EVALUATING THE REPRODUCTIVE HABITS AND THE BREEDING SEASON OF THE HOG-NOSED SKUNK (*CONEPATUS LEUCONOTUS*)

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ABSTRACT

I collected data to assess the times when the hog-nosed skunk, *Conepatus leuconotus*, is reproductively active and compared these times to its reported breeding season. I analyzed data from 752 museum collection specimens from six countries to determine sex ratio and discern in which months hog-nosed skunks had the highest activity, as indicated by collection date. Collection peaks were different for males and females, but were not exclusively seen during the reported breeding period. Testes measurements were analyzed for a smaller subset of 100 males from the United States collected throughout the year. Data were compared by collection month to determine if there was a significant difference in size of the testes and reported mating season. There was neither a difference in relative testis size among months, nor between the months of the reported breeding period and non-reproductive months. Reproductive behavior occurs outside the reported breeding period for *C. leuconotus*.

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INTRODUCTION

Skunks comprise a family of mammals (Mephitidae) that ranges from North and South America to some Malaysian Islands. With their characteristic black and white pelage and defensive scents, they are one of the most frequently encountered mammals in the United States. Skunks have a known association with a variety of zoonotic diseases, especially rabies. Because of the ability to spread rabies and their adaptation to urban environments, striped skunks (*Mephitis mephitis*) and their natural history have been well-studied. Conversely, less is known about the American hog-nosed skunk (*Conepatus leuconotus*), as it is a less frequently encountered species in the southwestern US. Hog-nosed skunks are easily distinguishable from striped skunks by the single, wide dorsal white stripe from the neck to the all-white tail and the absence of a small white stripe between the eyes. In addition to being slightly larger in size, the hog-nosed skunk also has a longer snout and forelimbs, along with lesser-developed carnassials and larger molars compared to other mephitids (Dragoo and Sheffield 2009). Previous researchers suggest that the foraging ecology in the hog-nosed skunk differs from that of other skunks with the former searching for prey by digging and rooting and the latter being more opportunistic. This likely alleviates some of the competition with other mesocarnivores. Hog-nosed skunks are currently not listed as federally or internationally endangered, but because of their reclusiveness and relative rarity, they are a species whose populations should be monitored in several states. Currently, they are a species of least concern in Texas, but many researchers note their decline throughout their range due to habitat loss and the introduction of exotic species, especially feral pigs (Meaney et al. 2006). A better understanding of this animal's biology is

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crucial in ascertaining its conservation status. Possible management and protection is reliant on a better comprehension of this skunk's feeding, behavioral, and reproductive habits.

Delayed implantation, also known as embryonic diapause, is a reproductive strategy in which further development of an early-stage embryo is halted and implantation in the uterus is suspended. As a result, the embryo remains in a state of dormancy for some period (Renfree and Shaw 2000). Development of the embryo is halted at varying stages depending on the species in which it is found. The size of the blastocyst delayed varies from 30-40 cells in some, such as the roe deer, to as many as 300-400 in many carnivores (Lopes et al. 2004). There are competing hypotheses explaining the ultimate utility of delayed implantation, however the most common idea suggests that seasonality plays an important role (Mead 1989). Delayed implantation is reasoned to align the birth of young with favorable environmental conditions, typically ensuring that young are weaned and have an optimal chance of survival, especially throughout the winter months. It may also enable solitary males to opportunistically mate, even if the conditions are not favorable, as finding a female may be difficult due to the animal's solitary lifestyle. Delayed implantation is known in over 100 mammal species across seven orders, representing less than two percent of mammalian species (Orr and Zuk 2014). It is widespread through mustelids, a related family in which skunks were previously grouped. About 34% of mustelids and mephitids have delayed implantation compared to only 0.05% of all other mammals that have this characteristic (Mead 1989). The family Mephitidae, which comprises skunks and stink badgers, was recently split from Mustelidae (Dragoo and Honeycutt 1997). The physiological mechanisms of delayed implantation are not fully understood, although there is evidence of photoperiod control, temperature dependence, and metabolic stress on the mother (Lopes et al. 2004).

The controlling mechanisms vary by family and are affected by various hormones likely influenced by photoperiod (Renfree and Shaw 2000). Mead (1971) found that in mustelids such as the western spotted skunk (*Spilogale gracilis*), delayed implantation is often terminated during lengthening photoperiods. Similar results have been shown in the mink, *Neovison vison* (Murphy and James 1974). Another hypothesis posits that terrestrial carnivores living in seasonal environments are more likely to benefit from this strategy (Ferguson et al. 1996). Additionally, temperature has been reported to play a role in delayed implantation, such as of that in the badger, *Meles meles* (Canivenc and Bonnin 1979). Finally, it has been reported that in some carnivore species, the mother's nutrition may lengthen the period of delayed implantation (Ferguson et al. 1996). Of the five species of skunks found in the United States, the western spotted skunk and the striped skunk are known to undergo varying degrees of delayed implantation, whereas the eastern spotted skunk does not (Mead 1968a. 1968b, Wade-Smith et al. 1980). Due to their relative rarity and limited distribution in the US, the remaining two members of the family, the hooded skunk (*Mephitis macroura*) and the hog-nosed skunk, have not yet been thoroughly examined to determine if they utilize this reproductive strategy (Dragoo and Sheffield 2009).

The reproductive biology of the striped skunk and both eastern and western spotted skunks is well-documented, whereas information regarding that of the hog-nosed skunk is lacking. Species of the two skunk genera, *Spilogale* and *Mephitis*, vary in gestation time and this variation may be attributed to delayed implantation (Dragoo and Honeycutt 1999). Western spotted skunks give birth to 2-5 kits, after a period of delayed implantation whereas eastern spotted skunks give birth to 2-5 kits after 50-60 days with little to no delayed implantation (Mead 1968a). Striped skunks give birth to about 5-8 kits after a gestation time of 59-77 days (Wade-Smith and Verts 1982). Hog-nosed skunks typically give birth to approximately 2-4 kits each litter and sexual maturity is reached around 10 to 11 months after birth (Hayssen et al. 1993). Female hog-nosed skunks are likely reproductively active in their first year, as 95% of 178 striped skunks were found to be pregnant or parous, of which 74% were one year of age (Greenwood and Sargeant 1994).

One of the earliest reports of the hog-nosed skunks' reproductive habits in Texas comes from Vernon Bailey's biological survey of Texas (Bailey 1905). He captured a single female on March 24 that contained one embryo (Bailey 1905). Taylor and Davis (1947) added more about the reproduction of the animal and reported capturing of 6 females in the spring of 1943. They suggested that breeding may take place in January or February, as they consistently found a litter size of 2-3 kits around late spring or early summer. They compared the gestation period of the hog-nosed skunk to other skunks and concluded that "Assuming a gestation period of two months or a little more as in other skunks, breeding must take place in late January or early February" (Taylor and Davis 1947). Robert Patton monitored several skunk species in the Trans-Pecos region of Texas, including hog-nosed skunks. Although hog-nosed skunks are rare, Patton noted lactating females in May and July and suggested the breeding season is likely in February and lasts about two months (Patton 1974).

Current literature estimates the hog-nosed skunk's gestation time at approximately two months, although these accounts lack additional reproductive information (Schmidly 2004). In contrast to this, captive male hog-nosed skunks have exhibited mating behaviors in November and captive females have exhibited a gestation period of more than 70 days (Dragoo and Honeycutt 1999). Also, there are accounts of intersexual denning behaviors outside of the reported breeding period (Brashear et al. 2015). These observations have led to uncertainty as to the reproductive patterns in hog-nosed skunks. Because some mephitids have delayed implantation (Mead 1968a, Wade-Smith et al. 1980), one possible explanation for the patters in hog-nosed skunks is some form of delayed implantation.

