Spatial Ecology of the Plains Spotted Skunk, *Spilogale interrupta*, in Texas

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Plains Spotted Skunk from Coryell County, Texas
Photo credit: J. Clint Perkins

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Executive Summary

The plains spotted skunk (*Spilogale interrupta*) is a species of concern in Texas and multiple studies propose its populations range-wide have declined significantly since the mid-20th century. The species is currently being considered for listing under the U.S. Endangered Species Act and, though the listing decision is still pending at the time of report finalization (August 2023), information presented herein was included in the listing decision. This report details a 30-month assessment of a plains spotted skunk population in Harris and Waller counties, Texas to address knowledge gaps including plains spotted skunk spatial ecology, occupancy, habitat associations, rest site selection, cause-specific mortality, and presence of toxicological organochlorine compounds.

We conducted this research in southeastern Texas in the Katy Prairie, part of the Western Gulf Coastal Plain ecoregion. This ecoregion was originally a coastal tall grass prairie; however, due to shifts in land use to agriculture, urbanization, and ranching, tallgrass prairie is now considered one of the least common landforms in the state. Our study sites were at two locations managed by the Coastal Prairie Conservancy. These locations comprised native and nonnative range lands, cultivated fields, stream corridors, and ephemeral wetlands.

We captured plains spotted skunks between January 2019 and August 2021. Animals were fitted with GPS transmitters that recorded at least four spatial relocations per 24-hour period. During the study we captured 50 unique individuals and attached transmitters to 46. Of these, we recorded spatial data on 30 (16 male, 14 female) sufficient for home range analysis.

Female spotted skunks had home ranges (95% minimum convex polygons-MCP) of 26.15 hectares (ha) across all seasons with a core area size (50% MCP) of 8.03 ha. In contrast, males had home ranges 5.8 times larger than females with 95% MCP of 152.35 ha and 2.64

times the core areas with 21.24 ha averaged across all seasons. Seasonal differences were noted with males having larger home ranges than females in spring and summer, but not fall and winter. Home ranges of males were largest in spring and similar for all other seasons. Females did not differ in home range size over the four seasons.

We utilized an array of camera traps to locate plains spotted skunks to increase success of live trapping efforts and to monitor locations of animals. In addition, data from camera traps was used to assess plains spotted skunk occurrence within varying land cover types, determine how plains spotted skunk occurrence was affected by presence of Macartney rose and cattle, and changes in spotted skunk occurrence due to management activities. At Warren Ranch, we recorded 189 spatially and temporally unique plains spotted skunk detections from 2019 through 2021. Eighty four percent of all detections occurred in a land cover type we classified as natural using a supervised classification of areal imagery.

We performed a hierarchical occupancy analysis using our camera trap dataset. We assessed detection probability, plains spotted skunk initial occupancy, and the probability that plains spotted skunks within discrete patches would either become extinct or colonize new patches. Our results suggest that as distance from camera stations to the nearest road increased, our ability to detect spotted skunks also increased. We were unable to disentangle whether the skunks were avoiding roads due to traffic or anthropogenic affects that created edges. We were unable to find support for environmental models that influenced both spotted skunk initial occupancy and patch colonization. Our most supported model indicated an inverse relationship between the proportion of natural land cover within a patch and the probability that skunks would become extinct with the patch. As natural land cover decreased, the probability of extinction increased. Moreover, we were able to directly link decreased natural land cover to anthropogenic activities. Portions of individual pastures were mowed as part of a management strategy to combat invasive species and shrub proliferation and one patch was converted to produce winter forage for cattle. Cumulatively, our results support the hypothesis that plains spotted skunk decline can be attributed to habitat loss and alteration.

Diurnal rest sites are essential resources for most carnivores as they provide shelter from the elements and protection from predation. We used radiotelemetry to locate plains spotted skunk rest sites and performed vegetation analysis of these sites as compared with randomly selected points to determine specific habitat associations used by skunks. From May 2019 through February 2021, we monitored rest site selection of 30 plains spotted skunks (13 females and 17 males). We recorded 652 total tracking events at 426 unique rest sites (212 female and 214 male). Macartney rose and southern dewberry brambles were the overhead cover at 90% of rest sites with Macartney rose further partitioned by clump height (75.4%, $n = 321$; 162 small, 134 medium, and 25 large). Cumulatively, Macartney rose brambles, southern dewberry brambles (14.6%, $n = 62$), and bunch grass (6.1%, $n = 26$) composed 96% of all rest sites at the site. A cover model ranked as the top model evaluated and included southern dewberry as overhead cover, height of visual obstruction, depth of grassland litter at the center of the rest site, total number of small Macartney rose brambles, and percentage of Macartney rose as ground cover. Relative to Macartney rose, the odds of a skunk selecting for southern dewberry as the overhead cover was 5-fold greater, contrary to the predicted positive influence of Macartney rose overhead cover in the cover hypothesis. Spotted skunks may have stronger selection for southern dewberry because it enhances their cryptic appearance and for the denser cover it provides that may reduce detection by predators.

There are few studies of cause-specific mortality in plains spotted skunks, but historical studies reported deaths primarily due to human activity such as fur-trapping, killing of nuisance animals, or by vehicle collisions. In locations with less anthropogenic influence, avian predators and mammalian predators are most important, though other natural causes are hard to determine. We recorded twelve plains spotted skunk mortalities (6 male, 6 female) and categorized seven as predation (4 avian, 2 mammalian, 1 unknown). Other mortalities were classified as natural (4) and unknown (1). A necropsy was performed on an adult male with a natural mortality. It likely died of a combination of hepatorenal insufficiency due to amyloidosis and cardiac mineralization.

