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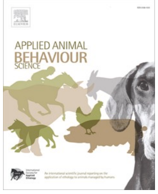

Surgical sterilization impacts on behavior of coyote pairs

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ABSTRACT

Coyotes (*Canis latrans*) involved in depredation of livestock, an act frequently resulting in human-wildlife conflict, often do so out of necessity for provisioning pups. Surgical sterilization methods such as vasectomy that preserve gonadal hormones have been successful in reducing depredation by free-ranging coyotes while allowing individuals to maintain territoriality and mate fidelity. However, use of these methods remain costly and ineffective for wide-scale use. Given the alternative proposal of using chemical sterilization techniques, we investigated whether the use of hormone-altering sterilization methods impacted behavior of captive coyote pairs (i.e., male-female pair bonds). Our objective was to evaluate behavior and reproductive hormones of mated coyote pairs that had received different surgical sterilization treatments. We assigned mated pairs of captive coyotes to different sterilization treatment groups (vasectomy, spay, neuter, ovary-sparing spay, and intact) and coded their behavior as the time spent in resting versus active (i.e., walking, running, scent communication, and aggressive interactions) behaviors. Additionally, hormone concentrations were analyzed to determine effectiveness of hormone-altering treatment, given the potential role of gonadal hormones in regulating behavior. The study was repeated across three breeding seasons. The top model comparing time spent active versus resting was the null model, although the model that included whether sterilization type altered hormones and year also had a ΔAIC of < 2.0. Testosterone concentrations between neutered and vasectomized or intact males was significantly different, indicating sterilization treatment was successful and the different sterilization techniques impact hormones differently; there were no statistical difference for estradiol or progesterone levels among female treatment groups. No sterilized pairs produced pups, but the intact pairs did. Although there are potentially some differences in behavior across sterilization treatment types, our results suggest sterilization of coyotes holds potential as a future management strategy as behavior did not differ among different treatments. Potential difference across years suggest further research is necessary to determine potential extraneous factors influencing behavior and the effect of treatment on territoriality on free-ranging coyotes.

1. Introduction

Around the world, human-wildlife conflict, particularly predator depredation of livestock, continues to be a recurring issue for coexistence initiatives. This is often because carnivores such as wolves (*Canis lupus*; Muhly and Musiani, 2009), lions (*Panthera leo*; Beattie et al., 2020), jaguars (*Panthera onca*; Carvalho et al., 2015), and coyotes (*Canis latrans*; Mitchell et al., 2004) compete with humans over resources in a continually changing ecological environment (Treves and Karanth, 2003). Conflict mitigation methods have included both lethal and non-lethal techniques, each with a varying level of success at reducing depredation rates (McManus et al., 2015). Carnivores play significant roles in the environment by controlling prey populations (Jones et al., 2016), emphasizing the need to adopt appropriate methods of conflict mitigation limiting potential unintended consequences in surrounding ecosystems (Mitchell et al., 2004).

In the United States, with coyotes responsible for the majority of predator-related losses of domestic livestock such as domestic sheep (*Ovis aries*), predator-livestock conflict remains a substantial concern for livestock producers, often leading to the lethal removal of adult coyotes to protect economic investments (USDA-APHIS, 2015). Lethal methods of controlling adult coyote populations, while appealing to livestock producers given the instantaneous outcome, can instead result in increased population numbers as reproduction among yearlings and overall litter size increases (Knowlton et al., 1999). This is referred to as compensatory reproduction. Additionally, nearby transient individuals

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lacking a pack or territory may take the place of removed individuals and continue hunting, further perpetuating the cause of human-wildlife conflict (Jaeger, 2004).

Depredation of livestock by coyotes typically peaks during the spring pup-rearing season (Jaeger, 2004; Till and Knowlton, 1983), with the majority of coyotes hunting larger prey items to provision pups (Harrison and Harrison, 1984; Sacks et al., 1999). This period of depredation often coincides with the lambing and calving seasons of domesticated ungulates, which represent the birth periods of the juvenile age class that is most vulnerable to depredation by coyotes and other carnivores (Green, 1994; Jaeger, 2004). For these reasons, management efforts may focus on controlling pup populations rather than adults to reduce the need for individuals to hunt larger prey species (Knowlton et al., 1999). Approaches to controlling livestock depredation have been demonstrated through denning, a process of lethally removing coyote pups to reduce the energetic needs of the pack (Till and Knowlton, 1983). Removal of coyote pups was similarly as effective at reducing sheep depredation as the removal of both offending breeding coyotes and their pups, signifying the importance of litters and pup provisioning in hunting behaviors of coyotes. Despite the success of denning in reducing depredation, the use of lethal techniques to reduce the number of coyotes present is not popular with the general public (Kellert, 1985; Slagle et al., 2017), highlighting the need for alternative non-lethal methods of removing pup provisioning to decrease depredation rates of livestock.