Some recent literature reviewing the general maintenance and evolution of delayed implantation have included information describing the phylogeny and the ancestral state of this trait (Johnson et al. 2000, Lindenfors 2003, Thom et al. 2004). In these phylogenetic trees, species are noted as having delayed implantation, direct implantation, or unknown. Hog-nosed skunks are listed as one of the many mustelids that do have delayed implantation, however, many other sources report that it is unknown whether or not they do utilize delayed implantation (Mead 1989, Dragoo and Honeycutt 1999, Schmidly 2004, Meaney et al. 2006, Dragoo and Sheffield 2009).

Mammalian carnivores living in seasonal environments are more likely to evolve delayed implantation compared with carnivores elsewhere (Ferguson et al. 1996). It has been also suggested that some life history traits are shared among those that exhibit delayed implantation and those that do not. These tradeoffs likely exist to decrease the risk of reproductive failure. Of the traits that differ among species, those species with delayed implantation are more likely to have smaller litter sizes than those without (Ferguson et al. 1996). Since hog-nosed skunks are distributed through much of Mexico and Central America, they are less likely to experience a difference in photoperiod and temperature changes in lower latitudes. Although Texas experiences fairly low seasonality as compared to high northern latitudes, it does experience a winter with temperatures below freezing and a reduction of food availability as well as variation in day lengths. Of the Musteloidea, hog-nosed skunks tend to have relatively small litter sizes. More recent studies dealing with delayed implantation (Lindenfors et al. 2003), especially within the superfamily Musteloidea, have suggested that this trait may have evolved in a basal caniform ancestor. Phylogenetic reconstruction strongly supports a basal monophyletic origin of delayed implantation. It has been suggested that this trait was either lost 12 times, or lost once and regained 11 times; these alternatives and all between are equally parsimonious (Lindenfors et al. 2003). Interestingly, all potential regains of this trait fall within the mustelid/mephitid group. It was also shown that both males and females of a mustelid species with delayed implantation were significantly larger in mass than their most closely-related sister species with direct implantation, suggesting that those with a larger mass are more likely to have delayed implantation (Lindenfors et al. 2003).

A common characteristic shared by males of many mammalian species in many polygamous mating systems is the enlargement of the testes during the breeding season, often due to sperm competition (Preston et al. 2003). With this increase, males benefit from having an increase in serum testosterone, increased sperm motility, and other functions that likely increase the mating success (Kaplan and Mead 1993). Larger testes allow more storage so that males have a higher chance to inseminate a female at multiple times. More storage may also allow a male to mate more often with a female, as paternity rate is hypothesized to be more proportional to number of copulations (Ginsberg and Huck 1989). In the hog-nosed skunk, the testis size would be expected to vary in accordance with the breeding season as in many other mammals. The testes would likely be largest during their breeding season and smaller outside of the breeding season.

I propose to collect data on the reproductive patterns of the hog-nosed skunk, *Conepatus leuconotus*, to determine the likelihood of this species utilizing delayed implantation as a reproductive strategy. Specifically, I will examine three sets of data to test the hypothesis that hog-nosed skunks reproduce at times other than the reported late winter season. First, I assembled and analyzed a data set of the collecting dates for *C. leuconotus* deposited in systematic collections. Collecting dates may reflect more frequent encounters by collectors with skunks when skunks are actively seeking mates during the year. For example, roadkilled *Mephitis mephitis* are more common during months when mating occurs (Dowler, pers. comm). Secondly, I examined seasonal variation in testis size to establish if a pattern suggests delayed implantation (Kaplan and Mead 1993). Third, I investigated observational data such as behavioral descriptions and photographs to determine validity of reports of reproduction occurring outside the reported breeding season in hog-nosed skunks.

MATERIALS AND METHODS

Various means of data collection were used to evaluate the reproductive habits of the hog-nosed skunk. Direct measurements of testis and collection dates were recorded from both salvaged roadkilled animals and retrieved from data in natural history collections (Appendix 1). Other data were assembled from previous studies on the animal, especially from those in central Texas. The imagery data were obtained from a study examining ecology of the hog-nosed skunks by Eric Pomposelli and behavioral data has come from observations of the animal made by myself and others researching hog-nosed skunks.

Roadkilled animals and collection specimens.— Specimens were used in analyses to find the sex ratio and the number of individuals collected by month to see if there were seasonal peaks in collection dates. I collected data from 752 records from throughout the hog-nosed skunk's distribution in six countries from 42 collections (Hafner et al. 1997). These specimens represented each month throughout the year. These data were found through the use of VertNet.org and iDigBio.org as well as data shared from other collections not found on those databases.

A subset of data from 616 hog-nosed skunks from five states was available from specimens collected as roadkilled animals or ones deposited in various collections across the USA. This subset was used for analysis of overall sex ratio of hog-nosed skunks from the USA. For analyses regarding skunks collected in the USA, all 616 samples were used. For the analyses regarding male and females and the month in which they were collected, 484 had the associated data required.

For analyses exclusively using males, only adults that had testis measurements, a total body mass, and a date on which the specimen was collected were used. Any roadkill specimens that had desiccation of the testis were excluded. Of the 616 specimens, 100 matched these criteria. Twenty-six male hog-nosed skunks were obtained by collecting roadkilled individuals across Texas and data from 73 male specimens came from records in the Angelo State Natural History Collection (ASNHC); one record was from the collection of Natural Science Research Laboratory at Texas Tech University. I measured and recorded testes with a ruler to obtain a length and a width in millimeters. The total testis size on specimen tags was usually recorded as a length and width measurement. The length has been multiplied by the width to obtain the testis measurement (Kaplan and Mead 1993). The mass of the animal was divided by the testis measurement to obtain a relative testis size as testes size scales with overall body size (Moller 1989). A subset of testes from the 26 males I collected was also weighed with the epididymis intact and without. Of each of these pairs, one testis was frozen at -80°C and the other was preserved in 10% formalin. For the testes percentage of body mass, the mass of the single testis was doubled. Whether testes were scrotal or nonscrotal was recorded for specimens collected or recorded from specimen tags, as scrotal males are often considered reproductive. To ensure that the data did not violate any assumptions, a Global Validation of Linear Models Assumption (GVLMA) and a Shapiro-Wilks test of normality were performed (Pena and Slate 2006). To compare both testicular size and relative testicular size, an ANOVA, a Type II ANOVA, and a permutational ANOVA were used. A Mann-Whitney U test was used when the data did not follow a normal distribution. Data were analyzed using RStudio version 0.98.1078 (R 2008).

Behavioral observations.—A study on denning ecology of hog-nosed skunks was conducted by Eric Pomposelli from April 2010 to October 2011 on a 3280-hectare plot on the Knickerbocker and Tweedy Ranches in Tom Green and Irion Counties, 24 kilometers west of San Angelo, Texas (31.256°N, 100.665°W). The area is between red prairie (to the east) and the semi-arid Edwards Plateau ecoregions of Texas and is characterized by rocky upland, juniper brush land, and mesquite scrubland habitats; Dove Creek forms a riparian zone. Live oak (*Quercus fusiformis*), juniper (*Juniperus pinchotii*), and mesquite (*Prosopis glandulosa*) trees, prickly pear cactus (*Opuntia spp.*), and native grasses comprise the dominant vegetation (Griffith et al. 2007).

Trapping and handling of animals followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011) and was approved by the Angelo State University Institutional Animal Care and Use Committee. Hog-nosed skunks were live captured by hand during nighttime spotlighting surveys and anesthetized using 10 mg (corresponding to 0.1 ml of the solution reconstituted to 100 mg/ml) of Telazol (Larivière and Messier 1996). Once anesthetized, ophthalmic ointment was applied to prevent ocular desiccation and standard measurements (total length, length of tail, length of hind foot, and length of ear) and mass, sex, and reproductive condition were recorded. A passive integrated transponder (Avid Identification Systems Inc., Norco, California) was inserted under the skin at the base of the neck, and each skunk was equipped with a 30g radiocollar (Telemetry Solutions, Concord, California), not exceeding 5% of the body mass.