No toxicological analysis for organochlorine pesticides and polychlorinated biphenyls (PCBs) has been previously performed for spotted skunks. We submitted liver samples from ten plains spotted skunks (three from Harris Co., Texas and seven from central South Dakota) and nine striped skunks from Harris Co., Texas for analysis of persistent organic pollutants. We detected three DDT metabolites including 4,4'-DDE present in six of seven plains spotted skunks from South Dakota (86%) and one plains spotted skunk from Texas (33%). Additionally, 2,4'-DDE was detected from two plains spotted skunks from South Dakota (29%) and 4,4'-DDD was detected from two additional plains spotted skunks from South Dakota (29%). We detected two chlordane congeners (Heptachlor-epoxide and oxychlordane) in 4 of 7 plains spotted skunks from South Dakota, but no detectable chlordane congeners were present in the three plains spotted skunks from Texas. PCB congeners were detected in every sample analyzed. Eighteen were detected from South Dakota plains spotted skunks with an average of 9.57 PCB congeners per skunk. Plains spotted skunks from Texas had 22 unique PCB congeners with an average of 15 congeners per skunk.

Despite our efforts to follow reproductive females and describe kit rearing, we were unable to observe young in reproductive dens. We monitored 19 females and provide notes on breeding ecology for 11. Four females were confirmed to have birthed at least once by visual confirmation of young, one female showed evidence of nursing, but kits were never observed, and four individuals showed signs of either being pregnant or having copulated, but kits were never observed. Three individuals were non-breeding at the time of capture.

Chapter 1: Plains spotted skunk background information

Introduction

By most accounts, plains spotted skunks (*Spilogale interrupta*) were abundant during the first half of the 20th century, though research during this time was limited to dietary study (Selko 1937; Crabb 1941), development (Crabb 1944), ecology (Crabb 1948), and taxonomy (e.g., Howell 1906). Research on spotted skunks during the remainder of the 20th century was sparse, at best, with a single systematic monograph (Van Gelder 1959), studies of reproductive physiology (Mead 1968 a, b, and others) and a radio-telemetry study conducted in 1981-1982 in southeastern Missouri (McCullough and Fritzell 1984). Despite its historical abundance, the plains spotted skunk experienced a range contraction and population decline that was identified as starting in the 1940s from examination of fur harvest records (Landholt and Genoways 2000, Gompper and Hackett 2005). As early as 1995, a retired mammalogist in Texas, Rollin Baker, recognized that plains spotted skunks along the Texas Gulf Coastal Plain had declined relative to survey work that he had performed periodically since 1938 (Baker 1995). In 2012, the United States Fish and Wildlife Service (USFWS 2012) issued a 90-day finding that indicated protection of the skunk may be warranted under the Endangered Species Act. The conservation concern for plains spotted skunks spurred a flurry of research, but the species is still understudied relative to many other North American carnivores (Jachowski and Edelman 2021).

Classification and Nomenclature

Plains spotted skunks are members of the family Mephitidae, separated as a distinct family from Mustelidae by Dragoo and Honeycutt (1997). Within the family, the genus *Spilogale* has seen multiple revisions, with as many as 14 species recognized (Howell 1906) to as few as two (Van Gelder 1959). The plains spotted skunk was originally described as *Mephitis putorius* by Rafinesque in 1820. The history of how Rafinesque came to name this species and the determination of the type locality being Chariton or Saline County, Missouri is available via Woodman and Ferguson 2021. In 1959, spotted skunks in the United States were recognized as a single species, *S. putorius* (Van Gelder 1959). Later, research by Mead (1968a, 1968b), revealed that spotted skunks in the western U.S. had delayed implantation and different breeding periods, relative to the eastern spotted skunks. On that basis, *S. gracilis* was separated from *putorius* as the western spotted skunk. The plains spotted skunk was then recognized as *S. putorius interrupta*, one of three subspecies of the eastern spotted skunk, with the others being *S. p. ambarvalis* from Florida and *S. p. putorius* from the Appalachian region north of Florida. Most recently, nucleotide sequencing studies by Shaffer et al. (2018) proposed that *S. p. interrupta* was genetically distinct from the other subspecies, and McDonough et al. (2022) performed additional molecular research that resulted in recognition of the plains spotted skunk as a unique species, *Spilogale interrupta*, one of seven species in the genus *Spilogale*.

<u>Common Names:</u> plains spotted skunk, prairie spotted skunk, spotted skunk, civet cat, civet.

Present Legal Status

<u>International:</u> Vulnerable. International Union of the Conservation of Nature as *Spilogale putorius* (Gompper and Jachowski, 2016).

<u>Federal:</u> Previously listed as a Category 2 species (listing as Threatened or Endangered may be warranted, but data are insufficient). United States Fish and Wildlife Service conducted a species status assessment and listing review of the species in summer 2022. Findings will be published in the Federal Register prior to September 30, 2023.

<u>State:</u> Texas Species of Greatest Conservation Need; S1-S3 Ranking (Critically Imperiled [S1], Imperiled [S2], or Vulnerable [S3]; TPWD, 2020).

Physical Description

Plains spotted skunks are diminutive, slender carnivores exhibiting a series of broken white dorsal stripes on an otherwise black pelage (Kinlaw 1995). Total length of adults in Texas average 515 mm for males and 473 for females and weight averages between 680 g (M) and 450 g (F; Schmidly and Bradley 2016). Their body shape is elongated with short limbs and plantigrade limb posture. Spotted skunks are known to be semi-arboreal and have multiple pads on the soles of the feet as well as slightly curved forefeet that possibly assist in climbing (Kinlaw 1995).

