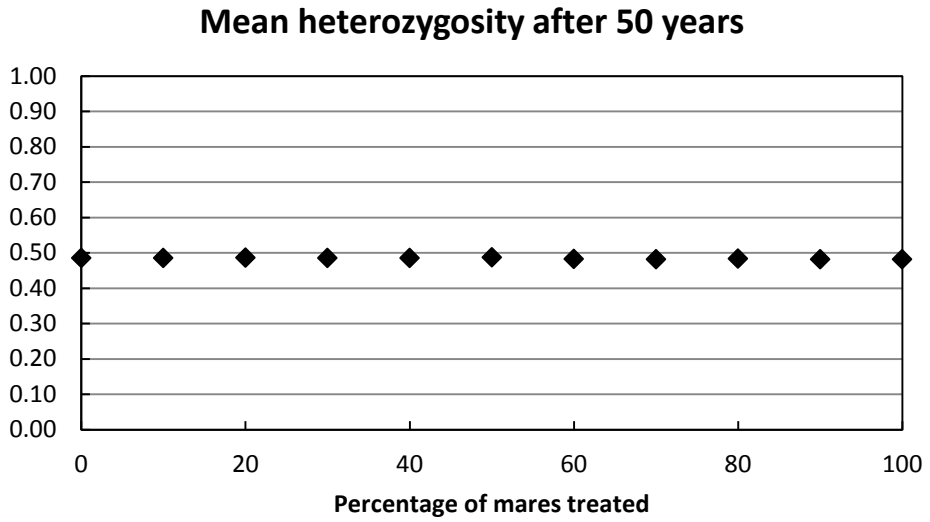


Figure 20. Initial population size = 750, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	962.21	882.4	866.81	832.94	790.31	832.37	794.81	730.52	778.33	701.12	625.35
Mean number of removals after 20 years	2796.8	2371.7	2191.6	1846.0	1667.5	1492.6	1328.4	1264.1	1078.9	951.3	870.4
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.48	0.48	0.48	0.48

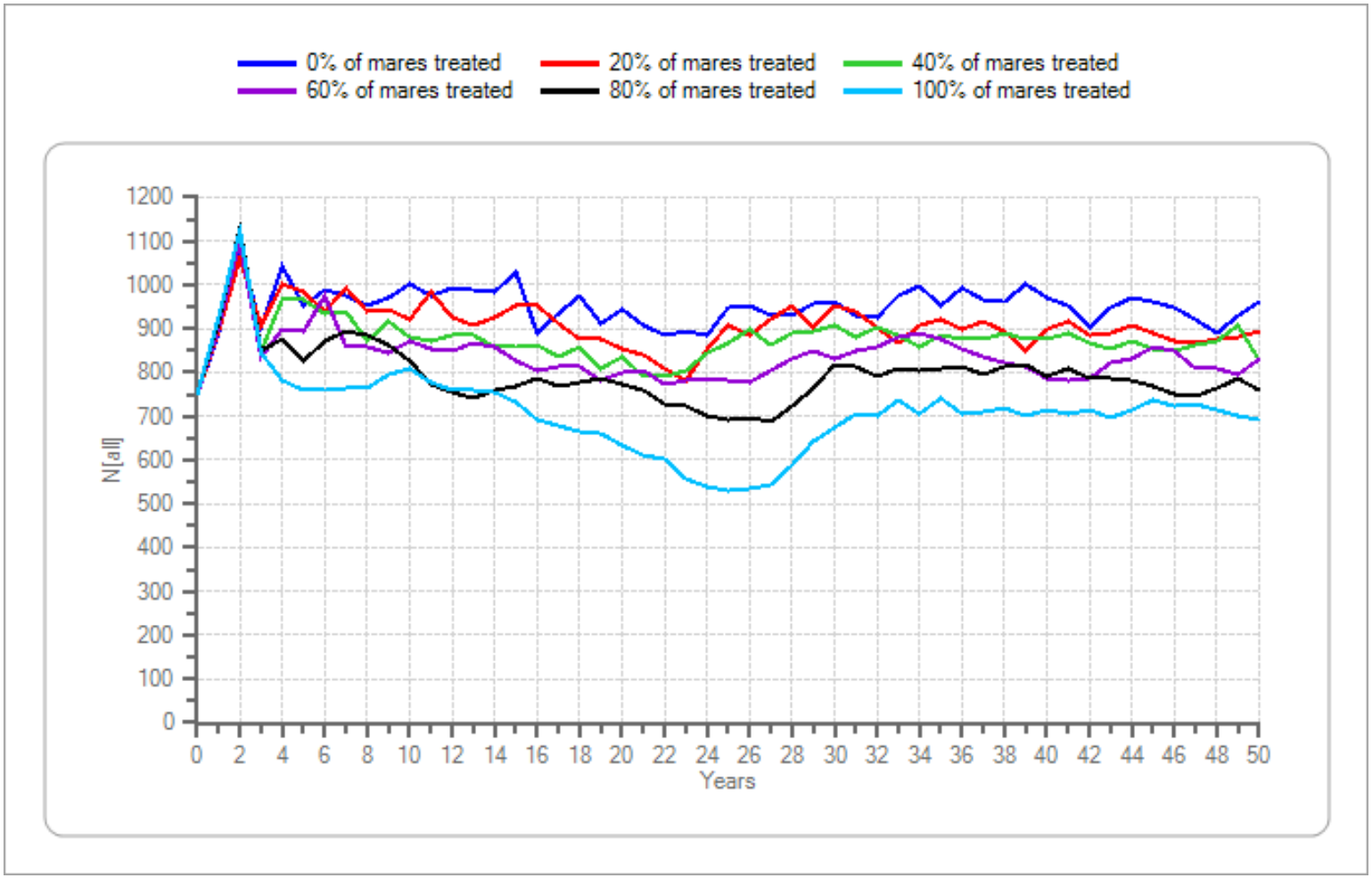
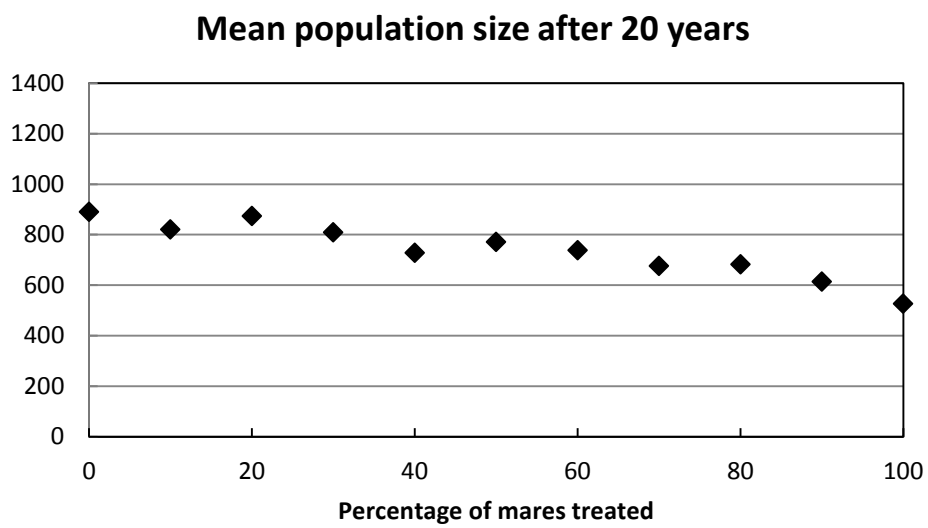
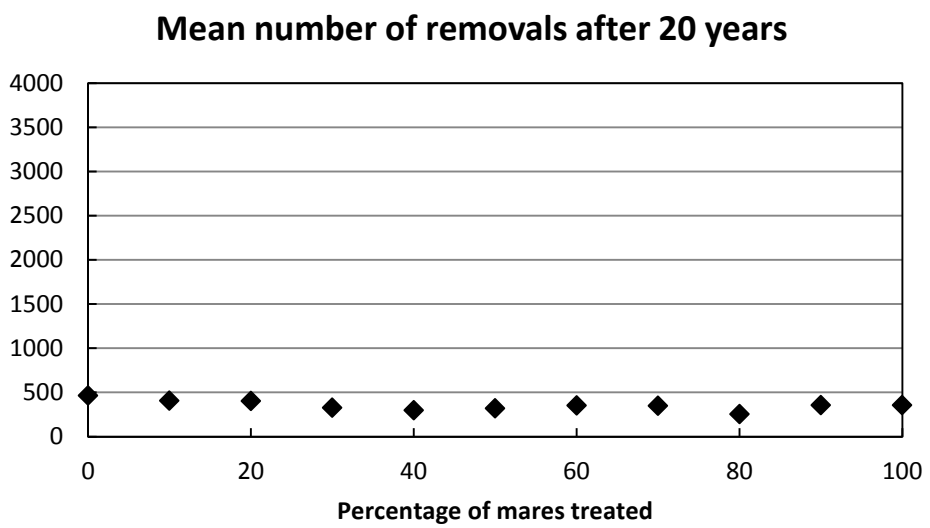
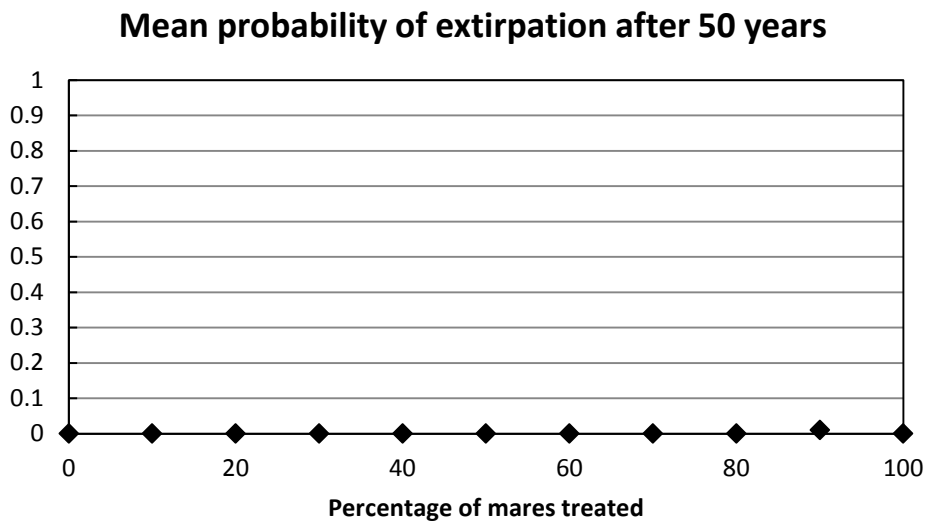


Figure 20. Initial population size = 750, lambda = 1.25, genetic diversity = low.—Continued







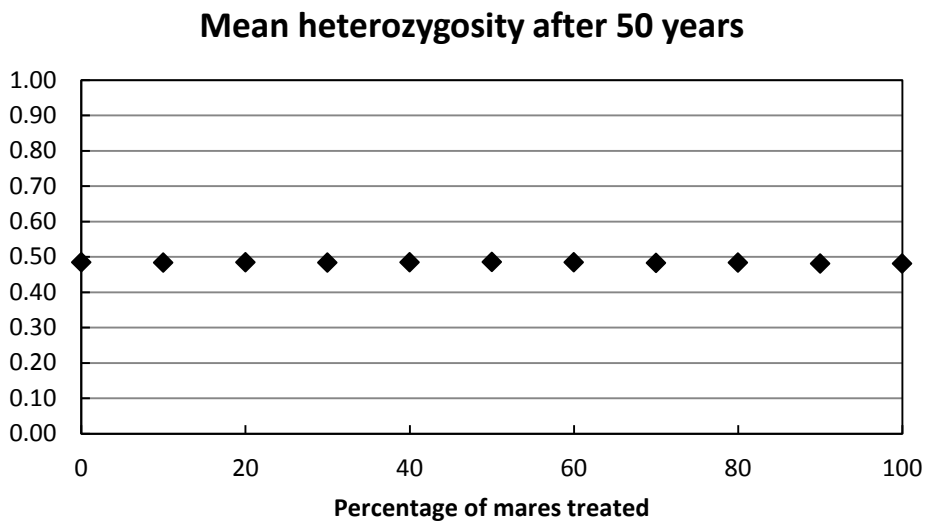


Figure 21. Initial population size = 1000, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	889.31	819.79	873.34	808.05	727.98	770.69	737.69	675.8	681.41	613.88	525.62
Mean number of removals after 20 years	462.82	404.15	400.39	327.35	297.35	318.94	349.73	348.62	252.02	354.25	356.61
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0.01	0
Mean heterozygosity after 50 years	0.48	0.48	0.48	0.48	0.48	0.49	0.48	0.48	0.48	0.48	0.48

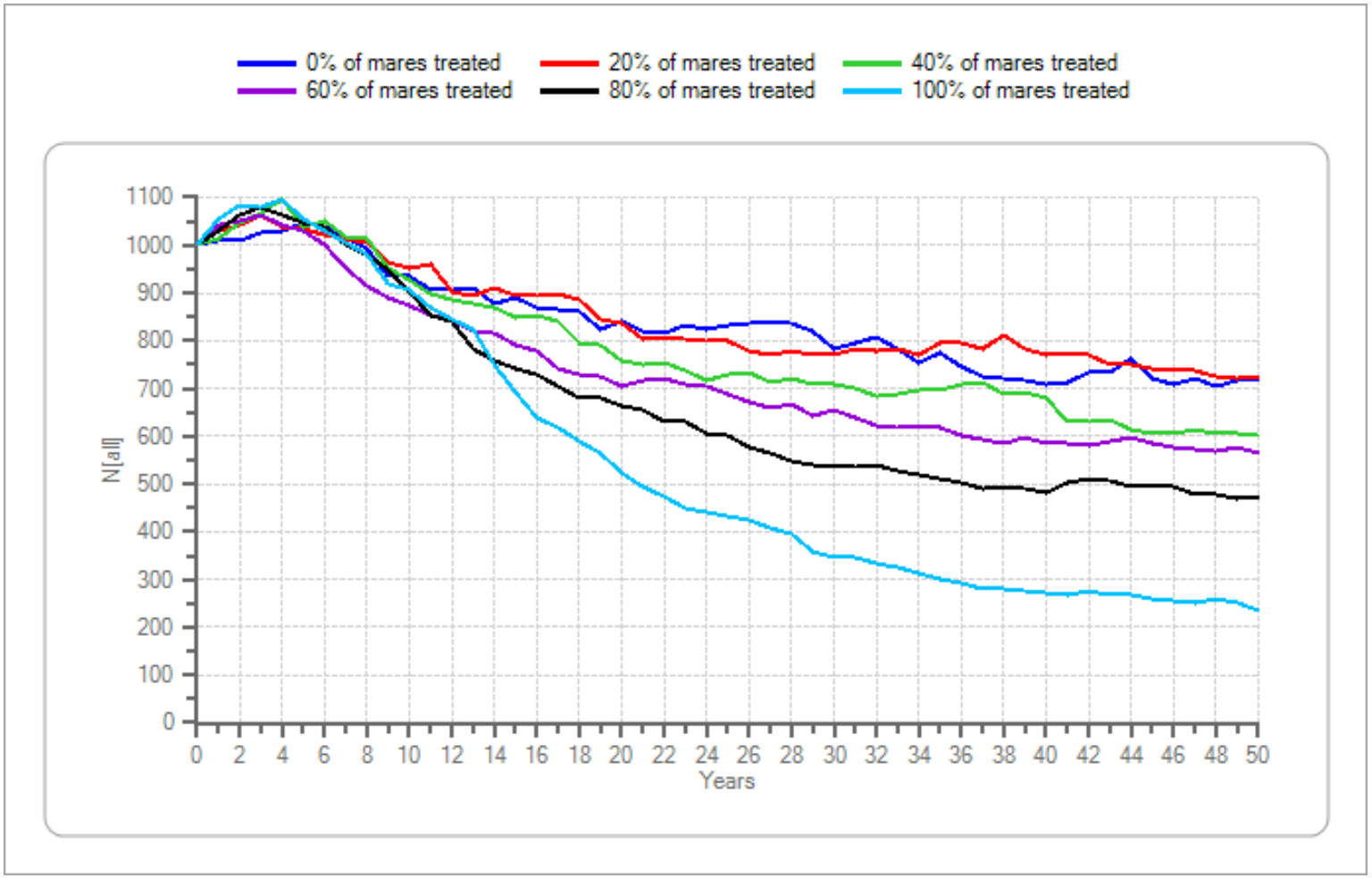
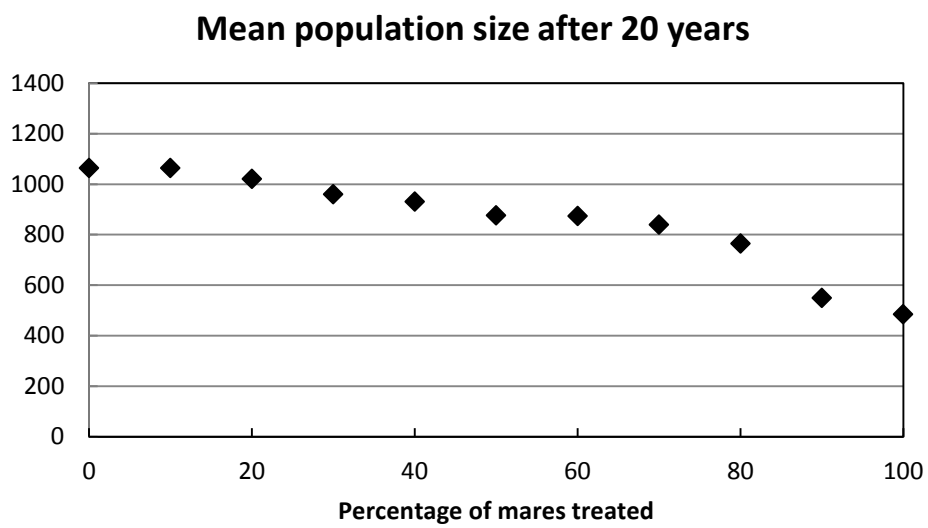
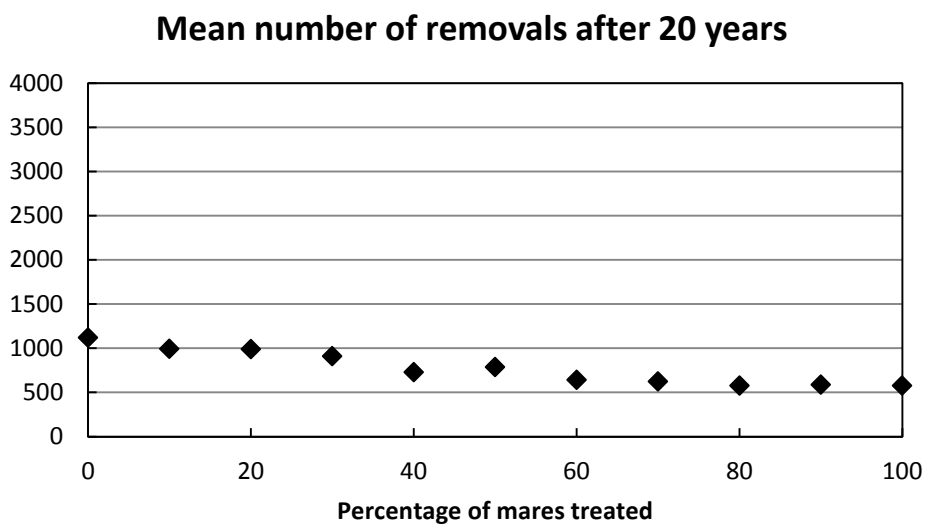
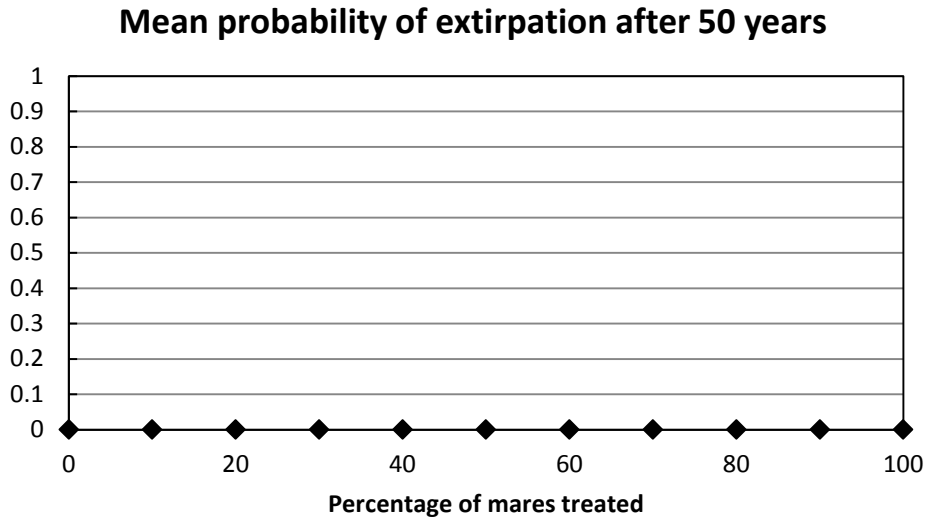


Figure 21. Initial population size = 1000, lambda = 1.05, genetic diversity = low.—Continued







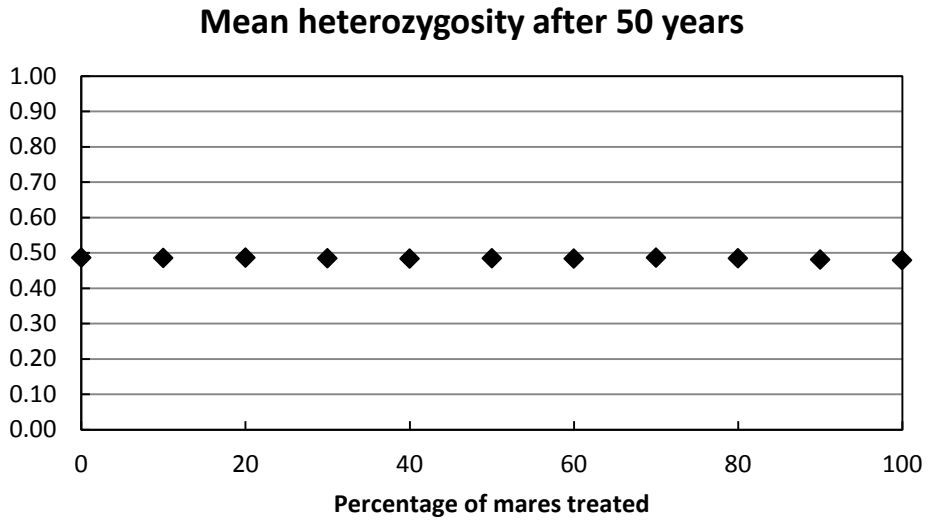


Figure 22. Initial population size = 1000, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1063.82	1064.02	1019.82	959.43	930.43	875.89	872.81	839.08	764.81	549.04	483.72
Mean number of removals after 20 years	1118.7	991.5	988.6	908.4	728.6	785.9	642.2	621.2	577.2	585.4	577.7
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.48	0.48	0.48	0.48	0.49	0.48	0.48	0.48

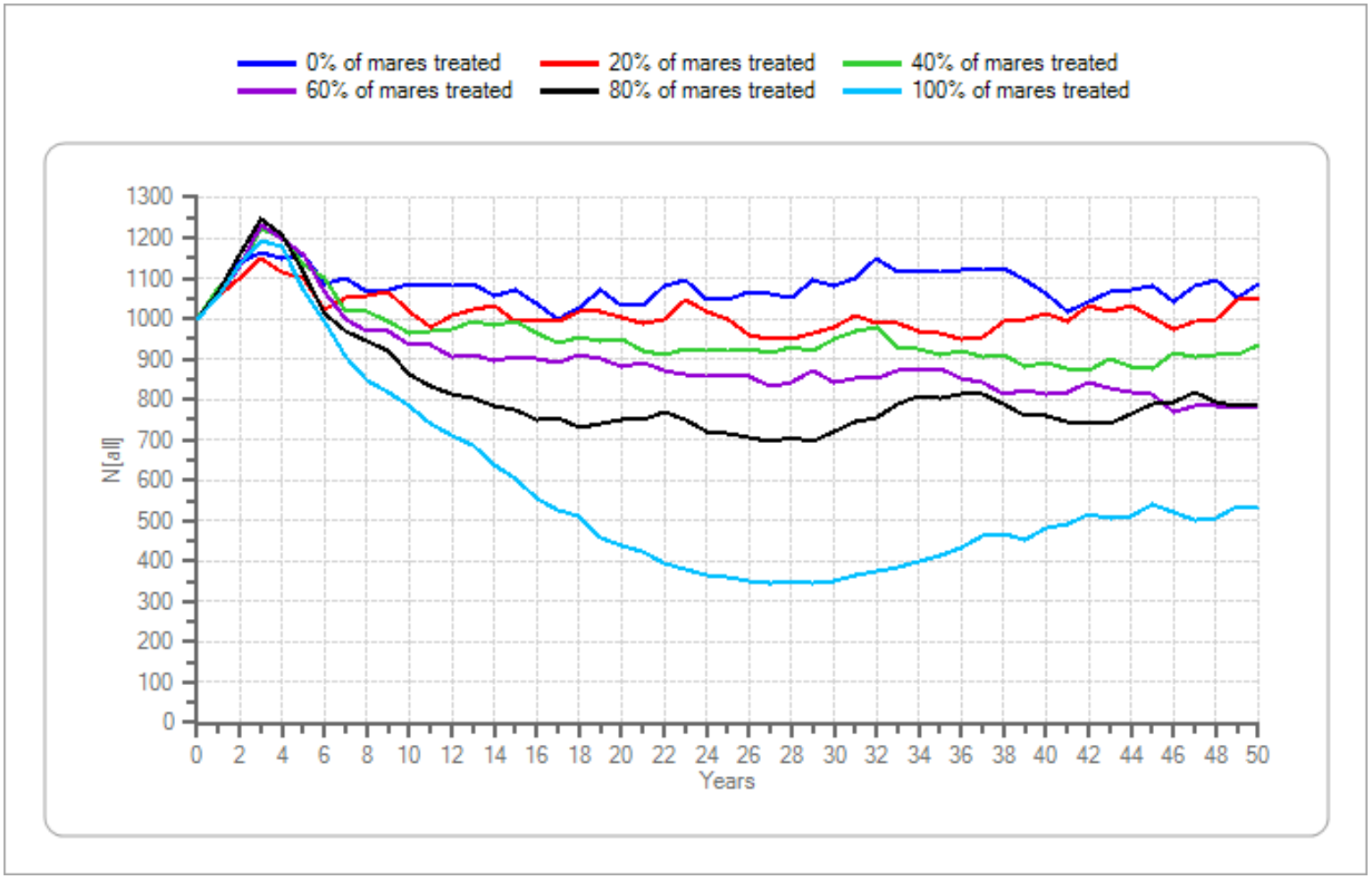
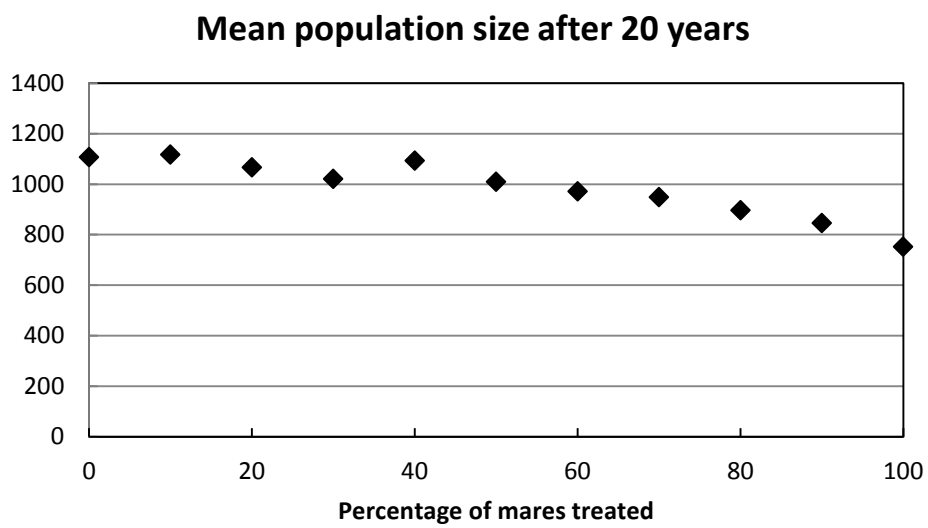
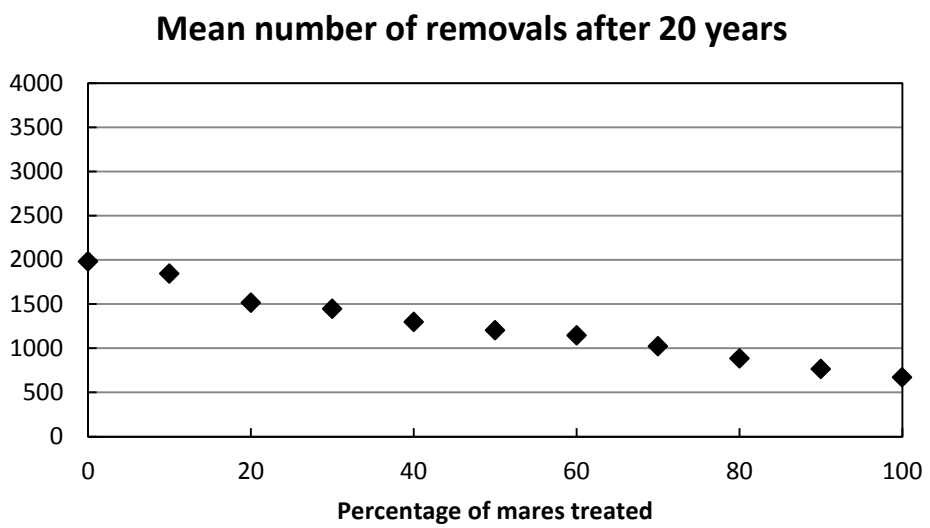
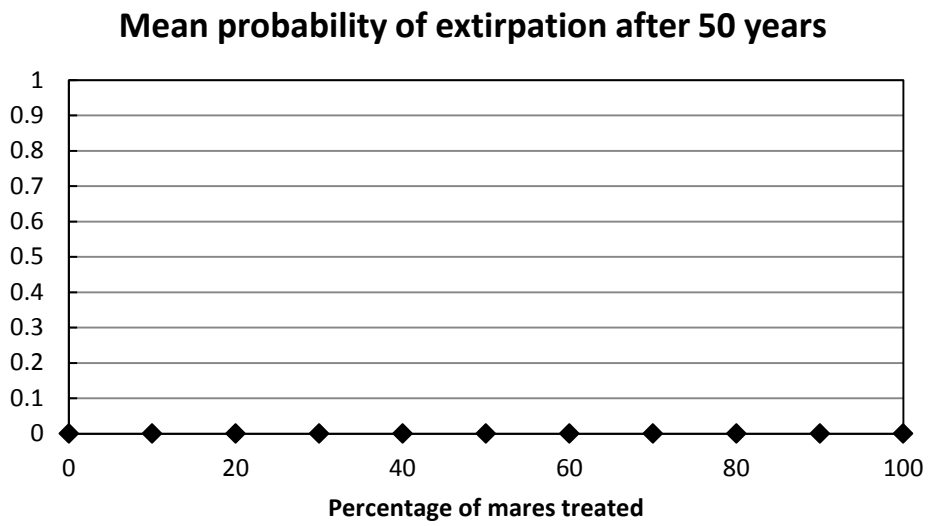


Figure 22. Initial population size = 1000, lambda = 1.10, genetic diversity = low.—Continued







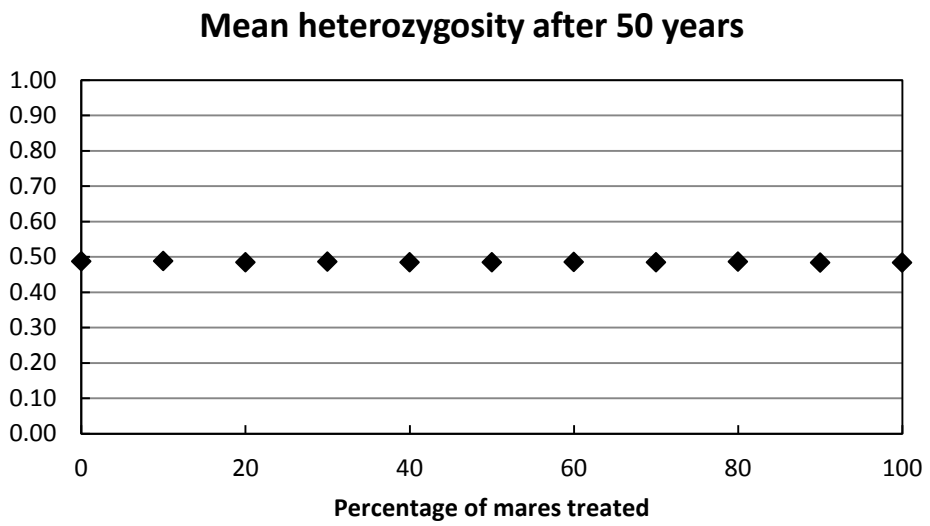


Figure 23. Initial population size = 1000, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1106.82	1117.06	1065.72	1020.7	1093.2	1008.49	970.31	948.69	895.72	845.6	752.22
Mean number of removals after 20 years	1979.8	1841.8	1512.9	1445.2	1296.8	1201.1	1143.0	1020.5	883.9	764.1	669.3
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.48	0.49	0.48	0.48	0.49	0.48	0.49	0.48	0.48

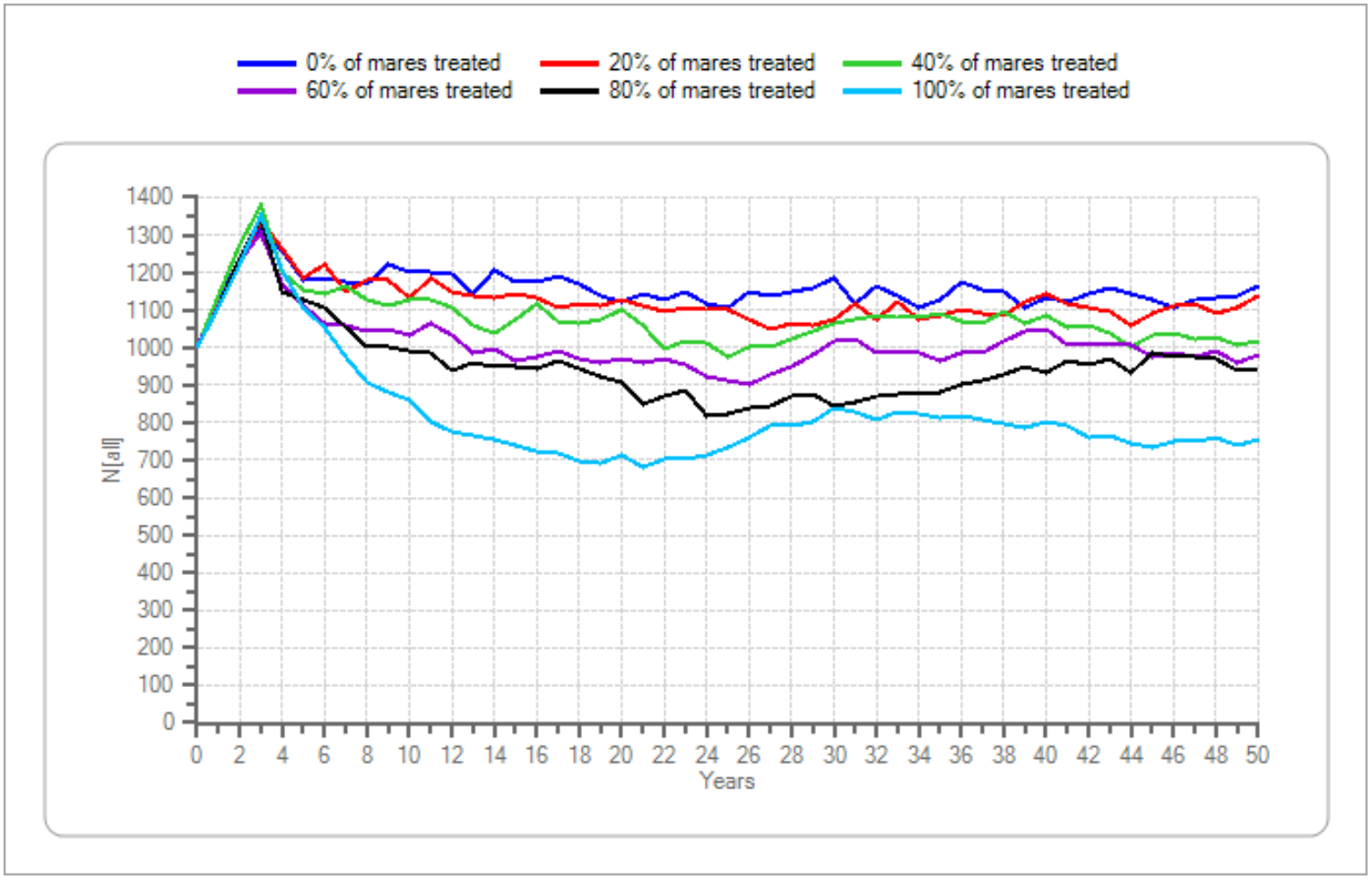
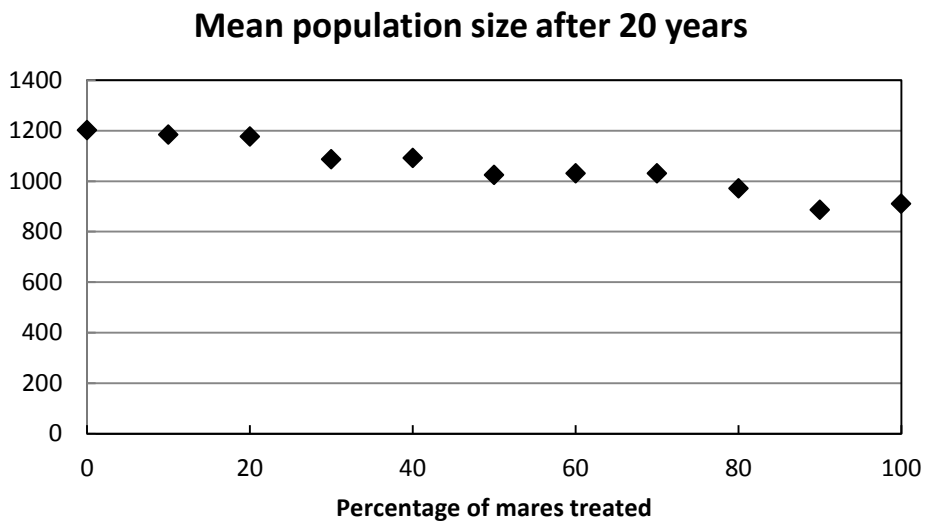
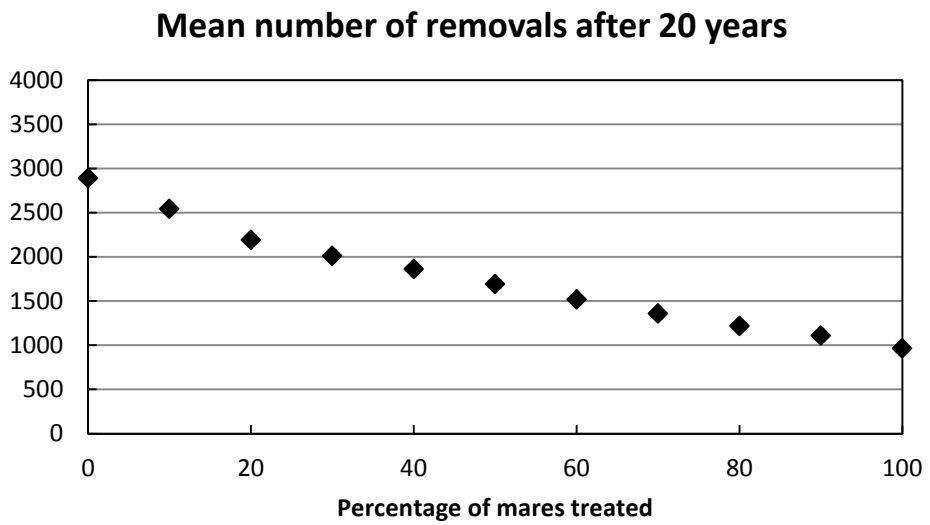
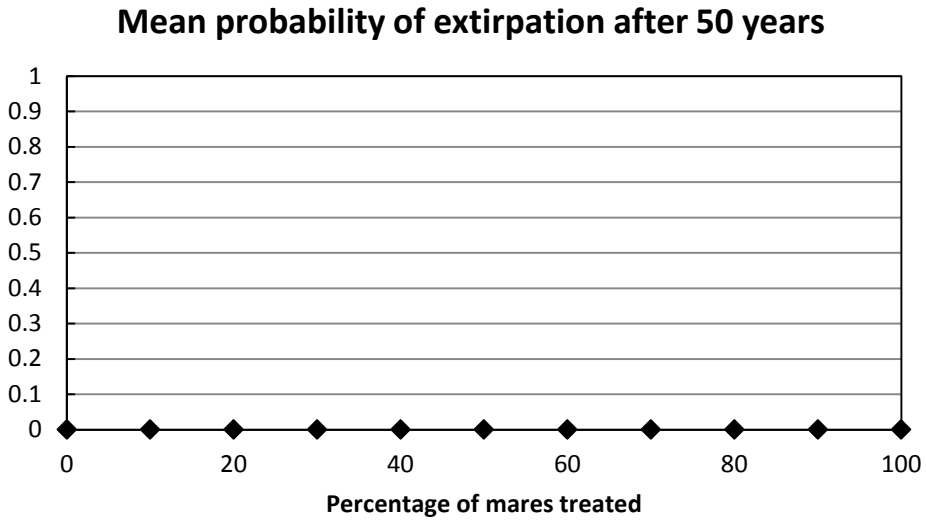


Figure 23. Initial population size = 1000, lambda = 1.15, genetic diversity = low.—Continued







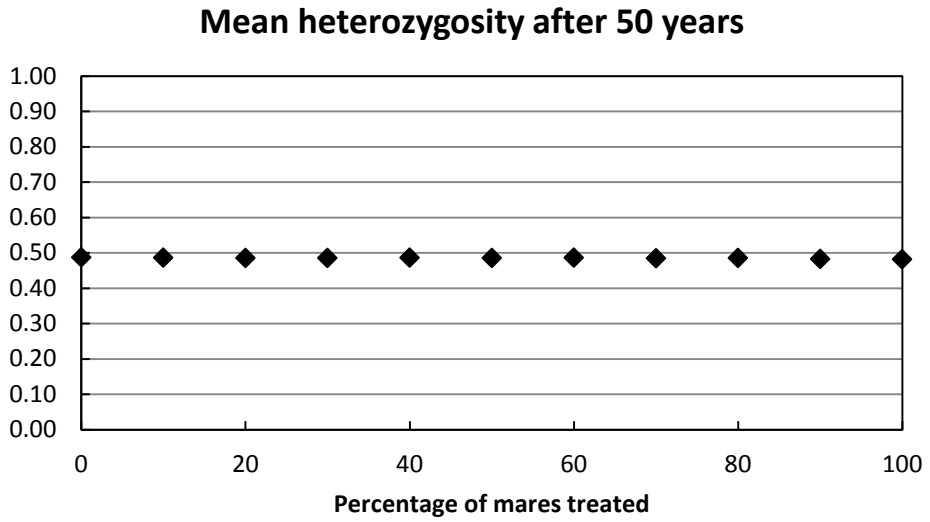


Figure 24. Initial population size = 1000, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1201.08	1184.02	1176.54	1085.77	1090.8	1024.31	1030.98	1029.85	970.74	885.67	910.27
Mean number of removals after 20 years	2890.6	2543.6	2191.6	2009.4	1861.7	1689.8	1515.9	1357.8	1215.4	1107.5	961.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.48	0.48	0.48

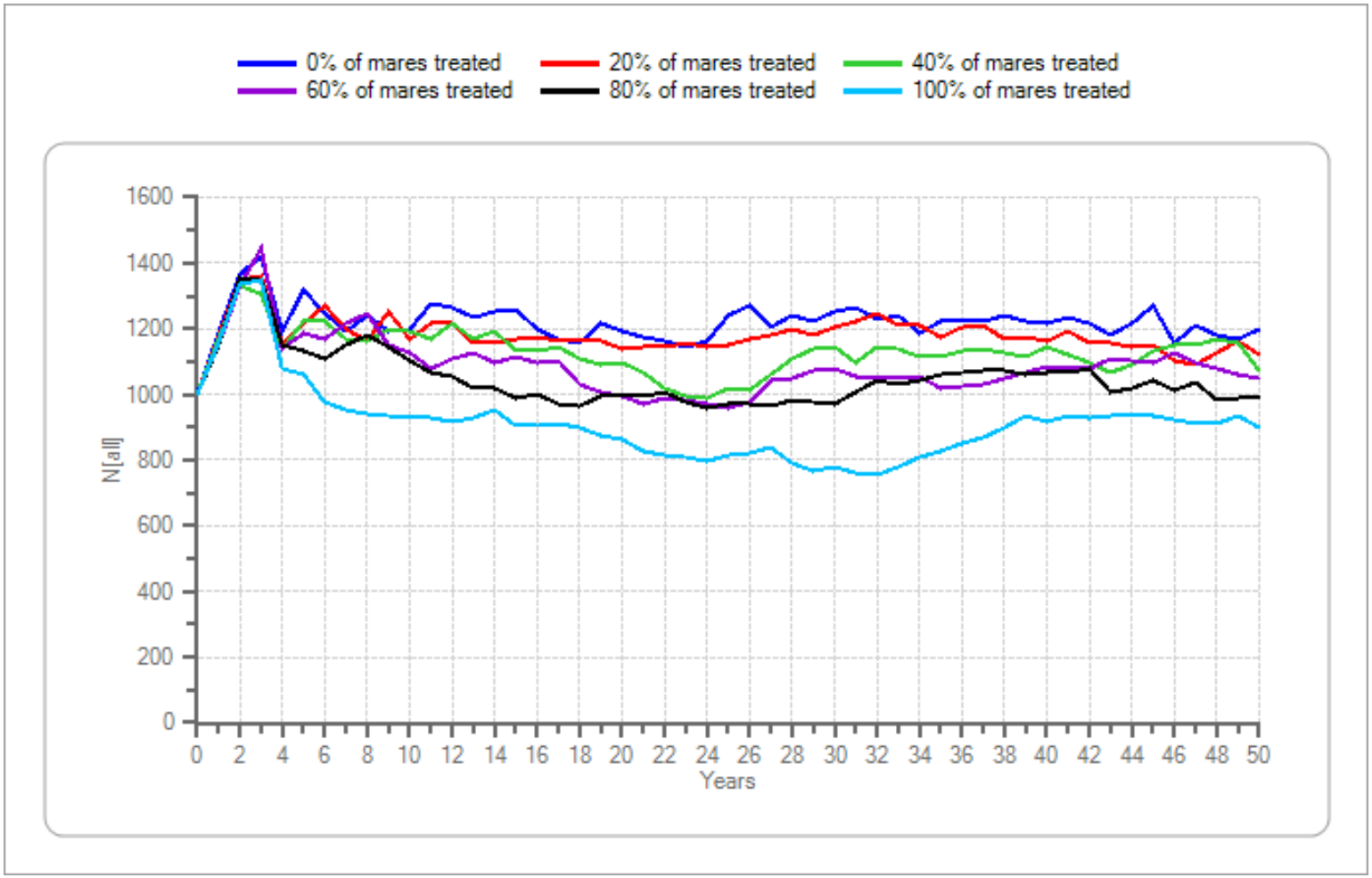


Figure 24. Initial population size = 1000, lambda = 1.20, genetic diversity = low.—Continued

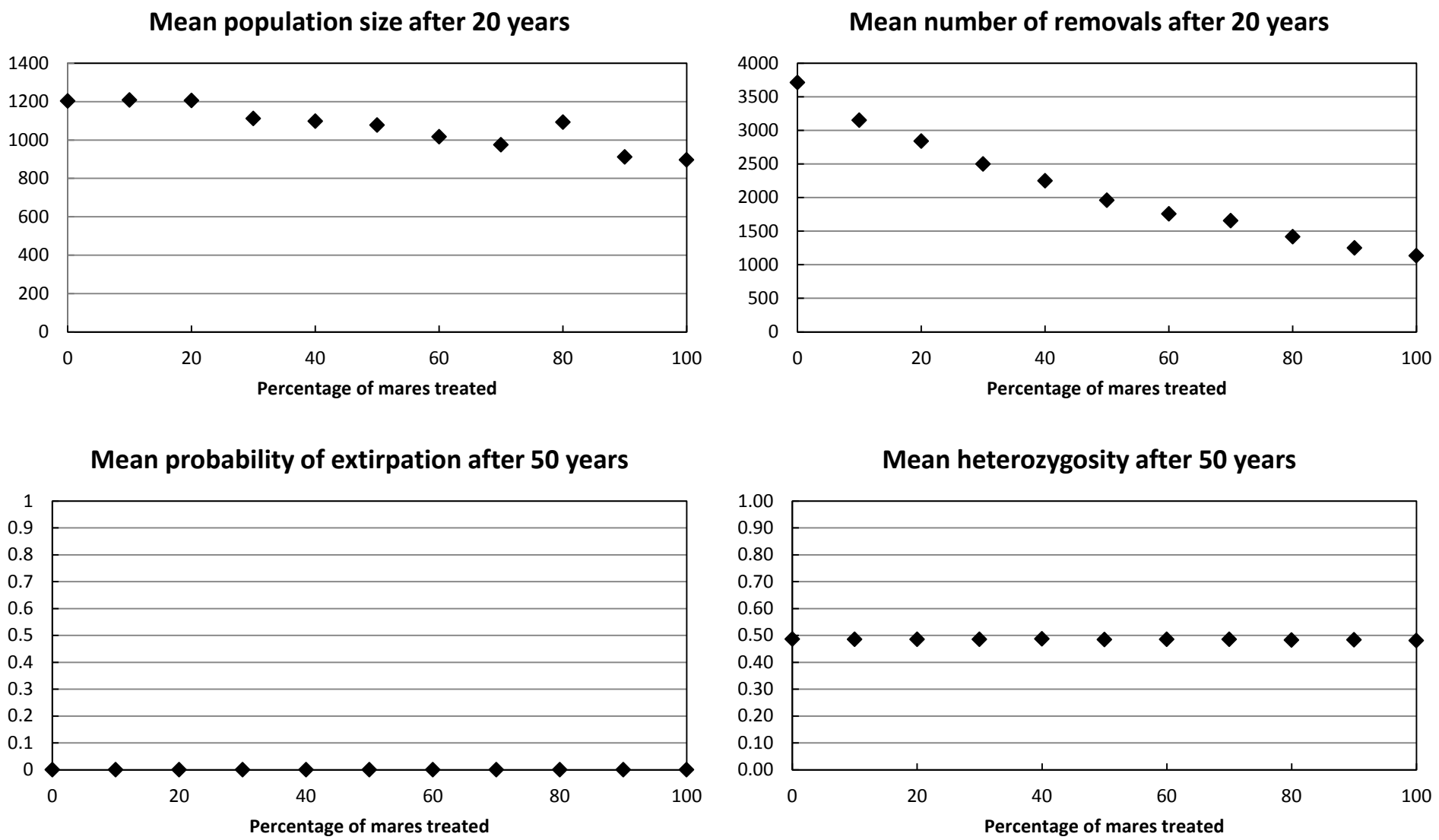


Figure 25. Initial population size = 1000, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1202.9	1207.36	1205.51	1111.97	1097.25	1076.74	1016.51	975.02	1092.2	911.9	896.56
Mean number of removals after 20 years	3713.4	3151.0	2838.8	2498.7	2250.0	1960.2	1756.7	1654.6	1417.1	1247.7	1131.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.49	0.48	0.48	0.48

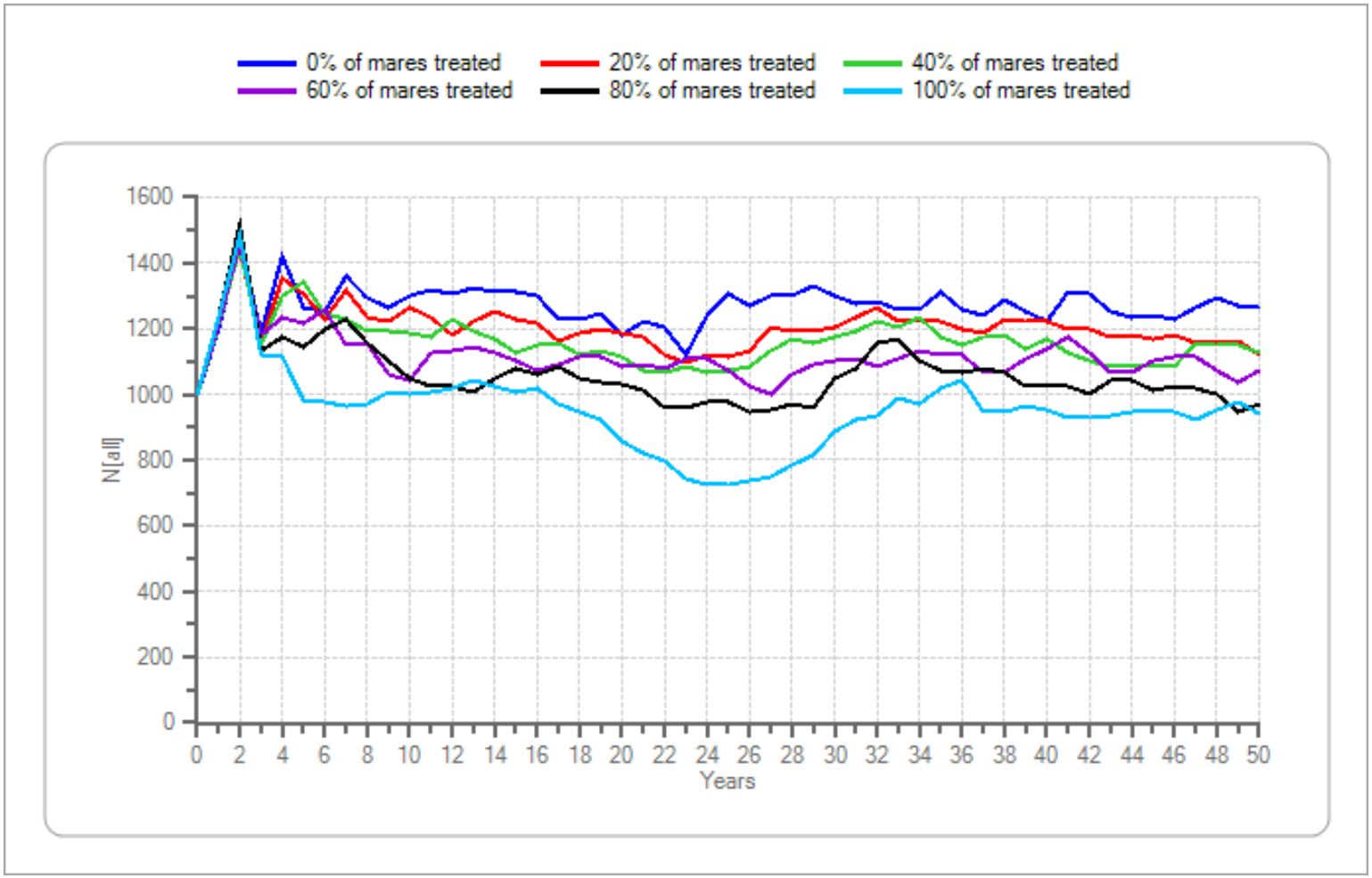
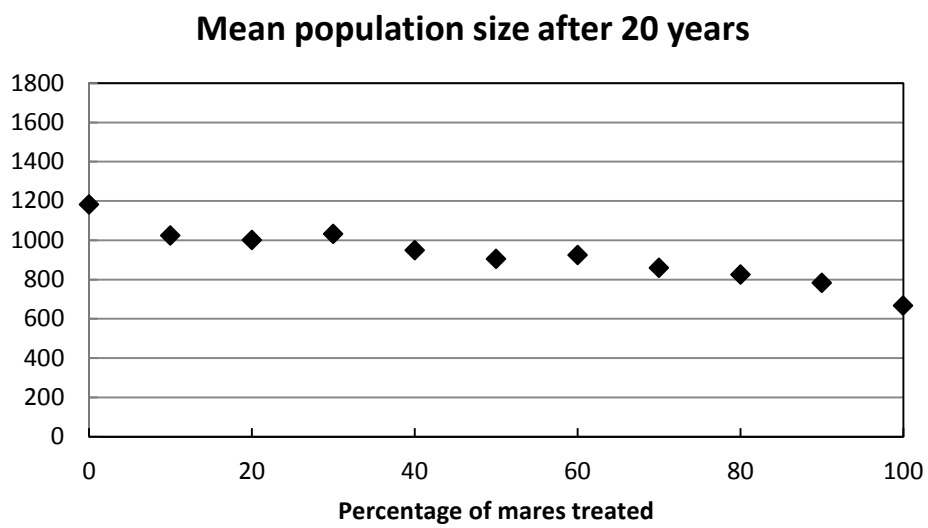
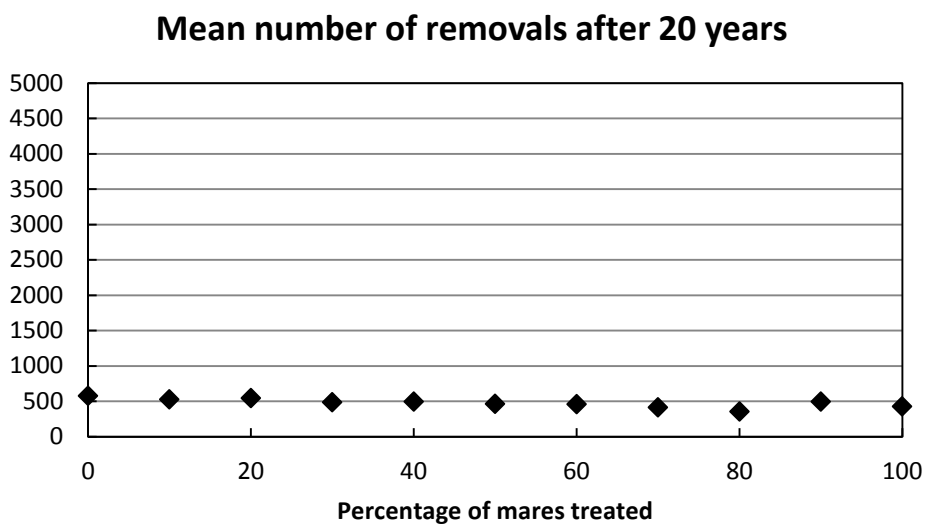
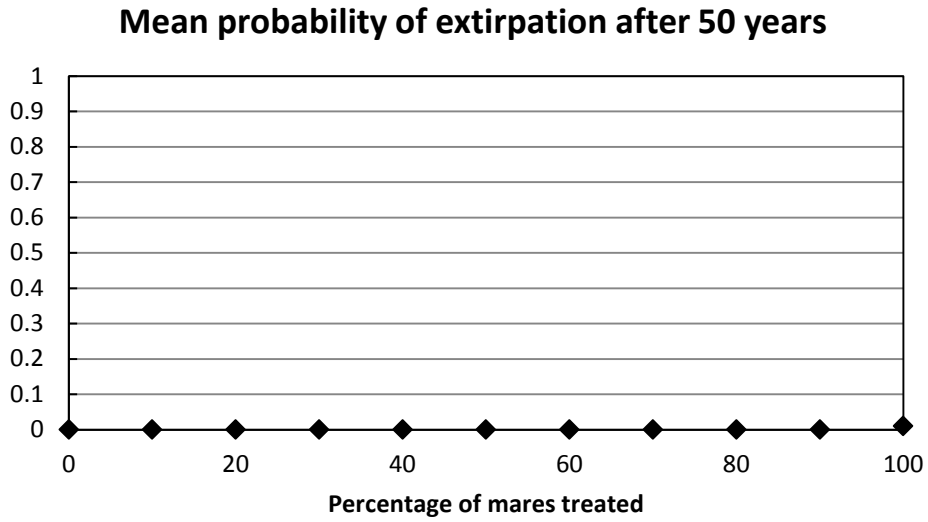


Figure 25. Initial population size = 1000, lambda = 1.25, genetic diversity = low.—Continued







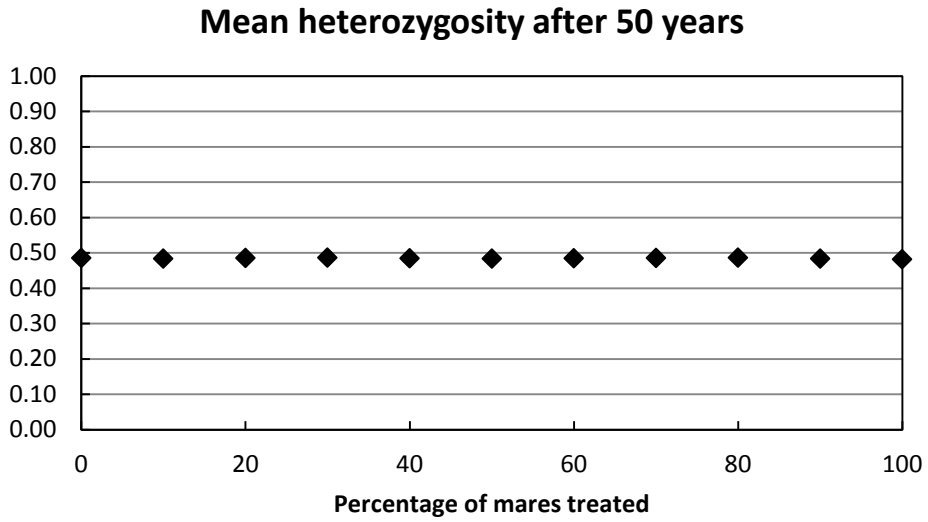


Figure 26. Initial population size = 1250, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1181.85	1024.21	1000.58	1032.15	948.63	904.73	923.82	859.2	824.58	782.46	666.31
Mean number of removals after 20 years	574.25	523.43	544.18	483.72	493.96	463.54	459.15	412.01	352.83	491.73	425.11
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.01
Mean heterozygosity after 50 years	0.49	0.48	0.49	0.49	0.48	0.48	0.48	0.49	0.49	0.48	0.48

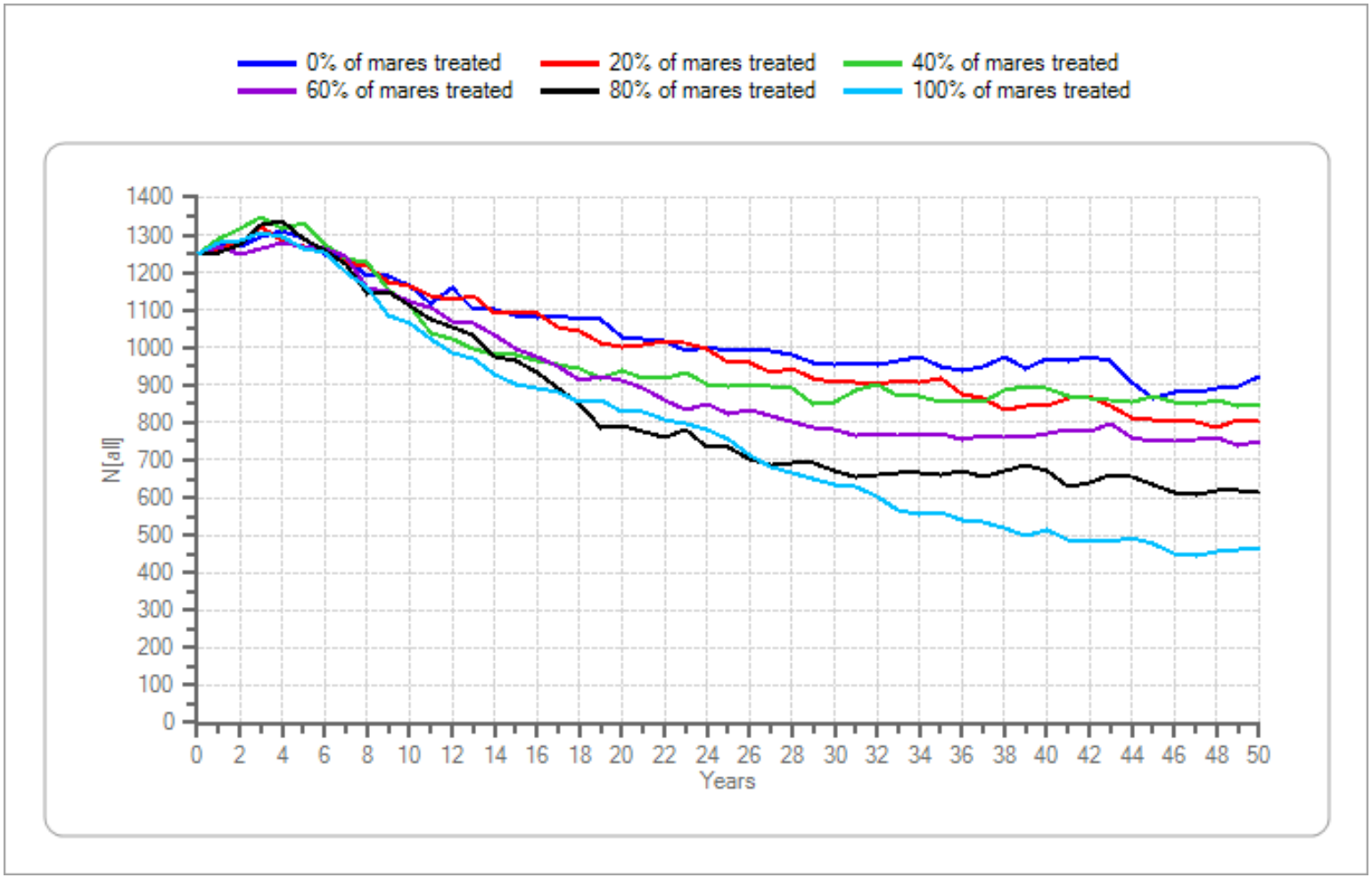
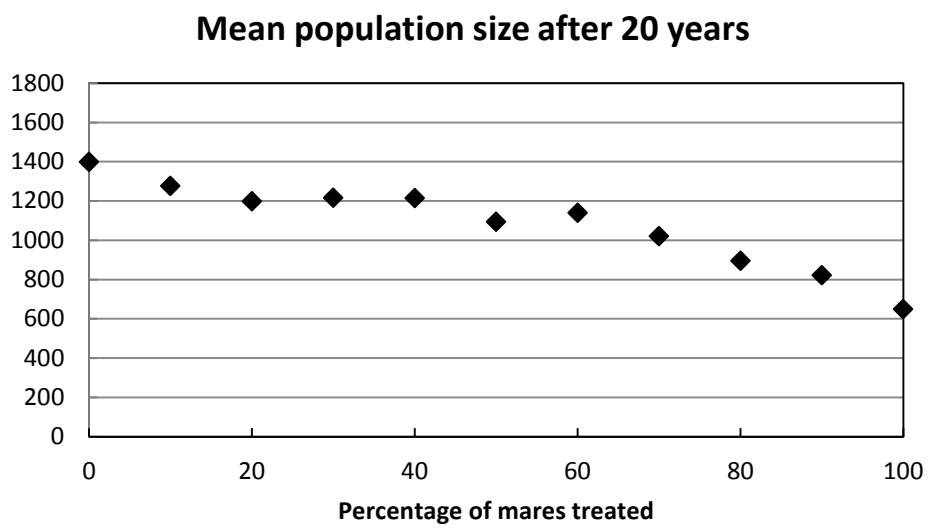
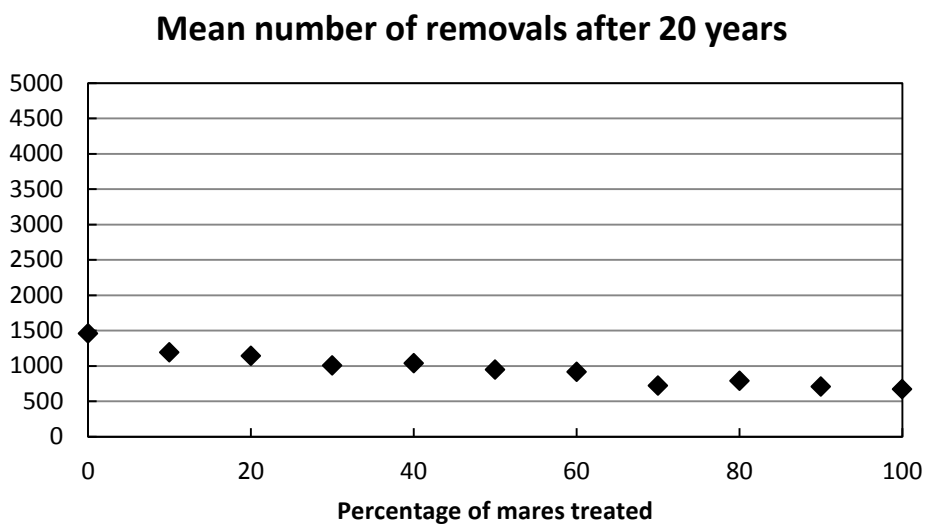
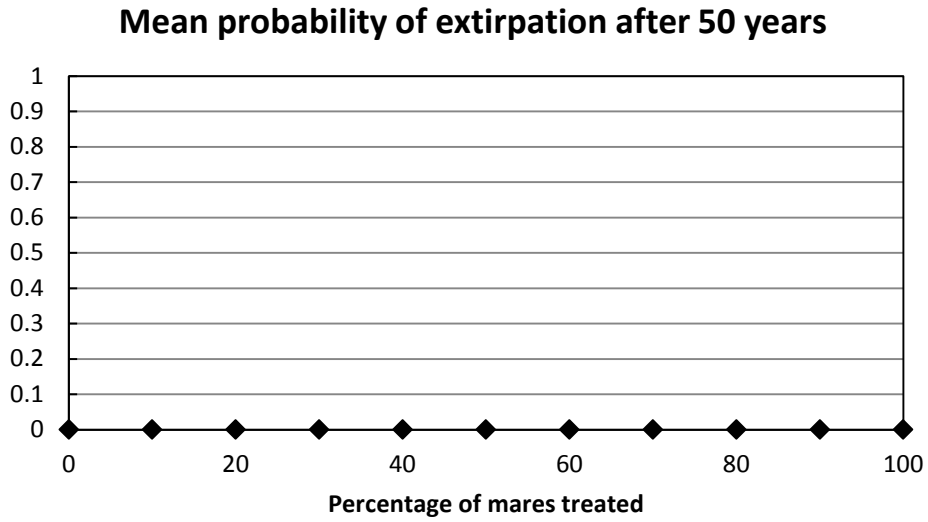


Figure 26. Initial population size = 1250, lambda = 1.05, genetic diversity = low.—Continued







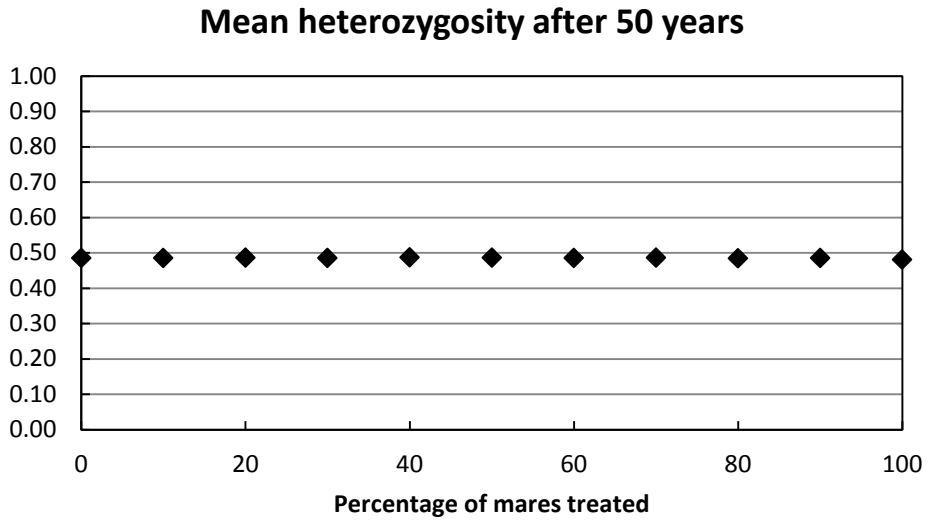


Figure 27. Initial population size = 1250, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1398.94	1275.26	1198.43	1215.27	1214.22	1094.1	1138.43	1019.54	893.99	821.65	649.09
Mean number of removals after 20 years	1458.6	1191.7	1140.2	1004.3	1038.6	948.0	915.8	720.1	788.2	708.3	672.1
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.48	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.48

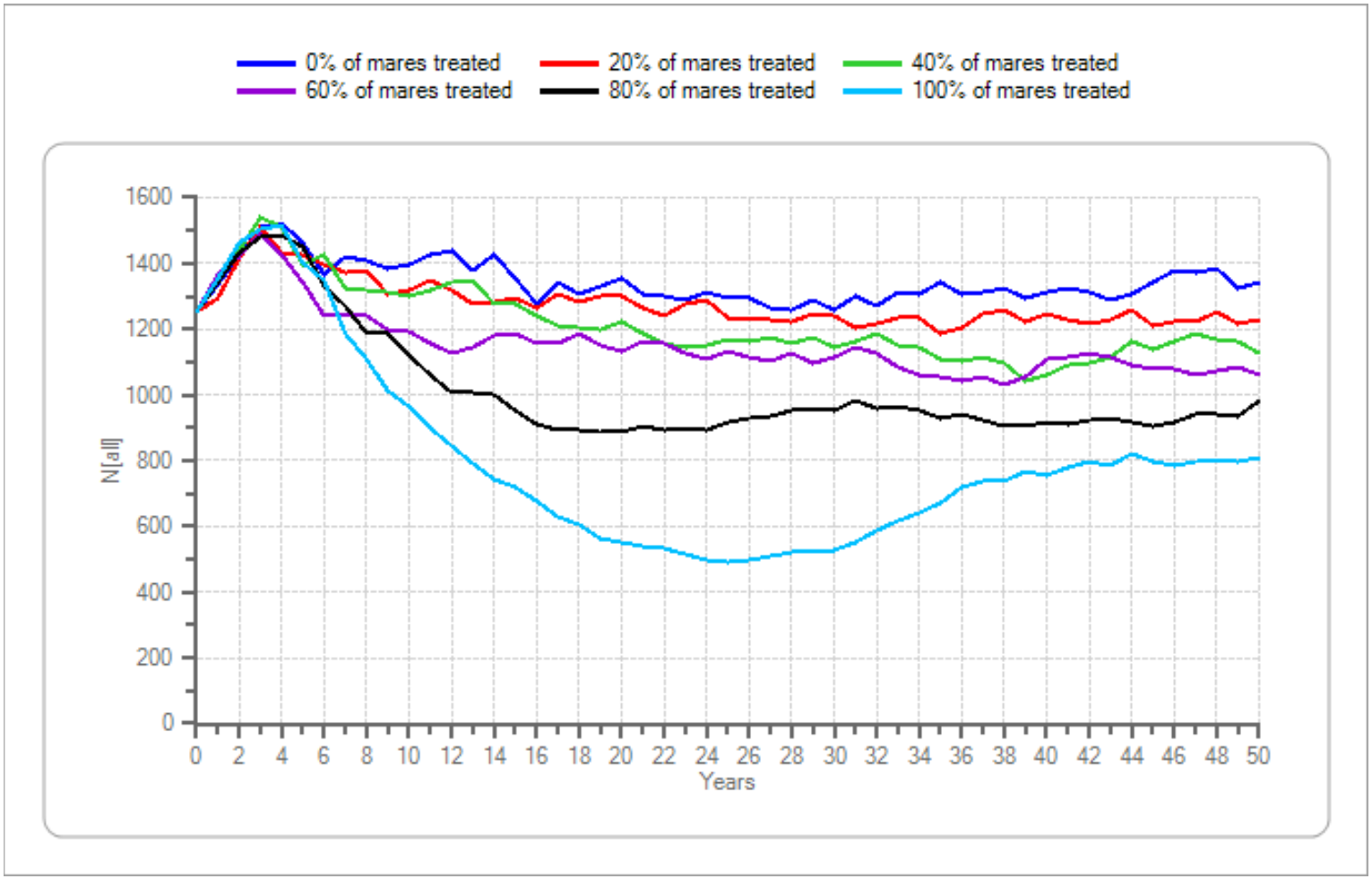
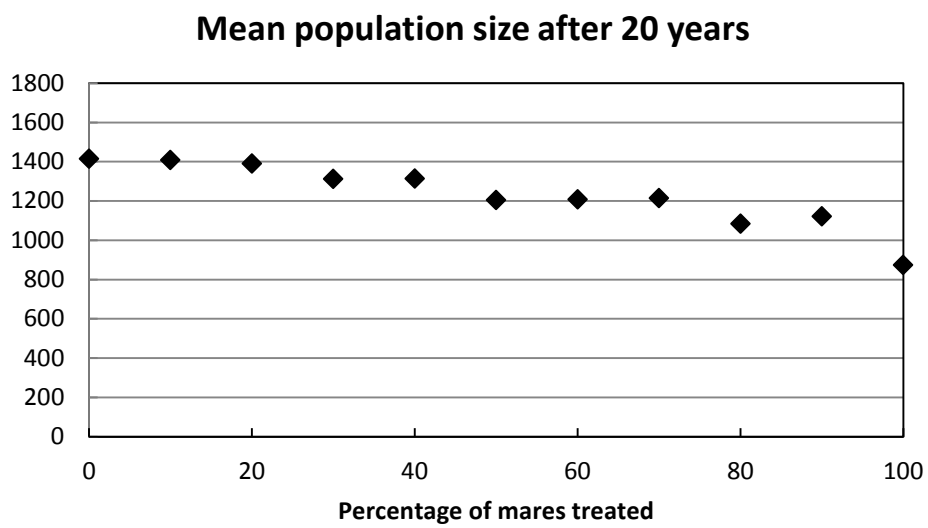
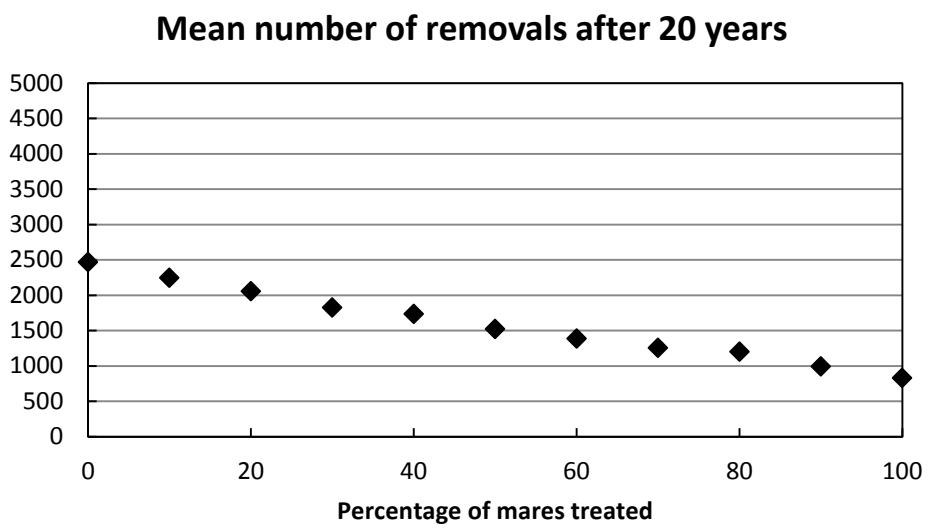
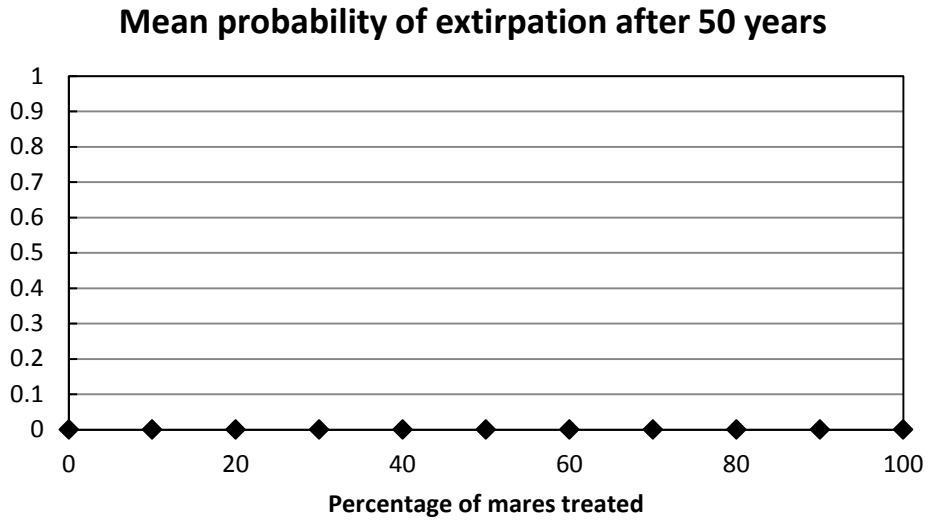


Figure 27. Initial population size = 1250, lambda = 1.10, genetic diversity = low.—Continued







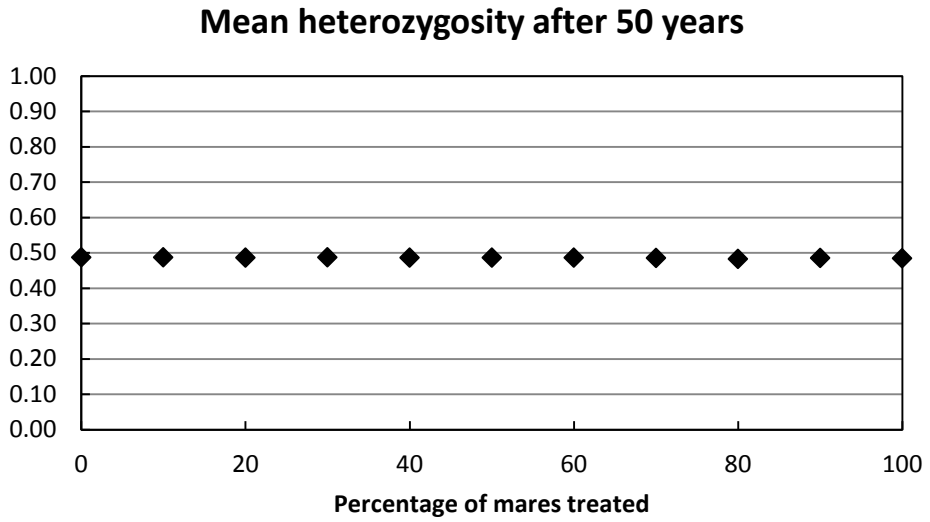


Figure 28. Initial population size = 1250, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1413.8	1407.5	1390.5	1311.6	1313.5	1204.9	1207.4	1213.3	1084.0	1120.9	874.2
Mean number of removals after 20 years	2465.8	2243.6	2057.3	1822.6	1733.3	1522.9	1385.8	1252.5	1198.7	990.3	829.2
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.48

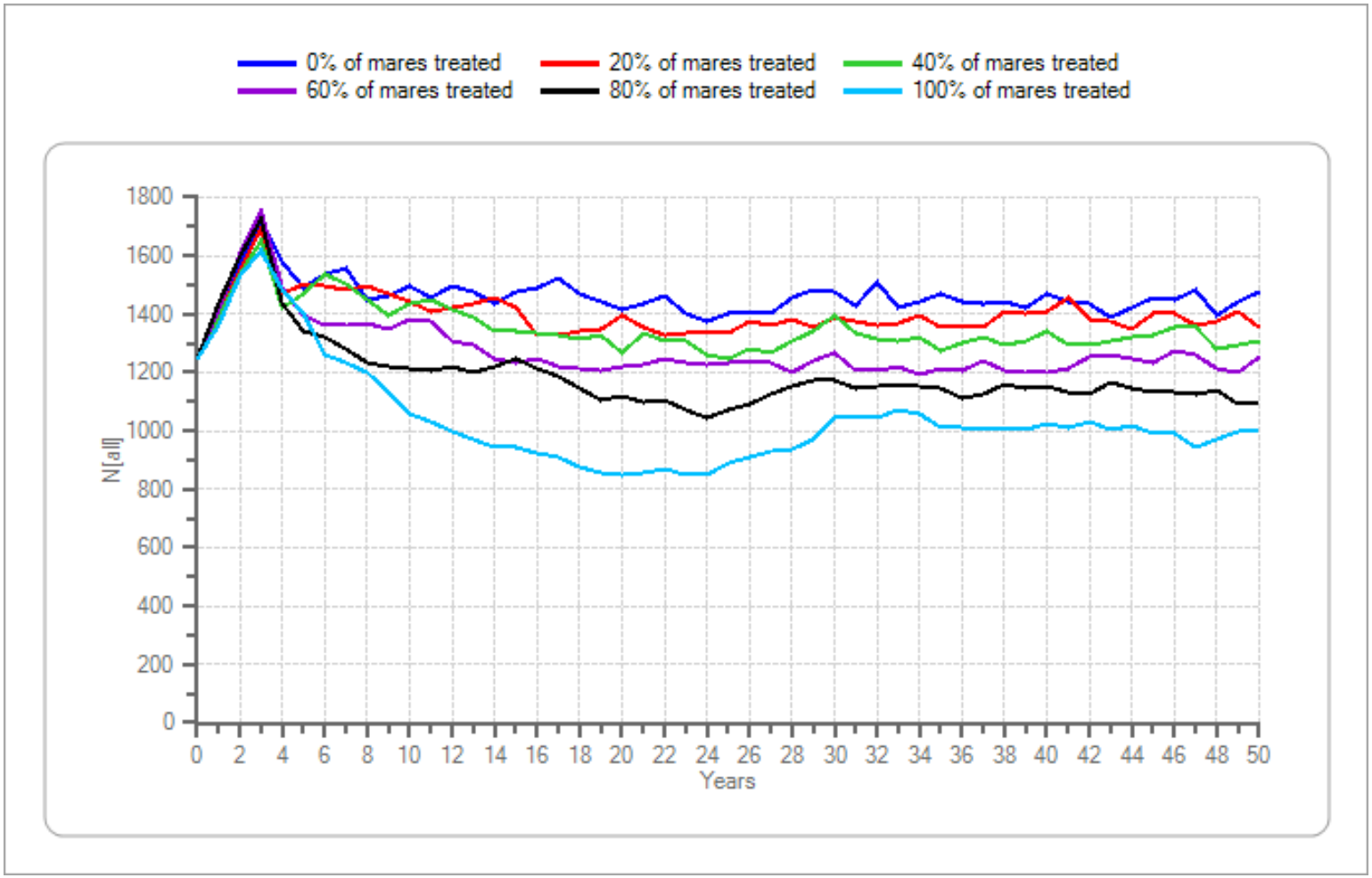
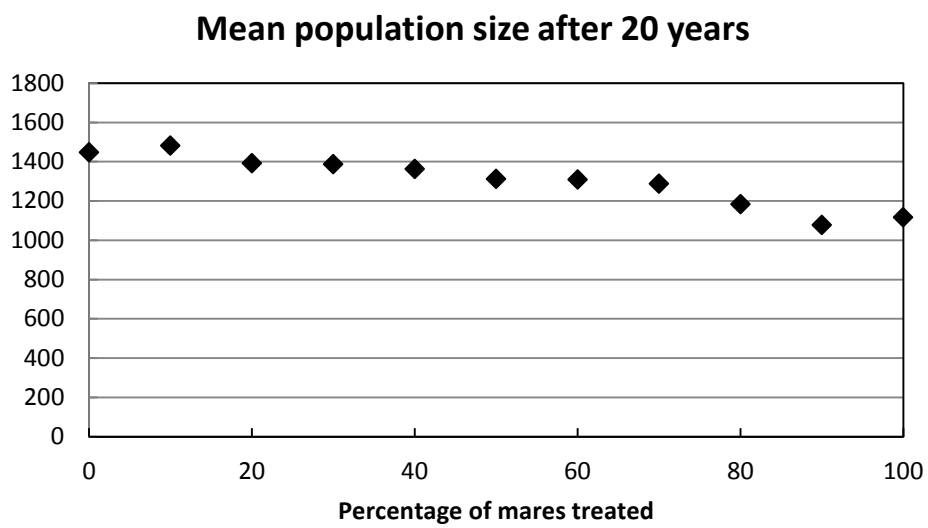
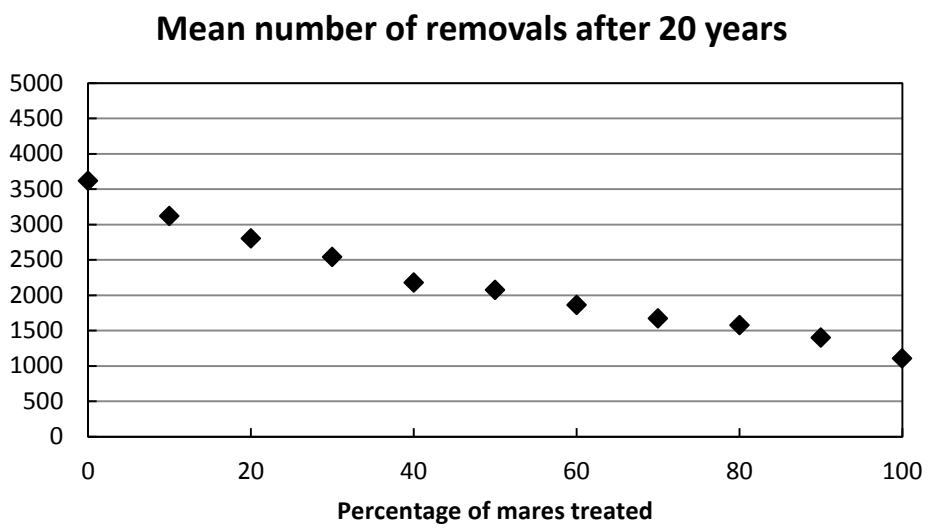
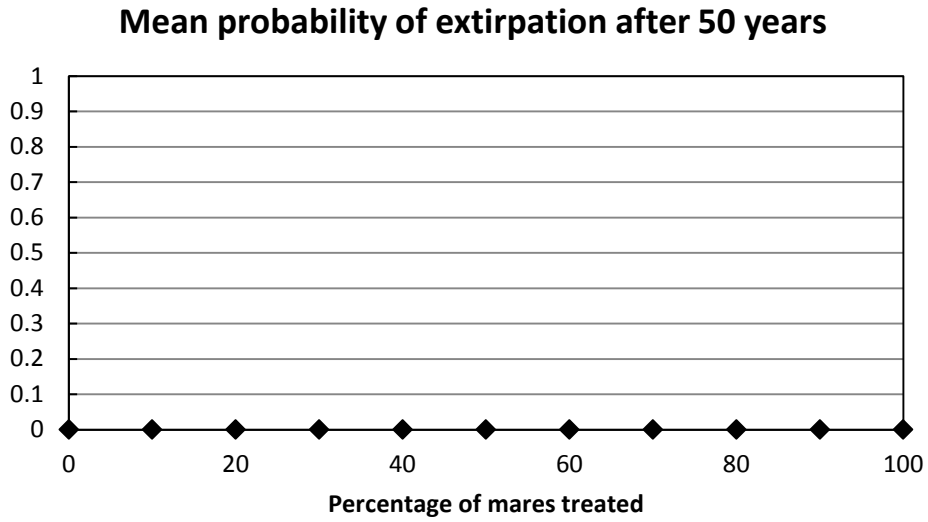


Figure 28. Initial population size = 1250, lambda = 1.15, genetic diversity = low.—Continued