Surgical sterilization methods such as vasectomy and tubal ligations have been used previously in free-ranging gray wolves (*Canis lupus*; Mech et al., 1996; Spence et al., 1999), red fox (*Vulpes vulpes*; Saunders et al., 2002), coyote-red wolf (*Canis rufus*) hybrids (Gese and Terletzky, 2015; Gese et al., 2015), and coyotes (Bromley and Gese, 2001a, a, 2001b; Seidler and Gese, 2012; Gese and Terletzky, 2015) as a means of non-lethal population control that also preserves gonadal hormones important in maintaining social and reproductive behaviors. Surgical sterilization of coyote packs helped reduce depredation in territories where breeding adults did not have pups to provision while also maintaining territory and mate fidelity (Bromley and Gese, 2001b). It also has been effective at preventing introgression of coyote genes into the endangered red wolf population (Fredrickson and Hedrick, 2006; Hinton et al., 2013; Gese et al., 2015).

While surgical sterilization of coyotes has been an effective management tool (i.e., minimizing introgression and reducing depredation rates), costs prohibit implementation as a large-scale non-lethal management tool given the requirement of surgery and use of anesthetics (DeLiberto et al., 1998; Massei and Cowan, 2014). Exploration of non-invasive methods of sterilization with the use of a hormone-altering chemical sterilant has alternatively been proposed (DeLiberto et al., 1998). However, understanding of how altering sex steroid hormones will affect canid behavior must be obtained before large-scale application of chemical sterilization can be implemented. This information must include whether there is continuation of pair-bond behaviors, pack cohesion, and social structures; research currently lacking sufficient experimental evidence throughout the literature (Asa et al., 2005; Mitchell et al., 2004).

Although rare among mammals, social monogamy, where an adult male and female remain associated behaviorally for the long-term through pair-bonds (Lukas and Clutton-Brock, 2013), is found in many wild canid species, including coyotes. Coyotes also partake in genetic monogamy or exclusivity in mating (Hennessy et al., 2012), with pairs maintaining and defending territories equipped with provisions such as dens, water, and food resources (Bekoff and Wells, 1986; Gese, 2001). Pair-bond behaviors between coyotes are crucial to an individual's fitness as well as the success of the pack through the continuation of territoriality and biparental care of young between mates (Carlson and Gese, 2010; Gese, 1998). Disruptions to social structures may lead to the breakdown of pair-bonds and subsequent social systems, resulting in a loss of territoriality against incoming packs, mating behaviors, and access to resources such as mates, space, and food (Gese, 1998, 2001).

While gonadal hormones are believed to play a role in modulating coyote behavior particularly during the breeding season as hormone levels become elevated, evidence is conflicting throughout the literature. Mating behaviors between pair-bonds were positively associated with increases in estradiol and progesterone levels in captive females during proestrus (Carlson and Gese, 2008). However, alterations in hormones of male captive coyotes using reproductive inhibitors were found to not alter behavior (Young et al., 2018), indicating potential extraneous factors influencing coyote behavior warranting further research.

Because effective chemical sterilization methods are not yet available for coyotes (Young et al., 2018), in this study, we relied on hormone-altering and non-hormone altering surgical sterilization treatments of captive coyotes to explore possible impacts on behavior that could ultimately impact pair-bonds. As previous studies have suggested that hormone-altering sterilization methods such as spay and neuter may alter mating behaviors (Asa and Valdespino, 1998), we hypothesized captive coyotes with hormone-altering sterilization treatment may experience alterations in behavior. Finally, gonadal hormone concentrations were analyzed to verify success of sterilization treatment and ensure hormones were altered within the timeframe of the study.

2. Materials and methods

2.1. Study area

All methodology was approved by the Institute for Animal Care and Use Committee at the U.S. Department of Agriculture (USDA) National Wildlife Research Center (QA-2716). The study used captive coyotes maintained at the USDA-Wildlife Services-National Wildlife Research Center's Predator Research Facility in Millville, Utah, USA (66.4 ha). The facility manages and cares for a colony of coyotes using methods to maintain wild behavior (Shivik et al., 2009). Approximately 100 adults are housed at the facility as male–female pairs in individual outdoor enclosures (0.1–1.0 ha in size) with natural earthen floors and native grass vegetation. The enclosures are surrounded by chain-link fencing and contain a manmade den box, two shade tables, and an *ad libitum* source of water. Individual coyotes breed at least once in their lifetime, with some breeding multiple times.