Distribution

Plains spotted skunks historically ranged from northern Tamaulipas, Mexico to southern Manitoba, Canada, westward from the Mississippi River through the Great Plains region of North America to central and northern Texas, Oklahoma, eastern Colorado, and Wyoming (Kinlaw 1995). Recent examination suggests a truncated geographic range, with local extirpations occurring at the northern and southern portions of the distribution (Perry et al. 2021). Within Texas, the species was recently confirmed to be present in five ecoregions (Perry et al. 2021); however, desert spotted skunks (*S. leucoparia*; previously *S. gracilis*) may co-occur with plains spotted skunks in counties on the eastern edge of the Edwards plateau. In these areas desert spotted skunks may be locally displacing plains spotted skunks (Jefferson et al. 2022).

Habitat Associations

There has been a paucity of research designed to identify associated habitats of plains spotted skunks. Those previous studies have focused on the forested regions of Arkansas (Lesmeister et al. (2009, 2013) or Missouri (McCullough and Fritzell 1984; Higdon and Gompper, 2021). In general, the species is considered grassland adapted on the prairies and plains; however, it is accepted that the plains spotted skunk has generalized macro-habitat requirements that includes a variety of land cover types. In addition to the prairie vegetation types of the Great Plains region, the species historically occurred within the tallgrass coastal prairies from southern Texas to central Louisiana, mixed oak-pine forests in the Ouachita Mountains of Arkansas and Oklahoma and the Ozark Mountains of Arkansas and Missouri (Kinlaw 1995), and mixed oak-ashe juniper stands of the Cross Timbers ecoregion of Texas (Avrin et al. 2021, Perkins et al. 2022).

Despite variation in utilized land cover types across the range of the species, a dependence on impenetrable ground cover or a closed canopy may be critical to plains spotted skunk persistence (Kinlaw 1995). In the Ouachita highlands of Arkansas and Oklahoma, plains spotted skunks are restricted to early successional forests with extensive understory comprised of shrubs or saplings (Branham and Jackson, 2021; Higdon and Gompper, 2020) and it was reported to avoid areas with mature timber largely devoid of ground cover due to management regimes for the red-cockaded woodpecker (Lesmeister et al. 2013). In the Texas Cross Timbers ecoregion, the species was found in stands of Ashe-juniper with associated rocky outcroppings (Avrin et al. 2021). Within the Western Gulf Coastal Plain ecoregion of Texas, spotted skunks have been found in native prairies with invasive brambles (Perkins et al. 2022).

History of Population Declines

Fur trapping harvest of plains spotted skunk from the 1930s and 1940s was in the hundreds of thousands of individuals in the central U.S. (Gompper and Hackett 2005; Gompper 2017). Despite the apparent decline in populations across much of their range by the mid-20th century, there was evidence that the species also experienced a northern and northeastern range expansion (Schantz, 1953; Scott, 1951; Swanson 1934). By the 1970s spotted skunks were uncommon to rare in parts of the Great Plains (Choate et al. 1973). One hypothesis suggested first by Van Gelder (1959) was that plains spotted skunks colonized the central Great Plains as the marshland prairies of the 1800s were drained, making the Great Plains more suitable for the skunks. Species abundance increased with anthropogenic changes, especially the widespread installment of small, sustenance-based farms in the late 19th and early 20th centuries. This led to more conducive habitat and a population boom occurred (Van Gelder 1959, Choate et al. 1973, Sasse 2021). With the widespread change to commercial farming practices in the mid-20th century, species decline was hypothesized to be a return to the previous norm. While not unfounded, this hypothesis is unlikely to remain true across the entire historic range of the species. There is little doubt that populations of plains spotted skunks are markedly lower than historic levels of the first half of the 20th century.

Whether this decline occurred over a matter of years or over decades is open for debate (Sasse 2021); however, there is growing concern that conservation efforts are needed for plains spotted skunks (Gompper and Jachowski 2016, Gompper 2017). Many state wildlife agencies currently list plains spotted skunks as endangered, threatened, or a species of concern (Gompper and Hackett 2005) and the species (considered within the eastern spotted skunk [*Spilogale putorius*]) is categorized as Vulnerable by the IUCN with a decreasing population (Gompper and Jachowski 2016). Also, there is little consensus on the cause of the decline. Arguments have been made for overharvest, disease, effects of pesticide use, alteration of predator guilds, and landscape changes, including large-scale monoculture farming (see Gompper 2017 for summary).

Chapter 2: Study Area

We conducted our study in southeastern Texas within the Western Gulf Coastal Plain ecoregion, an area characterized by flat topography, poor drainage, and wet soils (Griffith et al. 2007). Historically, this region was a coastal tallgrass prairie; however, extensive anthropogenic alteration has converted much of the original prairie to urban areas, rangeland, or agricultural lands. The dominant grassland vegetation in the region was little bluestem (*Schizachyrium scoparium*), gulf muhly (*Muhlenbergia capillaris*), brownseed paspalum (*Paspalum plicatulum*), and switchgrass (*Panicum virgatum*).

Within this ecoregion we focused on the Katy Prairie, a 40,873-ha remnant portion of coastal prairie located in Harris and Waller counties, Texas (Figures 1 and 2; Apfelbaum et al. 2019). Like much of the original Western Gulf Coastal Plain, the Katy Prairie has undergone extensive habitat loss and alteration primarily from urbanization by the expansion of the Houston metro area (Moore 2022) and secondarily by conversion to grazing lands and agricultural fields.