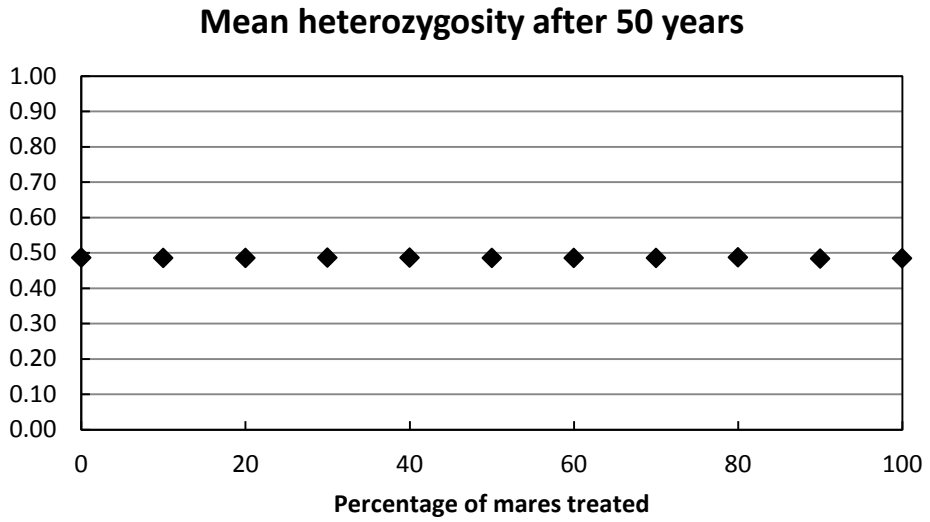


Figure 29. Initial population size = 1250, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1447.5	1481.5	1392.3	1386.1	1362.7	1312.3	1309.2	1287.2	1182.8	1077.5	1116.7
Mean number of removals after 20 years	3615.2	3121.2	2802.8	2538.6	2179.6	2071.5	1859.4	1671.5	1576.1	1398.1	1103.3
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.48	0.48

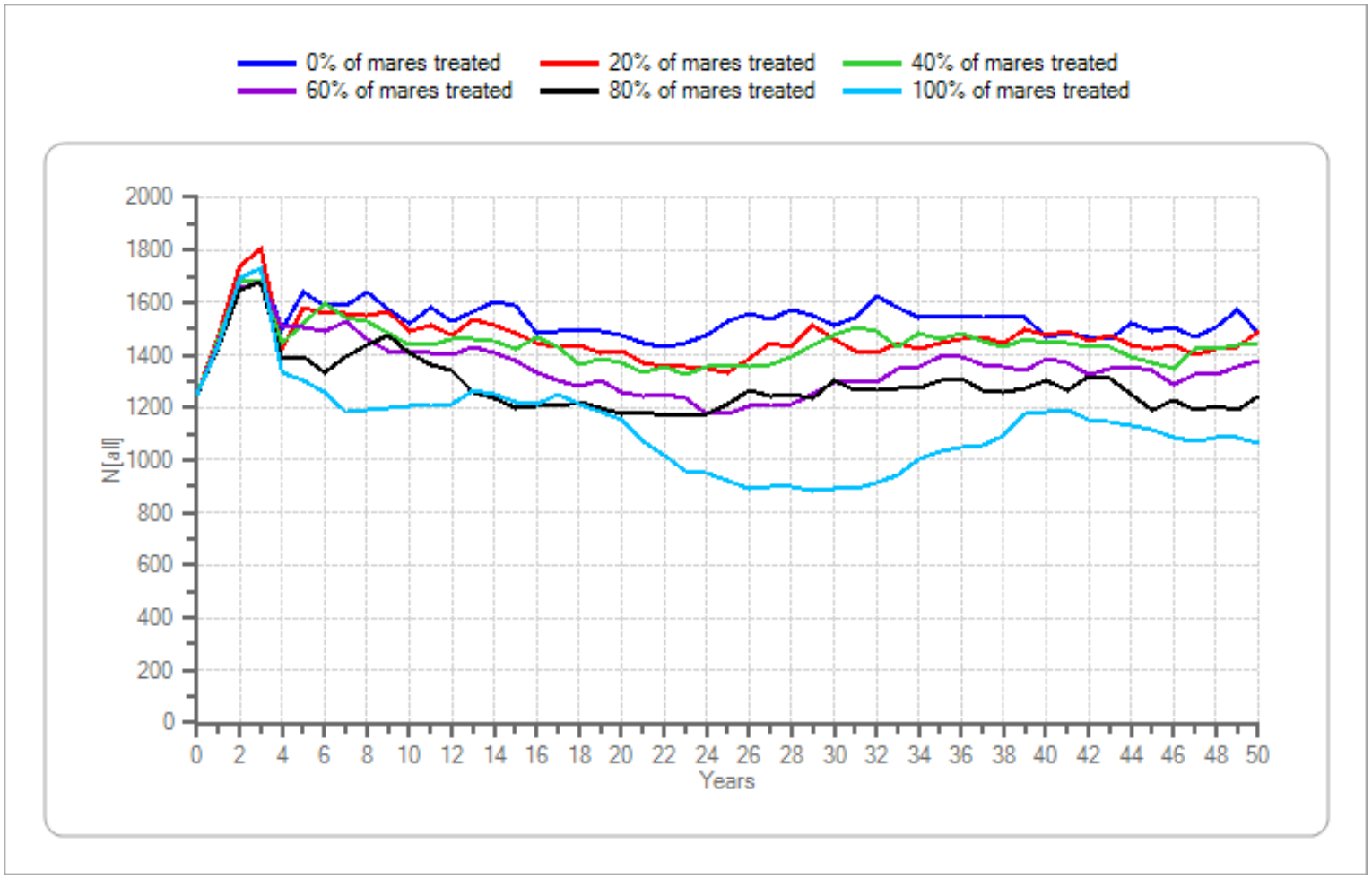
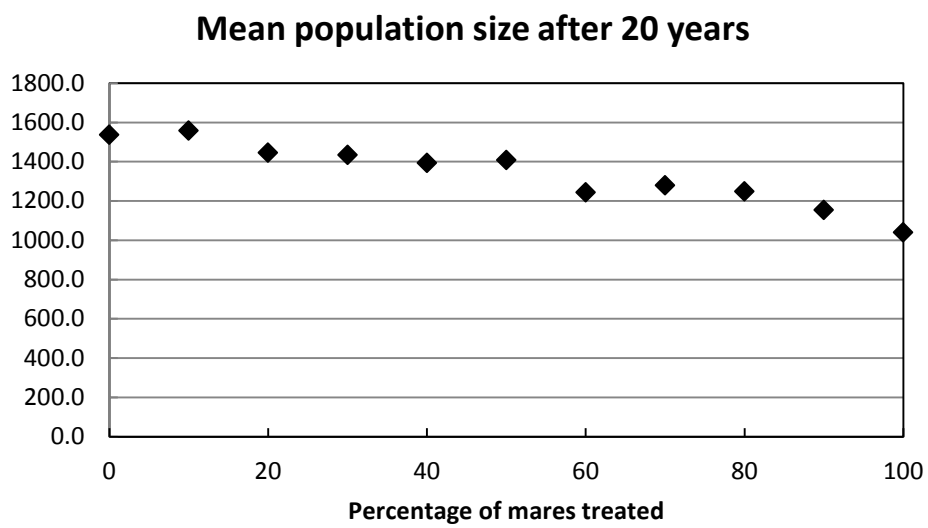
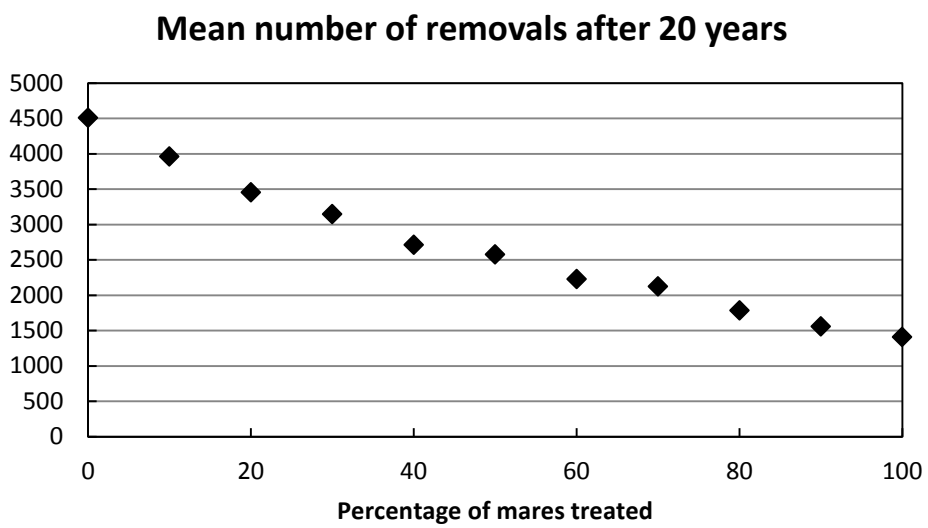
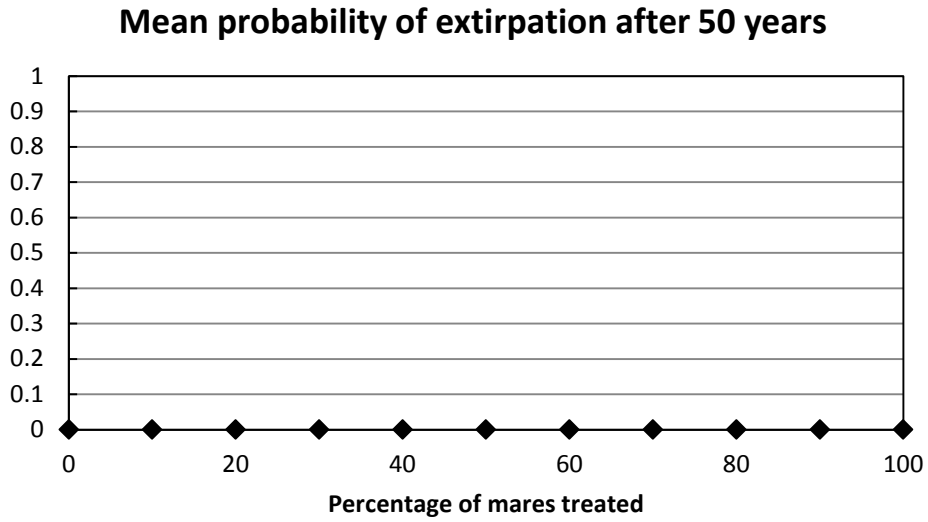


Figure 29. Initial population size = 1250, lambda = 1.20, genetic diversity = low.—Continued







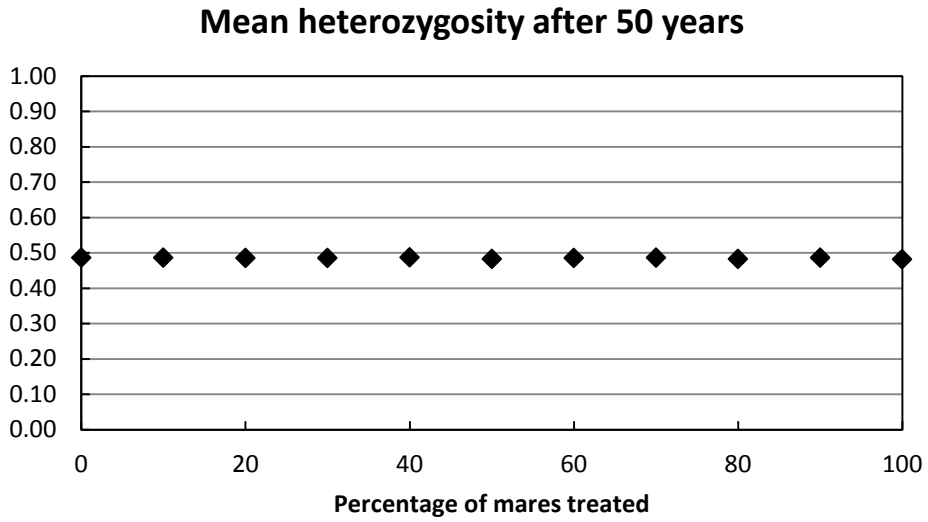


Figure 30. Initial population size = 1250, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1536.3	1557.9	1445.1	1433.6	1394.1	1407.8	1243.9	1279.1	1248.4	1154.4	1039.9
Mean number of removals after 20 years	4509.1	3960.3	3453.9	3146.1	2711.4	2577.9	2226.4	2122.1	1782.3	1559.0	1409.3
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.48	0.49	0.48	0.49	0.49	0.48	0.49	0.48

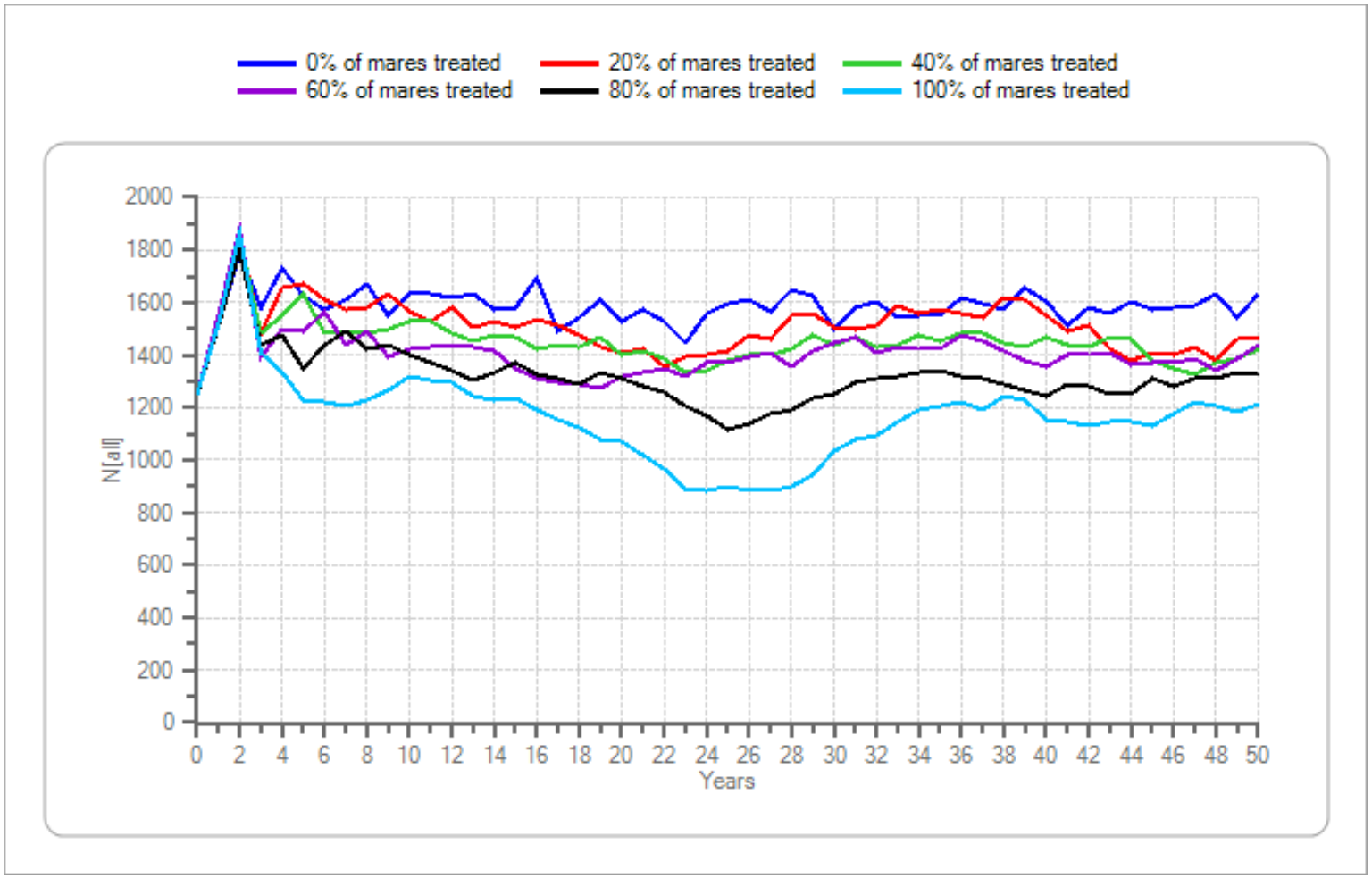
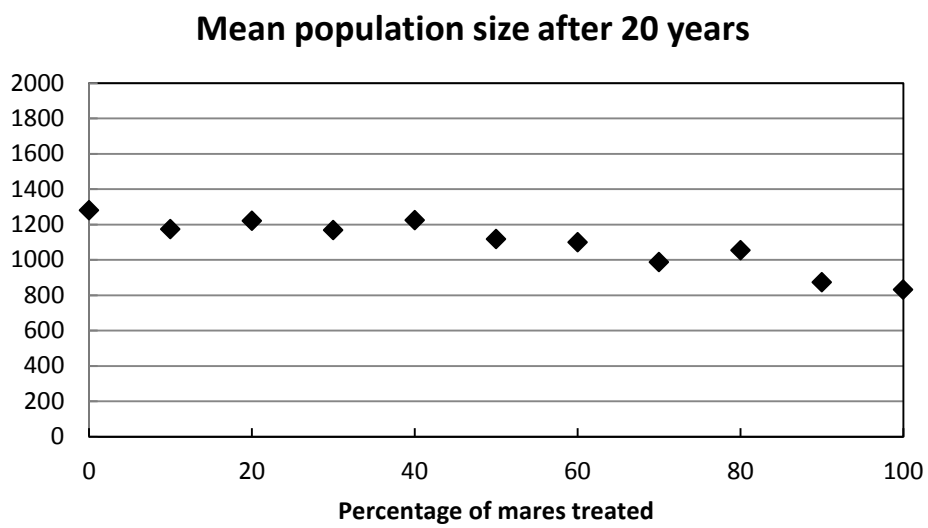
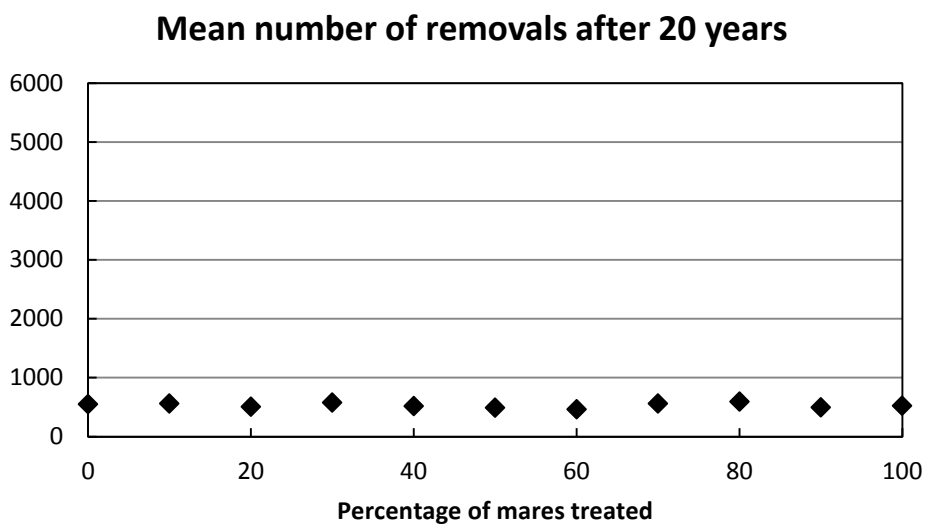
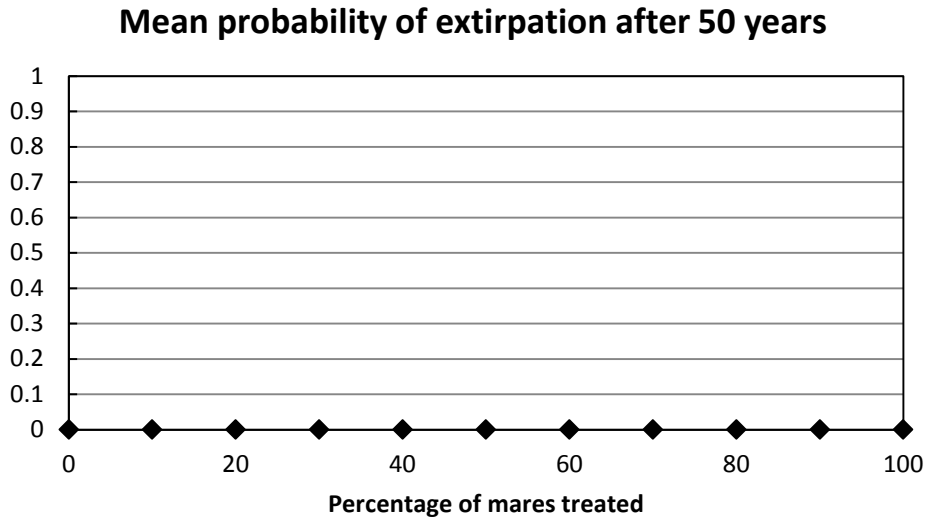


Figure 30. Initial population size = 1250, lambda = 1.25, genetic diversity = low.—Continued







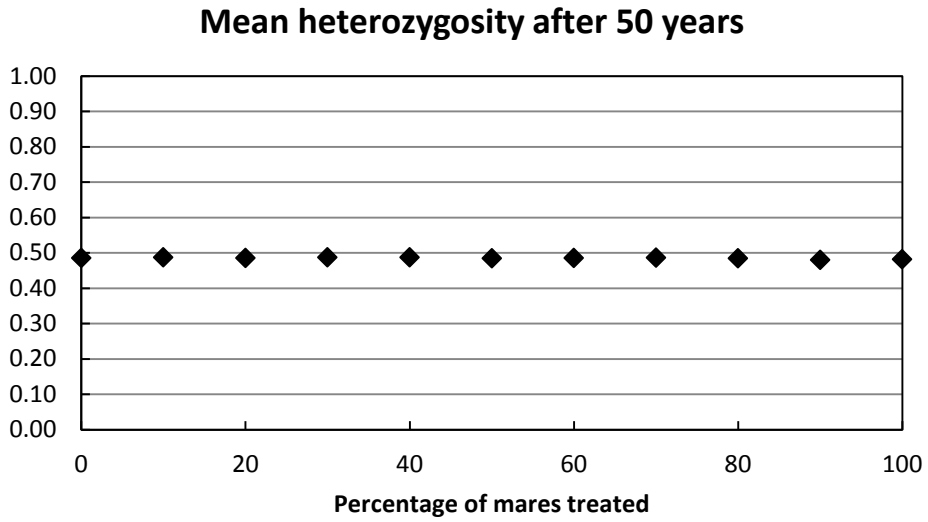


Figure 31. Initial population size = 1500, lambda = 1.05, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1279.4	1173.9	1220.8	1167.1	1224.9	1117.3	1098.7	987.4	1053.9	873.3	830.5
Mean number of removals after 20 years	547.92	557.55	504.35	573.75	514.62	487.4	459.94	558.95	591.28	496.54	524.2
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.49	0.48	0.48	0.48

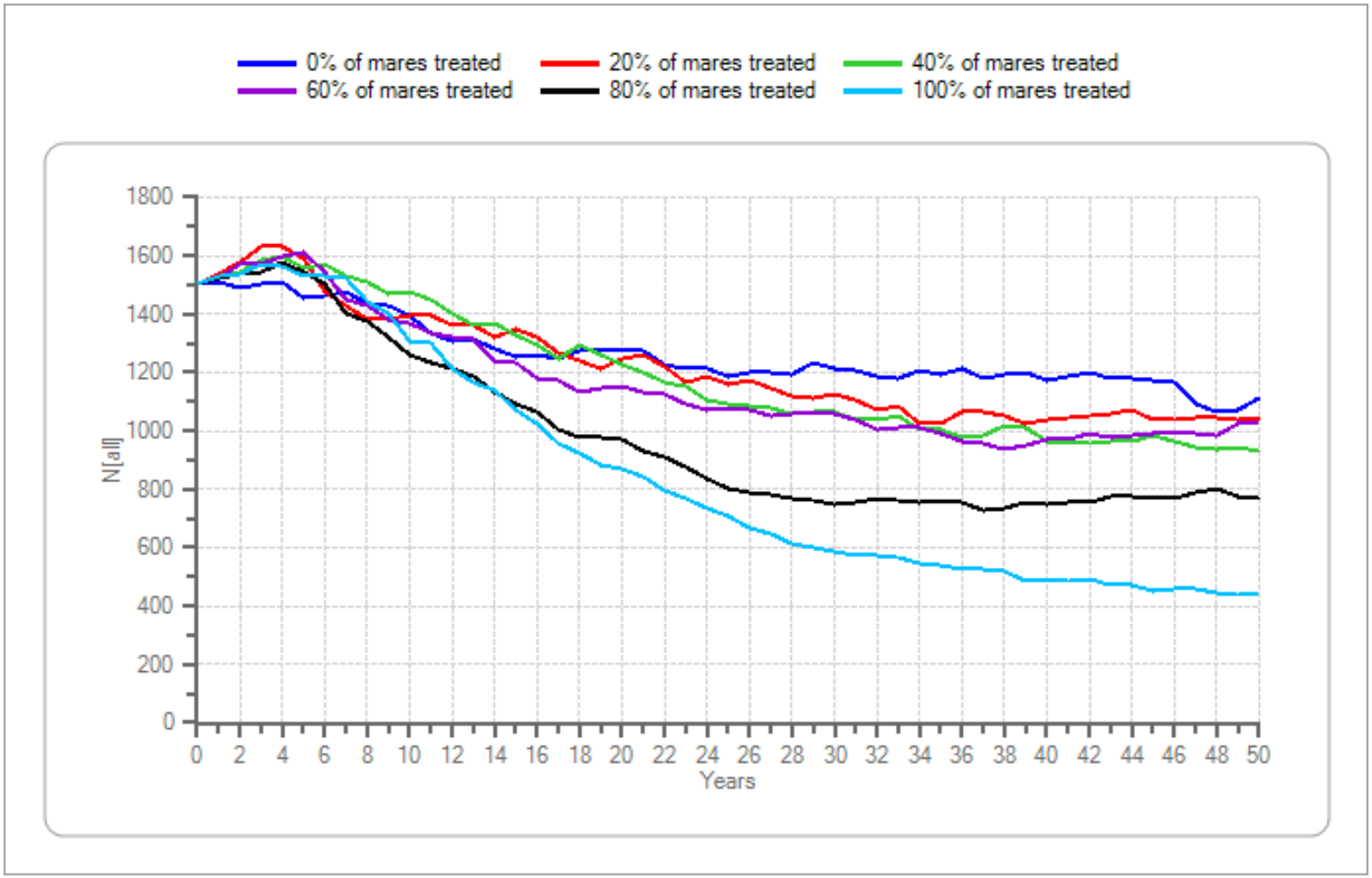
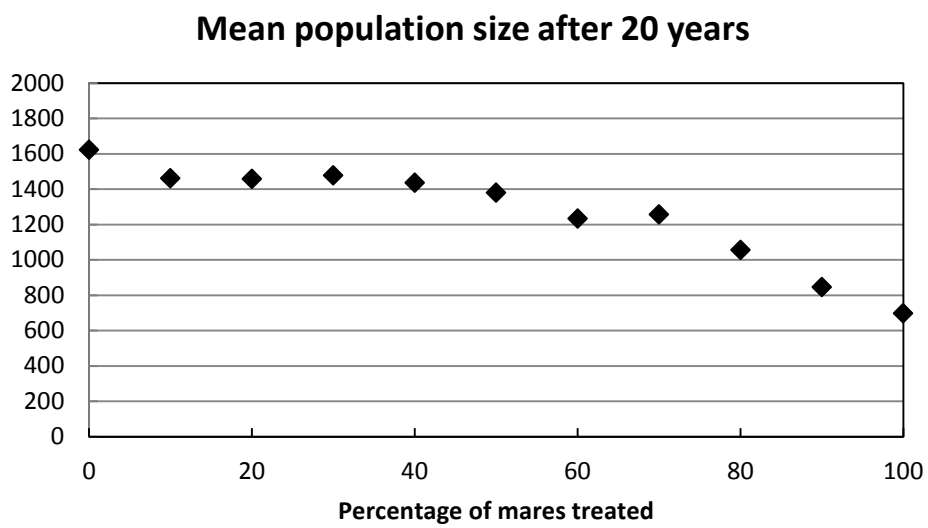
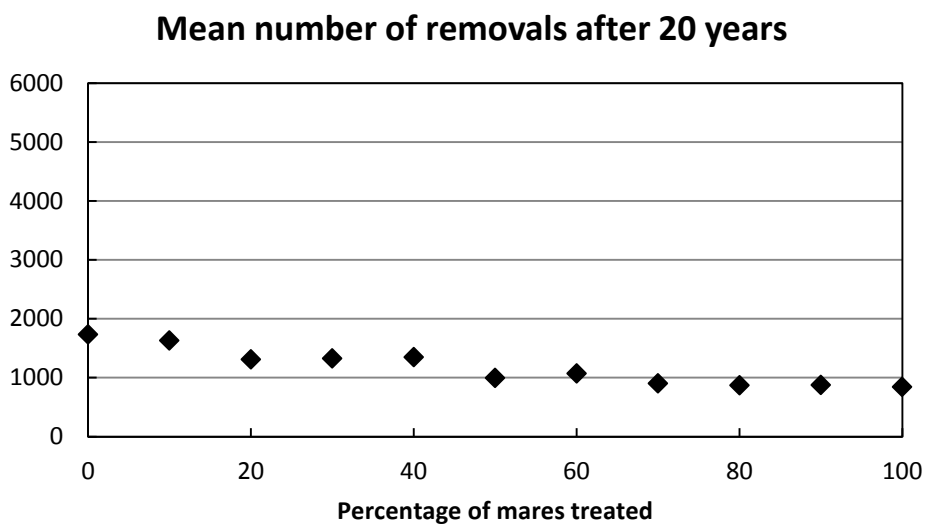
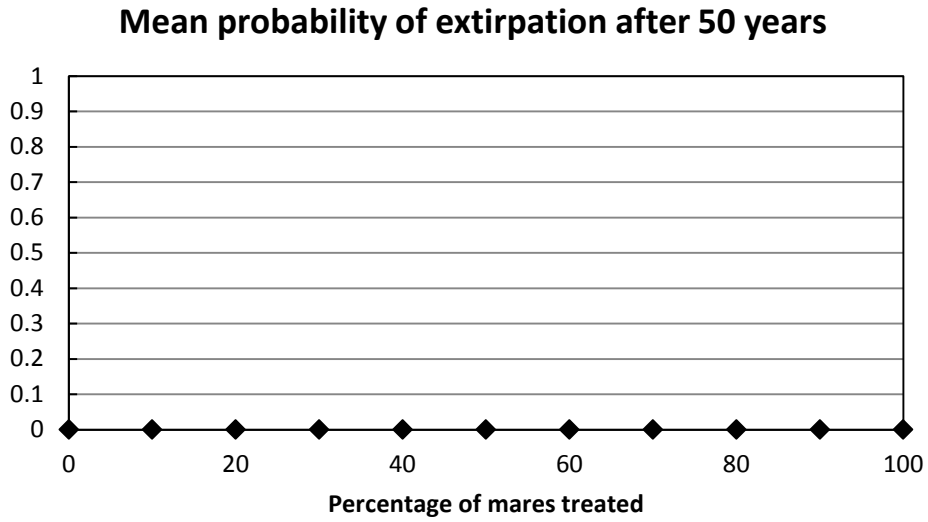


Figure 31. Initial population size = 1500, lambda = 1.05, genetic diversity = low.—Continued







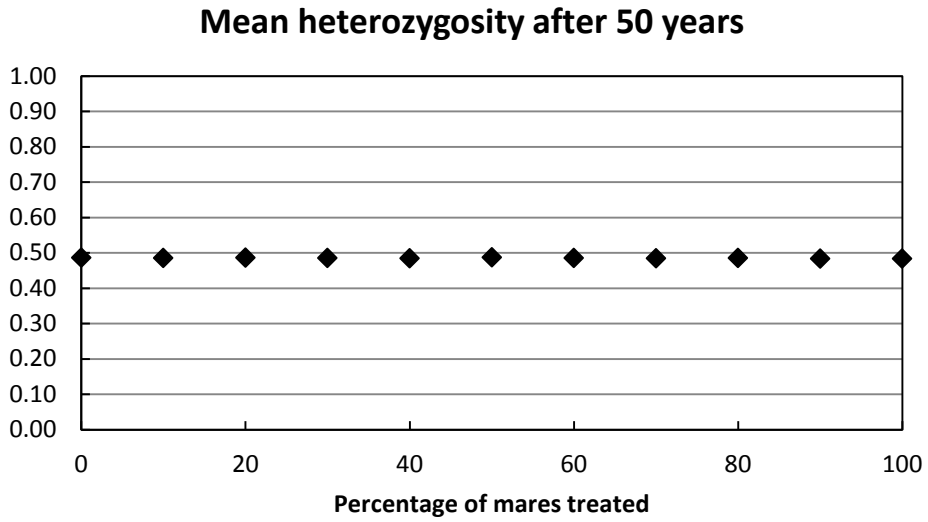


Figure 32. Initial population size = 1500, lambda = 1.10, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1622.8	1461.2	1457.5	1477.4	1435.9	1380.1	1232.4	1255.8	1054.9	846.0	697.3
Mean number of removals after 20 years	1735.0	1628.0	1308.3	1327.2	1348.6	995.9	1072.9	903.0	868.5	872.8	843.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.48	0.49	0.48	0.48	0.49	0.48	0.48

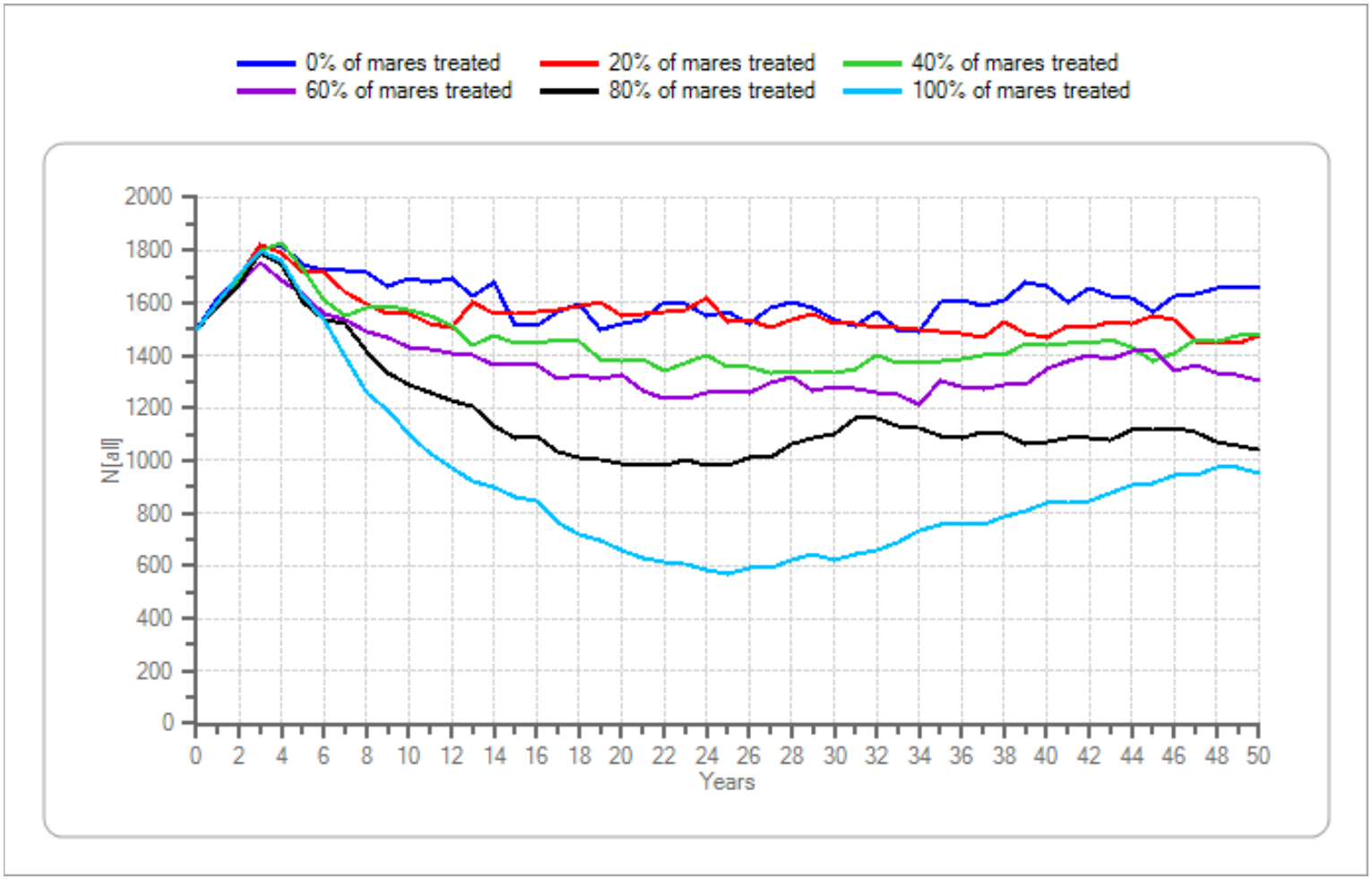
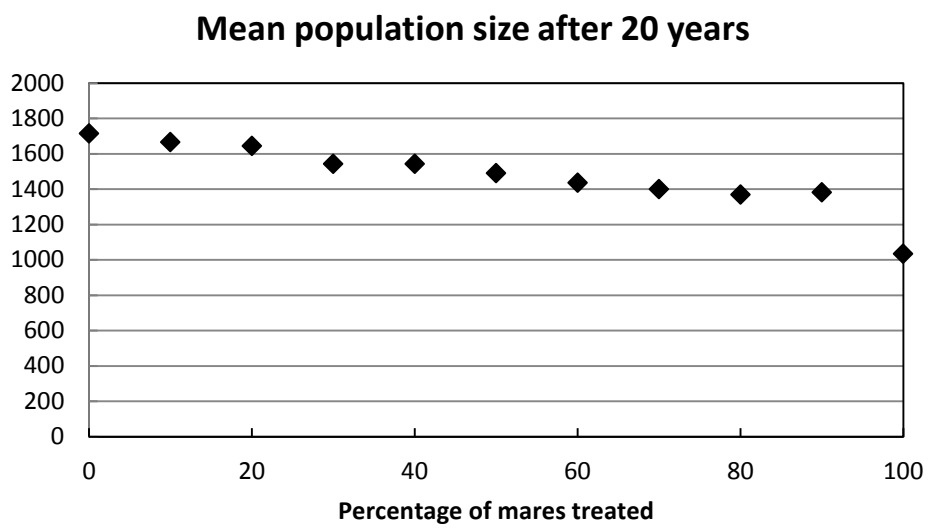
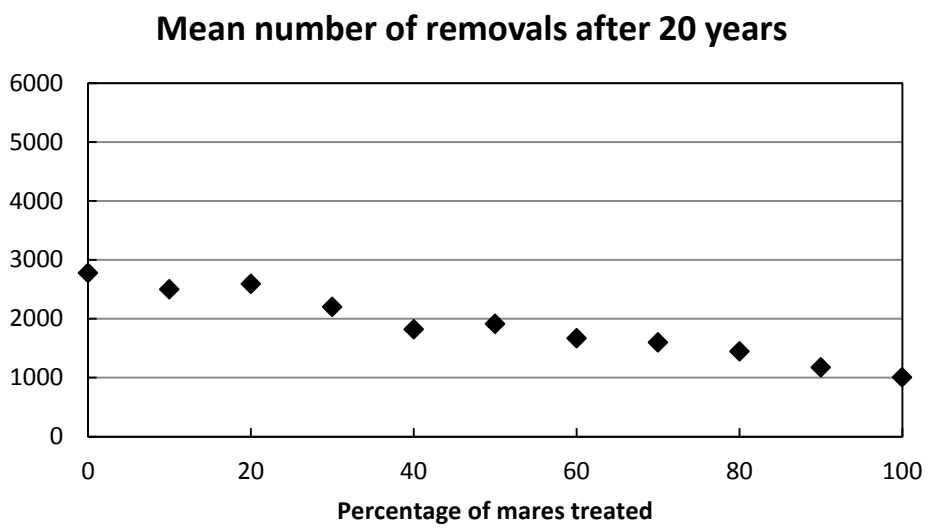
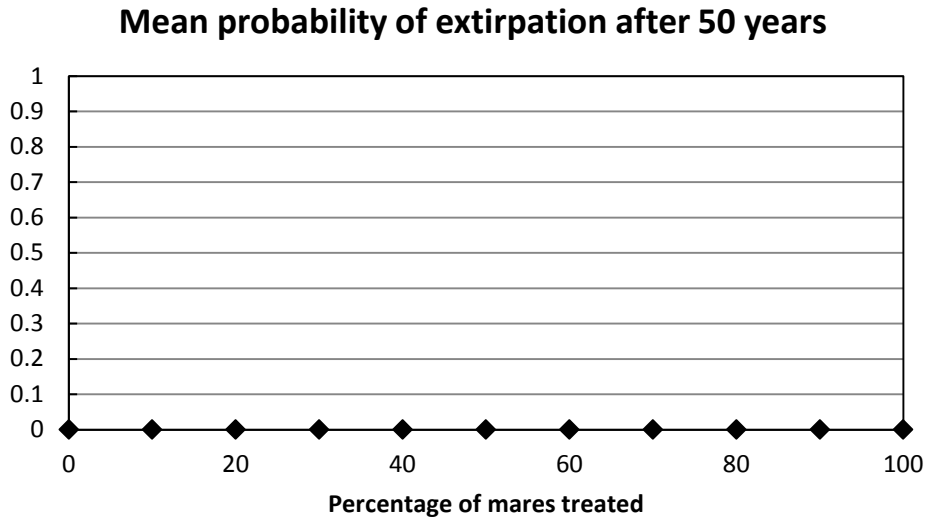


Figure 32. Initial population size = 1500, lambda = 1.10, genetic diversity = low.—Continued







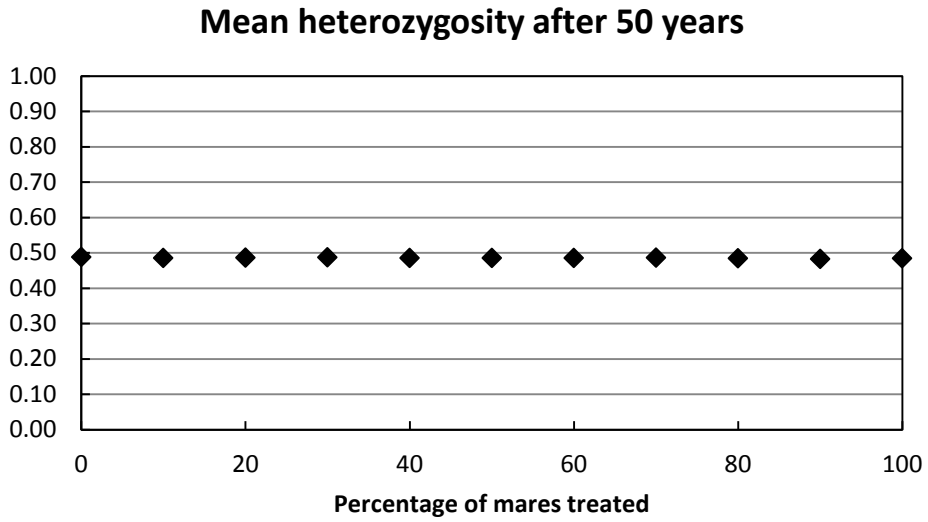


Figure 33. Initial population size = 1500, lambda = 1.15, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1714.8	1666.7	1643.6	1542.5	1542.7	1489.8	1436.2	1399.0	1368.2	1382.5	1033.5
Mean number of removals after 20 years	2777.7	2501.4	2591.7	2200.3	1822.5	1910.5	1669.9	1596.4	1444.0	1172.7	1004.9
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.48	0.48

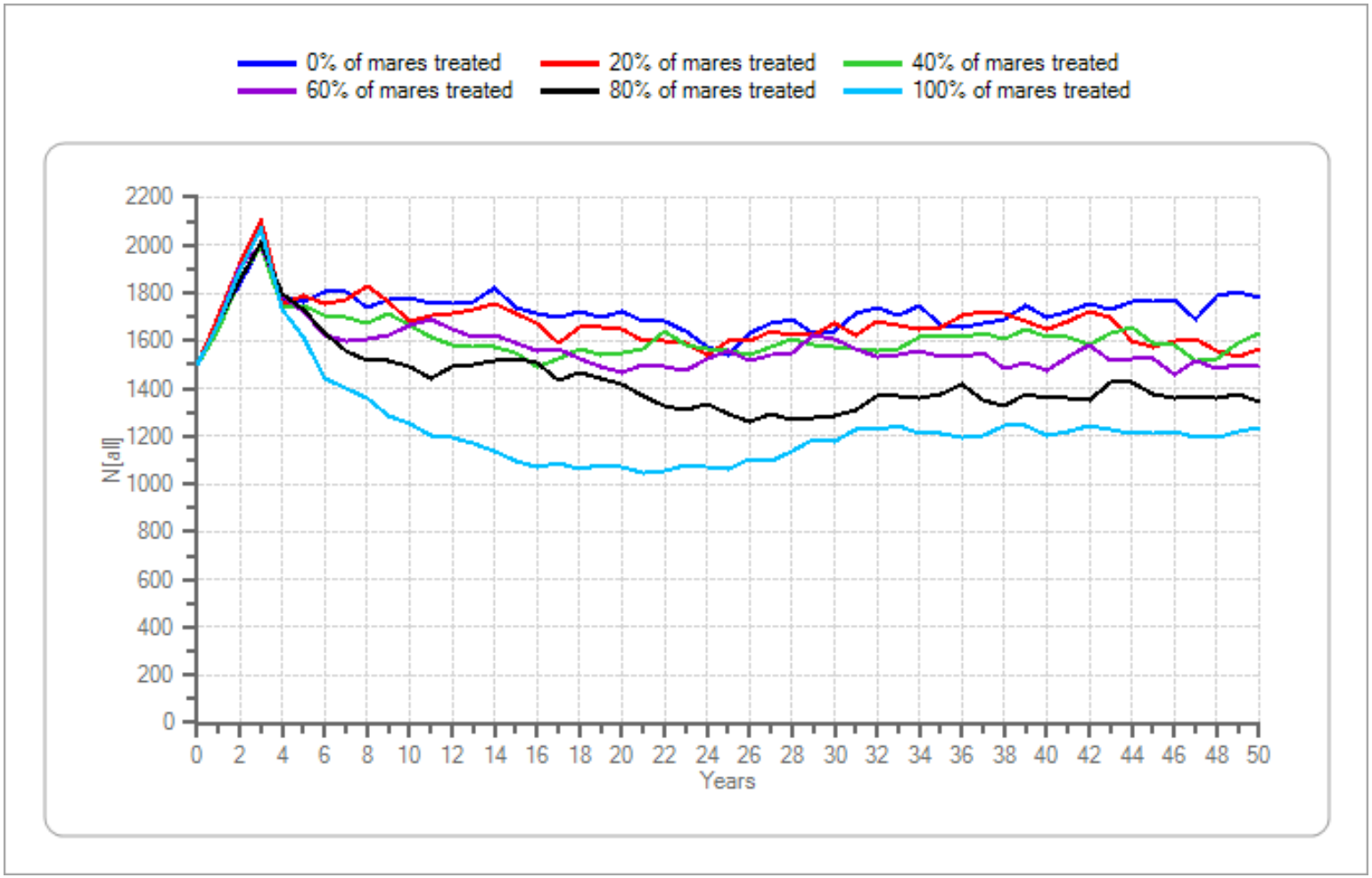
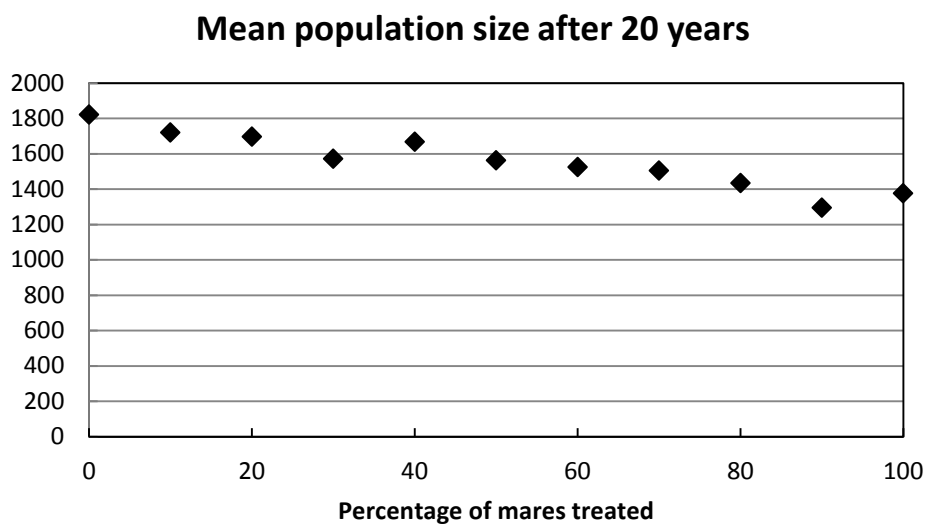
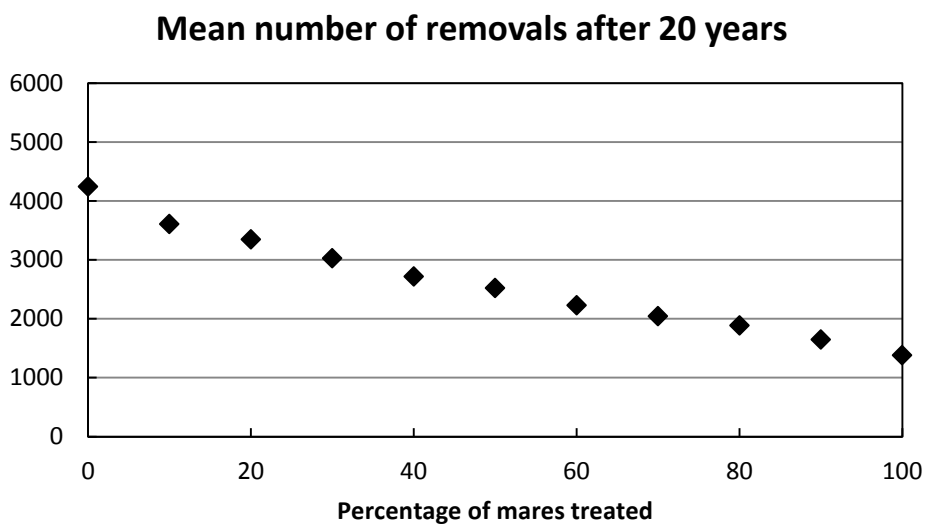
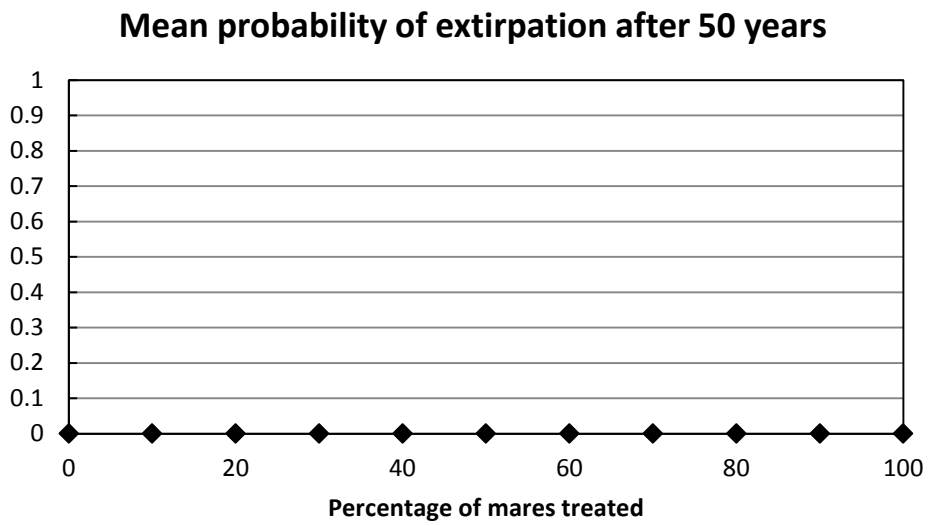


Figure 33. Initial population size = 1500, lambda = 1.15, genetic diversity = low.—Continued







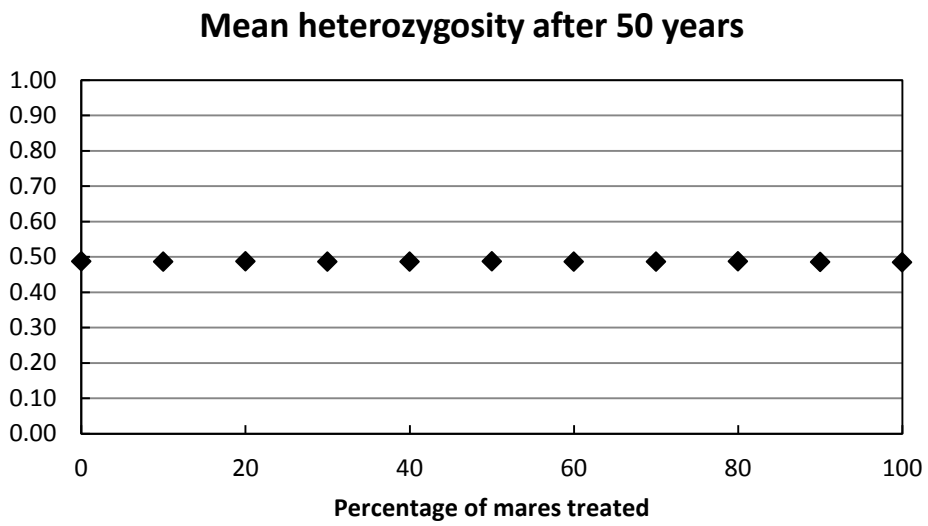


Figure 34. Initial population size = 1500, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1821.8	1719.5	1697.0	1572.3	1667.0	1563.1	1523.7	1505.0	1433.3	1294.1	1376.3
Mean number of removals after 20 years	4241.3	3605.67	3348.38	3026.93	2716.34	2518.97	2225.75	2043.6	1886.32	1648.08	1381.6
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.48

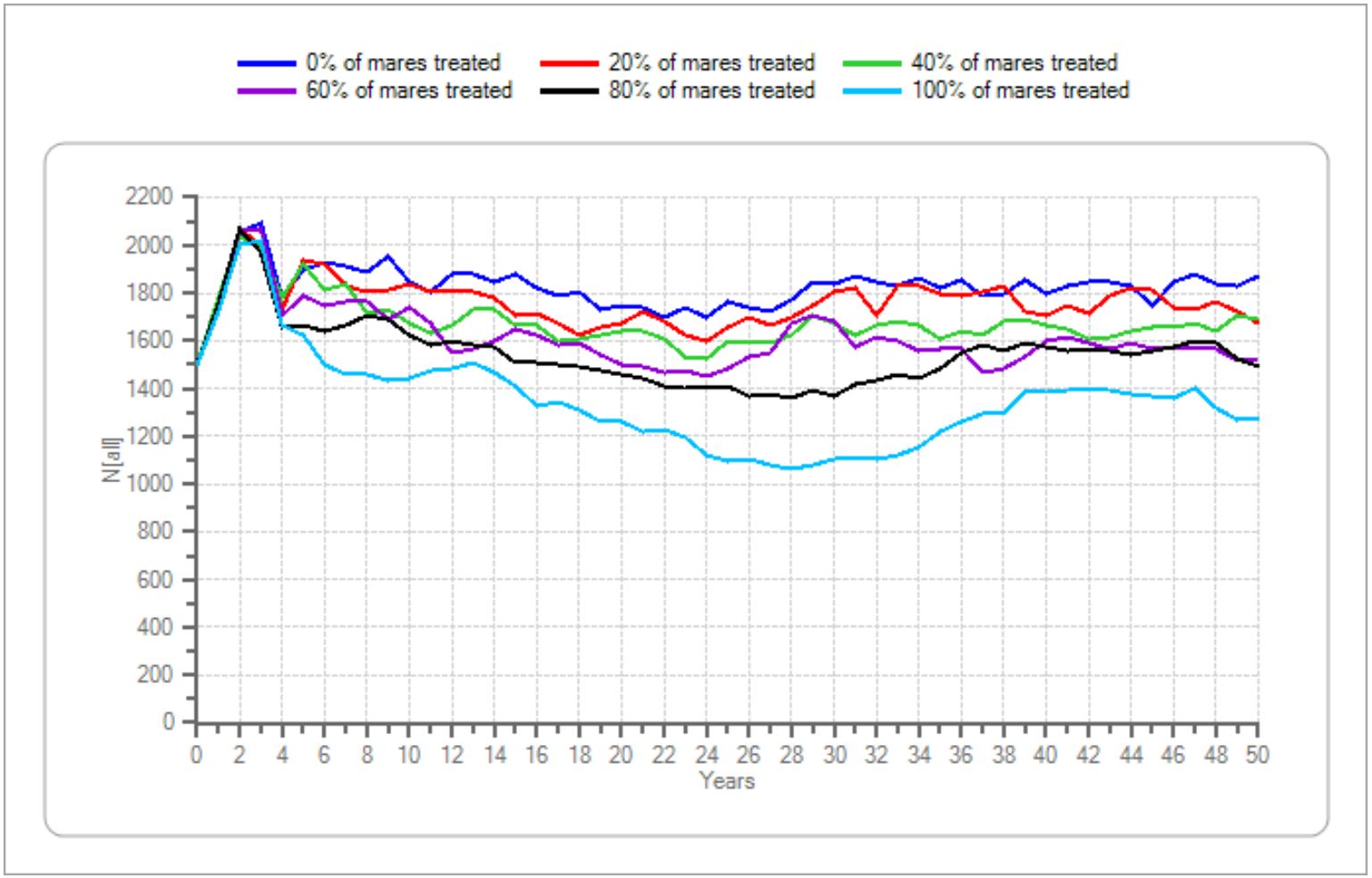
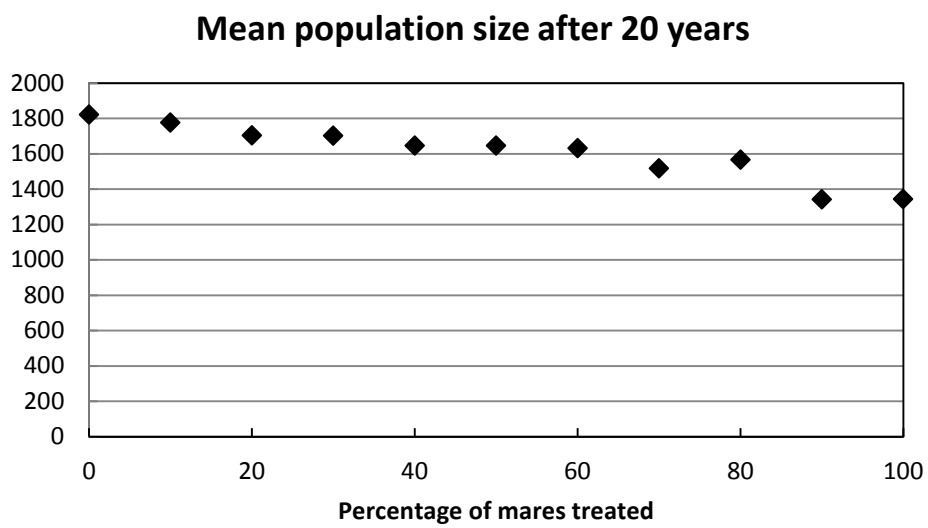
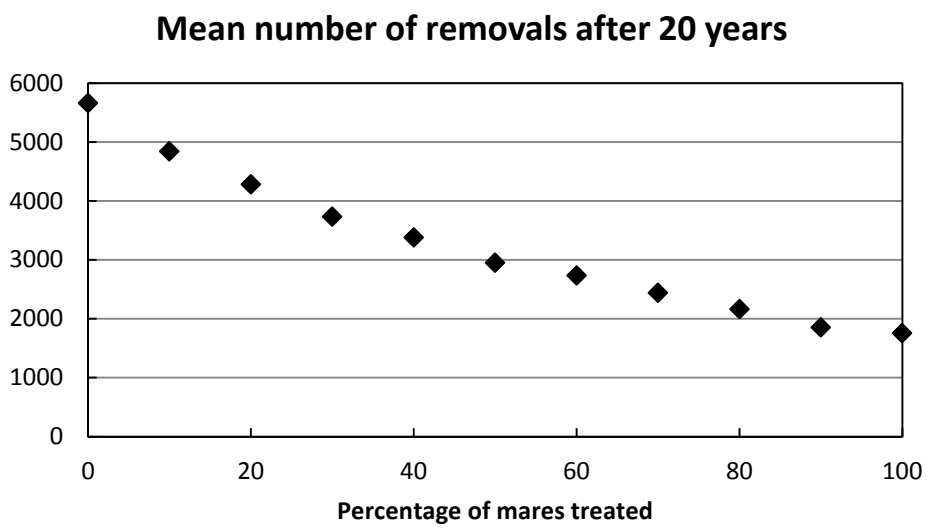
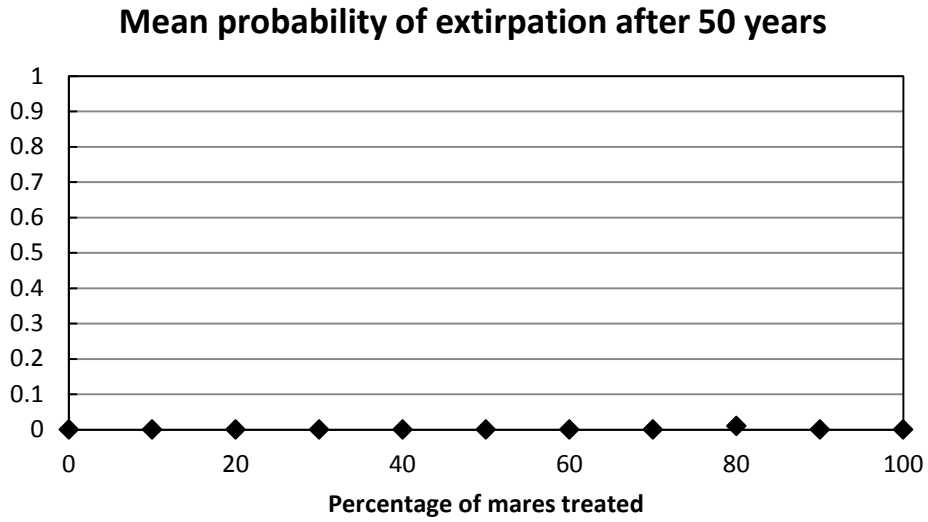


Figure 34. Initial population size = 1500, lambda = 1.20, genetic diversity = low.—Continued







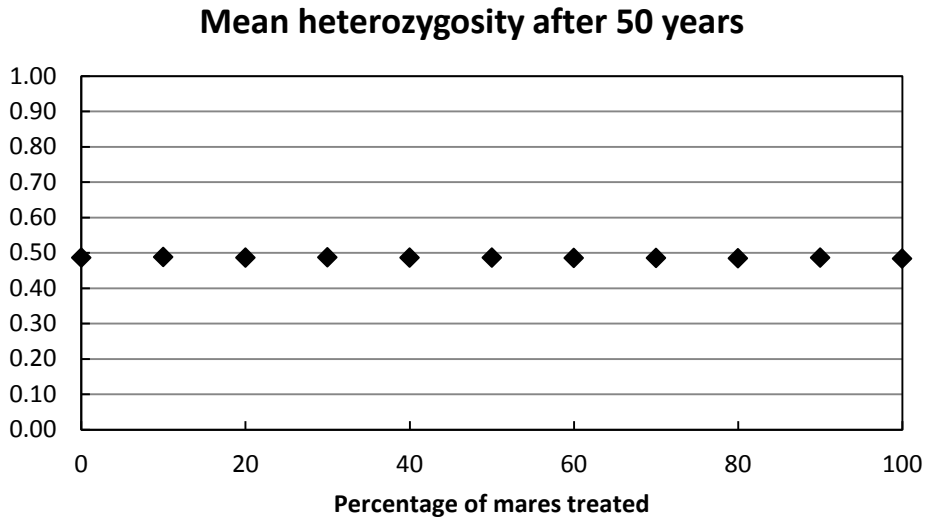


Figure 35. Initial population size = 1500, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1821.3	1775.6	1704.2	1702.5	1645.8	1645.7	1631.5	1516.9	1566.6	1342.5	1343.4
Mean number of removals after 20 years	5662.1	4837.4	4282.2	3732.1	3380.2	2949.7	2731.2	2438.5	2164.2	1852.6	1756.3
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0.01	0	0
Mean heterozygosity after 50 years	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.48	0.49	0.48

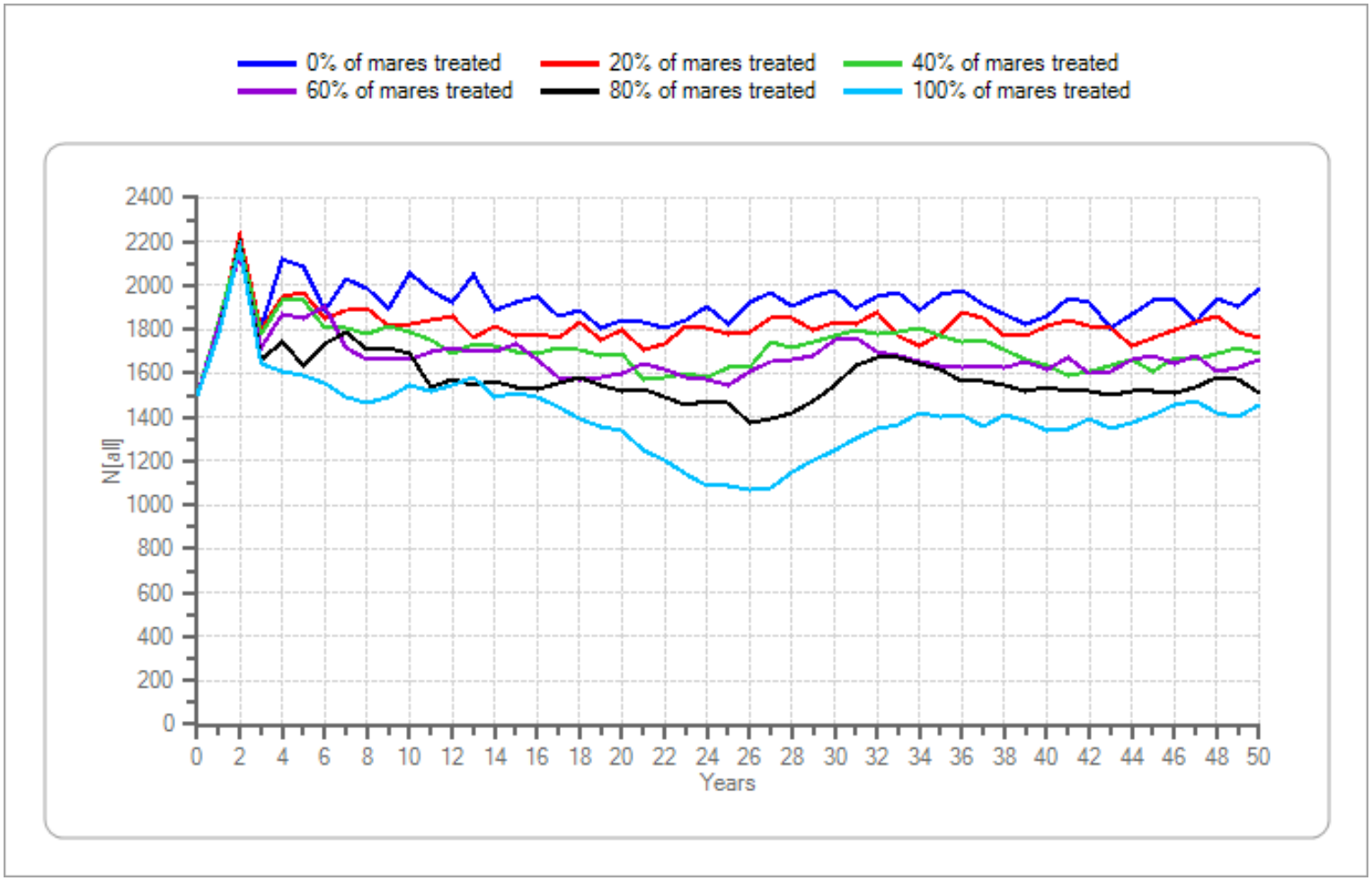
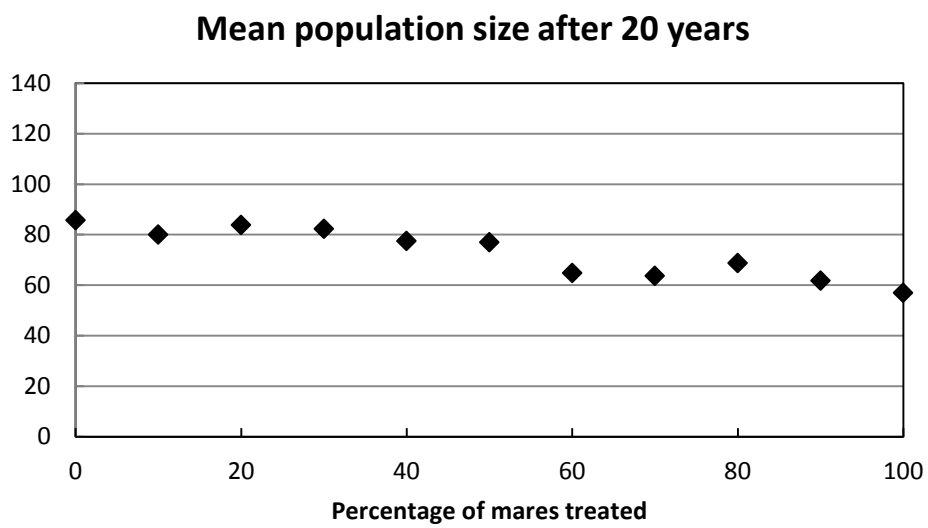
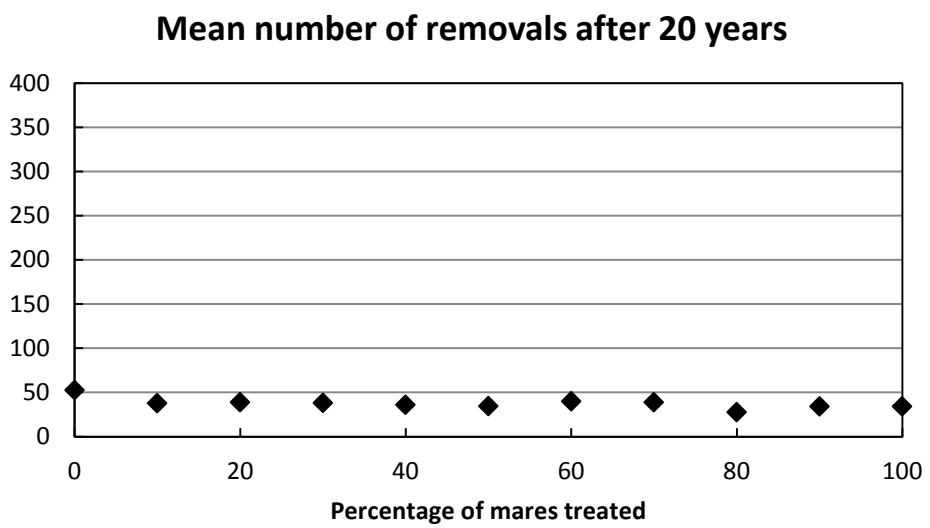
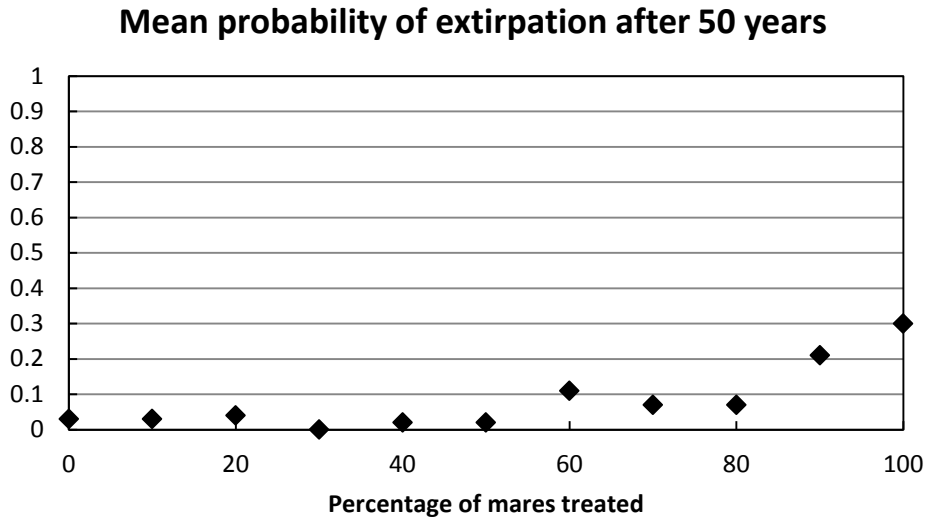


Figure 35. Initial population size = 1500, lambda = 1.25, genetic diversity = low.—Continued







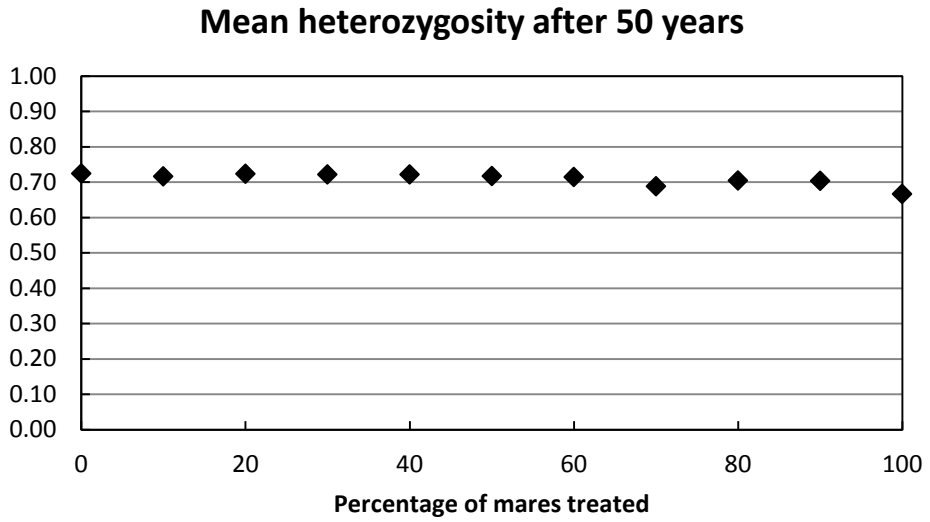


Figure 36. Initial population size = 100, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	85.65	80.04	83.81	82.32	77.46	76.91	64.82	63.57	68.7	61.78	56.86
Mean number of removals after 20 years	52.51	37.59	38.83	38.02	36.03	34.27	39.99	38.8	27.57	34.2	34.23
Mean probability of extirpation after 50 years	0.03	0.03	0.04	0	0.02	0.02	0.11	0.07	0.07	0.21	0.3
Mean heterozygosity after 50 years	0.72	0.72	0.72	0.72	0.72	0.72	0.71	0.69	0.70	0.70	0.67

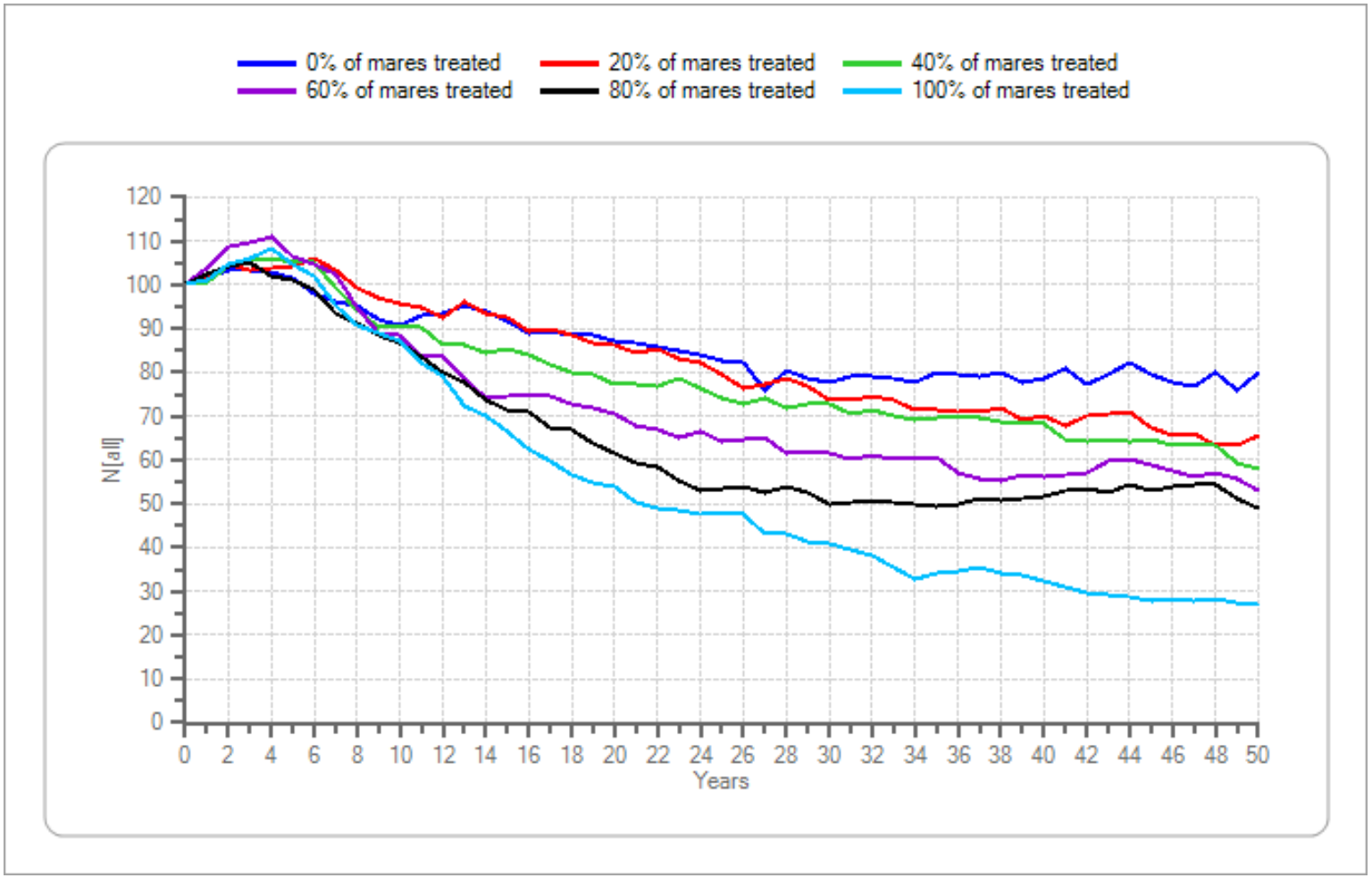
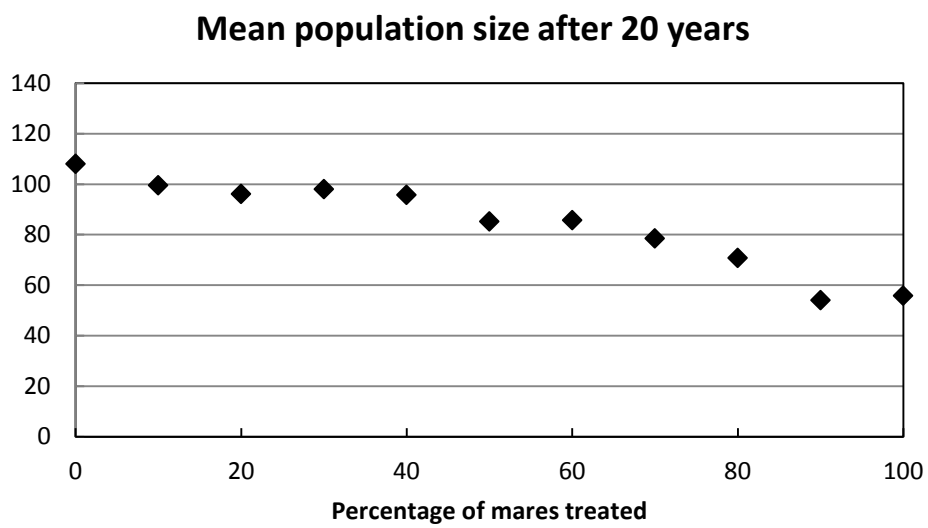
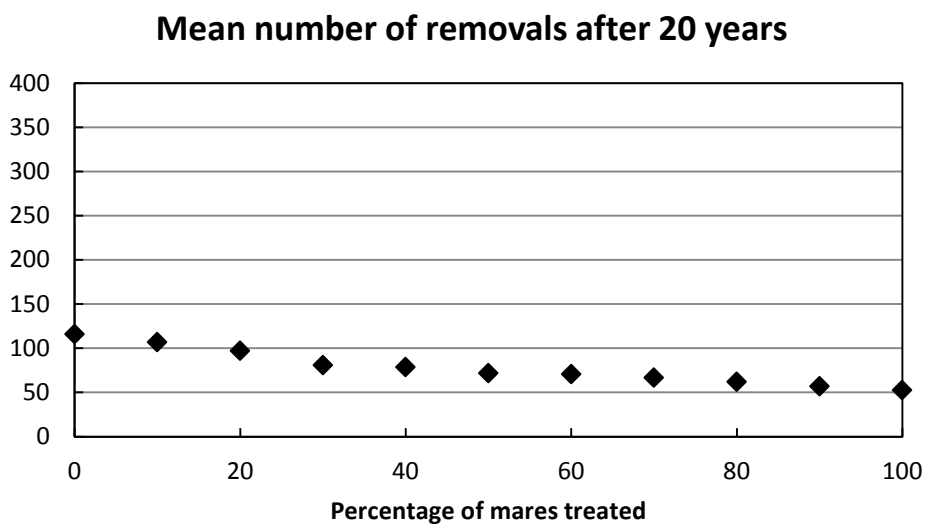
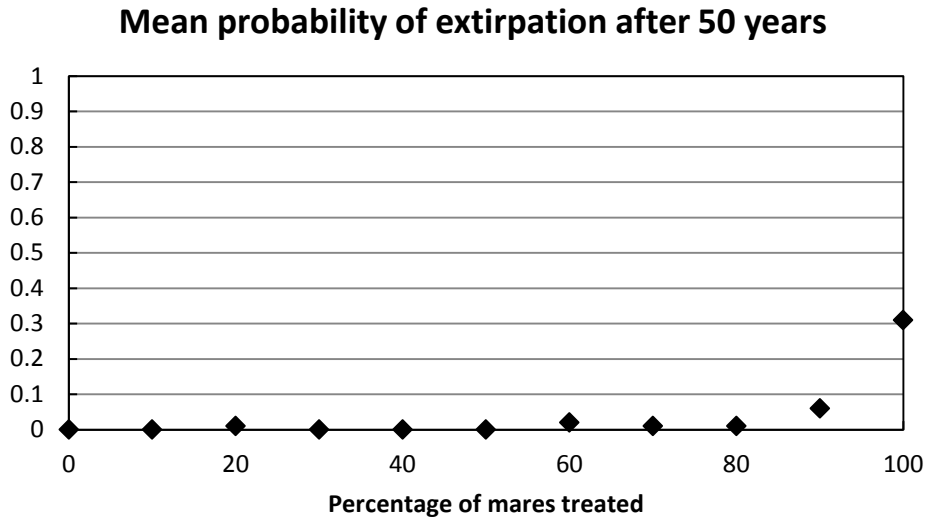


Figure 36. Initial population size = 100, lambda = 1.05, genetic diversity = high.—Continued







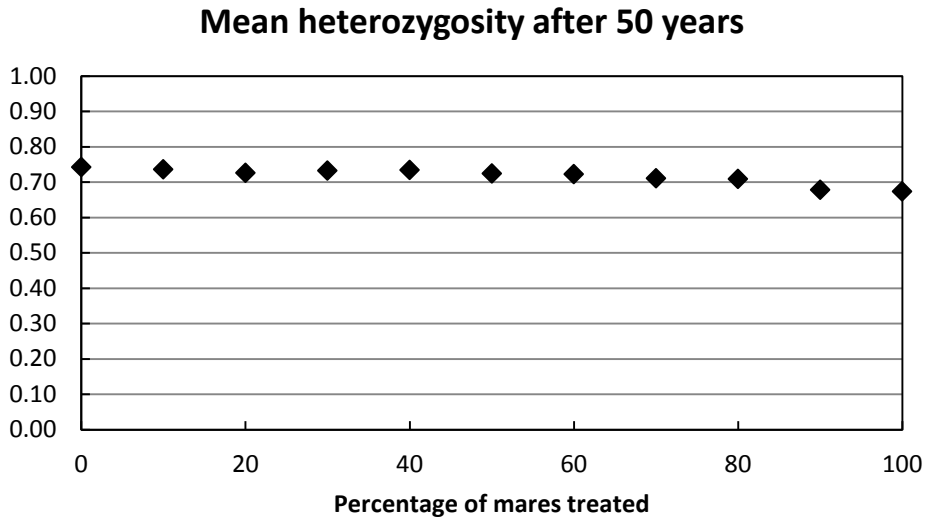


Figure 37. Initial population size = 100, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	107.97	99.44	96.12	98.03	95.64	85.17	85.71	78.45	70.71	53.94	55.76
Mean number of removals after 20 years	115.94	106.96	96.94	80.92	78.45	71.85	70.62	66.8	62.01	56.75	52.5
Mean probability of extirpation after 50 years	0	0	0.01	0	0	0	0.02	0.01	0.01	0.06	0.31
Mean heterozygosity after 50 years	0.74	0.74	0.73	0.73	0.73	0.72	0.72	0.71	0.71	0.68	0.67

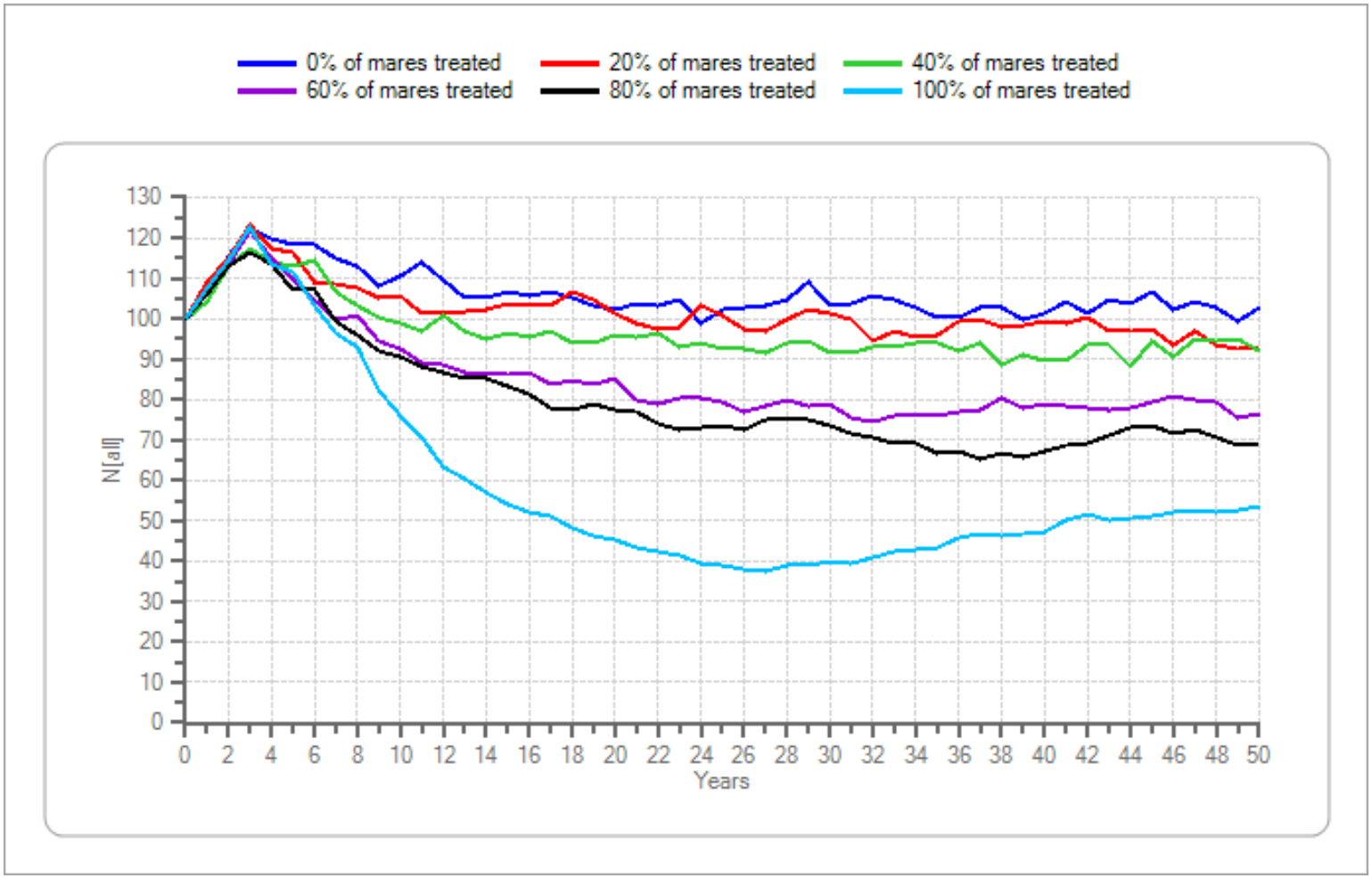


Figure 37. Initial population size = 100, lambda = 1.10, genetic diversity = high.—Continued

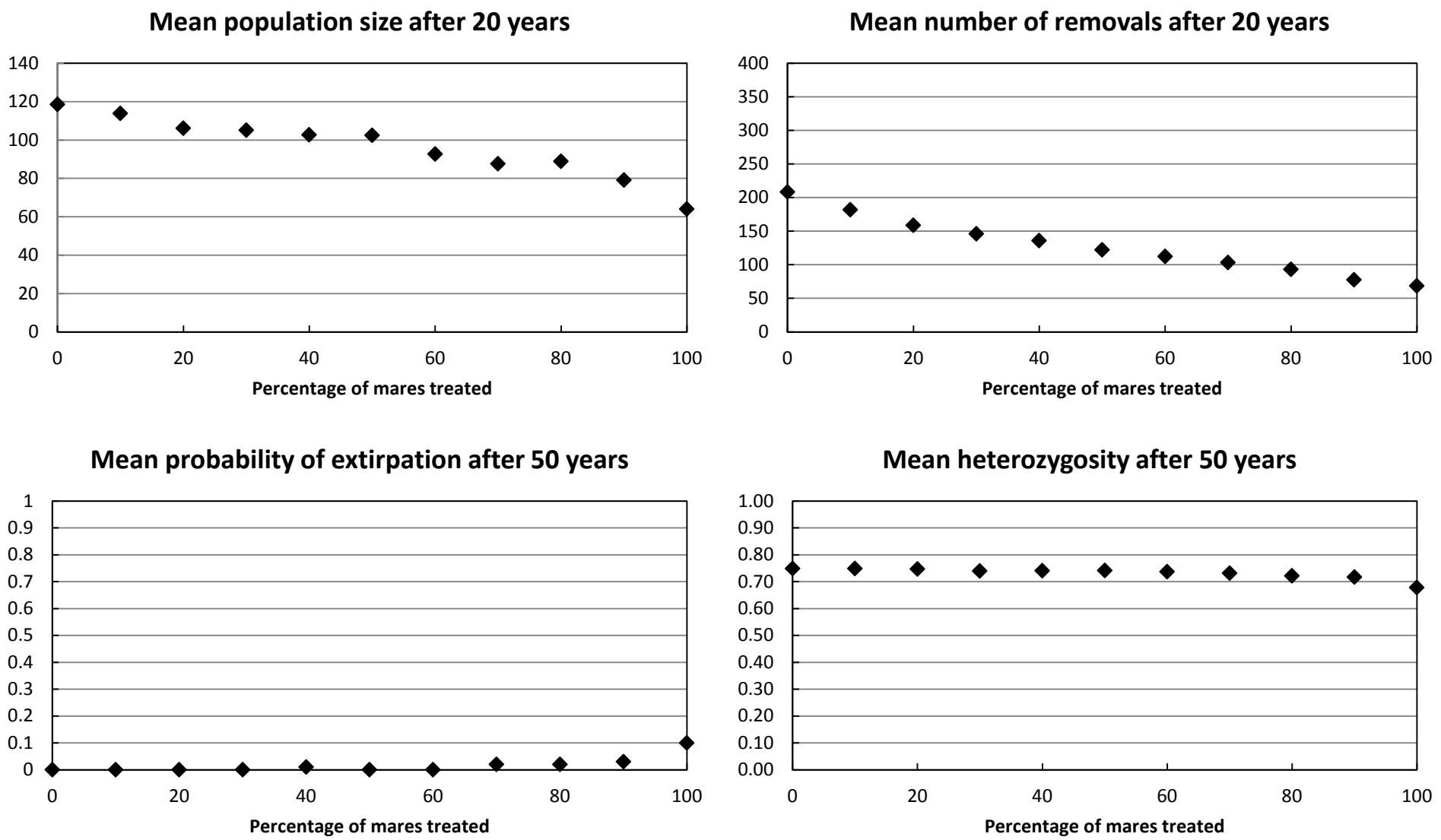


Figure 38. Initial population size = 100, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	118.53	113.82	106.1	105.09	102.72	102.37	92.64	87.6	88.79	79.15	63.95
Mean number of removals after 20 years	208.4	181.74	158.65	145.93	135.73	121.88	112.21	103.11	93.06	77.44	68.55
Mean probability of extirpation after 50 years	0	0	0	0	0.01	0	0	0.02	0.02	0.03	0.1
Mean heterozygosity after 50 years	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.72	0.72	0.68