2.2. Sterilization methods

To determine if sterilization of coyotes impacts behavior, 28 pairs of adult captive coyotes housed at the Predator Research Facility were chosen according to colony breeding management goals to participate in this study. Four treatment groups (vasectomy, spayed, neutered, and intact) were determined, with intact pairs (i.e., breeding pairs) serving as the experimental control group. As vasectomies are commonly used at the Predator Research Facility to manage the number of individuals in the colony, three individuals having previously received a vasectomy and their mates were chosen at random to participate in the vasectomy treatment group. Six additional pairs were selected from the pool of potential breeders in the colony to participate in the spay and neuter treatment groups, where one individual from each pair was randomly chosen to receive a sterilization treatment of either spay or neuter. In total, three males were neutered and three females were spayed. Additionally, three pairs of breeding coyotes were selected from the pool of potential breeders to participate in the intact treatment group, where they did not receive any sterilization treatment. To obtain our sample size, the study was conducted during three experimental sessions: Year 1 (2016–2017), Year 2 (2017–2018), and Year 3 (2018–2019). An identical subject selection process was conducted for all years, with the exception of an additional treatment group, namely ovary-sparing spay, being added at the beginning of Year 3. This was added because of health concerns for female coyotes raised by the attending veterinarian. Five different pairs were also selected for each treatment group rather than three during Year 3 as colony management goals allowed for a larger sample size (see Table 1 for a summary of year-by-treatment group sample sizes and corresponding power analysis results).

Sterilization treatments were conducted using a protocol that was pre-determined by the attending veterinarian, with both males and females receiving treatment after being anesthetized prior to surgery. Males receiving sterilization treatment underwent an orchiectomy (also known as neuter) while females underwent an ovariohysterectomy (also known as spay) or hysterectomy (also known as ovary-sparing spay). Following sterilization surgery, treated coyotes were monitored daily and housed individually without their mate in an outdoor pen to recover from surgery for a two-week period. Mating pairs were then put back together into clover and interaction outdoor pens (0.1 ha each) for behavioral observation for the duration of the study.

2.3. Behavioral observations

Behavioral observations were conducted on all coyote pairs before and throughout the breeding season (October through March during Year 1, November through January during Year 2, and January through March during Year 3). In Year 3, observations between October and December were unable to be collected due to a federal government shutdown. Individuals were observed for 15-minute focal sampling periods at random times throughout daylight hours to ensure visibility of behaviors. Each coyote was observed for behavioral samples during weekdays at least once every two weeks. Prior to every behavioral observation session, one individual from each pair was randomly selected to be observed during the given session. Behavioral data was, however, collected on both individuals in the pair throughout the breeding season to control for bias. The five major behavioral categories used for sampling included resting, active, scent communication, pair interactions, and aggressive interactions (Table 2); however, scent communication, pair, and aggressive interactions were scarcely observed and therefore incorporated into the active category for analysis. The type of observed behavior and duration of behavior (in seconds) the focal coyote exhibited was recorded during each focal observation. Those conducting observations were trained by the same person to ensure consistency in training across individuals. Monthly checks of behavioral observations were used to confirm interobserver reliability. Observers were also blind to the treatment assignments, albeit they likely were able to determine which pairs were in the intact group once pregnant females began to have large bellies (i.e., nearing the end of each session). Behavioral observations were conducted from a mobile observation blind only used for observations as not to elicit positive or negative behaviors.

Table 1
Sample size by surgical sterilization treatment and year for captive coyotes (*Canis latrans*) at the U.S Department of Agriculture, National Wildlife Research Center- Predator Research Facility, Logan, Utah, USA. Results of a power analysis for determining the minimum sample size necessary is included because we needed to use a minimal number of coyotes per treatment type and the captive coyotes are maintained at the USDA-Predator Research Facility for research purposes and sterilizing coyotes for this study needed to accommodate the colony management. Each sample size includes the animal that underwent the treatment and its mate pair. For example, there were 3 neutered males in year 1, and data were obtained from those males and their female mates for n = 6. Sampling occurred from October 2016 to March 2017, November 2017 to January 2018, and January 2019 to March 2019.

	Treatment Group				
Year	Intact	Neuter	Spay	Spay-ovaries	Vasectomy
1	8	6	6	NA	6
2	10	6	6	NA	6
3	8	10	10	10	10

Year	Effect Size	k	Significance Level	n*	Power
1	0.5	4	0.05	6	0.436
2	0.5	4	0.05	6	0.436
3	0.5	5	0.05	8	0.649

* most conservative, using the lowest n of any test group for that year.