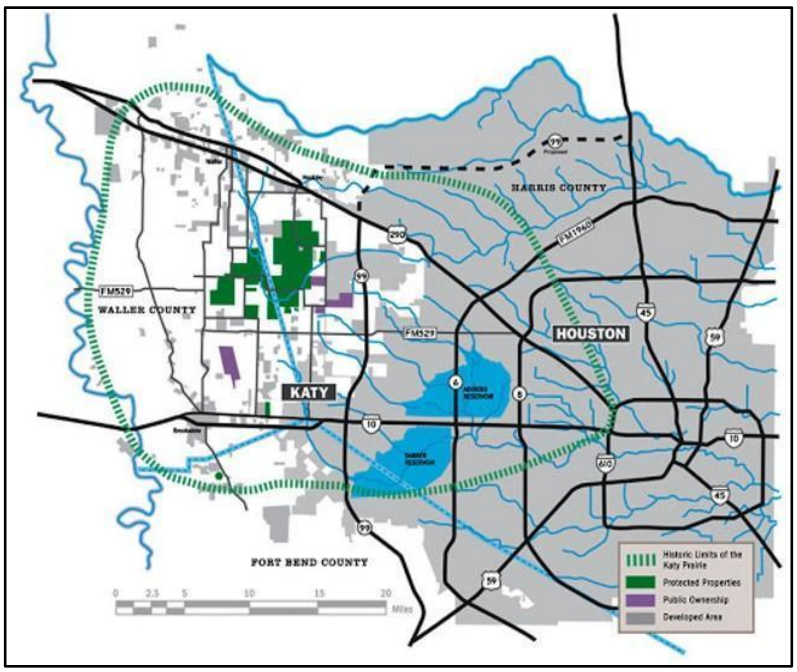

Figure 1. Historical extent of the Katy Prairie, location of Coastal Prairie Conservancy protected properties (green)in Harris and Waller counties, Texas, and extent of developed land cover. Map provided by Coastal Prairie Conservancy

Additionally, private lands in Katy Prairie have undergone decades of fire suppression, which has led to a proliferation of invasive species and brush such as deep-rooted sedge (*Cyperus entrerianus*), Chinese tallow (*Sapium sebiferum*), and Macartney rose (*Rosa bracteata*). Despite these changes, we located a robust population of plains spotted skunks within the prairie as part of a larger survey for the species in Texas (Perkins et al. 2022). Spotted skunks were detected at locations with conservation plans and detailed management strategies while crowd-sourced data indicated a wider distribution across the entire region.

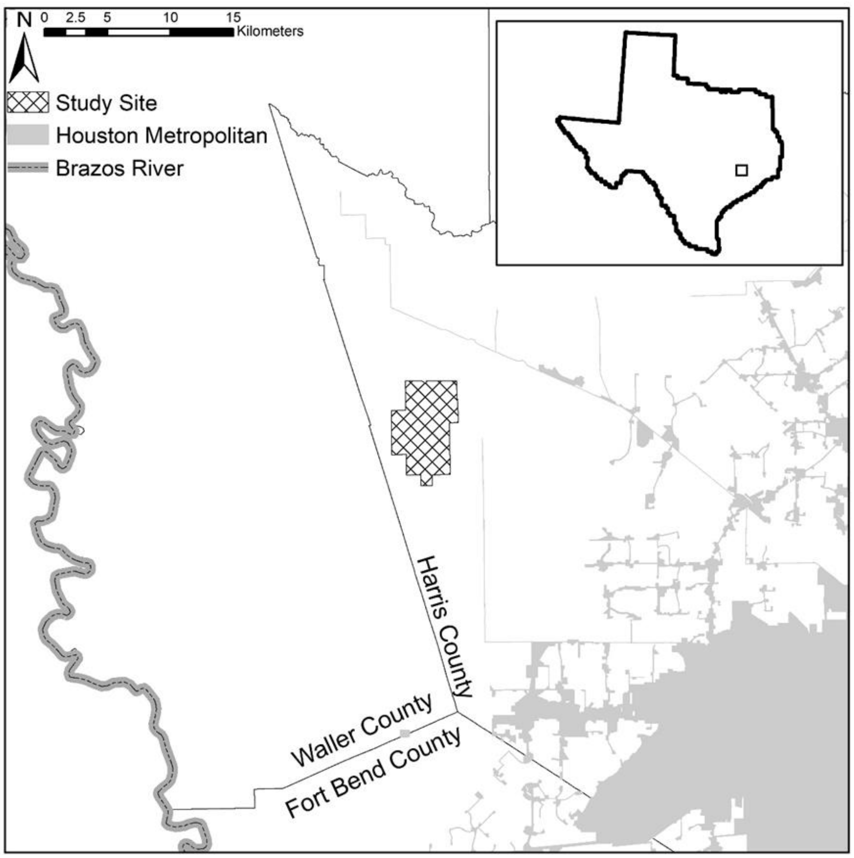

Figure 2. Location of our Warren Ranch study site in Harris County, TX. From 2019 – 2020, we monitored a population of plains spotted skunks to assess spatial demography, seasonal occupancy, and rest site selection.

We conducted research on two disjunct sites on the Katy Prairie both administered by the Coastal Prairie Conservancy (CPC), previously known as Katy Prairie Conservancy (CPC 2022).

The first site was Warren Ranch (Figure 3), co-owned by CPC and the Warren family trust in Harris County, Texas (Warren, 2020). Warren Ranch is a 2430-ha working cattle ranch managed to conserve native range land, mitigate invasive species infestation, and restore natural wetlands and streams. Warren Ranch is a mosaic of native and nonnative rangelands, hay fields, cultivated fields, restored stream corridors, and seasonal wetlands.

The second site, Coastal Prairie Office (KPC in Figure 2), is a contiguous amalgamation of smaller properties owned by CPC in eastern Waller County, Texas. This site is 1188 ha in size and land practices include sustainable cattle grazing, seasonal wetland impoundments for waterfowl, and the publicly available 22.2-ha Indiangrass Preserve and Ann Hamilton Trail, Field Office, and Native Seed Nursery. Management activities at CPC were like those at Warren Ranch with a notable exception being prairie restoration efforts in two pastures at the Coastal Prairie Office site during our research period.