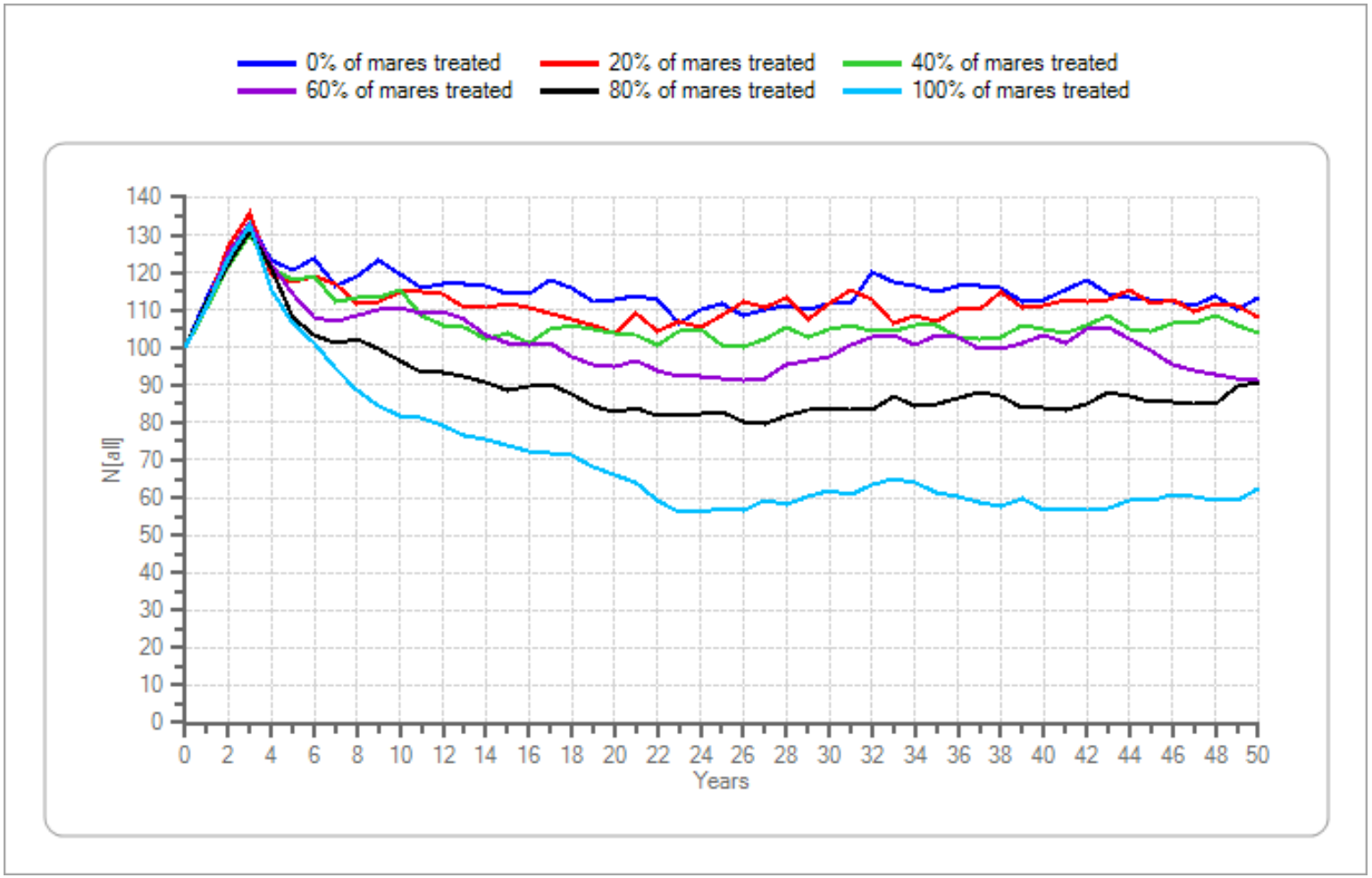


Figure 38. Initial population size = 100, lambda = 1.15, genetic diversity = high.—Continued

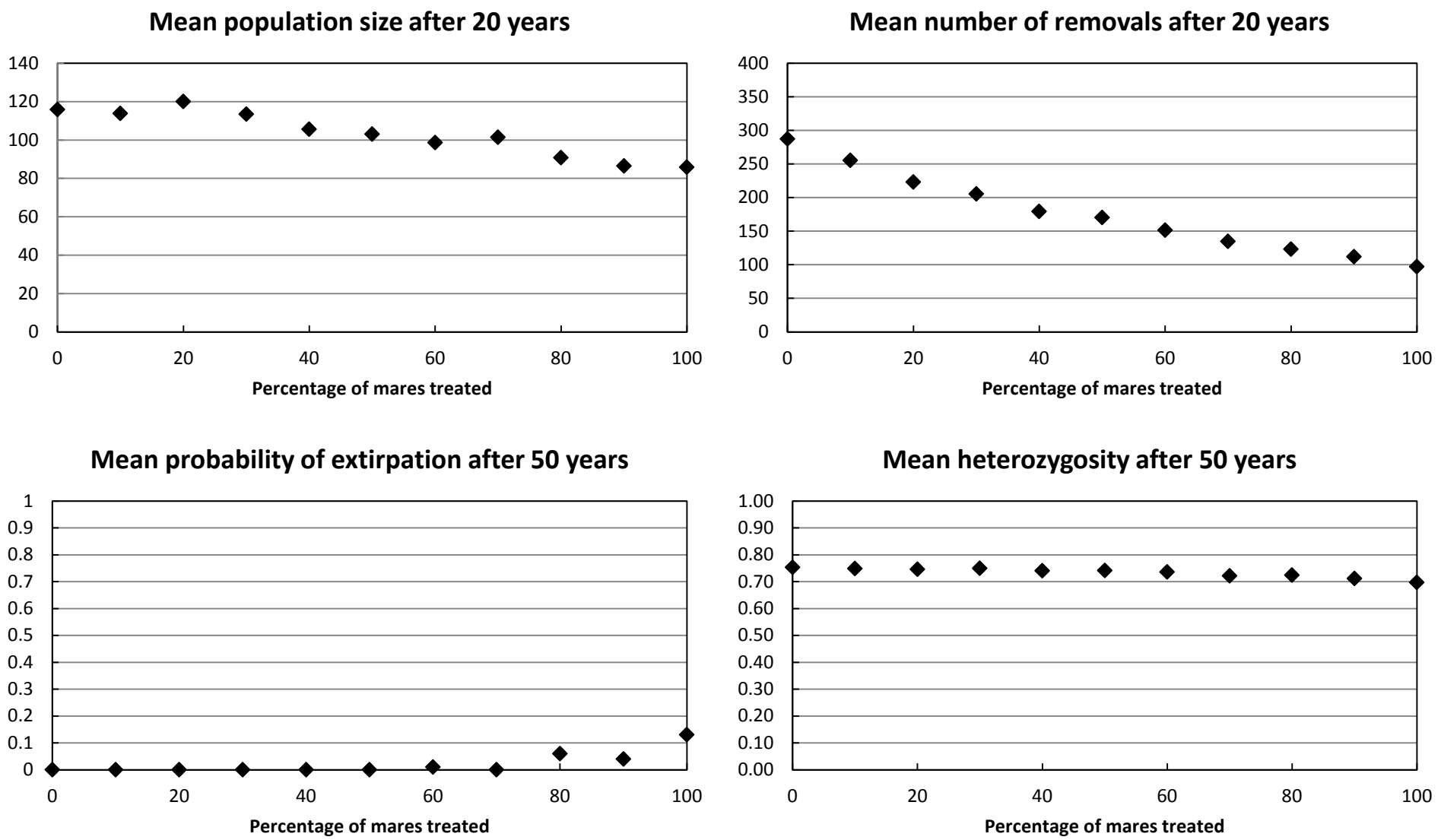


Figure 39. Initial population size = 100, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	115.87	113.82	120.06	113.47	105.64	102.99	98.66	101.46	90.77	86.41	85.86
Mean number of removals after 20 years	287.24	255.15	222.93	205.42	179.11	170.34	151.32	134.9	123.26	112.03	97.01
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0.01	0	0.06	0.04	0.13
Mean heterozygosity after 50 years	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.72	0.72	0.71	0.70

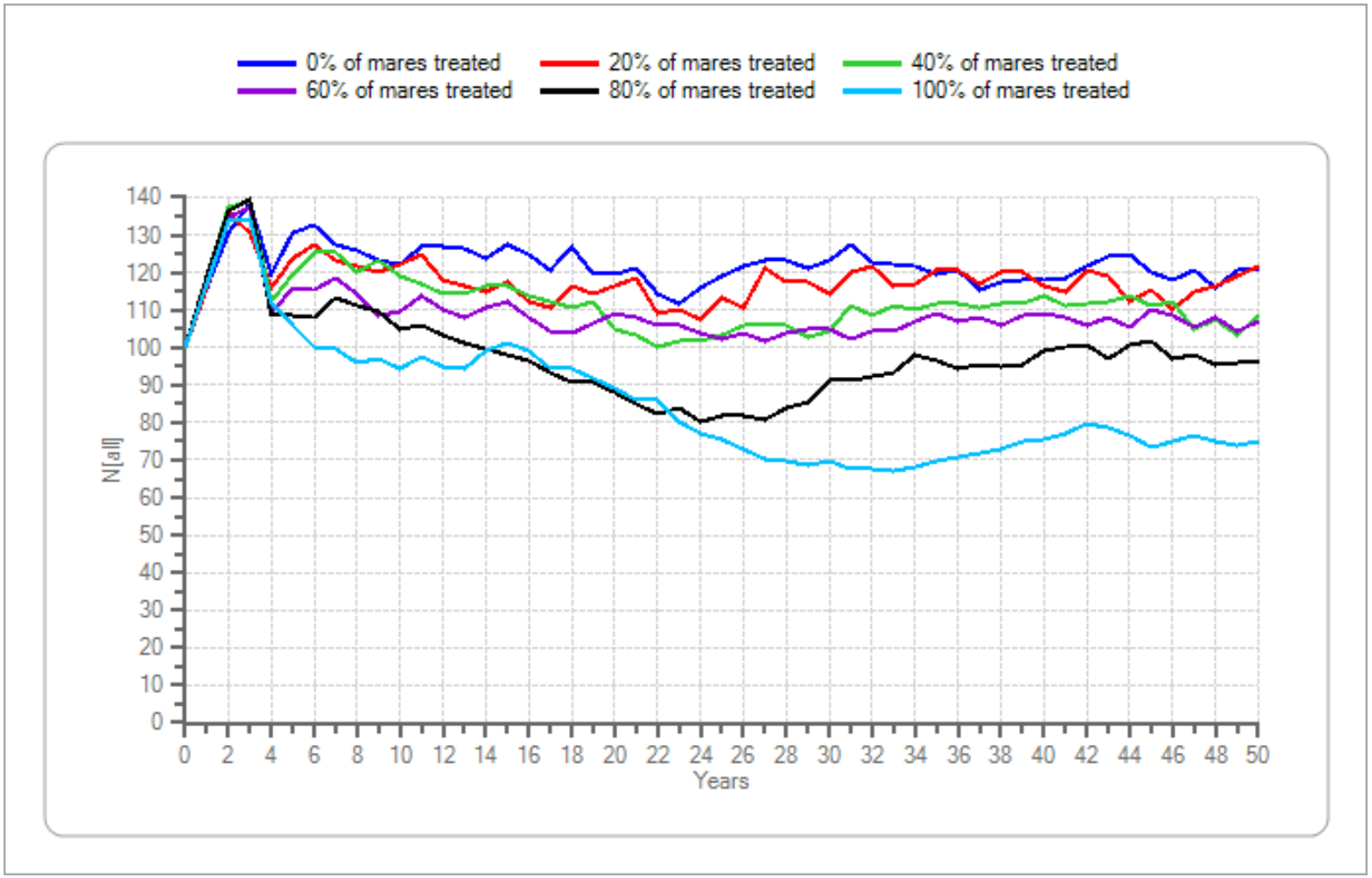


Figure 39. Initial population size = 100, lambda = 1.20, genetic diversity = high.—Continued

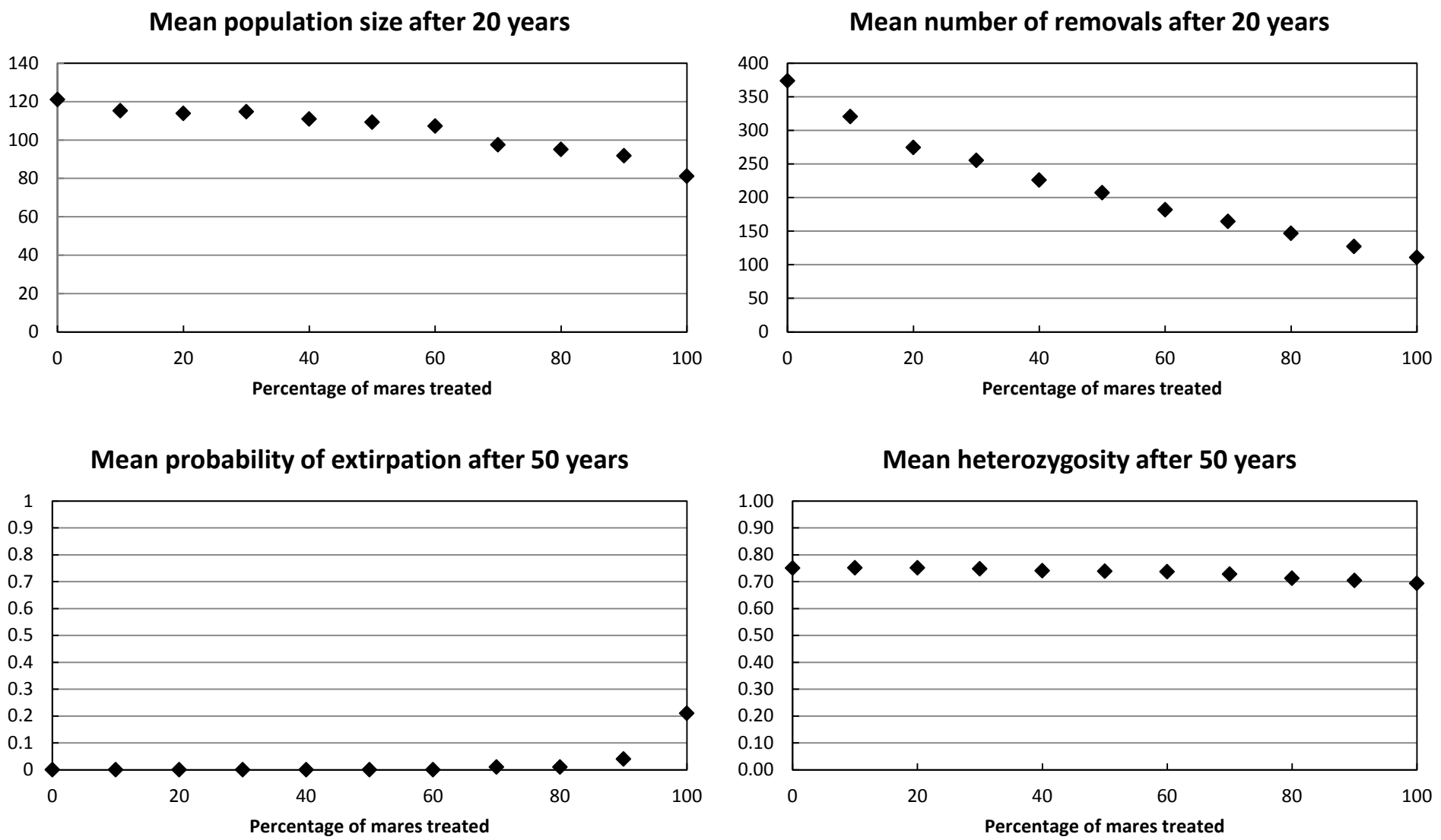


Figure 40. Initial population size = 100, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	121.02	115.17	113.84	114.66	110.88	109.29	107.18	97.48	95.07	91.82	81.06
Mean number of removals after 20 years	373.76	320.53	274.59	255.43	225.87	207.24	181.97	164.56	146.74	127.02	110.7
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0.01	0.01	0.04	0.21
Mean heterozygosity after 50 years	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.73	0.71	0.70	0.69

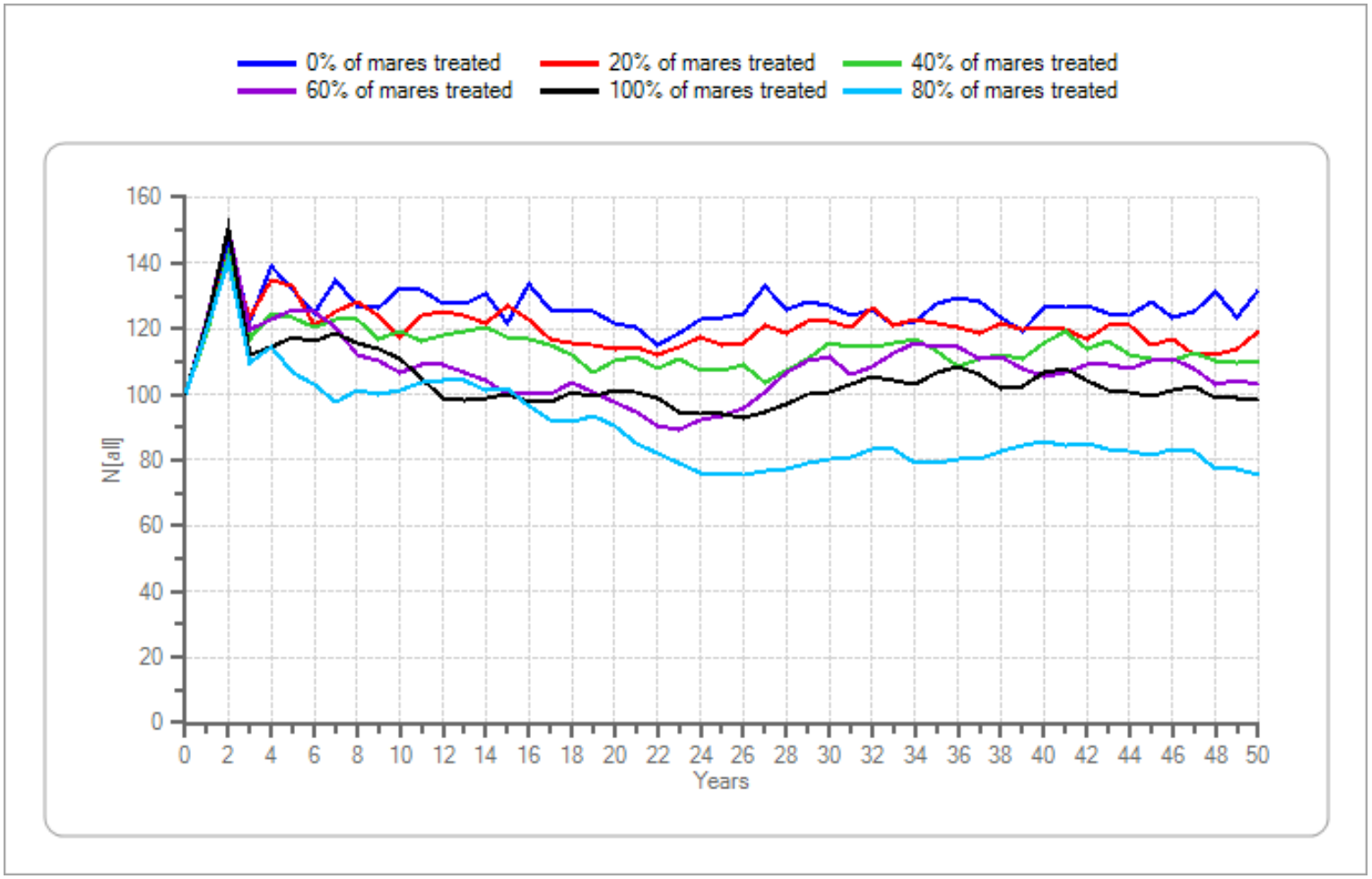
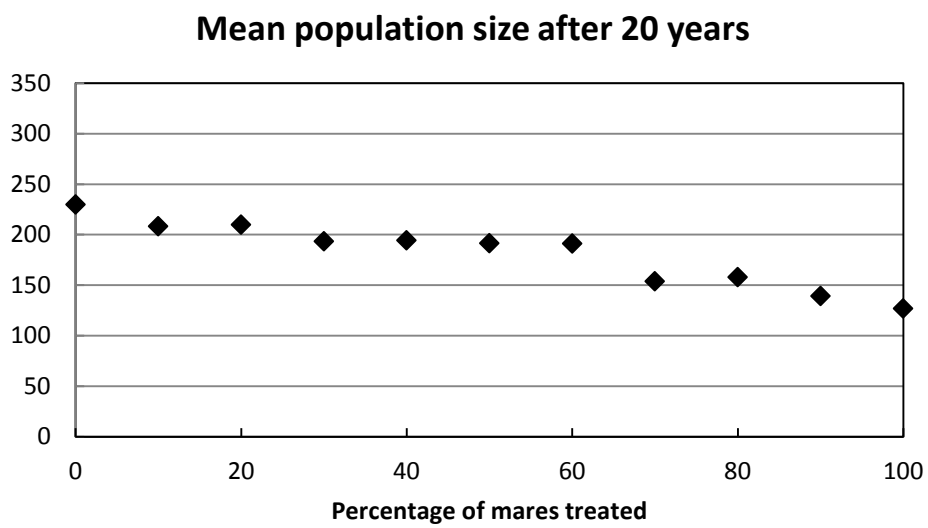
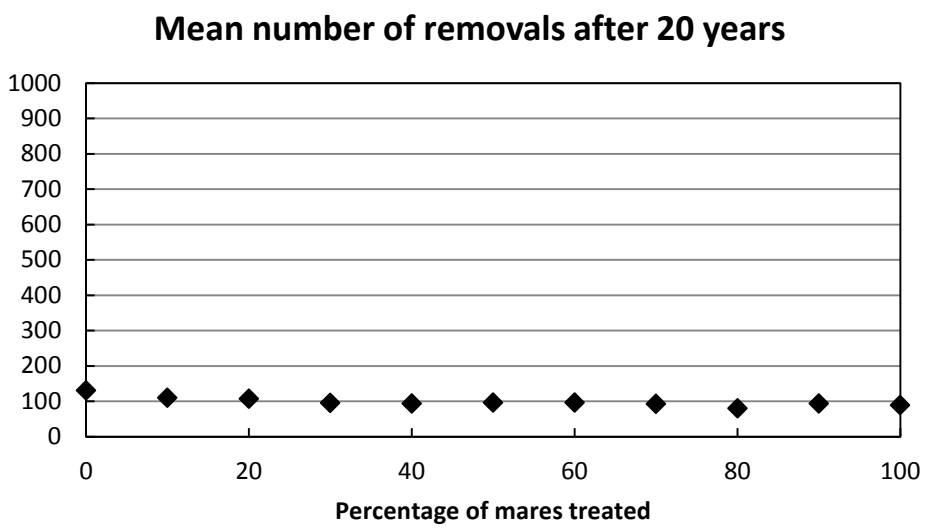
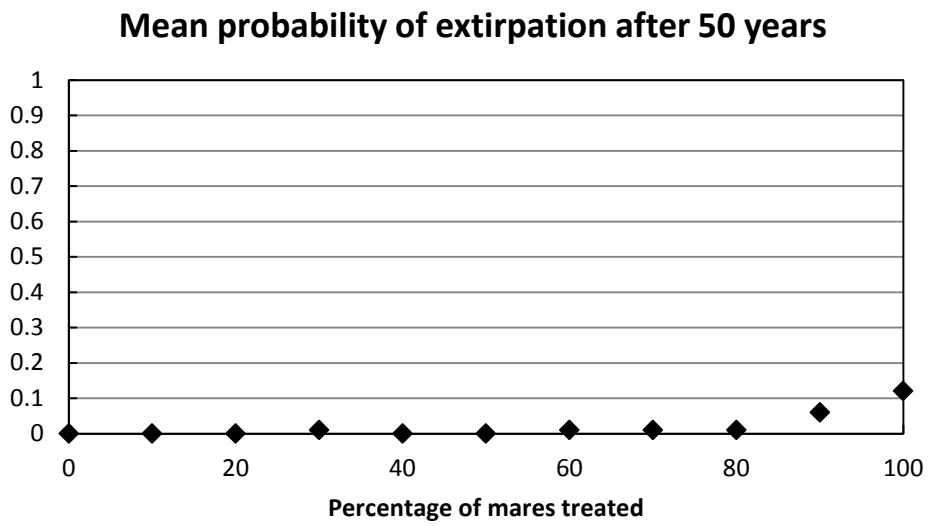


Figure 40. Initial population size = 100, lambda = 1.25, genetic diversity = high.—Continued







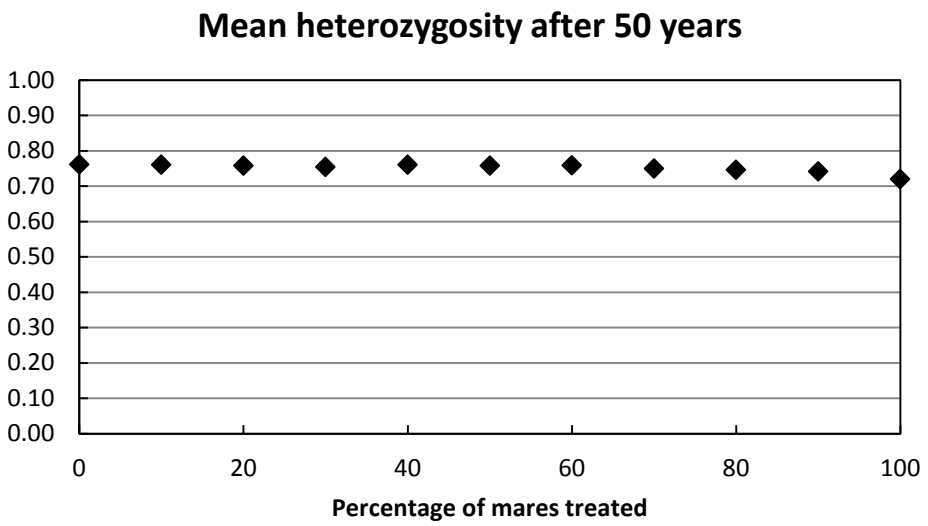


Figure 41. Initial population size = 250, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	229.82	208.28	209.81	193.14	194.37	191.52	191.17	153.65	157.95	139.22	126.74
Mean number of removals after 20 years	130.33	109.21	106.87	95.15	93.21	95.93	96.1	91.98	79.41	93.08	89.13
Mean probability of extirpation after 50 years	0	0	0	0.01	0	0	0.01	0.01	0.01	0.06	0.12
Mean heterozygosity after 50 years	0.76	0.76	0.76	0.75	0.76	0.76	0.76	0.75	0.75	0.74	0.72

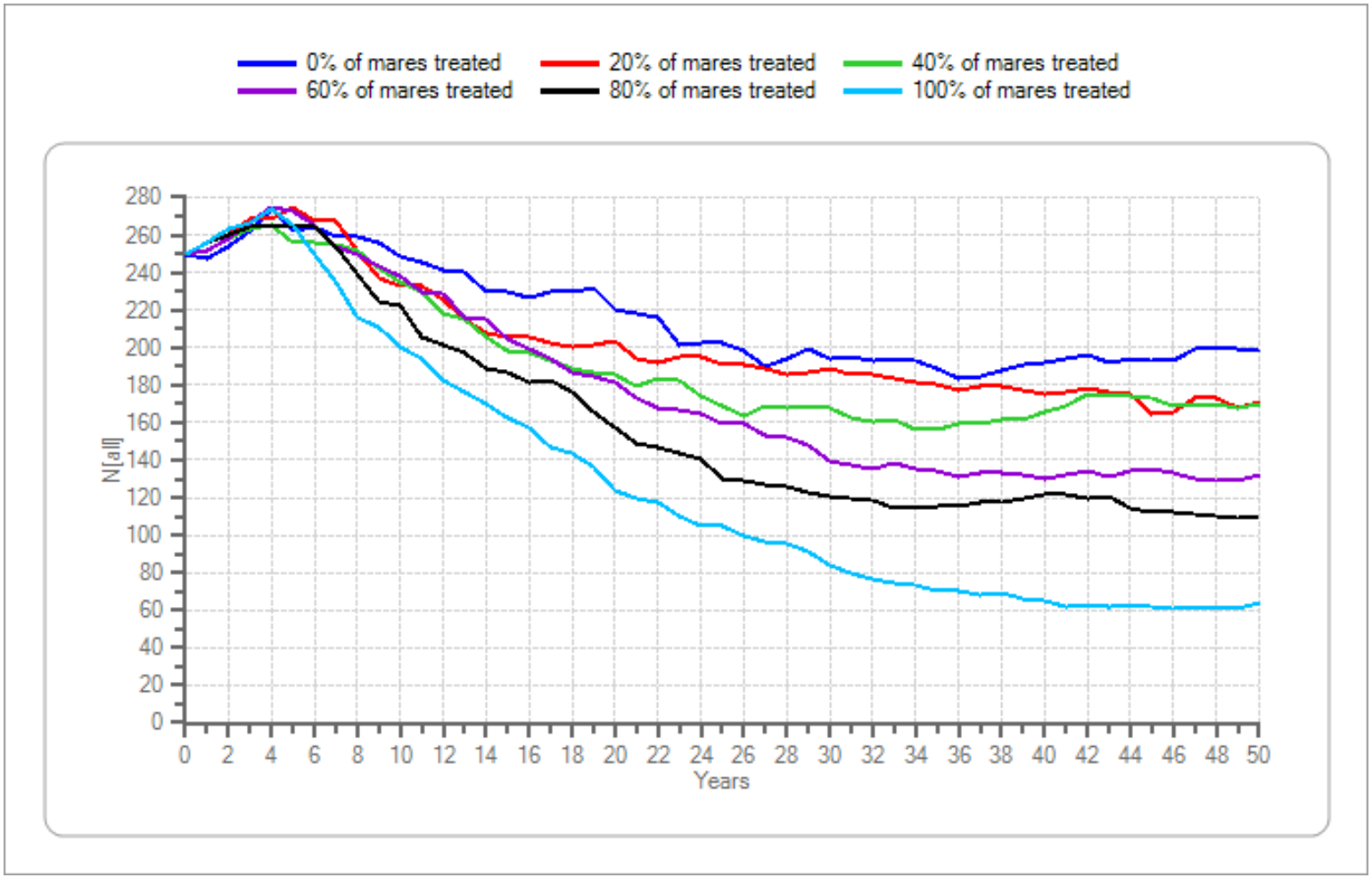
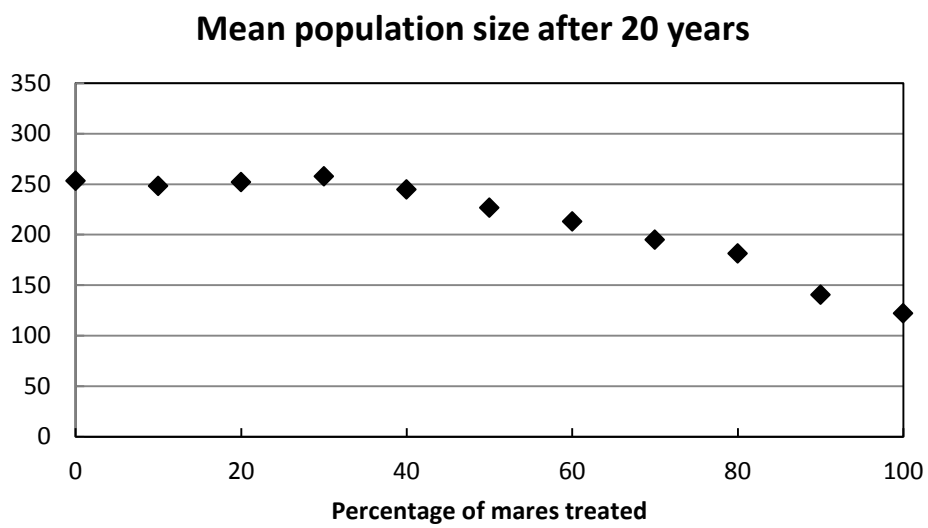
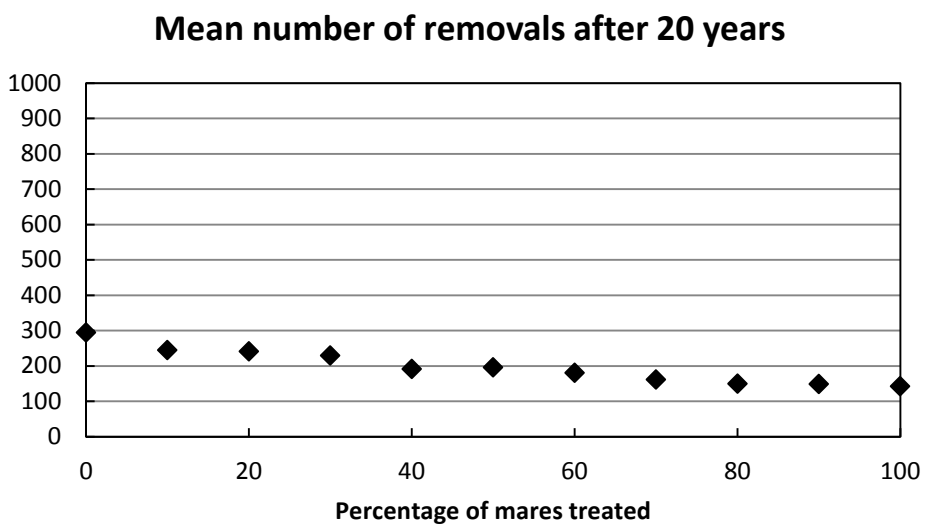
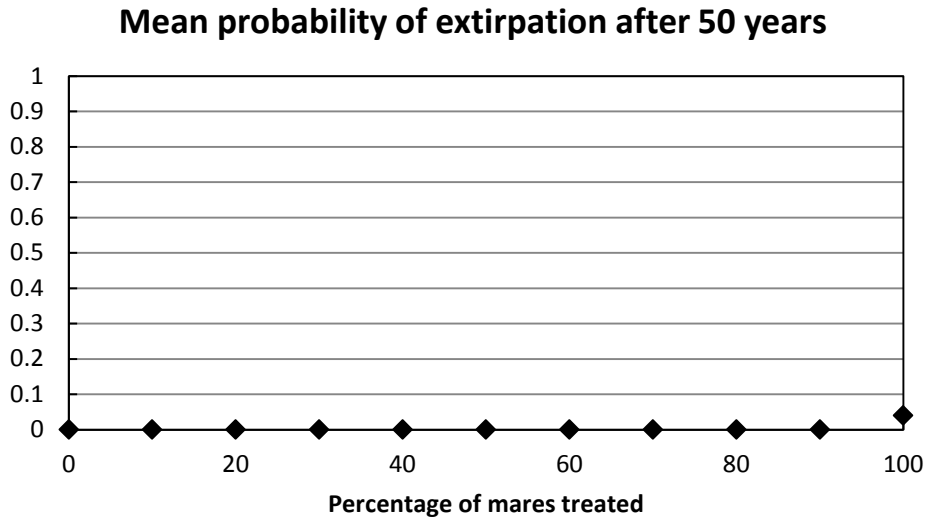


Figure 41. Initial population size = 250, lambda = 1.05, genetic diversity = high.—Continued







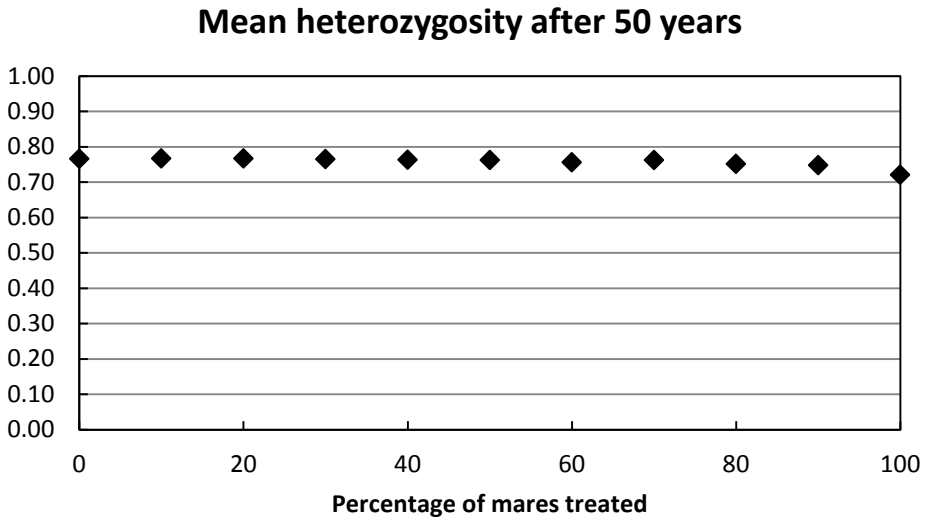


Figure 42. Initial population size = 250, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	253.29	248.13	251.86	257.66	244.53	226.55	212.8	194.92	181.23	140.26	121.89
Mean number of removals after 20 years	294.42	244.72	240.7	229.15	190.87	195.41	180.34	161.04	149.36	148.76	141.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.04
Mean heterozygosity after 50 years	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.75	0.75	0.72

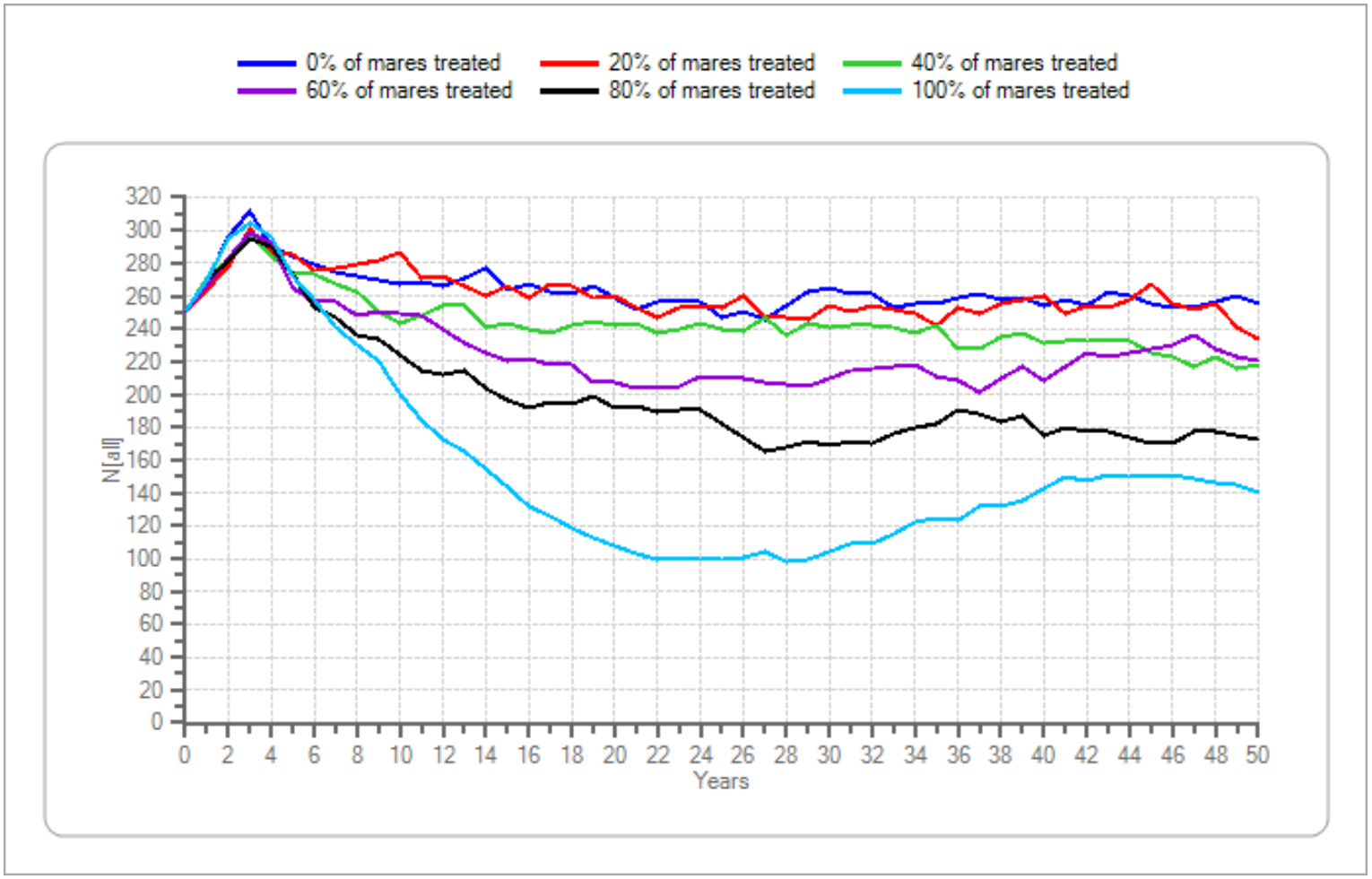
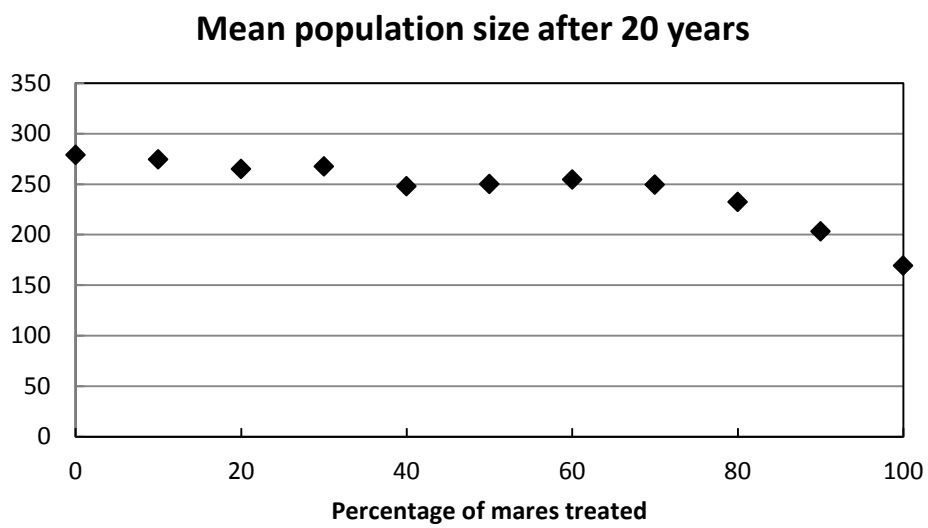
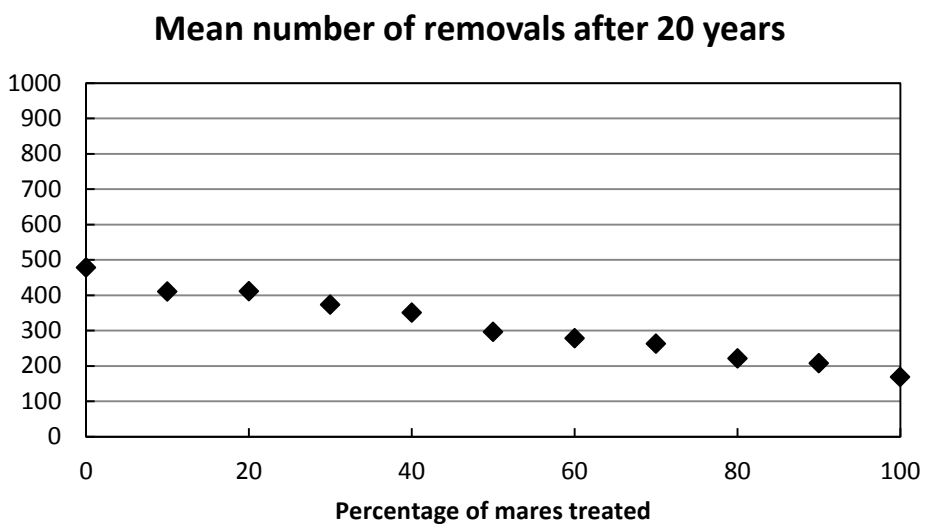
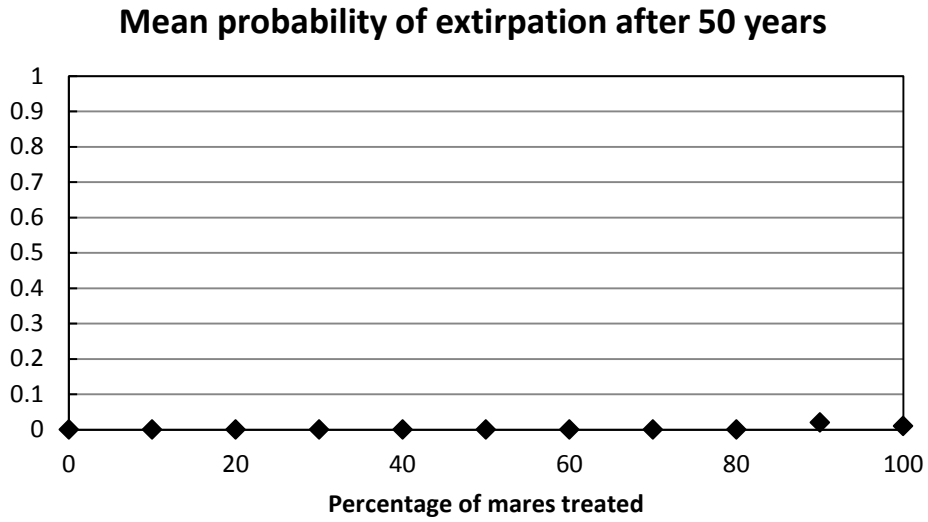


Figure 42. Initial population size = 250, lambda = 1.10, genetic diversity = high.—Continued







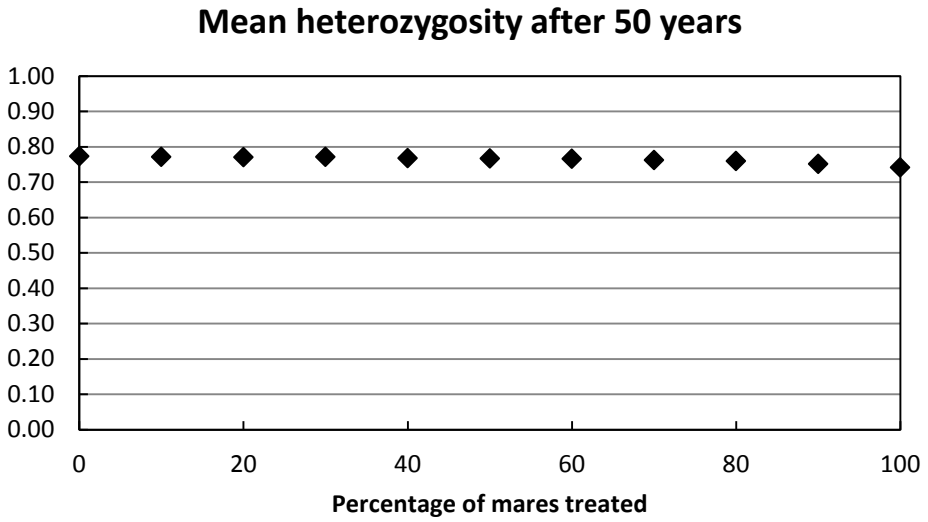


Figure 43. Initial population size = 250, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	278.95	274.39	264.77	267.48	247.95	249.86	254.3	249.36	232.19	203.01	169.11
Mean number of removals after 20 years	478.13	410.55	411.23	373.12	350.21	295.77	278.33	262.32	220.73	207.34	168.69
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0.02	0.01
Mean heterozygosity after 50 years	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.75	0.74

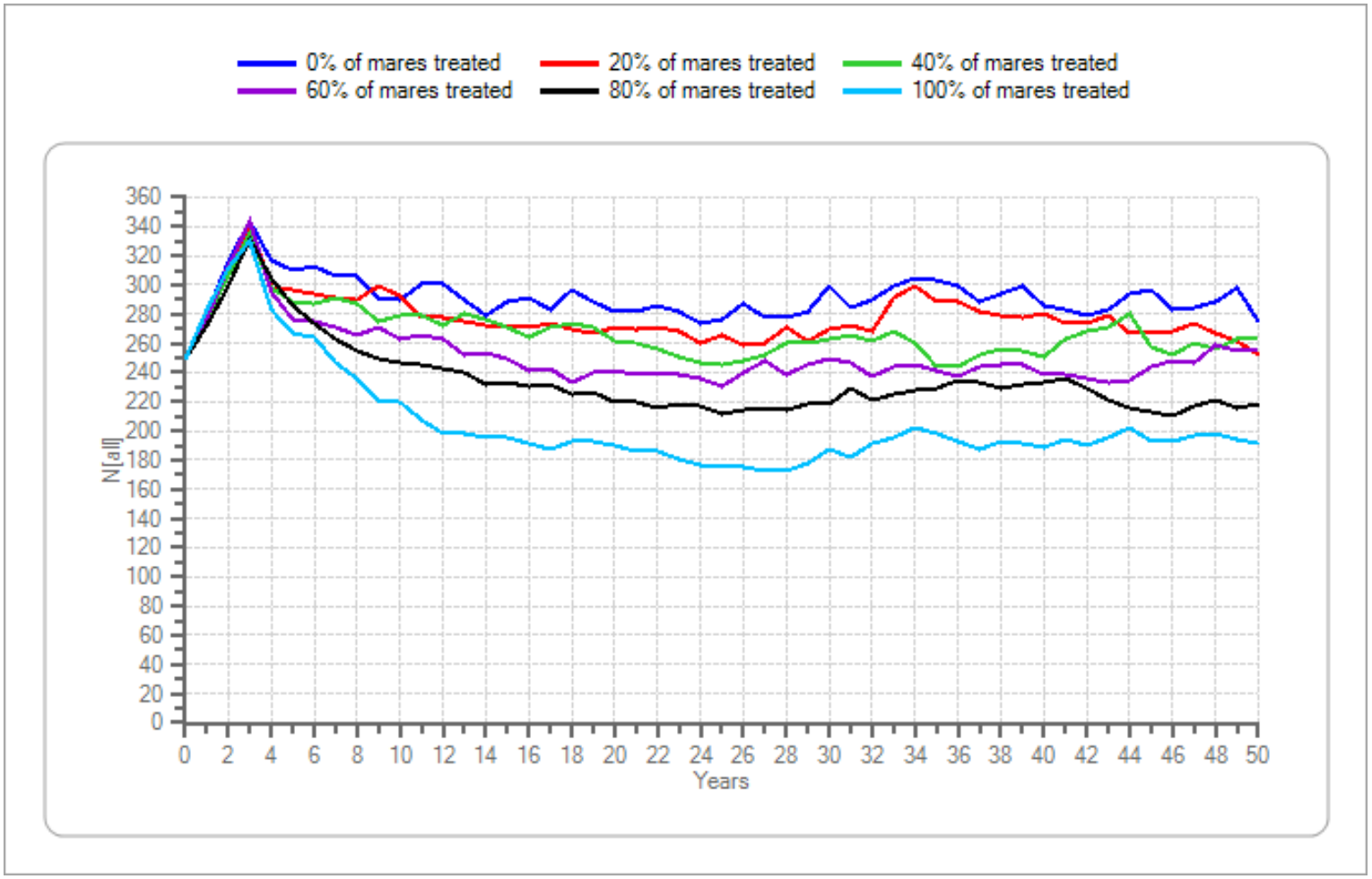
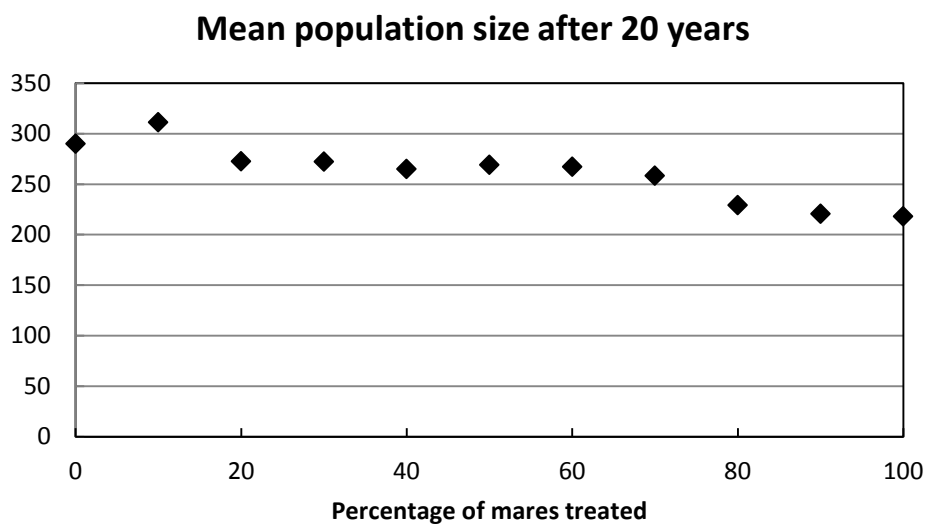
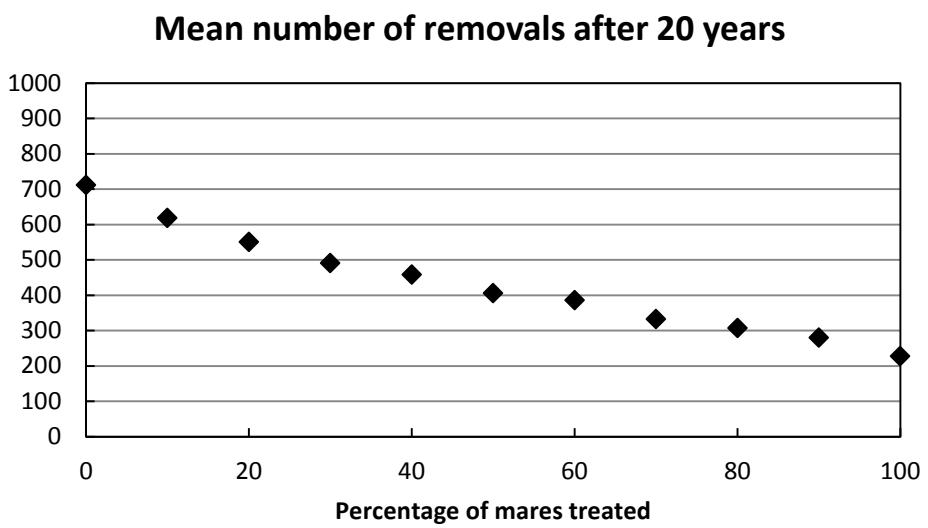
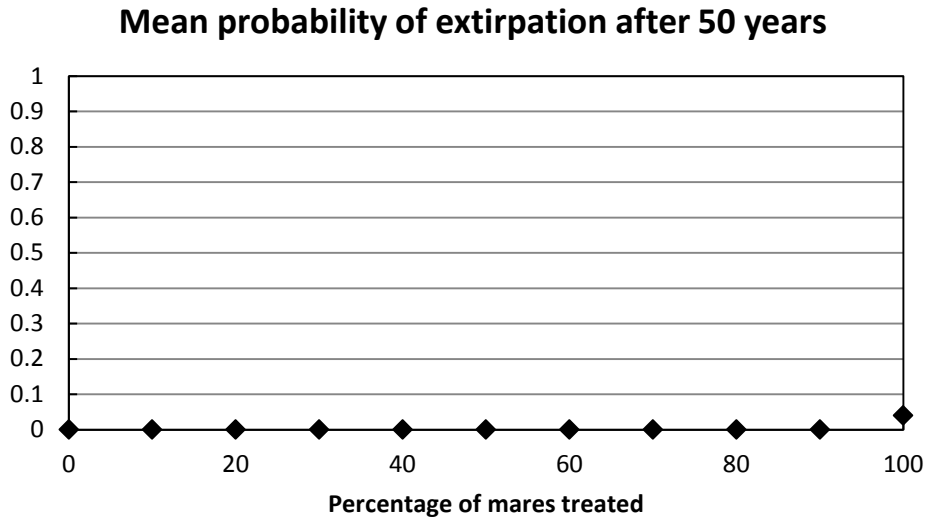


Figure 43. Initial population size = 250, lambda = 1.15, genetic diversity = high.—Continued







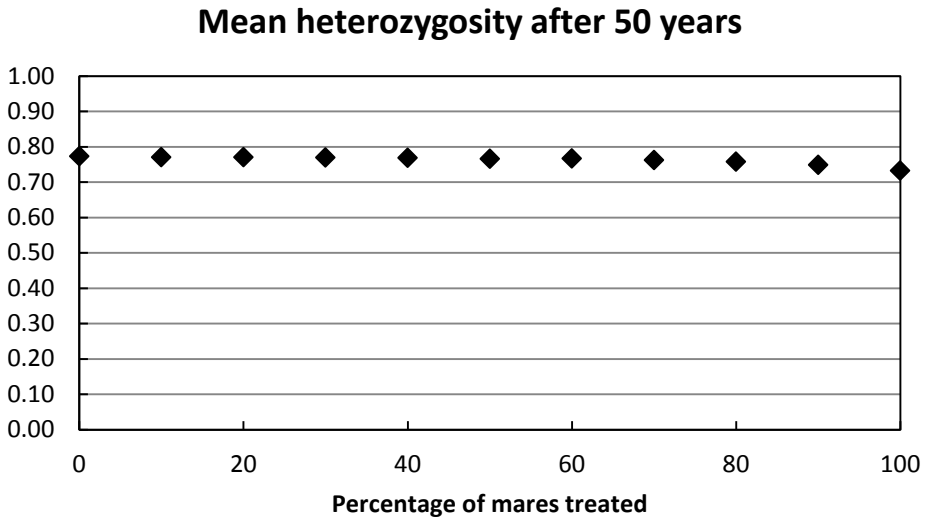


Figure 44. Initial population size = 250, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	290.06	311.21	272.6	272.05	264.99	269.08	267.14	258.33	229.2	220.46	218.12
Mean number of removals after 20 years	711.9	618.06	550.39	491.13	458.39	405.16	385.49	331.92	307.21	279.49	227.5
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.04
Mean heterozygosity after 50 years	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.75	0.73

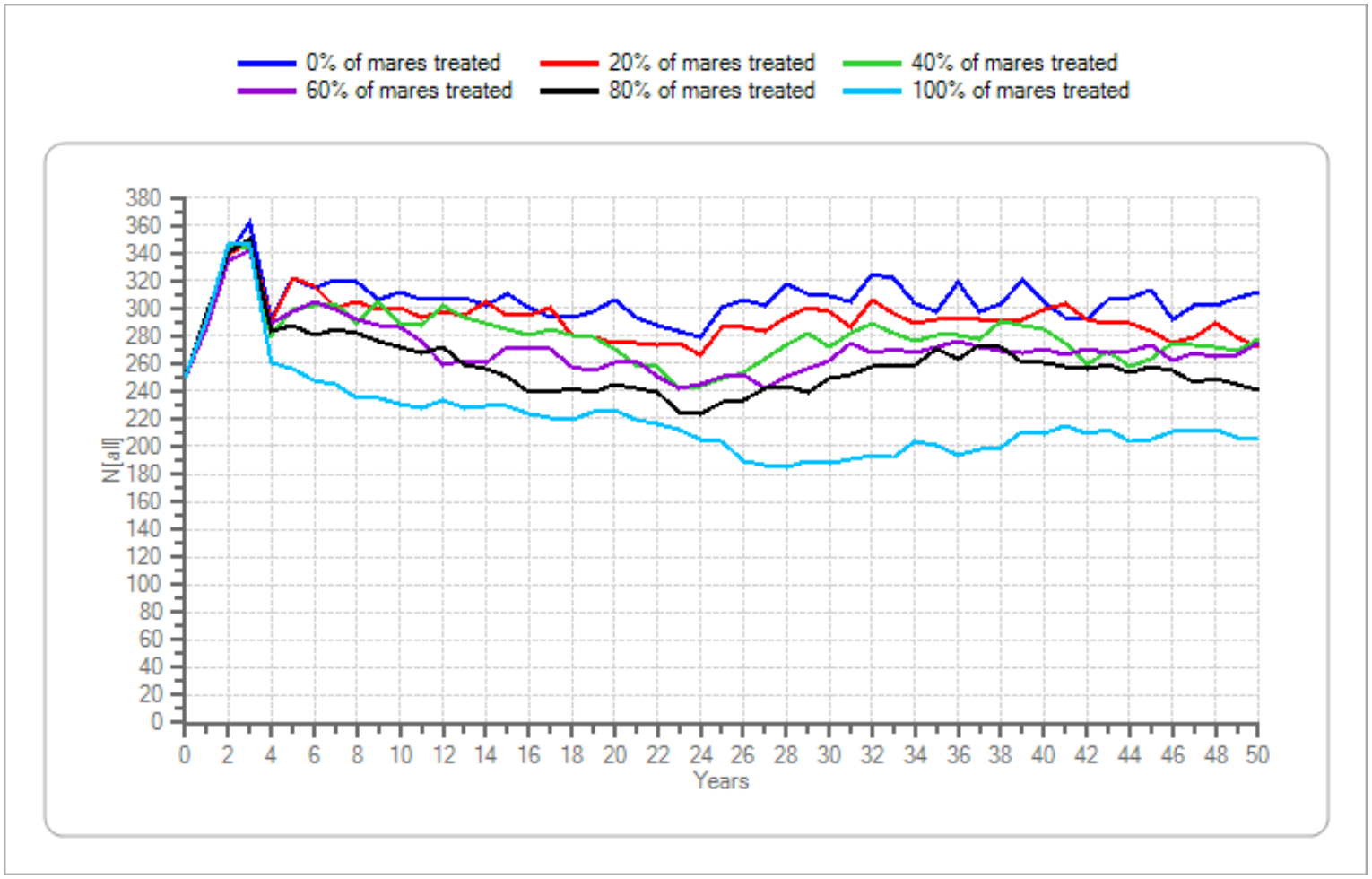
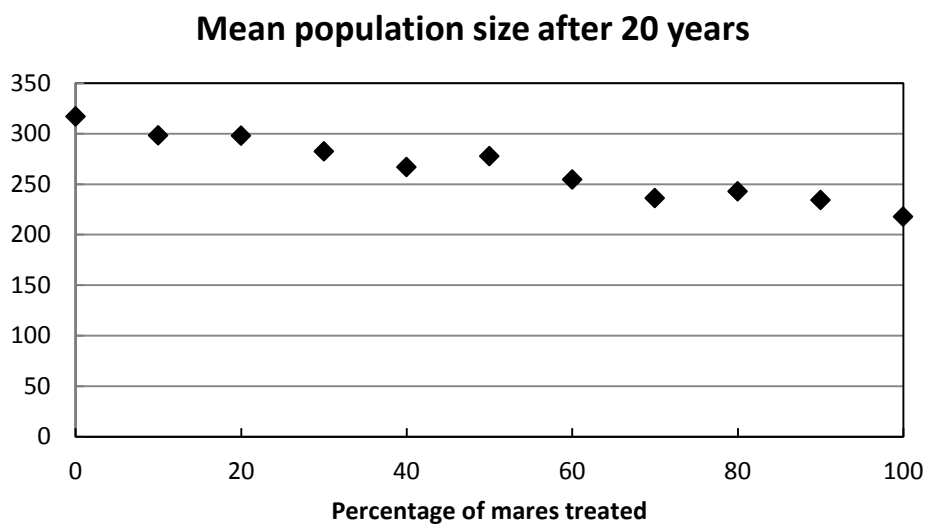
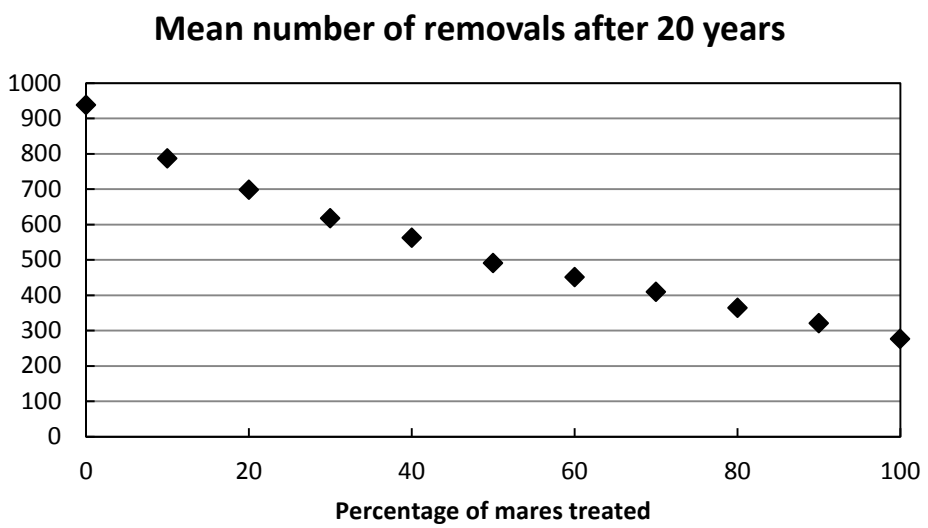
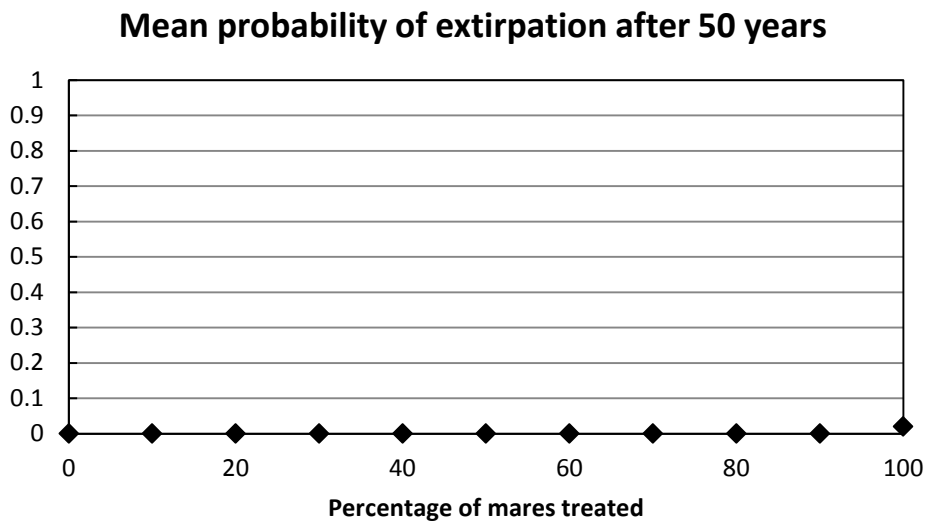


Figure 44. Initial population size = 250, lambda = 1.20, genetic diversity = high.—Continued







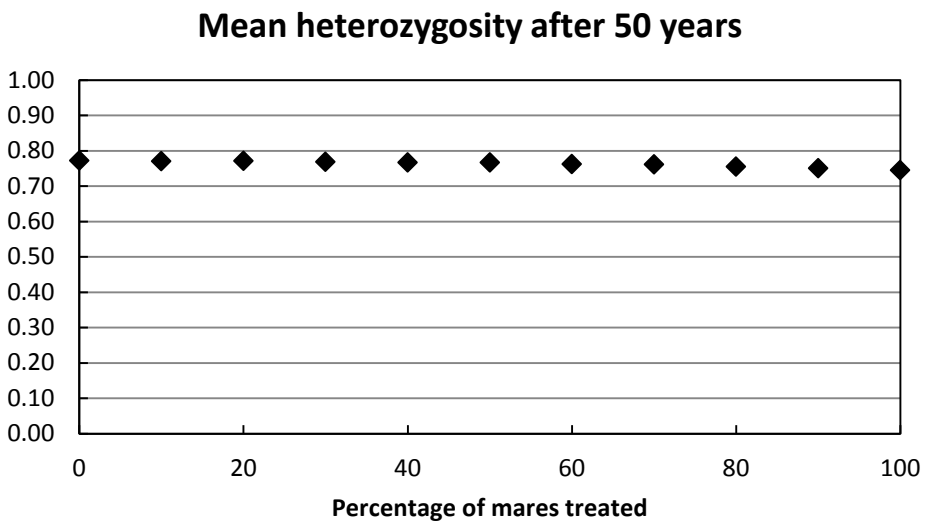


Figure 45. Initial population size = 250, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	316.89	298.09	297.94	282.24	266.75	277.65	254.56	236.14	242.76	234.27	217.59
Mean number of removals after 20 years	937.55	786.93	697.69	617.57	562.54	490.47	450.66	409.16	363.89	320.86	276.24
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.02
Mean heterozygosity after 50 years	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.75	0.75

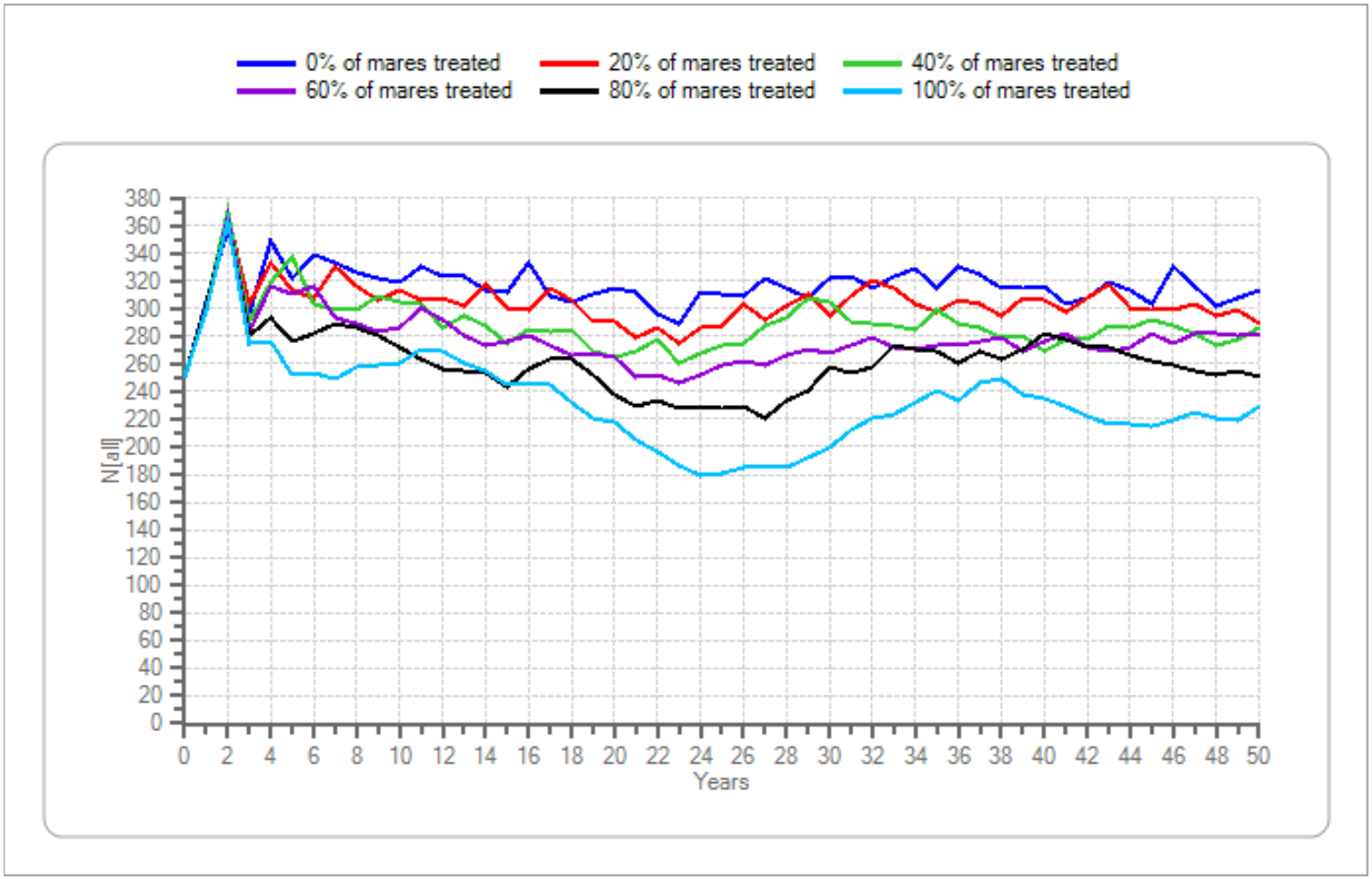
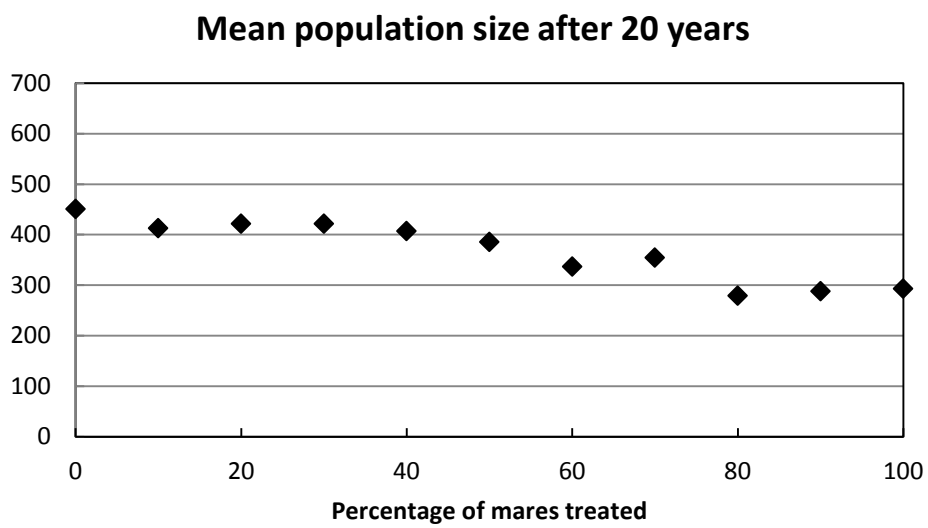
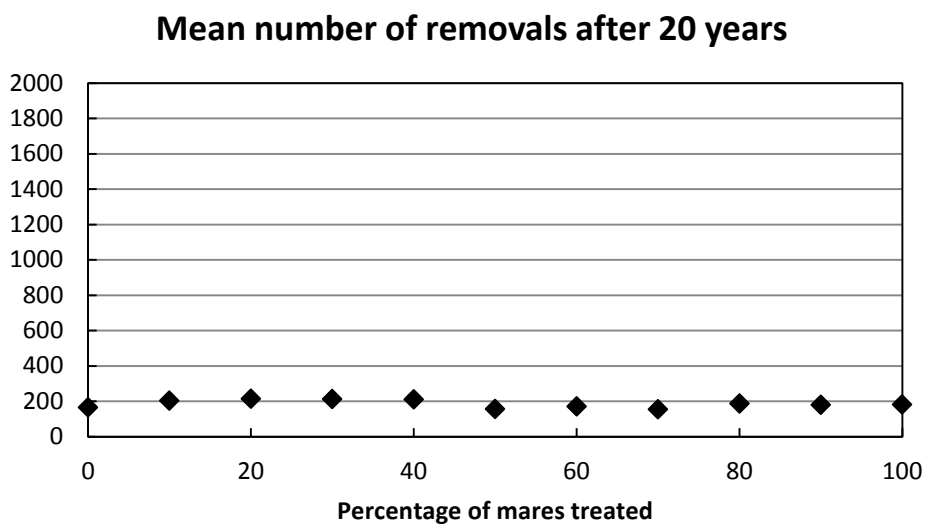
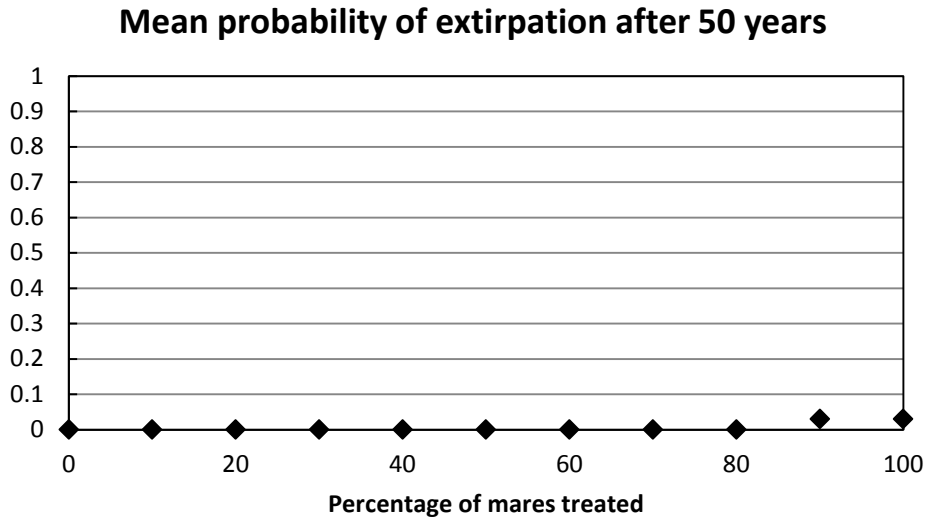


Figure 45. Initial population size = 250, lambda = 1.25, genetic diversity = high.—Continued







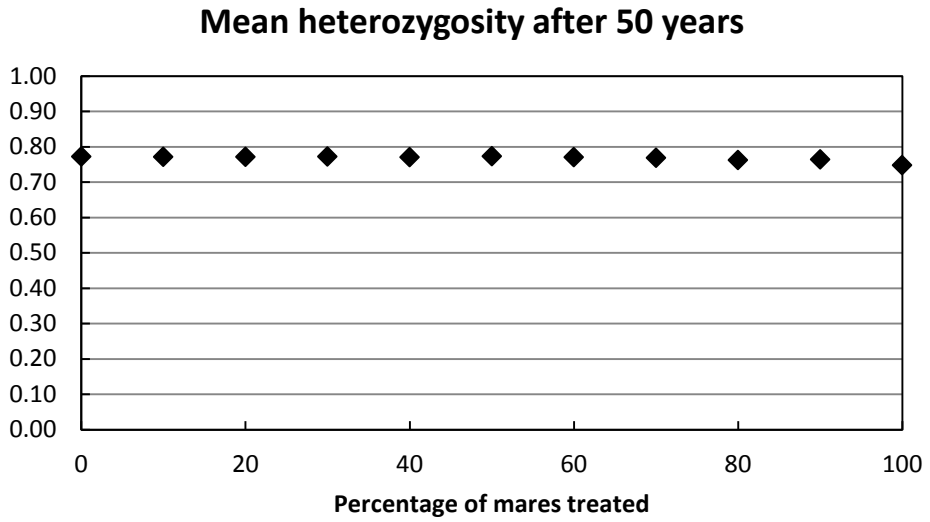


Figure 46. Initial population size = 500, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	450.57	412.67	421.58	421.47	407.01	385.44	336.29	354.49	278.69	287.98	292.7
Mean number of removals after 20 years	165.39	203.66	214.47	212.76	209.18	155.55	170.07	154.49	186.23	178.59	180.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0.03	0.03
Mean heterozygosity after 50 years	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.75

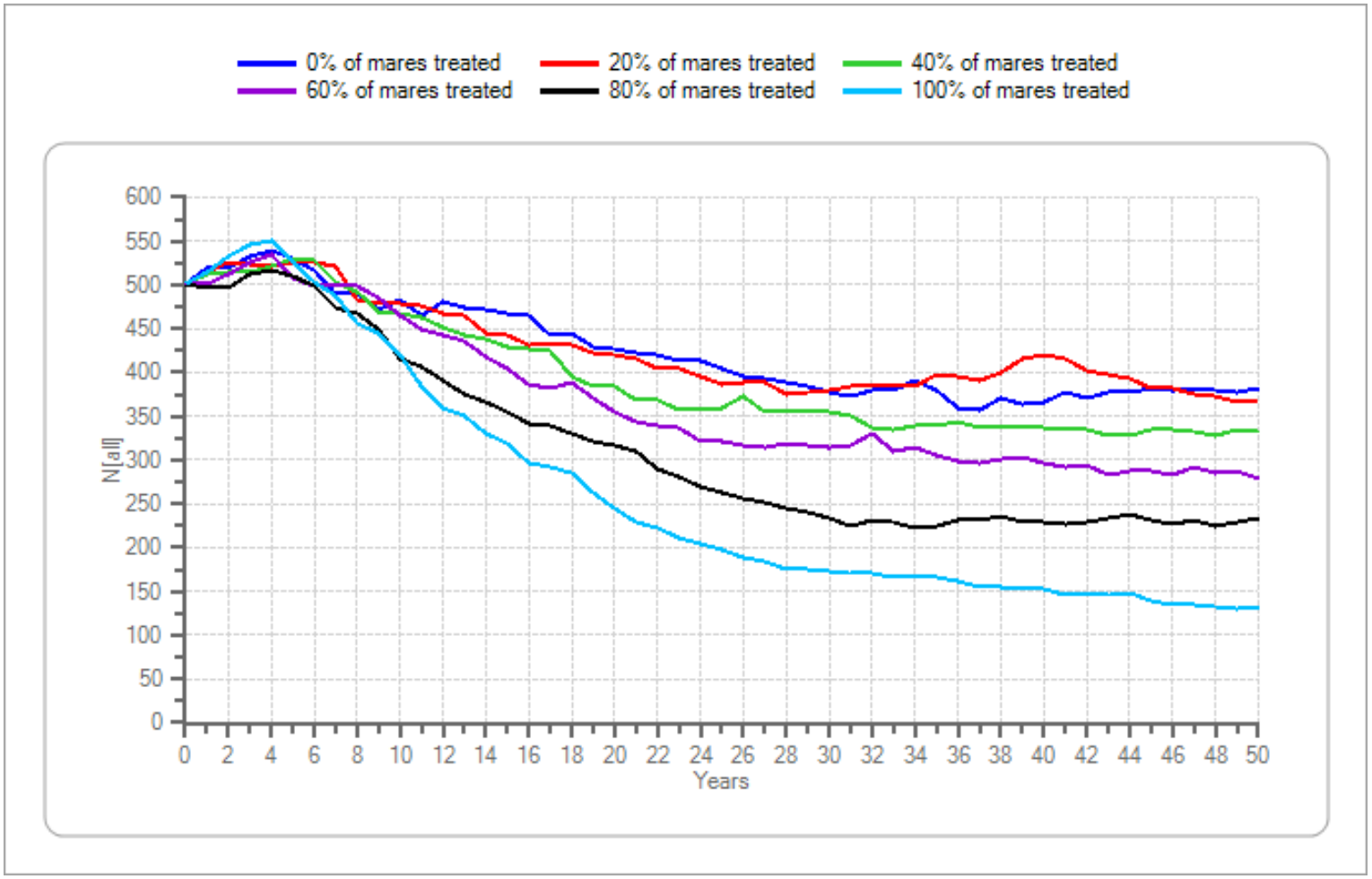
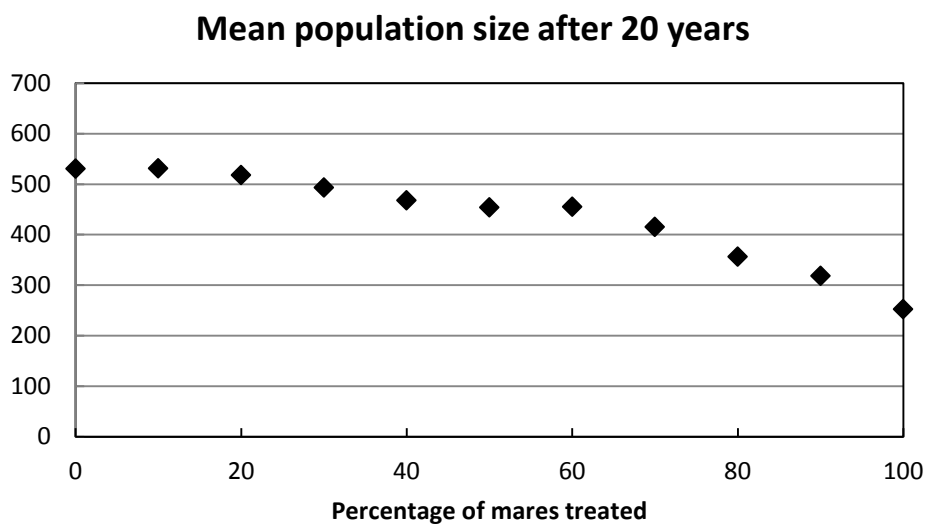
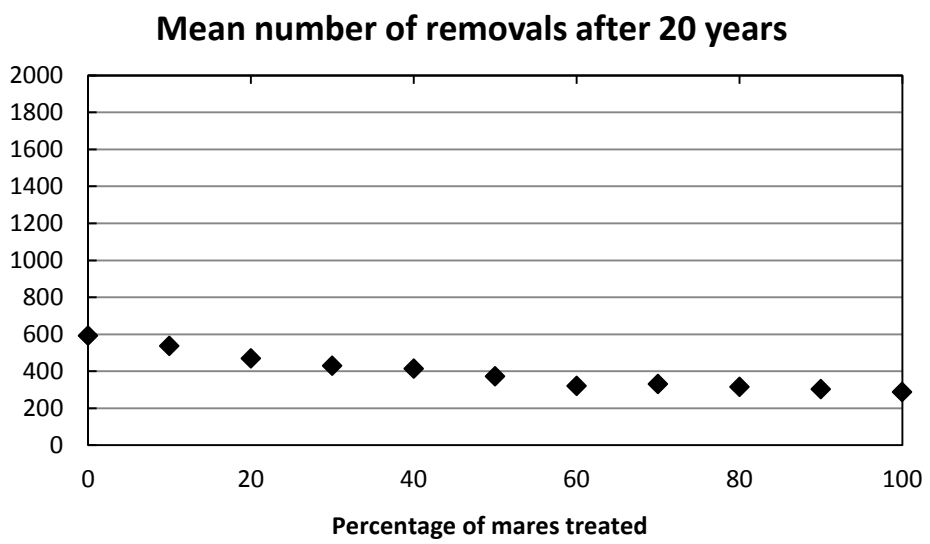
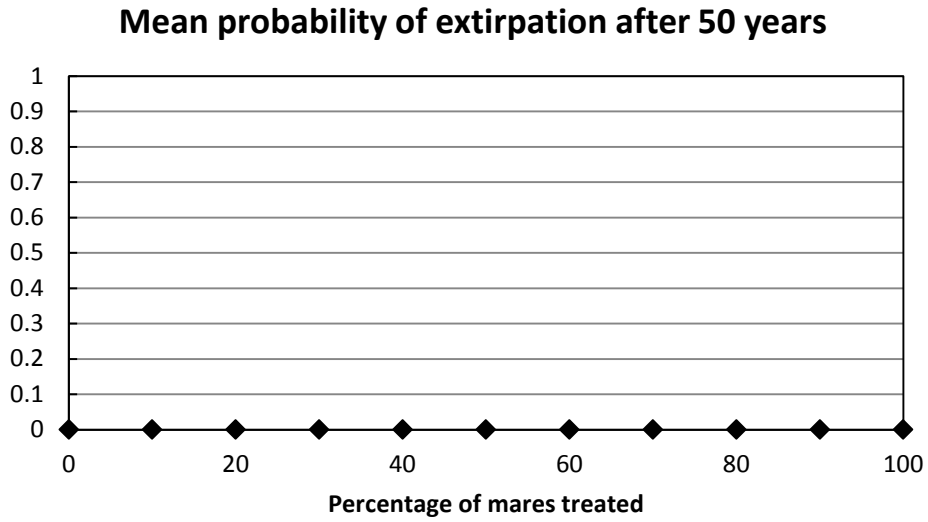


Figure 46. Initial population size = 500, lambda = 1.05, genetic diversity = high.—Continued







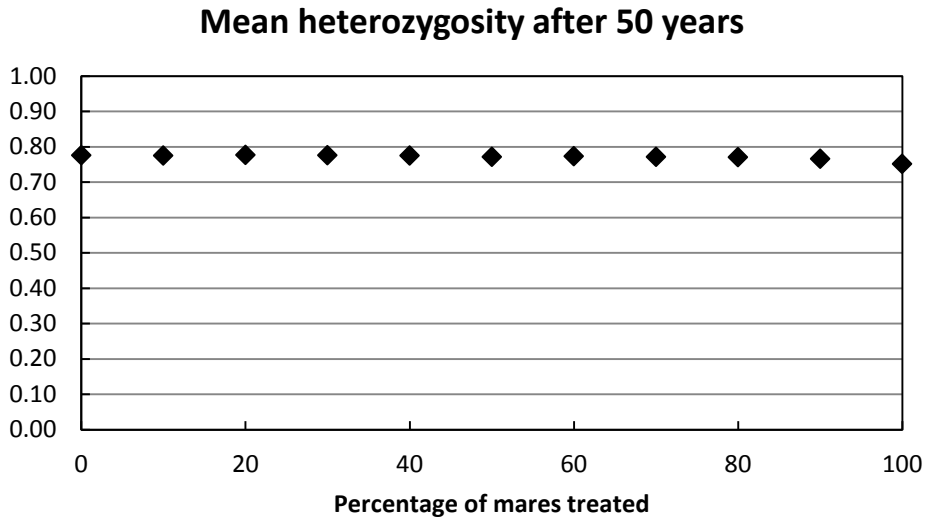


Figure 47. Initial population size = 500, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	530.61	530.85	517.52	493.18	467.76	453.59	454.75	415.17	356.16	318.2	252.43
Mean number of removals after 20 years	591.77	536.39	469.19	428.35	413.44	371.39	319.96	329.77	315.36	302.16	286.23
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.77	0.77	0.77	0.77	0.77	0.77	0.75