Because of the similarity in pelage coloration among individuals of *C. leuconotus*, a unique mark was made by dyeing the hair of some individuals with Nyanzol-D to help with later identification (Hoogland 1995). Skunks were released at the site of capture after recovering and becoming responsive. A combination of daylight radiotelemetry, 24-hr infrared-triggered camera traps, and nighttime field observations were used for this portion of the study. The skunks were radio tracked during the daylight in order to identify their den sites. Individuals were generally located a minimum of every three days during non-reproductive months (April - December) and 4 or more times per week during reproductive months (January - March). Moultrie Game Spy 6.0-Megapixel digital cameras (Birmingham, AL) and 1 Reconyx HC500 (Holmen, WI) were used for the majority of the study. Each image was time and date stamped so that behaviors could be associated with an exact time and date. A total of 2,559 camera trap nights were available for this portion of the study. Unpublished data and images from Eric Pomposelli were used and analyzed for this part of the study.

RESULTS

Sex ratio and collection months.— Records were found in museum collections throughout the hog-nosed skunk's distribution in six countries: USA (616), Mexico (124), Nicaragua (5), Guatemala (4), El Salvador (2), and Honduras (1). Sex was 401 males (53.33%), 185 females (24.60%), and 166 specimens with an unknown sex (22.07%). The unidentifiable specimens were likely indiscernible due to desiccation or decay as a roadkilled specimen or preparators failed to record sex of the animal. Without the unidentified sexes, the male to female ratio was 2.17:1. Records that included both sex and date of collection were used to address variation in specimen collection by month (Fig. 1). Highest peaks of specimen collection for males occurred in January, February, August, and October. Lowest peaks of collection occurred in April, May, June, July, and September (Fig. 1). For females, the highest peak month was June.

The higher latitude subset of 616 specimens from the United States was further analyzed because of the extra data associated with the collection record. Of the 616 specimens examined, 338 were males (54.90%), 146 were females (23.70%), and 132 unidentified (21.43%). For animals with known sex, the male to female ratio was 2.37:1. For the USA subset of specimens, peak months for male collection were January, February, March, August, October, November, and December with the two highest peaks in February and November (Fig. 2). For females, the highest peak month was again June. Two of fifteen females with reproductive data had embryos present. One collected on 22 March 2004 had four well-developed embryos present. Crown-rump lengths for these four were 127mm, 123mm, 124mm, and 138mm with mass of 23.5g, 25.5g, 25g, and 37g, respectively.

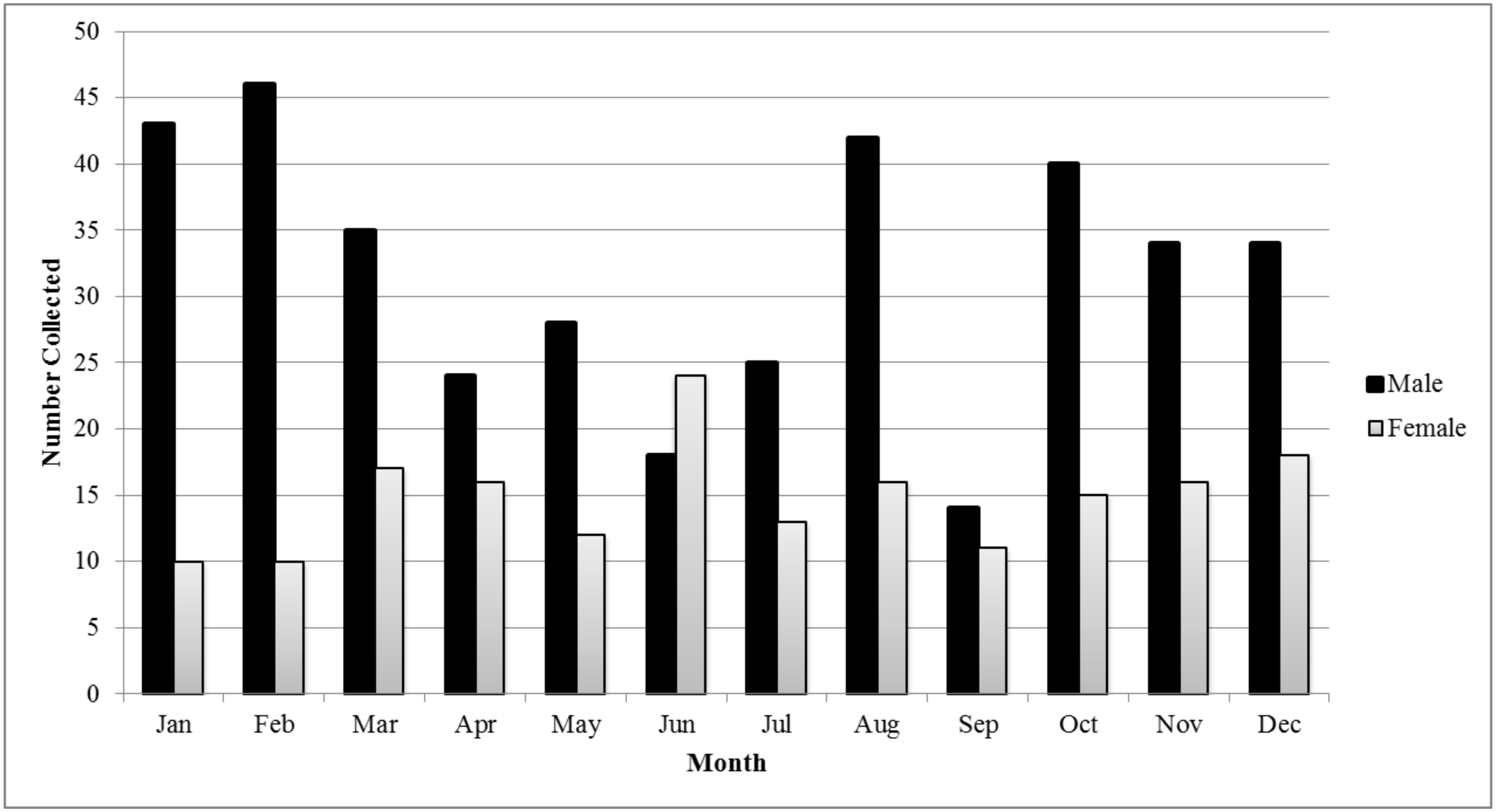

FIG. 1--Number of *Conepatus leuconotus* from 586 records in 42 collections by month in both the United States and five Central American countries. Those with unidentified sexes are excluded.