Land cover management at our sites was continuous throughout the project. Management generally occurred at the pasture level and varied among pastures with different land cover types. Natural rangeland pastures were composed of native grasses and forbs, grazing was minimal, and herds were rotated at least seasonally and sometimes bi-monthly. Management of cover within these pastures was by either prescribed fire or mechanical cutting conducted either yearly or on a multi-year rotation. The spatial extent of mechanically managed pastures ranged from ~ 100% to less than 33% of the individual pasture annually. Within nonnative rangeland Pastures (see chapter 4), herds were rotated less frequently, prescribed fire was never used, and mechanical manipulation occurred yearly. Agriculture land cover (winter forage and pastures undergoing prairie restoration) were heavily grazed when seasonally appropriate and underwent more than one mechanical manipulation per year. A second class of native rangeland pastures were present at our sites. These pastures were grazed at the same frequency as the natural pastures; however, these pastures were primarily managed by prescribed fire. There was a demarcation in seral stages based on when these pastures were most recently burned. Natural pastures last burned prior to 2016 had succeeded from a prairie or rangeland seral stage to a shrub-dominated seral stage with few native or nonnative grasses present. From the skunk's perspective, there was sparse ground cover in these pastures; however, there was complex mid and overstory cover. Natural pastures last burned after 2016, were in a prairie seral stage with high biodiversity of grasses and forbs and a lack of mid and over story cover. Additionally, there was a notable lack of accumulated prairie litter resulting in sparse ground cover for the skunks.

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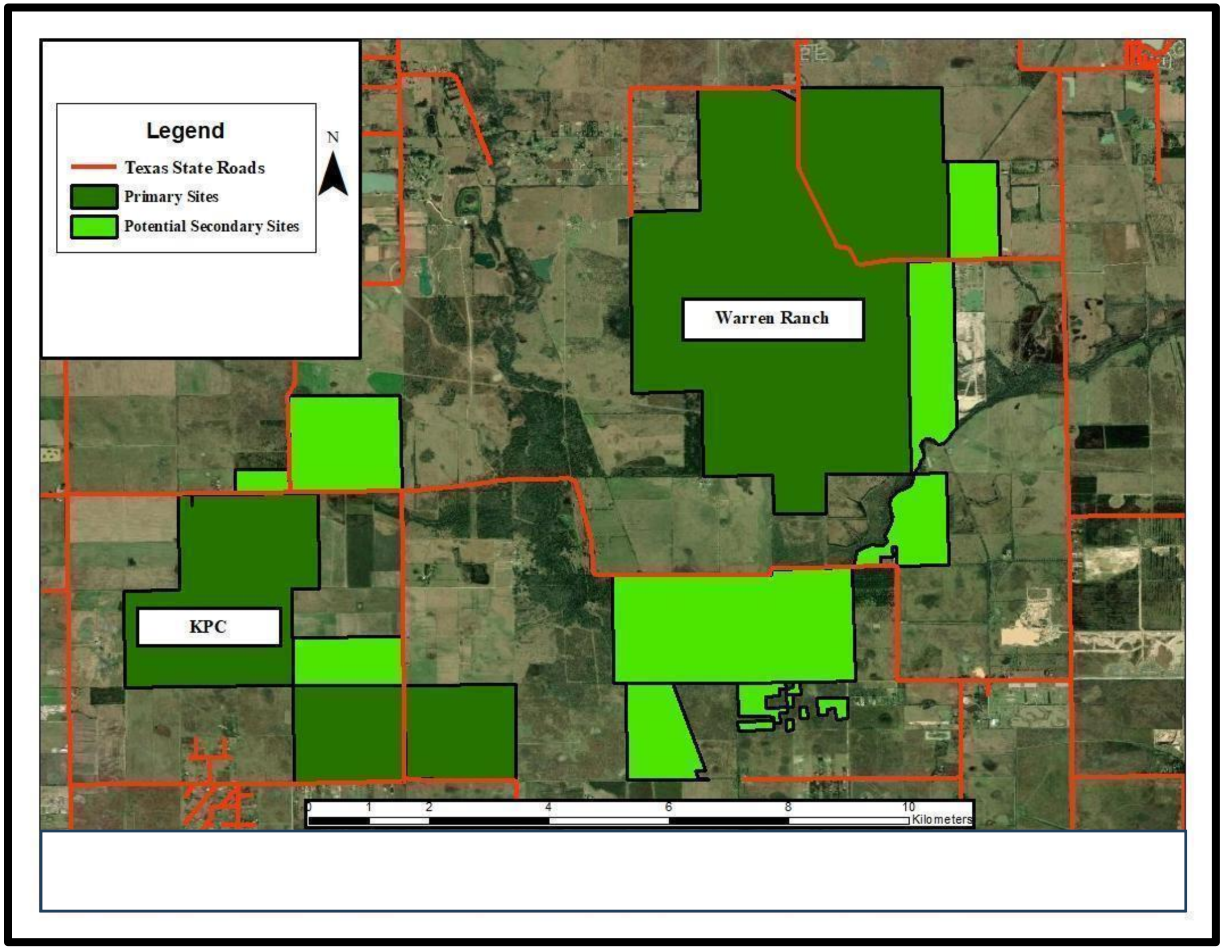

Figure 3. Map of *Spilogale* project survey locations in Harris and Waller counties, Texas. Warren Ranch and KPC were our primary study sites while additional CPC conserved properties are noted in light green.