Table 2
Five main behavioral categories, which were later collapsed into resting and active (all other behaviors), and description of each individual behavior used for behavioral observations of adult captive coyotes across all three years.

Category	Observed Behavior	Description of Behavior
Resting	Lying down	Mid-section of body in contact with ground
	Sit	Back part of body in contact with ground
	Stand	Stationary, upright position
	Vigilant	Lying down, but alert with head up
Active	Walk	Locomotion without in-air phase
	Trot	Locomotion with in-air phase
	Run	Locomotion with in-air phase where hind legs extend to meet or pass front legs
	Pace	Walking back and forth over the same, small area
	Self-groom	Lick own body
	Scratch	Scratch own body
	Eat	Consume solid food
	Drink	Consume water
	Bark	Short, loud vocalization often linked to aggression
	Howl	Aowwwwwwww
	Other	Any other behaviors, recorded in detail
Scent Communication	Mark urine dig	Dig-like behavior, typically with back legs after urinating
	Raised leg urinate	Urinate with hind leg lifted
	Squat urinate	Urinate in squatting posture, hind leg may be slightly lifted
	Overmark urinate	Urinate in same spot where other coyote urinated <5 min
	Defecate	Defecate
	Dig	Scratch soil/dirt
	Sniff site	Investigate soil/dirt/plant/etc.
Pair Interactions	Sniff mate	Investigate other coyote
	Play invitation	Stamp or bow forelegs or use forelegs to paw mate
	Play chase	Chase mate, non-aggressive
	Present	Female orients to male for mounting
	Attempt mount	Male attempts to mount female
	Mount	Male mounts female
	Tie	Mount is successful
Aggressive Interactions	Charge/lunge	Advance toward mate, ears typically back
	Growl	Growl at mate
	Gape	Open mouth, oriented toward mate
	Antagonistic chase	Chase mate, aggressive
	Submissive crouch	Crouch or semicrouch body position
	Submissive whining	Long and high-pitched, may accompany crouch
	Bite	Snapping jaws shut

2.4. Hormone analysis

During Year 3, fecal samples were collected once per week for eleven weeks (January through March). The day prior to feces sample collection, individual coyotes were fed separately, and their diet was marked with colored glitter to help identify fresh scats from each individual (Burns et al., 1995). Males received one color while females received a different color. Once feces samples were collected as part of daily husbandry, they were frozen until gonadal hormone concentrations (estradiol, progesterone, and testosterone) were able to be analyzed using enzyme-linked immunosorbent assays (ELISA) in the Endocrinology Lab located at the St. Louis Zoo.

2.5. *Fecal hormone extraction*

Approximately 0.5 g of wet fecal material was weighed then shaken overnight in 5 mL of a modified phosphate-saline buffer containing 50 % methanol (Shideler et al., 1993). Liquid extracts were decanted, and solids were removed through centrifugation at 4000 *g*. Supernatants were then frozen at −80 °C until assay. Fecal material was placed in a drying oven overnight at 100 °C.

2.6. *Fecal progestogen assay*

Fecal progestogens, androgens and estrogens were measured using commercially available EIA kits (DetectX © Progesterone EIA K025, DetectX © Testosterone EIA K032, DetectX © Estradiol K030, Arbor Assays). The detection limits were as follows: progesterone: 50–3200 pg/ml, testosterone: 40.96–10,000 pg/mL, estradiol: 39.06–10,000 pg/mL. Samples were diluted 1:10 or 1:100 with assay buffer, and all assays were run according to kit directions. Concentrations were determined as ng/mL, and then divided by the dry weight of the extracted feces to give the results as ng/g feces. All samples were assayed in duplicate. Mean intra-assay variation of duplicate samples was 8.8 % for progesterone, 7.4 % for testosterone, and 9.1 % for estradiol. Mean inter-assay variation of two quality control pools was 6.7 % for progesterone, 7.9 % for testosterone, and 8.2 % for estradiol.