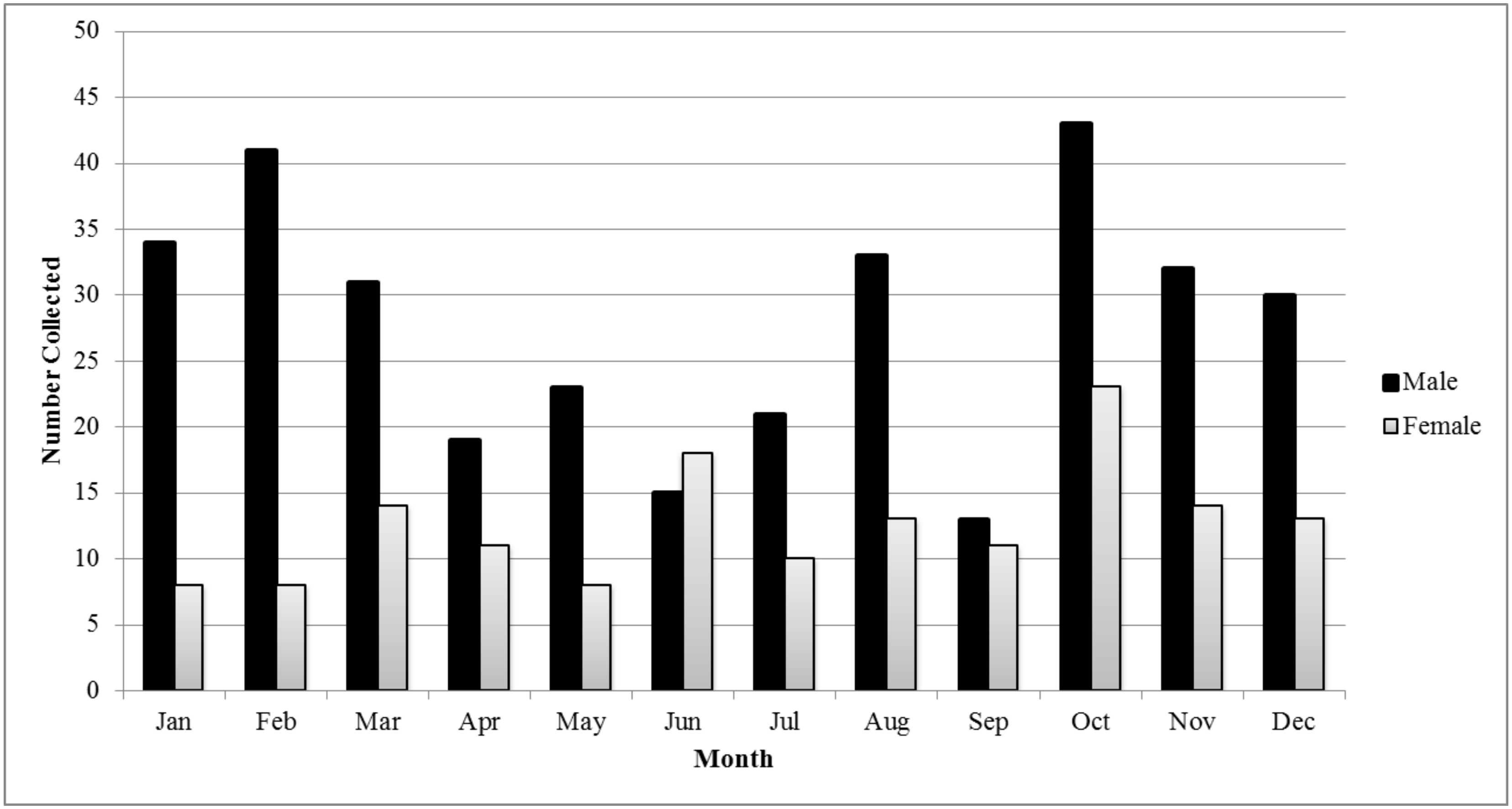

FIG. 2.-- Number of *Conepatus leuconotus* of each sex collected by month from 484 individuals in the United States. Those with unidentified sexes were excluded.

A female collected on 17 March 2016 had three embryos present measuring 29mm, 25mm, and 19mm with mass of 1.2g, 0.87g, and 0.47g, respectively. Of the 616, six specimens from the USA had no date associated.

Testicular size.—The testis size of 100 males from the USA subset was graphed against date to compare to that of Kaplan and Mead, 1993 (Fig. 3). Testis size was then compared to determine if the size (length × width) varied by month. The length times width measurement was first tested using an ANOVA for any difference among months and no significance was found (F=1.5613, P=0.12). Because there was no difference, a second ANOVA was then conducted to test if testis size varied with body mass. Data were normal and met all assumptions using a global validation of linear model assumptions (GVLMA global test, (P=0.89), so a Type II ANOVA was performed to test if testis size varied significantly with body mass (Fig. 4). This revealed that testis size (F=8.9117, P=0.004) did vary significantly with body mass. Because of its small sample size (n=2), the month of June may have had a stronger effect on the results of the test, but the exclusion of this month from the analysis still yielded similar results (F=11.4614, P=0.0012) in that the means still vary. Because of the significant relationship between testis size and body mass, other calculations were performed using mass divided by testis size giving a relative testis size (Fig. 4). A Type II ANOVA was then performed to compare variation of relative testis size by month. Results indicated no significant difference in relative testis size among the months (F=1.2874, P=0.26). A further test, a permutational ANOVA (10,000 iterations) was performed to ensure that the relative testis size did not significantly differ by month. This also yielded no significant difference (P=0.24) among months (Fig. 5). Overall body mass was compared across each month of the year with the exception of June, as its sample

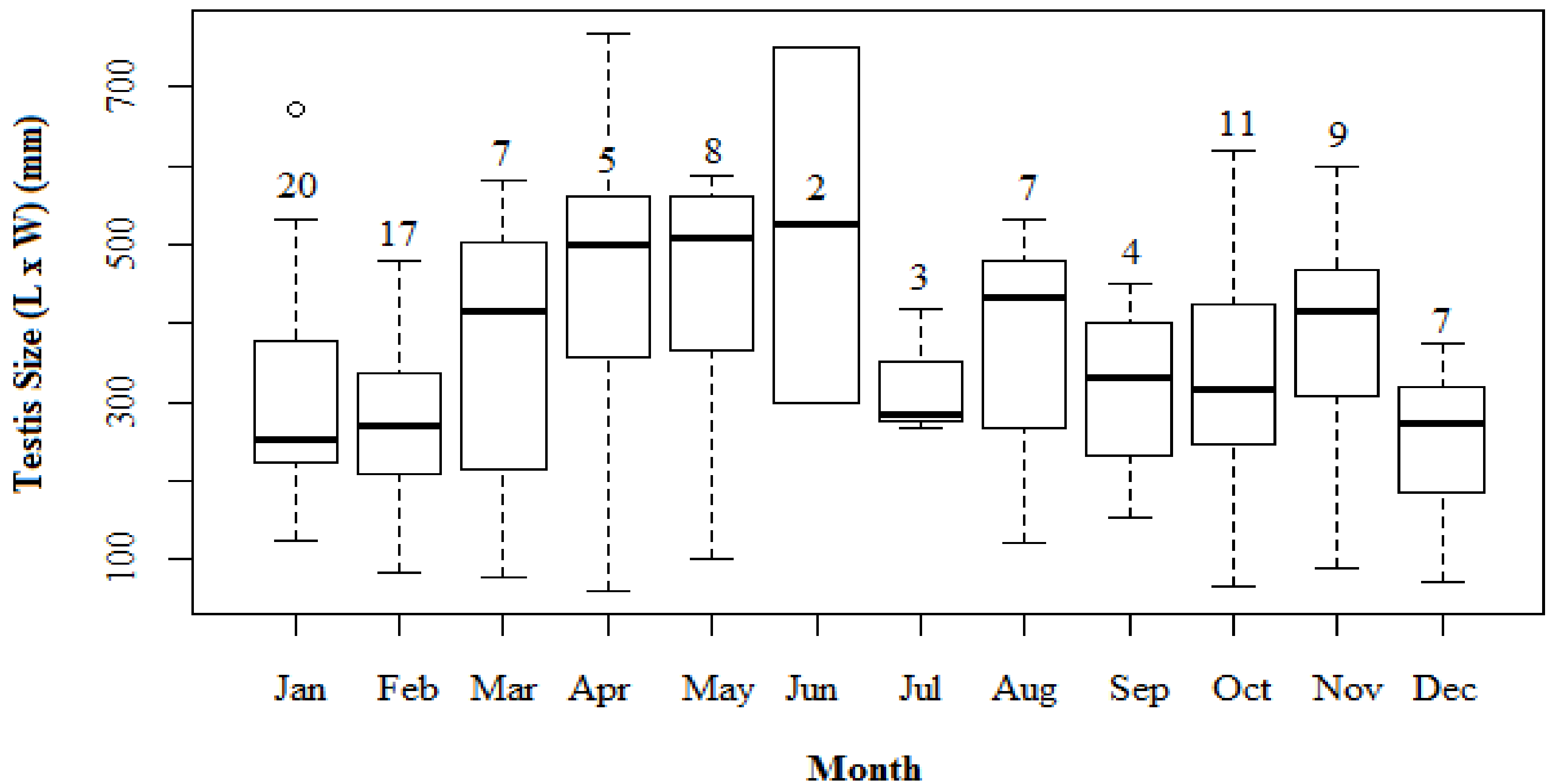

Fig. 3—Box-and-whisker plot of testis size (L × W) (mm) by month of 100 *Conepatus leuconotus* males. Sample size is given above each box. This comparison is made similar to that of Kaplan and Mead (1993) for a monthly comparison of testis size.