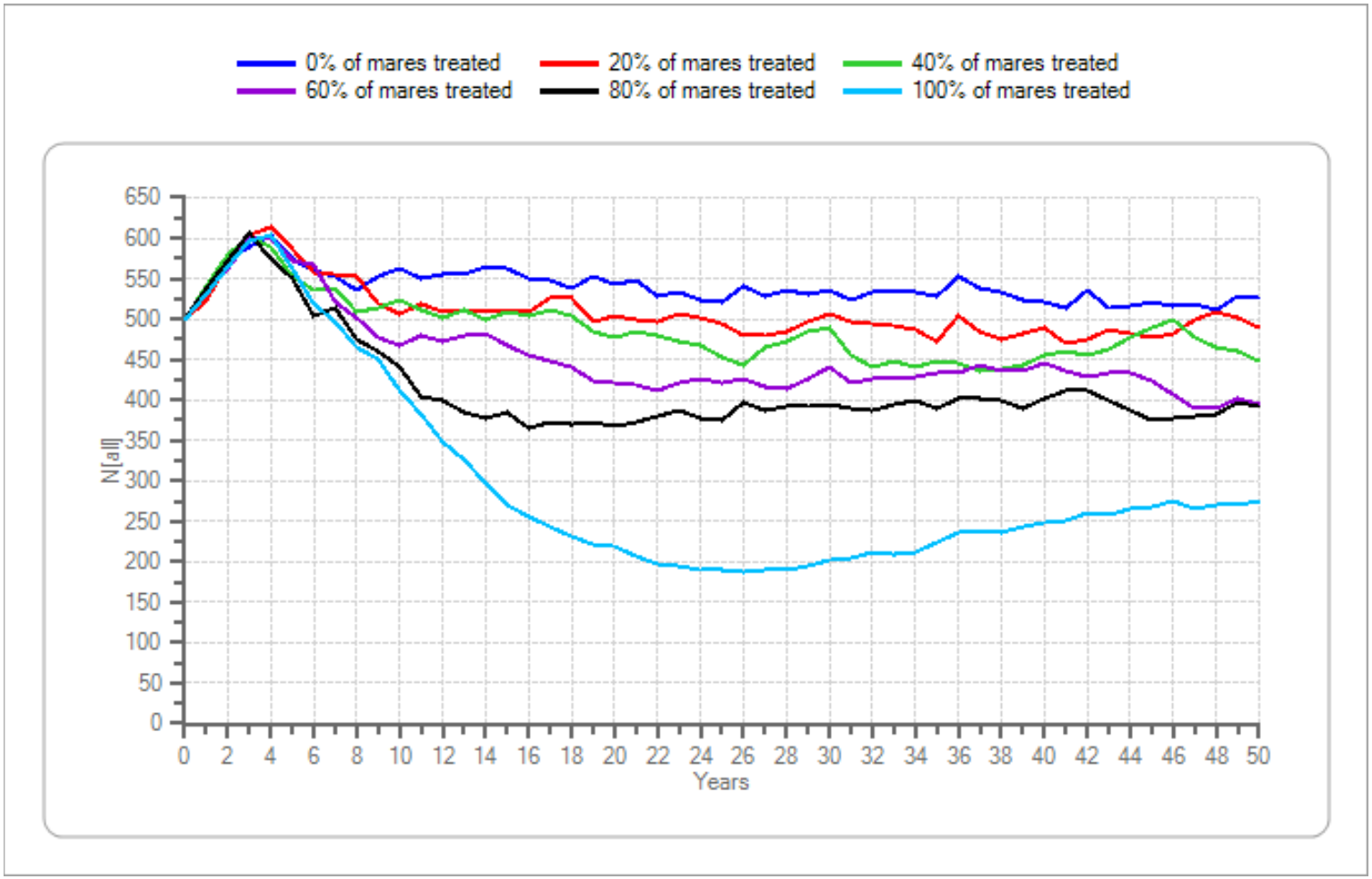
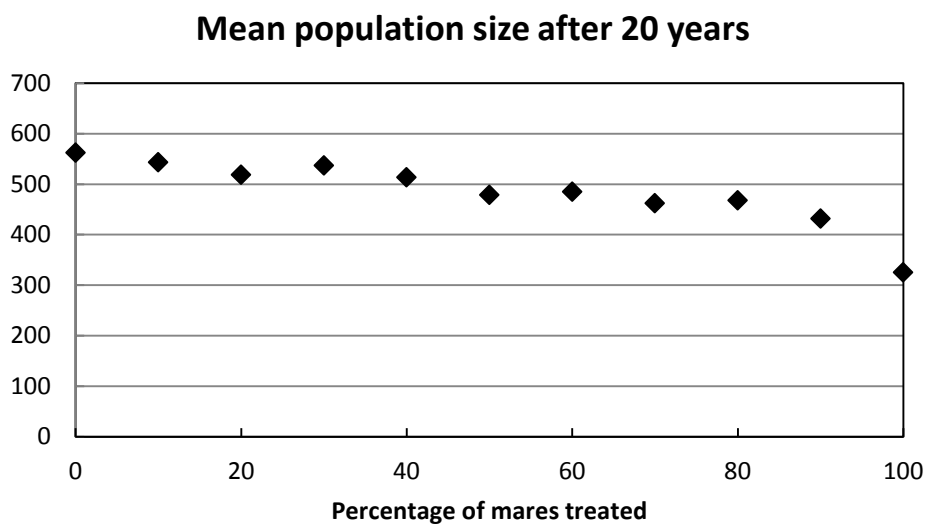
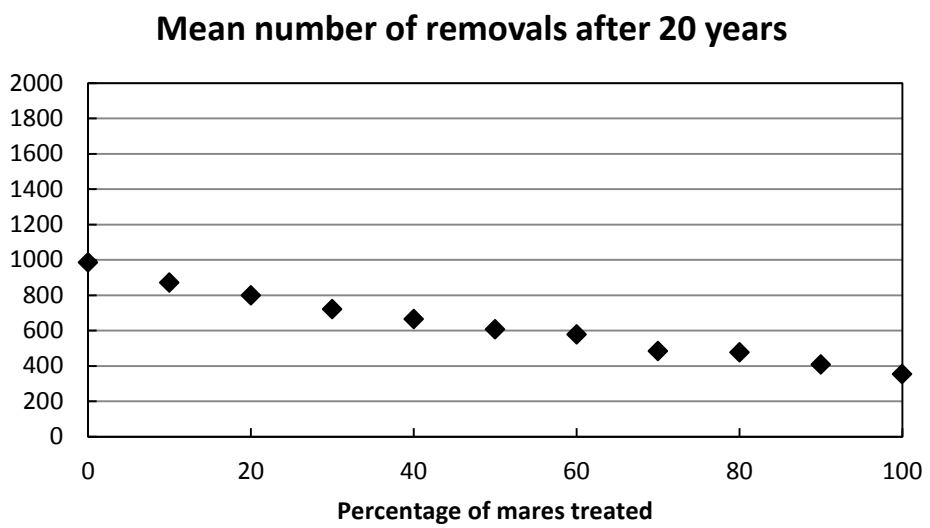
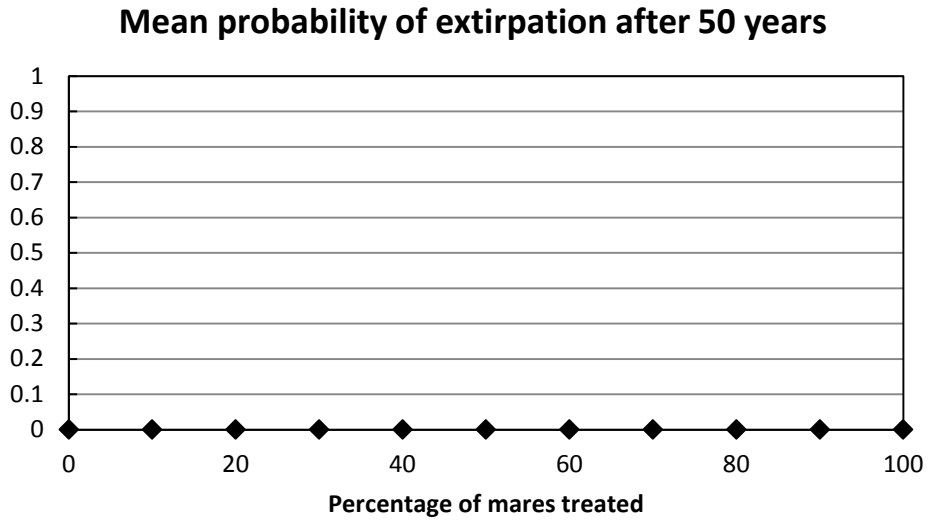


Figure 47. Initial population size = 500, lambda = 1.10, genetic diversity = high.—Continued







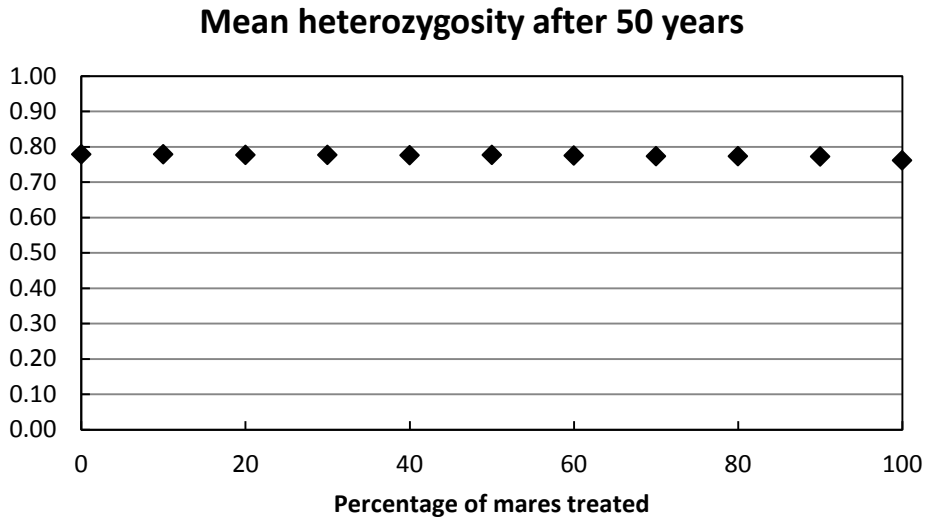


Figure 48. Initial population size = 500, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	562.32	542.84	518.23	536.63	513.12	478.55	484.51	462.25	467.67	431.71	325.38
Mean number of removals after 20 years	985.18	870.87	799.25	721.09	665.02	606.24	577.73	482.76	475.37	406.87	352.26
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.77	0.77	0.77	0.76

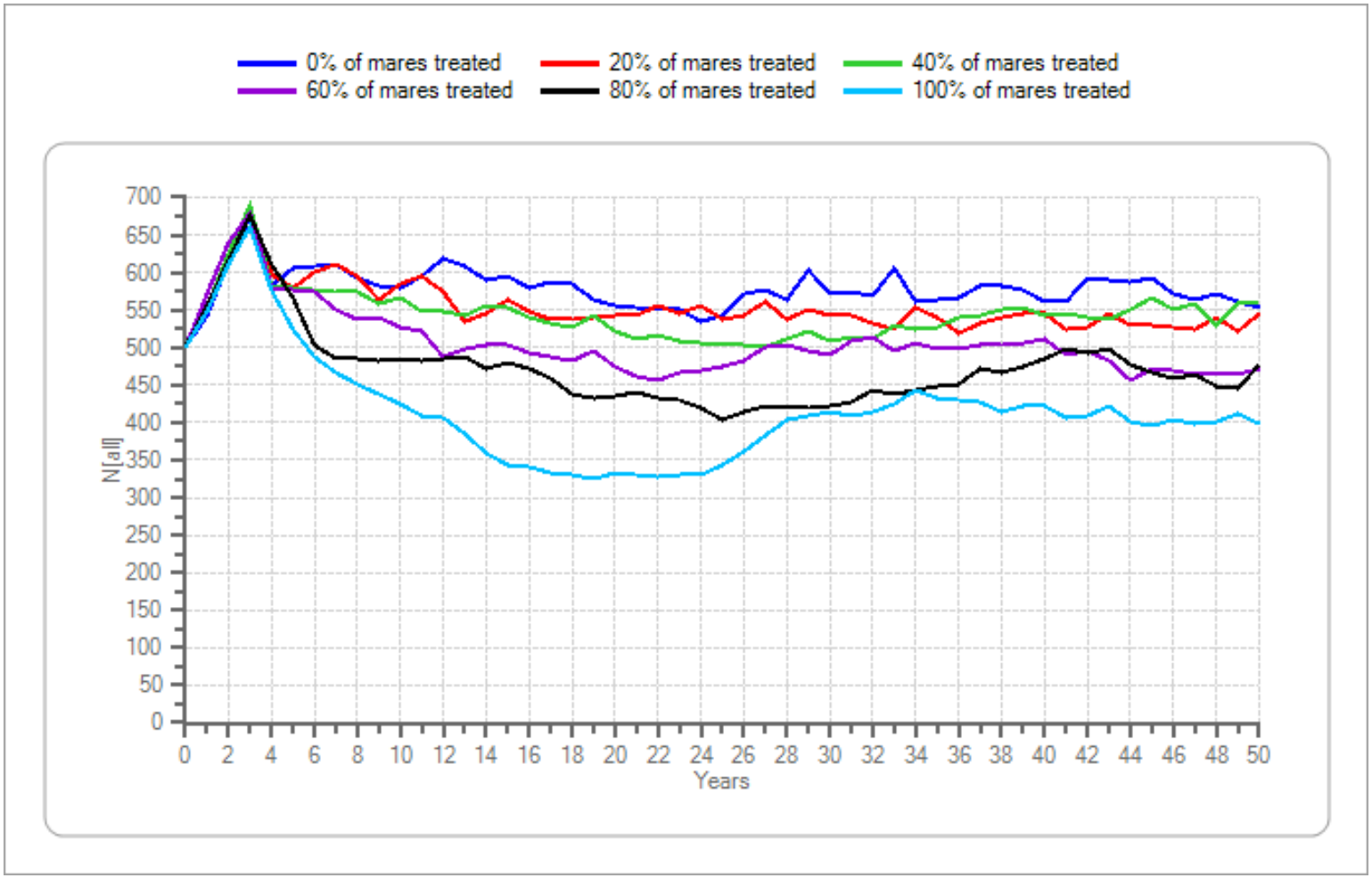
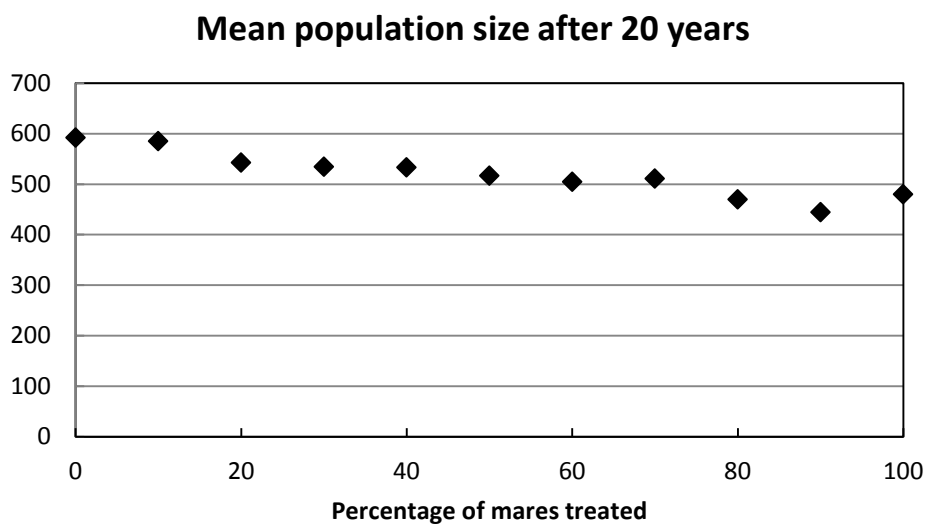
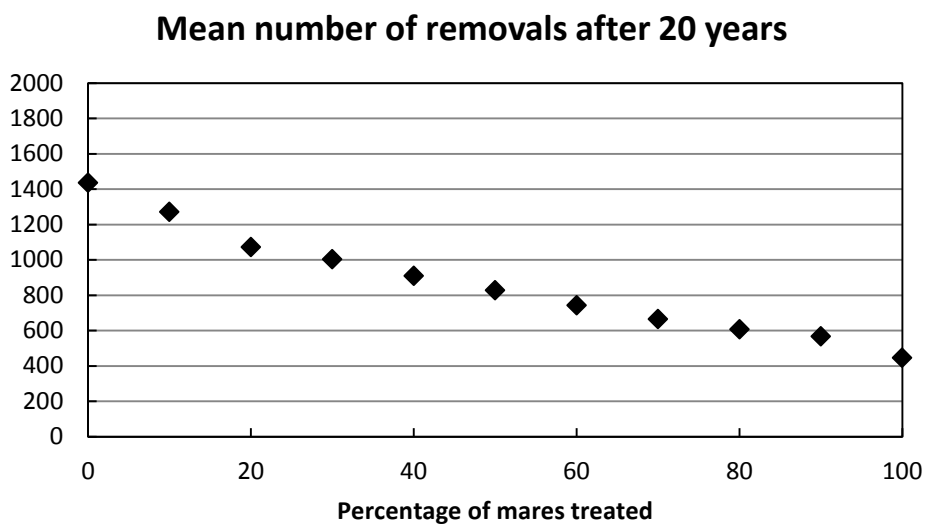
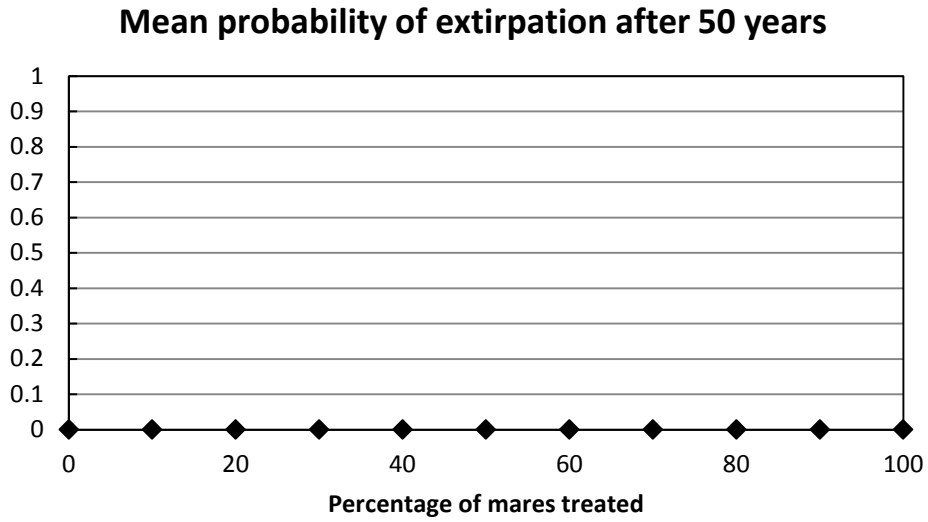


Figure 48. Initial population size = 500, lambda = 1.15, genetic diversity = high.—Continued







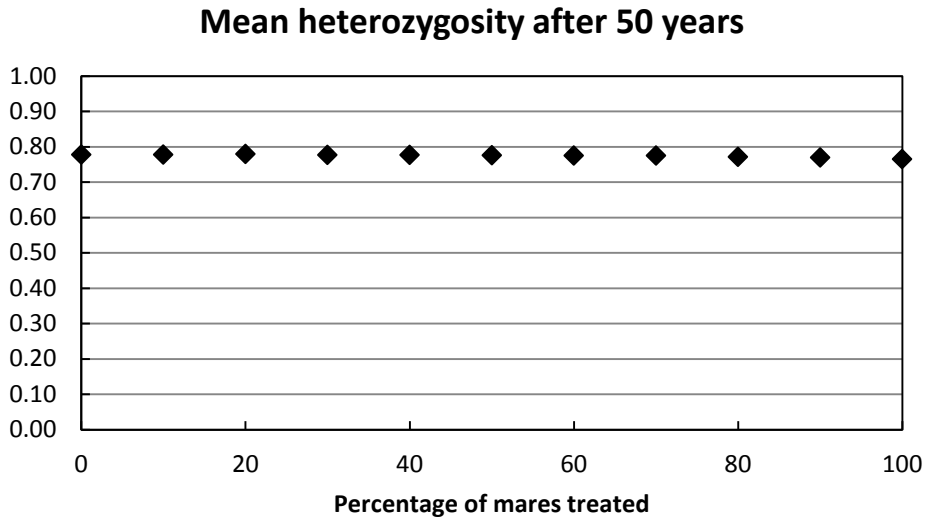


Figure 49. Initial population size = 500, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	591.83	584.93	542.73	534.42	532.9	516.29	504.75	510.67	469.76	444.23	479.71
Mean number of removals after 20 years	1435.1	1271.0	1072.6	1003.7	908.9	826.8	741.6	663.7	606.5	567.2	445.4
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.77	0.76

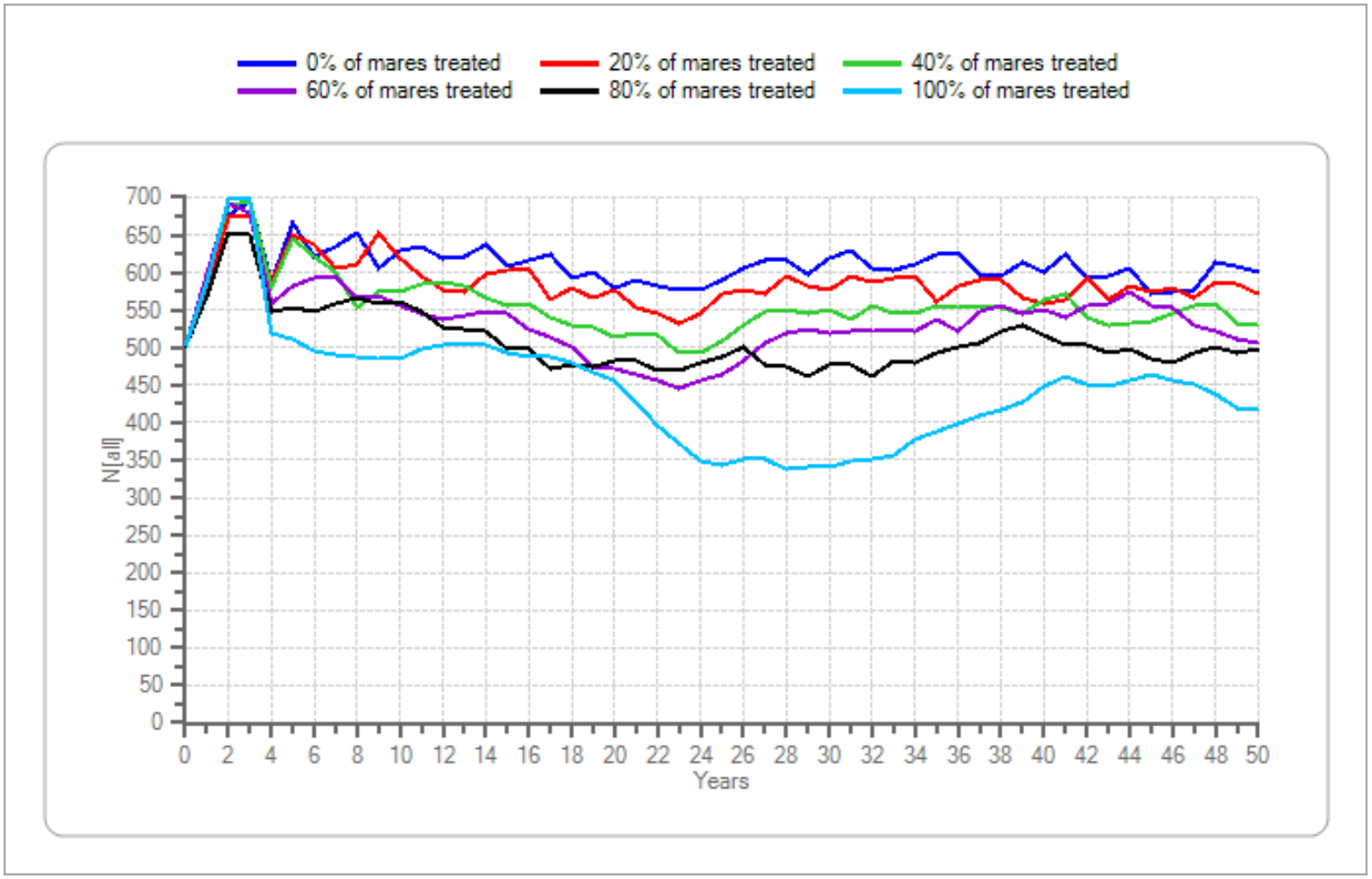
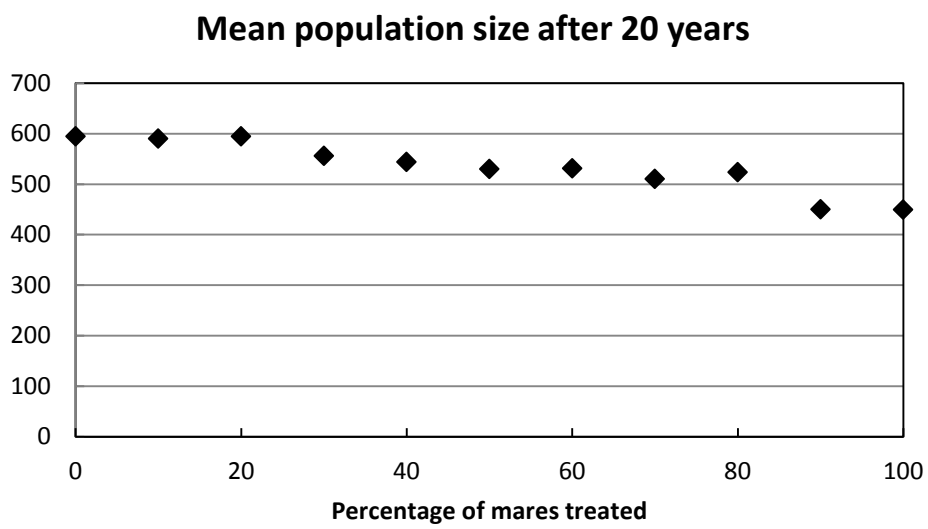
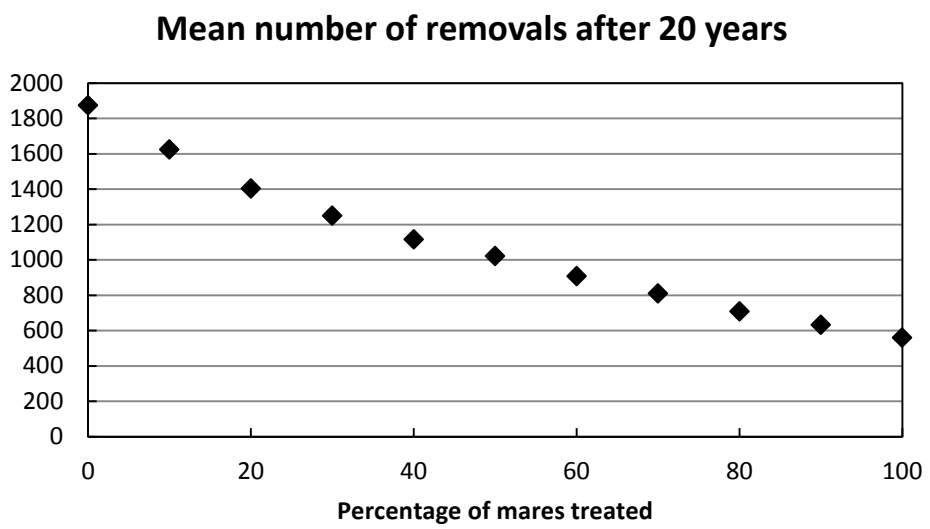
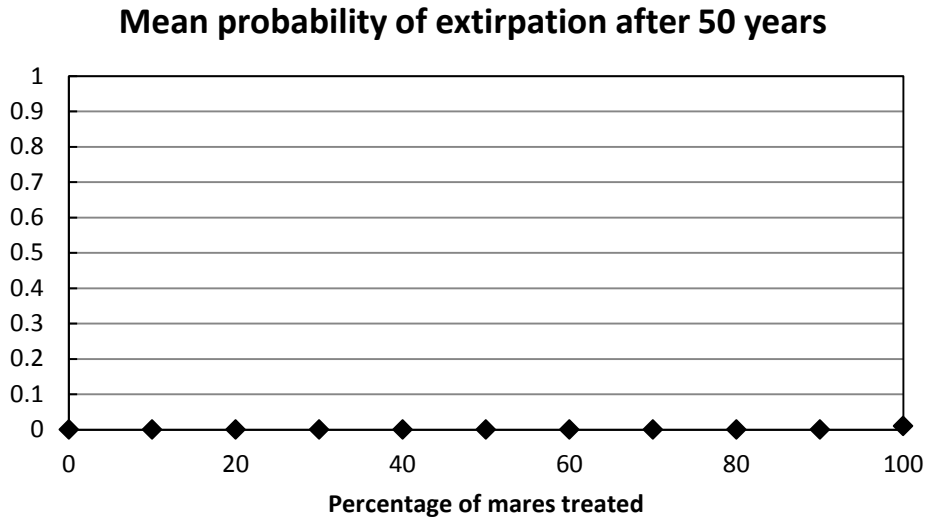


Figure 49. Initial population size = 500, lambda = 1.20, genetic diversity = high.—Continued







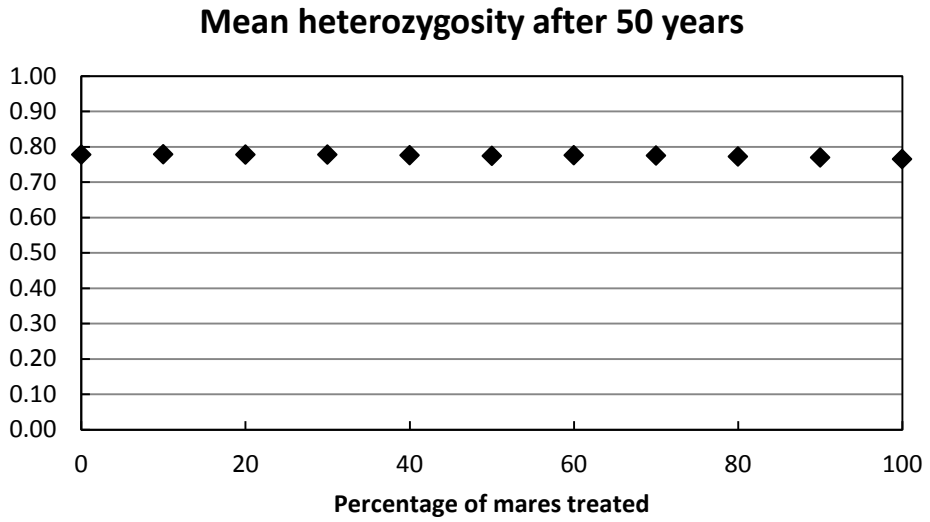


Figure 50. Initial population size = 500, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	594.45	589.9	594.5	555.72	543.85	529.95	531.28	510.02	523.65	449.97	449.55
Mean number of removals after 20 years	1874.5	1624.6	1402.6	1249.2	1116.2	1021.7	907.0	809.4	707.9	631.4	559.9
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.01
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.77	0.78	0.78	0.77	0.77	0.76

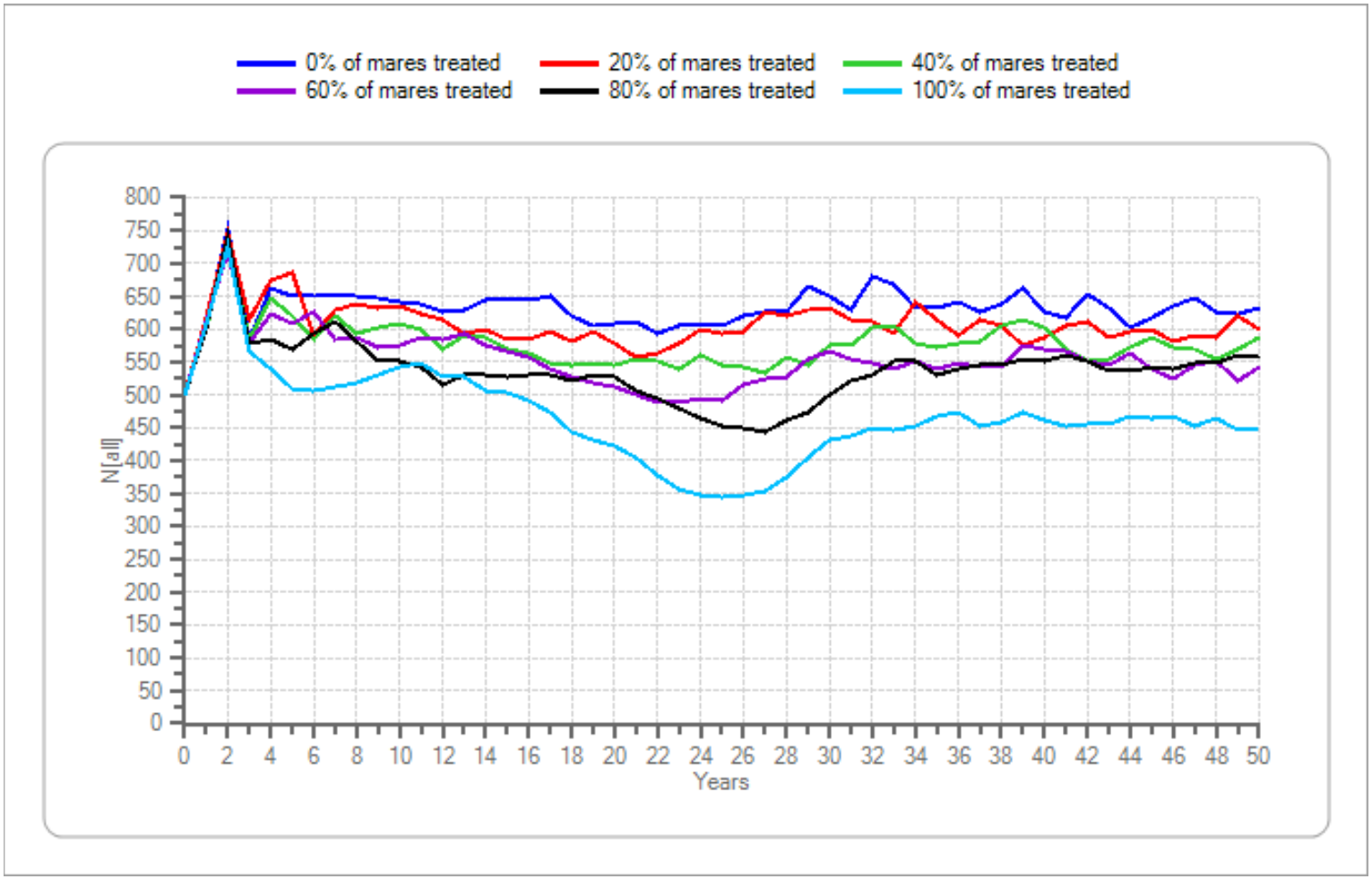
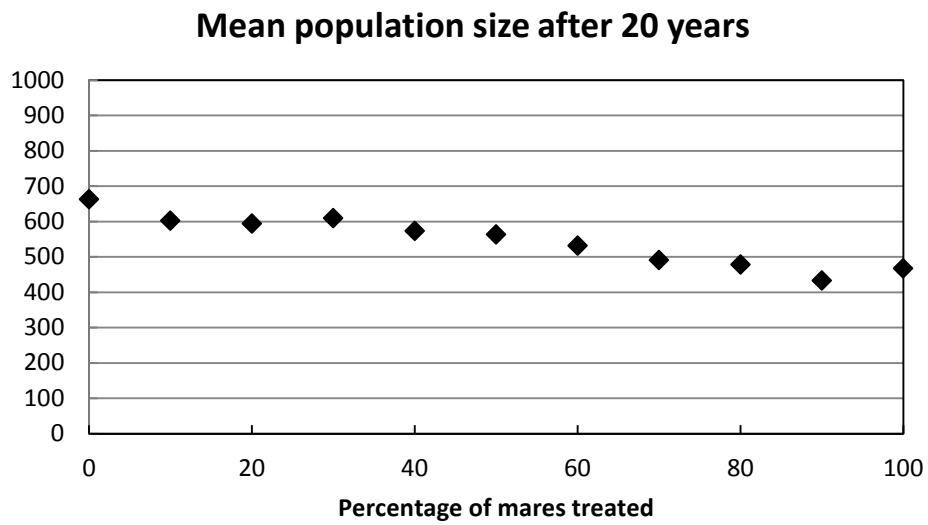
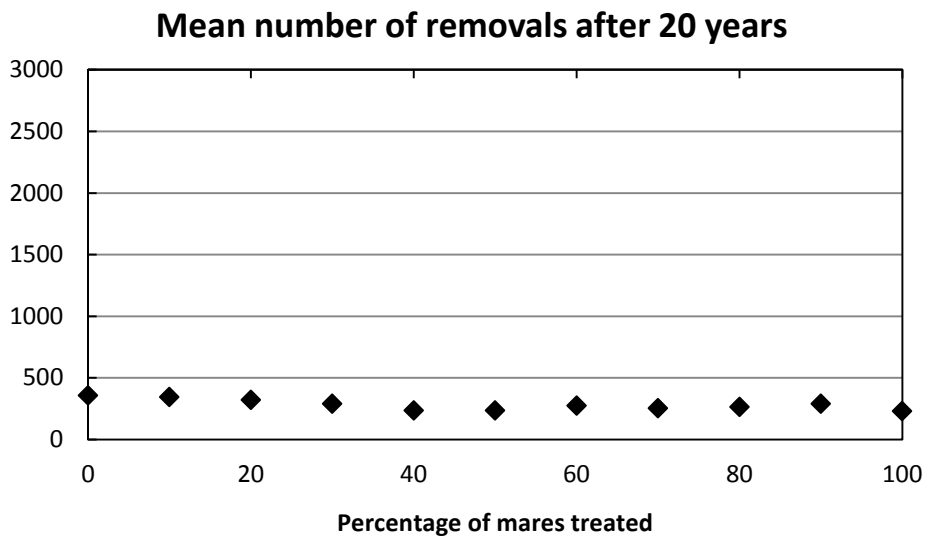
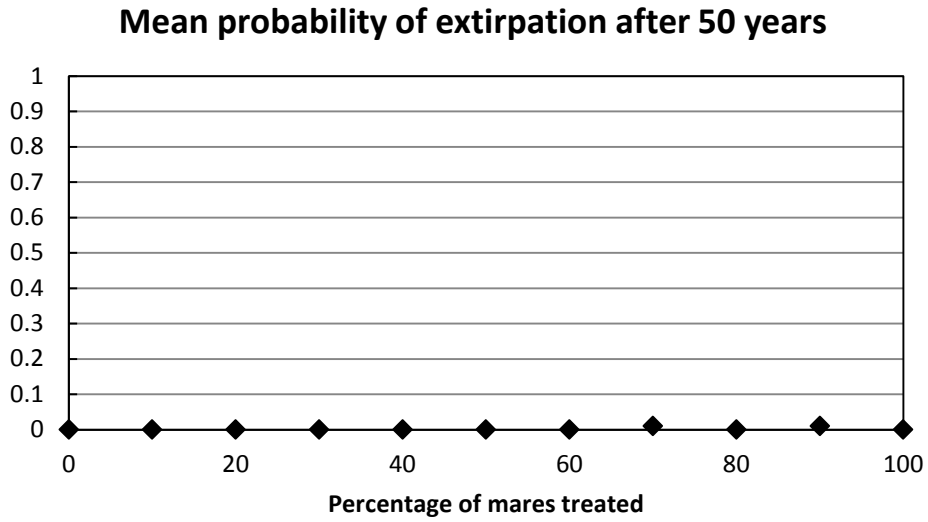


Figure 50. Initial population size = 500, lambda = 1.25, genetic diversity = high.—Continued







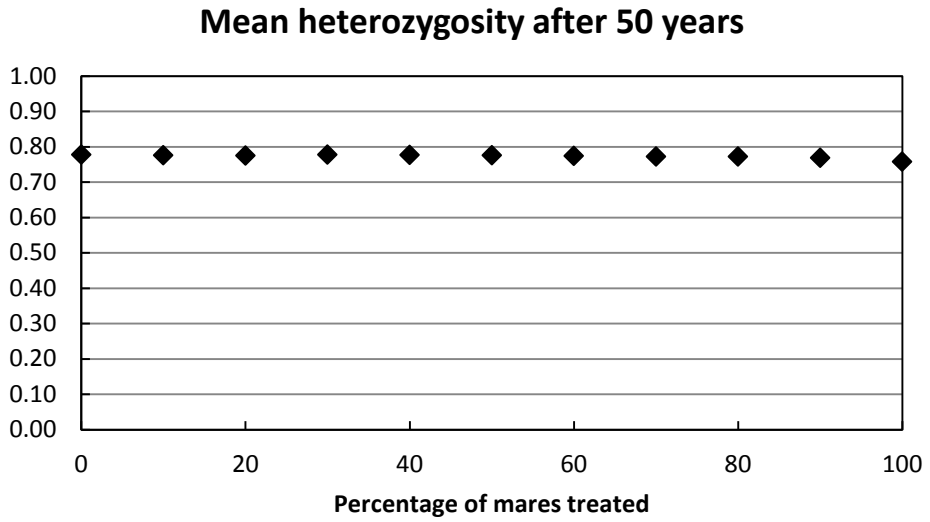


Figure 51. Initial population size = 750, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	662.85	602.11	594.21	609.04	573.37	563.41	531.5	490.61	478.09	432.79	467.39
Mean number of removals after 20 years	357.45	343.82	320.45	290.07	234.09	234	273.83	253.99	263.92	289.45	228.35
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0.01	0	0.01	0
Mean heterozygosity after 50 years	0.78	0.78	0.77	0.78	0.78	0.78	0.77	0.77	0.77	0.77	0.76

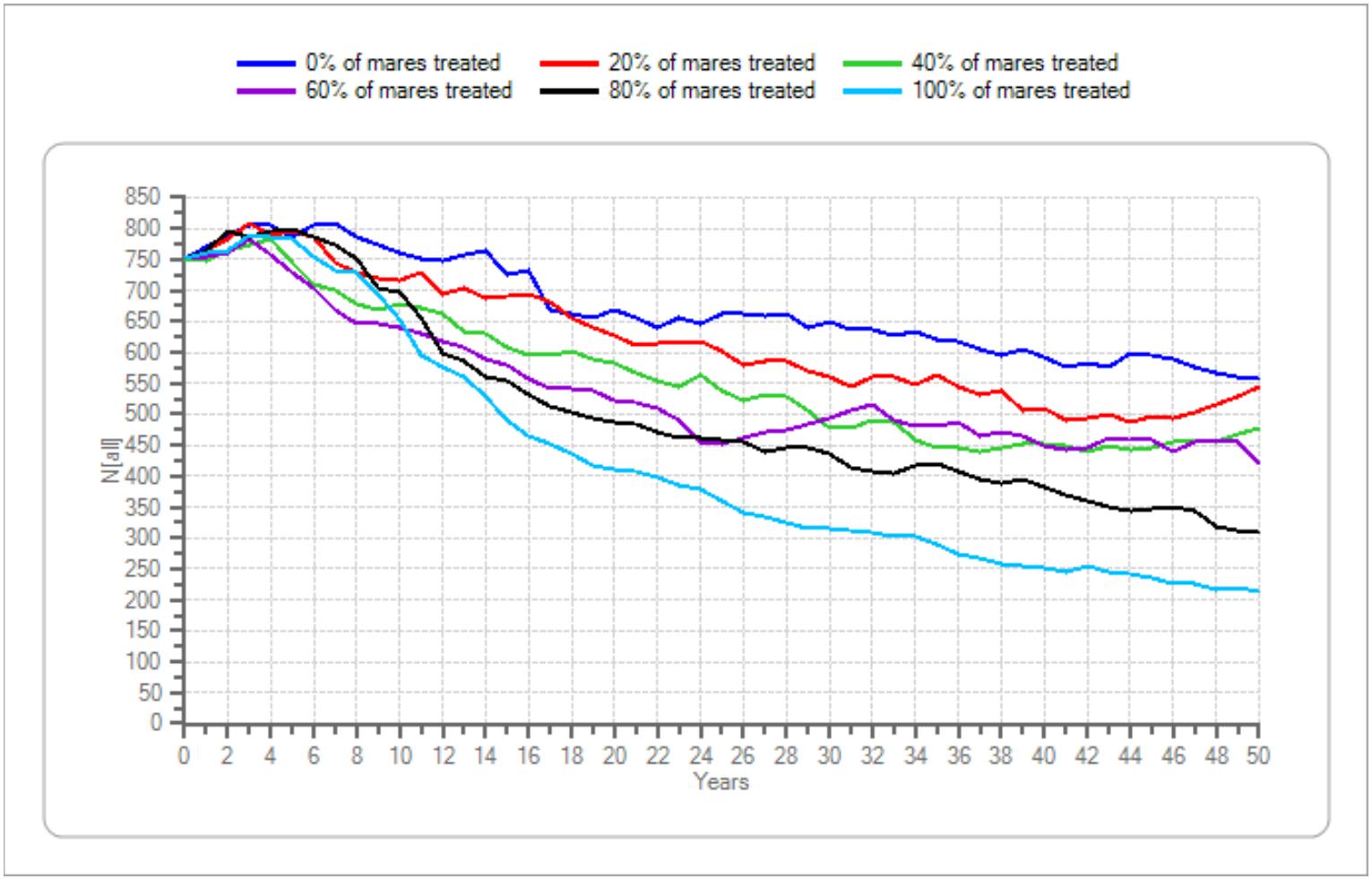
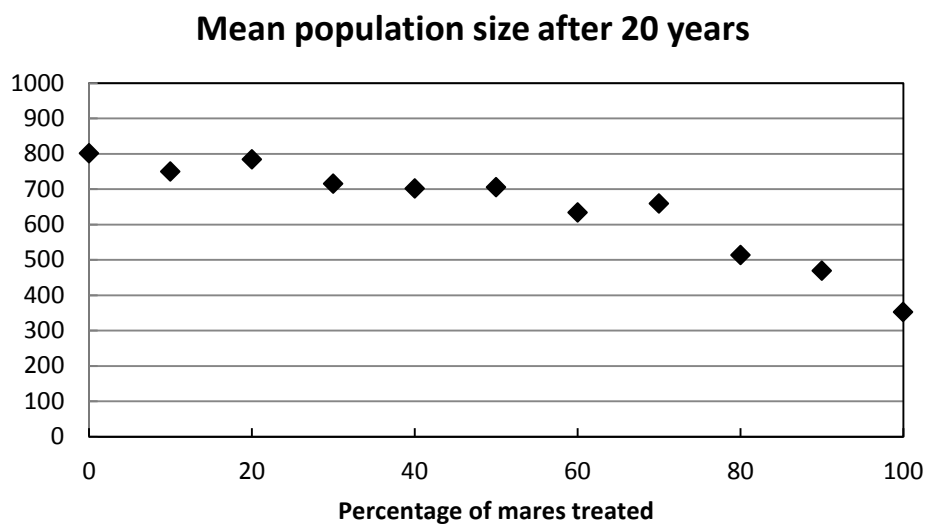
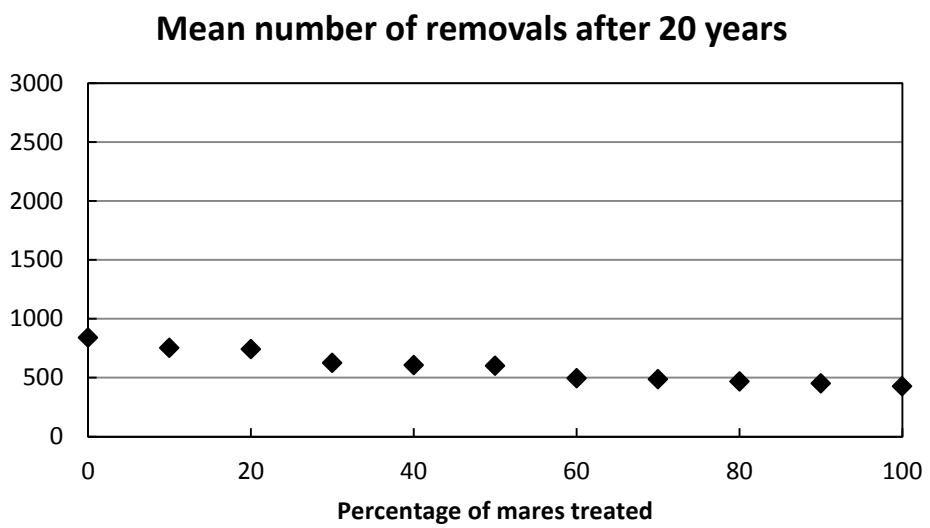
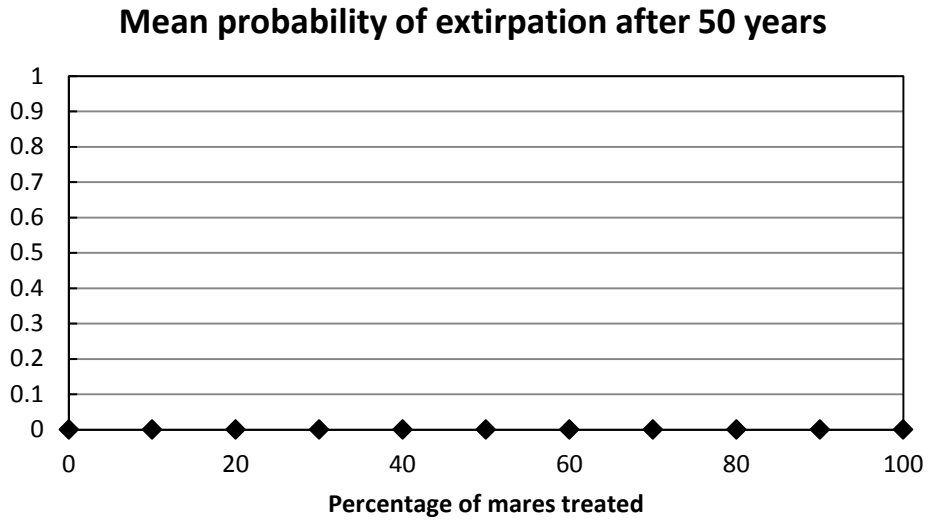


Figure 51. Initial population size = 750, lambda = 1.05, genetic diversity = high.—Continued







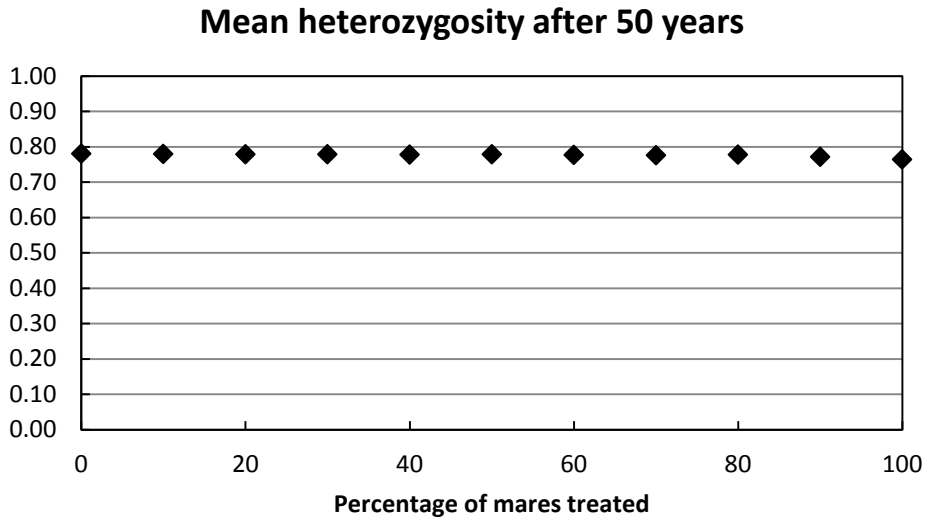


Figure 52. Initial population size = 750, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	800.78	749.94	784.34	714.78	701.29	705.25	633.52	658.99	513.24	468.63	351.92
Mean number of removals after 20 years	838.9	753.64	742.14	625.1	605.75	599.52	493.98	486.5	466.16	451.41	427.27
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.76

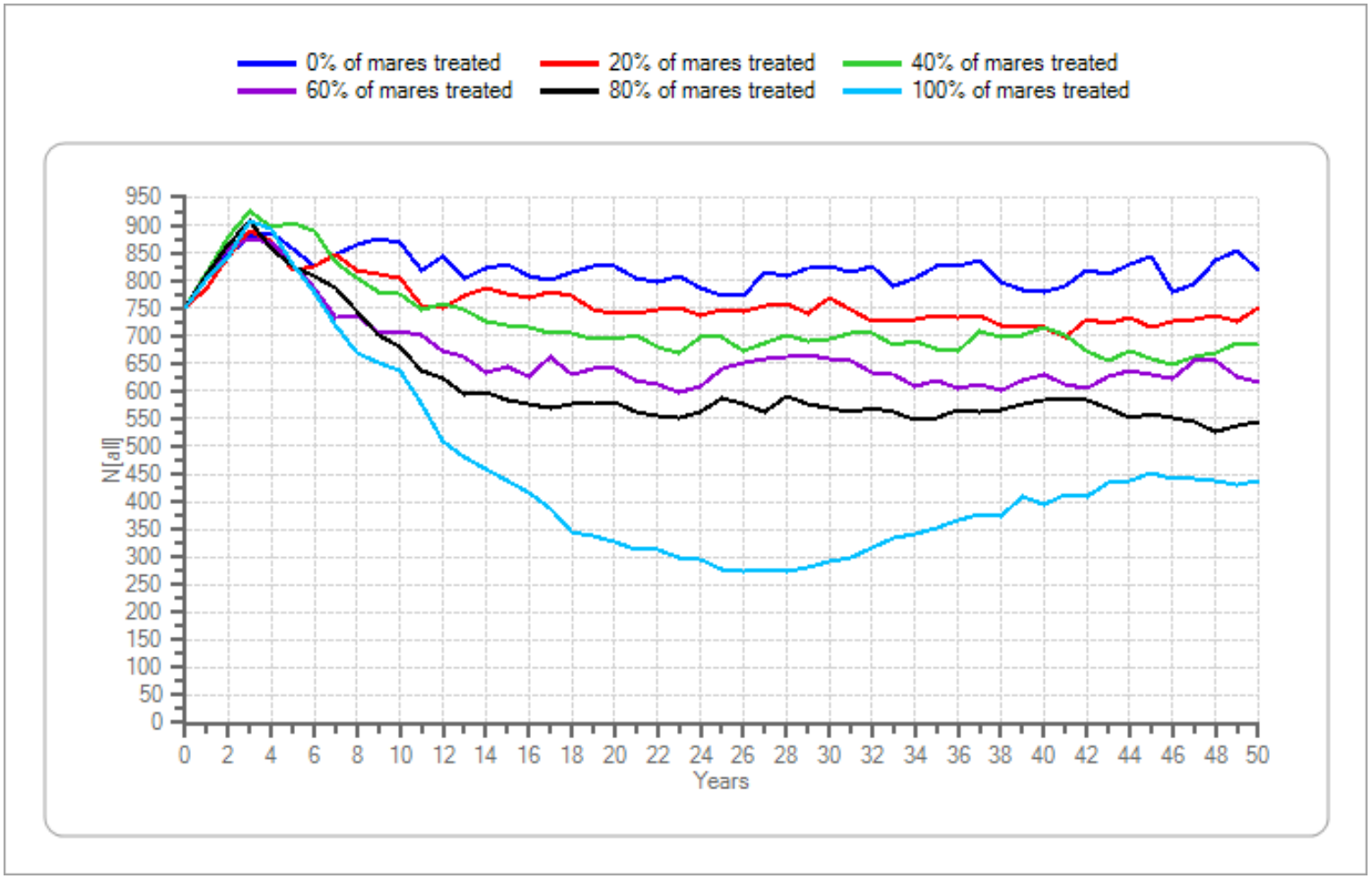
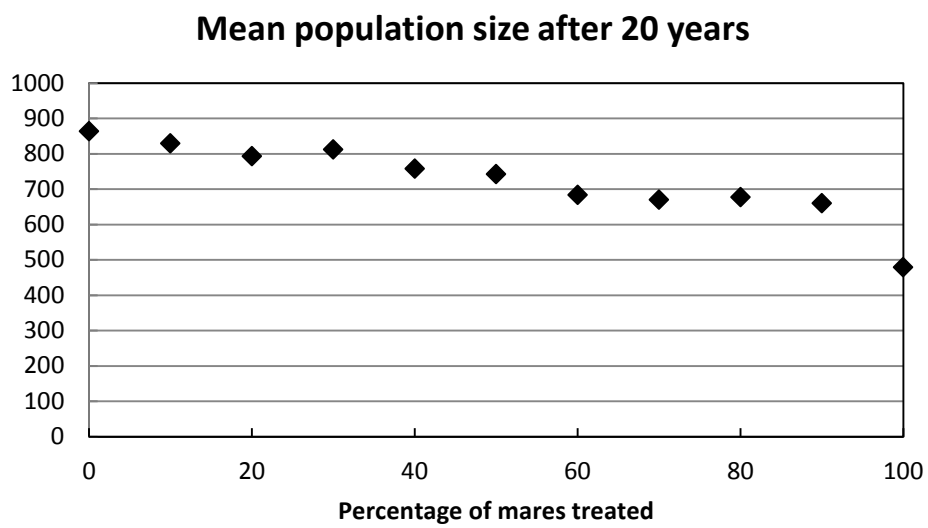
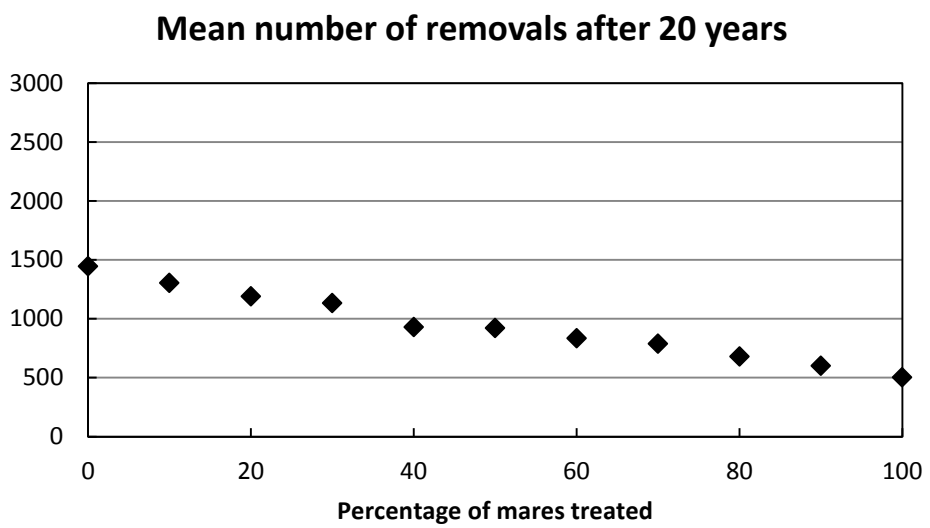
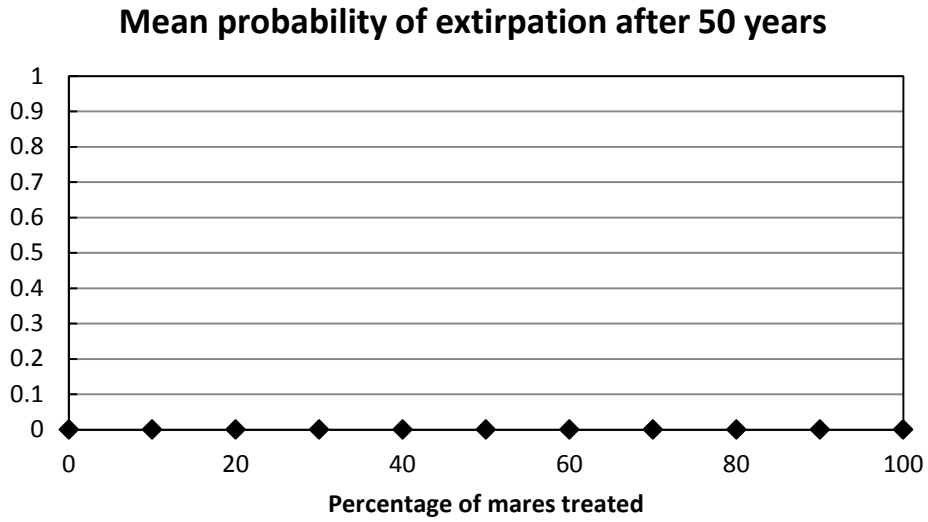


Figure 52. Initial population size = 750, lambda = 1.10, genetic diversity = high.—Continued







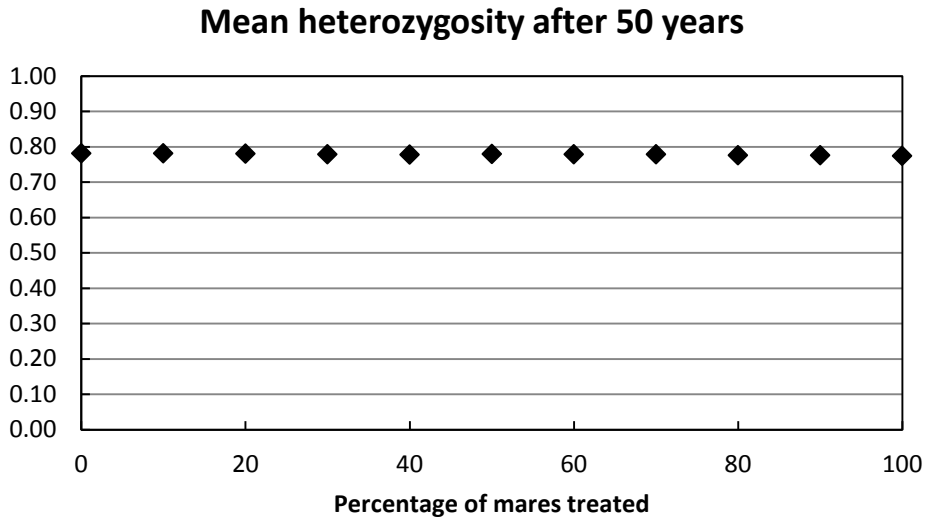


Figure 53. Initial population size = 750, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	863.26	828.87	793.09	812.32	758.17	742.73	683.78	670.07	677.22	660.32	478.72
Mean number of removals after 20 years	1444.8	1304.1	1189.2	1132.7	929.9	920.5	834.1	788.1	679.1	599.5	501.4
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

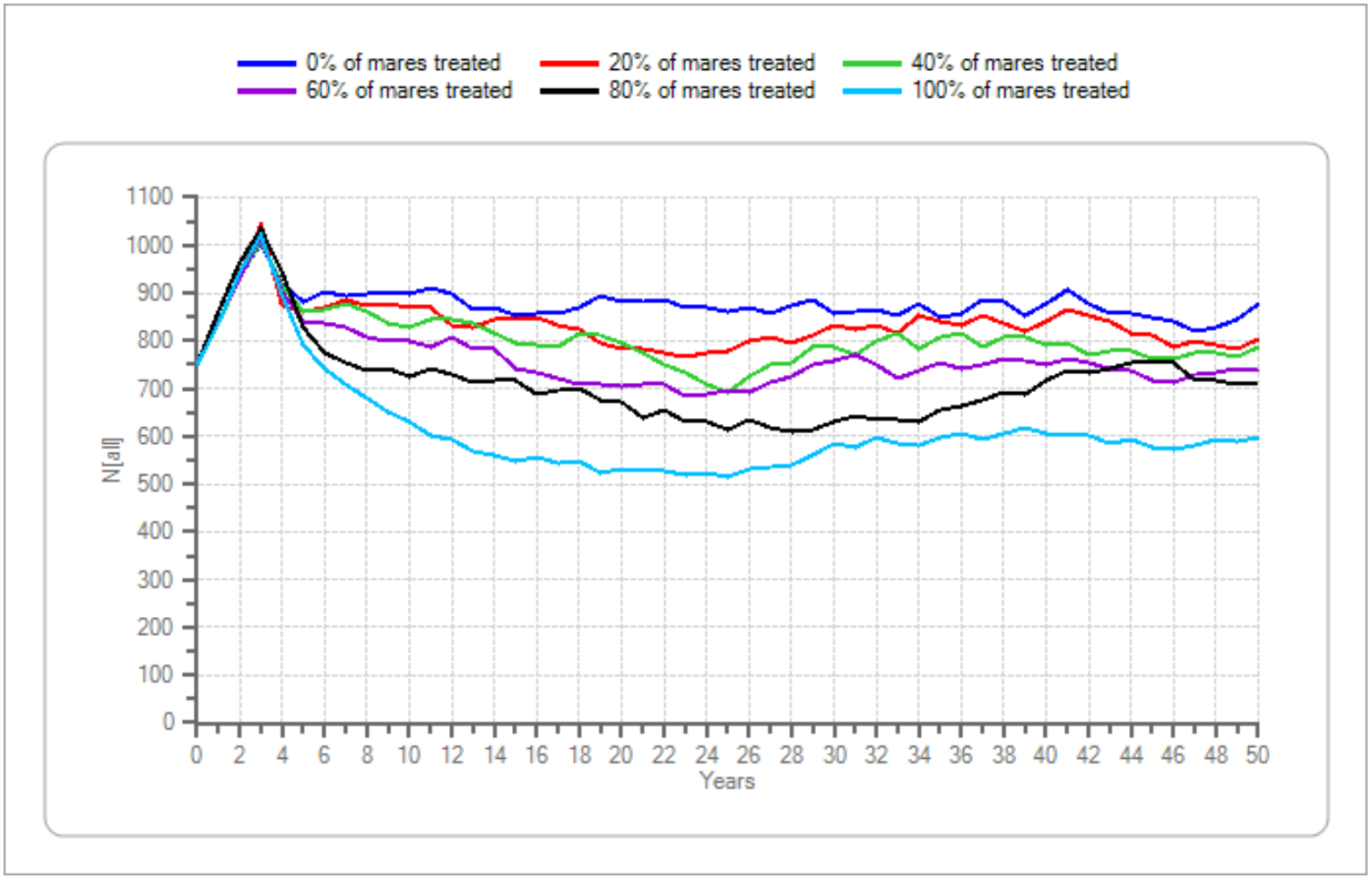


Figure 53. Initial population size = 750, lambda = 1.15, genetic diversity = high.—Continued

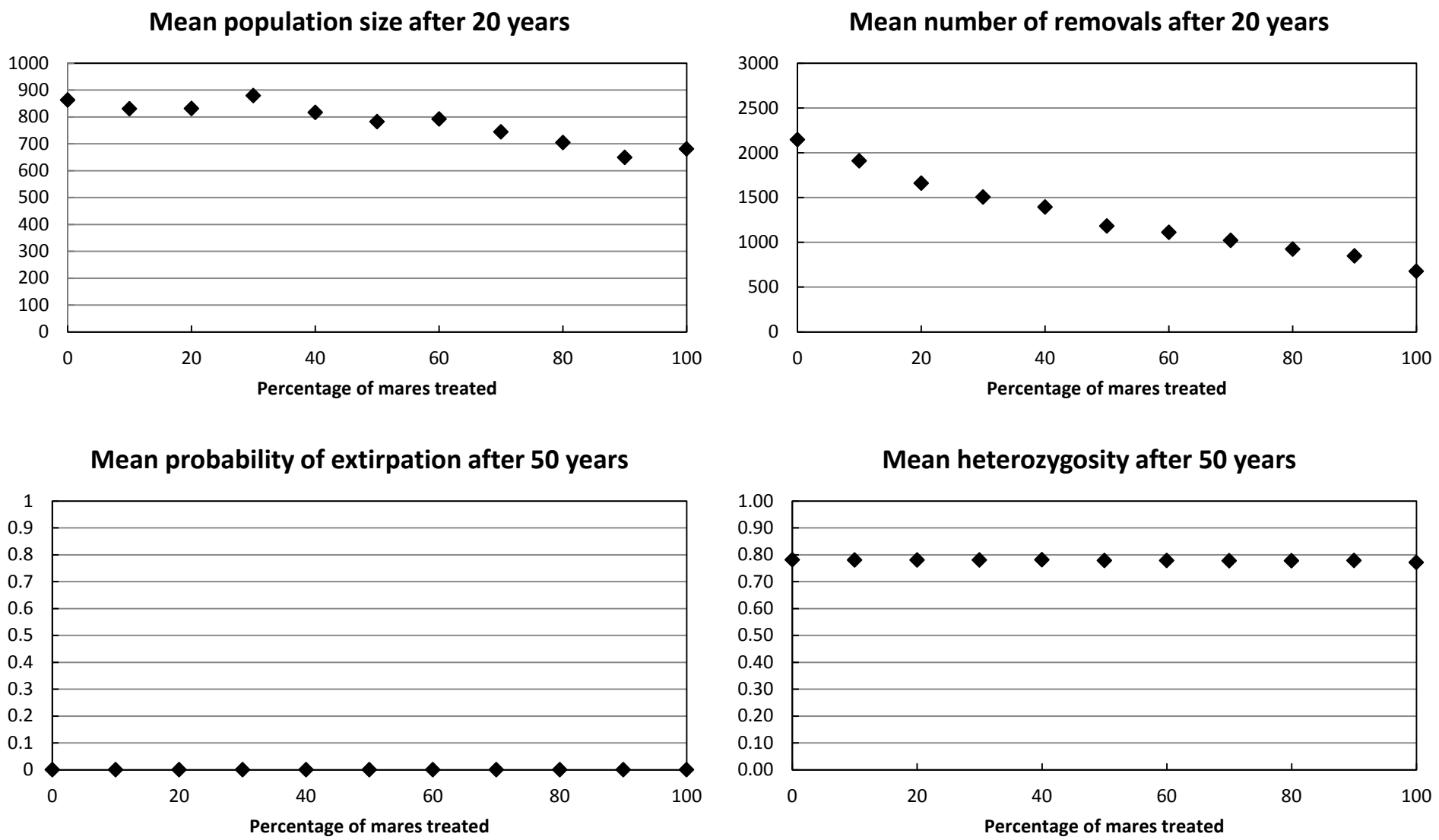


Figure 54. Initial population size = 750, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	862.48	830.01	830.72	878.81	816.6	782.19	791.89	743.94	704.05	649.05	680.91
Mean number of removals after 20 years	2146.1	1909.2	1659.2	1506.0	1394.6	1181.1	1109.7	1021.6	923.1	847.1	675.6
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

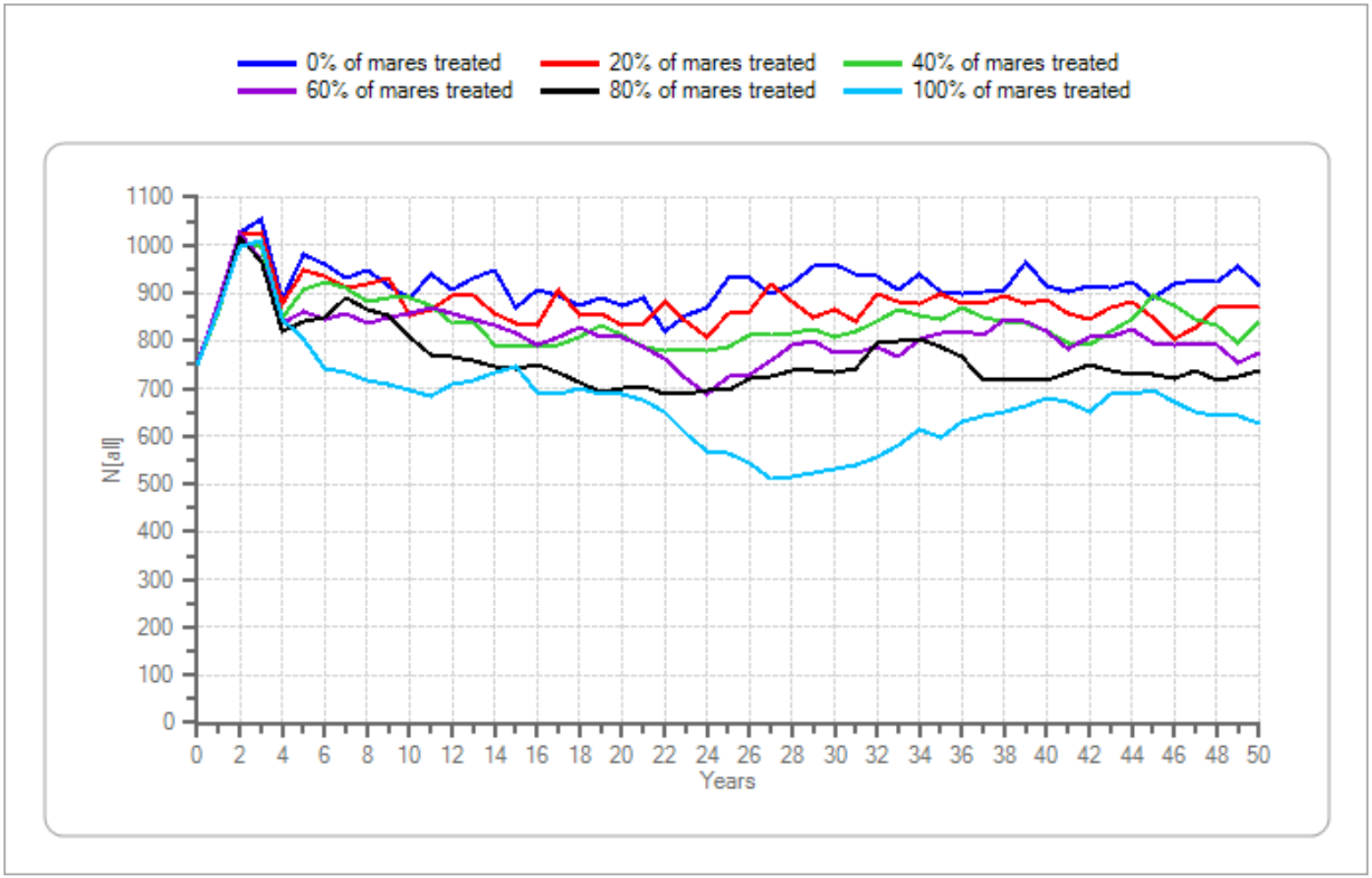


Figure 54. Initial population size = 750, lambda = 1.20, genetic diversity = high.—Continued

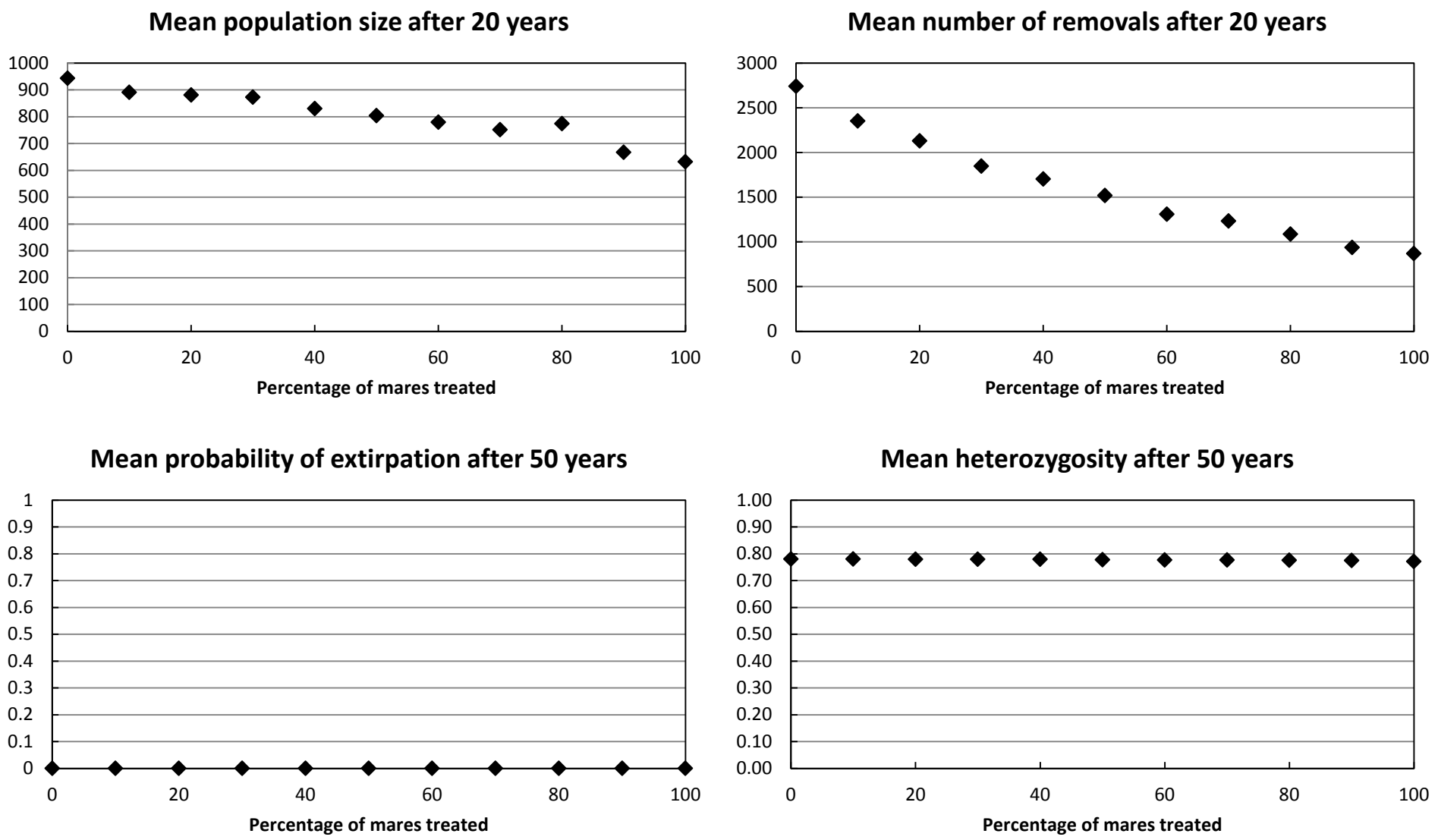


Figure 55. Initial population size = 750, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	943.03	890.54	880.47	872.85	830.01	804.21	779.9	751.3	773.8	666.84	631.64
Mean number of removals after 20 years	2741.1	2352.9	2128.9	1845.6	1703.7	1518.4	1309.1	1234.3	1086.5	937.0	868.1
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.77

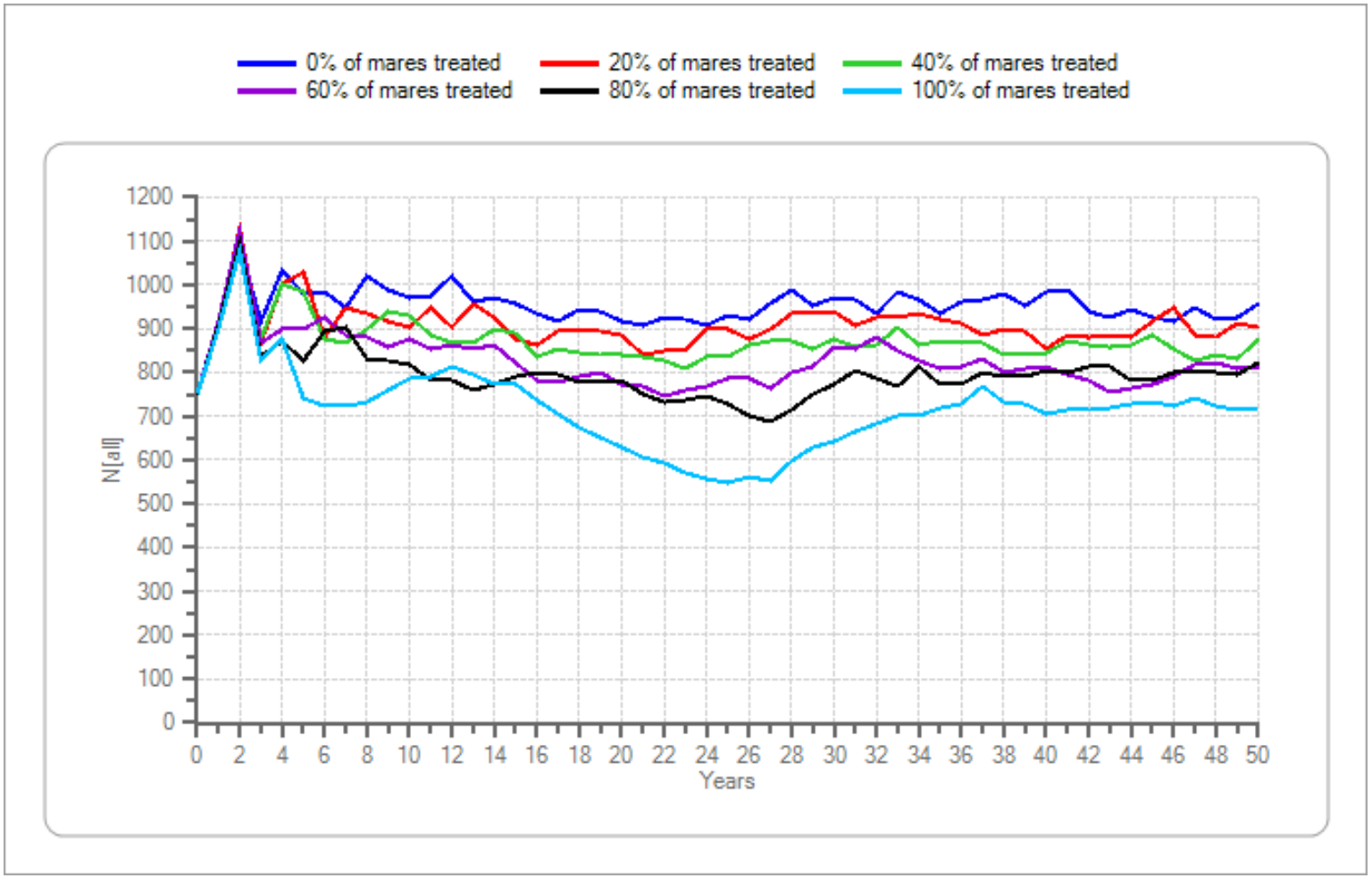
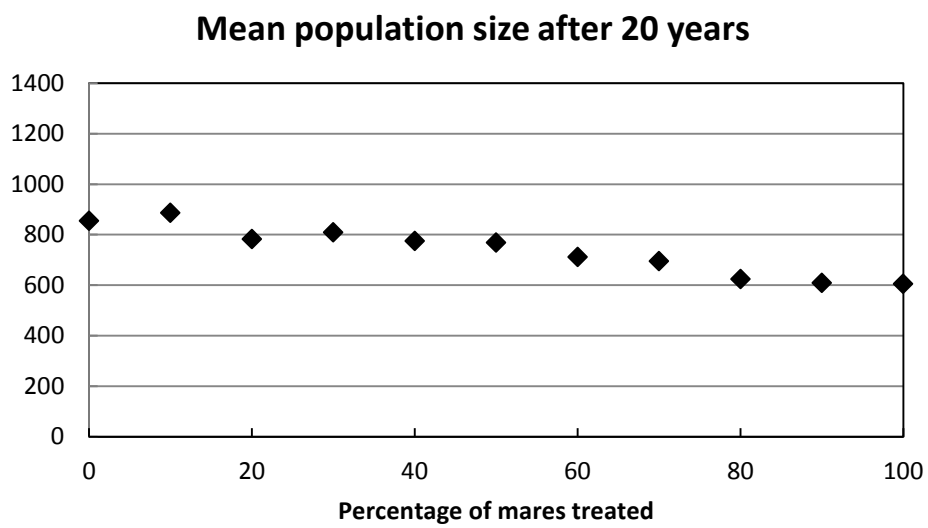
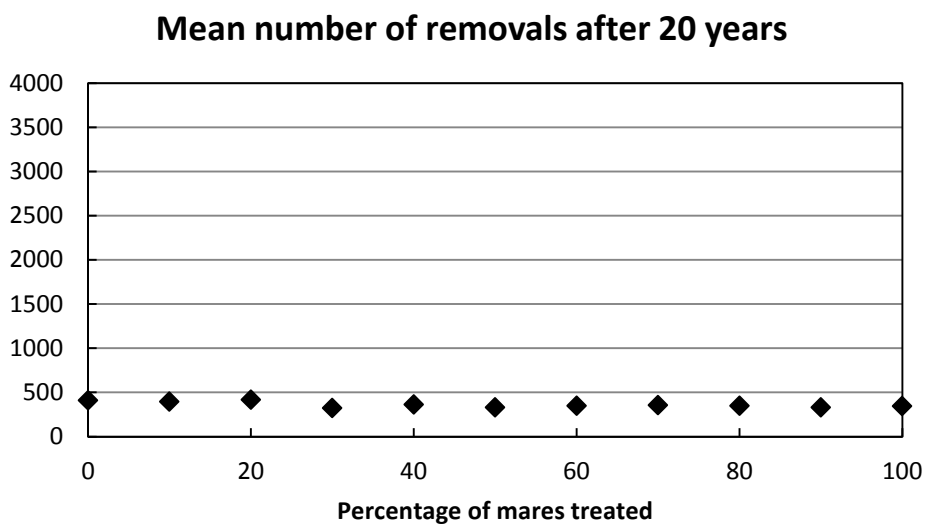
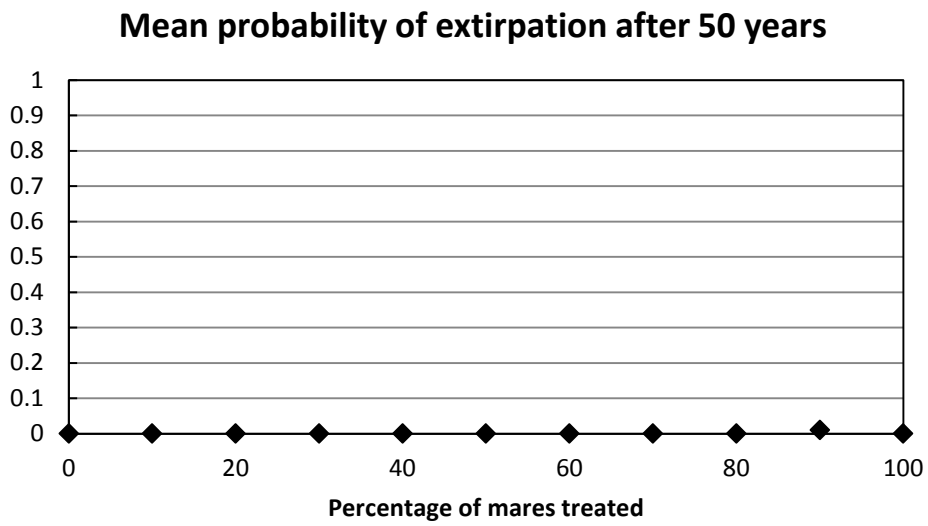


Figure 55. Initial population size = 750, lambda = 1.25, genetic diversity = high.—Continued







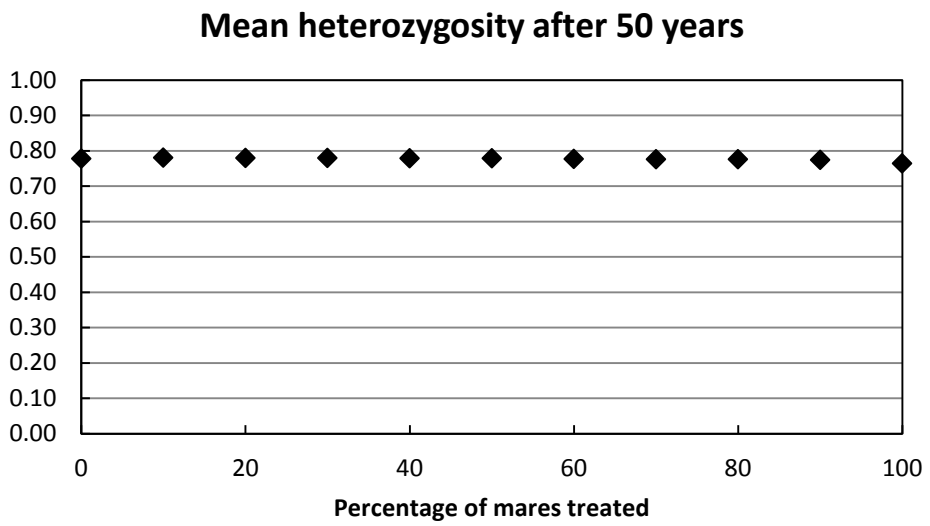


Figure 56. Initial population size = 1000, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	854.42	886.33	782.46	808.7	774.68	767.58	711.38	694.91	623.41	608.7	604
Mean number of removals after 20 years	408.46	393.28	416.19	321.57	362.43	331.22	346.92	353.96	346.09	328.18	344.26
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0.01	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.76

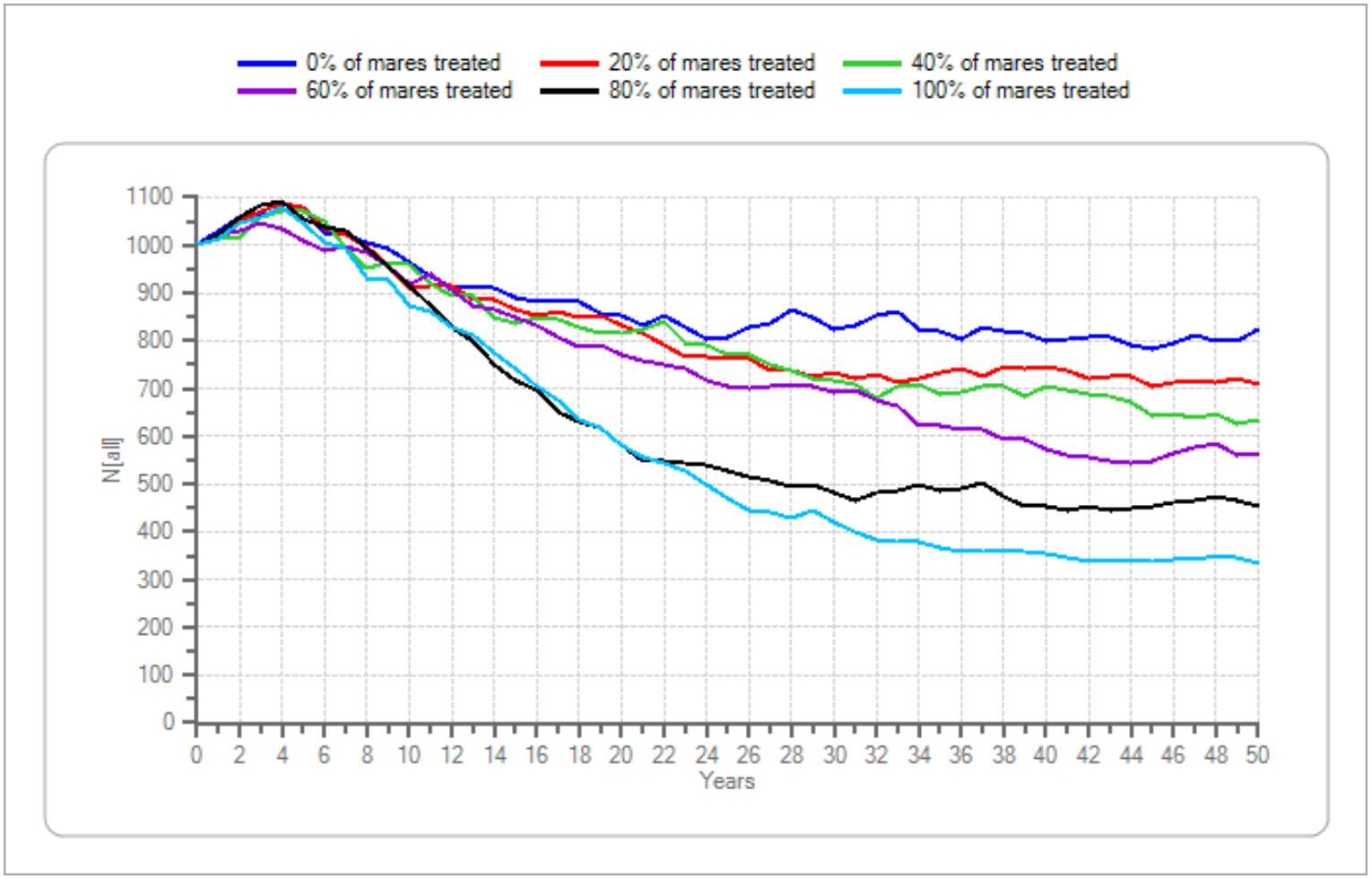
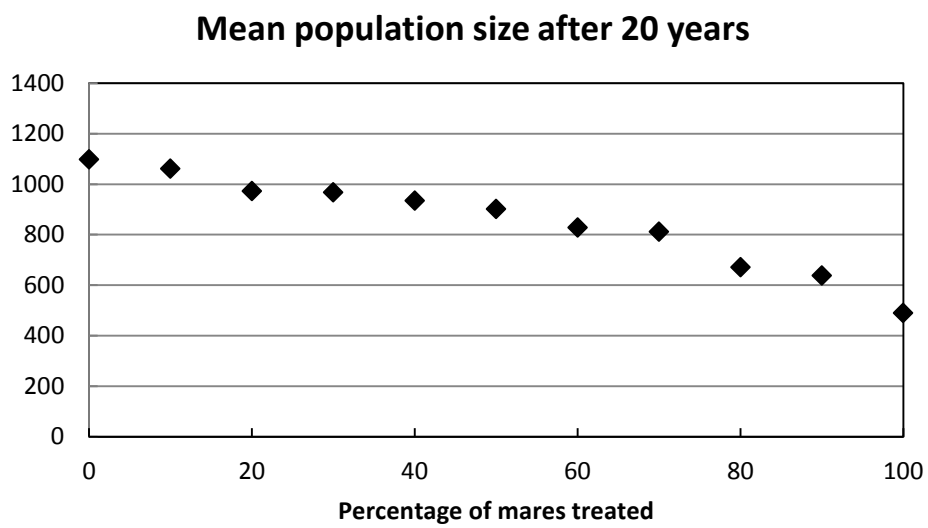
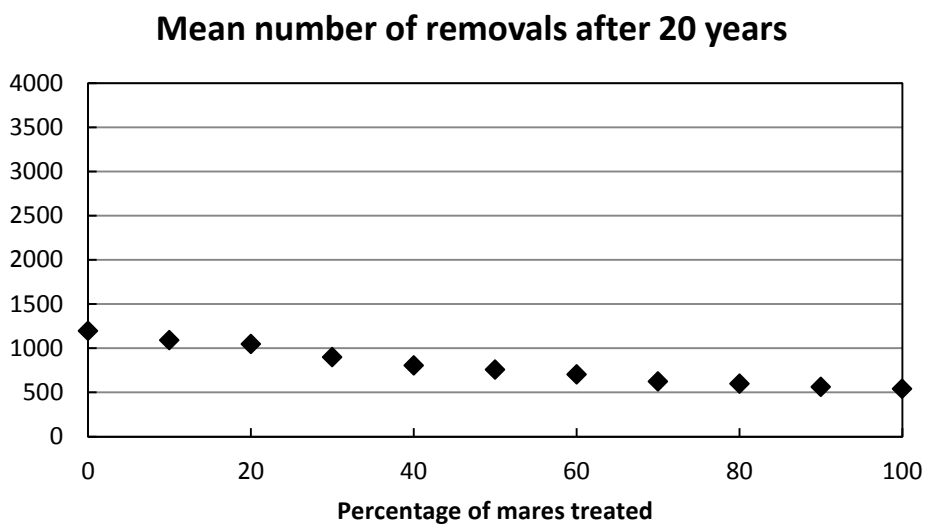
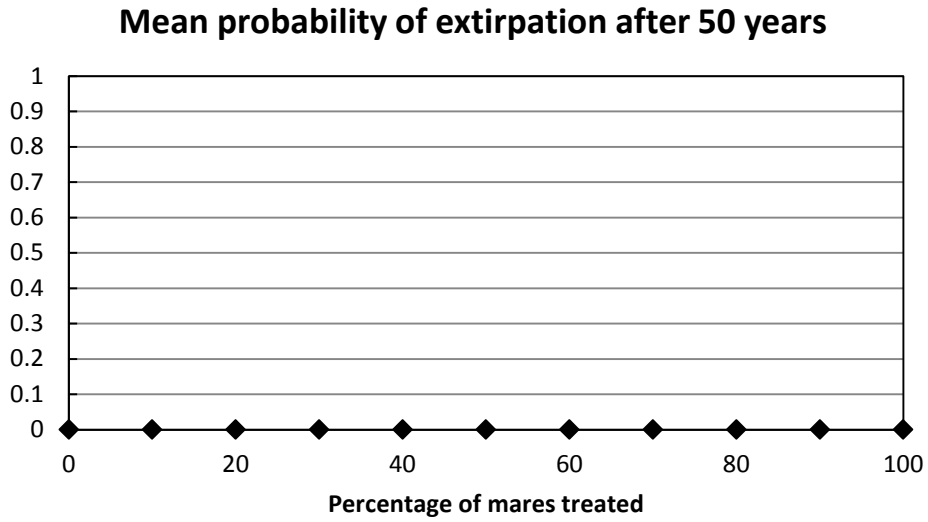