2.7. *Statistical analysis*

To determine whether coyote behavior differed among the various experimental groups, or if hormone alteration affected their behavior, a series of multivariate linear mixed models (Table 3) were performed using the *mmer* function in the *sommer* package (Covarrubias-Pazaran, 2018) in R 4.0.5 (R Core Team, 2021). Using this method, estimates for the bivariate response (i.e., time spent exhibiting active behavior and resting behavior) are simultaneously calculated and coefficients can be tested across the different responses. Fixed effects used in the competing models included: year in which behavioral observations were conducted, whether or not the sterilization method was expected to impact hormones (i.e., spay and neuter vs. other treatments and intact), sterilization group (including intact breeding pairs), and sex. As behavioral samples were collected repeatedly from the same individuals throughout the breeding season, all models considered each individual coyote as a random effect, nested within their uniquely identified mating pair. The null model, as well as models using interactions between fixed effects terms, was also considered in the suite of competing models which were then ranked using AIC for the selection criteria (Table 3). Any models within two AIC units of the top-performing model (lowest AIC) were further evaluated using the *emmeans* package (Lenth, 2021), where significant terms were obtained using Type III ANOVA methods, least-squares means were estimated, and pairwise contrasts were performed. Type I error was controlled, when necessary, using the Tukey method.

To detect significant differences in gonadal hormone concentrations among the different sterilization groups (including intact breeding individuals), three generalized additive mixed models (GAMMs) – each specific to the three assayed gonadal hormones – were conducted using the *gam* function in the *mgcv* package in R (Wood, 2004). Smoothing splines ("week") were utilized to help account for fluctuations in hormone levels over time, and individual animals were designated as random effects. As for the behavioral analysis, Tukey contrasts were again performed, this time using the *glht* function in the *multcomp* package after manually constructing the models' contrast matrices. The significance threshold was set at 0.05 for all statistical analysis.

3. Results

3.1. *Behavioral observations*

For the behavioral analysis, the null model resulted with the lowest AIC, followed closely by the model with the hormone-altered and year fixed effects (ΔAIC = 1.10, see Table 3). Although the top model had no fixed terms to inform its estimates, ANOVA testing between the two categories of behavior validated a significant difference between the

Table 3
Multivariate linear mixed models were performed using the *mmer* function in the *sommer* package (Covarrubias-Pazaran, 2018) in R 4.0.5 (R Core Team, 2021) for resting and active behavioral data from captive coyotes housed as mated pairs. Models with a delta (Δ) AIC value ≤ 2 are in bold.

Model_Fixed_Effects	AIC	Δ AIC	LogLik	K
Null model	**−6254.852**	**0.000**	**3129.426**	**15**
HormoneAltered + Year	**−6253.747**	**1.105**	**3134.874**	**33**
HormoneAltered + Sex + Year	−6245.901	8.951	3132.950	39
HormoneAltered	−6245.067	9.785	3126.533	21
SterTrtNum + Year	−6242.199	12.653	3135.099	51
SterTrtNum	−6237.733	17.119	3128.867	39
HormoneAltered + Sex	−6237.264	17.588	3124.632	27
HormoneAltered * Sex + Year	−6236.130	18.722	3130.065	45
SterTrtNum + Sex + Year	−6234.306	20.546	3133.153	57
HormoneAltered + SterTrtNum + Year	−6232.344	22.508	3132.172	57
SterTrtNum + Sex	−6229.865	24.987	3126.932	45
HormoneAltered + SterTrtNum	−6227.818	27.034	3125.909	45
HormoneAltered * Sex	−6227.543	27.309	3121.772	33
HormoneAltered + SterTrtNum + Sex + Year	−6224.512	30.340	3130.256	63
HormoneAltered * SterTrtNum + Year	−6223.907	30.945	3129.954	63
HormoneAltered + SterTrtNum + Sex	−6220.006	34.846	3124.003	51
HormoneAltered * SterTrtNum	−6219.411	35.441	3123.706	51
HormoneAltered * Sex + SterTrtNum + Year	−6214.554	40.298	3127.277	69
HormoneAltered * SterTrtNum + Sex + Year	−6214.211	40.641	3127.106	69
HormoneAltered * Sex + SterTrtNum	−6210.120	44.732	3121.060	57
HormoneAltered * SterTrtNum + Sex	−6209.714	45.138	3120.857	57
SterTrtNum * Sex + Year	−6208.413	46.439	3128.206	81
SterTrtNum * Sex	−6203.931	50.921	3121.965	69
HormoneAltered + SterTrtNum * Sex + Year	−6197.657	57.195	3124.829	87
HormoneAltered + SterTrtNum * Sex	−6193.162	61.690	3118.581	75
HormoneAltered * SterTrtNum * Sex + Year	−6189.396	65.456	3122.698	93
HormoneAltered * SterTrtNum * Sex	−6185.506	69.346	3116.753	81

***All models compared use the same random effects: 'CoyID', nested in 'MatePair'.

time coyotes spent performing active behaviors versus the time they spent resting ($P < 0.0001$). Further, coyotes were estimated to spend 620 s resting (95 % CI: 590–649) and 280 s in an active state (95 % CI: 250–310) for a typical 900-second focal observation (Fig. 1).