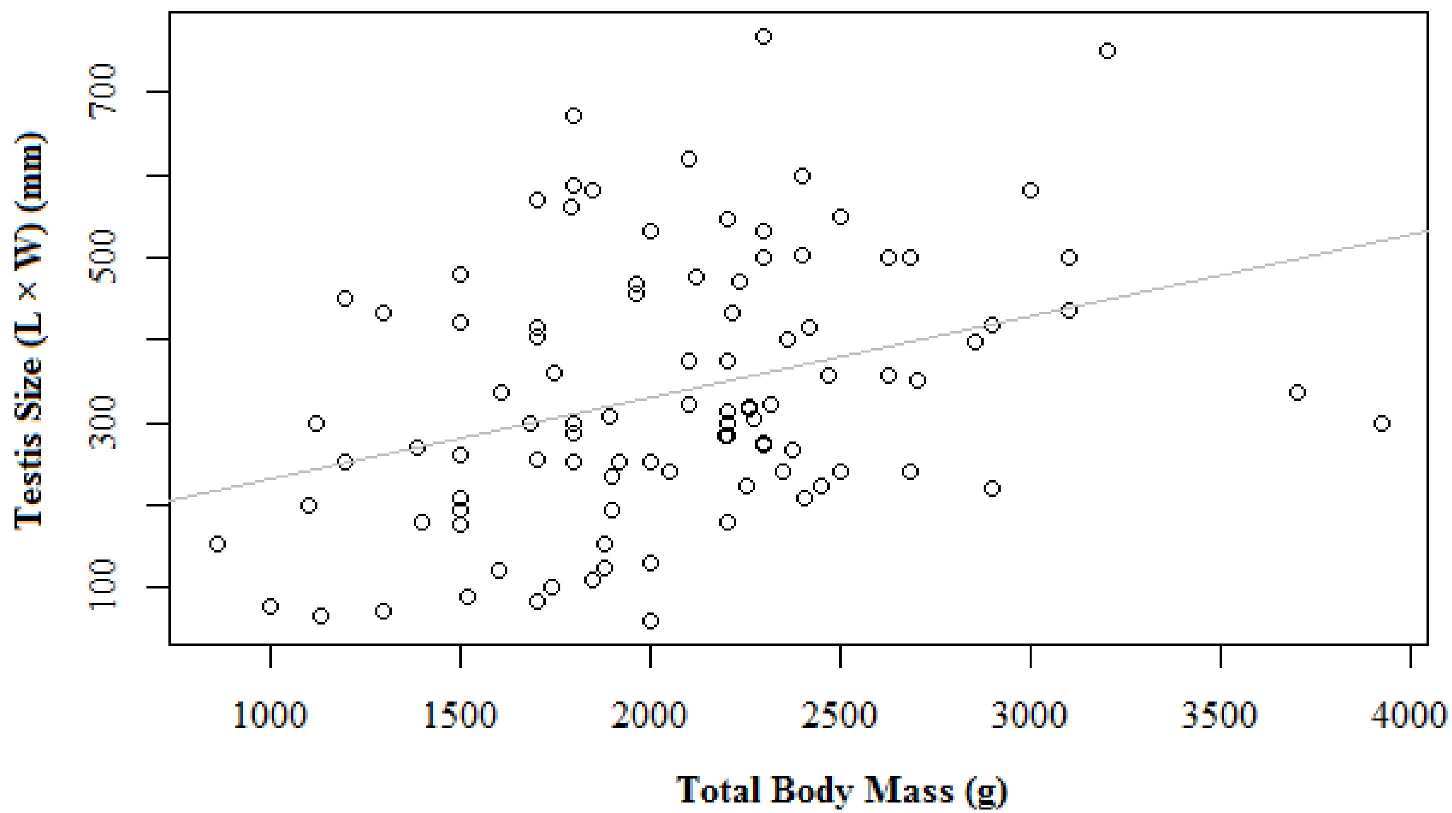

FIG. 4--Scatter plot of testis size of *Conepatus leuconotus* against total body mass with a linear best-fit line. Testis size does vary by body mass (Type II ANOVA table, F=8.9117, *P*=0.0037).