Figure 56. Initial population size = 1000, lambda = 1.05, genetic diversity = high.—Continued







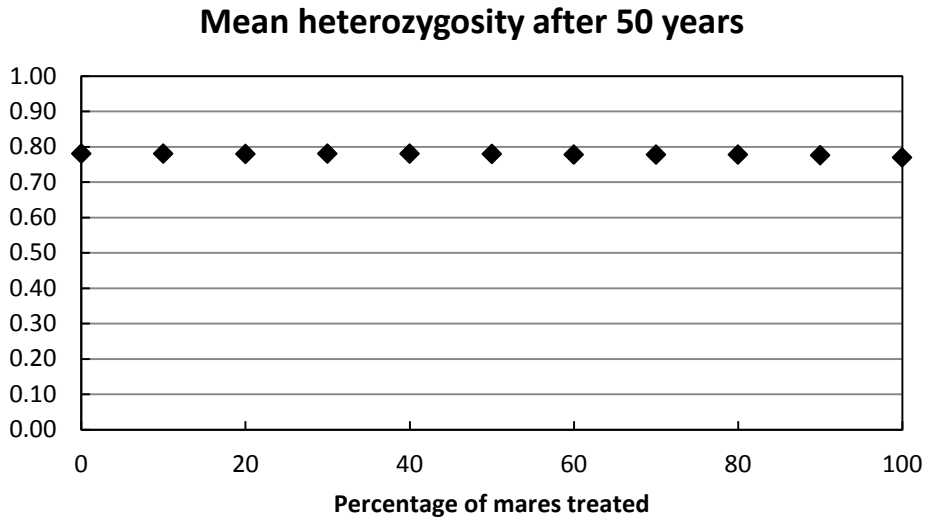


Figure 57. Initial population size = 1000, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1098.09	1061.14	972.15	967.57	933.76	901.35	828.11	810.67	669.9	638.13	489.1
Mean number of removals after 20 years	1196.9	1091.1	1048.1	899.6	804.5	755.5	702.3	621.7	597.8	560.3	541.2
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

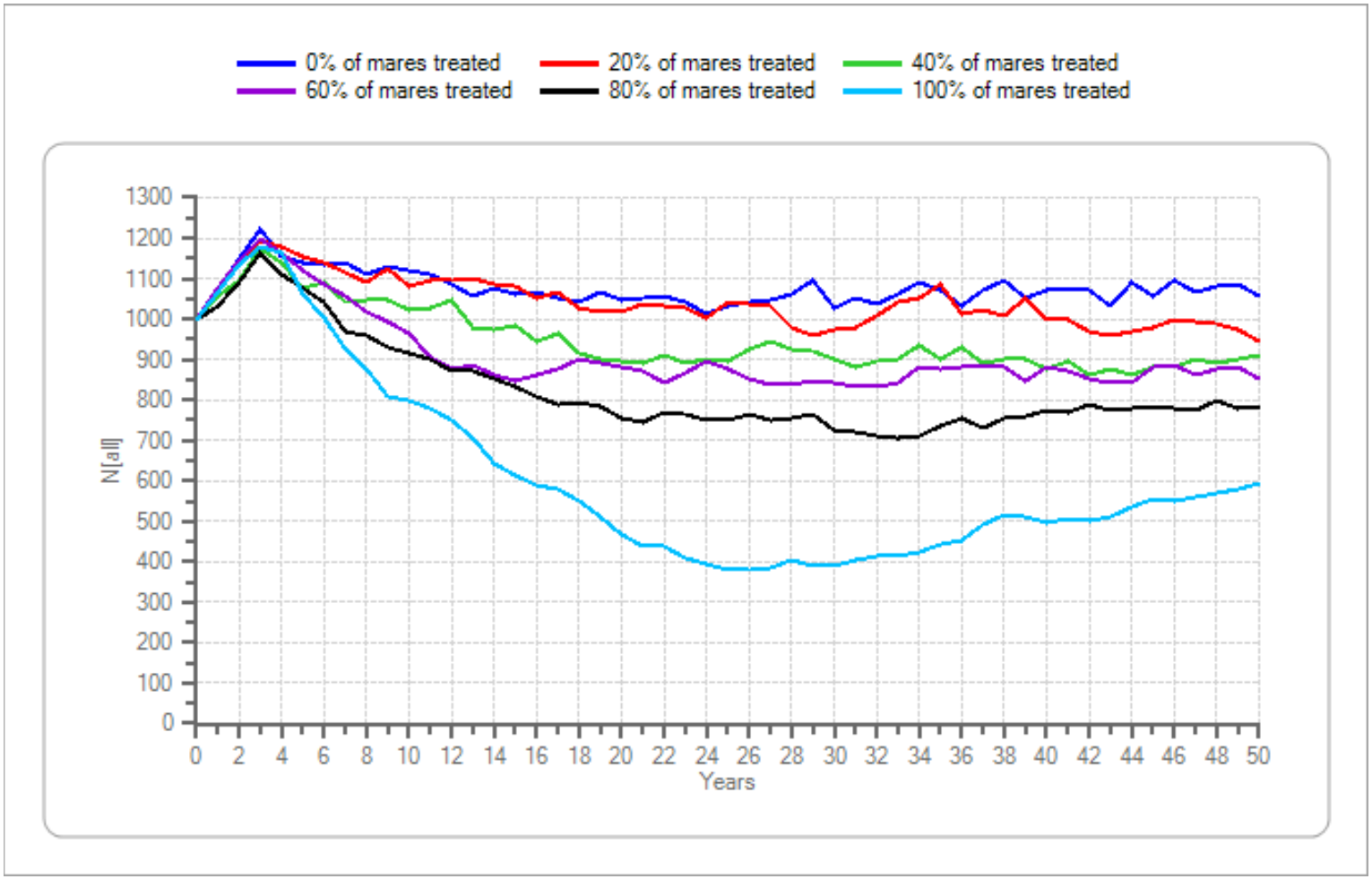
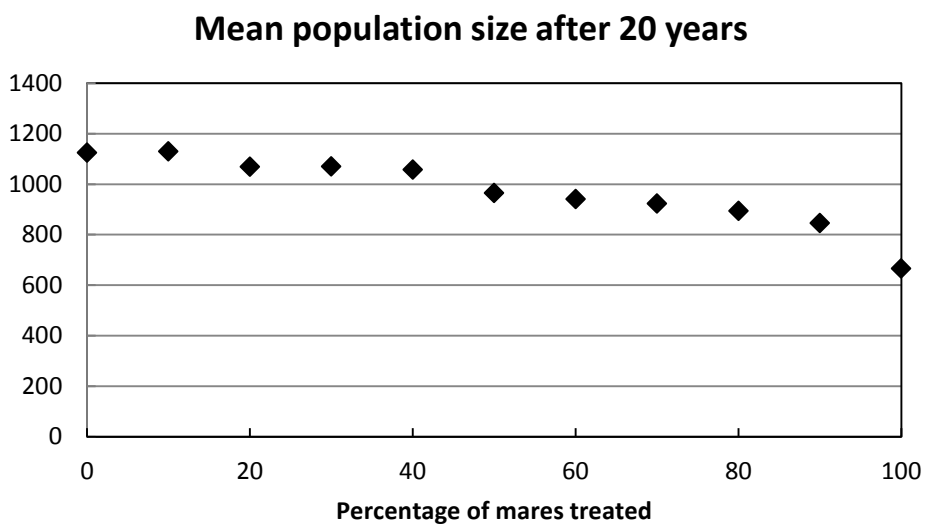
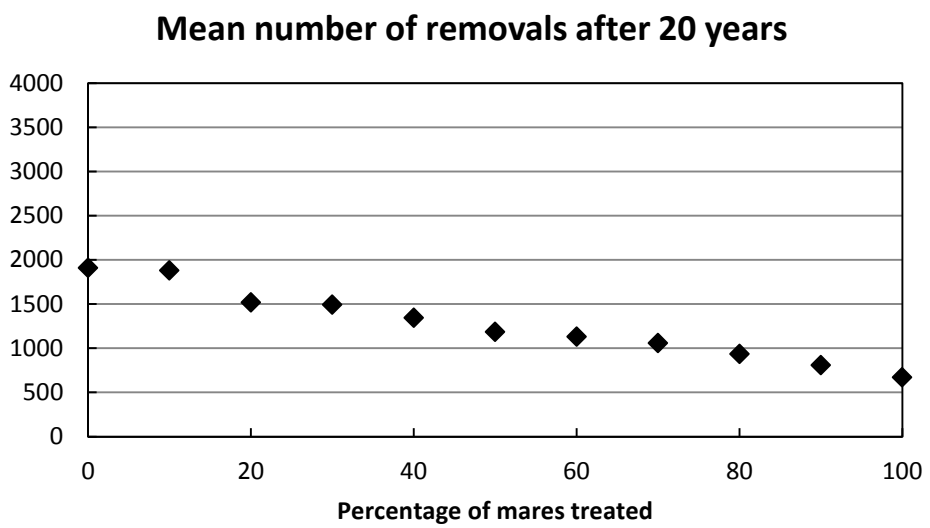
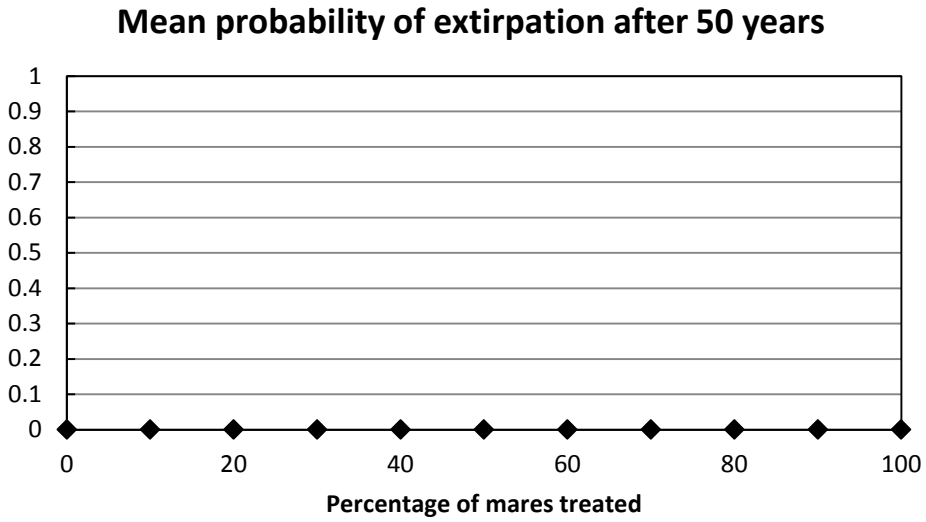


Figure 57. Initial population size = 1000, lambda = 1.10, genetic diversity = high.—Continued







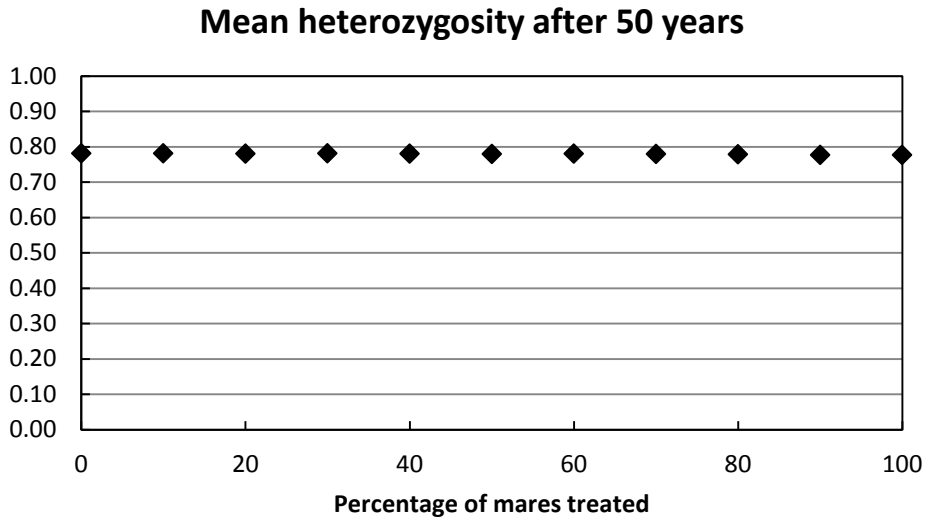


Figure 58. Initial population size = 1000, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1124.15	1128.74	1069.1	1069.36	1056.52	964.86	940	922.21	893.25	845.4	665.68
Mean number of removals after 20 years	1907.4	1878.5	1517.7	1492.3	1343.0	1182.7	1130.2	1058.3	934.0	808.2	669.0
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

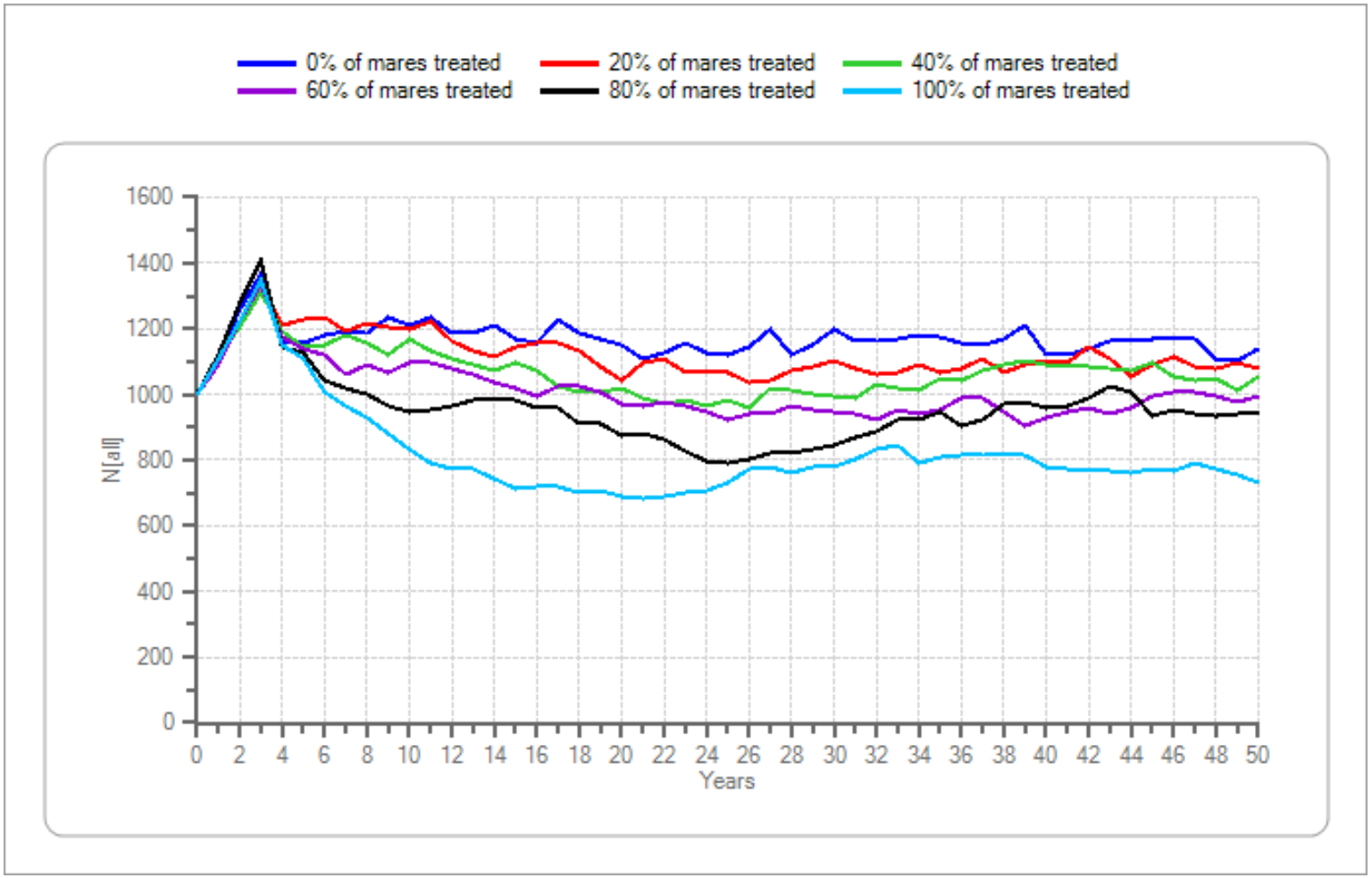
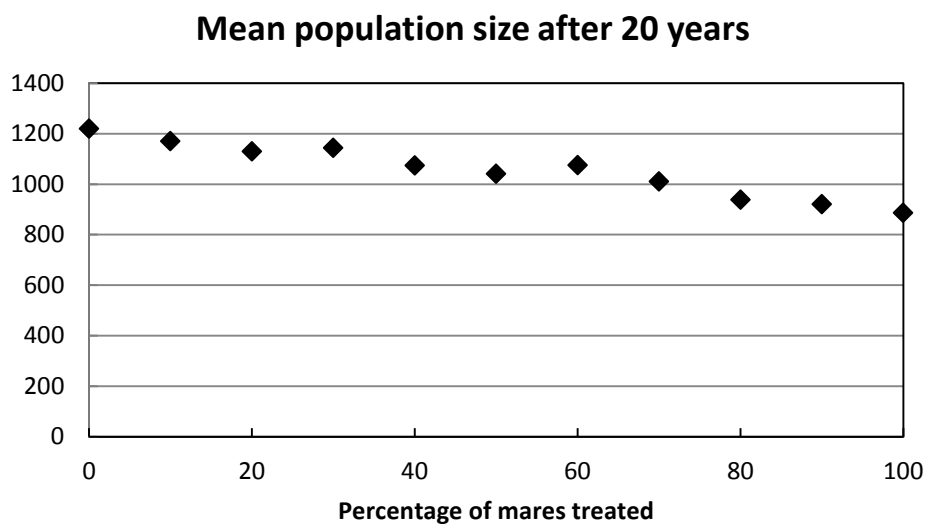
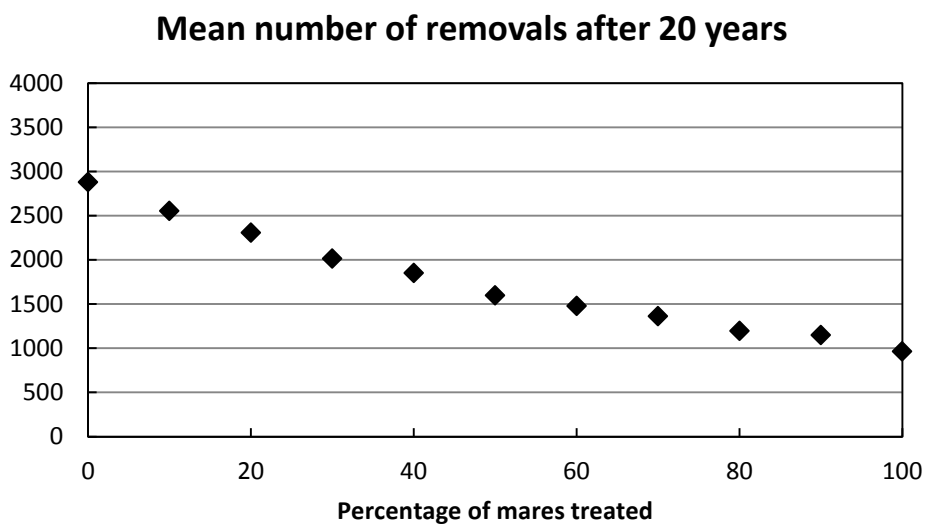
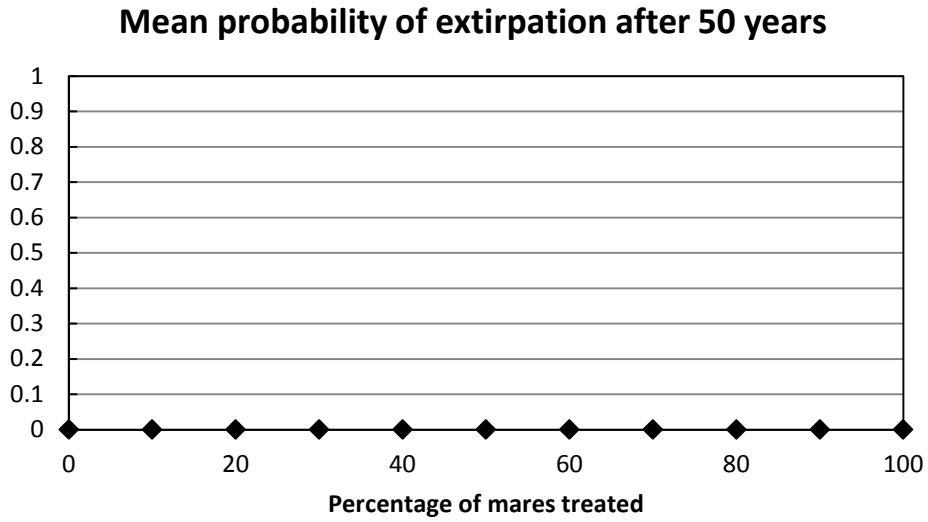


Figure 58. Initial population size = 1000, lambda = 1.15, genetic diversity = high.—Continued







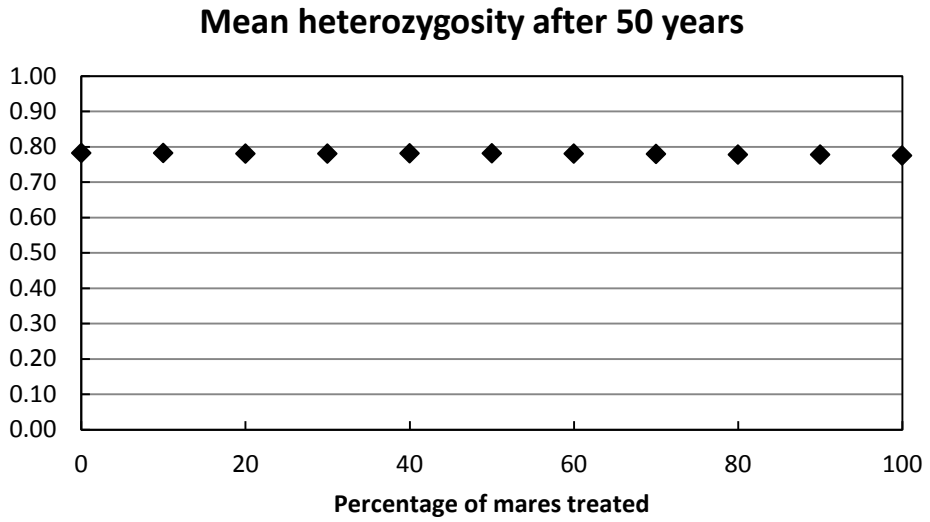


Figure 59. Initial population size = 1000, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1219.7	1169.6	1129.0	1143.8	1073.6	1041.0	1075.2	1009.6	938.2	920.0	886.5
Mean number of removals after 20 years	2877.8	2551.9	2307.0	2013.1	1851.7	1597.8	1478.2	1362.8	1196.4	1149.4	963.0
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

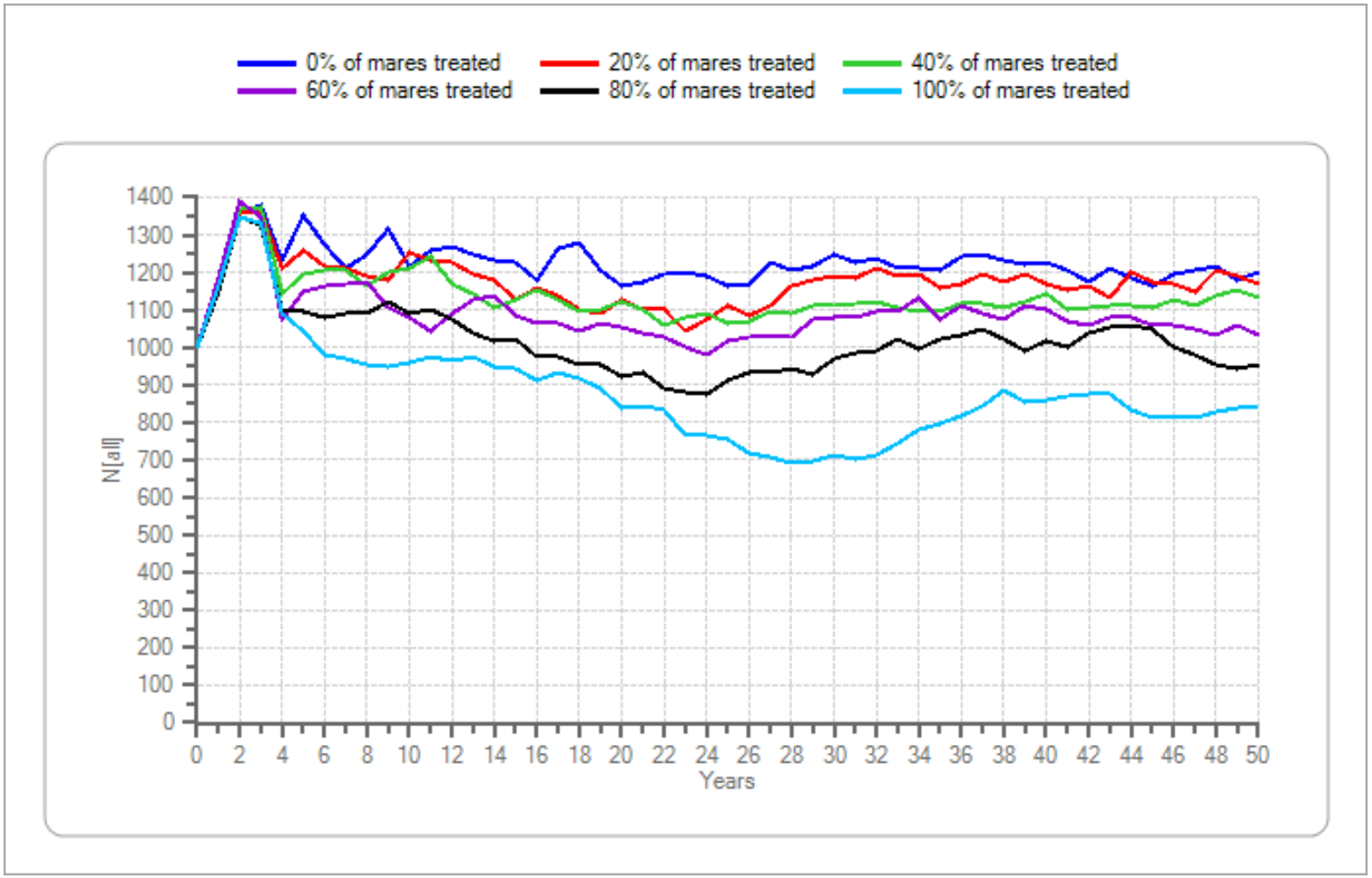
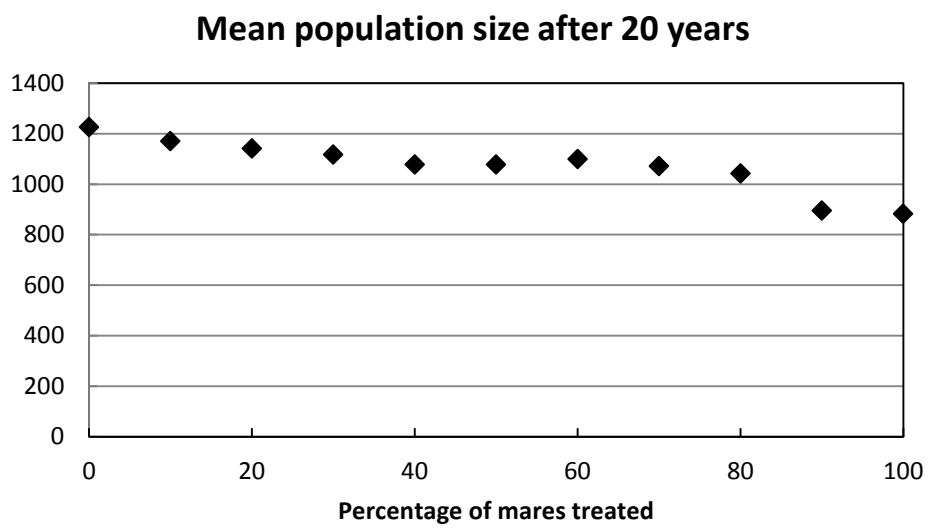
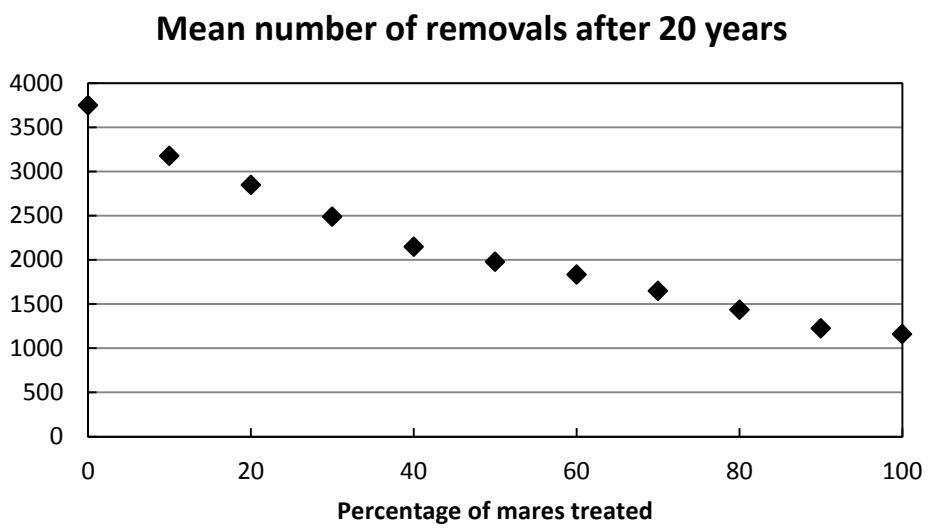
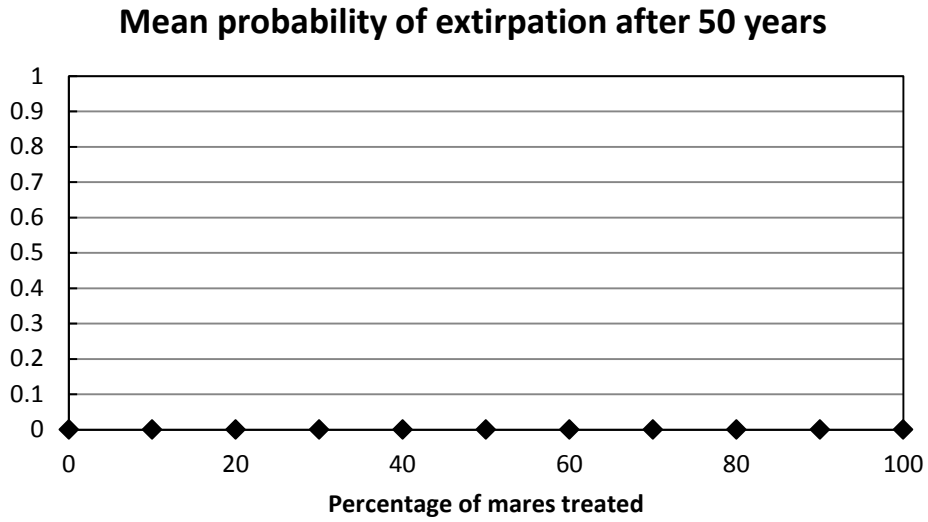


Figure 59. Initial population size = 1000, lambda = 1.20, genetic diversity = high.—Continued







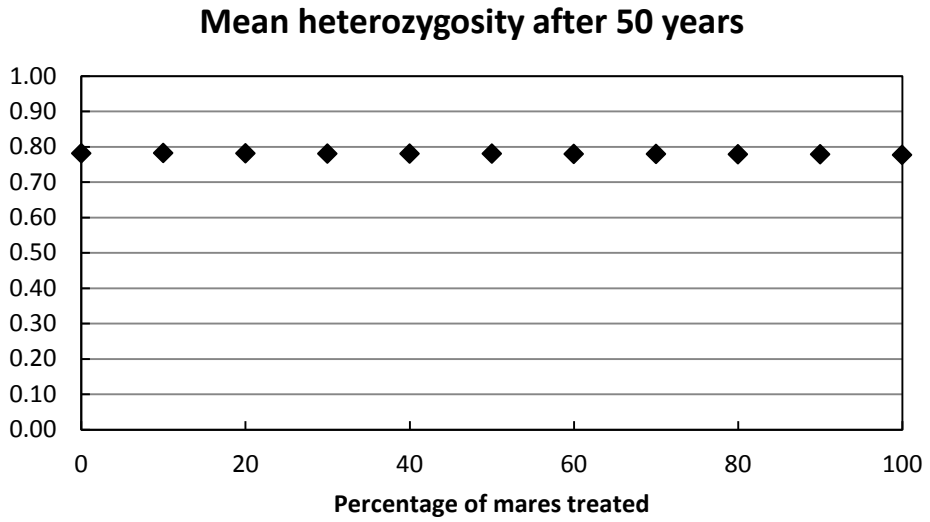


Figure 60. Initial population size = 1000, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1225.4	1169.7	1140.7	1117.1	1077.6	1076.7	1098.6	1071.1	1041.7	894.4	882.7
Mean number of removals after 20 years	3749.6	3175.7	2844.7	2489.0	2147.9	1978.4	1831.9	1649.2	1432.6	1223.8	1159.1
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

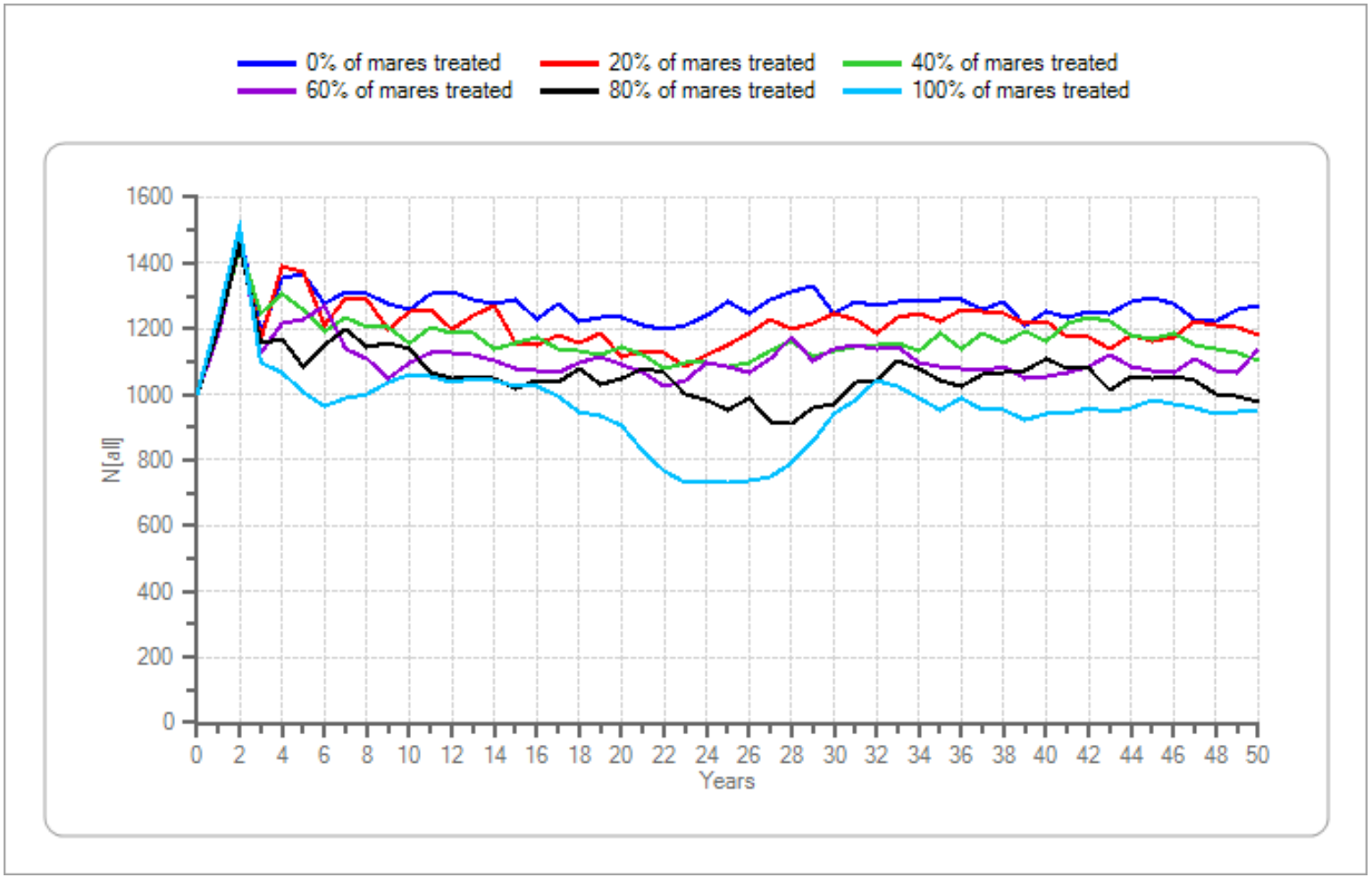
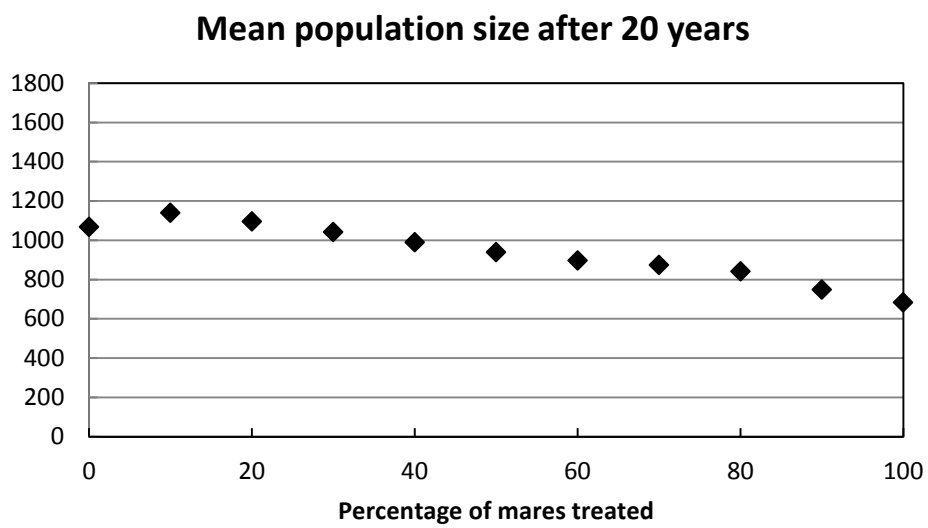
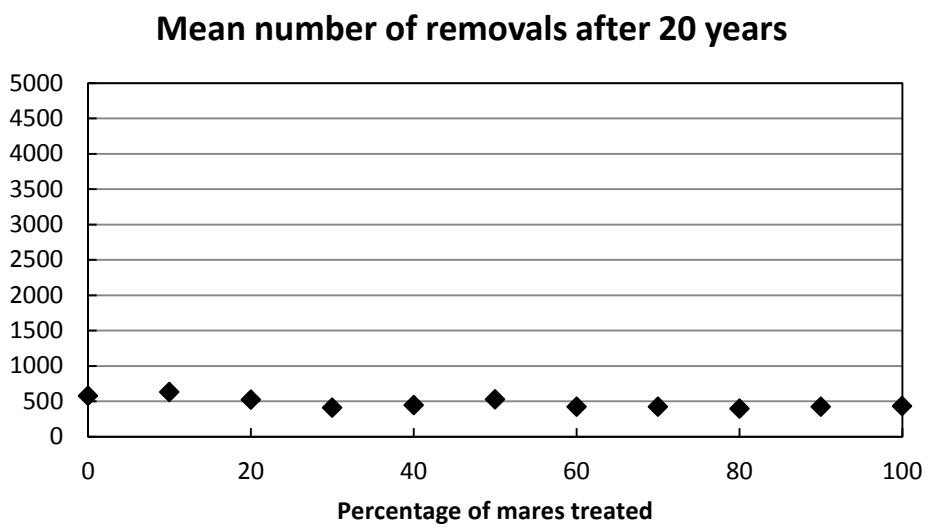
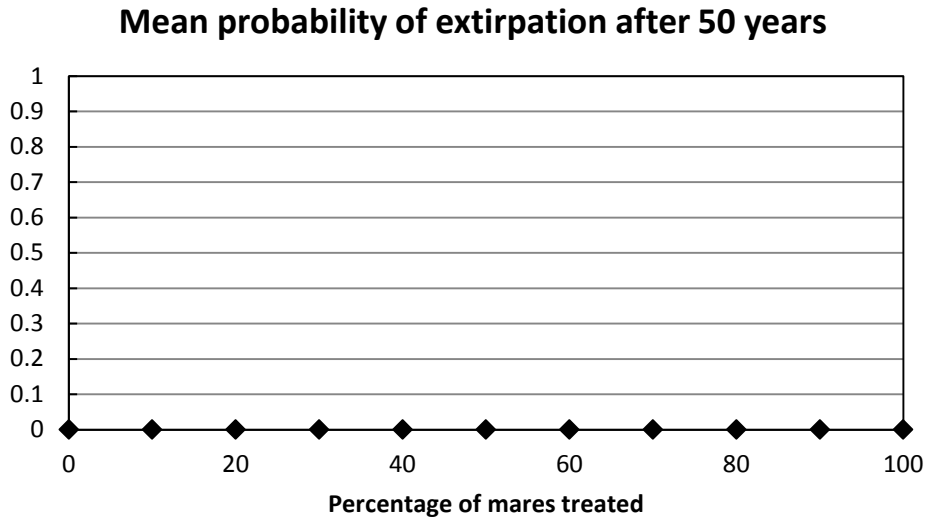


Figure 60. Initial population size = 1000, lambda = 1.25, genetic diversity = high.—Continued







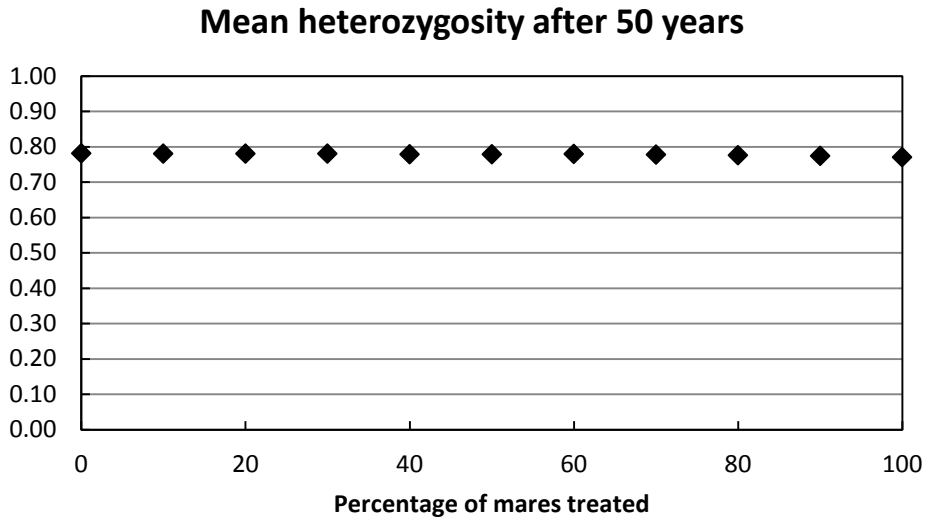


Figure 61. Initial population size = 1250, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1066.7	1139.5	1095.1	1041.0	989.2	938.4	896.1	874.0	841.0	747.5	683.3
Mean number of removals after 20 years	575.5	629.3	519.5	406.3	442.6	524.2	422.0	420.3	393.0	422.2	428.9
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.77

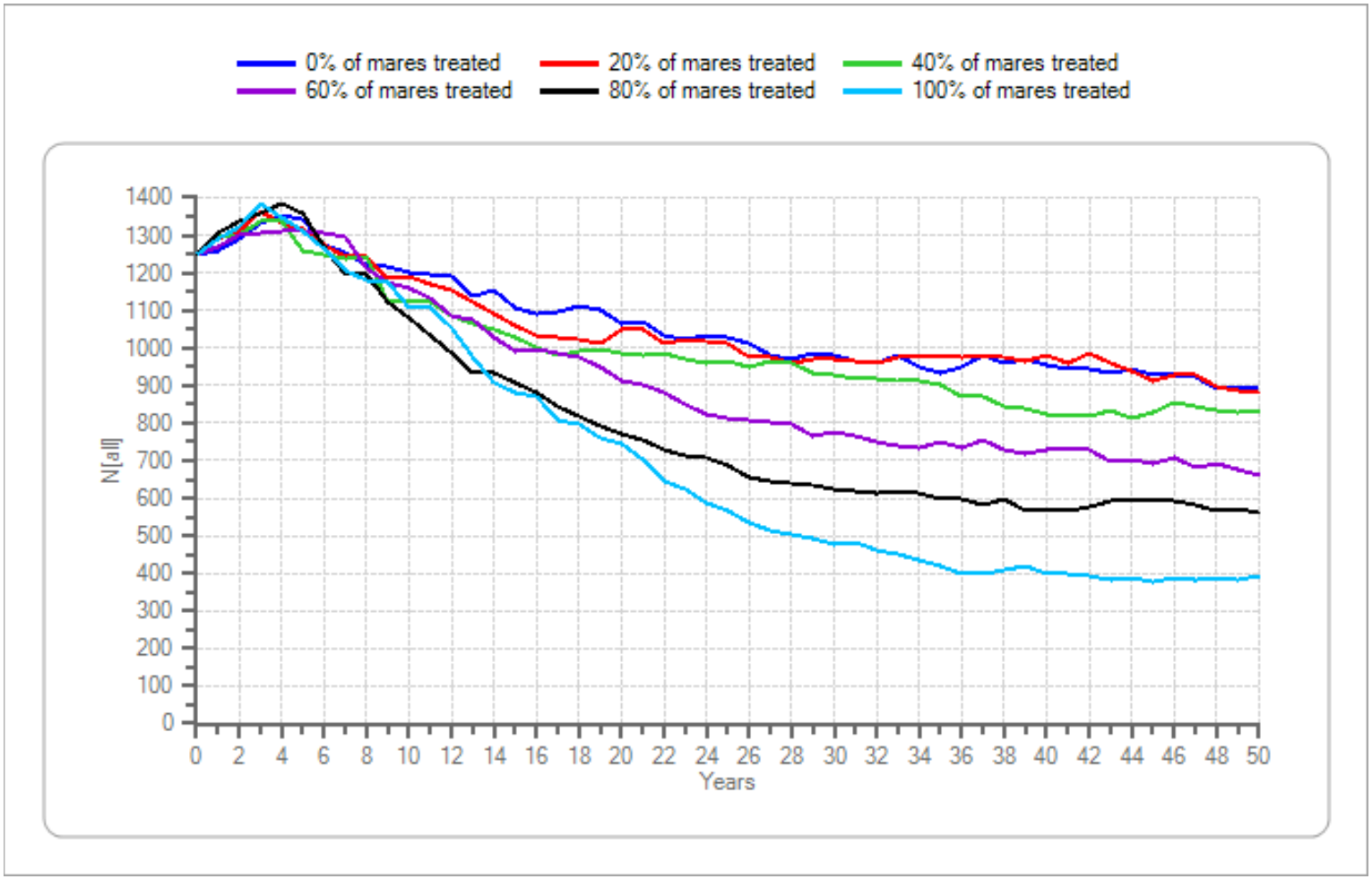
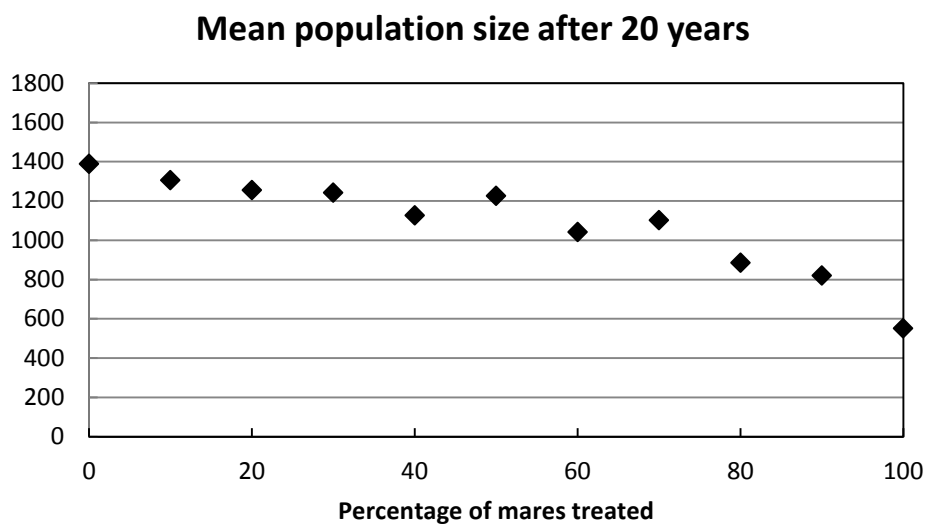
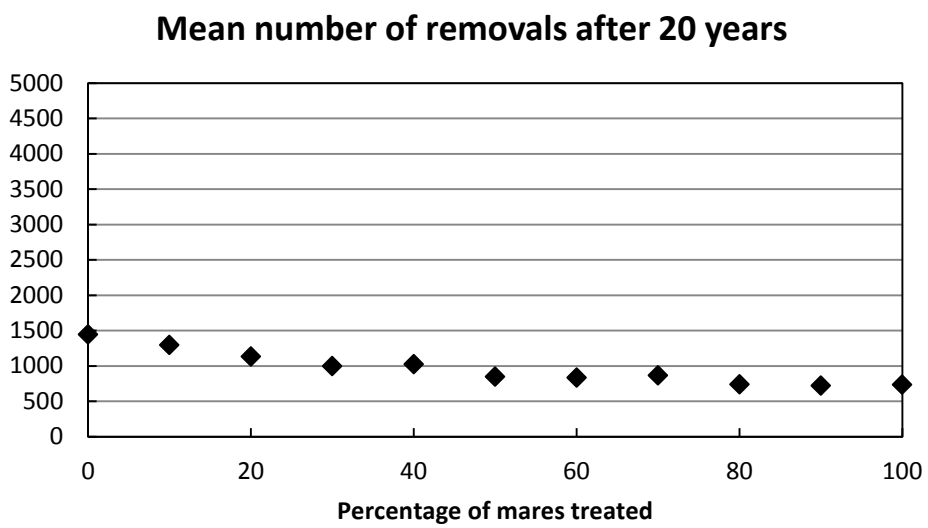
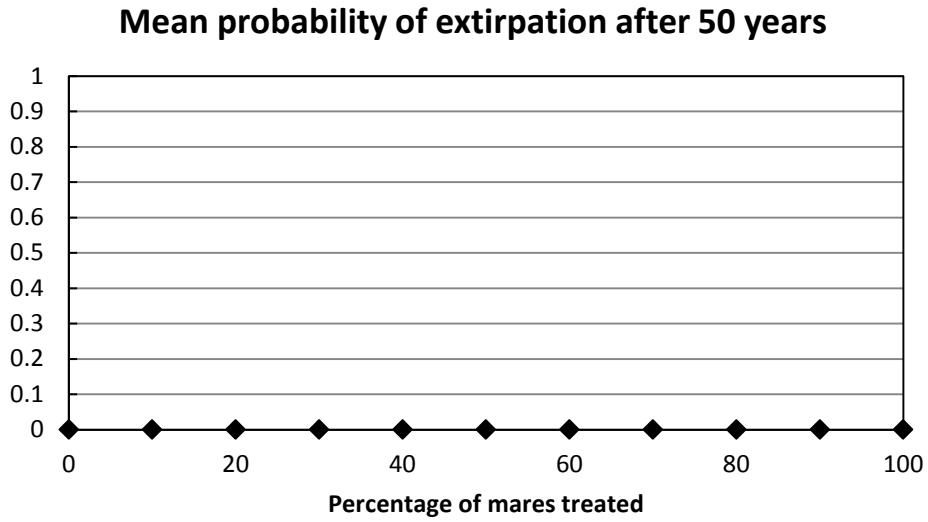


Figure 61. Initial population size = 1250, lambda = 1.05, genetic diversity = high.—Continued







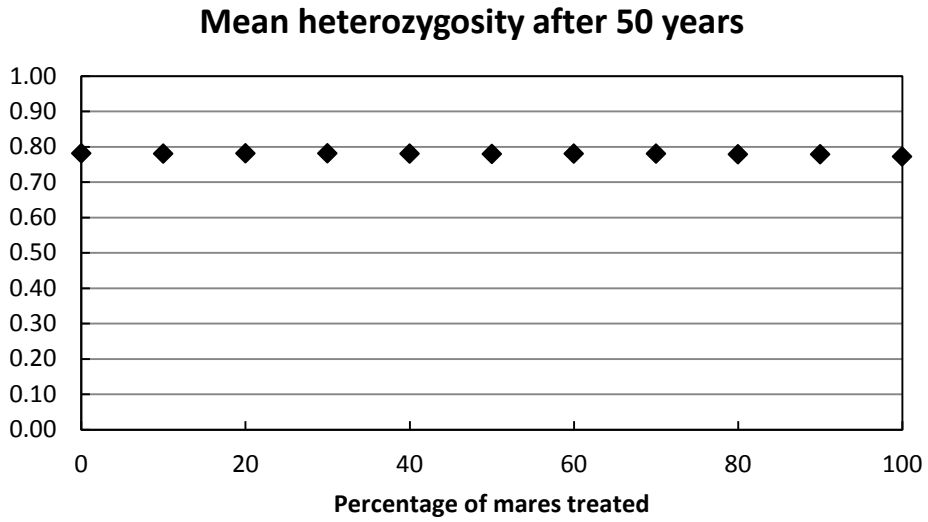


Figure 62. Initial population size = 1250, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1388.7	1304.7	1254.2	1242.3	1126.8	1225.7	1041.3	1101.1	885.0	820.1	551.3
Mean number of removals after 20 years	1442.2	1296.3	1129.6	995.3	1024.7	845.1	834.5	866.0	738.2	721.3	735.6
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

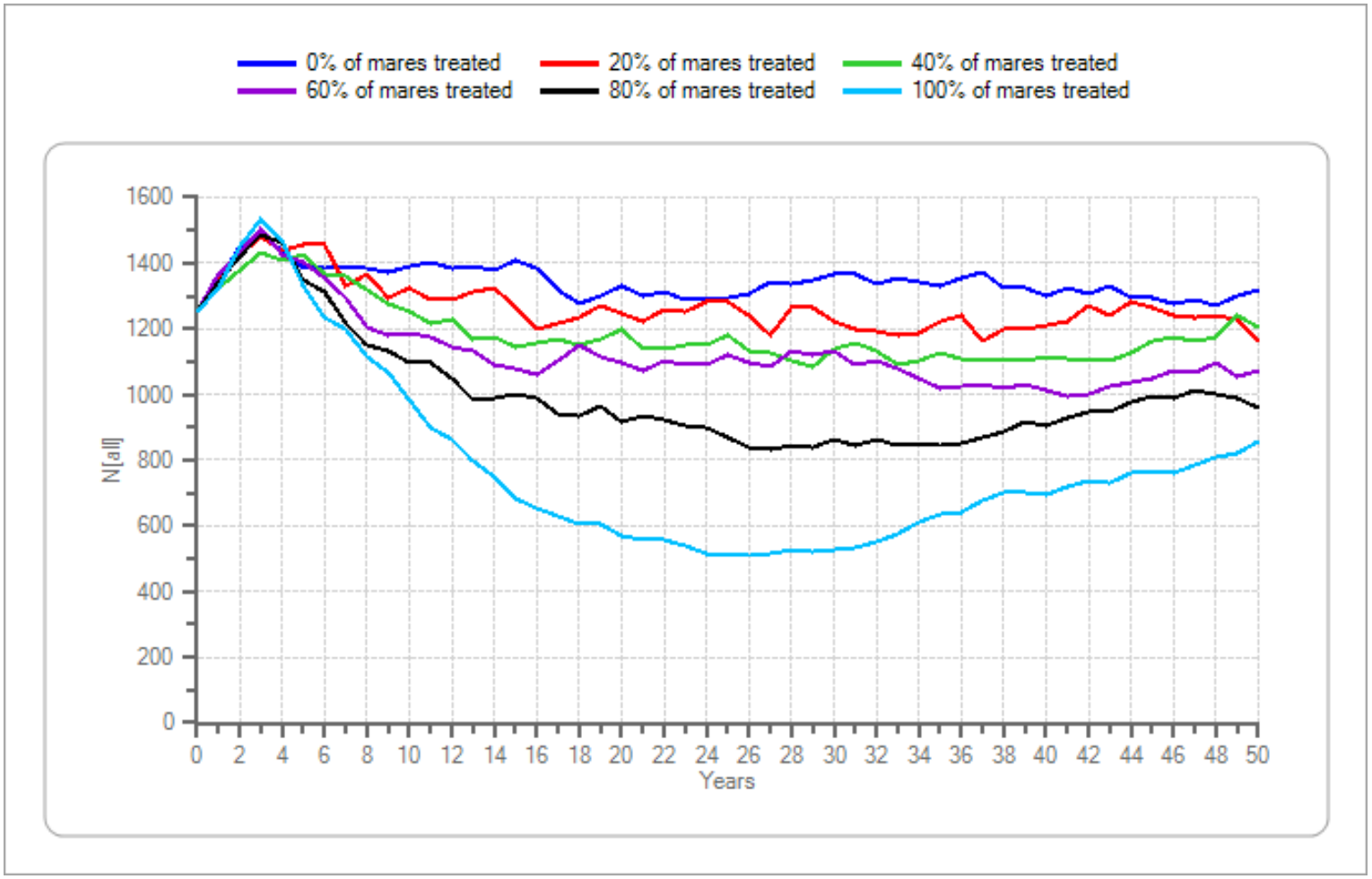
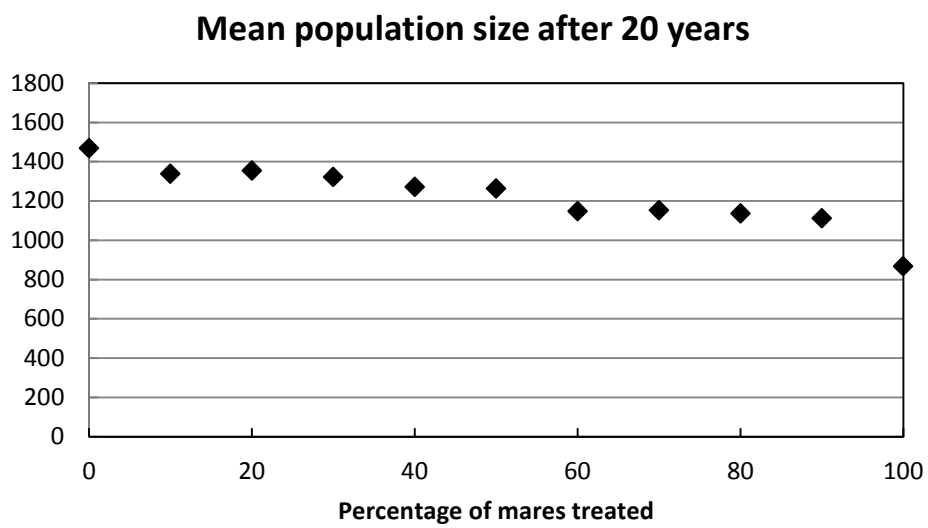
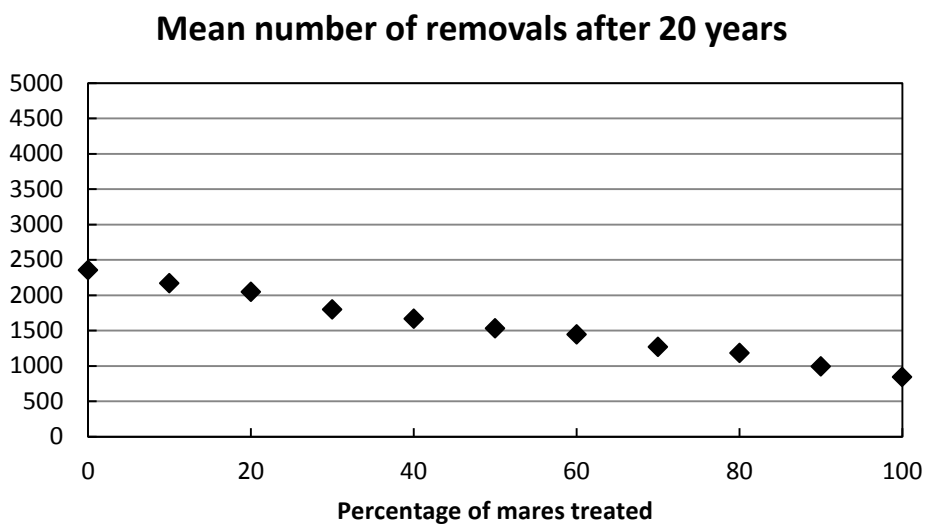
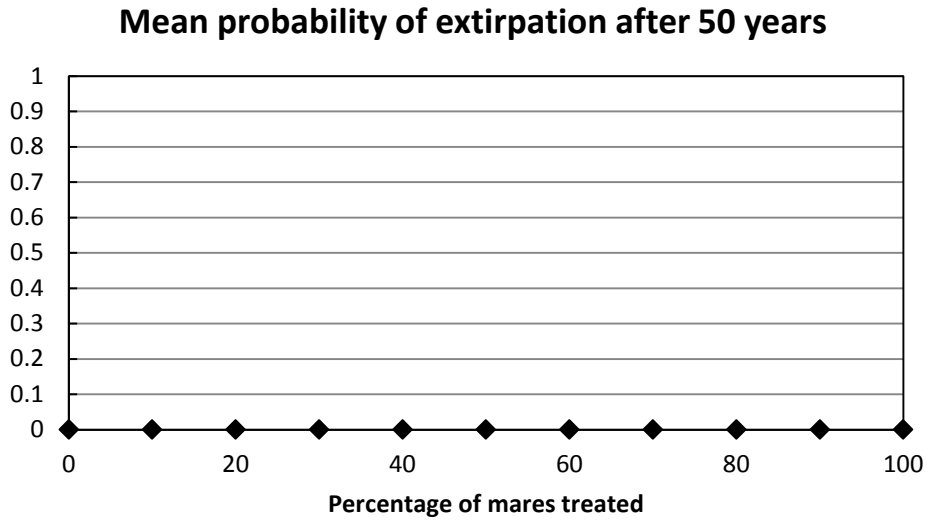


Figure 62. Initial population size = 1250, lambda = 1.10, genetic diversity = high.—Continued







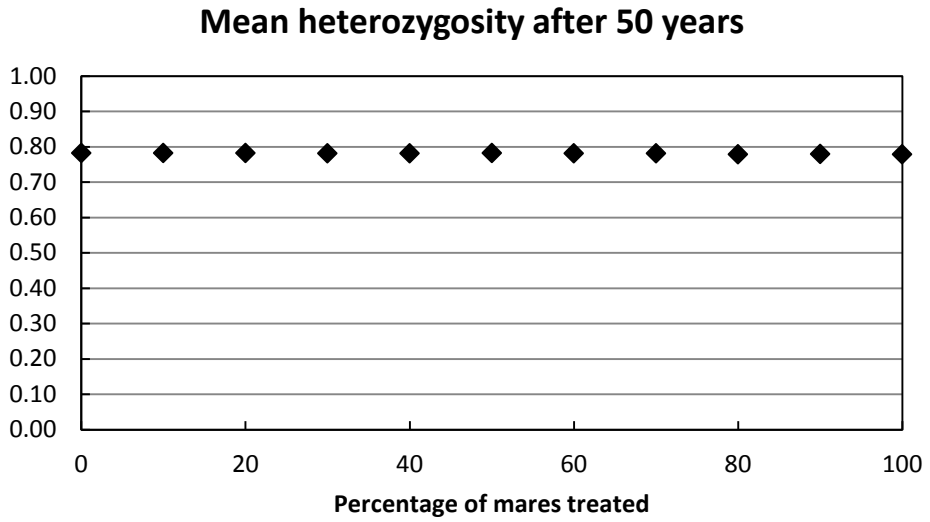


Figure 63. Initial population size = 1250, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1468.9	1338.2	1354.4	1321.2	1271.8	1263.7	1147.8	1151.5	1135.1	1112.2	866.6
Mean number of removals after 20 years	2353.0	2166.1	2044.2	1797.3	1667.0	1529.1	1445.7	1267.1	1179.7	991.6	840.9
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

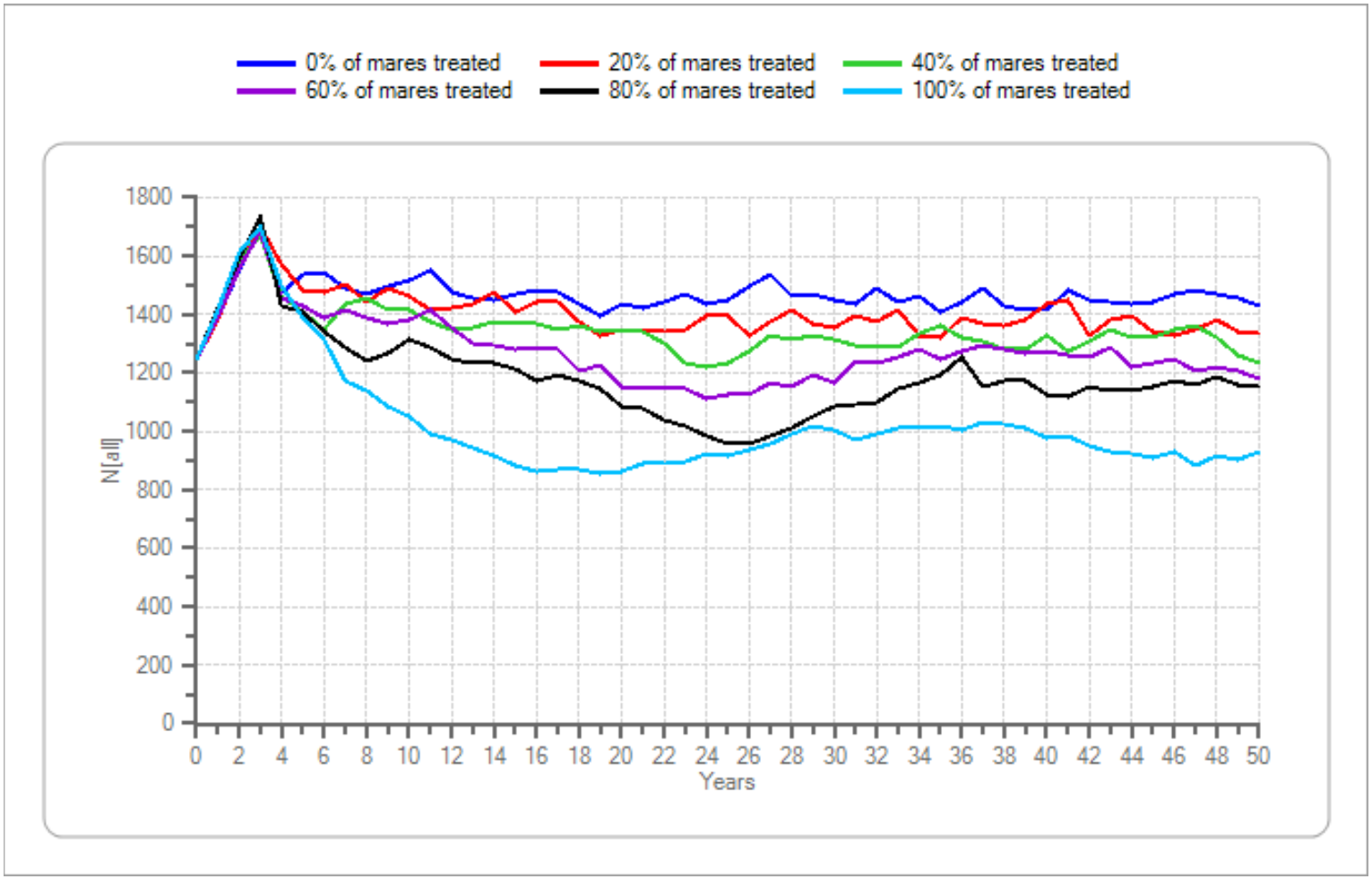
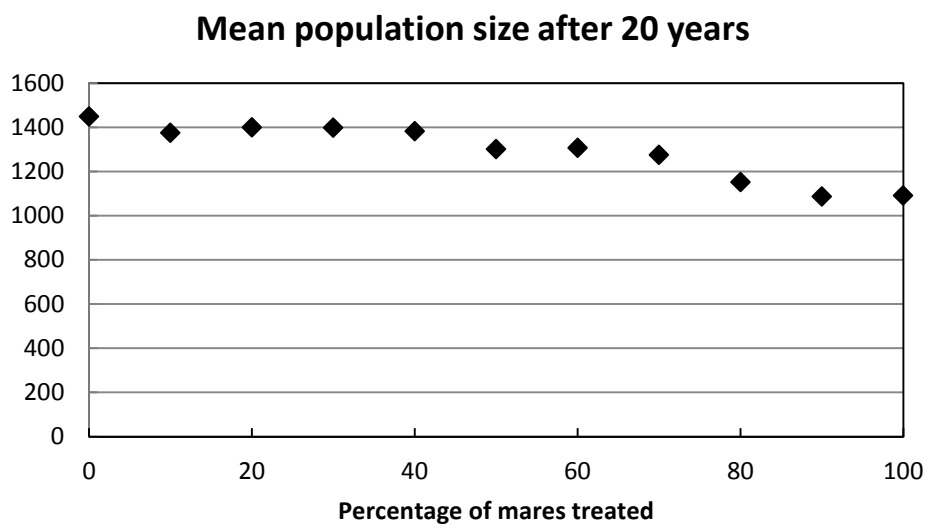
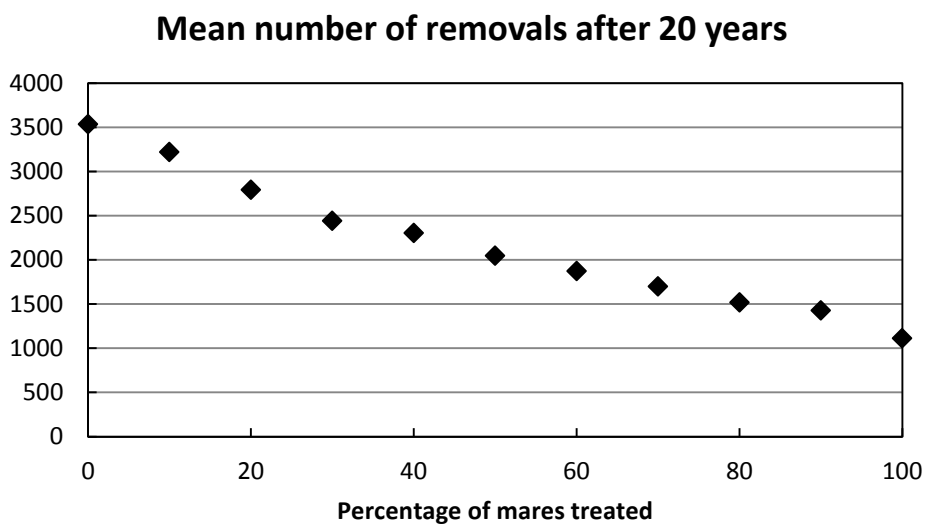
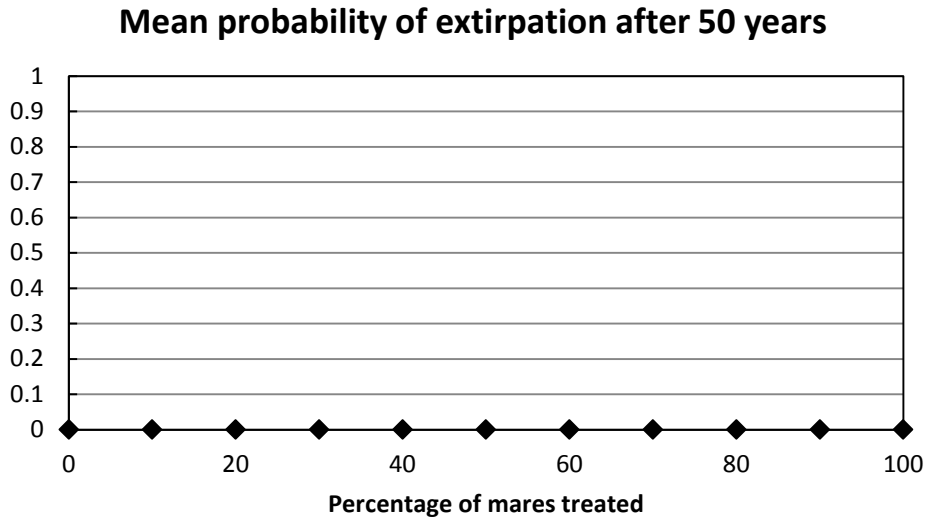


Figure 63. Initial population size = 1250, lambda = 1.15, genetic diversity = high.—Continued







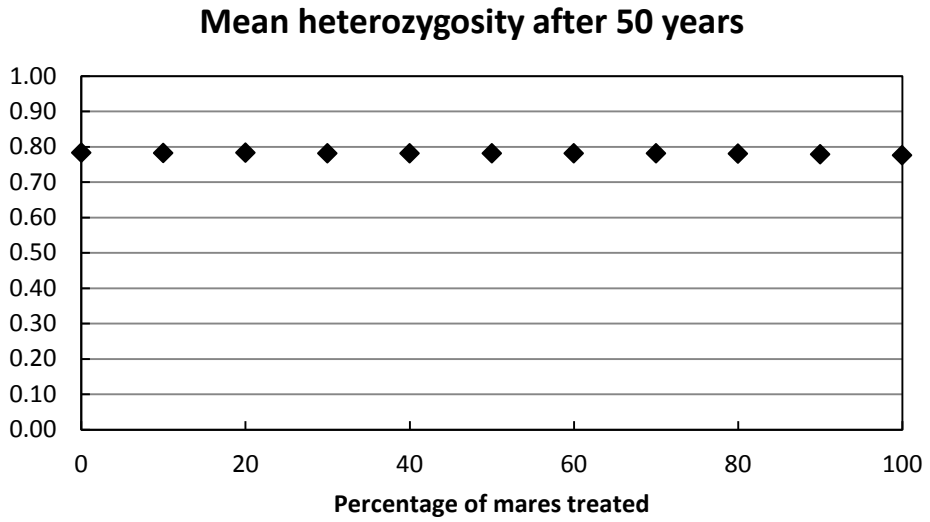


Figure 64. Initial population size = 1250, lambda = 1.20, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1449.1	1375.0	1398.9	1397.6	1381.6	1301.0	1306.8	1274.2	1152.3	1086.1	1090.1
Mean number of removals after 20 years	3536.0	3218.8	2791.6	2439.7	2302.0	2047.4	1874.1	1697.0	1517.3	1426.7	1110.3
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

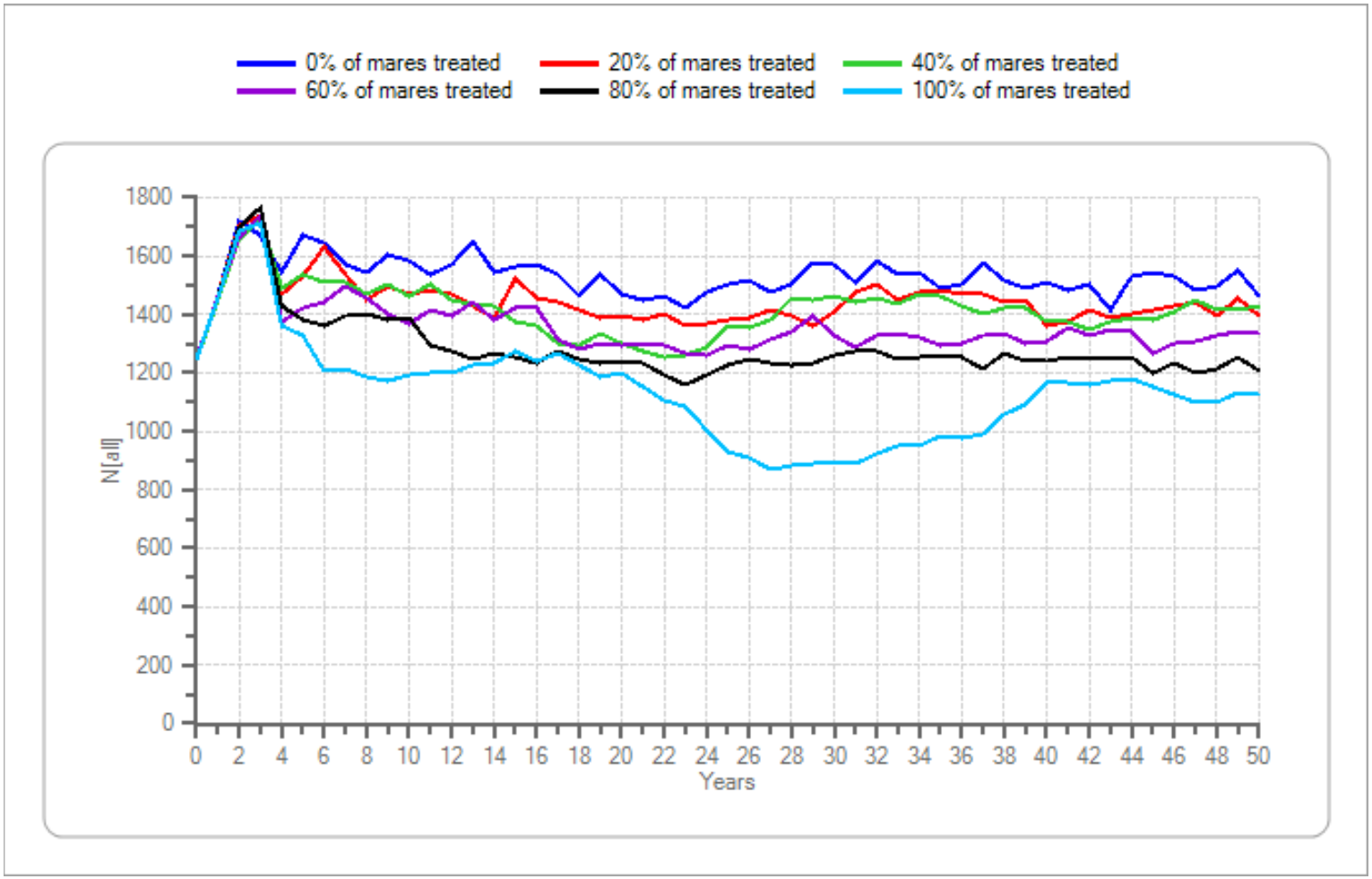
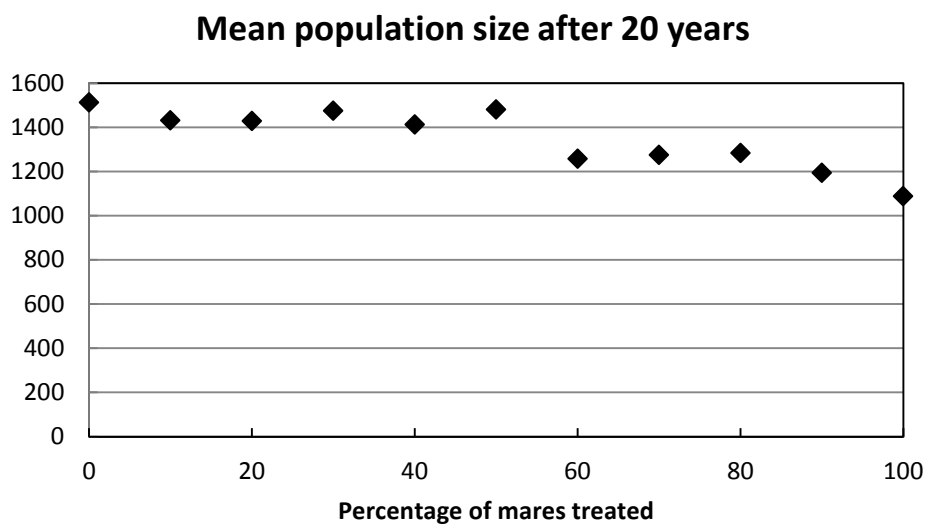
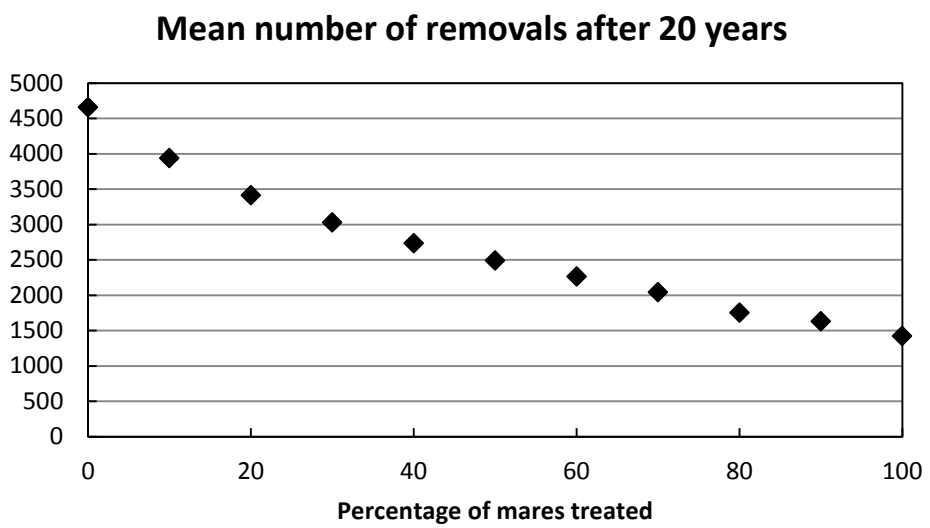
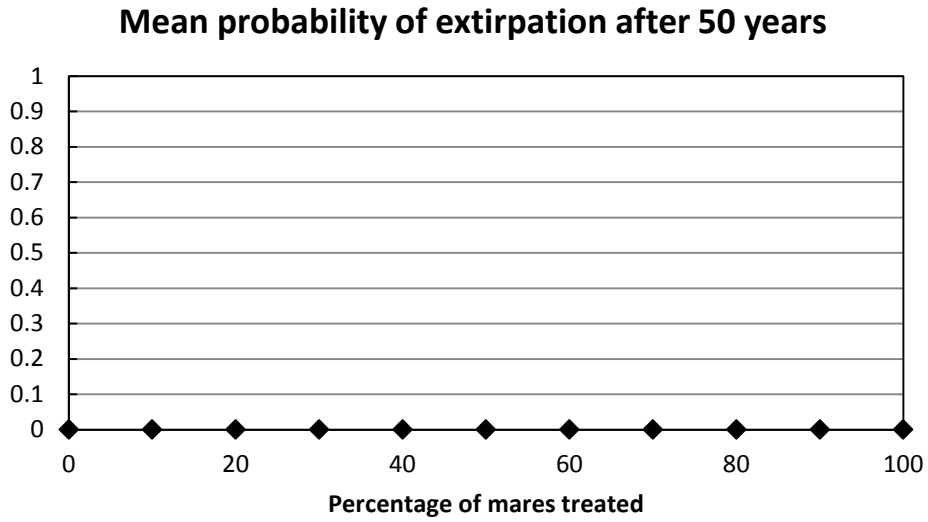


Figure 64. Initial population size = 1250, lambda = 1.20, genetic diversity = low.—Continued







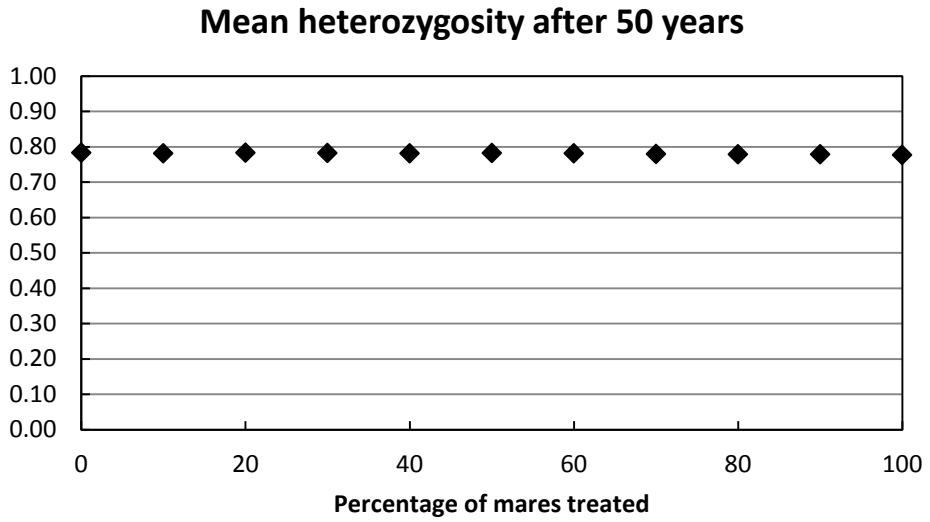


Figure 65. Initial population size = 1250, lambda = 1.25, genetic diversity = low.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1512.7	1430.9	1428.7	1474.7	1412.0	1480.7	1257.7	1274.9	1284.0	1193.1	1087.6
Mean number of removals after 20 years	4658.4	3937.6	3415.0	3030.1	2732.3	2491.5	2261.5	2041.8	1751.8	1629.4	1422.0
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

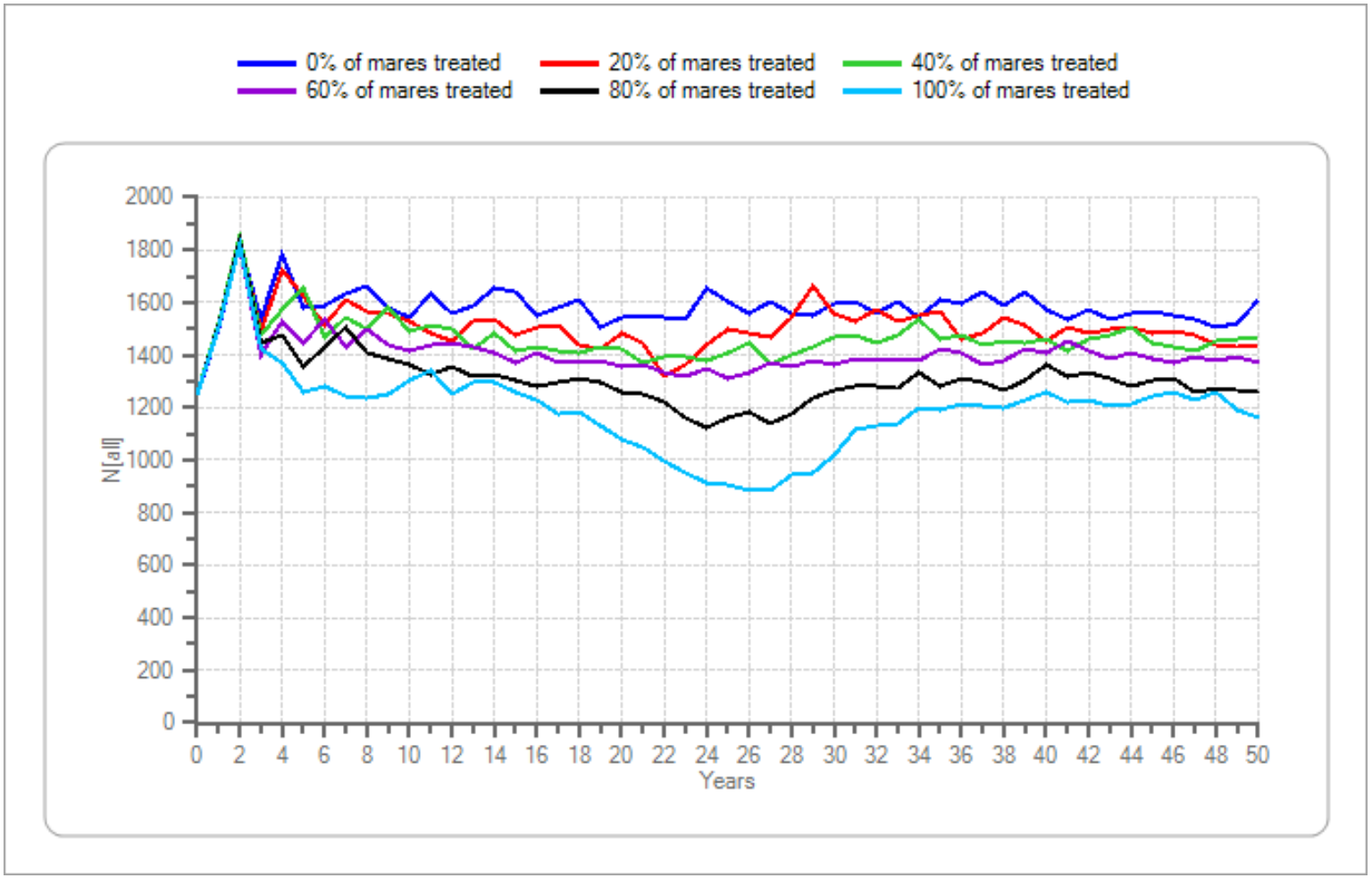
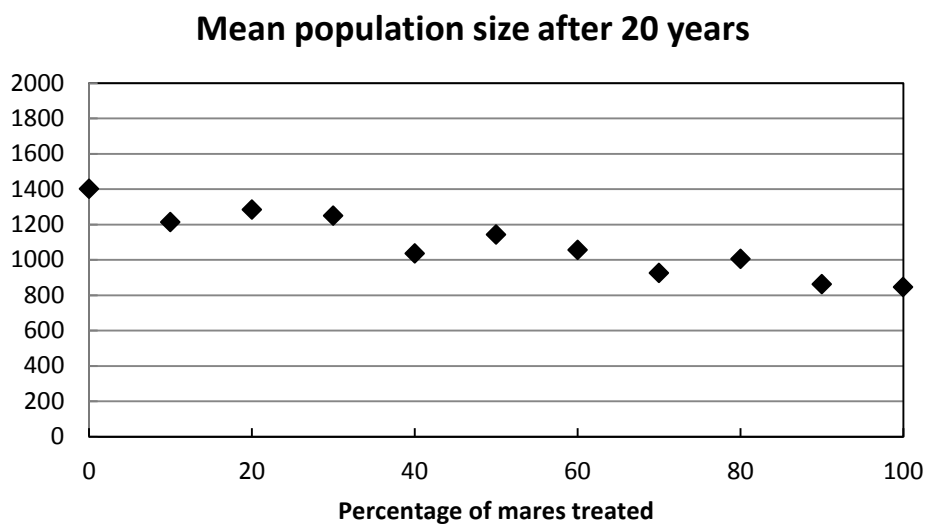
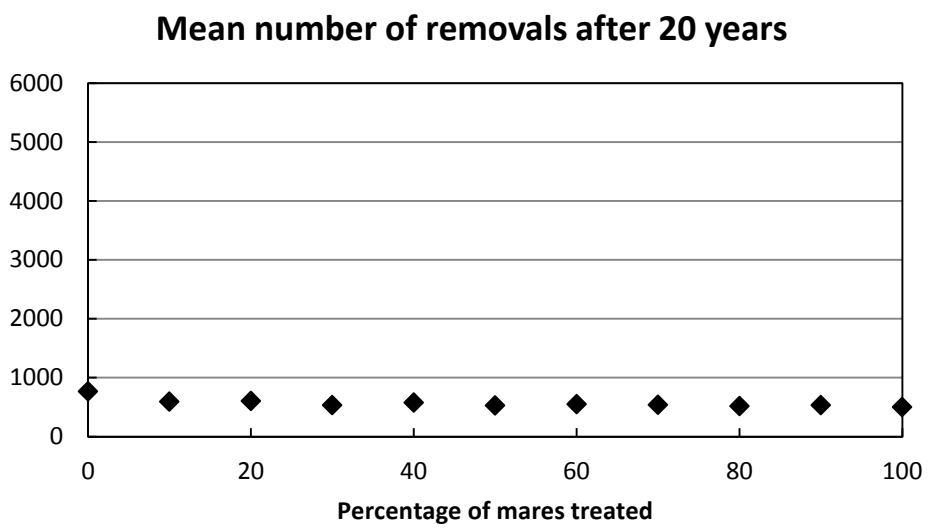
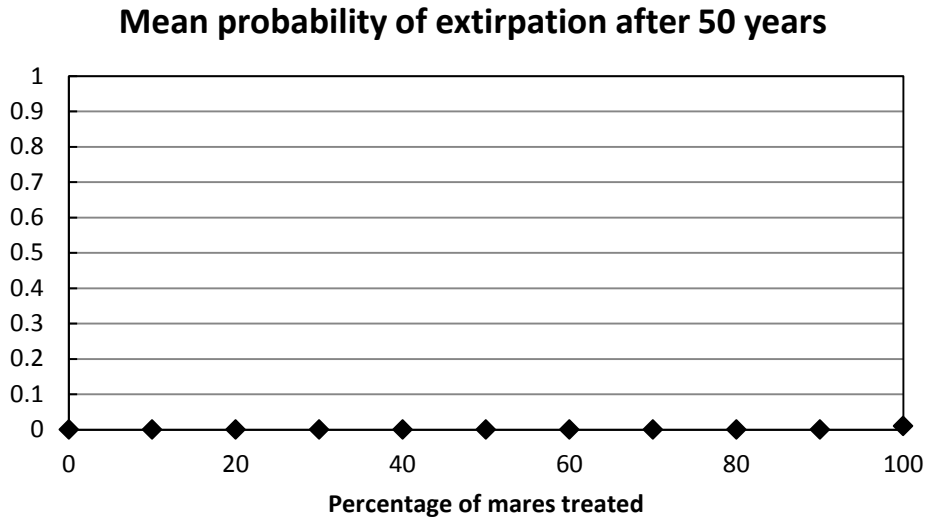