After employing a likelihood ratio test for mixed models, the second-ranked model was not significantly different from the top model ($\chi^2 = 10.90$, $\chi_k^2 = 18$, $P = 0.900$). Nonetheless, the fixed effects terms for the model were tested for significance and its marginal means were evaluated using pairwise contrasts. ANOVA results for the second-ranked model verified a significant difference between behavioral responses ($P < 0.0001$). When averaged over the levels of the hormone-altered and year effects, coyotes were estimated to spend 340 more seconds resting than engaging in active behavior (SE = 30.3, $P < 0.0001$). Behavior also significantly differed among the different years ($P < 0.0001$). All pairwise contrast tests resulted in significant differences when comparing behavioral responses of the third year to the first or second year ($P < 0.0001$, Fig. 2). Behavioral responses did not significantly differ between coyotes with or without altered gonadal hormones ($P = 0.3526$).

3.2. Hormone analysis

Testosterone levels were statistically significant among neutered males (beta estimate = -681.61; $P < 0.0001$), but not among those sterilized by vasectomy (beta estimate = 108.37; $P = 0.394$) relative to intact males (beta estimate = 704.63). Pairwise comparisons showed that neutered coyotes were estimated to have 681.6 ng/g less testosterone than intact males (SE = 127.9, $P < 0.0001$) and 790.0 ng/g less testosterone than vasectomized males (SE = 790.0, $P < 0.0001$) per fecal sample. Progesterone levels were not statistically significant for spayed (beta estimate = -1601.2; $P = 0.104$) or ovary-sparing spayed females (beta estimate = -506.6; $P = 0.661$) when compared to intact females (beta estimate = 3319.1). Similarly, estradiol levels were not significantly different for spayed (beta estimate = -35.64; $P = 0.241$) or ovary-sparing spayed females (beta estimate = -17.15; $P = 0.631$) when compared to intact females (beta estimate = 109.12).