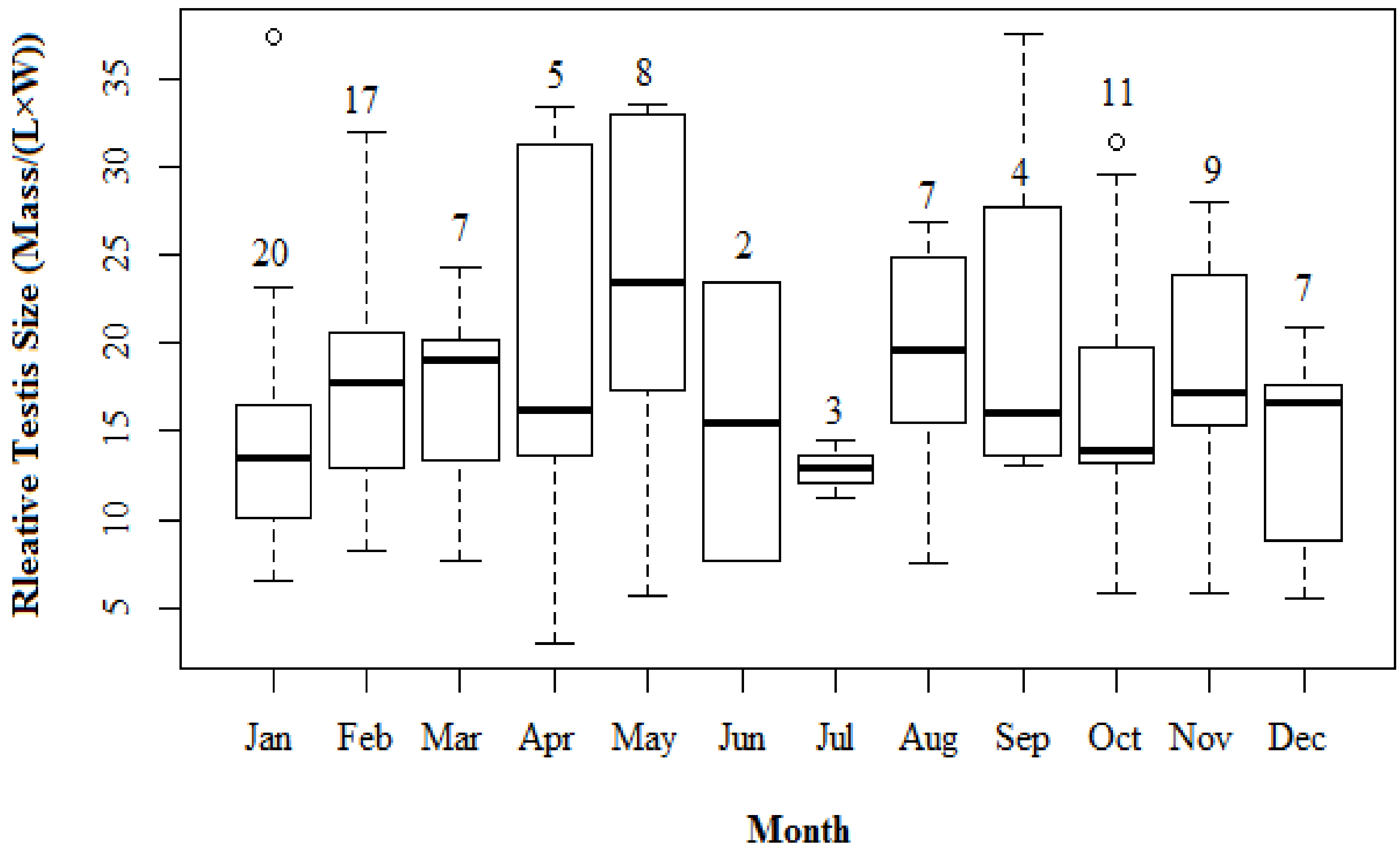

FIG. 5--Box-and-whisker plot shows no difference of relative testis size by month for 100 male *Conepatus leuconotus* sampled. The maximum and minimum and are represented as well as any outliers. Sample size for each month is given above each box.

size (2) was too small (Fig. 6). An ANOVA was conducted on the mass of the hog-nosed skunks by months, excluding June, and found that there was no significant difference in body mass by month (F=0.8995, P=0.1).

I compared testis size during the purported breeding season (January to March) to the non-breeding season (April-December). The data were not normally distributed (Shapiro-Wilk Normality Test, w=0.9179, P=0.01), thus a Mann-Whitney U test was used to compare the relative testis size by season. The relative testis size of specimens from the three reported breeding months, January, February, and March was combined and compared to that for the other nine months combined. No significant difference in testis size based on the reported breeding season and the non-breeding season (W=841.5, P=0.18) was found.

Of the 233 specimens, only 48 referenced the position of the testis as either scrotal or non-scrotal. The relative testis mass of scrotal males and the relative testis mass of non-scrotal males were compared using a permutational t-test (10,000 iterations). Surprisingly, there was no significant difference ($P=0.32$) between the two (Fig. 7). While the sample size of the testes position was small, the months with the highest percentage of scrotal males were January, December, February, and October. Sample sizes were too small to compare scrotal and nonscrotal males across all months as some were heavily represented and some not at all. The mass of the testis with the epididymis intact ranged from 1.2g to 4.9g. The average mass was 2.90g±0.27 (n=26). The mass of the paired testes without the epididymis ranged from 0.3g to 3.4g. The average mass was 1.71g±0.19. As a percentage of overall body mass, the testes ranged from 0.36%-0.39% with an average of 0.17%± 0.08. Data available across each month was insufficient and no statistical comparisons were able to be made.

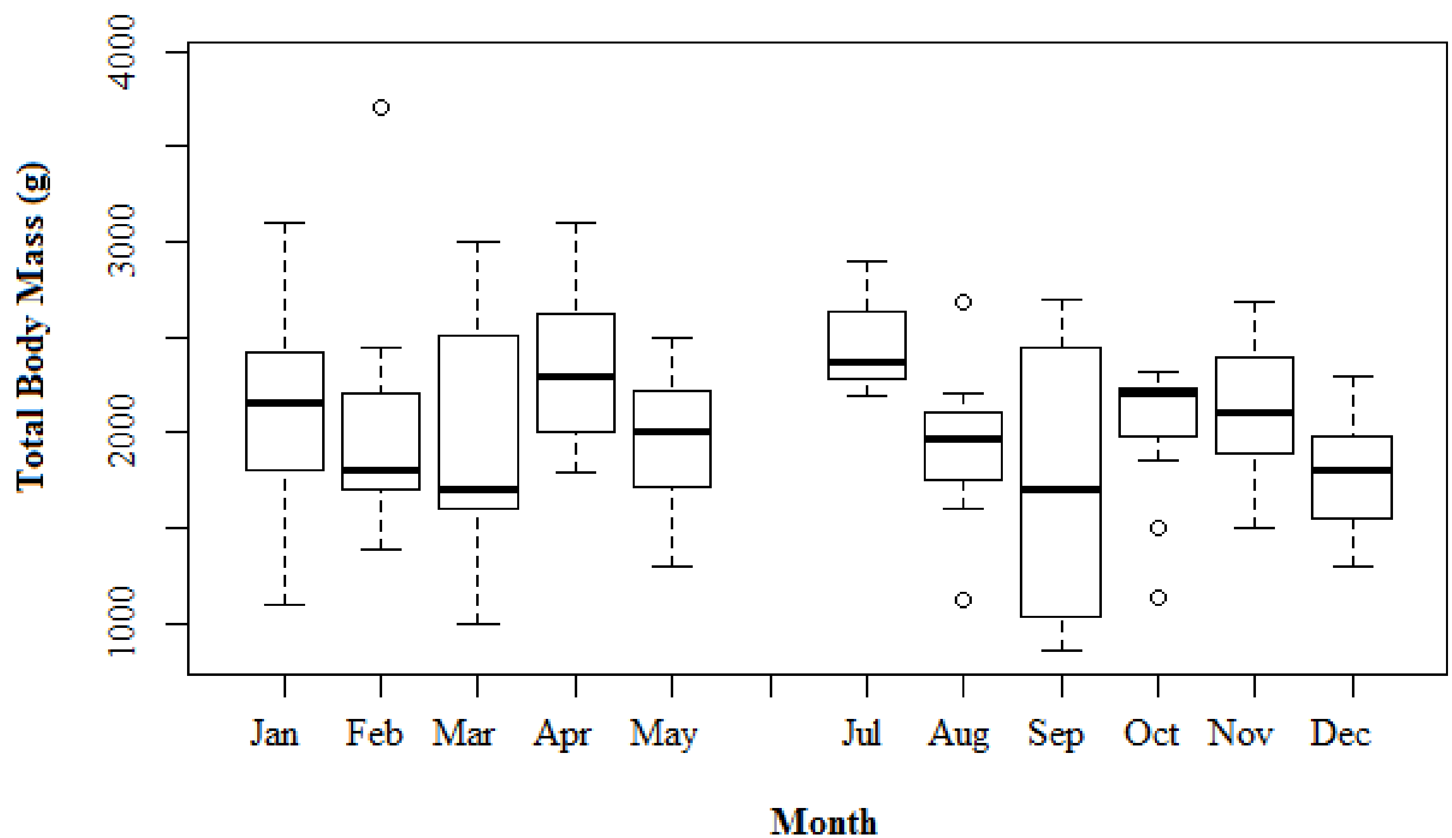

Fig. 6—Box-and-whisker plot shows no difference of total body mass for 98 male *Conepatus leuconotus* in each month. The month of June was excluded due to its small sample size. Sample size for each month is given above the box.