Figure 65. Initial population size = 1250, lambda = 1.25, genetic diversity = low.—Continued







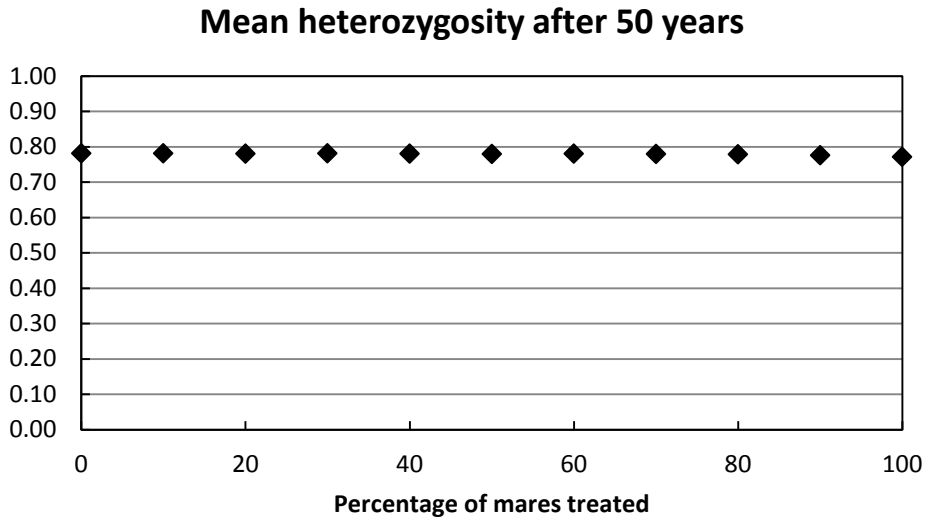


Figure 66. Initial population size = 1500, lambda = 1.05, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1401.3	1213.9	1283.2	1249.7	1035.4	1142.4	1056.3	925.6	1005.1	862.6	845.7
Mean number of removals after 20 years	764.8	589.6	604.0	530.4	578.3	527.0	547.6	539.9	516.7	534.7	497.8
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0.01
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

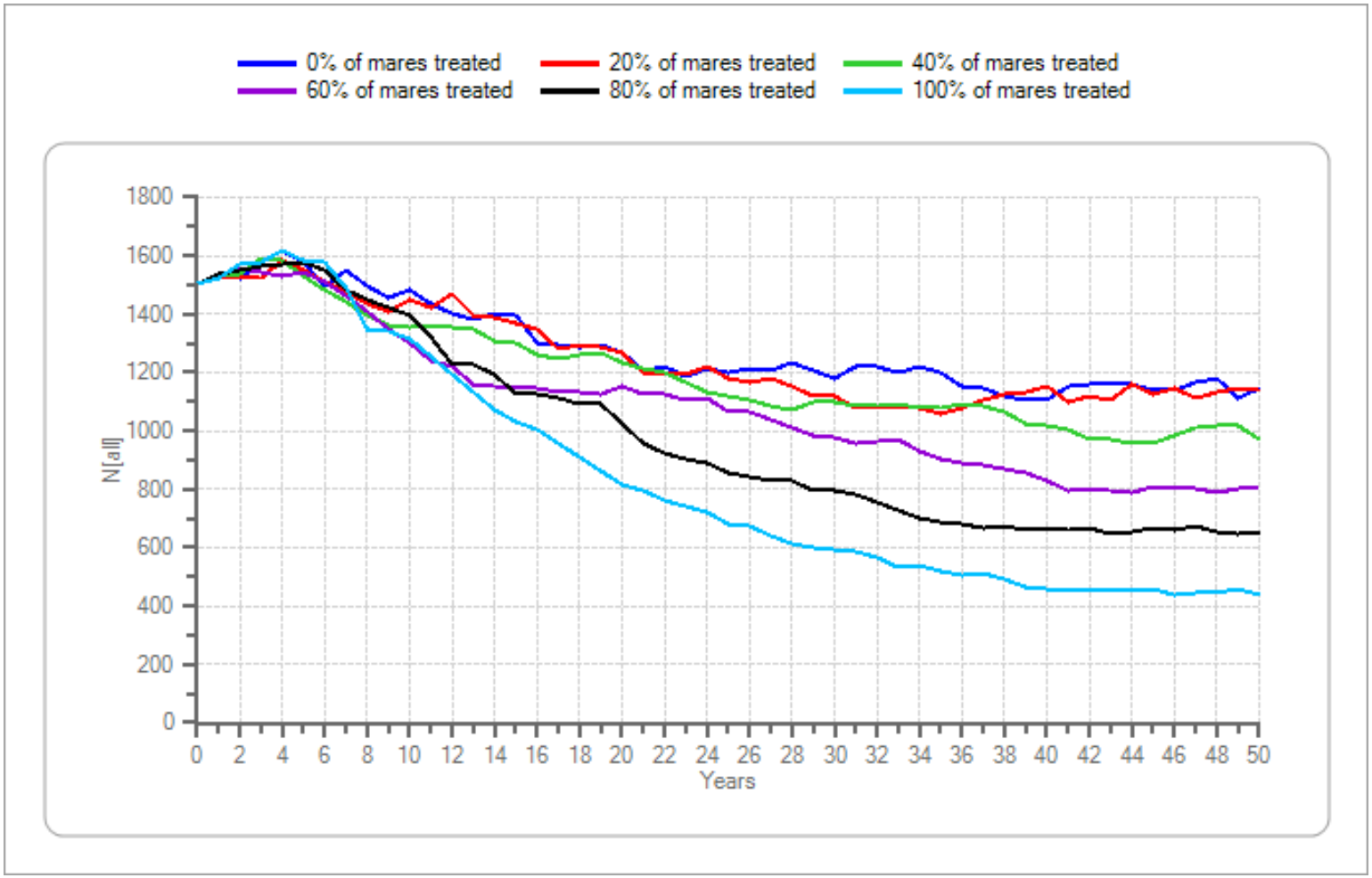
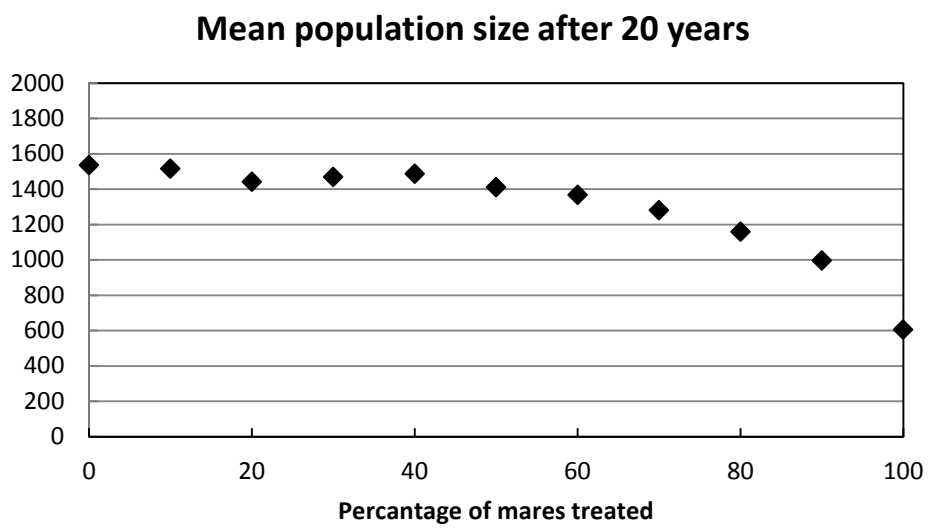
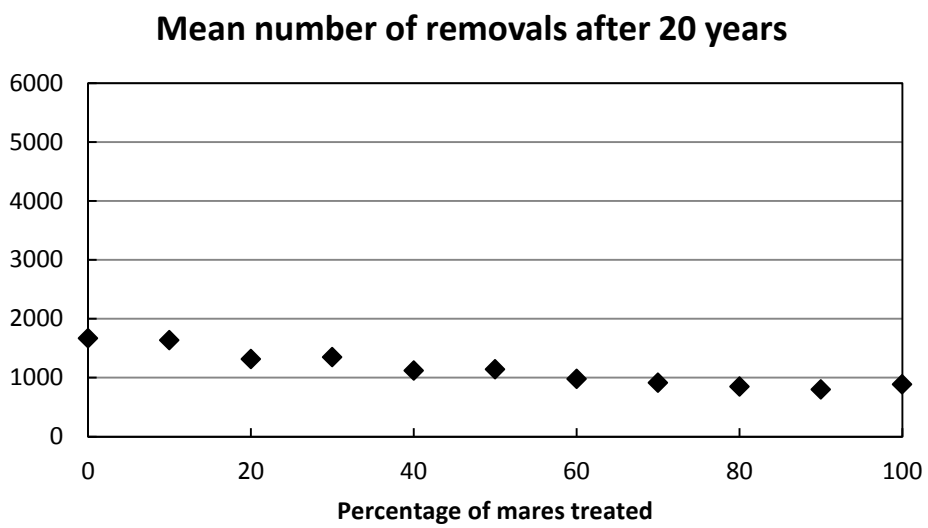
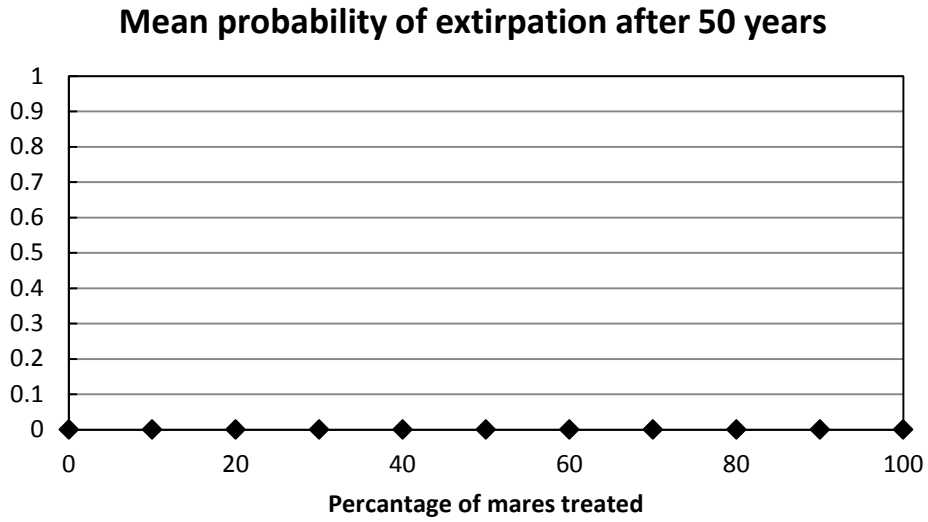


Figure 66. Initial population size = 1500, lambda = 1.05, genetic diversity = high.—Continued







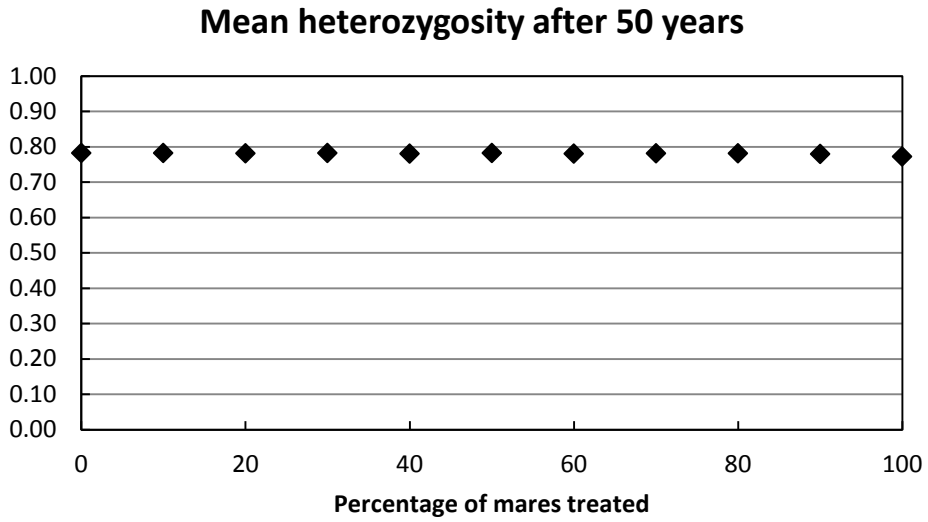


Figure 67. Initial population size = 1500, lambda = 1.10, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1534.7	1515.0	1441.5	1468.4	1487.3	1411.4	1367.4	1280.2	1158.3	996.3	604.3
Mean number of removals after 20 years	1669.4	1636.8	1316.9	1346.6	1118.7	1139.7	979.0	911.8	846.6	799.3	886.0
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.77

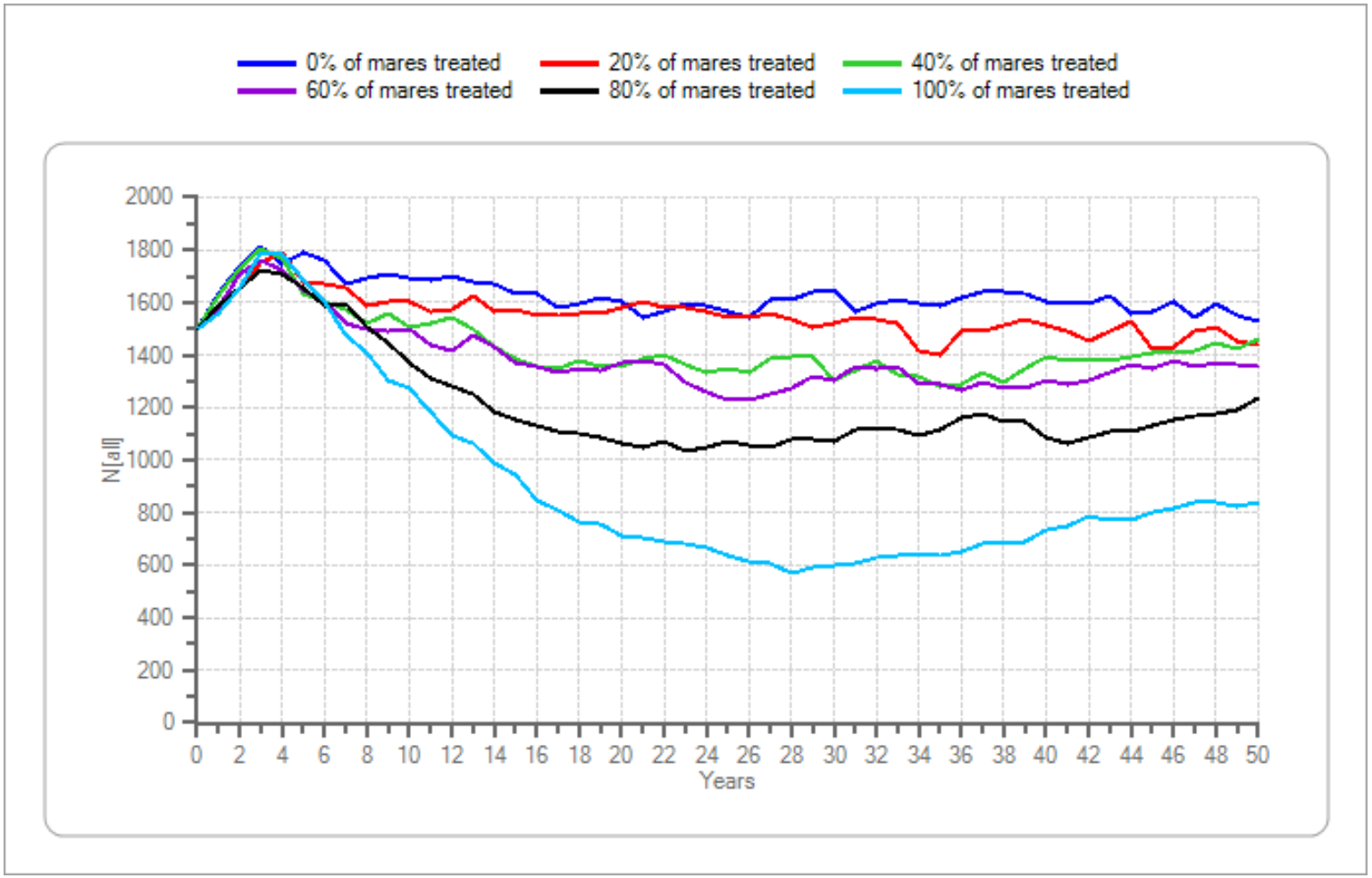
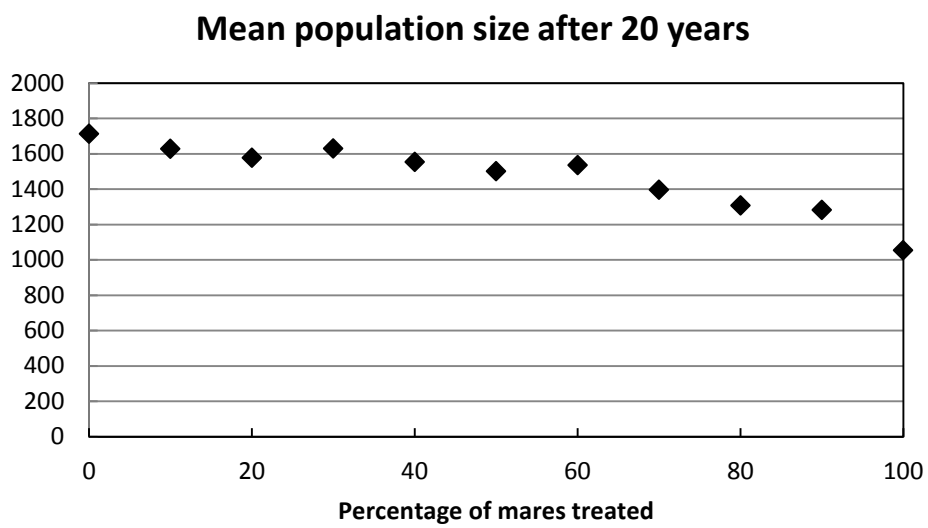
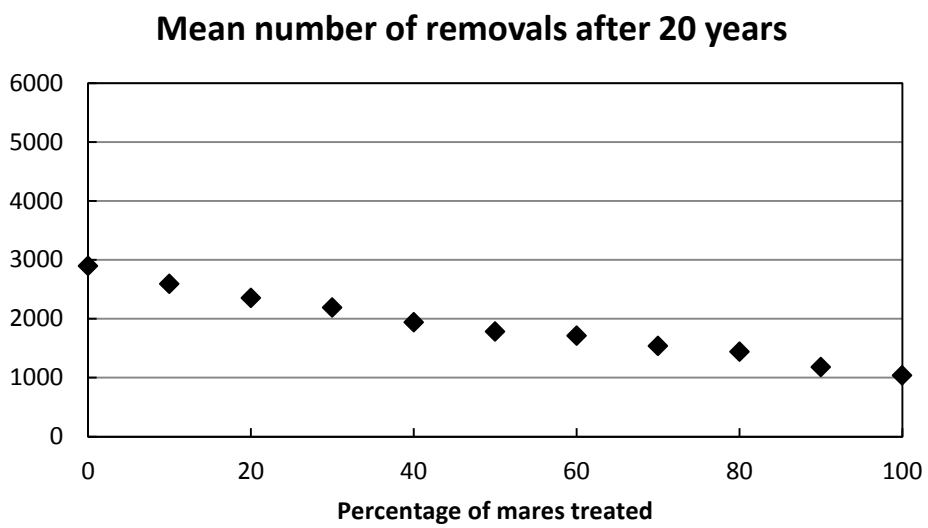
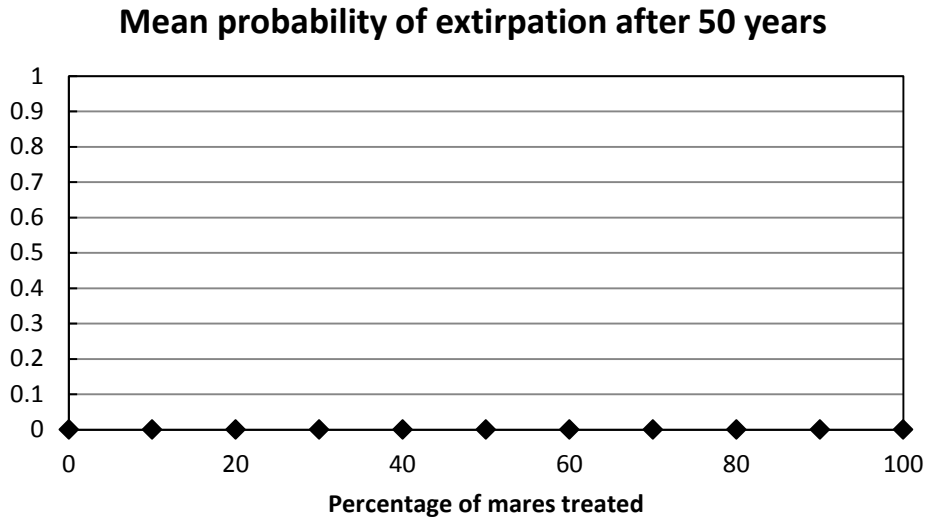


Figure 67. Initial population size = 1500, lambda = 1.10, genetic diversity = high.—Continued







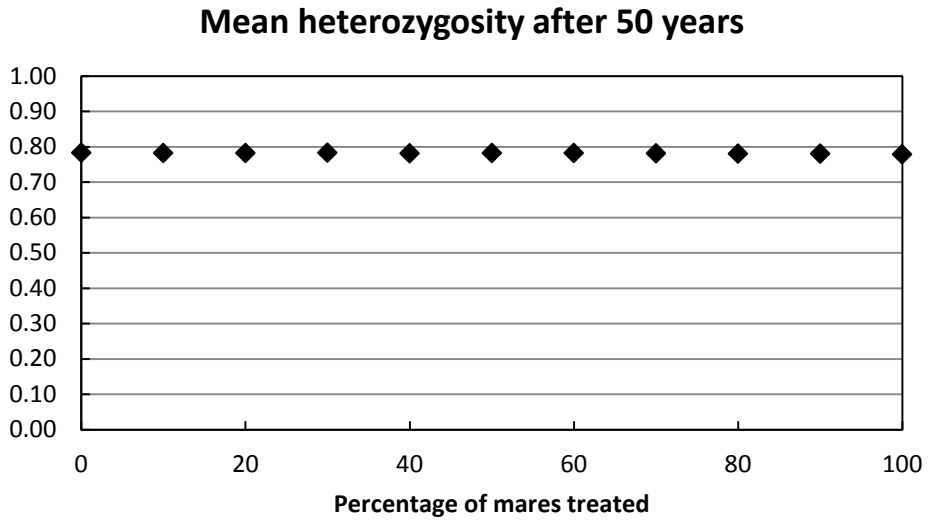


Figure 68. Initial population size = 1500, lambda = 1.15, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1712.0	1627.6	1576.6	1629.3	1553.6	1500.3	1535.1	1396.9	1306.5	1281.2	1053.2
Mean number of removals after 20 years	2896.0	2590.5	2349.9	2187.2	1938.7	1780.1	1713.2	1538.8	1441.8	1180.9	1038.4
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

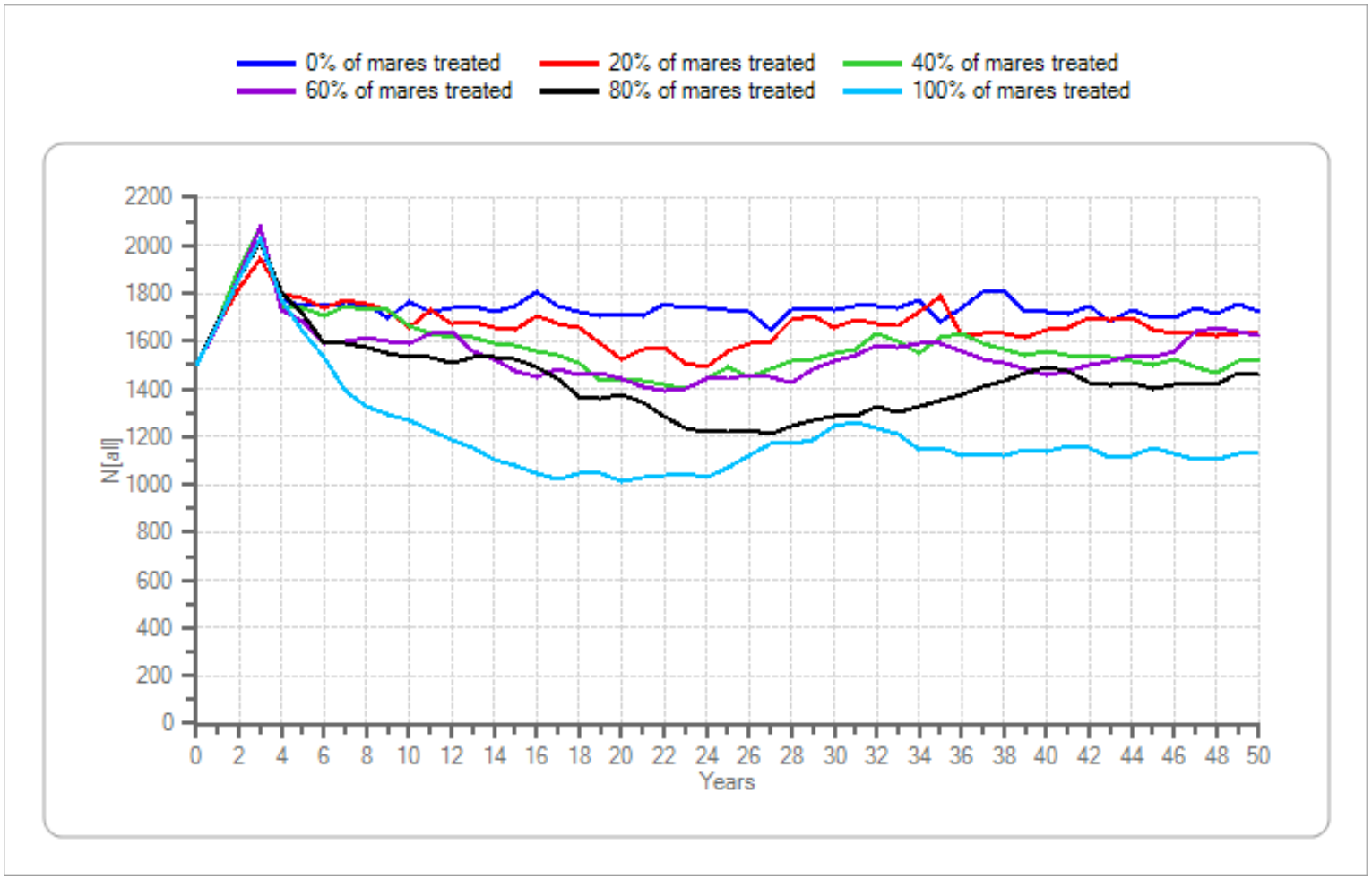
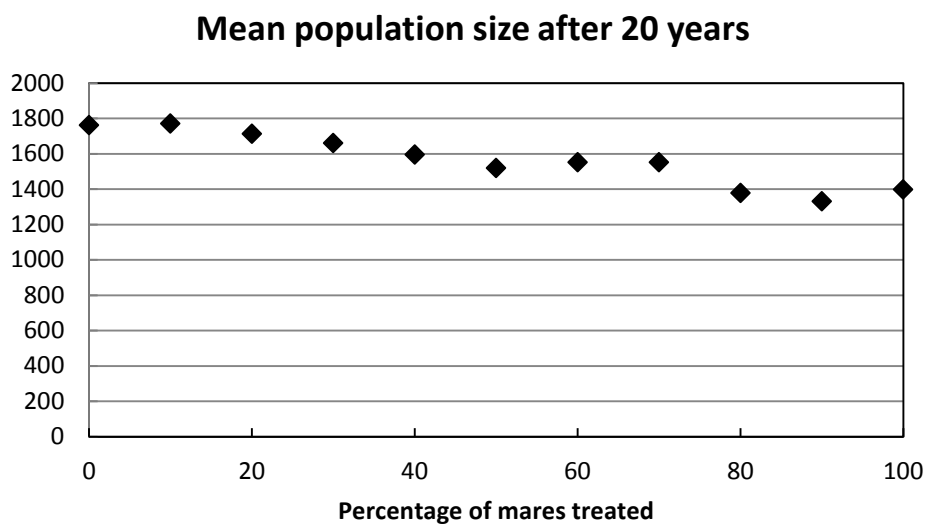
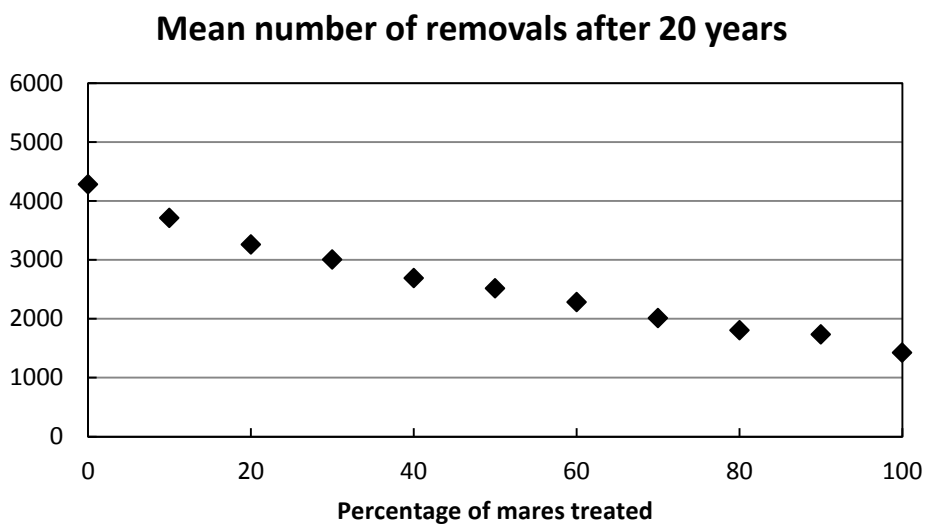
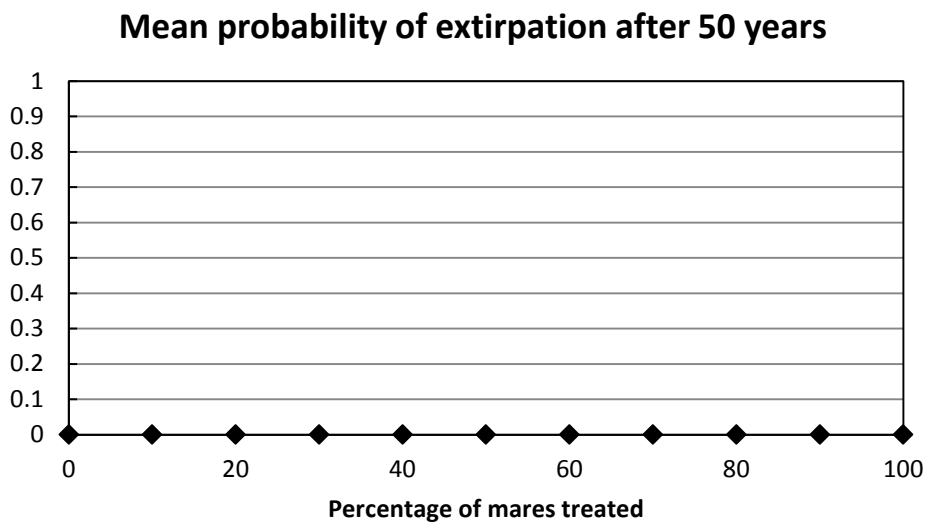


Figure 68. Initial population size = 1500, lambda = 1.15, genetic diversity = high.—Continued







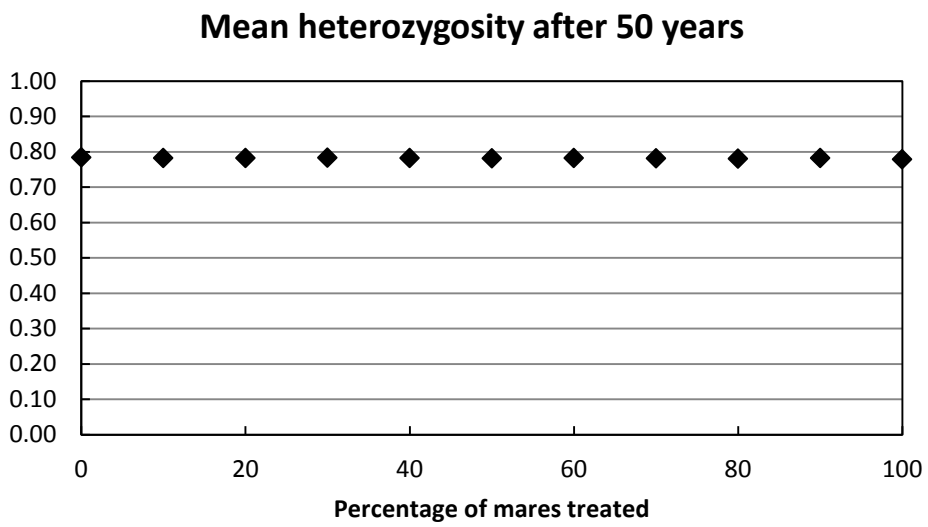


Figure 69. Initial population size = 1500, lambda = 1.20, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1761.4	1771.4	1712.8	1660.2	1595.1	1518.3	1551.7	1551.3	1378.4	1331.5	1397.5
Mean number of removals after 20 years	4280.8	3712.2	3260.2	3005.3	2689.8	2516.5	2281.8	2007.8	1802.1	1735.3	1423.0
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

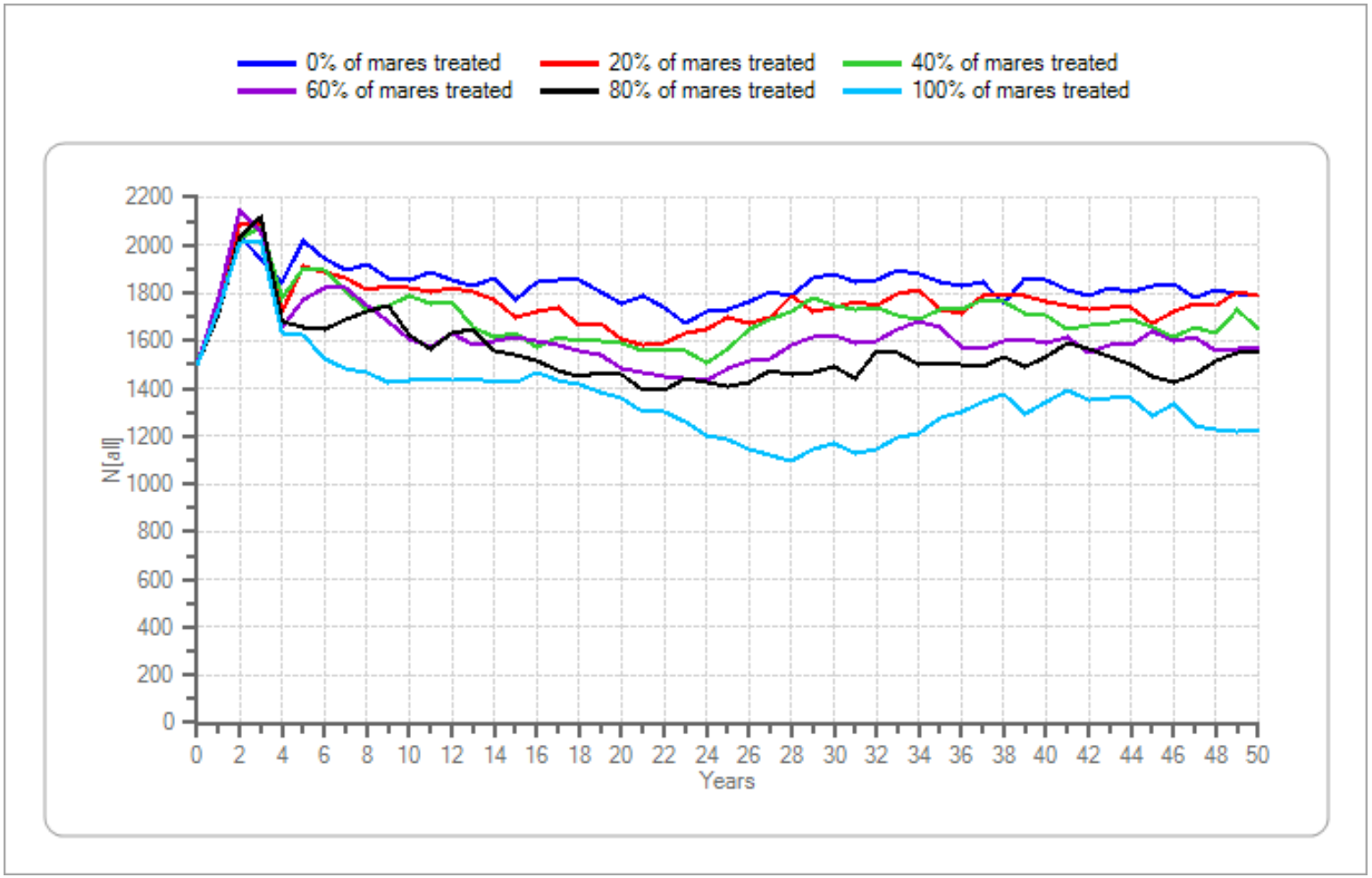
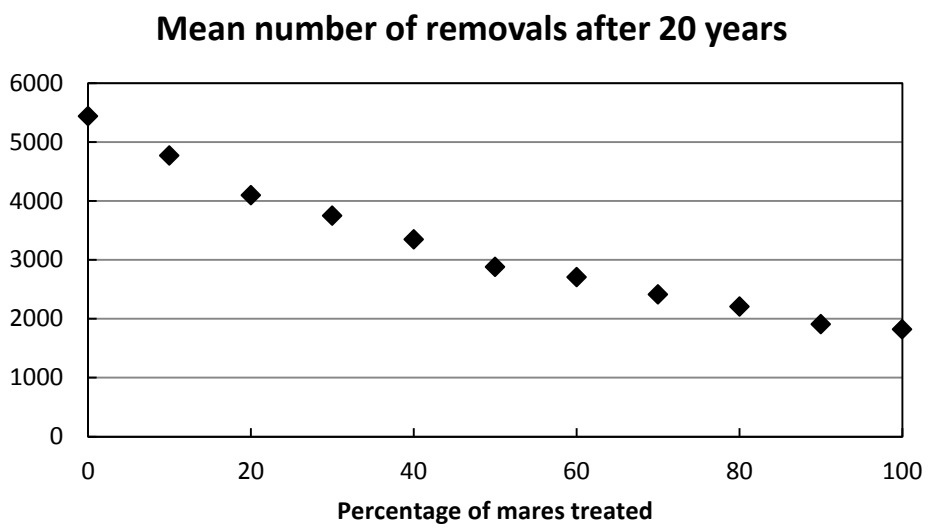
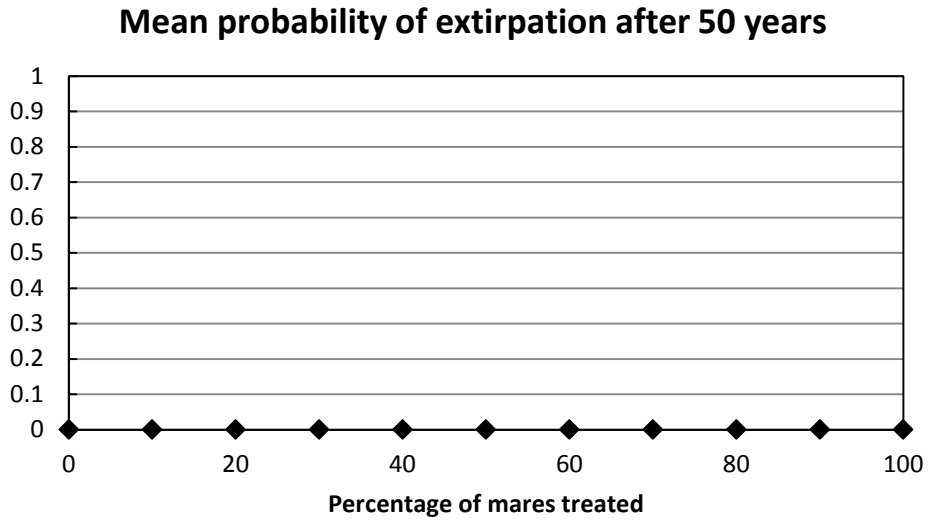


Figure 69. Initial population size = 1500, lambda = 1.20, genetic diversity = high.—Continued





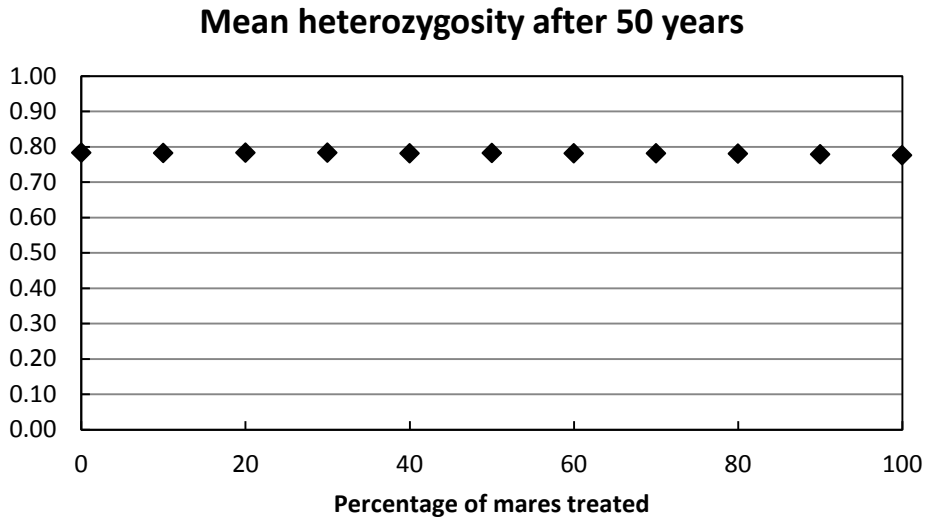


Figure 70. Initial population size = 1500, lambda = 1.25, genetic diversity = high.

Percentage of mares treated	0	10	20	30	40	50	60	70	80	90	100
Mean population size after 20 years	1902.9	1845.4	1761.8	1664.4	1620.0	1726.7	1585.1	1496.6	1543.0	1414.6	1267.5
Mean number of removals after 20 years	5436.7	4770.0	4094.4	3748.1	3344.8	2876.4	2706.6	2411.4	2203.6	1906.3	1821.2
Mean probability of extirpation after 50 years	0	0	0	0	0	0	0	0	0	0	0
Mean heterozygosity after 50 years	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

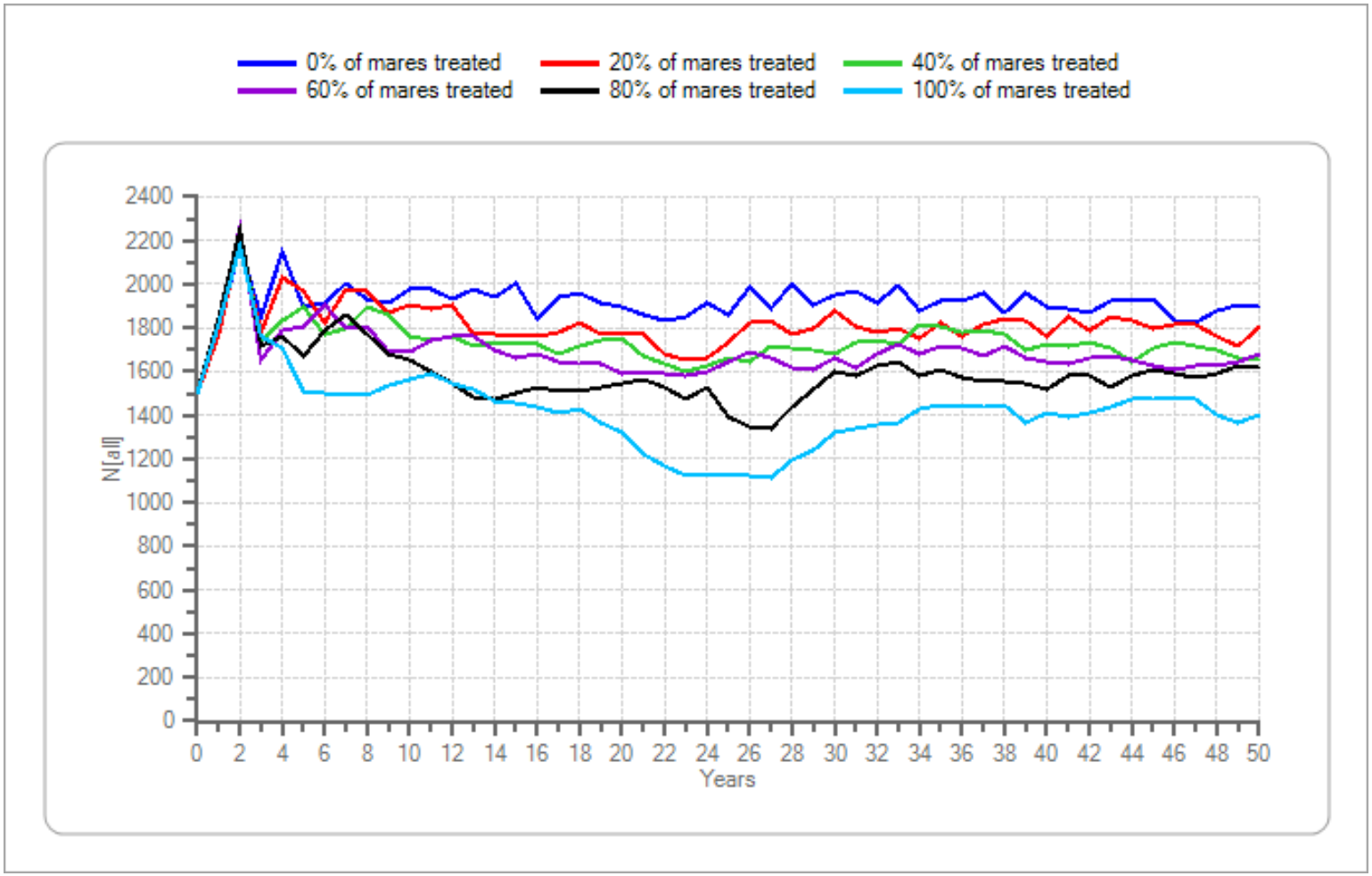
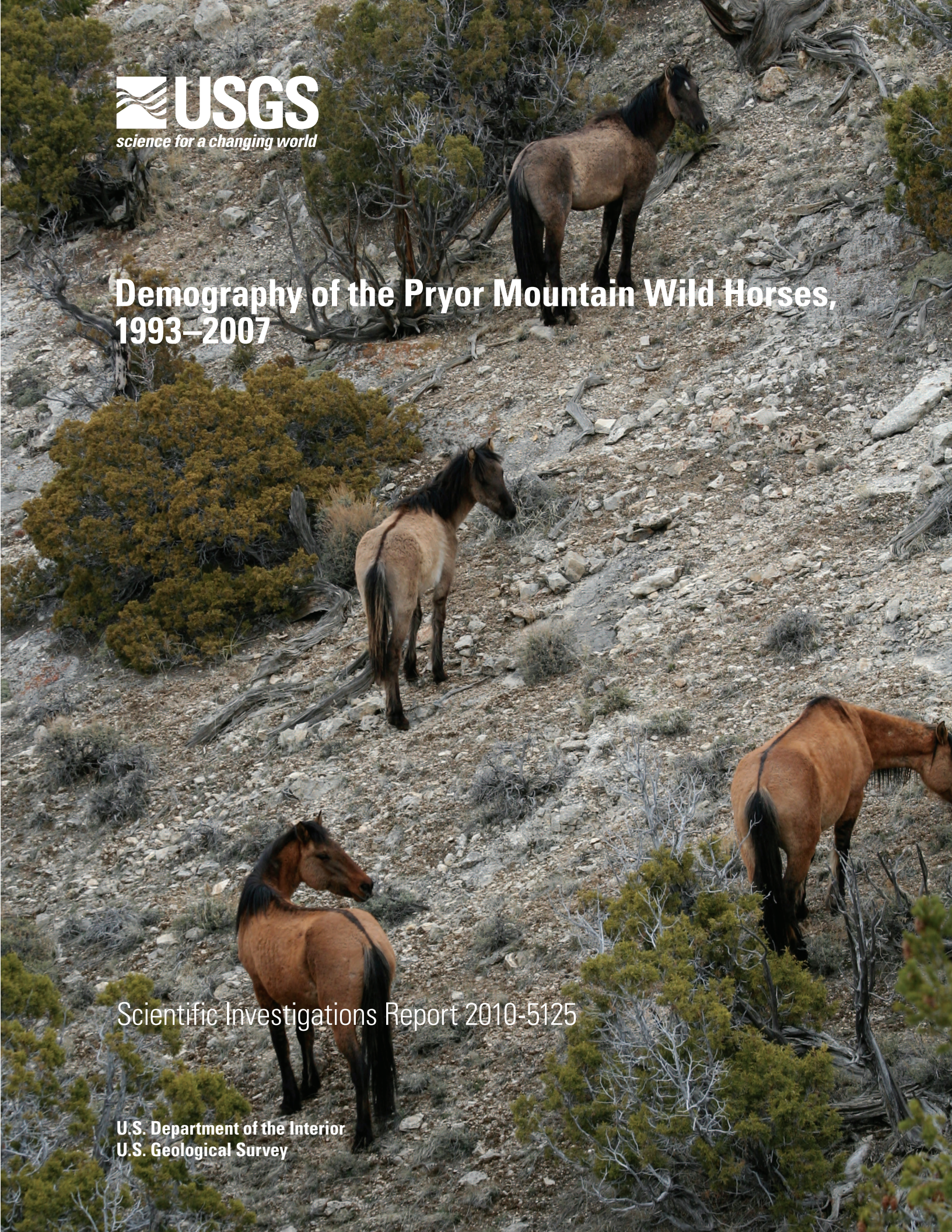


Figure 70. Initial population size = 1500, lambda = 1.25, genetic diversity = high.—Continued



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Demography of the Pryor Mountain Wild Horses, 1993–2007

Scientific Investigations Report 2010-5125

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By James E. Roelle, Francis J. Singer, Linda C. Zeigenfuss, Jason I. Ransom, Linda Coates-Markle, and Kathryn A. Schoenecker

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U.S. Department of the Interior
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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
yard (yd)	0.9144	meter (m)
	Area	
acre	0.4047	hectare (ha)
square mile (mi^2)	259.0	hectare (ha)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32$$

Acronyms Used in Report

AML	Appropriate Management Level
APHIS	Animal and Plant Health Inspection Service
BLM	Bureau of Land Management
FS	Forest Service
HMA	Herd Management Area
NCDC	National Climatic Data Center
NPS	National Park Service
PMWHR	Pryor Mountain Wild Horse Range
RAWS	Remote Automated Weather Station
USGS	U.S. Geological Survey

Demography of the Pryor Mountain Wild Horses, 1993–2007

By James E. Roelle,[1] Francis J. Singer,[1,2] Linda C. Zeigenfuss,[1] Jason I. Ransom,[1] Linda Coates-Markle,[3] and Kathryn A. Schoenecker[1]

Abstract

Wild horses (*Equus caballus*) at Pryor Mountain were studied by direct observation from 1993 through 2007. All horses present were individually identifiable on the basis of coat coloration, head and leg markings, gender, and band associations. Of the 609 horses either present prior to foaling in 1993 or born since, ages were precisely known for 491 (observed as a foal). Ages for 52 horses were estimated through tooth eruption and wear patterns, and for the remaining 66 horses through body size, morphology, and anecdotal evidence concerning when they were present on the range. At varying intensities, never less than 30 days per year, all horses were inventoried and their band associations noted. Foals were paired with dams based on observations of attachment during the early days and weeks of life. Year of death was determined by identification of the carcass where possible. In the absence of finding a carcass, an animal that was not observed for 2 years was considered to have died in the year that it went missing. Animals that were removed from the herd and mares that were part of a contraception study were excluded from calculations of survival and foaling rates, respectively, as appropriate.

The average prefoaling population over the 15 years of the study was 148.8 animals (range = 120–187), and the annual foal crop averaged 32.1 (range = 23–40). Large removals (19–60 animals) in four years helped maintain the herd at this level; apparent growth rate (calculated as though removals had not occurred) was 9.6 percent annually ($\lambda = 1.096$, range = 0.977–1.220). This annual growth rate is relatively low compared to that for many western horse herds, at least in part because of a decline in foal survival. Sex ratio of the foal crop varied widely among years, but pooled across years did not differ from 50:50. Sex ratio in the herd changed mostly as a result of removals. The average age of both males and females in the herd increased during the course of the study. Annual survival of males did not differ from that of females, nor did gender affect annual survival of foals. Pooled across years, ages, and sexes, the annual survival rate was 0.899. Annual foal survival rate was 0.697 and declined through time, with a tendency toward recovery in 2005–2007. Foal survival was higher in larger bands, but did not differ between foals born to primiparous and multiparous mares. A few 2-year-old mares produced foals; foaling rate (excluding contracepted mares and foals they produced) increased through age 10, remained high through age 15, and declined thereafter. Overall foaling rate for mares ≥3 years of age was 0.576 foals per mare, with no apparent trend during the period of our study. Foaling rate in years following gathers was somewhat lower than in other years. There was a positive relation between foaling rate and band size. Primiparous mares were somewhat less likely to foal in the following year than were multiparous mares. Most stallions that acquired a harem did so at age 5 or 6, and the average age of harem stallions increased during our study. Most harems had 1–3 mares ≥2 years of age, but harem size varied with age of the stallion, increasing through about age 11 and declining thereafter. About 6 percent of bands had a satellite stallion (≥5 years of age), but the mean number of mares did not differ between single- and multistallion bands. Most stallions left their natal band at age 2 or 3, but 17 percent remained with their natal band until age 4 or 5.

Foal survival rate was positively related to precipitation, suggesting a possible link to forage production and availability mediated through mare fitness. There also was evidence for density-dependent population regulation, as both population growth rate and survival rate were negatively correlated with population size from the previous year. These and other factors were not sufficient to stabilize the population during our period of study, however, as evidenced by the necessity for large removals in several years.

Introduction

The Pryor Mountain Wild Horse Range was created in 1968 by order of the Secretary, U.S. Department of the Interior, who directed that wild horses (*Equus caballus*) be managed in "a balanced program which considers all public values and without impairment of the productivity of the land" (Federal Register, Document 68-11056, Sept. 11, 1969;

[1] U.S. Geological Survey, Fort Collins, Colorado.

[2] Deceased.

[3] Bureau of Land Management, Wheat Ridge, Colorado.

Bureau of Land Management, 2006, p. 5). The Bureau of Land Management (BLM) was designated as the lead agency for management of wild horses, though the range also contains lands administered by the U.S. Forest Service (FS; Custer National Forest) and the National Park Service (NPS; Bighorn Canyon National Recreation Area). The Wild Free-Roaming Horses and Burros Act of 1971 (P.L. 92-195) further directed the FS and BLM to manage wild horses at Pryor Mountain and wild horses and burros (*Equus asinus*) wherever they occur "in a manner that is designed to achieve a thriving natural ecological balance on the public lands."

While laudable as general management goals, the conditions implied by phrases such as "a balanced program which considers all public values without impairment of the productivity of the land" and "a thriving natural ecological balance" have proven difficult to achieve for many wild horse herds. With the protection afforded by the Act and a dearth of natural predators, many wild horse herds can increase at annual rates of 15–25 percent (Eberhardt and others, 1982; Garrott and Taylor, 1990; Garrott and others, 1991b; Singer and others, 2000), thus threatening to exceed the carrying capacity of their ranges. BLM has addressed this problem largely through a gather and removal program in which excess horses are removed from the range and offered to the general public for adoption or sale to good homes. While hugely successful by some measures (BLM has adopted out more than 220,000 horses since 1971; *http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/wh_b_information_center/Fact_Sheet.html*), supply has exceeded demand, leaving nearly 32,000 horses in BLM corrals and long-term holding pastures. This approach is not embraced by all members of the public. Wild horse advocacy groups often favor a policy of minimum disturbance and intervention, arguing that there are too few, rather than too many, wild horses and that the gather and removal process is detrimental to horse health and well-being. On the other hand, ranchers and conservationists often see wild horses as competing with domestic cattle (*Bos taurus*) and native wildlife for forage, and would thus prefer that BLM reduce the size of many horse herds.

Given the contentious nature of the issues involved, BLM has a continuing need for the best scientific information available on the ecology and demography of wild horse herds. Since creation of the range in 1968, information on wild horses has been gathered nearly continuously by trained investigators and volunteers, making the Pryor Mountain horses perhaps the most studied herd in the Western United States. Furthermore, all of the horses at Pryor Mountain are individually identifiable (unique color markings), and many are of known (observed as a foal) or closely estimated (tooth eruption and wear patterns) age, making the resulting data dependable. The first study of horses at Pryor Mountain was initiated in 1970 by J.D. Feist, who focused largely on behavior (Feist, 1971), but also gathered some information on demography of the population (Feist and McCullough, 1975). Shortly thereafter, Hall (1972) performed an assessment of the herd and possible management alternatives. Perkins and others (1979) studied population dynamics, age distribution, and band composition in the Pryor Mountain herd from 1974 through 1978. Garrott and Taylor (1990), using information gathered by BLM personnel, reported on many aspects of herd demography from 1972 through 1986. F.J. Singer and several colleagues began studies at Pryor Mountain in the early 1990s, broadening their scope to include vegetation dynamics and conservation genetics as well as demography (Singer and Schoenecker, 2000). Prior to his untimely death in 2005, Singer also participated in work at Pryor Mountain on contraception and population estimation.

Here we report on the demographics of the Pryor Mountain wild horse herd from 1993 to 2007. The results contained herein represent a more detailed analysis of the data presented by Singer and others (2000) and an extension covering the next decade. While specific to the Pryor Mountain herd, and thus not necessarily representative of the other 180 Herd Management Areas (HMAs) overseen by BLM, we hope that our results will provide wild horse managers with useful insights as they continue to meet the challenges of managing wild horse herds in the Western United States.

Study Area

During the period of our study, horses actually used an area larger than the Pryor Mountain Wild Horse Range as originally designated. The northwestern and northern boundaries of our study area are not well defined, as there are no natural or manmade barriers to horse movement in that direction. As shown in figure 1, however, the study area encompasses about 22,300 hectares (ha). The following description of the area largely follows Singer and others (2000) and Ricketts (2004).

The study area lies along the Montana-Wyoming border at approximately latitude 45°4'N and longitude 108°19'W, 76 kilometers (km) south of Billings, Mont., and 14 km north of Lovell, Wyo. (fig. 1). The area is topographically diverse, dominated by narrow canyons and steep cliff faces. Elevations range approximately from 1,175 to 2,670 meters (m), with corresponding variation in climate and vegetation. Temperatures range from 40 ºC in the summer to -34 ºC in the winter. Mean annual precipitation ranges from 15 centimeters (cm) at lower elevations to nearly 70 cm at higher elevations, with much of the annual total falling in April–June. Midelevation ridges and plateaus often remain snow free or accumulate <10 cm of snow as a result of winds and southern and western exposures. Higher elevations often have winter snow accumulations of 1 m or more. In water years 1993–1998 (water year 1993, for example, runs from October 1992 through September 1993), mean annual precipitation at Lovell, Wyo. (*http://www.wrcc.dri.edu/summary/climsmwy.html*), was 17.2 cm, essentially identical to the 30-year average (Bureau of Land Management and others, 2008). From 1999 to 2004, however, severe drought conditions prevailed (mean annual precipitation = 12.3 cm). Prolonged drought can decrease plant health

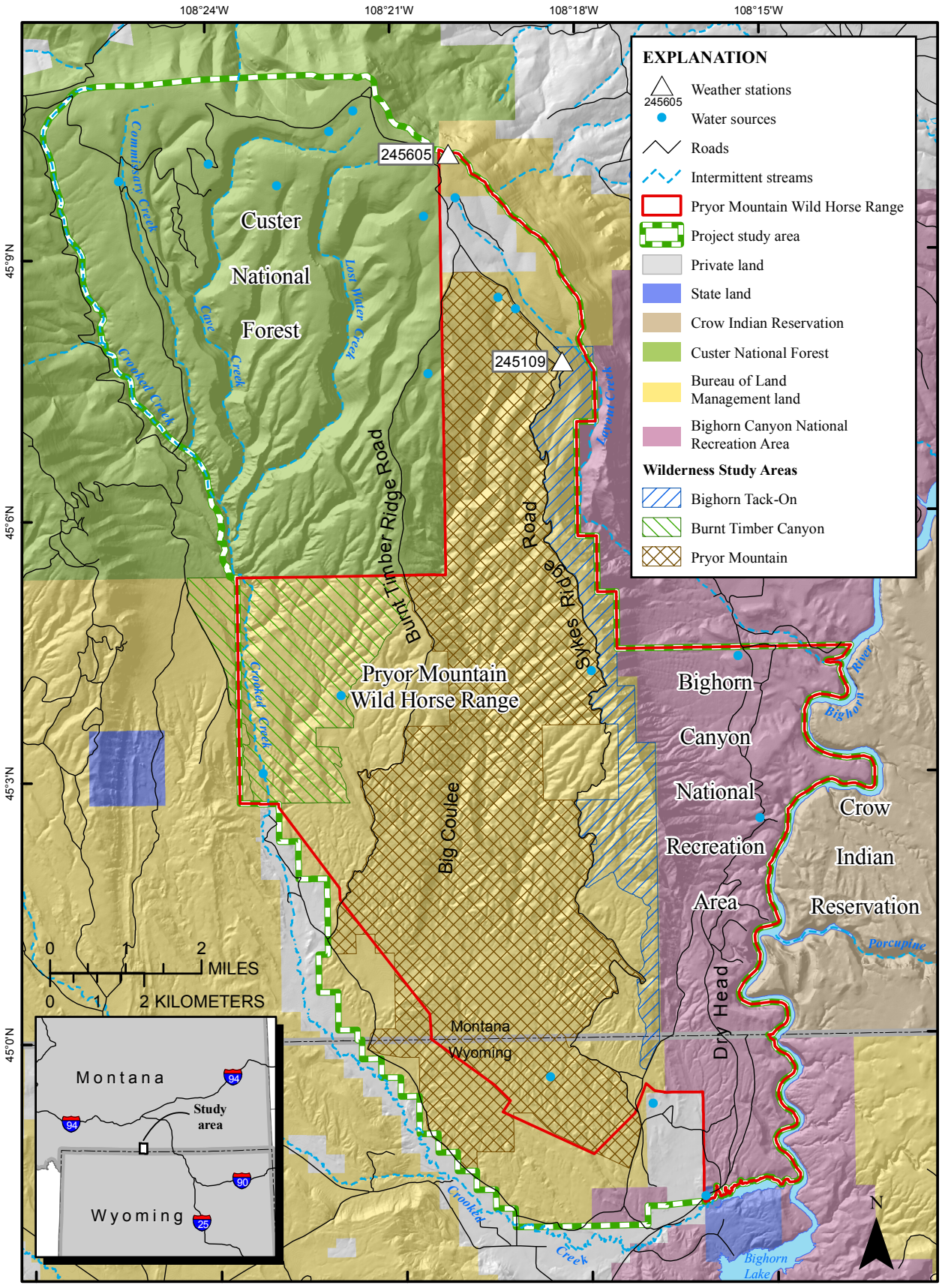

Figure 1. Location of the Pryor Mountain Wild Horse Range and study area.

and vigor and forage production. Temporary relief from the drought occurred in 2005 (annual precipitation = 21.2 cm) and 2007 (annual precipitation = 14.3 cm for 11 months, data missing for June) At least in 2005, greater precipitation probably resulted in improved forage production (Bureau of Land Management, 2006).

Vegetation in the study area changes dramatically with elevation. At the highest elevations, subalpine meadows (Idaho fescue, *Festuca idahoensis*; alpine timothy, *Phleum alpinum*; alpine bluegrass, *Poa alpina*; Ross sedge, *Carex rossii*; silky lupine, *Lupinus sericeus*) are interspersed with stands of subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*). At somewhat lower elevations, Douglas fir (*Pseudotsuga menziesii*) occurs with an understory of snowberry (*Symphoricarpos* spp.). Still lower is a shrub community dominated by big sagebrush (*Artemisia tridentata*), rubber rabbitbrush (*Ericameria nauseosus*), shrubby cinquefoil (*Dasiphora fruticosa*), Utah juniper (*Juniperus osteosperma*), and curl-leaf mountain mahogany (*Cercocarpus ledifolius*) and a variety of grasses and forbs such as bluebunch wheatgrass (*Pseudoroegnaria spicata*), threeawn (*Aristida purpurea*), needle-and-thread (*Hesperostipa comata*), and blue grama (*Bouteloua gracilis*). For a more complete description of vegetation communities on the Pryor Mountain Wild Horse Range, see Ricketts (2004).

Herd Behavior and Management

The social structure of the Pryor Mountain wild horse population consists of bands that include a harem stallion, occasionally along with a satellite stallion, plus adult mares and yearlings and foals of both sexes. In general, we use the term band to refer to all members of the group and harem to refer to mares ≥2 years of age. Males older than yearlings that have not acquired a band usually are found in bachelor groups. Distribution and movements of the bands largely are restricted by topography to the west (Crooked Creek drainage) and east (Bighorn Canyon), and fences of varying integrity to the north and south (fig. 1). The wild horses occur in three more or less discrete subpopulations (Burnt Timber Ridge, Sykes Ridge, and Dryhead). These areas are separated by Big Coulee (a deep canyon) and a long, nearly vertical cliff face. However, the horses are able to move between these areas at high elevations and below Big Coulee, as well as occasionally by way of trails on the cliff face. Varying numbers of horses also use U.S. Forest Service land to the north and west of the original horse range boundary. Interchange of horses between subpopulations occurs regularly, but not with high frequency. The horses move with the seasons, spending winters at low and middle elevations where snow is less deep and summering at higher elevations. Distribution is restricted by available water in summer, when the horses tend to congregate near water sources. Water has less effect on distribution in winter, when the horses use snow to meet their water needs.

In the past, the herd has been managed consistent with national selective removal policy for the BLM Wild Horse and Burro Program, removing mostly young animals and conserving the core breeding component of the population. In general, BLM defines Appropriate Management Level (AML) as the number of adult wild horses (6 months and older) determined through the agency planning process to be consistent with the objective of achieving and maintaining a thriving natural ecological balance and multiple-use relation (Bureau of Land Management, 2006). Initially, the AML for the Pryor Mountain herd was set at 115–127, but was revised to 85–105 horses in 1992. However, based on genetic research (Cothran and Singer, 2000; Gross, 2000a,b), from 1996 to 2006 BLM attempted to manage the herd at an average of 140 adult horses to help conserve genetic diversity (Bureau of Land Management, 2006). More recently, BLM has determined that management at an AML of 90–120 adult horses is necessary to protect and improve rangeland health (Bureau of Land Management, 2009).

The BLM has managed the size of the Pryor Mountain herd primarily through a gather, removal, and adoption program. In 1994, wranglers on horseback attempted to capture the entire herd by moving horses into corral traps. In 1997 and 2001, a helicopter was used to move most of the family bands (individual bachelors typically were not gathered) into corral traps; approximately 125 animals were gathered in each of these years. Smaller gathers aimed at removing excess bachelor stallions and yearling females were conducted in 2003 and 2006. The 2003 gather involved a helicopter to move groups containing target horses into a corral trap. The 2006 gather was conducted using bait trapping to lure the horses into a trap, after which they were transported by vehicle to a holding corral.

Since 2001, some mares in the Pryor Mountain herd have received fertility control treatments using the contraceptive porcine zona pellucida [PZP; see Kirkpatrick and Frank (2005) for a recent review of contraception in equids and other free-ranging animals] as part of a cooperative research project between BLM, the U.S. Geological Survey (USGS), and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS). In 2001, six mares (age 1–2) that had not yet reached breeding status were treated with PZP by hand injection during the gather. A booster inoculation was administered to three of these females in August 2002; the remainder had either died or could not be located to deliver the booster. Fertility control was subsequently applied to younger mares (age 1–2, 2002–2004) and older mares (>14 years, 2002–2004; >10 years after 2004) that had already contributed to the genetics of the population. After 2001, all inoculations (both primers and boosters) were delivered remotely by darting. Because of the timing of booster inoculations relative to the breeding season, the first contraceptive effects were expected in 2004. From 2004 through 2007, 20.0, 29.0, 10.8, and 30.8 percent of the mares (≥3 years old) in the herd were presumptively contracepted.

BLM also has attempted to manage the herd to adjust the sex ratio in favor of males (Bureau of Land Management, 2006) and occasionally has introduced a limited number of horses (both mares and stallions) from outside herds in order to supplement the herd genetically. Introduction of outside horses was discontinued in 1994.

Methods

Field Data Collection

All data reported in this study were gathered through direct observations of the herd by trained BLM and USGS employees and volunteers. From 1995 to 1997, efforts were focused on the peak foaling season, May 1 to June 30. From 1998 to 2007, daily observations were made during the peak foaling and breeding period, May 1 to August 30. This effort totaled approximately 100 hours of observation per week during 1995–1997 and 100–150 hours per week during 1998–2007. Semi-weekly to weekly observations were made March–April and September–November. Fewer observations were made during midwinter, and they were limited to accessible areas of the range, which varied from year to year. Observations were made from the ground during systematic surveys along roads and trails that traverse the study area. Ground observations were augmented with two helicopter flights each fall or winter, 1995–2001, and one flight in late 2006, as well as 20–25 days of horseback surveys throughout each year, 1995–2005.

Greater than 90 percent of the bands were located at least twice per week during the May–July period, and all bands usually were located at least once per week from April to mid-May and August to mid-October. Reduced effort in the remainder of the year resulted in less than one observation per week per band. Observers collaborated during the peak observation season to develop a weekly list of band composition and foal presence.

Wild horses were approached within 100–250 m and could be observed for periods of 2–6 hours with no apparent effect on their behaviors. All horses were identified individually based on color, markings, gender, and band associations since 1993. All stallions, mares, yearlings, and foals observed in bands, bachelor groups, or alone were recorded. Lead harem stallions were identified based on behavior as described in Feist and McCullough (1975), Oom and Santos-Reis (1986), and Berger (1986). Interchanges between bands by mares and stallions were recorded, as were returns to the original band.

Each horse in the population was assigned a unique number during data compilation. Of the 609 animals in the population from 1993 to 2007, horses whose birth year was known from foal records included 480 of the 481 foals born during 1993–2007 and 11 horses born prior to 1993. Ages of an additional 52 animals were estimated by a veterinarian based on tooth eruption and wear patterns (American Association of Equine Practitioners, 2002). Ages of the remaining 66 animals were based on body size and morphology and anecdotal evidence of years the animals were on the range. Death year was determined by identification of the carcass of the deceased individual or, if an individual was not observed for two consecutive years, the animal was considered to have died in the year it went missing. Number of foals produced was determined by observation as described above. It is possible that some foals were born and died without being seen; however, given the intensity of the observations, we believe that this was rare. Identification of an individual's dam was based on observations of attachment of foal to mare during the early days and weeks of the foal's life.

Analysis

We conducted all of our analyses on the basis of biological years, which we defined as just prior to foaling in one year to the same time the next year. Thus, the biological year 1993 encompasses the period from spring 1993 to spring 1994. Because we defined the biological year in this way, because we made no attempt to pinpoint when during a year a horse was born or died, and because we have the benefit of hindsight (other reported data were often projections), our data will in some cases not match exactly those presented elsewhere (for example, various Environmental Assessments prepared by BLM).

We calculated annual population growth using the expression $\lambda_t = N_t / (N_{t-1} - R_{t-1})$, where λ_t is the growth multiplier from year $t-1$ to year t, N is the population size, and R_{t-1} is the number of animals removed in the period $t-1$ to t. In this formulation, λ represents the apparent growth rate of the population had horses not been removed (Garrott and Taylor, 1990).

Survival rates were calculated as the number of animals present at the end of a year divided by the number present at the beginning of that year. Animals that were removed from the herd were excluded from survival calculations in the year they were removed. Foaling rates were calculated as the number of foals born (regardless of how long they lived) divided by the number of mares present. Mares that were presumptively contracepted and foals that they produced were excluded from calculations of foaling rate.

Band composition has been recorded on "horse lists" regularly (usually weekly) since 1996. These are lists of all the harem stallions present and all the members of their bands, plus lists of bachelor males and animals in transit between bands. To derive information on harem stallions and band composition, we used two of these lists for each year from 1996 through 2007, one from the breeding season (usually early June) and one from fall (September–November, usually the last list available for the calendar year). For each harem stallion present, we calculated the number of mares ≥2 years of age in the band for each of the two time periods and then

took the maximum of those two values as the number of mares held in the harem in that year. We calculated total band size in an analogous manner, and we used these same lists to derive information on bachelors and satellite stallions, which we defined as ≥5 years of age. We defined a satellite stallion in this way in an effort to distinguish true multistallion bands from cases in which a male simply remained with his natal band longer than usual.

We looked for evidence of density dependence in foaling rate, herd survival, foal survival, and population growth rate in the Pryor Mountain wild horse population using stepwise multiple regression with the current year's postfoaling population and the previous year's postfoaling population as independent variables. For these analyses, we used data from Garrott and Taylor (1990) for 1976–1986 (foaling rate, herd survival, and population growth rate), as well as data from our study for 1993–2007. We excluded data from the winter of 1977–1978, during which 51 percent of the population died as a result of heavy snow and ice accumulations (Garrott and Taylor, 1990), from the analyses of density dependence in herd survival and growth rate. We also excluded data from 2004, when 96 percent of the foal crop was lost, from the analysis of foal survival. Foal losses in 2004 are suspected to have been the result of predation by mountain lions (*Puma concolor*). We believe that these exclusions are justified because the biological processes operating in these years were clearly different from those operating in other years.

It would be desirable to have consistent, long-term data on annual forage production for comparison with demographic parameters, but we are unaware of such data for the study area. As a possible surrogate, we examined weather records from a number of stations in the area, reasoning that forage production in this semiarid environment likely is related to precipitation, even though large elevation differences across the study area make it unlikely that any single station will be representative of the entire area. In terms of location, the best available data are from a Remote Automated Weather Station (RAWS; Wild Horse Montana, NWS ID #245605, elevation 2,675 m), which was operated in the study area from October 1991 to July 1997 (*http://raws.dri.edu/wraws/emtF.html*) (fig. 1). Unfortunately, overlap between this period of record and the period of our study is minimal. A National Climatic Data Center Station (NCDC #484770, elevation 1,170 m) at Lovell, Wyo., has a much longer period of record (1948–present; *http://www.wrcc.dri.edu/summary/climsmwy.html*), but its lower elevation caused us to question its relevance to higher elevations in the study area. For the 7 years of data in common, however, there was a strong correlation between the two stations for both total annual precipitation ($R^2 = 0.921$, $P = 0.0006$) and the minimum of the monthly average minimum temperatures ($R^2 = 0.763$, $P = 0.01$). We therefore used data from the Lovell station and stepwise multiple linear regression to analyze relations between weather and herd survival rate, foal survival rate, and foaling rate (mares ≥3 years of age). Data on annual herd survival and foaling rate were available from both our study and the work of Garrott and Taylor (1990), but data on annual foal survival were available only from our study. For the reasons cited above, we again excluded 2004 from the analysis of foal survival.

Other investigators (Heitschmidt and others, 2005; Smart, 2005) showed that spring precipitation (April–June) is related to annual forage production in the Great Plains. We therefore divided precipitation data into spring (April–June), summer (July–September), and winter (October–March). For each year, we also calculated the mean of the monthly average minimum temperatures (December–February) and the mean of the monthly average maximum temperatures (April–September) as indices of winter cold and summer heat.

For herd survival, we used the covariates winter precipitation in the previous year, spring precipitation in the current year, and summer precipitation in the current year (hypothesizing that all might have a positive relation with forage production in the current year), winter precipitation in the current year (potential negative effect of snow accumulation on forage availability in the current year), and average monthly minimum temperature in the current year (potential negative effect on ability to meet energy demands). For foal survival, we used the same set of covariates and added average monthly maximum temperature (possible effect of heat stress on more susceptible foals). For foaling rate, we used the covariates summer precipitation in the previous year (potential positive relation with forage production during early pregnancy), winter precipitation in the previous year (potential negative effect of snow accumulation on forage availability), average monthly minimum temperature (potential negative effect on ability to meet energy demands), and spring precipitation in the current year (possible positive relation with forage production during late pregnancy).

All data were entered into Microsoft Excel® spreadsheets (Microsoft Corporation, Redmond, Wash.). Analyses were conducted in SAS® v. 9.1 (SAS Institute, Cary, N.C.) using standard statistical techniques as noted in the Results section.

Results

Population Size and Growth

Prior to foaling in spring 1993, there were 128 horses ≥1 year of age in the study area. From 1993 to 2007, 481 horses were born; thus, our study encompasses 609 individual animals. During the period of study, the prefoaling population averaged 148.8 animals (range = 120–187), the foal crop (number born) averaged 32.1 (range = 23–40), and the postfoaling population averaged 180.9 animals (range = 149–227) (table 1). As we calculated it, the postfoaling population represents the maximum number of animals that could have been present at any time during the year, as it assumes no mortality of foals or adults during the foaling period. The number of deaths per year apparently increased during 1999–2006, while the foal crop remained relatively constant throughout

Table 1. Size and growth rate of the Pryor Mountain wild horse (*Equus caballus*) herd, 1993–2007.

[Growth rate (λ) is calculated based on the postfoaling population. —, not applicable; SE, standard error of the mean.]

Year	Prefoaling population	Foals born	Postfoaling population	Deaths	Removals	λ
1993	128	23	151	1	1	—
1994	149	34	183	3	60	1.220
1995	120	29	149	7	0	1.211
1996	142	32	174	7	2	1.168
1997	165	35	200	15	46	1.163
1998	139	23	162	7	1	1.052
1999	154	38	192	27	0	1.193
2000	165	37	202	15	0	1.052
2001	187	40	227	31	46	1.124
2002	150	32	182	28	0	1.006
2003	154	30	184	32	7	1.011
2004	145	28	173	38	0	0.977
2005	135	36	171	19	0	0.988
2006	152	30	182	16	19	1.064
2007	147	34	181	9	0	1.110
Total	2,232	481	2,713	255	182	—
Mean	148.8	32.1	180.9	17.0	12.1	1.096
SE	4.18	1.30	5.14	3.02	5.36	0.0228

the period of study. The mean annual apparent growth rate, calculated as though removals had not occurred, was 9.6 percent (λ = 1.096, range = 0.977–1.220), or 10.5 percent if the year (2004) of heavy foal loss is excluded. Although the growth rate rebounded somewhat in 2006 and 2007, there was a significant (R^2 = 0.514, P = 0.004) overall decline during the period of study as determined by linear least squares regression (fig. 2).

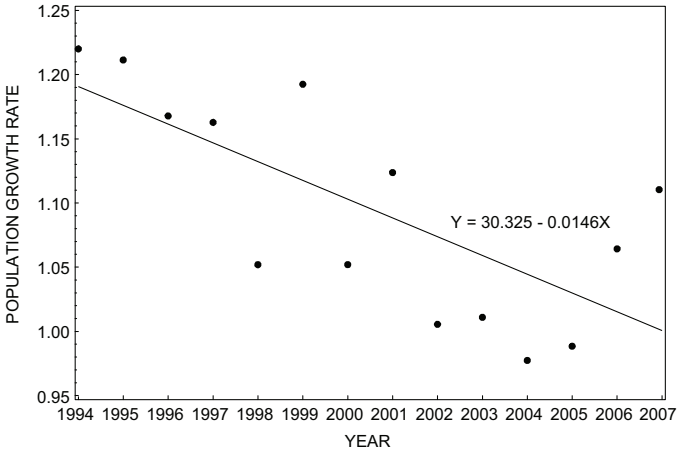

Figure 2. Trend in growth rate of the Pryor Mountain wild horse (*Equus caballus*) population, 1993–2007.

Removals

Major removals of wild horses occurred in 1994, 1997, 2001, and 2006, with smaller numbers of horses removed in four other years (table 2). For the entire period, adults (≥2 years of age) were removed most often (62.6 percent; n = 182), followed by yearlings (28.6 percent) and foals (8.8 percent). Across all age classes, slightly more males (56.6 percent; n = 182) were removed than females.

Sex and Age Structure

During the period 1993–2007, 477 foals of known sex were born; four foals disappeared before sex could be ascertained. Slightly more males (243) were born than females (234), but this difference was not statistically significant (χ^2 = 0.170, P = 0.68). On an annual basis, however, the proportion of males in the foal crop varied widely (range = 34.8–71.9 percent) (fig. 3). The proportion of males produced also varied by age of mare, declining from age 3 through about age 10, and then increasing in older mares (fig. 4).

The sex ratio of animals ≥1 year of age varied only moderately during the period of study, ranging from a high of 55.2 percent males in 1997 to a low of 44.2 percent males in 2000 and 2007 (fig. 5). The largest interannual changes in sex ratio occurred as a result of removals in 1994 (27 males, 33 females

Table 2. Removals of wild horses (*Equus caballus*) from the Pryor Mountain herd, 1993–2007.

	Foals		Yearlings		Adults		
Year	Males	Females	Males	Females	Males	Females	Total
1993	0	0	0	0	1	0	1
1994	8	6	7	7	12	20	60
1996	0	0	0	0	2	0	2
1997	2	0	11	1	20	12	46
1998	0	0	0	0	1	0	1
2001	0	0	10	6	8	22	46
2003	0	0	1	0	6	0	7
2006	0	0	4	5	10	0	19
Total	10	6	33	19	60	54	182

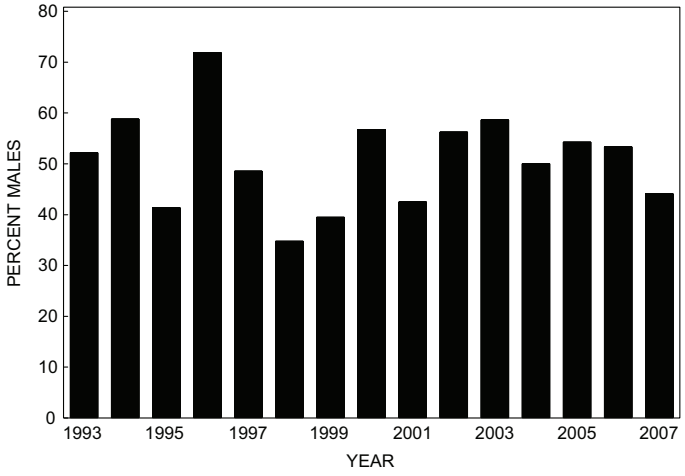

Figure 3. Sex ratio of wild horse (*Equus caballus*) foals born in the Pryor Mountain herd, 1993–2007.

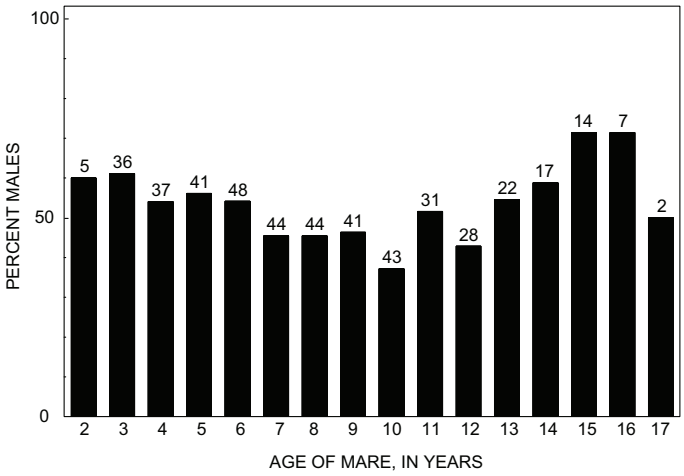

Figure 4. Variation in the sex ratio of wild horse (*Equus caballus*) foals by age of dam in the Pryor Mountain herd, 1993–2007. Values above the bars are number of foals born.

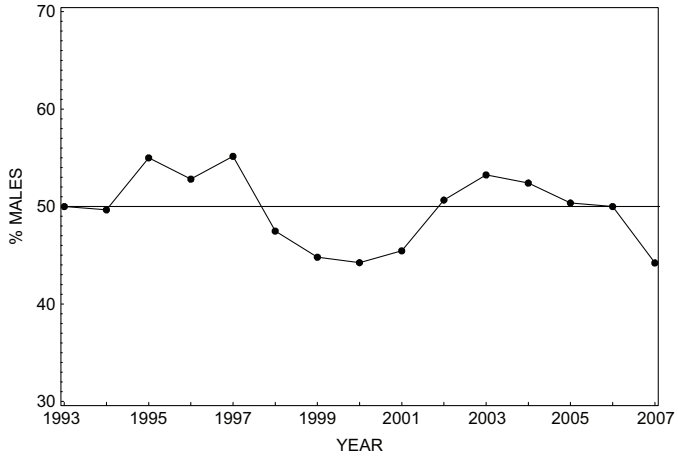

Figure 5. Annual sex ratio of adult (≥1 year old) wild horses (*Equus caballus*) in the Pryor Mountain herd, 1993–2007.

removed), 1997 (33 males, 13 females), 2001 (18 males, 28 females), and 2006 (14 males, 5 females).

The age structure of the Pryor Mountain wild horse herd, on the other hand, changed markedly between 1993 and 2007 (fig. 6*A*,*B*). In 1993, males in the herd averaged 4.4 years of age and females averaged 4.2 years. In 2007, the comparable values were 8.1 and 6.8 years, respectively. Both of these trends are statistically significant as judged by linear least squares regression (males, $P < 0.0001$, $R^2 = 0.911$; females, $P < 0.0001$, $R^2 = 0.832$).

Survival

Pooled across years, ages, and sexes ($n = 2{,}531$), the annual survival rate of horses on the study area was 0.899. Excluding four foals that disappeared before sex could be ascertained, survival of males (0.896, $n = 1{,}246$) did not differ from that of females (0.905, $n = 1{,}281$) ($\chi^2 = 0.486$,

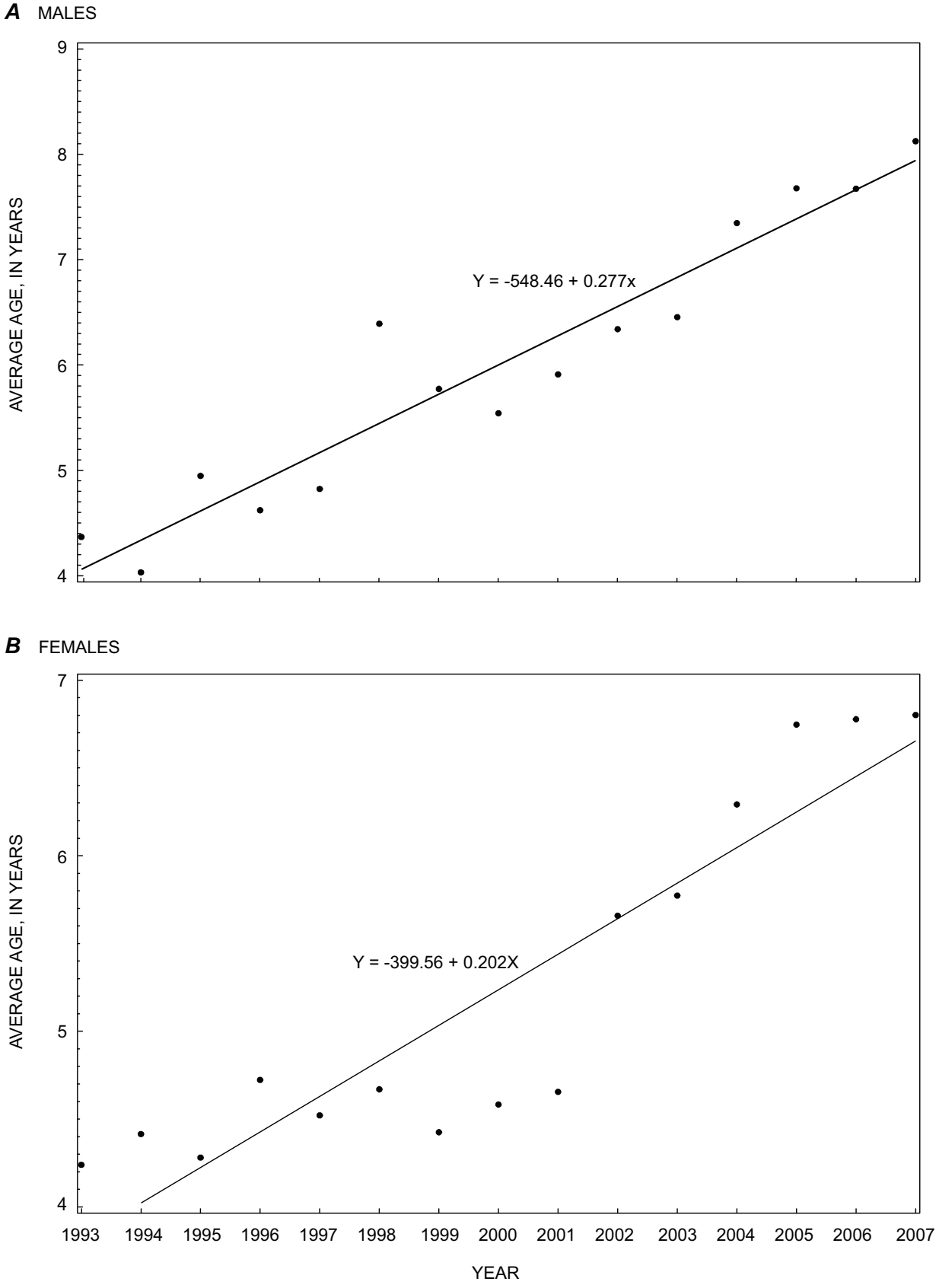

Figure 6. Average age of (*A*) male and (*B*) female wild horses (*Equus caballus*) in the Pryor Mountain herd, 1993–2007.