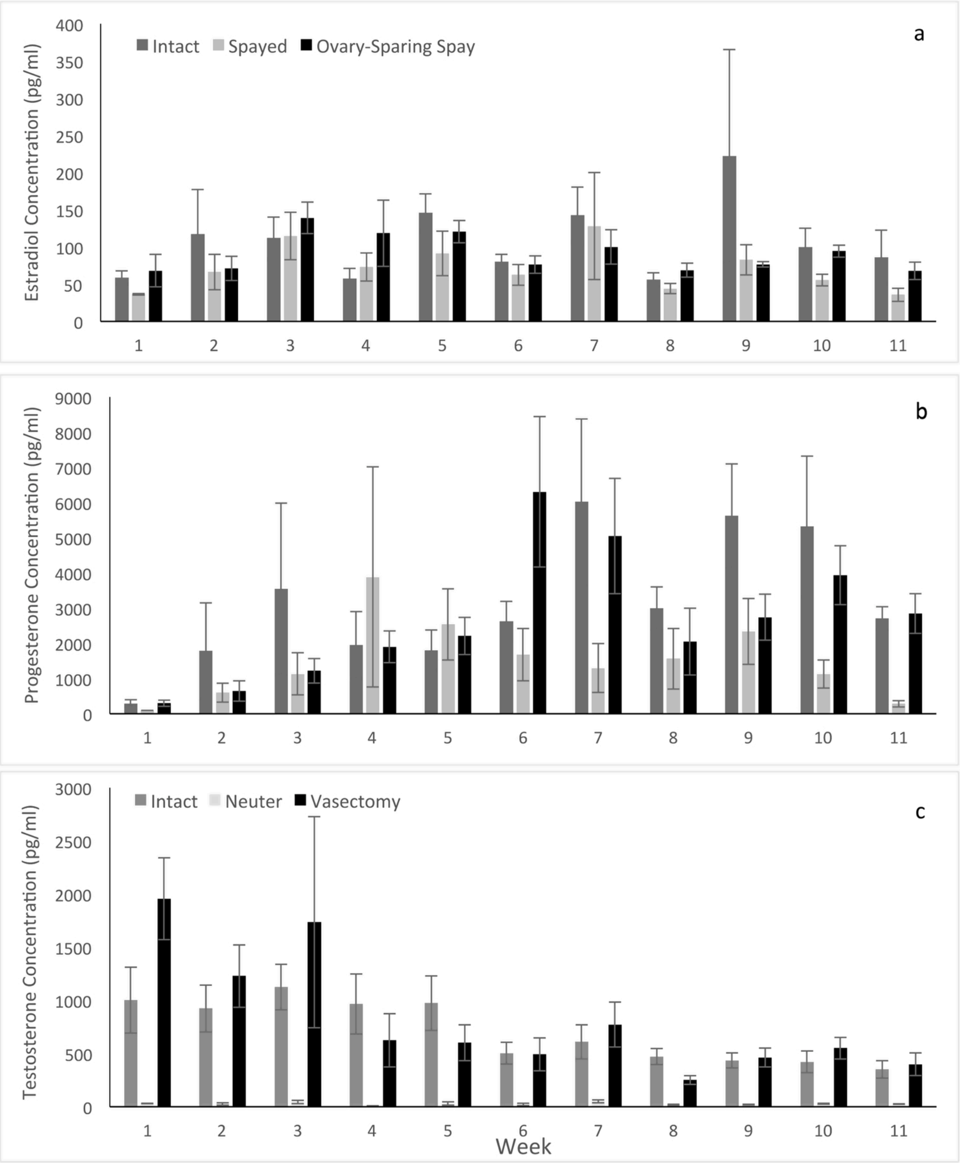

Fig. 1. Estradiol (a), progesterone (b), and testosterone (c) fecal concentrations (ng/mL) (+/- SE) of both treated adult coyotes and their mates housed at the U.S Department of Agriculture, National Wildlife Research Center- Predator Research Facility, Logan, Utah, USA. Fecal samples were obtained from 17 January to 15 March 2019.

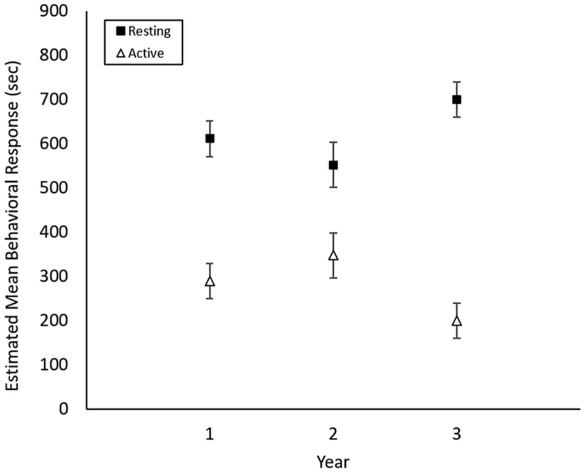

Fig. 2. Estimated mean behavioral response (in seconds) by year of adult coyotes housed at the U.S Department of Agriculture, National Wildlife Research Center- Predator Research Facility, Logan, Utah, USA spent engaged in resting and active behavioral categories. Error bars represent 95 % confidence intervals. Sampling occurred from October 2016 to March 2017, November 2017 to January 2018, and January to March 2019.

4. Discussion

There exists a lack of information over how alterations in gonadal hormones impacts coyote behavior when sterilization techniques are utilized to reduce provisioning needs of breeding coyotes. In this study, we observed no statistically significant differences in behavior of coyotes that underwent different sterilization surgeries or remained intact, even though at least one surgical procedure altered gonadal hormones (Table 3). Hormone alteration and year were, however, considered in determining coyote behavior given their inclusion in the second best-fit models (Table 3).

The results of our study were consistent with outcomes found by Bromley and Gese, 2001a, 2001b, as free-ranging coyotes receiving sterilization treatment (vasectomy and tubal ligation) did not experience modifications to pair-bond maintenance as a result of treatment. This is interesting because the surgical sterilization procedures used in Bromley and Gese, 2001a, 2001b did not alter gonadal hormones whereas at least one of the procedures in our study did – neutered males. Our observations were also consistent with Carlson and Gese, 2010, as estradiol steroid hormone treatment of captive female coyotes did not impact pair-bond behavior despite alteration of hormones. Interestingly, although there was a trend from highest to lowest levels of estradiol and progesterone from females that were intact to females that received the ovary-sparing spay and then females that were spayed, this trend did not result in statistical differences among females. This trend may help explain why our findings were contrary to predictions made by others suggesting alterations to hormones would impact pair-bond behavior (Asa et al., 2005; Mitchell et al., 2004; Seidler and Gese, 2012); we did not detect the alteration in hormones among females that was expected in spayed animals. It also indicates additional social, environmental, or physiological factors influence behaviors of coyotes in addition to sex steroid hormones.