Chapter 3: Spatial analysis of plains spotted skunk home range dynamics

Introduction

Globally, mesocarnivores or small carnivores (carnivores weighing less than 21.5 kg - Do Linh San et al. 2022) are imperiled with over half of all species in decline (Marneweck et al. 2021). Declines are attributed to anthropogenic change, including habitat alteration and removal of apex predators. A popular theory, mesopredator release, postulates that mesopredators (in North America, mesocarnivores and opossums) will experience range extension and localized population increase following the extirpation of apex predators (Ritchey and Johnson, 2009). Support for this theory is mixed, as less than half of published studies observed this effect and evidence from those that did often cannot be replicated (Jachowski et al. 2019). It is possible that interspecific interactions and idiosyncratic responses of species within mesopredator guilds are more important drivers of species composition than can be explained by trophic cascades. While some species have experienced range expansion in part due to apex predator removal or anthropogenic change (Peterson 1996), others are in immediate threat of extinction. Striped skunks (*Mephitis mephitis*), coyotes (*Canis latrans*), and raccoons (*Procyon lotor*) have become synanthropic (Allen et al. 2022, Schmidly and Bradley 2016). Other species, such as the Black-footed ferret (*Mustela nigripes*; Clark 1987) and the San Joaquin kit fox (*Vulpes macrotis mutica*), are imperiled because of anthropogenic activities (Fernandez-Sepulveda and Martin 2022). Moreover, mesocarnivores, because of their central location in food webs, may be suitable sentinels for changing ecosystem function and study of local mesopredator guilds may identify effects of anthropogenic change *in situ* (Marneweck et al. 2022).

Spotted skunks, *Spilogale* spp. are a genus of understudied carnivores with an uncertain conservation status that have received significant research attention in the 21st century

(Jachowski and Edelman, 2021). Recent focus has been on distribution (Cheeseman et al. 2021, Perry et al 2021), conservation status (Dowler et al. 2017), genetics and taxonomy (Shaffer et al. 2018, McDonnough et al. 2022), and denning ecology (Higdon and Gompper 2020) leaving finer ecological questions such as habitat selection and resource partitioning, unanswered. Moreover, many recent examinations have focused on the eastern spotted skunk (*Spilogale putorius*) or focused on plains spotted skunks (*Spilogale interrupta*) in forested regions (Lesmeister et al. 2008, 2009, 2012; Avrin et al. 2021) and interpretation and application of results to different regions or land covers may be problematic.

Within the Ouachita Mountains of Arkansas, plains spotted skunks exhibited seasonal and sex-based variation in home range size (Lesmeister et al. 2009). Males utilized larger home range sizes than females and exhibited seasonal variation in home range size that was associated with the spring breeding season. Females exhibited little seasonal variation in home range size. Home range size of male plains spotted skunks in the Ozark Mountains of Missouri exhibited similar seasonal trends (e.g., largest in the spring); however, this evidence was from four individual skunks, none of which were female (McCullough and Fritzell 1984). At second-order habitat selection (the home range of an individual skunk compared to the aggregate home ranges of all individuals), skunks in Arkansas consistently selected shortleaf pine vegetative communities with stands that were less than 30 years old (Lesmeister et al. 2009). Selection of shortleaf pine stands decreased as stand age increased leading authors to conclude that cover in the form of a closed canopy or dense underbrush are critical components of plains spotted skunk habitat. Similarly, desert spotted skunks (*Spilogale leucoparia*) in central Texas exhibited nocturnal use of overgrown mesquite pastures higher than what was available, avoided agricultural land cover, and avoided anthropogenic housing areas (Neiswenter and Dowler

2007). Thorne et al. (2017) reported that eastern spotted skunks (*Spilogale putorius*) occupied both younger-aged forest stands, and more mature forest stands along an elevational gradient with both stand types providing complex structure and cover.

Prior to population decline in the mid-20th century (Gompper and Hackett 2005), the ecology and management of plains spotted skunks was examined in southeastern Iowa (Crabb 1948). While this work is considered the seminal examination of the plains spotted skunk, researchers struggle to compare results to contemporary examinations. During the study, only relic portions of native prairie remained within the study unit and much of the study unit consisted of agriculture fields, grazed wood lots, pastures, and locations and structures associated with small-scale agriculture and livestock production. Plains spotted skunks were heavily associated with anthropogenic structures including barns, houses, other outbuildings, and locations used for seasonal hay and crop storage. This association seems two-fold as skunks selected diurnal rest sites within these structures and preyed upon commensal rodents, consumed cottontail rabbits lethally removed and left by farmers, and occasionally preyed on chickens and chicken eggs (Crabb 1941, Crabb 1948). While there is anecdotal evidence supporting the hypothesis that a commensal relationship between plains spotted skunks and traditional farms may exist in locations where the species persists (Fino et al. 2019, Perkins et al. 2022), most of the results from Crabb (1948) have never been replicated. Moreover, the plains spotted skunk may be extirpated in Iowa and throughout much of the Great Plains region (Perry et al. 2021).

No study of home range has been conducted in the Great Plains since Crabb's study conducted from 1939 – 1942. Our objectives were to examine plains spotted skunk seasonal home range and core area dynamics in a working coastal prairie rangeland.

Materials and Methods

In winter and spring 2019, we conducted live trapping surveys at 3 locations (Warren Ranch, Coastal Prairie Office, and Laas Ranch). Surveys at Warren Ranch yielded the most captures and individuals (n = 4), surveys at CPC resulted in 2 captures, and surveys at Laas Ranch resulted in no captures. Subsequently, all live capture and monitoring efforts were conducted solely at Warren Ranch.

The capture of plains spotted skunks occurred between January 2019 and August 2021. The primary capture method for both species was via Tomahawk live traps (Tomahawk Live Trap Co., Hazelhurst, WI) deployed in pastures of known skunk presence (Perkins et al. 2021). Spotted skunks were monitored with GPS transmitters (Lotek, Model Series Litetrack 10; Newmarket, Ontario, Canada) that weighed less than 10% of skunk's overall weight and rarely exceeded 5% (Sikes et al 2016). Transmitters were scheduled to record at least four spatial relocations per 24-hour period with fixes partitioned between nocturnal and diurnal periods at a 3:1 ratio. Transmitters were programmed with an active 2-hour VHF window allowing us to locate the skunk and download spatial data.