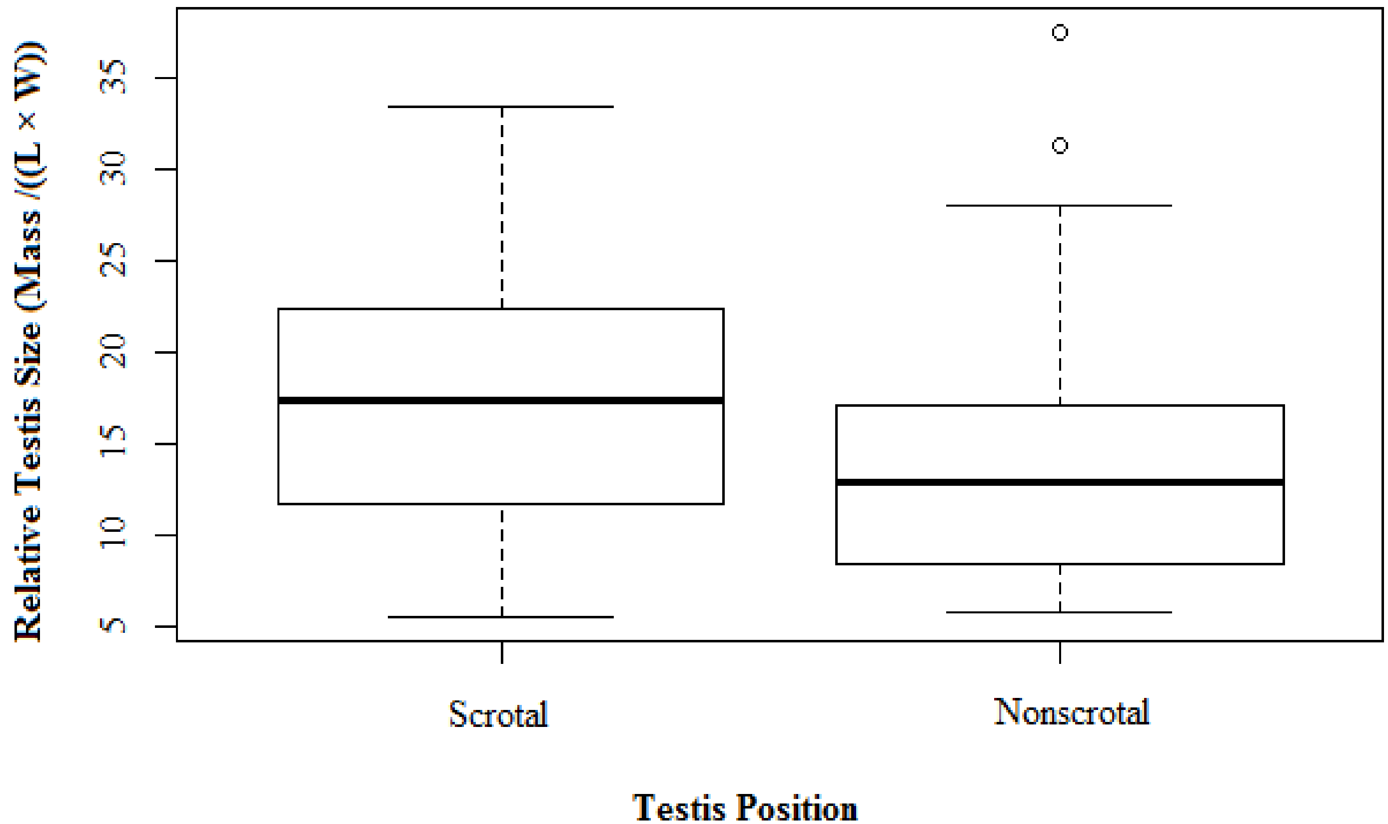

FIG. 7-- Comparison of relative testis size in scrotal and non-scrotal male *Conepatus leuconotus*. No significant difference was found. The maximum and minimum as well as any outliers are represented (n=48). Sample size for each group is given above each box.

Behavioral observations.-- During one of the spotlighting surveys, a collared female was detected on August 28, 2015 that was being closely followed by an uncollared individual. The uncollared animal was larger in size, but was not confirmed to be a male. On November 9, 2002, another observation was made of one male (that was later caught by hand) following another at a close distance. These actions suggested reproductive behavior (Dowler 2004).

Images from camera trapping revealed some instances of attempted reproduction of hog-nosed skunks. On 28 August, 2011, an image of a scrotal male with large testes was taken. On 30 August, 2010, a pair was captured together in an image at 07:18. The marked individual in the photo was a female, but the sex of the other was unconfirmed. It is unlikely that the sex of the unconfirmed individual was a female. Seven separate images of co-denning of a male and female were captured by the cameras. Co-denning of different males and females also occurred in December, and twice in August of 2008 (Brashear, pers. comm). On 29, August, 2010 an uncollared male was observed attempting copulating with a collared female outside of a den. On 12 and 13 September, 2010, multiple images were captured of a different pair consisting of a collared male attempting copulation with a collared female at the entrance of her den (Fig. 8). Beginning at 08:33, the male was first seen attempting copulation. Multiple attempts occurred throughout the day until the male finally left at 22:42. There were no images of copulation during the reported breeding season of the animal, however most copulation probably takes place away from den sites. Young were detected around den sites between April and August. The first image from a camera trap that showed a kit with a female outside of the den was on 21April, 2011. The last image

FIG. 8.-- Image from 12 September 2010, showing a collared male mounting and attempting copulation with a collared female outside of her den site. There were multiple images of attempted copulation from these two individuals. The marking on its back allowed this individual to be identified as a male.

of a kit with a female for the year was seen on 28 August, 2011.

DISCUSSION

Sex ratio and collection months.-- The majority of hog-nosed skunk specimens in the ASNHC, and presumably other collections, come from salvaged animals that had been struck by a vehicle. The male biased sex ratio likely reflects wider movements of males compared to females and thus, a great risk from vehicles on roads. This pattern is supported by home range studies on radio-collared individuals. Home range for male *C. leuconotus* ($\bar{X}$=177.9 ha) is significantly larger than that of females ($\bar{X}$=83.4 ha) in Texas (Brashear et al. 2015). Because of females' relatively smaller home range, it is not surprising that they are much less frequently encountered than males. Males, as in other solitary carnivores, may be moving further on average to increase the probability of mating with multiple females (Sandell 1989). In February and October there were 42 and 43 males (>13% each of the total male sample) collected, respectively. If hog-nosed skunks do mate exclusively during the suggested breeding season, then the number of males collected throughout the year would be expected to peak in January, February, and March, as they are likely searching for females.

There has also been high roadkill mortality in west-central Texas reported by others. In a roadkill skunk survey, highest rates for males were found in January, February, October, and November; however, females had no discernable peak (Ferguson, pers. comm). These data are consistent with the collection dates found throughout museum collections. Striped skunks in Texas are more frequently found as roadkill animals in February, which is believed to coincide with the breeding season (Dowler, pers. comm). In Kansas, there was a dramatic increase in number of striped skunks found dead along the highway (Smith-Patten and Patten 2008). This increase coincides with the striped skunks mating season, as February and March are months in which males have been known to travel great distances in search of receptive females. If male hog-nosed skunks follow a similar pattern to that of the striped skunks, then they may be actively searching for mates at peak collection times. Since prevalence seems to peak at two times of the year, males may be trying to mate with a female who had lost her pregnancy earlier in the season, thus giving her a second chance to conceive and increase fitness; however, the pattern for this would likely show a secondary peak in May or June.

Of the female specimens, only two were noted to have detectable embryos. Both of those were in March. This embryological stage is consistent with the findings by Bailey (1905), Patton (1974), and Davis and Schmidly (1994), but gives no insight on delayed implantation.

Testicular size.-- In many mammals, testicular size is generally dependent on the mating system. In those in which multiple males attempt to mate with multiple females, there is a tendency for a corresponding increase of testis size due to sperm competition, i.e. greater sperm production and larger ejaculates would be favored by selection leading to larger testis size. This is often seen in polygamous mating systems in which the animal has a breeding season. It is seen in many carnivores such as the coyote (*Canis latrans*) (Minter and Deliberto 2008), lynx (*Lynx lynx*) (Goritz et al. 2006) and the more closely-related badger, *Taxidea taxus* (Mead 1993) and western spotted skunk (Kaplan and Mead 1993). Testicular size can usually be an indicator of reproductive success (Preston et al. 2003). It is also predictive of ejaculate volume and serum testosterone, which are both likely to increase mating success (Moller 1989). The western spotted skunk, an animal with known delayed implantation, testis size greatly increases during its reproductive months, September to

November, and this also correlated with both ejaculate volume and serum testosterone (Kaplan and Mead 1993).