Throughout the study, coyotes spent most of their time participating in resting behaviors followed by active, scent communication, pair interaction, and finally aggressive interaction behaviors. In fact, scent communication, pair interactions, and aggressive interactions were so rarely observed that they could not be analyzed independently and were combined with other active behaviors for our analyses. This likely means that sterilization does not create intolerance or break down pair-bonds but future studies may want to increase sample size, time spent per focal sample, or focus more precisely on these rare behaviors as they are likely important for monitoring of pair-bonds. While the captive setting may have forced pair-bonds to be maintained, we have observed fighting among coyote pairs previously and have on a few occasions needed to separate mated pairs to avoid risk of injury to individual coyotes. Thus, the nature of being in captivity is unlikely to have prevented coyote behavior from changing if the surgical procedure affected behavior. Instead, that aggression remained low suggests coyote pairs were not trying to separate.

We observed statistically significant differences between experimental years, signifying potential extraneous factors influencing behavior. Observers utilized in the study differed between years, but the training each individual received remained consistent as each observer was trained by the same person. In terms of environmental changes, coyotes have been found to decrease activity during periods of high temperatures, high wind speeds, and low barometric pressure, suggesting weather patterns may have an effect on behavior (Madsen et al., 2020). Discrepancies between the sampling periods used each year may have also played a role in the differences seen across years. The government shutdown during one year of the study resulted in the start of behavioral observations being delayed until January, so that two fewer months of data were collected relative to the other years. This period of time is often when pair bonds are strengthened and most courtship behavior observed (Beckoff and Wells 1986), with mating more likely to occur during the time data was collected in all years (i.e., January and February). This may also have impacted the hormonal data analysis in that we may have missed the period (i.e., courtship) that hormones would have been most different among females. Even so, it is evident that behaviors among treatment groups did not differ across years.

As well as observing behavior, gonadal hormone concentrations of both treated coyotes and their mates were analyzed to determine effectiveness of sterilization treatment. In males, there was a statistically significant difference in testosterone levels between neutered and intact or vasectomized individuals, indicating success of sterilization treatment through the reduction of hormone concentrations. These differences in testosterone levels did not, however, translate into changes in behavior between hormone-intact and hormone-altered individuals. In females, there was not a statistically significant difference in progesterone or estradiol levels between treated individuals despite the removal of hormone-producing ovaries in spayed coyotes. Often, in the absence of gonads, the adrenal gland compensates by producing excess quantities of sex steroid hormones, potentially explaining the hormone fluctuations seen in spayed coyotes post-surgery (Fig. 2; Santen et al., 1980).

While the results of our study remain an important first step in understanding the behavioral impact of surgical sterilization of coyotes, it is important to note because this study was conducted on captive coyotes, we could not assess aspects of territoriality important to free-ranging coyotes. Sterilization techniques such as vasectomy and tubal ligation were found to not affect territoriality (Bromley and Gese, 2001a) and have been used as a management tool for maintaining coyote territories surrounding an endangered red wolf population (e.g., Gese and Terletzky, 2015), but hormones have been positively associated with urine-marking behavior (Asa et al., 1990). Given territoriality plays a large role in pair-bond behaviors, further investigation regarding the effect of hormone alteration on territoriality in free-ranging coyotes is necessary prior to implementation of chemical sterilization.

Understanding the effect hormone alteration has on pair-bond behavior between coyotes is crucial in determining the success of future contraception use in the sterilization of free-ranging coyotes. Surprisingly, the sterilization surgeries that removed gonads had different effects on males and females; neutered males had less testosterone than males that were intact or underwent a different procedure, whereas females showed no statistical difference among procedures or compared to those that remained intact. We had expected all gonadal-removing surgical procedures to alter hormones (Hart and Eckstein, 1997). While we found that spayed females showed the lowest estradiol and progesterone values, the timing of our sampling – only starting in January instead of at the start of courtship behaviors - may have prevented detection of measures that warranted statistical differences. Further research may be required in determining timing of hormonal fluctuations related to pair bonds and regarding additional factors besides gonadal hormones potentially influencing pair-bond behaviors and pair-bond maintenance between mates to ensure continuation of social structures and pack cohesion.

4.1. Conclusions

The use of non-lethal methods such as sterilization will be important moving forward for the conservation of not only individual species, such as the coyote, but also for the preservation of surrounding ecosystems. Disruptions to ecosystems may result in ecological cascades through the loss of large predators, triggering an increase in mesopredators and subsequent reduction of bird and small vertebrate populations (Crooks and Soule, 1999). Targeted usage of chemical sterilization techniques will be required to identify repeat, problematic coyotes depredating livestock or impacting sensitive species to properly manage for reducing conflicts. Improper usage of coyote control methods through broad-scale administration could potentially result in unintended environmental consequences (Mitchell et al., 2004), an area warranting further research to determine the overall population and ecological impacts.

Declaration of Competing Interest

The authors report no declarations of interest.

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