Workup

Upon capture, the live trap, trap cover, and skunk were placed in a clear plastic container and anesthetized by placing Isoflurane (Piramal Healthcare Limited, India, distributed by MWI, Boise, ID) on a paper towel, placing the paper towel into the container, closing the container with a lid, and visually monitoring the skunk until it showed signs of induction. Upon induction, the skunk was removed and given an intramuscular injection of TELAZOL® (Zoetis, Madison, NJ) or a generic equivalent (Lariviere and Messier 1996). Time from Isoflurane application to induction time was recorded. Subsequent timing of health monitoring of skunks was modified from previously reported methods (Lariviere and Messier 1996, M. Ben David pers. comm.).

Tracking and dataset aggregation

Skunks were tracked to a diurnal rest site weekly until skunk mortality, collar slip, the skunk traveled off the study site, project termination, or collar malfunction (Jefferson 2021, Hamilton 2022). During each tracking event, spatial data were remotely downloaded from GPS transmitters. For each skunk, all spatial data from all collars were combined into a single dataset and all invalid fixes were removed. Demographic data, season, and a unique identifier were added to the dataset. Next, the cumulative dataset was uploaded and displayed in ArcGIS Pro, all fixes with a High Dilution of Precision (HDOP) greater than 10.0 were removed to reduce location error (Lewis et al. 2007), all relocations were displayed, and any relocations associated with skunk capture were manually removed. For final data aggregation, we retained all remaining points from an individual skunk that was monitored for at least 30 days per season.

Home range and core area estimation

Our primary purpose was to assess home range and core area size; as such, use of a minimum convex polygon (MCP) is an appropriate spatial estimator. We created a two-dimensional home range (95%) and core area (50%) estimates per season of individual skunk's utilized distribution using the adehabitat package in program R (Calenge 2006). Estimation of a species home range provided an area estimate by which 95% is traditionally considered the home range and 50% is considered the core area that is more frequently used than the remainder of the home range (Burt 1943). We used an analysis of variance to assess differences in home range and core area among seasons and between sexes. Post hoc comparisons were performed using a pairwise t-test with a Bonferroni correction. Prior to analysis, home range and core area estimates were log transformed and assumptions of normality (Shapiro - Wilks) and homogeneity of variances (Levene's Test) were evaluated.

Demographic analysis

Differences in home range and core area were analyzed between sexes and among seasons. Collars were not affixed to pre-dispersal juveniles but were attached to post-dispersal sub-adults in fall 2019 and 2020. Spotted skunks are considered to reach adult status in the winter post parturition (Crabb 1944, Kinlaw 1995). Using this designation to assign a categorical age classification would either require use of an arbitrary date to change the individual from sub-adult. As we observed no dispersal movements in sub-adults during the winter season and collected spatial data on only eight sub-adult skunks, we combined adult and subadult skunks into a single dataset without an age classification.

Results

Our live trapping surveys resulted in the capture of 50 unique spotted skunks. Initially, trapping surveys were conducted in pastures of historical spotted skunk presence (Dowler et al. 2017, Perkins et al. 2022), with traps arrayed as transects, and baited only with chub mackerel. While these methods did capture spotted skunks, the result was a 6:0 male bias. Starting in summer 2019, live trap surveys were conducted in pastures with recent spotted skunk detection by camera trap, traps arrayed as grids, and traps alternately baited with chub mackerel or "sweet bait" (Perkins et al. 2021).

Cumulatively, we captured 50 unique plains spotted skunks, attached GPS transmitters to 46 individual skunks and recorded spatial data from 30 individuals (16 M: 14 F) sufficient for analysis (Table 1). Female skunks contributed 32 individual seasons with an average home range size across all seasons of 26.156 ha (95% MCP) and an average core area size (50% MCP) across all seasons of 8.038 ha (Table 1). Male skunks contributed 35 individual seasons with an average home range size across all seasons of 152.359 ha (95% MCP) and an average core area size (50% MCP) across all seasons of 21.245 ha. Across all seasons, male home range sizes were

5.8 times larger than female home range sizes and male core area sizes were 2.64 times larger than females.

Table 1. Seasonal home range (95% MCP) and core area (50% MCP) for plains spotted skunks monitored in Harris County, Texas; 2019 – 2021.

Sex		Winter	Spring	Summer	Fall	Global
Male (95%)						
	n	11	11	7	6	35
	$\bar{x}$	107.775	329.597	58.166	19	152.359
	SE	51.79	57.03	12.48	5.43	31.71
	Range	8.96 - 554.59	43.11 - 586.67	21.12 - 118.56	8.42 - 44.54	8.42 - 586.67
Female (95%)						
	n	8	9	10	5	32
	mean	33.03	22.714	26.512	20.641	26.156
	SE	15.29	3.12	5.67	6.75	4.29
	Range	6.534 - 135.62	12.77 - 41.53	8.21 - 60.85	3.95 -37.53	3.95 - 135.62
Male (50%)						
	$\bar{x}$	12.976	44.325	11.860	5.039	21.245
	SE	5.28	11.03	2.65	0.95	4.51
	Range	1.482 - 43.21	7.66- 129.65	2.38 - 23.8	0.77 - 8.43	0.77 - 129.65
Female (50%)						
	mean	7.461	5.360	10.754	8.351	8.038
	SE	2.86	0.59	2.83	2.54	1.18
	Range	1.51 - 21.31	2.67 - 8.72	2.48 - 30.125	0.95 - 16.2	0.95 - 30.125