In another carnivore that experiences delayed implantation, the American badger, the testes of males had a clear increase in weight during the breeding season, March to June (Wright 1969). During non-reproductive periods, the animals' testes regressed and remained inactive. Implantation is expected to occur in early February. The discernable peaks for the badger coincide with the three months of estrus that females experience each year. The implantation of the late-stage embryo then is delayed until early February (Wright 1969).

Additionally, Schulte-Hostedde et al. (2003) have postulated that males with larger testes produce more testosterone; this leads to an increase in lean muscle mass. Because males tend to have more muscle mass than females, it may be a sexually selected trait and a secondary consequence of large testis size (Shulte-Hostedde et al. 2003). It would be metabolically costly to maintain large testes throughout the year, especially in an animal that may have difficulty finding food during certain parts of the year. In some mustelids, such as the European badger, it has been reported that adult males of the species are lighter during their active breeding season (Relexans and Canivenc 1967). If the hog-nosed skunk did utilize delayed implantation, it may be expected to find males with the lowest body mass during the months in the active breeding period. No change in body weight was found.

Hog-nosed skunks that are actively breeding and searching for mates would be expected to have a larger testis size. My data showed that the relative testis size did not vary significantly by month. This could imply that males are actively searching for mates at other times of the year than their reported breeding season.

Testis size of this species may be dependent on the effects of photoperiod. The pineal gland and its associated hormones, such as melatonin, may be important for seasonality of reproduction and the testicular cycle, as reported for the western spotted skunk (Berria et al. 1990). In mink, an animal with well-documented delayed implantation, removal of the pineal gland resulted in a non-terminable diapause (Bonnefond et al. 1990).

Scrotal testes are usually an indicator of active reproduction in mammals, whereas testes are nonscrotal during other seasons. The scrotum maintains temperature of the testes lower than the body which typically prevents a decrease in spermatogenesis and sperm motility (Hansen 2009). In those mammals that have distinct reproductive seasons, testes are scrotal during those periods and nonscrotal during other parts of the year. In some small mammal groups, position of testes in males is an accurate indicator of reproductive status with 87%-94% accuracy (McCravy and Rose 1992). It would likely be metabolically wasteful for males to retain scrotal testes year round. With many accounts for scrotal male hog-nosed skunks in various times of the year, especially in fall and late winter, it is plausible that males may be trying to actively mate during these times. It is interesting to note that relative size of the testes did not differ in the position of the testes. This inconsistency may be attributed to a low sample size, as only 498of the 177 male specimens referenced the position of the testes. Additionally, specimen preparators likely vary in their ability to determine scrotal from nonscrotal animals. This problem may be compounded by difficulties in assessing animals that had been run over by a vehicle. Collection data can be helpful in understanding natural history aspects. With more complete data regarding the position of the testes, it may be likely to distinguish an active breeding period. A larger sample size with each month represented, preferably by a single observer, would prove useful in determining testes position during the mating season.

Behavioral observations.— Seven total mating attempts on two separate occasions were recorded by remote cameras at den sites. Because copulation is probably more likely to happen away from the den sites, camera traps are very limited in their ability to record actual reproductive behaviors. The images reported herein do provide some record of the mating behavior outside of the reported mating season. To date, there is only one other instance that has been reported in reproductive behavior outside of the reported mating season of *Conepatus leuconotus* in a captive pair in November (Dragoo and Honeycutt 1999).

Mate guarding strategies are common in males, especially those in polygynous mating systems (Sherman 1989). The observations of one hog-nosed skunk following closely to another may suggest courtship behavior. The close following of the other animal was akin to that of a mate-guarding strategy often used to ensure paternity (Parker 1984). In the cases reported herein, sex of the animals could not be confirmed, but the size discrepancy in the animals may be indicative of sex. All of these observations took place outside of the breeding season and suggest that males may be attempting copulations at times other than their reported breeding season.

The use of assays to detect different levels of associated hormones, such as progesterone in females, would provide insight on the time of estrus of females and may even help identify when an early-stage embryo is present (Bernard 1991). Hormone regulation of delayed implantation likely varies among taxa in which it is found and is not yet fully understood, but the detection of prolactin, among other hormones, at lower levels may signify a female with an unimplanted early-stage embryo (Murphy et al. 1981).

Three lines of evidence suggest that hog-nosed skunks may breed beyond the reported season. Testis size did not show a peak during months reported as the breeding season. Obviously, scrotal males were observed in late summer. Secondly, activity of males, as indicated by collection date, did not have a single pea in late winter, corresponding to the reported breeding season. Third, sightings of presumed pairs in fall and photographic images showing copulation in late summer suggest late summer and fall breeding. These observations are consistent with some degree of delayed implantation, as in other skunk species. Further studies will be required to ascertain the degree of delayed implantation, if at all. Observations of a captive breeding colony have proven successful in understanding the physiology of reproduction as in *Spilogale* (Mead 1968 a, b) and would likely contribute to the knowledge of the hog-nosed skunk's reproductive biology.

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APPENDIX I

List of countries and collections from where data from specimens of *C. leuconotus* are found.

Locality	Number from locality	Collection	Number from collection	Locality	Number from locality	Collection	Number from collection
El Salvador	2	UMMZ	2	New Mexico	47	CAS	1
Guatemala	4	LACM	3	New Mexico		MSB	27
Guatemala		TCWC	1	New Mexico		UFMNH	1
Honduras	1	AMNH	1	New Mexico		UMMZ	1
Nicaragua	5	KUM	4	New Mexico		USNM	15
Nicaragua		TCWC	1	New Mexico		UTEP	2
Mexico	124	AMNH	4	Oklahoma	2	OMNH	1
Mexico		KUM	48	Oklahoma		USNM	1
Mexico		LACM	13	Texas	507	ACUNHC	5
Mexico		LSUMZ	3	Texas		AMNH	7
Mexico		MSB	11	Texas		ASNHC	232
Mexico		MSUMR	1	Texas		BMNH	6
Mexico		OMNH	5	Texas		BYU	1
Mexico		TCWC	11	Texas		CCMNS	1
Mexico		TTU	1	Texas		CM	1
Mexico		UMMZ	25	Texas		CSUTC	3
Mexico		USNM	1	Texas		CUMZ	1
Mexico		YPMVZ	1	Texas		DaMNH	2
Arizona	49	KUM	4	Texas		FHSM	1
Arizona		LACM	1	Texas		FLMNH	9
Arizona		MSB	20	Texas		FMNH	8
Arizona		TTU	1	Texas		FWM	5
Arizona		UMMZ	4	Texas		HPU	11
Arizona		USNM	17	Texas		KUM	10
Arizona		UTEP	1	Texas		MCZ	5
Arizona		YPMVZ	1	Texas		MSB	9
Colorado	11	DMNS	11	Texas		MSUMR	1

Appendix I. *Continued*

Locality	Number from locality	Collection	Number from collection	Locality	Number from locality	Collection	Number from collection
Texas		MVZ	3	Texas		TMM	1
Texas		MWSU	17	Texas		TTU	43
Texas		REDPATH	1	Texas		TXST	2
Texas		SHSU	1	Texas		UMHB	1
Texas		SRSU	6	Texas		USNM	53
Texas		TAMUCC	2	Texas		UTA	1
Texas		TARL	7	Texas		UTEP	5
Texas		TCWC	45	Texas		WWF	1