$P = 0.486$), nor was the difference between the sexes significant for foals ($\chi^2 = 2.500$, $P = 0.114$, $n = 461$). Data from both sexes were therefore pooled for calculations of survival by year.

Survival by year varied from a high of 0.993 in 1993 to a low of 0.780 in 2004 (table 3), when 27 of 28 foals were lost, with predation by mountain lions suspected as the cause (Bureau of Land Management, 2006). For the period of our study, there was a trend toward decreasing foal survival (fig. 7; $P = 0.020$, $R^2 = 0.353$), but there was no trend in survival of animals ≥1 year old ($P = 0.282$, $R^2 = 0.088$). Foal survival increased in the most recent three years of our study, however.

More than one half of the horses that died from 1993 to 2007 were foals, and annual foal survival pooled across years was 0.697 (table 4). Survival increased gradually from age 0 to age 3, remained high through age 15, and then declined gradually. Horses ≥18 years of age were pooled for this analysis because of small sample sizes in these age classes. The oldest animal recorded during the period of study was a male that died at age 26 in 2005. Nine horses lived to be 20 years of age or older, seven of which were males. Four horses (3 males, 1 female) age 20 and 21 were present on the range at the end of our study.

As judged by logistic regression, there was a positive relation between the probability of a foal surviving and the mean (average of summer and fall in a given year) number of members in the band ($n = 393$ foals in 1996–2007; Wald $\chi^2 = 14.964$, odds ratio = 1.244 with 95 percent CI = 1.114–1.390, $P = 0.0001$). Thus, for example, the regression yields a predicted foal survival rate of 0.457 (95 percent CI = 0.351–0.567) for a band size of two, and a predicted survival rate of 0.757 (95 percent CI = 0.688–0.815) for a band size of eight.

We also examined survival of foals in relation to parity of their dams—primiparous (those giving birth to their first foal) compared to multiparous (those that had given birth one or more times). For this analysis, we excluded all mares born before 1993 (since parity could not be determined with certainty) and any foals they produced. We also excluded mares that were presumptively contracepted or were present postcontraception (hereafter referred to as treated and posttreated) and foals that they produced. For the remaining subset of foals, there was no difference in survival based on parity of the dam ($n = 189$, $\chi^2 = 0.957$, $P = 0.328$).

Table 3. Annual survival rates of Pryor Mountain wild horses (*Equus caballus*), 1993–2007.

[Horses that were removed were excluded from survival calculations in the year of their removal.]

Year	n	Deaths	Survival
1993	150	1	0.993
1994	123	3	0.976
1995	149	7	0.953
1996	172	7	0.959
1997	154	15	0.903
1998	161	7	0.957
1999	192	27	0.859
2000	202	15	0.926
2001	181	31	0.829
2002	182	28	0.846
2003	177	32	0.819
2004	173	38	0.780
2005	171	19	0.889
2006	163	16	0.902
2007	181	9	0.950
Total	2,531	255	0.899

Foaling Rates

For ease of comparison with other studies, we calculated foaling rates of mares ≥2 years of age, ≥3 years of age, and ≥4 years of age. Treated and posttreated mares were excluded from these calculations, as were foals born to unknown dams. Pooled across years, productivity was 0.501 foals/mare (range = 0.254–0.705) for mares ≥2 years of age, 0.576 foals/mare (range = 0.300–0.795) for mares ≥3 years of age, and 0.597 foals/mare (range = 0.311–0.795) for mares ≥4 years of age (table 5). Regression analysis showed a slight increase in foaling rate of mares over time (for mares ≥3 years of age, $P = 0.088$), but variability was high ($R^2 = 0.208$).

From 1993 to 2007, 464 of the 481 foals produced in the study area were born to 118 known or highly probable dams (table 6), an average of 3.93 foals per mare. Many of the dams represented in table 6 are still producing foals; thus, the data represent only a single point in time. Twenty-seven dams that each produced seven or more foals accounted for a total of 237 foals, or 51.1 percent of the production by known dams. Of the 54 dams that have been monitored since birth (that is, those born in 1993 or later) and have not been contracepted, 6 (11.1 percent) first produced a foal at age 2, 29 (53.7 percent) at age 3, 14 (25.9 percent) at age 4, 3 (5.6 percent) at age 5, and 2 (3.7 percent) at age 7. The average age at first foaling for these dams was 3.4 years. Age-specific fecundity was 0.048 foals per mare for 2-year-old mares, increased nearly monotonically through age 10, remained high through age 15, and dropped rapidly thereafter (table 7). The maximum age at which any mare gave birth during the study period was 17 years, although that mare was presumptively contracepted and thus is not represented in table 7. A single set of twin foals (both females) was born during the period of our study, to a 6-year-old mare in 1994.

We used logistic regression to examine the probability of foaling in years following a gather as compared to other years for three age groups of mares: 2 years old, 3 years old, and ≥4 years old. Foal production (yes or no) was the dependent variable and year was the independent variable, and we used a specific hypothesis test (or contrast) to compare the mean probabilities of foaling. For this analysis we considered

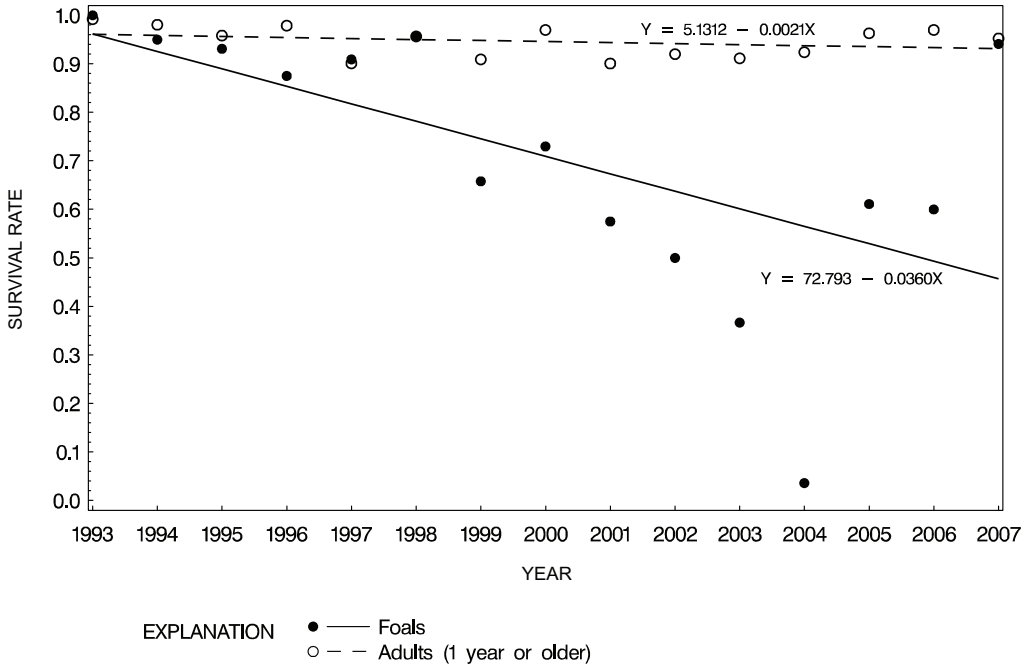

Figure 7. Trend in annual survival of Pryor Mountain wild horses (*Equus caballus*) for adults and foals, 1993–2007.

Table 4. Age-specific survival rates of Pryor Mountain wild horses (*Equus caballus*), 1993–2007.

[Horses that were removed were excluded from survival calculations in the year of their removal.]

Age	Males			Females			Total		
	n	Deaths	Survival	*n*	Deaths	Survival	*n*	Deaths	Survival
0	233	77	0.670	228	60	0.737	[1]465	141	0.697
1	115	9	0.922	137	12	0.912	252	21	0.917
2	91	5	0.945	108	9	0.917	199	14	0.930
3	77	3	0.961	89	2	0.978	166	5	0.970
4	72	1	0.986	84	5	0.941	156	6	0.962
5	70	1	0.986	87	4	0.954	157	5	0.968
6	67	4	0.940	76	3	0.961	143	7	0.951
7	60	2	0.967	73	0	1.000	133	2	0.985
8	60	1	0.983	69	0	1.000	129	1	0.992
9	56	1	0.982	65	1	0.985	121	2	0.984
10	53	0	1.000	57	4	0.930	110	4	0.964
11	49	3	0.939	48	3	0.938	97	6	0.938
12	38	2	0.947	40	5	0.875	78	7	0.910
13	34	1	0.971	32	4	0.875	66	5	0.924
14	33	1	0.970	27	1	0.963	60	2	0.967
15	30	1	0.967	22	2	0.909	52	3	0.942
16	30	1	0.967	18	4	0.778	48	5	0.896
17	26	4	0.846	9	2	0.778	35	6	0.829
≥18	52	12	0.769	12	1	0.917	64	13	0.797
Total	1,246	129	0.896	1,281	122	0.905	[1]2,531	255	0.899

[1] Includes four foals that disappeared before sex could be ascertained.

Table 5. Annual foaling rates of Pryor Mountain wild horse (*Equus caballus*) mares, 1993–2007.

[Treated and posttreated mares in 2004–2007 and foals that they produced were excluded, as were foals born to unknown dams.]

Year	Mares ≥ 2 years old			Mares ≥ 3 years old			Mares ≥ 4 years old		
	Foals	Mares	Foals/mare	Foals	Mares	Foals/mare	Foals	Mares	Foals/mare
1993	15	59	0.254	15	50	0.300	14	45	0.311
1994	32	64	0.500	32	59	0.542	30	50	0.600
1995	23	46	0.500	23	42	0.548	20	38	0.526
1996	32	51	0.627	30	44	0.682	27	40	0.675
1997	34	66	0.515	33	50	0.660	29	43	0.674
1998	23	55	0.418	23	48	0.479	22	43	0.512
1999	38	70	0.543	37	52	0.712	34	46	0.739
2000	37	76	0.487	36	61	0.590	27	44	0.614
2001	40	88	0.455	40	75	0.533	34	60	0.567
2002	32	63	0.508	32	57	0.561	28	53	0.528
2003	30	65	0.462	29	56	0.518	29	50	0.580
2004	28	52	0.538	28	48	0.583	28	48	0.583
2005	31	44	0.705	31	39	0.795	31	39	0.795
2006	23	42	0.548	23	42	0.548	23	37	0.622
2007	21	35	0.600	21	29	0.724	21	29	0.724
Total	439	876	0.501	433	752	0.576	397	665	0.597

Table 6. Total foals born to Pryor Mountain wild horse (*Equus caballus*) mares, 1993–2007.

[Includes only foals born to known dams. Many of the mares represented are still producing foals.]

Foals / Mare	Number of mares	Total foals	Percent of total foals
1	43	43	9.3
2	12	24	5.2
3	10	30	6.5
4	5	20	4.3
5	16	80	17.2
6	5	30	6.5
7	9	63	13.6
8	6	48	10.3
9	4	36	7.8
10	2	20	4.3
11	4	44	9.5
12	1	12	2.6
14	1	14	3.0
Total	118	464	100

only the gathers conducted in 1994, 1997, and 2001 in which most of the family bands were gathered; in 2003, only groups containing animals targeted for removal were gathered, and in 2006 a subset of the herd was captured by bait trapping. For mares 2 or 3 years old, the data were too sparse to allow calculation of the maximum likelihood estimate in the logistic regression (for example, in some years no mares in these age groups produced foals). For mares ≥4 years old the probability of foaling was lower in years following a gather than in other years ($P = 0.03$). The absolute value of this difference was small, however. The mean predicted foaling rate in years following a gather was 0.522 ($n = 3$ years, 134 mares) as opposed to 0.622 ($n = 12$ years, 531 mares) in other years.

We also used logistic regression to examine the probability of a mare foaling as a function of number of members in the band in summer and found a positive relation ($n = 577$ mare-years, Wald $\chi^2 = 24.122$, odds ratio = 1.254 with 95 percent CI = 1.146–1.372, $P < 0.0001$). As an example, the regression yields a predicted probability of foaling of 0.427 (95 percent CI = 0.347–0.511) with a band size of two, as compared to a probability of foaling of 0.743 (95 percent CI = 0.682–0.797) with a band size of eight.

Table 7. Age-specific fecundity of Pryor Mountain wild horse (*Equus caballus*) mares, 1993–2007.

[Treated and posttreated mares in 2004–2007 and foals that they produced were excluded, as were foals born to unknown dams. —, not applicable.]

Age	Number of foals	Number of mares	Foals/mare
0	0	234	0.000
1	0	156	0.000
2	6	124	0.048
3	36	87	0.414
4	34	81	0.420
5	37	72	0.514
6	43	69	0.623
7	41	68	0.603
8	44	70	0.629
9	42	69	0.609
10	43	59	0.729
11	31	48	0.646
12	28	40	0.700
13	22	30	0.733
14	14	23	0.609
15	14	20	0.700
16	4	9	0.444
17	0	4	0.000
≥18	0	3	0.000
Total	439	1,266	—

In addition, we compared the probability of foaling in the next year for primiparous and multiparous mares. Primiparous females were less likely to foal ($\chi^2 = 27.29$, $P < 0.0001$) in the year following their first foal (proportion foaling = 0.26, n = 43) than were multiparous females (proportion foaling = 0.72, n = 110). On the other hand, multiparous females were somewhat more likely to foal ($\chi^2 = 2.60$, $P = 0.107$) if they foaled in the previous year (proportion foaling = 0.72, n = 110) than if they did not foal in the previous year (proportion foaling = 0.61, n = 76).

Harem Stallions and Band Composition

During the 12 years (1996–2007) for which information was available, the number of bands varied from 29 to 38 (table 8), with an increasing trend through time (least squares regression, $P = 0.012$, $R^2 = 0.48$). There was no relation, however, between number of bands and band size (least squares regression, $P = 0.88$); that is, band size did not decline significantly as the number of bands increased. Bands were held by 64 different stallions during this period, the youngest being age 3 and the oldest being age 23 (fig. 8). The vast majority of bands, however, were held by stallions aged 5–17 (fig. 8; n = 399). A single stallion gained his first harem at age 3, but 18 of the 29 harem stallions (62.1 percent) for which we have records since birth (born in 1993 or later) acquired their first harem at age 5 or 6 (fig. 9). As might be expected from the increasing age of all males in the herd (fig. 6A), the average age of harem stallions also increased through time (table 8) (least squares regression, $P < 0.0001$, $R^2 = 0.82$).

Harems with as many as six breeding-age females (≥2 years old) were recorded occasionally, but more than 86 percent of the harems contained 1–3 breeding-age females (fig. 10; n = 399). Harem size varied by age of stallion, increasing from age 3 to about age 11 and declining thereafter (fig. 11; n = 399). There were four somewhat anomalous cases in which a male was listed as a harem stallion but the harem size was zero (fig. 10). In two of these cases there were no harem members at all when the observations were recorded. These represent harems in flux, with one or more mares sometimes with the stallion and sometimes not. In the other two cases the only band members were the harem stallion and a yearling female, and we counted only females ≥2 years old in our calculations of harem size.

Of 399 band-year combinations for which we have observations, only 25 (6.3 percent) involved satellite stallions, which we defined as a male ≥5 years old that was associated with a harem stallion. Three pairs of stallions that lived in the same band for 6, 6, and 5 years accounted for 17 of these 25 associations. The remaining eight associations lasted only 1 or 2 years. In only one case were three stallions involved, and that association was maintained for a year or less. Two of the long-term associations (≥5 years) involved stallions that were the same age (7 through 12 years of age) or 1 year apart (harem stallion 5 through 9 years of age; satellite stallion 6 through 10 years of age). The third long-term association included a harem stallion 17 through 22 years of age and a satellite stallion 7 through 12 years of age. The mean number of mares (≥2 years old) did not differ between single- and multistallion bands (2.2 mares in both cases), although 5 of the 25 multistallion bands had 4–6 mares.

Of the 180 male foals born from 1993 through 2003 (those born after 2003 would have been only 3 years old or younger at the end of our study), 124 died or were removed before reaching age 4 and thus had virtually no chance of becoming a harem stallion. An additional 17 died or were removed at age 4 or 5 and thus had a much reduced chance of becoming a harem stallion. Three died at age 6, and two were removed at age 7 without having held a harem. Of the original 180 male foals born, 29 became harem stallions. Of the remaining five animals, two were still bachelors at the end of our study (ages 4 and 6 in 2007), two briefly held harems (at ages 4 and 8) but reverted to bachelor status, and one was a satellite stallion in his natal band (at age 5).

Table 8. Number of wild horse (*Equus caballus*) bands, mean age of harem stallions, mean harem size (mares ≥2 years old), and mean band size (all members) in the Pryor Mountain herd, 1996–2007.

[—, not applicable.]

Year	Number of bands	Mean age of harem stallions	Mean harem size	Mean band size
1996	29	8.76	1.90	5.14
1997	31	9.68	2.06	5.23
1998	31	10.26	1.87	4.90
1999	31	10.71	2.68	5.74
2000	31	11.10	2.68	5.87
2001	38	10.71	2.39	5.24
2002	34	11.06	1.94	4.59
2003	36	10.89	2.06	4.53
2004	34	11.21	2.06	4.03
2005	35	12.11	2.11	4.20
2006	35	13.00	2.20	4.66
2007	34	11.97	2.38	5.18
Total	399	—	—	—

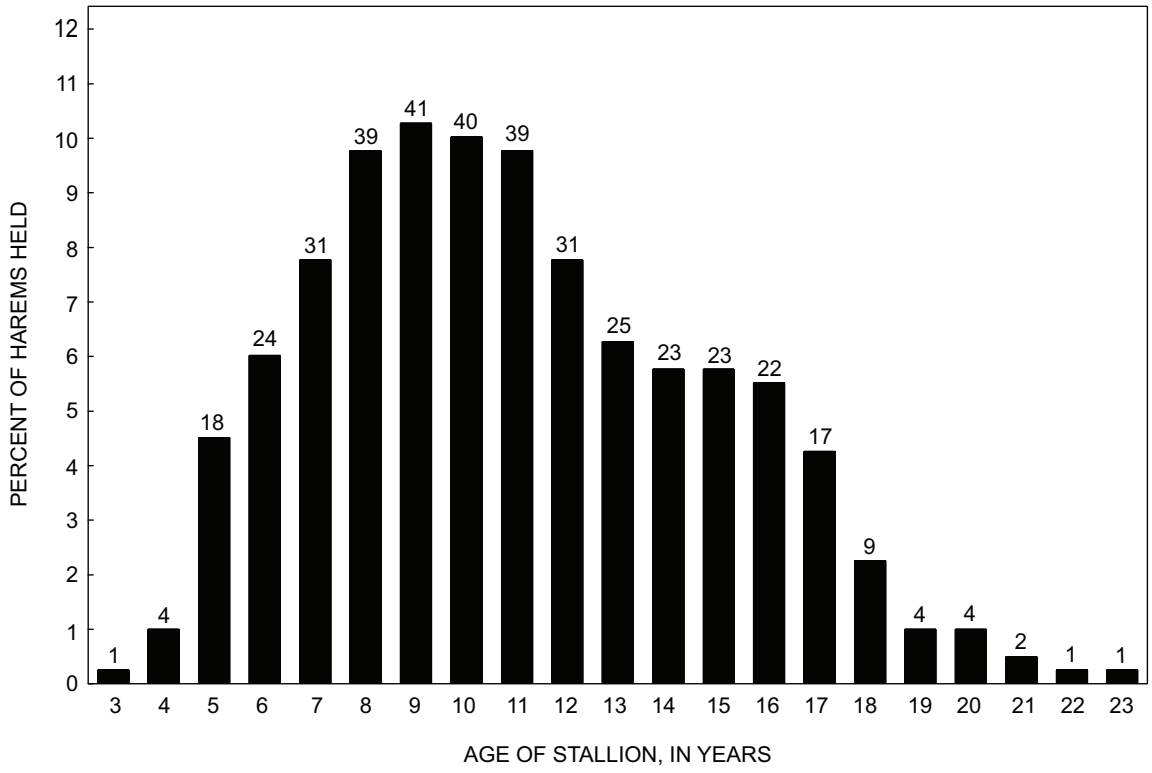

Figure 8. Age of wild horse (*Equus caballus*) stallions holding harems in the Pryor Mountain herd, 1996–2007. Values above the bars are number of harems.

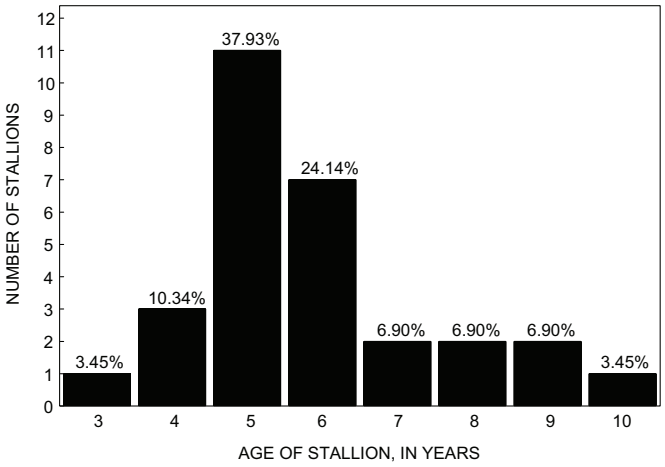

Figure 9. Age at which wild horse (*Equus caballus*) stallions first held a harem in the Pryor Mountain herd, 1996–2007. Values above the bars are percent of the total stallions represented on the chart.

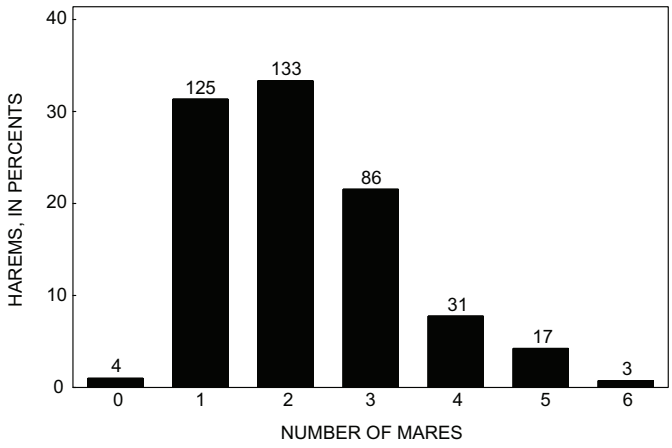

Figure 10. Frequency distribution of wild horse (*Equus caballus*) harem size in the Pryor Mountain herd, 1996–2007. Values above the bars are number of harems.

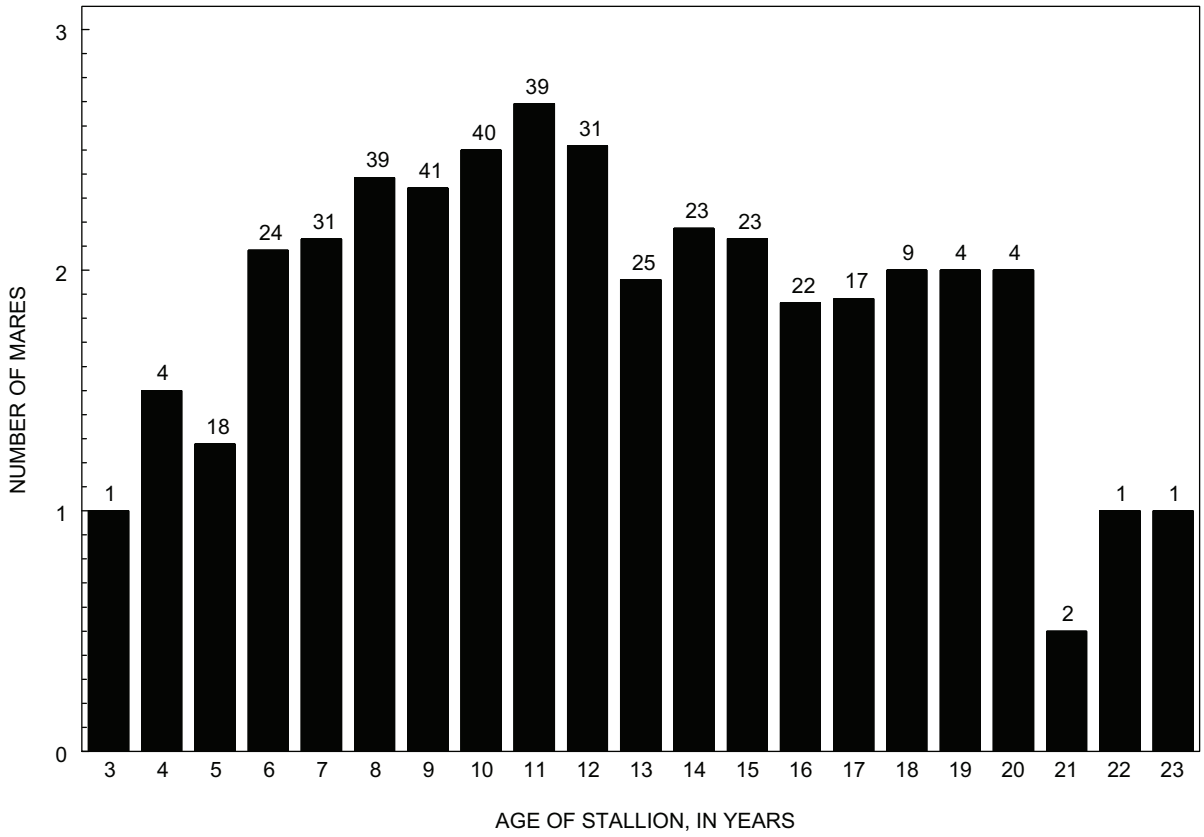

Figure 11. Mean number of wild horse (*Equus caballus*) mares held relative to age of harem stallions in the Pryor Mountain herd, 1996–2007. Values above the bars are number of harems.

Bachelors

The number of bachelor males in a wild horse herd changes through the course of a year, as young males are expelled from or leave bands, middle-aged males (formerly bachelors) gain bands, and older males gain and lose bands. In the Pryor Mountain herd, the number of bachelor males in summer (early to mid-June) varied between 16 and 36 during the period of study (fig. 12). The largest interannual change occurred between 1997 and 1998, in part because of the removal of 18 bachelors in fall 1997. In total, 40 bachelors were removed from the herd from 1996 through 2007. Pooled across years, 188 of 328 bachelors (57.3 percent) were 2 through 5 years of age (fig. 13).

To examine the question of when young stallions leave their natal bands and become bachelors, we used a subset of our data that included all males born in 1996 or later (thus ensuring that we had complete information on their band associations since birth). Of the 59 males that were born in 1996 or later and reached bachelor status (many died or were removed before becoming bachelors, others were born too late in the study period to have an opportunity to become bachelors), 4 (6.8 percent) became bachelors at age 1 (though one subsequently returned to his natal band), 32 (54.2 percent) at age 2, 13 (22.0 percent) at age 3, 7 (11.9 percent) at age 4, and 3 (5.1 percent) at age 5.

Population Regulation

Density Dependence

Regression analyses showed no relation between the foaling rate of mares ≥3 years of age and population size in either the current year or the previous year ($P \geq 0.143$). Foaling rate of 2-year-old mares may have declined somewhat with larger population in the previous year ($n = 13$ years, $P = 0.090$), but variability was high ($R^2 = 0.239$). Other young age classes (3–5 years) did not show similar relations, nor did combinations of these age classes.

Overall herd survival was related negatively to the previous year's postfoaling population (fig. 14A) ($n = 23$ years, $P = 0.006$), but there was a great deal of variability ($R^2 = 0.305$). Foal survival, however, was unrelated to population size ($n = 13$ years, $P > 0.15$). There also was a relation between population growth rate and the previous year's postfoaling population (fig. 14B) ($n = 23$ years, $P = 0.002$, $R^2 = 0.382$). This is perhaps not surprising, because survival rate is an important component of population growth rate.

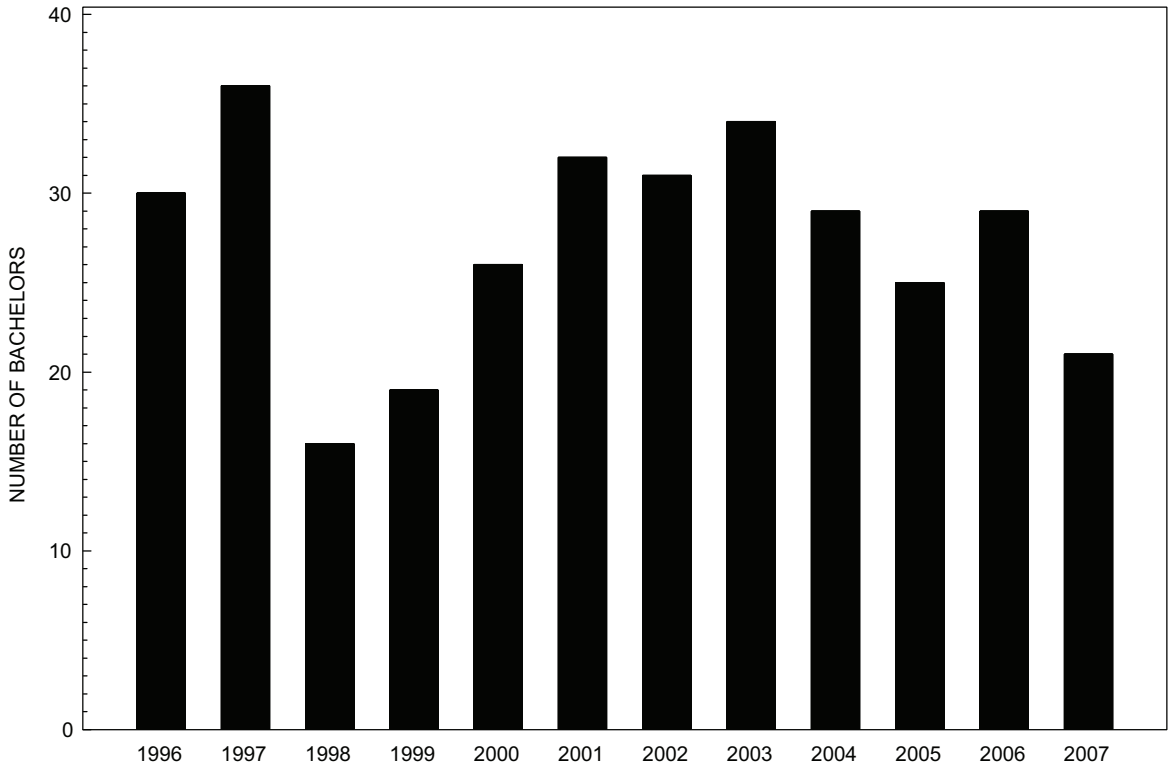

Figure 12. Number of bachelor wild horses (*Equus caballus*) in the Pryor Mountain herd, 1996–2007.

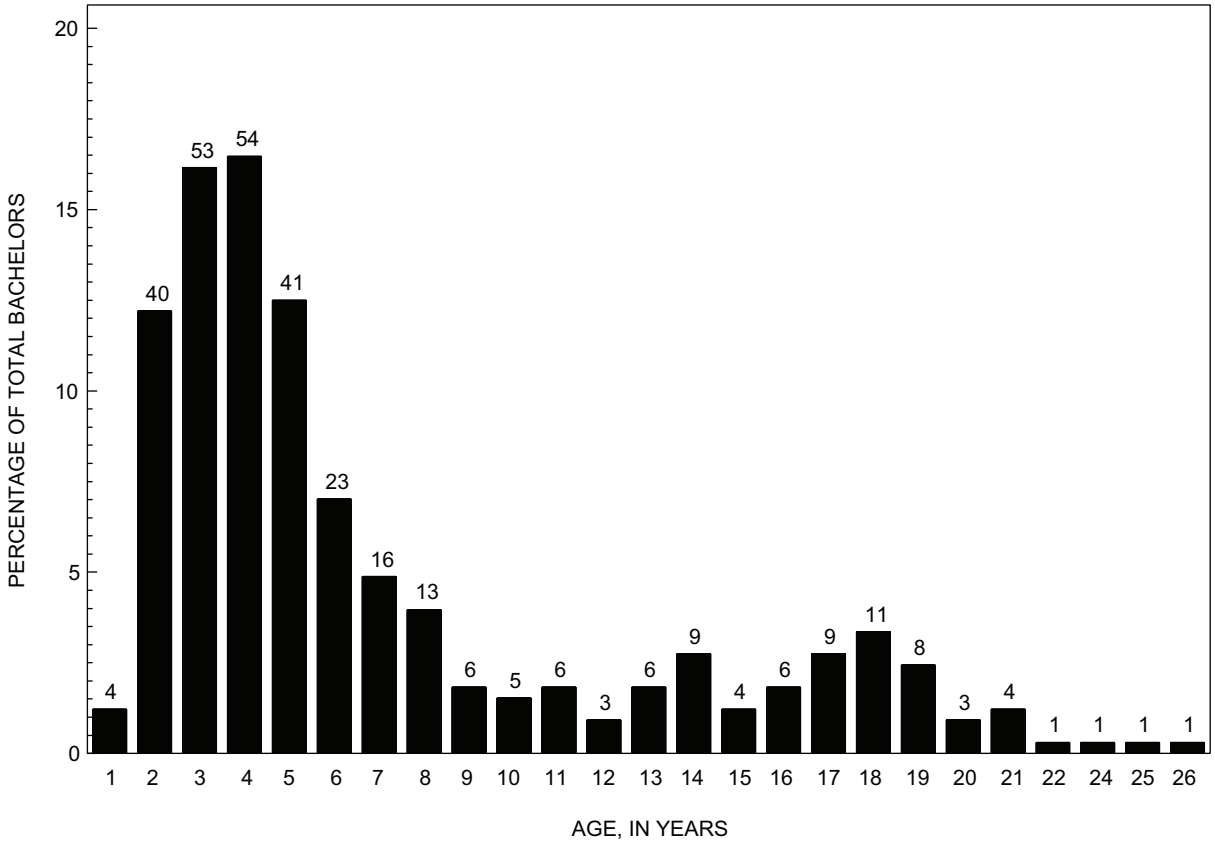

Figure 13. Age distribution of bachelor wild horses (*Equus caballus*) in the Pryor Mountain herd, 1996–2007. Values above the bars are number of bachelors.

Weather

None of the covariates that we used entered the stepwise multiple regression model for herd survival ($P > 0.15$; table 9). For foal survival, both summer and winter precipitation in the current year met the criterion for entry in the model ($P < 0.15$), and both were related positively to foal survival. In the case of winter precipitation in the current year, the positive relation was contradictory to our hypothesis. In combination, these parameters accounted for about 81 percent of the variability in foal survival. In terms of mare foaling rate, only the mean of the monthly average minimum temperature in the previous winter entered the model, and the relation accounted for only about 15 percent of the variability.

Discussion

Population Size and Growth

From 1993 to 2007, the prefoaling (adult) population averaged about 149 animals, only slightly more than the target population of ≈140 adults established on the basis of genetic considerations (Bureau of Land Management, 2006). Removals and a declining survival rate for foals appear to have been the primary factors keeping the population at this level. Contraception also may have played a role if it prevented the birth of any foals. However, it seems likely that the effect of the contraceptive program on population growth was small, given that relatively few mares were treated and that those mares were not in the most productive age classes.

The subject of population growth in wild horse populations has been controversial since early reports (for example, Cook, 1975) of rates as high as 30 percent annually. Other investigators challenged the accuracy of such estimates, and several used simulation modeling in combination with the scant data then available for wild horses and extrapolation of data from other ungulates to show that growth rates of 20–30 percent seemed highly unlikely given apparently reasonable assumptions about model parameters (Conley, 1979; Wolfe, 1980). Frei and others (1979) suggested that the apparently inflated growth rates might be an artifact of improved aerial census techniques and greater experience of observers.

As additional data were accumulated, however, it became apparent that initial assumptions regarding population parameters, particularly survival rates and age of mares at first reproduction, were probably conservative for many wild horse herds (Wolfe, 1986). Eberhardt and others (1982) calculated rates of increase of 20 and 22 percent for two herds

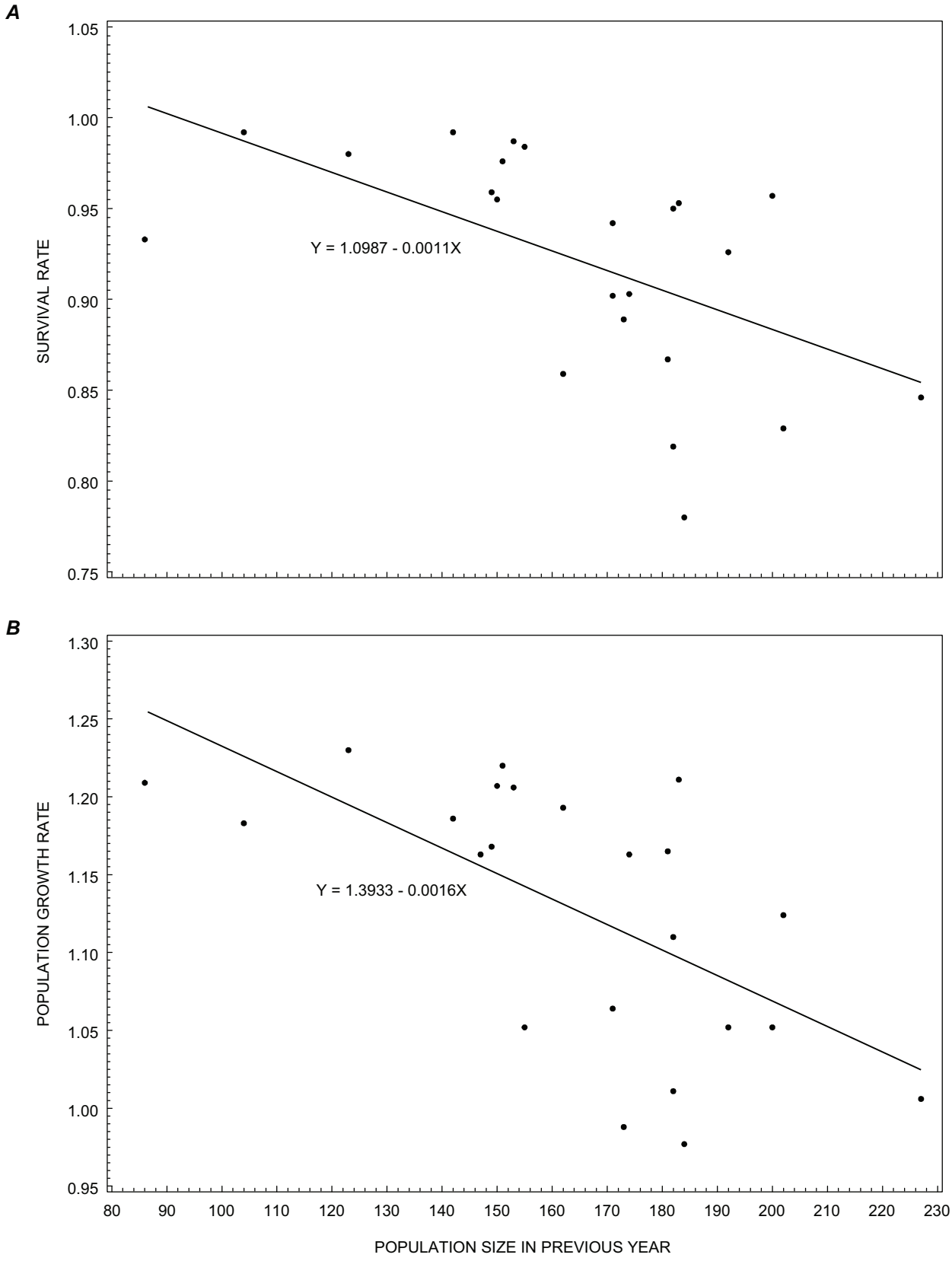

Figure 14. Relation of (*A*) survival and (*B*) population growth rate to the previous year's population for wild horses (*Equus caballus*) in the Pryor Mountain herd. Includes data from this study and from Garrott and Taylor (1990), excepting the abnormally severe winter of 1977–1978.

Table 9. Results of stepwise multiple regressions between wild horse (*Equus caballus*) herd survival, foal survival, and foaling rate (mares ≥3 years of age) and various weather parameters for the Pryor Mountain herd.

[Includes data from Garrott and Taylor (1990) and this study for herd survival and mare foaling rate, but only from this study for foal survival. *n*, sample size; SE, standard error of the estimate; R^2, coefficient of determination; *P*, probability; —, no variables entered the model; SUMP, summer precipitation in the current year; WINP, winter precipitation in the current year; LMNT, average monthly minimum temperature (December–February) in the previous year.]

Population parameter	*n* (years)	Covariate	Parameter estimate	SE	Partial R^2	Model R^2	*P*
Herd survival	19	—	—	—	—	—	—
Foal survival	10	SUMP	0.164	0.041	0.71	0.71	0.002
		WINP	0.117	0.059	0.10	0.81	0.090
Foaling rate	19	LMNT	0.008	0.005	0.15	0.15	0.105

in Oregon and, by comparing pre- and postremoval populations with known removals, showed that the aerial counts used to derive the growth rates must have been reasonably accurate. Berger (1986, p. 76), based on a 5-year study of horses in the Granite Range of Nevada, reported an average annual growth rate of 31 percent. [Note, however, that Berger (1986, p. 77) also reported an exponential growth rate of 0.188, which is equivalent to a finite growth rate of only 20.7 percent annually.] Wolfe (1986), using data from an unspecified number of BLM herds (>40,000 animals), calculated an annual growth rate of 14 percent for the period 1981–1983 and 15 percent for 1983–1985. Garrott and others (1991b) reported an unweighted mean growth rate of 21 percent (range = 15–27 percent) for 12 western herds. High rates of population growth are not limited to western horse populations. Franke Stevens (1990), for example, reported that the horse population at Rachel Carson National Estuary, made up of four small islands off the coast of North Carolina, increased from 44 animals in 1984 to 68 animals in 1986, from which we calculated an annual growth rate of 24.3 percent.

Reported growth rates of wild horse herds have not been universally high, however. Keiper and Houpt (1984), for example, stated that the horse population on Assateague Island National Seashore grew from 42 to 80 animals during the period 1975 to 1982, which represents an average annual growth rate of 9.6 percent. Goodloe and others (2000) found an average annual growth rate of 4.3 percent (range = 3.0–6.8 percent) for horses on Cumberland Island, Georgia, from 1986 to 1990 and suggested that the low rates likely were the result of density-dependent response to limited forage. Greger and Romney (1999), studying a horse population at the Nevada Test Site (now the Nevada Wild Horse Range), found an average growth rate of -7.0 percent, which they attributed to very low foal survival as a result of predation by mountain lions.

The first multiyear study of horses at Pryor Mountain was that of Perkins and others (1979). While they did not calculate population growth rates, they presented information sufficient to do so for two years. Based on information in their table 1, we calculated growth rates of 7.5 and 16.0 percent for 1976 and 1977, respectively, for the postfoaling population. Garrott and Taylor (1990) reported growth rates at Pryor Mountain for 1977–1986 with a mean of 11.9 percent, but 17.8 percent if a year with heavy winter mortality (51 percent of the population died) was excluded. Singer and others (2000) found a mean growth rate of 17.6 percent at Pryor Mountain from 1992–1997.

Our mean annual growth rate (9.6 percent; 10.5 percent if the year of heavy foal loss is excluded) for the period 1993–2007 is thus toward the lower end of the range reported for the Pryor Mountain and other herds. The declining trend in growth rate through time appears to be a result of decreasing survival of foals, though both foal survival (2005–2007) and growth rate (2006–2007) increased in recent years. Contraception also may have played a small role in reducing the average growth rate, though it is difficult to separate the effects of multiple factors.

Sex and Age Ratios

Studies at Pryor Mountain and elsewhere have found foal sex ratios favoring both males and females, with the pattern sometimes switching for the same herd in different time periods. At Pryor Mountain, for example, Feist and McCullough (1975) reported that 54.3 percent of 35 foals were males, Singer and others (2000) found that 58.3 percent of "surviving" foals were males (sample size not given), but Perkins and others (1979) found 48.5 percent males among 103 foals. Similarly, Keiper (1979) reported 52.6 percent males among 190 foals on Assateague Island, but Keiper and Houpt (1984) found 46.6 percent males in 103 foals born at the same location. In many of these studies with relatively small sample sizes, of course, the results did not differ significantly from parity, which also is true of most studies with larger samples, including ours. Speelman and others (1944), for example, found 49.4 percent males in 568 foals born to domestic mares raised under range conditions in Montana. Garrott (1991), who

studied the sex ratio of more than 10,000 foals removed from 74 BLM herds, found no significant difference from parity in 65 of those herds; the foal sex ratio favored females in seven herds and males in two herds. Interestingly, one exception to this pattern is found in the study by Garrott and Taylor (1990) at Pryor Mountain, who reported a sex ratio of 39.7 percent males ($P < 0.001$) in a sample of 330 foals from 1972 to 1986. Garrott and Taylor offered no explanation for this result, but they did comment that other data from their study might suggest low forage availability. Other investigators have hypothesized that females in poor condition (as through low forage availability) should produce fewer male offspring (Trivers and Willard, 1973).

The foal sex ratio did not differ from parity during the course of our study, and there was no overall difference in survival between the sexes. Thus, apart from minor variations resulting from each foal crop entering the population, consistent departures from a 50:50 sex ratio are the result of removals. This is evidenced by the fact that the largest interannual changes in sex ratio occurred following the larger removals. The drop in the proportion of males in the herd in 2007 was because of the removal of 14 males and 5 females in 2006, but also because natural mortality in 2006 consisted of 13 males and only 3 females. The increasing age of the herd also is mostly explained by removals; for all animals removed during the period of study, the average age was 3.2 years for females and 2.7 years for males. This is in keeping with BLM's management strategy of retaining harem stallions and core breeding-age mares (6–10 years) on the range (Bureau of Land Management, 2006). Low survival of foals in 2003 (36.7 percent) and 2004 (3.6 percent) also reduced the proportion of young animals in the herd and thus increased the average age. To the extent that it prevented any mares from foaling, the contraceptive program also would have contributed to increasing age of the herd, but the magnitude of any such effect is unknown.

Survival

The most striking feature of the survival data from our study is the general decline in foal survival in 1993–2004, followed by an apparent increase in the next 3 years. It would be tempting to attribute at least the declining part of this pattern to increased observational effort and hence increased detection of neonatal loss. We do not believe this to be the case, however, because there was no trend in the number of foals produced through time (linear least squares regression, $R^2 = 0.06$, $P = 0.38$), and because all animals present in the herd in 1993 and all foals born since then have been accounted for. Predation by mountain lions is suspected as the cause of low foal survival in 2004 and perhaps in 2003, though only four foals were confirmed to have been taken by mountain lions (Bureau of Land Management, 2006). Three mountain lions were removed from the area by hunters in winter 2004–2005 (Bureau of Land Management, 2006), which might in part explain the increase in foal survival observed in 2005–2007. It also seems possible that predation began on a smaller scale much earlier than 2003, but we know of no evidence to support this hypothesis. Predation by mountain lions has been shown to be an important factor in foal mortality in at least one herd (Turner and Morrison, 2001) and has been implicated in others (Salter, 1979; Greger and Romney, 1999). Wolves (*Canis lupus*), when present, are also suspected of preying on wild horses (Salter, 1979; Oom and Santos-Reis, 1986). Another possible contributor to the observed pattern in foal survival is drought. The drought began in 1999, the same year as the first real drop in foal survival, and was somewhat ameliorated in 2005 and 2007, at which time foal survival increased. The results of our regression analyses between precipitation and foal survival would seem to support this explanation. On the other hand, the initial response to reduced forage availability would likely be a decline in body condition of mares and foals, and such a decline was not observed during the early years of the drought (LC-M).

Overall, survival rates from our study for 1993–2007 are similar to those reported by Perkins and others (1979), but lower than those reported by Garrott and Taylor (1990) and Singer and others (2000) for the same study area (table 10). The foal survival rate reported by Garrott and Taylor, however, probably is an overestimate because it covers the period from midsummer to midsummer; neonatal mortality, which often constitutes the majority of foal mortality (Boyd, 1979; Keiper, 1979; Berger, 1986; Monard and others, 1997; Goodloe and others, 2000), likely was missed. Age of the herd also may account for some of the difference between our survival rate and that calculated by Garrott and Taylor (1990). At the end of their study in 1986, about 22 percent of the herd was ≥11 years of age, whereas the comparable number for 2007 was 39 percent. Perkins and others (1979) and Singer and others (2000) did not provide sufficiently detailed age data to make similar comparisons. Survival rates from our study were also moderate compared to those reported previously for other horse herds in North America (table 10).

Relations between foal survival and band size have not often been reported in the literature on wild horses, and the few available results are equivocal. We found that foals from larger bands tended to survive better than those from smaller bands. Working on Camargue horses in France, Feh (1999) found that there was less foal mortality in bands with an alliance of two stallions (3.2 percent), than in single-stallion bands (22.0 percent). Multiple stallions often are associated with larger bands. Cameron and others (2003), on the other hand, found that among Kaimanawa horses in New Zealand more foals died in multistallion bands (survival = 80 percent, $n = 30$) than in single-stallion bands (survival = 94 percent, $n = 50$), but the difference was not quite significant ($P = 0.056$). Similarly, evidence for a relation between mare age or parity and foal survival is equivocal. We found no relation between foal survival and parity of the mare. Keiper and Houpt (1984) reached the same conclusion, but only 10 foals died in the course of their study. In contrast, Cameron and

Table 10. Survival rates reported for North American wild horse (*Equus caballus*) herds.

[Data are arranged in order of increasing foal survival. Sample size (*n*), when available, is given in parentheses. NA, data not available; PMWHR, Pryor Mountain Wild Horse Range.]

Source and location	Survival rate (*n*)				Comments
	Foals	Yearlings	Adults	Herd	
Greger and Romney (1999), NV	0.12	NA	NA	NA	Foal survival is a mean for 8 years and is a maximum estimate because some neonatal mortality probably went undetected.
Turner and Morrison (2001), CA and NV	0.32	0.88	0.92	NA	All data represent means for 10 years.
Goodloe and others (2000), GA	0.600 (105)	NA	0.923 (263)	NA	Adult rate is for mares only, probably ≥3 years old.
This study, (PMWHR)	0.697 (465)	0.917 (252)	0.949 (1,814)	0.899 (2,531)	
Perkins and others (1979), PMWHR	0.75	NA	NA	0.903 (579)	Foal survival is a mean for 4 years. Herd survival is a weighted mean calculated from Table 1, Perkins and others (1979).
Boyd (1979), WY	0.824 (51)	NA	NA	NA	Data for 1978 only.
Singer and others (2000), PMWHR	0.87	NA	NA	Males, 0.965 Females, 0.975	Not clear that data reported for the herd actually includes all animals.
Siniff and others (1986), Pine Nut, NV	0.875 (40)	NA	0.972 (72)	NA	Adult rate is a weighted mean for 2 years representing only mares, probably ≥2 years old
Siniff and others (1986), Pah Rah, NV	0.877 (73)	NA	0.975 (81)	NA	Adult rate is a weighted mean for 2 years representing only mares, probably ≥2 years old
Keiper and Houpt (1984), MD and VA	0.883 (86)	NA	NA	NA	Foal rate is a weighted mean for 8 years.
Pellegrini (1971), NV	0.905 (21)	NA	NA	NA	Data are for 1969. However, in 1968 no foals were observed, and in 1970 only one foal was observed.
Berger (1986), NV	0.917 (120)	0.969 (64)	0.951 (329)	0.945 (513)	Herd rate calculated from data on the three age groups.
Garrott and Taylor (1990), PMWHR	0.968 (249)	0.977 (173)	0.962 (865)	0.965 (1,287)	Excludes data from a severe winter. Adult and herd rates calculated from Table 2, Garrott and Taylor (1990). Foal rate measured from midsummer to midsummer and may be an overestimate.

others (2000) found significant differences in mortality of foals born to mares 3–5 years old (18 percent mortality), 6–8 years old (3 percent), and ≥9 years old (9 percent).

Foaling Rates

Foaling rates of mares at Pryor Mountain most often have been low to moderate when compared to those reported for other North American herds (table 11). In fact, the rate of 0.432 foals/mare for mares ≥4 years of age calculated from Feist and McCullough (1975) is among the lowest for any herd, although their study was primarily behavioral and lasted only 1 year. In addition, they attempted to classify mares aged 1, 2–3, and ≥4 years without the benefit of examining tooth eruption and wear, and they admit that some classification errors probably occurred. If there was a tendency to misclassify 3-year-old mares as being ≥4 years old, the resulting foaling rate would be biased low. Garrott and Taylor (1990), however, reported similarly low rates, and mares in their study were aged by dental characteristics. As noted above, Garrott and Taylor (1990) reported on foals present in midsummer; some neonatal loss likely was missed, which would tend to bias foaling rates low. Perkins and others (1979) did not report age-specific foaling rates of mares, but for the three years of their study for which data are complete (1975–1977), the foal crop was 15.1 percent of the prefoaling population (weighted average, $n = 411$ animals ≥1 year of age). The comparable value during our study was 21.6 percent (table 1, $n = 2{,}232$ animals). These values, of course, are affected by the sex and age structure of the herd. Similarly, Singer and others (2000) did not report age-specific foaling rates in sufficient detail to be included in table 11. They did, however, show rates of 0.107 foals/mare for mares of age 2, 0.54 for age 3, 0.67 for ages 4–11, and 0.46 for ages ≥12 in the period 1992–1997. Comparable values from our study (1993–2007) were 0.048, 0.414, 0.588, and 0.636, respectively. Thus, it appears that foaling rates at Pryor Mountain have varied considerably through time, with those from our study being intermediate.

Several investigators have suggested that forage availability can affect the condition of feral mares and thus foaling rates (Green and Green, 1977: Nelson, 1978; Berger, 1986; Siniff and others, 1986; Garrott and Taylor, 1990). Hall (1972) described range conditions at Pryor Mountain as being poor to extremely poor through much of the area. Feist and McCullough (1975) reported a low foaling rate in 1971, as did Garrott and Taylor (1990) in the first 3 years (1976–1978) of their study. Furthermore, Garrott and Taylor (1990) suggested that low foaling rates likely were a result of poor forage, also noting that the foaling rate increased markedly in 1979 following loss of 51 percent of the herd in the severe winter of 1977–1978. Singer and others (2000) reported somewhat higher foaling rates than were observed in the earlier studies and also noted that former managers who visited Pryor Mountain in 1997 remarked about the overall improvement in "plant condition." Again, our values for foaling rate appear to be intermediate. It also may be relevant that mean annual precipitation in the biological years covered by the study of Singer and others (17.55 cm; 1992–1997, all years included regardless of missing data) was greater than that for the remainder of our study (13.79 cm; 1998–2007).

In addition to its effects on foaling rates of adult animals, forage availability may affect age of first reproduction by mares. Feist and McCullough (1975), Perkins and others (1979), and Garrott and Taylor (1990) all worked at times when forage production may have been poor at Pryor Mountain and all found that mares first foaled at age 3. Singer and others (2000), on the other hand, at a time when forage production may have been somewhat better, found that 10.7 percent of 2-year-old mares produced foals; the comparable value from our study was 4.8 percent. First foaling at age 2 (Salter and Hudson, 1982; Berger, 1986; Lucas and others, 1991) and age 3 (Boyd, 1979; Keiper and Houpt, 1984; Goodloe and others, 2000) has been reported for other herds, with the rate for 2-year-olds varying both among herds and within herds in different years (Garrott and others, 1991a). In at least two studies, low foaling rates in young mares have been attributed to high rates of fetal and neonatal loss (Lucas and others, 1991; Linklater and others, 2004).

Other factors that have been shown or hypothesized to affect foaling rate include gathers, band stability, and parturition history of the mare. In the case of gathers, we found a small but statistically significant effect, with foaling rate being lower in years following gathers. It must be remembered, however, that we did not design an experiment specifically to test a hypothesis about the effect of gathers. In particular, we had to assume that the majority of mares were affected by the gather (either actually moved into a trap or significantly disturbed by helicopter activity) in each of the three years that we considered to be gather years. Given the nature of the gathers in those three years, we believe that such an assumption is reasonable. Other evidence for an effect of gathers on foaling rate is equivocal. Hansen and Mosley (2000) studied two herds, one in Idaho and one in Wyoming, and found no difference in foaling rate between mares ungathered, gathered and released, or gathered and adopted. The ungathered samples were small, however, consisting of only 14 mares in each herd. Ashley and Holcombe (2001), on the other hand, found that in one of the two years of their study, ungathered mares foaled at a higher rate than did those that were gathered and released or gathered and removed. They found no evidence for an effect of a gather in the other year.

Evidence for an effect of band stability on mare foaling rate is more consistent. Berger (1983) found that mares in bands with resident stallions produced foals at a higher rate than those in bands taken over by a new stallion. Kaseda and others (1995), working on Misaki horses in Japan, reported that mares in "stable consort relationships" had higher reproductive success than those that changed stallions. Similarly, Goodloe and others (2000) found that mares that changed bands had a lower probability of foaling than those that did

Table 11. Foaling rates reported for North American wild horse (*Equus caballus*) herds.

[To the extent possible, data are arranged in order of increasing production by mares ≥3 years old. Sample size (*n*), when available, is given in parentheses. NA, data not available; PMWHR, Pryor Mountain Wild Horse Range.]

Source and location	Foals/mare (*n*)			Comments
	Mares ≥2 years old	Mares ≥3 years old	Mares ≥4 years old	
Siniff and others (1986), Pine Nut, NV	0.385 (104)	NA	NA	Combined data for 2 years. Age of mares inferred from other information.
Feist and McCullough (1975), PMWHR	NA	NA	0.432 (81)	Some animals may have been aged incorrectly. Includes two foals born to 3-year-old mares.
Greger and Romney (1999), NV	NA	0.360	NA	Unweighted mean for 5 years.
Garrott and Taylor (1990), PMWHR	NA	0.488 (600)	NA	Calculated from Table 3, Garrott and Taylor (1990).
Wolfe and others (1989), NV	0.432 (206)	NA	NA	Calculated from Table 5, Wolf and others (1989), for lactating mares.
Boyd (1979), WY, 1979	NA	0.526	0.545	Sample sizes not given.
Turner and others (1992), CA and NV	NA	0.537	NA	Unweighted mean for 6 years.
Keiper and Houpt (1984), MD	NA	0.571	NA	Unweighted mean for 8 years.
Nelson, (1978), NM	NA	NA	0.553 (38)	
This study, PMWHR	0.501 (876)	0.576 (752)	0.597 (665)	
Keiper (1979), MD	NA	0.614	NA	Unweighted mean for 5 years.
Salter and Hudson (1982), Alberta	0.521 (71)	0.621 (58)	NA	Calculated from Table I, Salter and Hudson (1982).
Seal and Plotka (1983), OR	NA	0.626 (99)	NA	Foaling rate based on lactating mares from Table 4, Ashley and Holcombe (2001).
Ashley and Holcombe (2001), Granite Range, NV, 1994	NA	NA	0.714 (28)	Calculated only for ungathered horses.
Ashley and Holcombe (2001), Granite Range, NV, 1997	NA	NA	0.741 (54)	Calculated only for ungathered horses. Age of mares inferred from other information.
Wolfe and others (1989), WY	0.535 (172)	NA	NA	Calculated from Table 5, Wolfe and others (1989), for lactating mares.
Garrott and others (1991a), NV	0.584 (901)	NA	NA	Foaling rate based on lactating mares gathered in June. Calculated from Table 1, Garrott and others (1991a).
Keiper (1979), VA	NA	0.744	NA	Unweighted mean for 4 years.
Ashley and Holcombe (2001), Garfield Flat, NV, 1997	NA	NA	0.824 (17)	Calculated only for ungathered horses. Age of mares inferred from other information.
Boyd (1979), WY, 1978	NA	0.781	0.863	Sample sizes not given.
Siniff and others (1986), Pah Rah, NV	0.670 (109)	NA	NA	Combined data for 2 years. Age of mares inferred from other information.

not. Data on mare interchanges are available from the Pryor Mountain herd, but have not yet been analyzed.

The question of whether parturition history of the mare affects foaling rate has been of interest since early investigators reported what they perceived to be a tendency to foal in alternate years (Green and Green, 1977; Nelson, 1978). This question has been addressed in a number of ways and results have varied. Seal and Plotka (1983) found that 68.4 percent (n = 76) of pregnant mares also had a foal at their side or were lactating, and Wolfe and others (1989) found no difference in pregnancy rates for lactating and nonlactating mares in six different populations. Neither of these studies, however, addressed the question of whether lactating and nonlactating mares are equally successful in bringing their pregnancies to term. On Assateague Island, 10.2 percent of 49 mares followed for 4 years foaled in alternate years, whereas 55.1 percent foaled in either 3 or 4 years (Keiper, 1979). Similarly, Keiper and Houpt (1984) could not see any tendency to foal in alternate years among 14 mares followed for 8 years. At Pryor Mountain, in both our study and that of Garrott and Taylor (1990), primiparous mares were less likely to foal in the next year than were multiparous mares. Cameron and Linklater (2000), on the other hand, found that the probability of foaling in a given year was reduced significantly if the mare had a foal in the previous year. Thus, while not universal, it appears that foaling in one year can sometimes reduce the probability of foaling in the subsequent year, particularly in primiparous mares.

Harem Stallions and Band Composition

Comparisons among band or harem sizes reported by various investigators are hampered by inconsistencies in defining what constitutes a harem member, or failure to do so at all. We defined harem members as mares ≥2 years of age, and found that the mean harem size was 2.2 mares with a range of 1.9 to 2.7 over 12 years; the number of bands ranged from 29 to 38. In other studies at Pryor Mountain, Feist and McCullough (1975) reported only average band size (5.0 including all members, not just mares), but they found 44 bands and 106 females ≥2 years of age, so the average harem size was likely somewhere near 2.4 mares. Note that the total population during the single summer of their study was about 225 animals, which probably explains the somewhat larger number of bands. Perkins and others (1979), working on a population that ranged from 95 to 168 animals (1974–1978), found 19–27 bands with a mean size of 4.9 (total animals), but did not report harem size in terms of number of mares. During a period (1992–1997) when the mean population was 161 animals, Singer and others (2000) reported an increase in the number of bands (18 to 31) and a decline in the number of mares (age unspecified) per harem from four to two. They attributed these trends to an increasing proportion of males in the population, death or waning vigor of a number of older males, and removal of members from the bands of several stallions aged 10–15 years. Investigators working on other herds have found similar or perhaps slightly larger average harem sizes. Mean harem size on Cumberland Island was 1.8 mares (Goodloe and others, 2000); the authors did not specify the age of mares included, but only mares ≥3 years old produced foals. Greger and Romney (1999) reported an average harem size of 2.4, which included all females ≥1 year of age, excluding daughters. Berger (1986, p. 130) found an average harem size of 3.1 mares with a range of 2.73–3.67 across years; age of mares was not specified but, because 2-year-old females reproduced, mares ≥2 years old may have been included. In Misaki horses (Japan), the annual average harem size ranged from 1.8 to 5.3 mares ≥2 years old; one stallion in one year had a harem of nine mares, and 55.0 percent of 60 harems (14 harem stallions) observed from 1979 to 1994 had four or more mares (Kaseda and Khalil, 1996).

In our study, 62.1 percent of harem stallions first acquired a harem at age 5 or 6. Other investigators have found comparable results: Berger (1986, p. 142), age 5–6; Kaseda and Khalil (1996), age 4–6; Asa (1999), age 5; Singer and others (2000), mean age = 6 years. In the last two years of our study, the mean age of harem stallions was 13 and 12 years, which is remarkably similar to the mean of 12.8 years reported at Pryor Mountain by Perkins and others (1979). Hall (1972), however, reported a mean age of 7.8 years, which is more similar to the data from the early years of our study. Age structure of removals is undoubtedly an important determinant of such results.

During our study, 6.3 percent of bands had multiple stallions. Feist and McCullough (1975) did not mention whether any of the 44 bands present during their study had multiple stallions. Perkins and others (1979) found only two multistallion bands in the 19–27 bands present during the 4 years of their study; if the mean number of bands present was, say, 23 per year, multistallion bands would have made up about 2.2 percent of the total. These percentages are lower than those reported for other herds: Goodloe and others (2000), 10.7 percent, n = 177; Nelson (1978), 11.8 percent, n = 17; Berger (1986, p. 129), 12 percent, sample size not given; Salter and Hudson (1982), 17.4 percent, n = 23; Asa (1999), 28 percent and 33 percent in 2 years, n = 18 and 9; Miller (1979), 23–45 percent for 3 years, n = 12–18; Bowling and Touchberry (1990), 39.1 percent, n = 69; Franke Stevens (1990), 50 percent, n = 12; Green and Green (1977), 45 percent and 54 percent in 2 years, n = 53 and 67, respectively. Kirkpatrick and Turner (1986) suggested that differences in proportions of bands with multiple stallions may be related to population density and sex ratio, with a greater ratio of males to females tending to lead to multistallion bands, and noted that some of the reported proportions may be high as a result of inaccurate aging of the animals based on body size [for example, Green and Green (1977)].

Bachelors

We have information on the total number of bachelors, but not on individual groups, which makes comparisons with other studies at Pryor Mountain difficult. Hall (1972) reported that the average size of bachelor groups was three, with individuals ranging in age from 1 to 5 years, and that older stallions usually lived alone after losing their harems. Feist and McCullough (1975) found 23 bachelor groups with a mean of 1.8 animals per group. Perkins and others (1979) found six bachelor groups, all with 2–3 individuals ranging in age from 3 to 8 years, as well as six older stallions that lived alone. With allowances for varying population size, our results do not appear to be greatly different from those found at other times at Pryor Mountain.

Most evidence suggests that males leave or are expelled from their natal band when they reach sexual maturity, which usually occurs at age 3 and occasionally at age 2 (Angle and others, 1979; Kirkpatrick and Turner, 1986). Our data show a somewhat different pattern. A few males left their natal band at age 1, most (54.2 percent) left at age 2, but 17 percent remained with their natal band until age 4 or 5. Of the three individuals that remained in their natal bands until age 5, two subsequently acquired harems and one was still a bachelor (6 years old) at the end of our study. Interestingly, one of these stallions took over his natal band, at which point his dam left the band for another. It seems possible that instances of stallions remaining with their natal band until age 4 or 5 account for some of the reports in other studies of a high proportion of multistallion bands.

Population Regulation

Several investigators have suggested a relation between forage availability and foaling rate, but few studies have been long enough and had sufficient variation in population size to allow detection of density-dependent effects. Berger (1986, p. 97) found little evidence for density dependence in his data from the Granite Range and suggested that the confounding effects of weather were responsible. He did, however, find some evidence for a decline in foaling rate with increasing population size in data from Keiper and Houpt (1984). Singer and others (2000) found that population growth rate at Pryor Mountain declined with increasing population size, but this result was clearly dominated by data from a single year [Singer and others (2000), fig. 5]. They did not find evidence for a density-dependent effect on either foaling rate or survival rate. Jenkins (2000) examined data from the Granite Range and Pryor Mountain herds, both having individually identified animals, as well as five additional herds where population size was estimated using aerial surveys. In all seven cases, he found that population growth rate decreased with increasing population size, but only one was statistically significant ($P \leq 0.05$). However, his meta-analysis of the entire data set indicated that the probability of the same result through chance alone was only 0.001.

Our results are similar to those of both Singer and others (2000) and Jenkins (2000) in that we also found that population growth rate declined with increasing population size, albeit population size from the preceding year, which suggests that lag effects may be important. Furthermore, we found similar evidence for a negative relation between overall herd survival and population size, and perhaps some weaker evidence that the foaling rate of 2-year-old mares is affected by population size. We did not, however, find evidence for a density-dependent effect on foal survival, perhaps in part because Garrott and Taylor (1990), whose study encompassed a range of population sizes, did not provide annual foal survival rates.

We used population size as a surrogate for population density in these analyses, as did all of the authors cited in this section. In doing so, we made the implicit assumption that the area used by horses did not change significantly through the course of the study. In the case of the Pryor Mountain herd, however, horses probably made increasing seasonal use of Forest Service lands north and west of the original wild horse range. It seems likely that any density-dependent effects would have been more pronounced had this increase in use of Forest Service land not occurred.

In all cases where we found evidence for density dependence, variability was high, suggesting that other factors also may be important in regulating the Pryor Mountain wild horse herd. Assuming that the cause of mortality was correctly identified, mountain lion predation may have had a dramatic effect on foal survival in at least one year. In the latter years of our study, contraception also may have had some effect on foaling rate, though it seems likely that this effect was small given that relatively few mares were treated and that they were not in the most productive age classes. One of the most important regulatory mechanisms appears to be precipitation, which we found to be strongly related to foal survival. Both density dependence and precipitation effects are consistent with an interpretation of forage availability per individual as an important parameter. All of these factors combined, however, were not sufficient to stabilize the population during our period of study, as evidenced by the necessity for large removals in several years.

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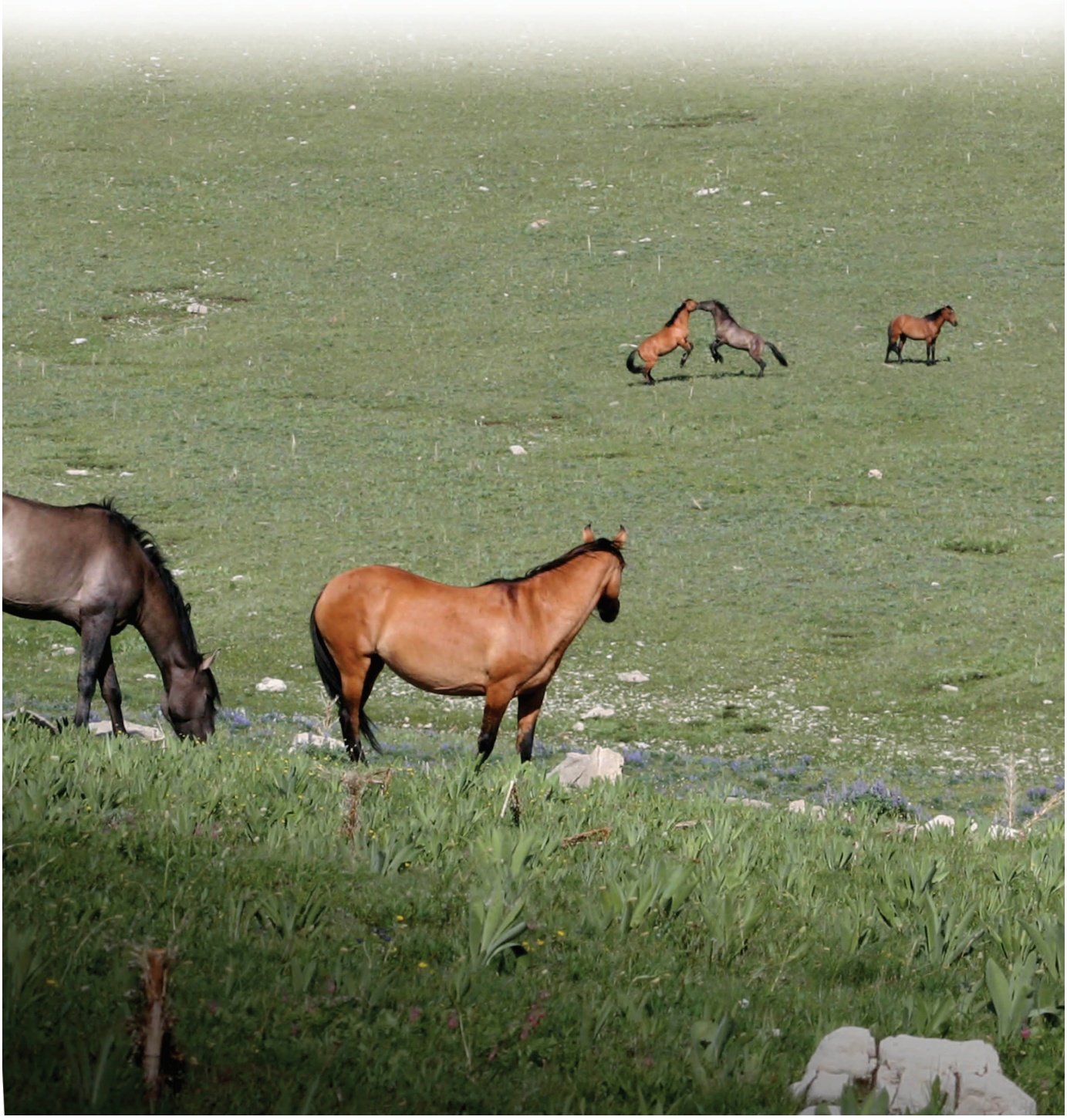

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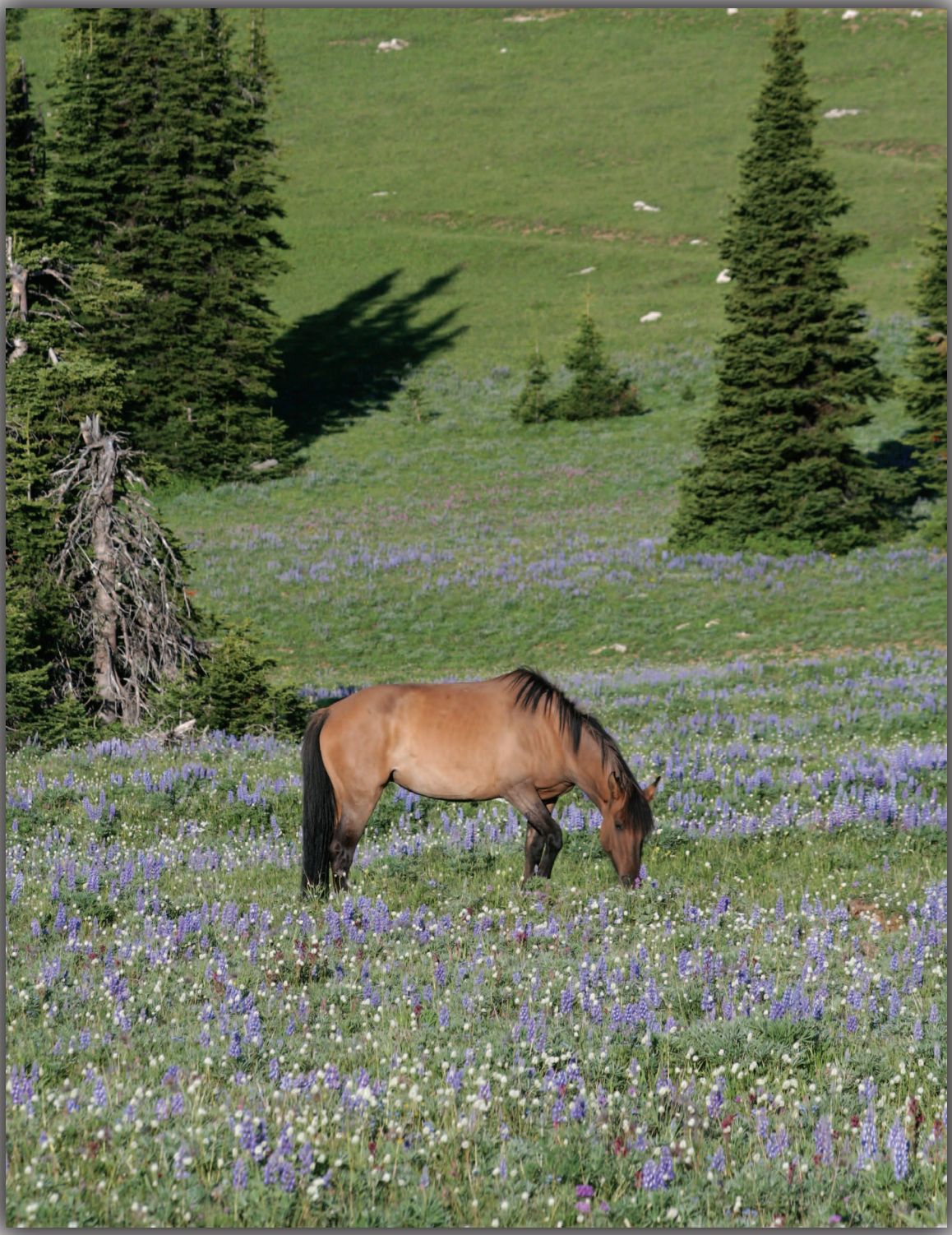

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Original Research

Laparoscopic Ovariectomy Diminishes Estrus-Associated Behavioral Problems in Mares

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ABSTRACT

Synthetic progestins, including altrenogest, are employed to modify undesirable estrus-related behavior or poor performance in mares. Bilateral laparoscopic ovariectomy (BLO) is another means of management. Our objective was to compare the perceived improvement in mares with estrus-related problems administered altrenogest to BLO. Owners of mares with estrus-related problem(s) that had undergone BLO after altrenogest treatment completed a telephone survey. They rated the behavioral or performance improvements with altrenogest and after BLO on a Likert scale (1 = no response; 5 = complete resolution). They also compared improvement with altrenogest and following BLO (1 = better response with altrenogest; 2 = comparable responses; 3 = better response with BLO). Likert scores for the two treatments were compared using the Wilcoxon signed rank test. The association between altrenogest and BLO scores was compared using Spearman rank-order correlation. Owners or trainers identified a variety of estrus-related problems in 20 mares meeting the inclusion criteria. Median scores for BLO were significantly higher than those for altrenogest ($P = .008$). The association of the perceived response to altrenogest and BLO was positive but relatively weak ($\rho = 0.288$). In a substantial proportion of mares with estrus-related problems, a salutary response to altrenogest was associated with a favorable response to BLO; however, some mares showing limited improvement with the progestagen still responded favorably to BLO. In this series, BLO was perceived by owners or trainers to be at least as effective as altrenogest administration as a means of diminishing a number of estrus-related behavioral and performance problems.

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1. Introduction

Trainers involved in a variety of disciplines have observed variation in the athletic performance of some mares that appear related to the estrous cycle [1–3]. Estrus-related behavioral or performance problems include difficulties during riding or training, back or flank pain, aggressiveness to other horses or people, frequent urination, colic signs, and undesirable estrous behavior during competition or training [2–5].

A variety of synthetic progestagens have been used in efforts to control undesirable behavior or impaired performance related to estrus [3,6]. Among the most popular is altrenogest (Regu-Mate; Merck, Whitehouse Station, NJ). Altrenogest is effective in eliminating estrous signs within 2–3 days in mares treated during estrus and will continue to suppress behavioral estrus with daily administration [1,6]. Despite recommendations to the contrary, this progestagen is reputedly used for prolonged periods in athletic mares without ill effects [7]. The associated cumulative expense, the necessity for daily administration, and potential deleterious effects after unintentional percutaneous absorption in women are disadvantages of its use.

Bilateral ovariectomy is regarded by some as a treatment of the last resort for mares with estrus-related problems

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that fail to respond favorably to pharmacologic treatment [3,6]. Complications after conventional open ovariectomy, although uncommon, can be grave and include serious hemorrhage, infection, adhesions, herniation, and evisceration [8,9]. Hooper et al [5] reported the elimination of objectionable estrus-related behavior in 14 of 17 mares ovariectomized via colpotomy. Improved performance after surgery was observed in a subset of 10 of 12 treated mares used for athletic purposes.

It is widely held that morbidity and mortality accompanying surgery are reduced when mares are ovariectomized using laparoscopic techniques [10], and bilateral laparoscopic ovariectomy (BLO) is increasingly popular for treatment estrus-related problems in working mares. The efficacy of BLO to reduce estrus-associated behavior and performance problems has not been firmly established, although favorable results have been reported in 70%–85% of cases where the procedure has been conducted for this purpose [11–16]. Similarly, the value of a favorable response to altrenogest to predict the efficacy of subsequent BLO is unknown. Critics of using the behavior- or performance-modifying effects of progestagen therapy to predict improvements after bilateral ovariectomy indicate that exposure of a mare to progesterone or one of its analogs is not equivalent to removal or absence of an estrogen influence [3,6]. Nonetheless, collective experience has led to the clinical impression that substantial improvements in the behavior and performance of mares with altrenogest administration are mirrored by those after BLO. Thus, the objectives of this study were to compare behavioral improvement in mares with altrenogest therapy to improvement after BLO and to determine if the behavioral or performance improvements realized with altrenogest were predictive of the response to BLO.

2. Materials and Methods

2.1. Case Selection

The medical records of mares that had undergone a BLO for estrus-related behavioral or performance problems were retrieved. Inclusion criteria included (1) mares had to have undergone treatment with altrenogest for a minimum duration of 2 weeks at some time before surgery; (2) at least some reduction in objectionable behavior(s) had to have been observed with altrenogest treatment (supporting the of an estrus-related problem); (3) had to have had grossly normal ovaries; and (4) follow-up was available. Mares that had been treated with other progestagens (e.g., medroxyprogesterone acetate [Depo-Provera; Pfizer, New York, NY]) but not altrenogest were not included.

2.2. Surgery

Bilateral laparoscopic ovariectomy was conducted in a manner previously reported [17]. Briefly, following fasting for 15–18 hours, mares were confined in treatment stocks with cross-ties and sedated with an IV loading dose of 3 μg/kg of detomidine followed by a continuous IV infusion (12 mg detomidine diluted in 250 mL of sterile polyionic fluids) to effect. Both flanks were clipped in a conventional fashion. Local anesthesia was effected using injections of 5–10 mL of 2% mepivacaine at the intended portal sites.

A 10-mm telescope portal was created in the left 17th intercostal space, at the level of the palpable ventral extremity of the tuber coxa and insufflation with carbon dioxide (6–10 mm Hg) was used to augment working space. A pair of 5-mm instrument portals was placed in the paralumbar fossa. The mesovarium was desensitized with 20–30 mL of local anesthetic injected at three to five locations dorsal to the ovary and tip of the uterine horn using an endoscopic injection needle. Subsequently, the mesosalpinx was elevated from its position on the lateral side of the ovary and dissected cranially to the point at which it fuses with the mesovarium using an electromechanical dissector (SonoSurg; Olympus KeyMed, Center Valley, PA). The dissector was used to sever the proper ligament, separating the ovary from the tip of the uterine horn, and dissection was continued craniad until a relatively narrow pedicle of mesovarium remained. A hand-tied ligating loop of one polydioxanone was placed on the pedicle containing the principal vasculature of the ovary. After transection of the remaining pedicle, the left ovary was passed to the right side of the abdomen between the urinary bladder and the body of the uterus. A similar portal arrangement was established on the right side of the mare, and the ovarian pedicle was transected using the technique described previously. Both ovaries were removed by morcellation through the right flank after enlargement of an instrument portal to 1.5 cm (electromechanical morcellator; Rotocut; Karl Storz, Tuttlingen, Germany).

2.3. Follow-up

A questionnaire was prepared that was used to conduct a telephone survey of individuals whose mare(s) had undergone BLO after management of objectionable behavior or impaired performance following one or more courses of daily altrenogest administration for a minimum duration of 2 weeks. Owners were asked to identify the principal behavioral and/or performance problem that had prompted medical and then surgical management, as well as any other perceived behavioral abnormalities. They rated the degree to which each of altrenogest therapy and BLO eliminated the principal behavioral or performance problem on a Likert scale (1 = no response; 5 = complete resolution). They also compared the quality of response to altrenogest and BLO (1 = better response to altrenogest; 2 = comparable response to both; 3 = better response to BLO). Owners were also requested to identify any complications that resulted from BLO.

2.4. Statistics

Likert scores were reported as medians and ranges. Owners' opinions regarding the relative efficacy of the two treatments were compared using the Wilcoxon signed rank test. The association between altrenogest treatment and BLO was assessed using Spearman rank-order correlation.

3. Results

Twenty mares met the four inclusion criteria and were included in the study. Mares ranged in age from 3 to 15 years with an average age of 9.4 years. Principal estrus-related problems, identified by owners or trainers, were some or all of kicking, bucking, or rearing (six mares); colic during estrus or ovulation (four mares); refusal to work during estrus (three mares); aggressiveness to other horses, people, or both (three mares); stallion-like behavior (one mare); undesirable estrus behavior during work (one mare); flank pain during estrus (two mares). Commonly, owners reported that their mares demonstrated additional behaviors to those previously mentioned to a lesser extent than the primary one. Before surgery, the mean duration of altrenogest administration was 4.7 months (range, 2 weeks–12 months). Dosage ranged from the recommended dose (0.044 mg/kg daily) to four times the recommended dose. Five mares underwent treatment with medroxyprogesterone acetate in addition to altrenogest on at least one separate occasion.

The median scores for owners' perception of resolution of the principal problem with altrenogest and BLO were 4 (range, 2–5) and 5 (range, 2–5), respectively. The perceived improvement in undesirable behavior or suboptimal performance after BLO was statistically significantly better than that for altrenogest therapy ($P = .008$). The association of responses to altrenogest and surgery was positive but weak ($\rho = 0.288$). Owners' opinions regarding the effects of both altrenogest and BLO on resolution of secondary problem(s) were virtually identical to those provided for the primary problems.

Of the 20 mares, six (30%) showed estrus signs at least once after surgery; in two mares (both aged 11 years), this was transient and the behavior ultimately disappeared. In the remaining four mares (mean age, 7.3 years [range, 3–10 years]), mild estrus signs persisted.

Complications related to the procedure were rare. Incisional swelling that resolved without treatment was reported for one mare, and two mares experienced mild colic signs shortly after discharge from the hospital. In both these mares, symptoms abated with treatment with nonsteroidal anti-inflammatory drugs.

4. Discussion

The results of this study provide evidence that BLO for the management of a variety of estrus-related behavioral and performance problems compares favorably with altrenogest therapy. Indeed, 95% of owners were of the opinion that BLO resulted in similar (four of 20) or greater (15 of 20) resolution of the primary problem than was achieved with medical therapy. A single mare had a better response to altrenogest than BLO. The principal problem for this 11-year-old mare was kicking and lack of regard for handlers. After surgery, the mare no longer kicked; however, she continued to slam into the wall of her stall. This behavior was not considered sufficiently problematic to resume treatment with altrenogest.

A variety of undesirable behaviors can accompany estrus in mares but clearly establishing a cause–effect relationship is challenging and is often a diagnosis made by exclusion. Obviously, for effective treatment of these problems, it is important to confirm that behavioral or performance problems are associated with estrus or the estrous cycle. Suppression of estrus with altrenogest was used to establish that estrus or ovulation was the source of colic symptoms in two mares [4]. We anticipated that a beneficial effect of progestagen administration on other putative estrus-related abnormalities would aid in establishing an association of undesirable behaviors with estrus and might portend a favorable response to BLO. For this reason, mares that failed to evince improvement in signs with altrenogest therapy, indirect evidence of a problem not related to estrus or cyclicity, were not included. (Parenthetically, four mares with altrenogest scores of 1 [no noticeable improvement] were excluded from the statistical analysis. Their median BLO score was 4.5 [range, 2–5]).

Commonly, owners' assessments of the effects of medical and surgical treatment paralleled one another. Interestingly, although scores for improvements observed with altrenogest and BLO were infrequently widely disparate, a number of owners observed only minor amelioration of signs with progestagen administration and perceived obvious improvement or complete resolution of the primary problem after BLO. The frequency of this phenomenon was sufficient (four of 20 mares) to result in a positive but weak Spearman correlation coefficient; thus, we were unable to strongly support the contention that the response to altrenogest is predictive of that to BLO. A stronger association of perceived improvements with medical and surgical treatment might be evident with a larger sample size.

One inclusion criterion included a course of treatment specifically with altrenogest before surgery. A number of mares that received just medroxyprogesterone acetate before BLO were not included. It has been shown that, like some other synthetic progestagens, medroxyprogesterone is relatively ineffective as a progesterone analog in mares because of weak binding to the progesterone receptor [18]. This purported lack of pharmacologic efficacy for mares given only medroxyprogesterone was considered a potential confounding factor, prompting their exclusion. Parenthetically, with rare exception, owners noted little or no improvement in signs with medroxyprogesterone, and those that had tried both altrenogest and medroxyprogesterone were generally of the view that the response to altrenogest was clearly superior.

It is important to recognize that bilateral ovariectomy does not consistently eliminate behavioral estrus in all mares. In this study, owners reported residual estrous behavior in six mares (30%), paralleling previous reports in which estrous behavior of reduced intensity was observed in 35% of 22 mares [5] and in one of five ovariectomized mares [19]. In this study, persistent estrous behavior appeared not to be age related; the mean age for mares continuing to show some estrous behavior after surgery was 2 years younger than the overall average. Residual estrous behavior in ovariectomized and anestrus mares was hypothesized to be because of an extragonadal source of sex steroids [20]. Another explanation for the phenomenon is that in mares, unlike other species, the absence of

estrous behavior is more closely associated with the presence of progesterone than the absence of estrogen [21]. This latter explanation is supported by the observation of estrous behavior in some seasonally anovulatory mares where both estradiol and progesterone levels are minimal [22]. Regardless of the reason for this phenomenon, it is prudent to advise owners that estrus-like behavior, albeit of a reduced intensity, may be observed in some ovariectomized mares.

Although not a specific objective of the study, the observation of very few complications reported by owners whose mares underwent BLO adds support to the clinical impression that laparoscopic ovariectomy in the standing sedated mare is a safe and reliable procedure associated with limited morbidity [10]. Smaller incisions, avoidance of the risks of general anesthesia, better local anesthesia, and visually monitored control of hemorrhage are widely accepted advantages of the technique and are likely contributory to the recent growth in popularity of this method of ovariectomy in mares.

This study, by virtue of inherent design limitations, is of limited scope. The results are based on the opinions of horse owners and trainers and are subjected to the fidelity of their memories, particularly for mares treated early in the study period. As for any study involving the use of a survey, the possibility of a placebo effect must be acknowledged. Second, establishing the association of objectionable behaviors to estrus or cyclicity is difficult, particularly without concomitant endocrinologic evaluation over the course of one or more estrous cycles. We attempted to establish the association of the behaviors and estrus by careful and repeated questioning of the owner or trainer with respect to the estrus-related nature of the principal problem, in addition to excluding mares that failed to improve with altrenogest therapy. Finally, as mentioned, mares did not uniformly undergo preoperative endocrinologic screening nor were ovaries submitted for histopathologic evaluation. Thus, despite using the inclusion criterion of palpably normally sized ovaries, it is possible some of the mares had an incipient granulosa theca cell tumor, which could have resulted in the observed undesirable behavior(s). Despite these shortcomings, that 95% of this sample of owners or trainers perceived an equal or better response to BLO compared with altrenogest suggests that BLO should be considered for the management of undesirable behavior(s) in mares that can be established to be related to the estrous cycle with some confidence.

5. Conclusions

A substantial proportion of estrus-related behavioral and performance problems that evince a salutary response to altrenogest showed similar or superior behavioral improvements after BLO. In addition, a number of mares demonstrating suboptimal improvement in objectionable behaviors with altrenogest treatment had a favorable response to BLO. These data suggest that for at least some mares with behavioral problems referable to the estrous cycle, BLO appears to be as effective as altrenogest. A clearer statistical association of the responses to the two treatment modalities, enabling clinicians to predict improvement after ovariectomy, might be better established with a larger sample size and the attendant collection of endocrinologic data to more firmly establish the link between undesirable behaviors and hormonal status of affected mares.

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ORIGINAL ARTICLE

Influence of caudal epidural analgesia on cortisol concentrations and pain-related behavioral responses in mares during and after ovariectomy via colpotomy

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Abstract

Objective: To determine the influence of epidural detomidine and morphine on serum corticosteroid concentrations and pain-related behavioral responses in mares during and after ovariectomy via colpotomy.

Study design: Blinded prospective study.

Animals: Nine university-owned mares.

Methods: Five of 9 horses received caudal epidural detomidine hydrochloride (0.01 mg/kg) and morphine sulfate (0.1 mg/kg) prior to surgery. All horses received local anesthetic around the ovarian pedicle, 0.02 mg/kg butorphanol IV at the start of the procedure and after first ovary removal, were sedated as required throughout the procedure, and were monitored for leg lifting, grunting, and abdominal tensing. Horses were monitored hourly for pain postoperatively. Heart rate was recorded every 4 hours, and photographs were taken to assess pain according to the horse grimace scale (HGS). Control group horses (n = 4) were treated with butorphanol (0.02 mg/kg IV) every 4 hours for 24 hours postoperatively. All horses received oral phenylbutazone 18 hours postoperatively. Serum cortisol was measured prior to the procedure, after first and second ovary removal, and 8 and 24 hours postoperatively.

Results: No differences were detected between horses receiving caudal epidural detomidine and morphine and those that received systemic opioids. A decrease in HGS score occurred after phenylbutazone administration.

Conclusion: Administration of caudal epidural detomidine and morphine resulted in similar pain-related behavior and corticosteroid concentrations as did administration of systemic butorphanol every 4 hours for 24 hours postoperatively.

Clinical significance: Caudal epidural detomidine and morphine may mitigate the requirement for frequent systemic opioid administration after a potentially painful procedure.

1 | INTRODUCTION

Ovariectomy is a routine surgery in the mare and can be achieved via colpotomy, median, paramedian, or laparoscopic approaches.[1] Colpotomies have become less common with the advent of laparoscopic surgery; however, ovariectomy via colpotomy offers an economical and practical alternative to the laparoscopic approach. Colpotomies require less surgical

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equipment and, in our experience, take less time to perform because of reduced preparation and suturing and lack of external incisions. Postoperative complication rates have been reported to be 4%.[2-4] Because of the small amount of equipment required and lack of external incision requiring care, colpotomies offer a useful method for sterilization of feral or wild equids, especially when facilities are limited. A laparoscopy-assisted colpotomy approach has been described that eliminates the requirement for a large external incision to remove the ovary; however, costly equipment is still required.[5]

During ovariectomy, local anesthetic is routinely applied to the mesovarium or directly to the ovary to mitigate pain.[6] However, this treatment does not completely eliminate sensation in the ovary or caudal tract, nor does it prevent straining, a response that may complicate the procedure.[3] Caudal epidural with anesthetics reduces straining by causing desensitization of the pudendal, middle and caudal rectal, and caudal cutaneous nerves, which are responsible for sensation of the external genitalia.[3,7] However, caudal epidural analgesia has no effect on pain from the ovary and increases the risk of ataxia and recumbency.[3,7] Caudal epidural with analgesics, such as opioids and α-2 agonists, effectively modulates pain during ovariectomy and may also reduce postoperative pain.[3,7] Depending on volume and location of administration, analgesics have been shown to have effects that extend to coccygeal, sacral, lumbar, and thoracic nerves and that may extend as far cranially as thoracic nerves 8-15.[7]

Systemic opioids such as butorphanol have profound analgesic effects but may lead to complications, such as central nervous system excitation, bradycardia, ataxia, and gastrointestinal stasis.[8,9] In addition, clearance of butorphanol is rapid because it has a half-life of 2 hours.[10] In contrast, epidural administration of hydrophilic opioids (such as morphine), when accompanied by α-2 agonists, has a duration of analgesia of up to 24 hours and less systemic absorption, which translates to reduced side effects.[11]

The first objective of this study was to evaluate the effect of the addition of caudal epidural detomidine and morphine during ovariectomy via colpotomy versus systemic sedation and analgesia alone during the procedure. The second objective was to evaluate the effectiveness of caudal epidural detomidine and morphine administered before surgery in controlling pain in the first 24 hours postoperatively vs repeated systemic use of opioids, which is the standard procedure in our hospital after ovariectomy via colpotomy. Drawing on our clinical experience using caudal epidural detomidine and morphine, we hypothesized that there would be a significant reduction in horse pain-related behaviors intraoperatively in the epidural group and no appreciable differences in behavior or corticosteroid concentrations postoperatively between treatment groups.

2 | MATERIALS AND METHODS

Nine university-owned mares (8 mixed quarter horse type breed, 1 African quarter horse) undergoing ovariectomy via colpotomy as part of another study (IACUCs 2012-0237 and 2015-0282) were used. Horses ranged in age from 9 to 30 years (median, 22) and weighed 420-570 kg (median, 500). None of the horses had a history of reproductive illness. A third party randomly assigned the horses to epidural or control groups by denoting group A as the epidural group and then using www.random.org to randomly assign numbers 1 through 9 to 1 of 2 groups, A (epidural) or B (control). Other than the clinician performing the epidural injection, all investigators were blinded to treatment group assignment during the surgery. Five horses (epidural group) received detomidine hydrochloride and morphine sulfate via caudal epidural prior to surgery, and the remaining horses (control group) received postoperative butorphanol injections (every 4 hours, IV).

2.1 | Surgical procedure

Feed was withheld for 12 hours prior to the surgical procedure, but access to water was not restricted. Approximately 30 minutes prior to the start of the procedure, ampicillin sodium (10 mg/kg IV), gentamicin sulfate (6.6 mg/kg IV), and flunixin meglumine (1.1 mg/kg IV) were administered through direct venipuncture. Horses were placed in stocks and sedated with acepromazine (0.05 mg/kg IV) and detomidine (0.006-0.018 mg/kg IV).[12] The epidural site was clipped and aseptically prepared in all horses. In the horses assigned to the epidural group, a board-certified equine surgeon performed a caudal epidural with detomidine (0.01 mg/kg) and morphine (0.1 mg/kg) diluted with saline to a total of 0.03 mL/kg, as previously described.[7] Briefly, the first intercoccygeal vertebral space was identified as the most cranial movable intervertebral space when the tail was lifted. The skin and subcutaneous tissue were then desensitized with local injection of 1-2 mL of 2% lidocaine, after which a 3-inch, 18 gauge Tuohy needle was introduced at a 10-degree forward angle into the epidural space. Location in the space was confirmed by using the "hanging drop" technique. A polyamide epidural catheter (Perifex continuous epidural anesthesia set; Braun Medical, Bethlehem, Pennsylvania) was introduced and advanced 20 cm cranial in the epidural space (measured prior to introduction) to the approximate level of S2-S5, depending on the size of the horse. Analgesia was then administered, and the catheter was withdrawn. In all horses, the prepared area was covered with a sterile gauze bandage so that treatment was not revealed to those blind to treatment. The veterinarian performing the surgery then reentered the room and conducted a standard ovariectomy via

colpotomy as previously described.[3] Butorphanol (0.02 mg/kg IV) was administered to all horses at the beginning of the procedure and again after the first ovary was removed. Regardless of group assignment, each ovarian pedicle was blocked before ovary removal by holding a sponge, soaked in 50 mL of 2% lidocaine, around the ovarian pedicle and applying pressure for 3 minutes. The placement of the ecraseur was started approximately 3 minutes after application of local anesthetic. Horses were additionally sedated as required during the surgery with detomidine or with xylazine at the discretion of the surgeon. Choice and dose of sedative was based on maintaining an appropriate surgical plane of sedation as determined by horse head height and reaction to environmental stimulus, with regard to the expected remaining duration of the procedure. Horse reactions, including leg lifts (any time the hoof left the ground but not associated with adjusting her stance), grunting of any duration, and abdominal tensing were recorded throughout the procedure by a dedicated blinded person who was also assigned to record any other potential complications, such as excessive hemorrhage, inability or reduced ability to perform the procedure as expected, or uncontrolled pain.

2.2 | Postoperative management

Horses were observed postoperatively by hospital personnel every hour for 3 days after surgery for changes in attitude, appetite, and manure production, for signs of pain such as tail swishing, teeth grinding, trembling, flank watching, pawing, obtunded attitude, or persistent recumbency. If at any observation there were signs of pain, the clinician was to be contacted to initiate rescue analgesia. Rescue analgesia was 5 mg butorphanol administered intramuscularly (IM). Heart rate was recorded every 4 hours for the first 24 hours postoperatively. Full physical examinations were performed every 12 hours for 3 days. All horses received procaine penicillin (22,000 IU/kg IM) twice daily, gentamicin (6.6 mg/kg IV) once daily, and phenylbutazone (2.2 mg/kg orally, starting 18 hours postoperatively) twice daily for 3 days postoperatively. Horses in the control group received butorphanol (0.02 mg/kg IV) every 4 hours for the first 24 hours after surgery.

2.3 | Cortisol assay

Blood samples were drawn from the jugular vein prior to the start of the procedure, after each ovary was removed, and at 8 and 24 hours postoperatively. Heparinized samples were centrifuged at 4000 × *g* for 5 minutes, and serum was collected and frozen at −80°C until the cortisol assay could be performed. Serum cortisol levels were later determined by using a solid phase, competitive chemiluminescent enzyme immunoassay as previously described.[13]

2.4 | Facial grimace scoring

The profile of each horse was photographed every 4 hours for 24 hours postoperatively, with t = 0 being the conclusion of surgery. Photographs were randomized and 4 blinded persons individually scored the images by using the horse grimace scale (HGS).[14] The HGS incorporates 6 facial action units: stiffly backward ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin, and strained nostrils and flattening of the profile.[14] Each category is scored from 0-2. The sum of the 6 categories results in the HGS score, with 0 being the least painful and 12 being the most. The 4 individuals who scored the photographs were given detailed instructions on how to use the HGS, and a composite HGS score was made by averaging the results from the 4 scorers. The HGS scores were evaluated over time (at t = 4, 8, 12, 16, 20, and 24 hours) and between treatment groups.

2.5 | Statistical analysis

Commercial statistical software (SAS version 9.4; SAS Institute, Cary, North Carolina) was used. Repeated-measures analysis of variance was used to determine whether each outcome variable differed significantly by treatment group over time, with horse considered a random effect. This analysis was performed in PROC MIXED (SAS Institute) with an autoregressive correlation structure; $P \leq .05$ was considered significant.

3 | RESULTS

All 9 procedures were performed without complication (including no excessive hemorrhage, inability to perform the procedure, or uncontrolled pain during the procedure or in the postoperative period), and all mares were returned to their herd after the 3-day hospitalization. The median duration of surgery was 49 minutes (range, 22-74). There was no significant difference in duration of surgery between the 2 groups (49-68 and 22-74 minutes for control and epidural groups, respectively). The procedure was started immediately after epidural administration or equivalent waiting period in the control group. The total volume administered to the epidural group varied with horse weight but was approximately 15 mL. Time between the 2 intraoperative butorphanol administrations (at the beginning of the procedure and after the first ovary was removed) ranged from 11 to 44 minutes (median, 30). In both treatment groups, additional α-2 agonist sedation was administered as required. Three horses (1 in the control group and 2 in the epidural group) received additional detomidine, with a median dose of 0.004 mg/kg; 6 horses (3 in the control group and 3 in the epidural group) received additional xylazine, with a median dose of 0.2 mg/

TABLE 1 Horse movement and additional sedation administered during ovariectomy via colpotomy for horses in the epidural and control groups

Horses by group	Abdominal tensing episodes, n	Abdominal tensing duration, s	Grunts, n	Leg lifts, n	Additional sedation, mg/kg
Epidural					
1	14	22	7	1	0.3 xylazine
2	7	9	0	2	0.2 xylazine, 0.002 detomidine
3	29	178	0	1	None
4	13	16	0	0	0.3 xylazine, 0.006 detomidine
5	7	45	0	1	None
Control					
6	13	13	0	0	None
7	14	15	0	9	0.2 xylazine
8	15	26	5	3	0.15 xylazine
9	5	13	0	3	0.5 xylazine, 0.004 detomidine

kg (Table 1). The proportion of mares receiving additional sedation and the dosage of additional sedation did not differ between treatment groups.

3.1 | Movement

Frequency of abdominal tensing, leg lifts, or grunts in either group was too low to draw a statistical conclusion (Table 1).

3.2 | Heart rate

One mare in the epidural group was excluded from heart rate analysis after being diagnosed with atrial fibrillation according to results of electrocardiogram in the immediate postoperative period. In the remaining mares, there was no significant difference in heart rate postoperatively between treatment groups (control group median, 50 beats per minute [bpm]; epidural group median, 48 bpm) or over time.

3.3 | Cortisol

There were no significant differences in serum cortisol concentrations between treatment groups, either overall or within time points. There was a significant ($P < .001$) change in serum cortisol concentration over time, with the lowest concentration preoperatively (control median, 6.6 μg/dL; epidural median, 3.8 μg/dL) and the highest concentration 8 hours postoperatively (control median, 8.2 μg/dL; epidural median, 9.0 μg/dL; Figure 1).

3.4 | Facial grimace scoring

There was no significant difference in HGS scores between treatment groups (Figure 2). Across both treatment groups there was a significant effect of time ($P = .01$). Median HGS scores were highest at t = 16, with a median control score of 4.4 and a median epidural score of 4 (control range, 3.3-5.3; epidural range, 2.5-4.3), and lowest at t = 20, with a median control score of 2.5 and a median epidural score of 2.3 (control range, 2-2.8; epidural range, 1.8-3.5). It should be noted that t = 18 is when horses received phenylbutazone.

4 | DISCUSSION

Our findings in this study lead us to conclude that administration of caudal epidural detomidine and morphine in

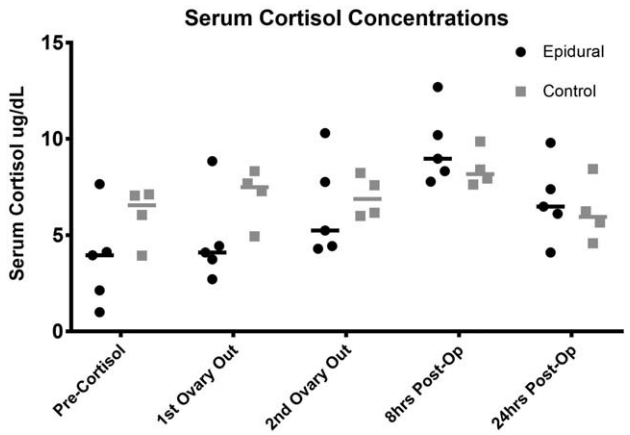

FIGURE 1 Serum cortisol concentrations of horses that did (epidural) or did not (control) receive detomidine and morphine via caudal epidural before ovariectomy via colpotomy. Time points include precortisol, prior to the start of surgery; 1st ovary out, after the first ovary was removed; 2nd ovary out, after the second ovary was removed; 8 hours postoperatively; and 24 hours postoperatively. The data points represent serum cortisol concentration (μg/dL) for each horse at each time point, and the bars represent the median

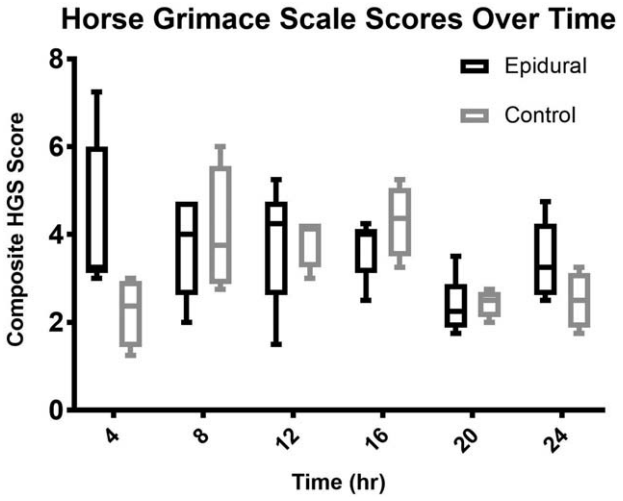

FIGURE 2 Postoperative HGS scores over time for horses that did (epidural) or did not (control) receive detomidine and morphine via caudal epidural before ovariectomy via colpotomy. Photographs of the horses were evaluated by 4 individuals blinded to treatment group. Boxes represent median and interquartile range (25th to 75th percentile), with the whiskers illustrating the minimum and maximum scores. HGS, horse grimace scale

addition to systemic opioids does not change frequency or intensity of signs of pain during ovariectomy and appears to provide analgesia equivalent to that of repeated opioid treatment in the postoperative period. The lack of difference between groups is likely due to achievement of adequate analgesia using our standard pain management protocol.

It is difficult to determine the amount of pain mitigation directly attributable to either epidural or opioid treatment during surgery or in the postoperative period in the absence of an untreated control group. However, our experience with this procedure (over 50 ovariectomies via colpotomy by KH) is that ovariectomy is a notably painful procedure and that mares show extreme discomfort in the first 24 hours postoperatively if analgesia is not administered. Thus, for ethical reasons, a control group that did not receive intraoperative sedation and analgesia or that was left untreated in the postoperative period was not used. This is a limitation of this study.

Pain is difficult to measure objectively in the horse.[15,16] One objective measure to assess pain is serum cortisol, which increases in the horse secondary to stress and pain and can be used as a comparative measure of pain response between individuals and over time.[17,18] That cortisol increased significantly at 8 hours but not intraoperatively was not surprising because systemic cortisol concentration peaks 2 hours after stimulus.[19] In addition, systemic administration of detomidine during the surgical procedure likely resulted in downregulation of sympathoadrenal activity, causing a temporary decrease in serum cortisol.[19,20] At 8 hours postoperatively, the control group had already received a dose of systemic butorphanol; lack of difference between groups at this time suggests that epidural detomidine and morphine were as effective as systemic butorphanol for postsurgical analgesia.

As another measure of pain, we used the recently reported HGS. We chose to use this system because it is rapid to perform, observers can be trained quickly to score with good inter-observer repeatability, and it has been shown to be effective and reliable for assessing pain in horses.[14,21] The significantly reduced HGS scores postoperatively at 20 hours compared with 16 hours in both groups may have been associated with phenylbutazone administration at 18 hours, which is our normal protocol after ovariectomy via colpotomy.[22] Lack of difference in heart rate postoperatively at 20 hours compared with 16 hours suggests that the HGS may have improved sensitivity compared with heart rate for the detection of a change in mild pain in horses.

We had hypothesized that addition of epidural analgesia to the standard sedation and analgesic regimen used during ovariectomy would decrease the pain responses noted and the requirement for additional sedative administration; however, it did not. Preparation for the surgery was started immediately after epidural administration, so it is possible that the epidural analgesia had not reached maximum efficacy during surgery. Time to onset of analgesia after administration of epidural morphine has been reported to be from 20 minutes to 6 hours; however, when morphine was administered in combination with detomidine, onset of action was more rapid (as short as 10 minutes after administration), and duration of action was potentiated for as long as 24 hours.[7,23,24] We used a relatively low detomidine dose in the epidural; it is possible that a higher dose may have produced a greater intraoperative effect. It is also possible that the presence of systemically administered butorphanol (κ-agonist and a partial μ-antagonist) may have antagonized the epidural morphine (μ-agonist) during surgery because opioids are known to interact when administered concurrently, regardless of the route of administration.[25,26] However, the extent to which this may occur has not been proven and may not be marked enough to alter the effect of the epidural morphine, which is highly conserved in the epidural space.[11]

The effect of the epidural on intraoperative pain would have been clearer had this been the sole analgesic during the surgery. However, because we were unsure of the level of analgesia afforded by the epidural alone, we hesitated to include a control or epidural group that did not receive systemic analgesics. In addition, administration of sedation before and during surgery maintained blinding to treatment group by the surgeons and the person noting pain responses during the procedure. The additional intraoperative sedation was not standardized in the dose or drug used, which is a potential limitation of the study. The operating clinician,

who was blind to the mare group, chose the drug and dose based on the level of the mare's sedation and the amount of time until the end of surgery. If the colpotomy incision had already been made, xylazine was typically used because of the shorter duration of action. One factor that may relate to the lack of difference in sedative administration between groups is that horse head height and reaction to environmental stimulus, rather than level of analgesia, was largely used by the operating clinician; these parameters would have been minimally affected by epidural administration of detomidine and morphine.

The doses of butorphanol used in the postoperative period of this study were low compared with some older reports but were similar to previously reported doses of butorphanol used during ovariectomy.[27,28] We administered the butorphanol at the interval recommended by the manufacturer (every 4 hours) because analgesic effects have been shown to last over this period. Using our current clinical practice of walk-by monitoring every hour, we verified that none of the horses in either group showed the signs that we had designated for rescue analgesia.

We limited the number of horses in the study to 9 (4 in the control group, 5 in the epidural group) because this study was performed concomitantly with a study for which the ovaries were being removed for examination. We believed that performing additional ovariectomies for the sake of validating a measure of pain control would be unethical.

In conclusion, our findings indicate that caudal epidural analgesia with detomidine and morphine do not provide additional analgesia during surgery in mares also given the standard sedative/analgesic regimen but that they do provide analgesia equivalent to that of systemic butorphanol administration in the postoperative period. Although administration of a caudal epidural is technically more challenging than administration of systemic butorphanol, it significantly decreases the requirement for postoperative handling and repeated IV or IM injections and, thus, may be especially applicable in situations such as sterilization of feral or wild equids in which repeated handling is problematic. The HGS proved easy to apply and, because handling is not required to evaluate HGS, this may also be applicable for assessment of feral equids. Our results lead us to conclude that use of caudal epidural analgesia should be considered by the practitioner when performing ovariectomy via colpotomy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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Pattern of focal γ-bursts in chess players

Grandmasters call on regions of the brain not used so much by less skilled amateurs.

The brain's medial temporal lobe structures are thought to be important for the initial formation of long-term memory[1,2], and active memory is indicated by bursts of γ-band activity in these and other areas of the association cortex[3,4]. Here we use a new technique of magnetic imaging to compare focal bursts of γ-band activity in amateur and professional chess players during matches. We find that this activity is most evident in the medial temporal lobe in amateur players, which is consistent with the interpretation that their mental acuity is focused on analysing unusual new moves during the game. In contrast, highly skilled chess grandmasters have more γ-bursts in the frontal and parietal cortices, indicating that they are retrieving chunks from expert memory by recruiting circuits outside the medial temporal lobe.

The 'chunking' theory of chess playing[5] suggests that expert memory is based on a large database of chunks in long-term memory. A chess grandmaster studies and practises for at least 10 years to learn more than 100,000 patterns (memory chunks). Consequently, grandmasters can 'recognize' the key elements in a problem situation much more rapidly than amateur players. Experts differ not only in the extent of their knowledge, but also in its organization. High-level processing elements, such as structuring knowledge and planning, assist in accessing the respective chunks[6].

We tested 20 male players (aged 42 ± 14 years), each with more than 10 years of tournament and training practice. Ten professional grandmasters scored between 2,400 and 2,600 on Elo's chess-skill rating scale[5]; amateur players ranked 1,700 and above. Magnetoencephalographic recordings were made while subjects played against a computer and were scanned in the 5 seconds after each move by the computer program for focal γ-bursts (20–40 Hz; Fig. 1a). Examination of single slices indicates pronounced activity in the region of the perirhinal and entorhinal cortex, hippocampus and related structures in amateur players, but not in grandmasters.

There was a strong negative correlation ($r = -0.84$) between the relative share of dipoles in these structures and Elo chess skill (Fig. 1b). The correlation was also significant when only the 12 players who had lost in the game were included in the analysis (draws and wins were achieved only by grandmasters). There was no relationship between the length or complexity of the game and the Elo score.

These marked differences in the distribution of focal brain activity during chess playing point to differences in the mechanisms of brain processing and functional brain organization between grandmasters and amateurs. Lesions in structures that are activated in amateur players impair recent memory while leaving remote memory intact[2]. Grandmasters seem to rely more on remote than on recent memory.

High-level processing elements[6] may also have contributed to the observed differences. The chunking theory of memory states that the number and nature of chunks that chess experts can hold in long-term memory can be used to predict chess performance[6]. Our results indicate that the activation of expert memory chunks produces focal γ-band activity in the neocortex, whereas amateur players primarily encode and analyse new information, tasks that activate the medial temporal lobe and the hippocampus. It is possible that these structures play only a transitional role during the establishment of expert memory in the neocortex.

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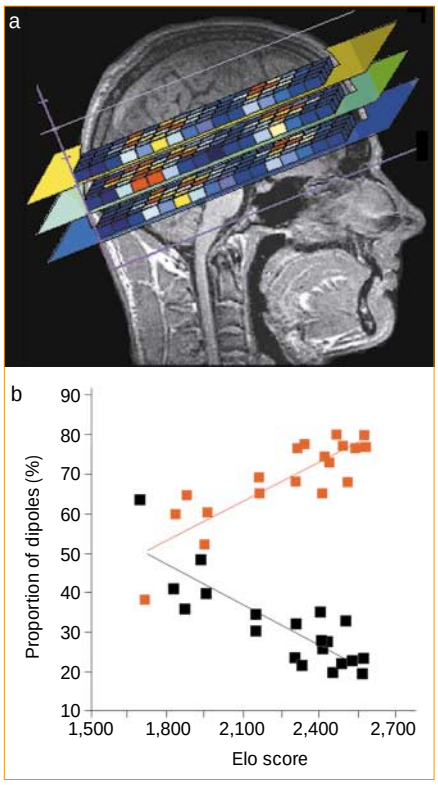

Figure 1 Focal γ-band activity in the brains of chess players. **a,** Determination of equivalent-current dipole density for inferior 'slices' through the hippocampus and medial temporal areas in an amateur player's brain. Increasing dipole density is indicated by a colour scale from dark to light blue, to yellow, to red. **b,** Relationship between chess-playing skill (Elo rating scale) and the relative share of dipoles located in medial temporal lobe structures (black) and in the frontal and parietal cortices (red). Amateur players show more focal γ-bursts in the medial temporal lobe than grandmasters, who show more activity in the frontal and parietal cortices.

Anabolism

Low mechanical signals strengthen long bones

Although the skeleton's adaptability to load-bearing has been recognized for over a century[1], the specific mechanical components responsible for strengthening it have not been identified. Here we show that after mechanically stimulating the hindlimbs of adult sheep on a daily basis for a year with 20-minute bursts of very-low-magnitude, high-frequency vibration, the density of the spongy (trabecular) bone in the proximal femur is significantly increased (by 34.2%) compared to controls. As the strain levels generated by this treatment are three orders of magnitude below those that damage bone tissue, this anabolic, non-invasive stimulus may have potential for treating skeletal conditions such as osteoporosis.

A common perception of bone adaptation is that mechanical signals must be large to influence morphology[2]. The peak signals that result from natural vigorous activity cause microdamage to bone material and require repair[3]. For example, peak strains of 2,000–3,000 microstrain are typically induced during locomotion[4], stimulating osteoclasts and osteoblasts to remove and then replace damaged tissue[5].

We have departed from this repair-mediated hypothesis by proposing that extremely small strains (for example, those that arise from muscle contraction during less vigorous but more frequent activities such as maintaining posture) are strong determinants of bone morphology[6].

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We examined the regulatory potential of extremely small (0.3*g*, where *g* is the Earth's gravitational field), high-frequency (30 Hz) mechanical accelerations by subjecting the hindlimbs of adult (6–8-year-old) female sheep (Warhill, intact ewes) to a ground-based vertical oscillation[7] for 20 min per day for 5 days a week. When the animals were not being treated, they joined the controls to roam freely over a pasture area. We used strain gauges attached to the animals' tibia bone to calibrate the device: these showed that the peak-to-peak amplitude of the strain generated was about 5 microstrain, which is 0.1% of the strain magnitude that is known to cause yield-failure in bone[8].

After 1 year of this mechanical-stimulation regime, the density of trabecular bone in the proximal femur, as quantified by computer tomography, was 34.2% greater in experimental sheep than in controls ($P < 0.01$; Table 1). This strong anabolic response was substantiated by undecalcified bone histology of the same region, which revealed a 32% increase in trabecular bone volume, a 45% increase in trabecular mesh number (Fig. 1) and a 36% reduction in mesh spacing, indicating an increase in the mean width of each trabecular element and the addition of new trabeculae.

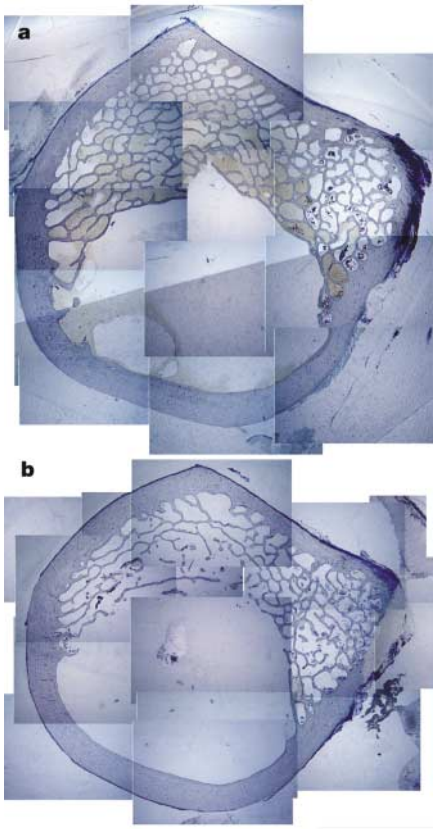

Figure 1 Montages of photomicrographs of the proximal sheep femur used for static histomorphometric evaluation after 1 year of exposure (20 min per day) to a 0.3*g*, 30-Hz mechanical stimulus. **a, b,** There is 32% more trabecular bone in the proximal femur of experimental animals (**a**) compared with age-matched controls (**b**) ($P < 0.04$).

Table 1 Proximal-femur parameters of control and stimulated sheep

	Control	Experimental	Difference	P
Animal mass (kg)	71.1 ± 7.1	70.3 ± 9.4	−1.1%	n.s.
Total density (gm cm^{-3})	466 ± 60	496 ± 53	+6.5%	< 0.1
Trabecular density (gm cm^{-3})	169 ± 37	227 ± 56	+34.2%	< 0.01
Bone volume/total volume (%)	15.2 ± 4.1	20.1 ± 4.8	+32%	< 0.04
Trabecular spacing (μm)	1,170 ± 124	756 ± 97	−36%	< 0.02
Trabecular number (trabeculae mm^{-2})	0.82 ± 0.16	1.19 ± 0.18	+45%	< 0.01
Bone-formation rate (μm^2 mm^{-1})	8.4 ± 12.7	17.9 ± 16.3	+113%	< 0.2
Mineralizing surface (%)	2.6 ± 0.16	6.34 ± 5.14	+144%	< 0.1

Animal mass and envelope-specific bone density (determined by quantitative computer tomography) of the proximal femur after 12 months of low-level mechanical stimulation. Also shown are indices of static and dynamic histomorphometry of the proximal femur. Although 'whole-bone' parameters of the proximal femur show only a limited tendency to be influenced by mechanical stimuli ($P = 0.1$), the increase in treated animals compared with controls is over 30% for trabecular bone alone ($P < 0.01$). One control was lost over the course of study for reasons not associated with the protocol. Nine animals were evaluated in the experimental group, with eight controls. All evaluations were made without knowledge of whether the animals were control or experimental.

We found that this low-level mechanical stimulation increased the rate of bone formation 2.1-fold ($P < 0.2$) and the mineralizing surface 2.4-fold ($P < 0.1$). This anabolic effect was highly specific to cancellous (porous) bone, as there was no significant histomorphometric change in any of the cortical bone parameters. We detected no difference in any bone index in the radius of either control or experimental animals (for example, mineral density was 0.6% less than controls; not statistically significant), indicating that the anabolic effect was specific to the region of the skeleton that was subjected to the mechanical signal.

Mechanical strain in the skeleton is a product of functional load-bearing — as seen, for example, in the mandible of the macaque[9] and the tibia of the alligator[10]. In addition to the large-amplitude strains typically associated with functional activity, a strain signal, much less than 5 microstrain in amplitude, arises through muscular activity in the frequency band 10–50 Hz (ref. 11). Generation of this small-amplitude, high-frequency muscle 'vibration' persists through even such passive activities as standing.

Skeletal morphology may therefore be sculpted by omnipresent, low-level muscle activity as well as by the peak impacts inherent in load-bearing. In terms of clinical relevance, the strong bone-generating capacity of these small signals suggests that biomechanical intervention might help to strengthen bone in osteoporosis sufferers without the side-effects associated with pharmacological treatment. In addition to being non-invasive and inducing a therapeutic response from the bone tissue itself, low-intensity mechanical signals incorporate all aspects of a complex remodelling cycle[12] and ultimately stimulate formation of lamellar bone[13] to improve bone quantity and quality.

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Vision

Realignment of cones after cataract removal

Through unique observations of an adult case of bilateral congenital cataract removal[1], we have found evidence that retinal photoreceptors will swiftly realign towards the brightest regions in the pupils of the eye. Cones may be phototropic, actively orientating themselves towards light like sunflowers in a field.

For 40 years, one of us (P.D.) lived with an unusual pupil configuration caused by congenital bilateral cataracts, first diagnosed at three years of age. The cataracts were managed by using a twice-weekly application of atropine, which dilated P.D.'s pupils to produce roughly annular (ring-shaped), clear regions around the dense nuclear cataracts. Because his optics were so poor (he was far-sighted, astigmatic and incapable of accommodation), P.D. squinted continuously as an adaptation, particularly for close work. His eyelids thus horizontally cropped each annulus at the top and bottom, leaving two clear entry points for light

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Contraceptive efficacy of priming and boosting doses of controlled-release PZP in wild horses

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Abstract

Context. At some sites, wild horse populations have been effectively and non-invasively regulated using remote darting with porcine zona pellucida (PZP) immunocontraceptive vaccines. However, this model has not been widely applied to wild horse herds in western USA, many of which are difficult to access because they roam large areas and are wary of people. Single-treatment, multi-year contraceptive vaccines would significantly broaden the scope for successful contraceptive management.

Aims. The aims of the present field studies were to (1) test the contraceptive effectiveness and longevity of primers incorporating PZP–adjuvant emulsions plus PZP and adjuvant in controlled-release pellets ('PZP-22'); and (2) compare the contraceptive effectiveness and duration of PZP–adjuvant emulsion-only boosters with those of PZP-22 boosters when administered by hand or remote darting to PZP-22-primed mares.

Methods. Wild horses in herd management areas in Colorado and Utah, USA, were rounded up in 2008 and in Utah again in 2012. Previously untreated females received a single hand-injection of PZP–emulsion plus controlled-release PZP pellets ('PZP-22' vaccine) and were then released. In Autumn 2010, 50 Colorado females treated in 2008 were booster-darted with either the PZP-22 vaccine or the PZP emulsion alone. In Utah, 57 previously treated females rounded up in 2012 received hand-injections of either the PZP emulsion–pellet vaccine or the PZP emulsion alone. Maternity was established through field observation of nursing and other close associations.

Key results. Effectiveness of initial controlled-release PZP treatments over 2 years was low relative to previous trials. However, boosters delivered by dart or by hand to PZP-22-primed mares yielded high levels of contraceptive effectiveness for 3 years, with no consistent difference between simple PZP-emulsion boosters and boosters incorporating controlled-release pellets.

Conclusions. Priming mares with PZP-22 extends the efficacy of a subsequent PZP booster to at least 3 consecutive years.

Implications. Regulation of wild horse populations may be achieved with existing contraceptive agents by developing models and management plans that account for the increased longevity of repeated contraceptive treatments.

Additional keywords: feral horse, immunocontraception, porcine zona pellucida, remote delivery.

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Introduction

The 1971 *Wild Free Roaming Horse and Burro Act* (P.L. 92-195) assigned primary responsibility for managing wild horses and burros occupying public lands to the US Bureau of Land Management (BLM), with secondary responsibilities to the US Forest Service (USFS) for managing the species on USFS lands. In 1978, prompted by growth of the herds subsequent to passage of the *Act*, Congress authorised the BLM and USFS to gather, remove and make available for adoption by the public wild horses and burros deemed excess relative to appropriate management levels set by public land use planning processes (*Public Lands Improvement Act*, P.L. 95-514).

Through the fiscal year (FY) 2015, the BLM had adopted out more than 239 000 wild horses and burros to the public (BLM 2016*a*). However, the rate at which horses have been adopted has failed to keep up with the number of horses removed from the range, especially since FY2001, resulting in increases in wild horse numbers in captive-holding facilities and on the range. As of March 2015, more than 45 000 formerly free-roaming wild horses were being maintained in short- and long-term holding facilities at a budgeted cost of US$62 million for FY 2016 (BLM 2016*b*). In addition, the 67 027 wild horses and burros estimated to be living on the range as of 1 March 2016 significantly exceeds BLM estimates of a system-wide

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appropriate management level (AML, the number of horses and burros defined by the BLM as the number that can thrive in balance with other public-land resources and uses) of 26 715 (BLM 2016*b*). While BLM estimates of AML can be challenged, it is clear that the system is unsustainable as currently being operated.

Both BLM documents and outside reviews have repeatedly identified on-the-range fertility control as a plausible option for reducing the rate of growth of wild-horse herds, thereby reducing pressure on the system of removal and adoption as well as the range itself. In response, BLM has been funding research into practical, safe, and effective wild-horse contraception since the 1970s (Turner and Kirkpatrick 1982; Plotka *et al*. 1988, 1992; Turner *et al*. 1997, 2007; National Research Council 2013). Several potentially suitable immunocontraceptive agents have emerged from BLM-funded research, including porcine zona pellucida (PZP) and GnRH vaccines (Killian *et al*. 2008; National Research Council 2013). Porcine zona pellucida has the deepest research history, having been previously tested and applied for several years at Assateague Island National Seashore, MD, and elsewhere (Kirkpatrick *et al*. 1990; Kirkpatrick and Turner 2002, 2003, 2008; Ransom *et al*. 2011; National Research Council 2013).

In a minority of BLM herd management areas (HMAs), wild horses are approachable enough so that contraceptive vaccines can be delivered directly by dart (Ransom *et al*. 2011; Naugle and Grams 2013). However, in others, horses are too wary to approach closely enough for darting, and are thinly dispersed over wide areas (Naugle and Grams 2013). In these areas, either gathers or trapping must be used to make horses accessible for treatment. Where accessing horses for treatment is challenging, the availability of a multi-year, single-treatment contraceptive vaccine would be enormously helpful in effectively and substantially reducing foaling rates and, consequently, rates of population growth.

Evidence for multi-year effectiveness of contraceptive vaccines is mixed. Killian *et al*. (2008) showed that single doses of SpayVac® (Immunovaccine Technologies, Halifax, NS, Canada) and GonaCon™ (USDA/NWRC, Fort Collins, CO, USA) significantly reduced fertility in a small sample of captive horses for up to 4 years. Gray *et al*. (2010) showed that GonaCon-B™ and a formulation of PZP with Adjuvac™ (USDA/NWRC, Fort Collins, CO, USA) reduced fertility in free-roaming Nevada mares for 3 years, although efficacy was modest for both vaccines (reducing fertility from control levels by 25–59%). Turner *et al*. (2001) showed that a single dose of native PZP plus PZP prepared in controlled-release microspheres produced a 1-year reduction in fertility comparable to that of two doses of native PZP administered 3 weeks apart. However, the microspheres tended to clump in the syringe and proved difficult to handle under field conditions (Turner *et al*. 2002). In a subsequent field trial at Clan Alpine HMA, NV, a single injection of 65 µg PZP emulsified in Freund's complete adjuvant (FCA) plus 410 µg PZP and 875 µg QA-21 adjuvant in controlled-release pellets reduced fertility in treated mares by 91%, 73%, and 38% from control levels in Years 1–3 after treatment (Turner *et al*. 2007).

On the basis of the results of the Turner *et al*. (2007) trial and supporting data on captive and non-captive mares (Turner *et al*. 2002; Liu *et al*. 2005), BLM initiated wider use of the PZP emulsion plus PZP/QA-21 pellet vaccine (dubbed 'PZP-22,' based on antibody titer concentrations displayed in Liu *et al*. 2005; National Research Council 2013), treating between 500 and 1000 mares annually between 2007 and 2013. However, the Clan Alpine study was not replicated, and information on the efficacy of BLM administrations at both the individual and population levels was anecdotal. Also lacking are any studies of the effectiveness and longevity of PZP boosters administered to mares primed with PZP-22.

As a part of a larger study to further evaluate the individual- and population-level effectiveness of PZP-22, and to establish the effectiveness and longevity of PZP boosters administered to mares that had previously been primed with PZP-22, we conducted field studies in two BLM herd management areas.

Materials and methods

Study areas

Sand Wash Basin Herd Management Area, Colorado

The Sand Wash Basin (SWB) HMA comprises ~638 km^2 located ~72 km west of Craig, CO, USA. Elevation ranges from 1900 to 2660 m. SWB receives 18–30 cm of annual precipitation, and the climate is typical of the cold deserts of the Rocky Mountain Region, with warm summers and very cold winters. Vegetation types within the HMA include sagebrush (*Artemesia spp*), bunchgrasses, saltbush (*Atriplex* spp.), and pinyon–juniper woodlands. Horses, livestock and wildlife at SWB rely for water on a combination of developed wells, undeveloped springs and seeps and artificial reservoirs.

According to BLM aerial-survey estimates, there were ~463 wild horses on the SWB HMA and 50 wild horses off HMA. Our initial ground inventory after the BLM gather in October 2008 indicated the presence of 194 horses (K. Grams and A. Rutberg, unpubl. data). The appropriate management level at SWB is 163–362 (BLM 2017).

Cedar Mountains Herd Management Area

The Cedar Mountains (CM) HMA comprises 727 km^2 and is located ~80 km west of Salt Lake City, UT, USA. Approximately 425 km^2 within the HMA is part of a congressionally designated candidate wilderness area; vehicles and permanent structures are not permitted within the designated wilderness area. Elevations vary from 1400 to 2460 m. Annual rainfall in the lower elevations averages 20–25 cm, and 25–38 cm of rainfall is received in the upper elevations. Desert shrubs and bunchgrasses are common in the lowlands, with sagebrush and juniper woodlands dominating higher elevations. Water is seasonally scarce, and is concentrated in dry summer months at cattle troughs and a few natural springs.

Our ground-based population inventory indicated that there were at least 608 horses on CM when the study began in 2008, with additional horses on adjacent military lands that had not yet been documented. Initial estimates by BLM based on their aerial survey indicated that 191 remained after the December 2008 BLM gather, although subsequent ground-based population inventories indicated that there were at least 265 horses present post-gather. The appropriate management level for CM is set by the BLM at 190–390 (BLM 2017).

Horse identification

Beginning in 2008, information was documented in the field and then entered into a database. This working document included sex, colour, face and leg markings, unique descriptions, mane and tail description, year of birth and the subpopulation in which the horses lived. Each horse was assigned an ID number to establish individual identity. Horses, including bachelors, were grouped by band in the areas in which they were most recently found. The horse list was updated weekly to reflect any changes or additions and was used in the field to help identify horses. Horses were individually recorded in the Wild Horse Identification Management System (WHIMS, US Geological Survey, Fort Collins, CO, USA). Information from the horse list was placed into this database along with individual pictures of each horse. A photo catalogue allowed for viewing each horse, with the pertinent information to that horse.

Horses that were identified during the study but were not observed within the HMA were excluded from the analysis.

PZP treatments

Wild-horse mares were gathered by the BLM and restrained in squeeze chutes for PZP treatments at Sand Wash Basin on 23 October 2008, and at Cedar Mountains on 17 December 2008 and 25–28 February 2012. Porcine zona pellucida treatments were also administered remotely by dart at SWB from 11 September to 17 November 2010. Thus, boosters were administered at SWB 2 years after the initial treatment, and at CM 3.2 years after the initial treatment.

Hand-injections

In October 2008 and December 2008 respectively, 62 mares (2+ years old) at SWB and 70 mares (2+ years old) at CM were gathered and treated with hand-injections of PZP-22, consisting of one priming injection of 100 µg PZP in 0.5-mL phosphate buffer solution (PBS) emulsified in 0.5 mL of modified FCA (mFCA, Calbiochem, LaJolla, CA, USA) (following Kirkpatrick *et al*. 1990), and a second injection of three heat-extruded lactide–glycolide polymer pellets (polymers from Lactel/Durect, Pelham, AL, USA) containing a total of 450 µg of PZP and 900 µg of QA-21 (Antigenics, Lexington, MA, USA), a saponin adjuvant, engineered to release at 1, 3 and 12 months (Turner *et al*. 2008). The 1- and 3-month pellets each contained 100 µg of PZP and 200 µg QA-21, and the 12-month pellet contained 250 µg PZP and 500 µg of QA-21. Both injections were delivered intra-muscularly into the hip, the PZP–mFCA emulsion with a hand-held syringe fitted with an 18-gauge needle, and the pellets with a trocar fitted with a 14-gauge needle. In the squeeze chute, the horses were also freeze-branded on the hip with a marking that identified them as PZP-treated mares. In February 2012, 85 previously untreated mares at CM were similarly treated and released. Of these mares, 23 were 2 years old, and 62 were ≥3 years old.

Booster trials

To examine the relative effectiveness of the PZP-22 booster when hand-injected, 58 mares that had been treated at CM in 2008 and recaptured for treatment in February 2012 received a hand-injection of either PZP-22 (with Freund's incomplete adjuvant (FIA, Calbiochem, LaJolla, CA, USA) substituted for mFCA, 'PZP-22–FIA'; $n = 39$) or the PZP–FIA booster alone ($n = 19$).

A parallel test of the PZP-22 booster when delivered remotely, by dart, was conducted at SWB. From 11 September to 17 November 2010, 34 mares received PZP-22–FIA delivered in a Pneu-dart® (Williamsport, PA, USA) dart designed with a rod within the 1.5-inch needle, to simultaneously inject both the emulsion and the dart (see Walter *et al*. 2012 for a similar device). Another 16 mares received only the PZP–FIA emulsion by dart (Kirkpatrick *et al*. 1990). Two dart projectors were utilised for short and longer distances. The Dan-Inject CO2 pistol (Børkop. Denmark) projected darts at a distance of 10–18 m. The Pneu-Dart X-Caliber Gauged CO2 projector rifle projected darts at a distance of 17–45 m. Depending on the tractability of the animal, the Dan-Inject was primarily used for close range.

Observations

Observational data were collected beginning in 2008, focusing on the months from June to October in SWB and April through to December at CM. Observations were conducted from the ground, usually by one but sometimes by two observers. Depending on distance, observations were made through binoculars or 20–60 × 80 spotting scope.

For each band encountered, observers identified all known horses present, described any new horses in the band that had not previously been described, tabulated the age–sex composition of the group, and, using maps and field notes, noted the location of the band. Mares were matched with foals within 2 weeks of birth, as indicated by persistent proximity and nursing. Within limits of visibility and accessibility, the observer also estimated and recorded body condition scores on a 1–5 scale (after Rudman and Keiper 1991) and noted any apparent injuries, lameness, abscesses and other injection-site reactions, or other signs of ill health, for each band member, as well as any reproductive activity or mortalities that occurred.

Data analysis

Foaling data inferred from observations of foal–mare associations and behaviour were compiled by mare age (when age data were available) and PZP treatment status. The relationship between age and foaling rates was examined using the linear regression function of IBM SPSS®-22, using study site, age and age squared as independent variables (modified from Ransom *et al*. 2011). Foaling rates were compared among treatment groups and between treatment groups and untreated mares by year after treatment, using Pearson's chi-square tests or, if sample sizes were too small, by two-tailed Fisher's exact tests. Sham controls were not used, because Turner *et al*. (1997) had previously demonstrated that fertility rates among sham controls and untreated controls were virtually identical.

The proportions of untreated and pretreatment mares of known age that foaled in a given year were calculated for each site. Foaling data comprised 170 foals born to mares of known age (2–17 years old) in 311 mare-foaling opportunities at SWB between 2008 and 2014, and 193 foals born to mares of known age (2–24 years old) in 376 mare-foaling opportunities at CM

between 2008 and 2015. Because fewer records existed for older mares, ages were consolidated into groups of at least 10 mares in each age category, assigning the median age to each group. Sample size per age group ranged from 12 to 154.

Because of uniformly low foaling rates among untreated 2-year-old females (see below), all comparisons between treatment groups and untreated controls were performed using mares 3 years old and older at the time of foaling. The criterion for rejecting null hypotheses was set at $\alpha = 0.05$.

Results

Effects of age on likelihood of foaling

In both study sites, 2-year-old females were less likely to foal, then older females (Fig. 1). Site, as an independent variable, was not a significant predictor of foaling rate ($P = 0.824$), and was eliminated from the analysis. Because the form of the relationship between age and fertility appeared to differ at both sites, each site was analysed separately. At CM, the probability of foaling followed a fairly strong parabolic relationship with age ($R^2 = 0.817$, $F_{2,5} = 11.14$, $P = 0.014$; Fig. 1*a*). However, at SWB, the age–foaling relationship was flat for mares older than 2 years old relative to CM, and the parabolic relationship was not significant ($R^2 = 0.580$, $F_{2,5} = 3.46$, $P = 0.114$; Fig. 1*b*).

Initial treatments with PZP emulsion and controlled-release pellets

Sample sizes for observation of foaling in treatment groups showed only slight attrition during the study, whereas samples of untreated controls observed for foaling generally grew from year to year as new individuals in the population were identified or were recruited as foals born into the herd during the study aged into the 3+ years age group (Table 1).

In all three priming treatments (CM 2008 and 2012 and SWB in 2008), foaling rates were statistically indistinguishable between treated and untreated mares during the foaling period immediately after treatment, defined as Year 0 (reflecting pretreatment pregnancy rates; Fig. 2). Significantly lower foaling rates were found among mares ≥3 years old in treated mares in Year 1 at CM in the 2008 ($\chi_1^2 = 29.04$, $P < 0.0001$; Fig. 2*a*) and 2012 ($\chi_1^2 = 24.99$, $P < 0.0001$; Fig. 2*b*) treatments, but not in the 2008 SWB treatments ($\chi_1^2 = 3.4$, $P = 0.065$; Fig. 2*c*), relative to untreated controls. In Year 2, foaling rates in treated mares were significantly lower than those in untreated mares for both the 2008 and 2012 CM treatments ($\chi_1^2 = 5.94$, $P = 0.015$; $\chi_1^2 = 5.57$, $P = 0.018$) and the SWB treatments ($\chi_1^2 = 6.2$, $P = 0.013$). Summed over the two post-treatment years, foaling

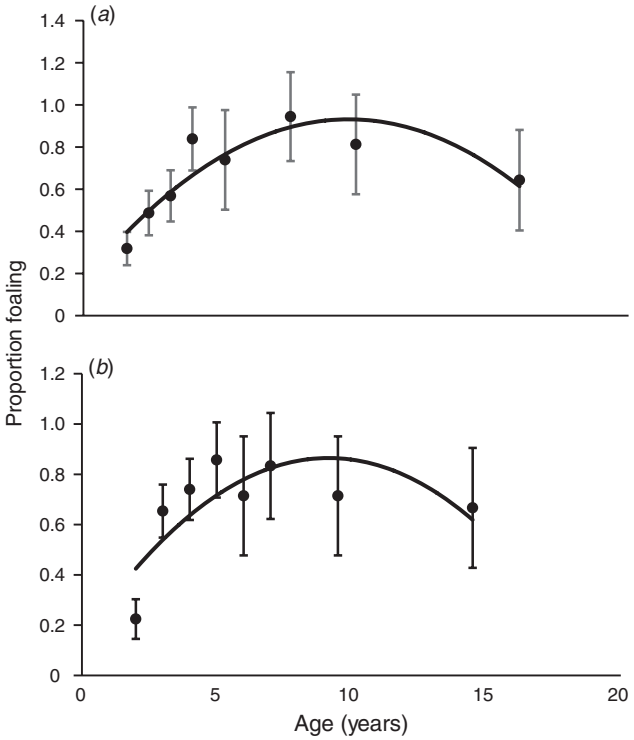

Fig. 1. Proportion of untreated and pretreated mares foaling by age. $N > 10$ for each age category. Error bars represent 95% CI. (*a*) Cedar Mountains, UT, USA, 2008–15 ($Y = -0.005X^2 + 0.126X + 0.165$, $R^2 = 0.817$, $F = 11.14$, d.f. = 2,5, $P = 0.014$). (*b*) Sand Wash Basin, CO, USA, 2008–14 ($Y = -0.009X^2 + 0.157X + 0.144$, $R^2 = 0.580$, $F = 3.46$, d.f. = 2,5, $P = 0.114$).

Table 1. Number of mares 3+ years old for which foaling data were obtained in each treatment group at Cedar Mountains (CM) Herd Management Area, Utah, USA, and Sand Wash Basin (SWB) Herd Management Area, Colorado, USA

At CM, 39 mares received PZP-22–FIA boosters and 19 received PZP–FIA boosters. At SWB, 34 mares received PZP-22–FIA boosters, and 16 received PZP–FIA boosters

Year	Cedar Mountain HMA			Sand Wash Basin HMA		
	PZP-22 primer	PZP boosters (total)	Untreated control	PZP-22 primer	PZP booster (total)	Untreated control
2008	70 hand-treated	–	–	62 hand-treated	–	–
2009	69	–	61	62	–	15
2010	69	–	63	61	50 dart-treated	18
2011	68	–	73	61	50	22
2012	85[A] hand-treated	58 hand-treated	38	–	50	52
2013	83	57	44	–	49	68
2014	80	56	56	–	28[B]	107
2015	–	55	94	–	–	–

[A]Twenty-three (23) mares that were 2 years old at the time of treatment were not included in Year 0 (2012) foaling-rate calculations.

[B]Twenty-one (21) mares were lost to the study because they received additional PZP treatments in 2013. Of the 28 remaining in the study, 17 had received PZP-22 boosters and 11 had received PZP emulsion only.

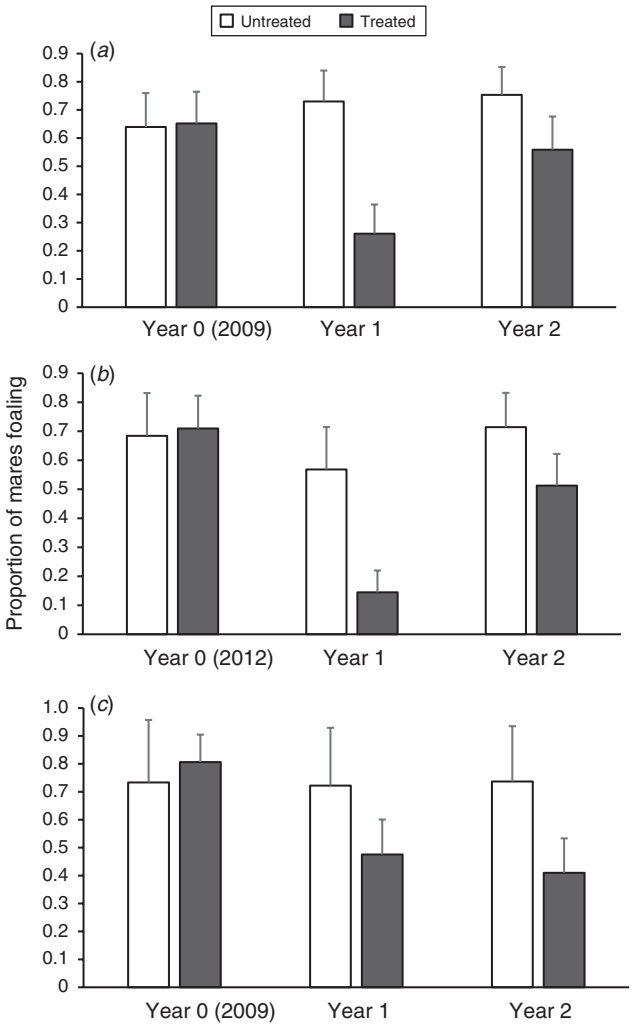

Fig. 2. Foaling rates in mares ≥3 years old. Error bars represent 95% CI. (*a*) Cedar Mountains herd-management area (HMA), UT, USA, following December 2008 administration of porcine zona pellucida (PZP)-22 primers by hand. Year 0 = 2009. (*b*) Cedar Mountains HMA, UT, USA, following February 2012 administration of PZP-22 primers by hand. Year 0 = 2012. Twenty-three PZP-22-treated females that were 2 years old in 2012 were excluded from the Year 0 analysis. (*c*) Sand Wash Basin HMA, CO, USA, following October 2008 administration of PZP-22 primers by hand. Year 0 = 2009.

rates among treated mares were reduced by 39% at SWB in 2008, by 45% at CM in 2008, and by 50% at CM in 2012.

No injection-site reactions were observed in mares after priming via hand-injection.

PZP booster treatments

At both SWB and CM, PZP boosters were associated with significant reductions in foaling rates among mares ≥3 years old every year for 3 years after treatment (Fig. 3).

At SWB, foaling rates in mares boosted with PZP by remote darting averaged 25.2% over 3 years (range 22.0–32.1%) versus 74.9% (65.4–79.4%) in untreated mares (Fig. 3*b*), i.e. foaling rates in booster-treated mares were reduced by 66% relative to those of untreated mares. In each year, foaling rates were significantly lower in treated mares than in untreated mares (Year 1, $\chi_1^2 = 19.46$, $P < 0.0001$; Year 2, $\chi_1^2 = 34.94$, $P < 0.0001$; Year 3, $\chi_1^2 = 20.00$, $P < 0.0001$). Mares darted with PZP-22–FIA showed lower foaling rates (averaging 17.8% across 3 years) than did mares darted with PZP–FIA emulsions alone (averaging 39.5% across 3 years), but there were no significant differences in foaling rates between the two treatment groups in any year.

Injection-site reactions were seen in 32 of 47 darted SWB horses (68.1%) observed in the weeks following darting. Of these reactions, 5–8 were draining abscesses (uncertainty owing to observation quality), of which at least five had resolved by the last time the horse was observed in the 2010 field season (15–52 days after darting). The remainder were swellings and granulomas averaging 2.75 cm (s.d. = 0.87 cm) in diameter. The two treatment types did not differ from one another in either the frequency of swellings and granulomas or in the frequency of draining abscesses.

At CM, hand-injected PZP boosters were also highly effective, with foaling rates of treated mares across 3 years averaging 14.9% (ranging from 7.0% to 20.0%) versus 67.5% (range 56.8–71.4%) for untreated females ≥3 years old, i.e. foaling rates in booster-treated mares were reduced by 78% relative to those of untreated mares (Fig. 3*a*). Treated mares had significantly lower foaling rates than did untreated controls in all years (Year 1, $\chi_1^2 = 30.09$, $P < 0.0001$; Year 2, $\chi_1^2 = 30.38$, $P < 0.0001$; Year 3, $\chi_1^2 = 35.03$, $P < 0.0001$). At CM, no consistent differences in efficacy emerged between the two treatment groups, and there was no significant difference in foaling rates between the PZP-22–FIA and the PZP–FIA-only group in any treatment year. No injection-site reactions were observed in hand-boosted mares.

Discussion

Mares hand-injected with PZP-22 primers generally demonstrated lower foaling rates than did untreated control mares. However, the effectiveness and longevity of PZP-22 were much lower at both CM and SWB than was reported in Turner *et al*. (2007) for wild horses in Clan Alpine. Several differences in manufacturing and protocol may account entirely or in part for the differences.

(1) *Treatment month of administration.* The 1-, 3- and 12-month pellet-release times were engineered to optimise effectiveness of vaccines delivered December to February. The highest 2-year efficacy of primers in the present study occurred when vaccinations were delivered in February (CM 2012), followed by vaccinations delivered in December (CM 2008), with those delivered in October (SWB 2008) being least effective. However, even with optimal administration timing, the efficacy levels observed in these trials following Year 1 did not match those observed at Clan Alpine, suggesting that timing alone is not a sufficient explanation for the differences.

(2) *Differences in timing of antigen release*. Subsequent *in vitro* testing of the heat-extruded controlled-release pellets of batches used in this trial was undertaken to ascertain whether changes in pellet-release characteristics originally described in Turner *et al*. (2007) and Turner *et al*. (2008)

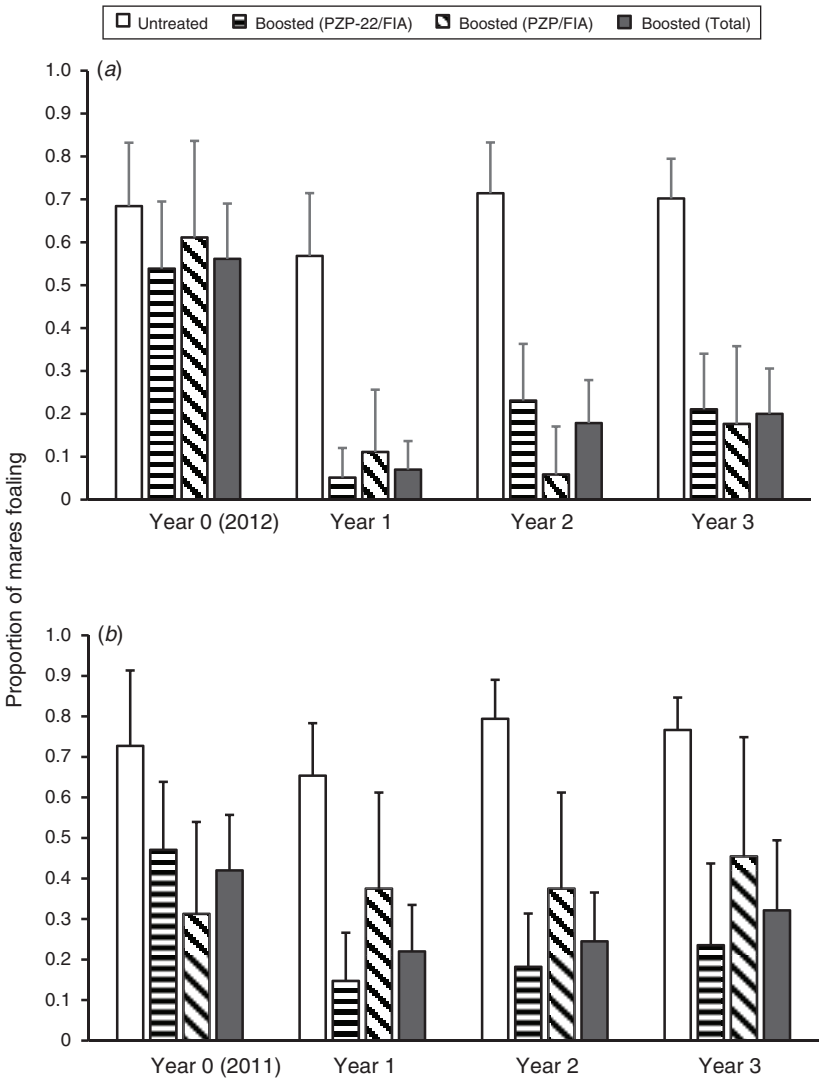

Fig. 3. Foaling rates in mares ≥3 years old. Error bars represent 95% CI. (*a*) Cedar Mountains herd-management area (HMA), UT, USA, following February 2012 administration of PZP-22–FIA or PZP–/FIA boosters by hand. Year 0 = 2012. (*b*) Sand Wash Basin HMA, CO, USA, following Autumn 2010 administration of PZP-22–FIA or PZP–FIA boosters by dart. Year 0 = 2011. Note that the Year 0 (2011) foaling rates for treated mares are the same as those for Year 2 in Fig. 2*c*.

contributed to suboptimal PZP-22 performance. Comparison of release characteristics of pellets produced before and after 2007 revealed that post-2007 pellets of the 12-month delay type released prematurely and more gradually than was expected (J. W. Turner, unpubl. data). Possible contribution of polymer-batch and pellet-manufacture variations to this circumstance are under investigation.

(3) *Method of injection.* PZP-22 at Clan Alpine was delivered by jab stick, and both the emulsion and pellet components were delivered together at the same injection site, whereas emulsion and pellets were delivered by two separate injections in all hand-injection protocols of PZP-22 followed in the present study. Although being an implausible explanation for the differences, this hypothesis also receives weak support from the improved PZP-22 efficacy relative to the PZP–FIA emulsion associated with dart delivery in booster trials (described below).

(4) *Difference in adjuvant.* PZP vaccine prepared before 2005 utilised FCA, and, thereafter, mFCA was used. Similar anti-PZP titer responses to primer-dose PZP treatment with these two adjuvants have been reported (Lyda *et al*. 2005). However, contraceptive efficacy was not determined, and it is possible that the antibody type and quality generated by these two adjuvants differed sufficiently to influence the immune memory generated by pellet-based boosters (M. Wooten, pers. comm.). In light of the limited future for FCA (related to tissue reactions and false-positive TB tests) and the promise of alternative multi-target adjuvants (e.g. Lu *et al*. 2015), comparison of mFCA with such novel adjuvants to address the above issue may be warranted.

Although the initial PZP-22 treatments tested in the present study showed disappointing effectiveness, a single PZP booster (either the PZP–FIA emulsion or PZP-22–FIA) effectively reduced fertility across three consecutive years. Whether delivered by dart, as at SWB in 2010, or by hand, as at CM in 2012, PZP boosters reduced foaling rates in treated mares by roughly 65–72% relative to untreated control mares over the 3 years. We do not know, because the studies had to be ended 3 years after boosting, whether some contraceptive effectiveness might persist into a 4th year.

Most previous research on PZP in western wild horses has presented data only on the effectiveness and longevity of the initial treatment (Turner *et al.* 1997, 2001, 2007). Working on wild horses at Little Book Cliffs Wild Horse Range, CO, USA, and the Pryor Mountain Wild Horse Range, MT, USA, Ransom *et al*. (2011) examined the relationship between the number of consecutive years of treatment (up to 4 years) with PZP emulsion boosters and the interval between the last booster and the first foaling. This interval ranged from 7 to 18 months, with some trend towards longer intervals as the number of consecutive years of treatment increased, although the sample size for 3 and 4 consecutive years of treatment was very small and the trend was not significant. In their study of reversibility of PZP treatments of wild horses at Assateague Island National Seashore, MD, USA, Kirkpatrick and Turner (2002) showed that most mares receiving two initial injections and up to one subsequent annual booster returned to fertility within 1 year, whereas mares receiving three or four consecutive years of treatment experienced delays of 3–4 years in return to foaling. Although our measures of the longevity of vaccine effectiveness differ from those used in other studies, namely annual foaling rates of treated mares relative to those of controls, rather than interval between last booster and first foaling, as used by Ransom *et al*. (2011) and Kirkpatrick and Turner (2002), our results appear to parallel those for Assateague mares receiving three or more consecutive years of treatment. Thus, even if the initial effectiveness of PZP-22 was low relative to previous studies, the combination of a PZP-22 primer and a single PZP booster appears to have yielded effectiveness comparable to three or more years of consecutive treatments (i.e. 4+ injections) of Assateague mares initially treated with the PZP–mFCA emulsion primer.

Our study failed to show that the addition of the controlled-release pellets to the PZP emulsion increased the efficacy and longevity of the booster. At CM, addition of pellets in hand-administration did not increase the efficacy or longevity of the PZP emulsion over the 3 years of the study. Dart delivery of the booster was associated with a doubling of efficacy across all 3 years at SWB, but because the sample is small, this test warrants replication.

Conclusions

Long-term management of wild horse populations with a non-invasive reversible contraceptive will require repeated treatments over the life of the wild mare. Thus, we believe that the research emphasis placed on the effectiveness and longevity of the primer vaccination may be misguided. The data presented here, as well as in Kirkpatrick and Turner (2008), suggest that population models should be constructed that allow for increased longevity of vaccine effectiveness with successive boosters. In practice, the initial application of contraceptives to a wild horse herd may produce only a modest reduction in population foaling rates, but sustained contraceptive applications over time could result in a much greater reduction in population foaling rates, even with current technology.

At Assateague, the initial management step was to treat every mare with a single primer of PZP–FCA emulsion (Kirkpatrick and Turner 2008). Although the primer alone was only somewhat effective as a contraceptive, subsequent boosting yielded increased effectiveness, and, ultimately, a reduction in herd numbers. This lesson needs to be applied in Western herds.

Also encouraging is the demonstration of management flexibility in PZP-22 application. First, the data suggest that the interval between initial and booster treatments, i.e. 2 years at SWB, and 3.2 years at CM, does not obviously influence effectiveness or longevity of the booster over the range of times tested. Second, the SWB data indicate that PZP-22–FIA can be effectively delivered remotely, at least matching the effectiveness of the PZP–FIA emulsion. However, until technical improvements and additional tests show that the controlled-release pellets significantly increase booster efficacy, managers can employ the much less costly PZP–FIA emulsion as a booster following administration of the PZP-22 primer.

Continuing to improve vaccine longevity is essential. The more individual mares are darted, the more difficult they become to re-treat (Kirkpatrick and Turner 2008; Naugle and Grams 2013). As the controlled-released pellets are refined, re-darting can be performed less frequently, making contraceptive control more cost effective.

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Range Relationships of Feral Horses with Wild Ungulates and Cattle in Western Alberta

R.E. SALTER AND R.J. HUDSON

Abstract

Spatial and foraging relationships between feral horses and coexisting ungulates were studied in the foothills of western Alberta. Distribution patterns of horses were compared to those of cattle, elk *(Cervus elaphus)*, deer *(Odocoileus hemionus* and *O. virginianus)*, and moose *(Alces alces)* using indices of spatial and habitat use overlap. Horses were more ubiquitous in their distribution than any other ungulate and utilized sites also used by other species. Lack of behavioural interactions and dietary differences suggested ecological separation of horses from deer and moose. Although horses and elk both used dry grasslands during winter and spring, competition for forage was minimal due to the low number of elk present. During spring horses occupied some areas later preferred by cattle but range use was not excessive prior to the turn-out of cattle. There was little contemporaneous spatial overlap of horses and cattle even though their summer diets showed 66% overlap. Potential for competition appeared highest between horses and cattle but grazing relationships were complex.

The growing body of knowledge concerning the ecology of feral horses includes several comparative studies on diets of horses, domestic stock and native ungulates (Hubbard and Hansen 1976; Hansen and Clark 1977; Hansen et al. 1977; Olsen and Hansen 1977). A commonly expressed rationale is the need to determine carrying capacities for various combinations of herbivores. However, dietary overlap is important only if accompanied by spatial overlap, if shared foods are in short supply, or if one herbivore limits access of another to a preferred food source. We report here results of a study to determine the extent of spatial, habitat use and dietary overlap between feral horses and other ungulates in western Alberta, and to determine levels of range utilization.

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Study Area

The study was conducted within 200 km^2 of the outer foothills of the Rocky Mountains approximately 30 km west of Sundre, Alberta. The predominant topographical feature was a series of roughly parallel, forested ridges rising to a maximum altitude of 1,900 m ASL. Lodgepole pine *(Pinus contorta)* dominated the forest cover. Meadow and shrub vegetation on a moisture gradient from flooded to dry covered approximately 20% of the study area, primarily in the valley bottoms and on south-facing slopes. Nine forested habitats, seven meadow/shrub habitats, and one miscellaneous (disturbed) habitat were identified (Salter 1978).

The area supported over 200 resident feral horses during the study. Harvestable populations of white-tailed deer, mule deer, and moose also were present year-round, although no estimates of numbers are available. A primarily cow-calf elk population estimated at approximately 50 animals used the area as winter-spring range. Cattle were present between June 15 and October 15 at a permitted stocking rate of 1,500 AUM.

Methods

Study Design

We considered late winter to be the critical period for survival of ungulates in this area (Salter and Hudson 1979), and thus focused on this period when determining spatial relationships.

Supplementary data on year-long distribution patterns of horses and native ungulates were obtained in summer, concurrent with data on cattle distribution. Literature available at the time of the study suggested forage competition would be most likely among the grazing ungulates (horses, cattle and elk) and thus only these species were included in dietary determinations. All quantitative data were obtained within two areas (total 30 km^2) considered representative of the study area as a whole.

Occupational Patterns

Occupational patterns were delineated by inspection of 983 temporary macroplots, each 15 m in diameter and spaced at 100-m intervals along straight line transects. Transects were located to provide representative coverage of major habitat types. Approximately one half of the plots were examined during January-March 1976, providing information on winter distribution of native ungulates and horses, while the remainder were examined from July-August 1976 for data on distribution of horses and cattle. Habitat type and presence/absence of sign of each species were recorded. Horse pellet groups were aged as spring type (amorphous, bleached and insect-riddled), fresh (moist, green), or unknown during the July-August surveys.

Food Habits

Diets were determined through analysis of fecal fragments. Fifty horse fecal samples were collected each month during 1976. Seventy-five fresh elk fecal samples were obtained during January-March (25 each month). Two hundred samples of cattle feces, representing deposition from mid-June to the date of sampling, were obtained in October. Each sample consisted of approximately 10 g of material. Composited samples were analyzed by the Composition Analysis Laboratory, Colorado State University.

Range use

An extensive survey method (Anderson and Currier 1973) was used to examine zones of utilization on all meadows within the areas covered by the macroplot surveys.

During October 1976 each meadow was traversed on foot in a zig-zag fashion, with sufficient coverage to determine the gross pattern of utilization. Use zones representing five classes of herbage utilization were mapped on enlarged aerial photographs and levels of use, stubble heights, and plant community types were recorded for each zone. A 70 ha area that constituted an important winter-spring range for elk and feral horses and a summer range for cattle was re-mapped on June 1, 1977, to establish level of use by feral horses and native ungulates during spring; remaining meadow areas also were re-examined (without mapping) during late May-early June, 1977.

Statistical Analysis

Distribution of sign on the macroplots was used as a measure of habitat occupancy. All analyses were based on presence/absence of sign (as opposed to absolute abundance) to avoid the assumptions of equal visibility and equal rates of disappearance among habitats and among species. Distribution in relation to habitat availability was examined using chi-square tests. A coincidence index and an association index (Dice 1945), and a coefficient of association (C_8: Hurlbert 1969), were used to evaluate the extent of mutual use of plots by horses and each other herbivore. Coincidence indices provide a measure of how much of the combined area used by two species sustained mutual use (i.e., what proportion of sites used by either species a or b were used by both). Association indices indicate the degree of overlap (i.e., what proportion of sites used by a were used by b, and vice-versa). C8 values provide a frequency independent measure of association.

Diets of horses were compared to those of cattle and elk using Kulcyznski's similarity index (Oosting 1956), which indicated what percentage of a pair of diets was identical, and Spearman's rank order correlation coefficient (Nie et al. 1975), which indicated to what degree forages were chosen in the same order by a pair of herbivores. One or both of these procedures have been used in previous studies of the interactions of feral horses with other ungulates (Hubbard and Hansen 1976; Hansen and Clark 1977; Hansen et. al. 1977; Olsen and Hansen 1977) and thus provide a standard means of comparison.

Results

Horse-Deer Relationships

On a year-long basis horses used 87% of sites used by deer (primarily mule deer, although some white-tailed deer were present), while deer used 33% of sites sustaining use by horses (Fig. 1). However, during winter horse sign occurred on only 25% of sites used by deer. Deer sign occurred on plots in white spruce woods much more frequently than expected, and in dry grasslands and mixed shrub and dwarf birch meadows much less frequently than expected, contrasting with the pattern of winter habitat selection by horses, which avoided white spruce woods and used meadow habitats in proportion to availability (Table 1).

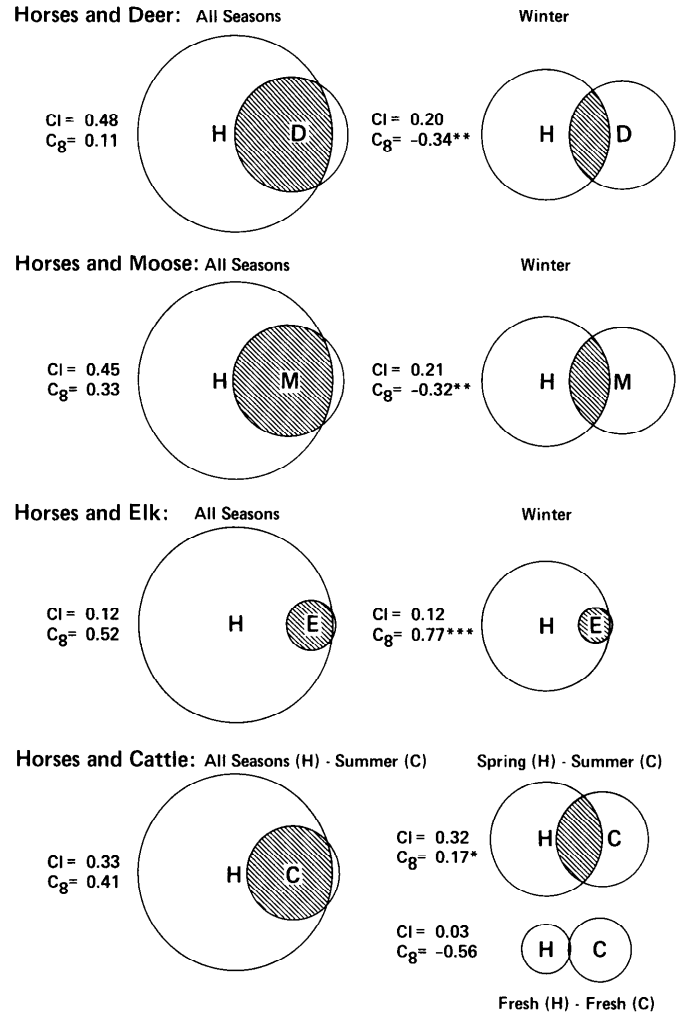

Fig. 1. *Diagramatic representation of spatial overlap of feral horses with native ungulates and cattle. Circles are drawn to scale based on frequency of occurrence of sign in macroplots examined during January-March and July-August, 1976. Proportions of plots receiving mutual use (calculated as association and coincidence (CI) indices) are represented by cross-hatched areas. Each C_8 value provides a frequency indepdent measure of association that was evaluated by chi-square probability of obtaining a greater x^2 value give as: *0.05≥p>0.01; **0.01≥p>0.005; ***p≤0.005). C_8 can vary from -1 to +1, a zero value indicating that two species are associated as expected by chance. Coincidence and association indices can vary from 0, representing no association, to +1, representing complete overlap.*

Table 1. Distribution of feral horse and native ungulate winter sign by habitat type.[1]

Habitat type	Percent of sampled plots in habitat type (n=490)[2]	Percent of plots containing sign in habitat type			
		Deer (n=126)	Moose (n=126)	Elk (n=14)	Horse (n=185)
Dwarf birch meadow	5.3	0.0⁻	7.1	14.3	6.5
Mixed shrub meadow	8.5	2.4⁻	18.2⁺	0.0	11.4
Willow thicket	2.6	0.8	6.3⁺	0.0	3.8
Dry grassland	7.5	0.8⁻	5.6	50.0⁺	9.7
Dwarf birch thicket	3.2	0.8	7.9⁺	0.0	4.3
Mesic pine woods	1.2	2.4	0.0	0.0	0.5
Dry pine woods	14.4	15.1	19.0	7.1	6.0⁻
Black spruce woods	3.0	1.6	2.4	0.0	3.8
White spruce woods	7.1	18.2⁺	4.0	0.0	2.2⁻
Poplar woods	4.2	3.2	0.8	14.3	7.6⁺
Mixed woods	12.8	15.9	12.7	14.3	14.0
Alder thicket	26.8	35.7	15.1⁻	0.0	27.0
Forested watercourse	1.8	1.6	0.0	0.0	2.7
Total χ^2		54.6	53.0	44.5	29.0

[1]Based on presence/absence of tracks or pellet groups in plots examined during January-March 1976. Habitat types contributing 10% or more to significant total x^2 values are underlined and marked ⁺ or ⁻ to signify preference or avoidance by each species. Value of χ^2 at p=0.05 is 26.3.
[2]Other habitats each comprising less than 0.5% of the total sample were sedge meadow, silver-berry thicket, alpine fir woods and disturbed sites.

Dietary overlap of horses and mule deer was not determined during this study, but reports from other areas indicate that little similarity would be expected. In three studies conducted in Colorado, annual diet similarity ranged from one to 11% and was usually less than 5% (Hubbard and Hansen 1976; Hansen and Clark 1977; Hansen et al. 1977).

Horse-Moose Relationships

On a year-long basis horses used 90% of sites used by moose, but overlap during late winter decreased to 25% (Fig. 1).

Although moose preferred mixed shrub meadows, dwarf birch thickets and willow thickets—habitats also frequented by horses (Table 1)—observations suggest separation of the two species on the basis of food selection. Moose were most commonly observed in February, when they browsed during morning in extensive shrubby meadows. Although meadow areas also were utilized for winter feeding by horses, different foods were chosen. The lack of direct evidence of browsing by horses was confirmed by fecal fragments analysis, sedges and grasses proving to be the most important dietary constituents (Table 2). An observation on February 12, 1976, of an adult moose and five horses feeding within 25 m of each other in a mixed shrub meadow—the moose on shrubs protruding above the snow and the horses pawing for sedges and grasses—suggests ecological separation of these two species during winter even in shared habitats.

Moose rely primarily on browse throughout the year, although grasses occur in the spring and summer diet (Banfield 1974). No comparative studies of horse and moose diets have been reported, but Storrar et al. (1977) found significant ecological separation of the two species in central British Columbia and believed that this was due primarily to differing food habits.

Horse-Elk Relationships

Horses used 93% of sites occupied by elk, whereas elk utilized only 6% of sites used by horses (Fig. 1), reflecting the wider dispersion and greater numbers of the latter within the study area. A similar situation occurred in winter, when there was a highly significant association between the two

Table 2. Percentages of plants in seasonal diets of feral horses, elk, and cattle.

Forage category	Late winter diets (Jan.-Mar.)		Summer diets (June-Oct.)	
	Horse	Elk	Horse	Cattle
Timber oat grass *(Danthonia intermedia)*	1	1	3	5
Tufted hair grass *(Deschampsia caespitosa)*	1	–	2	7
Hairy wild rye *(Elymus innovatus)*	28	19	26	7
Fescue *(Festuca* spp.)	15	21	23	41
Bluegrass (*Poa* spp.)	1	–	4	2
Sedge (*Carex* spp.)	35	9	34	27
Wire rush (*Juncus balticus*)	4	–	2	<1
Cinquefoil-avens (*Potentilla-Geum* spp.)	<1	2	<1	3
Lodgepole pine (*Pinus contorta*)	5	22	1	<1
Willow (*Salix* spp.)	<1	19	–	<1
Horsetail (*Equisetum* spp.)	3	<1	<1	–
Moss	3	–	<1	2

Other foods (<3%) in horse, elk and cattle diets were *Koeleria cristata* and *Schizachne purpurascens;* in horse and elk diets *Astragalus frigidus, Picea* spp., *Shepherdia canadensis-Elaeagnus commutata* and lichen; and horse and cattle diets *Agrostis scabra, Agropyron* spp., *Bromus* spp., *Calamagrostis* spp., *Stipa* spp. and *Vicia americana.*

Table 3. Percentages similarities and rank correlations of seasonal herbivore diets.

Diets	Percentage similarity	RHO values	p values[1]
Horse winter–elk winter	51	+0.38	0.011
Horse spring–cattle summer	67	+0.57	0.001
Horse summer–cattle summer	66	+0.57	0.001

[1]Two-tailed test.

species. During this time elk exhibited a preference for dry grassland habitat, as shown by a higher than expected frequency of sign (Table 1) and by observational data. Nine sightings of elk involving some 84 animals were made during February and March, all but two of these of herds feeding on south-facing grassland slopes. Horses were observed feeding on the same slopes at this time (22 sightings, January-March). Evidence of mutual use of winter habitat also was found in two small dwarf birch meadows, where it appeared that elk had followed horses into the area to feed in the same craters. Due to the paucity of elk sign in the study area as a whole, it was not possible to determine the extent of this relationship, or whether it represented commensalism or competition. Winter diets of the two species had some similarity (Tables 2, 3). Hairy wild rye *(Elymus innovatus),* fescues (*Festuca* spp.), and sedges (*Carex* spp.) were important shared constituents. Both coniferous and deciduous browse were important in elk diets but were little used by horses.

The level of dietary overlap between horses and elk was comparable to that occurring in other areas. In Wyoming, horse diets overlapped with those of elk by 52% in winter, 26% in spring, 14% in summer and 70% in fall (Olsen and Hansen 1977), while in northwestern Colorado dietary overlap of the two species averaged 42% on an annual basis (Hansen and Clark 1977).

Horse-Cattle Relationships

Over 90% of sites utilized by cattle in summer had received prior use by horses (Fig. 1). About 40% of these sites were used by horses in spring; the remaining 50% were used in winter or during an undetermined season. However, there was little overlap during the time when cattle were actually present, the coincidence and association indices being low and the C_8 value negative (although not statistically significant) when only fresh feces were considered.

Cattle utilized the dwarf birch thicket, willow thicket and dry grassland habitat types more frequently than expected, and the alder thicket types less frequently than expected from their availability within the study area (Table 4). All habitats except dwarf birch meadow were used to some extent. The general pattern of avoidance of the wettest habitats and concentration on dry, open sites, including roadsides, was confirmed by direct observation of cattle.

Horse and cattle diets during the period of cattle occupancy overlapped by nearly 70% (Table 3). Fescues and sedges together constituted over 50% of the horse diet and nearly 70% of the cattle diet during this time; the two diets differed chiefly in that horses ate more hairy wild rye (Table 3), indicating a greater use of forested habitats. However, open habitats—chiefly mixed shrub meadows—appeared to supply most of the forage utilized by horses throughout the summer.

In order to determine whether horses ate preferred cattle foods in spring, prior to arrival of cattle on the study area, comparisons were made between the April-May diet of horses (Salter and Hudson 1979) and the late June-early October diet of cattle. This pair of diets was 67% identical, although the order of the two most important forages was reversed—sedges being most important in the horse diet and fescues being most heavily utilized by cattle.

The high levels of similarity between the diets of horses and cattle agree with results of other studies of these two species (Hubbard and Hansen 1976; Hansen and Clark 1977; Hansen et al. 1977; Olsen and Hansen 1977).

Effects of Combined Range Use

By late summer 75% of nonforested habitat was used at a safe or lower level, most of the remaining 25% receiving heavy utilization (Table 5). This pattern varied according to habitat type. Dry grassland clearly received the heaviest use

Table 4. Distribution of feral horse and cattle pellet groups by habitat type.[1]

Habitat type	Percent of sampled plots in habitat type (n=493)	Percent of plots containing all age pellet groups in habitat type	
		Cattle (n=92)	Horse (n=417)
Dwarf birch meadow	2.4	0.0	2.4
Mixed shrub meadow	6.3	9.8	7.0
Willow thicket	1.6	8.7$^+$	1.0
Dry grassland	4.1	15.2$^+$	4.8
Dwarf birch thicket	2.2	12.0$^+$	2.6
Dry pine woods	20.4	15.2	19.2
Black spruce woods	2.4	2.2	2.9
White spruce woods	4.3	3.3	3.8
Poplar woods	3.9	6.5	4.3
Mixed woods	16.9	16.3	18.4
Alder thicket	32.9	5.4$^-$	31.4
Disturbed sites	1.0	4.4	0.7
Forested watercourse	1.4	1.1	1.4
Total χ^2		150.5	5.0

[1]Based on presence/absence of pellet groups in plots examined during July-August 1976. Habitat types contributing 10% or more to significant total χ^2 values are underlined and marked $^+$ or $^-$ to signify preference or avoidance by each species. Value of χ^2 at $p = 0.05$ is 21.0.

Table 5. Degree of grazing utilization on nonforested habitats, mid-October, 1976.

Habitat type	*Percent of habitat in use class*[1] None-very light	Light	Safe	Heavy	Severe	Habitat area (ha)	Percent of total
Dwarf birch meadow	73.3	20.8	3.9	2.0	0	68.9	17.5
Sedge meadow	34.7	38.8	22.4	4.1	0	4.9	1.2
Mixed shrub meadow	8.2	55.7	19.1	11.6	5.4	115.1	29.2
Willow thicket	0	0	100.0	0	0	20.8	5.3
Dry grassland	3.1	3.9	44.9	47.2	0.8	122.9	31.2
Dwarf birch thicket	9.9	39.9	25.4	24.9	0	60.7	15.4
Miscellaneous	0	0	0	83.3	16.7	0.6	0.2
Total area (ha)	71.4	109.3	117.2	88.7	7.3	393.9	100.0
Percent of total	18.1	27.7	29.8	22.5	1.8	99.9	

[1]None-very light: 0-15% of herbage biomass removed; light: 16-35% removed; safe: 36-65% removed; heavy: 66-80% removed; severe: >80% removed, plants grubbed, trampling damage evident.

and dwarf birch meadows the lightest, although grazing occurred in all habitats. Combined grazing by horses and cattle caused localized damage along stream courses and around both natural and artificial salt licks (category Miscellaneous, Table 5).

With the exception of some stream courses, wet and mesic habitats generally received limited utilization in summer, in direct contrast to the situation for dry grasslands, dwarf birch thickets and willow thickets—all preferred cattle habitats—which together received the heaviest grazing use. At the end of the summer grazing season, nearly half of the dry grasslands were heavily grazed and nearly all had received some degree of use. Although residual biomass was not measured, stubble heights (usually less than 5 cm) indicated that forage available on these areas over winter had been severely reduced. This also was true to a lesser extent for the closely associated dwarf birch thicket type.

In contrast, intensive examination of an important winter-spring range (and cattle summer range) showed that utilization of new growth was none to very light over 95% of the 70 ha area just prior to the cattle turn-out date. Concentration of foraging activity in other areas resulted in localized grazing and trampling damage (primarily in wet habitats) during spring, but evidence of spring grazing was found on less than 5% of total meadow habitat.

Discussion

A primarily descriptive approach was adopted in this study toward determination of spatial, habitat use and dietary relationships of feral horses with coexisting herbivores. It is recognized that when viewed superficially indications of spatial and food habits overlap may be taken as evidence either for or against competition (Sale 1974; Hudson et al. 1976). Overlap may be functionally synergistic, at least in some grazing systems (Bell 1971). Although measures of resource use overlap can only indicate the degree of similarity between species, and do not measure competition directly, the degree of competition and the amount of overlap are directly related when food is in short supply (Sale 1974). Thus, the extent of actual or potential competition can only be inferred when the use of food and space, the level of range utilization and the behaviour and physical condition of the animals are considered together rather than in isolation (e.g., Wilkinson et al. 1976). In the present study, evidence of competition was sought from the condition of the range and from behavioural interactions, interpreted in light of knowledge of spatial and dietary relationships.

Association and coincidence indices revealed the basic spatial relationships between feral horses and other ungulates. Perhaps the most interesting finding was the high frequency of occurrence of multi-season horse pellet groups as compared to those of other species. This may indicate a broad niche and non-specialist strategy of habitat occupation by horses, although their seasonal distribution was decidedly non-random. Diagrammatic representation of spatial relations showed that, on a year-long basis, horses overlapped extensively onto the areas used by all other species. However, consideration of seasonal distribution indicated that much of this overlap was noncontemporaneous.

Strongly expressed competitive interference (i.e., one species blocking another's access to a limited resource) or exploitation (depletion of the food resource by one species) would influence the interpretation of spatial relationships. Behavioural observations provided little evidence for competition through interference in this grazing system (cattle, mule deer, elk and moose all were observed feeding in proximity to feral horses without interaction), but dietary similarities and spatial overlap indicated the potential for exploitation of elk and cattle foods by horses. In contrast, feral horses would be not expected to exploit the food resource of browsing herbivores (moose, mule deer and white-tailed deer), which utilize herbage primarily during early growth stages in spring and summer (Banfield 1974).

The ability of elk to subsist on browse (Kufeld 1973) makes the importance of forage competition between elk and horses difficult to evaluate. Some shared winter foods (hairy wild rye, sedges) were abundant within extensive habitats (Salter 1978). Horses and elk both also used fescues during winter, but although these grasses were in short supply due to previous heavy grazing the extent of competition for them was minimized by the low numbers of elk present. Elk and horses (and to a lesser extent mule deer, white-tailed deer, and moose) utilized succulent green herbage during April-May, but the low incidence of grazing in nonforested habitats indicated that food was being produced more rapidly than it was consumed; in this situation no competition for food occurs (Sale 1974).

Similarly, spring grazing by horses did not deplete ranges preferred later in the season by cattle, but certain common use areas (primarily dry grasslands and dwarf birch thickets) were overgrazed by autumn. Heavy use of these habitats indicated that competition for some forages may have occurred late in the cattle grazing season.

Fescues were likely the key species in competitive

relationships; of the major shared forages they were the only ones confined largely to the heavily used dry grassland and dwarf birch thicket habitats. Their depletion also may have affected subsequent (winter) distribution and food selection by both horses and elk, but whether there was any effect on population productivity could not be determined. All other shared forage species were widespread and were not overutilized during any season of the year.

While these conclusions are valid for the patterns of resource use documented during the study, the complex and labile nature of range relationships needs to be emphasized. For example, the use of previously grazed areas by several species in spring illustrated a feedback effect of previous grazing resulting in increased availability of succulent forage early in the next growing season. Conversely, large areas of meadow habitat of apparently similar species composition were ungrazed throughout the year. The stability of this grazing pattern (i.e., the period over which certain areas are heavily grazed, while others remain ungrazed) could not be determined from the information available. This would be influenced by extrinsic factors such as location of artificial salt licks, alterations in cattle distribution through increased management, and control of feral horse numbers.

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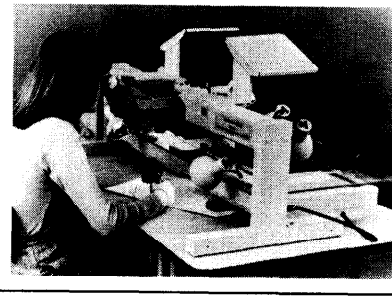

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