After a log transformation, home range size and core area estimations were confirmed to be normally distributed with homoscedastic variances. There was a significant difference in home range size between sexes, but significance depended upon season, $F_{(3,59)} = 5.9029$, $P = 0.001$). Male home range sizes were larger than females in spring ($F_{(1,18)} = 70.09$, $P < 0.001$) and summer ($F_{(1,15)} = 7.386$, $P = 0.0159$) but not in winter ($F_{(1,17)} = 2.62$, $P = 0.124$) and fall ($F_{(1,9)} = 0.044$, $P = 0.839$). Female home range estimates were similar among seasons ($F_{(1,30)} = 0.286$, $P = 0.597$). Male home range sizes varied among seasons ($F_{(3,31)} = 13.64$, $P < 0.001$) with larger spring season home ranges compared to winter ($T_{(20)} = -3.805$, $P < 0.001$), summer ($T_{(16)} = 4.967$, $P = 0.005$), and fall ($T_{(15)} = 7.881$, P < 0.001). All other seasonal comparisons of size were nonsignificant (P = range of 0.167 to 1).

We observed a significant difference between plains spotted skunk core area size between the sexes, but significance varied between seasons ($F_{(3, 59)} = 4.8779$, $P = 0.004$). Female core area estimates were similar across all seasons ($F_{(3,28)} = 0.5738$, $P = 0.638$). Male core area estimates varied among seasons ($F_{(3,31)} = 8.657$, $P < 0.001$) with larger core area estimates in spring compared to winter ($T_{(20)} = 3.418$, $P = 0.009$), summer ($T_{(16)} = 3.12$, $P = 0.044$), and fall ($T_{(15)} = 4.614$, $P < 0.001$). All other male core area seasonal comparisons (winter compared to summer and fall; summer compared to fall) were non-significant (P = range of 0.419 to 1). Male core area estimates were larger than female core area estimates in spring ($F_{(1,18)} = 36.77$, $P < 0.001$) with similar sized estimates in winter ($F_{(1,17)} = 1.308$, $P = 0.269$), summer ($F_{(1,15)} = 0.235$, $P = 0.635$) and fall ($F_{(1,9)} = 0.216$, $P = 0.635$).

Discussion

Plains spotted skunk home range and core area estimates were related to both season and sex. Female plains spotted skunks exhibited slight variation in either estimate across seasons, while male home range sizes were largest in spring and smallest in fall. Comparable results were previously reported from Arkansas (Lesmeister et al. 2009); however, home range estimates were consistently larger across all seasons and both sexes in Arkansas. Minimum convex polygon estimates for home range are widely reported in the literature and the debate about comparisons with kernel density estimates continues (Laver and Kelly 2008); however, methodology aside, we propose two hypotheses for our smaller overall home range sizes. Our study site was a fragmented matrix of land cover types that plains spotted skunks both selected and avoided. Spatially, most utilized patches were either partially surrounded by patches that the skunks avoided or by county or ranch roads. It is likely that these patches and roads provided barriers to movement that the skunks were unwilling or unable to traverse resulting in constrained home range sizes.

Second, based upon capture rates it is possible that plains spotted skunk density was lower in the Ouachita Mountains compared to our site (Hackett et al. 2007, Perkins et al. 2021). A meta-analysis of carnivore home range sizes and population densities suggested a trend of smaller home range sizes and increased density along a natural to urban habitat gradient (Šálek et al. 2015).

As in Arkansas, we also propose that male skunks from late winter through early summer exhibit extra home range excursions in search of mating behavior. During our study we monitored five male skunks continuously from winter through summer and three male skunks continuously from winter through spring. All eight individuals exhibited at least one extra home range excursion over 1-km during the spring season. Moreover, we hypothesize that at least six males first captured in spring were exhibiting extra home range excursions at time of first capture and returned to an established home range after monitoring was initiated. These six males, within 1 to 4 weeks, were in the hypothesized previously established home range area on either adjacent, inaccessible properties or on different portions of Warren Ranch. We also observed a single male translocate to a new home range during the breeding season. At initial capture (December 2020), S032 was categorically aged as an adult older than 1.5 years. It was then monitored through August 2021. In mid-March, S032 evacuated its established home range and moved southward on Warren Ranch. During the next month S032 roamed the central portion of the ranch without remaining in a single pasture for more than a week. By May, the skunk had established a new home range after which we observed two additional home range excursions, one in late May and one in mid-June.

Due to the necessity of repeated capture of monitored individuals, we were able to estimate the breeding season of our population by observation of male (testes abdominal or descended) and female breeding conditions (pre or post copulation, pre or post parturition). We observed males with descended testes from mid-February through early June. Our earliest observation of neonates and female den defense was the 26th of May. Gestational estimates for plains spotted skunks range from 50 to 65 days (Mead 1968a, Kinlaw 1995) suggesting a successful copulation in mid-March. Additionally, we confirmed a pre-parturition female as late as 16 July and a previous young of year female with signs (bite marks on the neck and head, lacerations near the vagina) of an attempted copulation on 06 June. While anecdotal, these observations provide additional evidence suggesting that the increase in male home range size during the spring was due to the search for breeding opportunities.

Female plains spotted skunks exhibited insignificant variation in home range size across seasons. Female plains spotted skunks in Arkansas exhibited a marked, albeit non-significant increase in home range size during the spring season. Female eastern spotted skunks in mountainous regions have been shown to select den sites based upon their pre and post parturition status (Thorne and Ford 2022). Cumulatively, we monitored eight individual female skunks during a spring season. Four of these females were confirmed to have birthed during the spring season while three were confirmed to be pre-parturition and exhibited similar pre-parturition behaviors as those with confirmed birthing events. Three of the four females exhibited a home range shift during spring, potentially to select a den as a birthing location. The fourth evacuated an established home range, established a new home range over a kilometer away during late spring, maintained that home range during the summer, and returned to the previous home range in fall. During mid to late spring, these females were excavating burrows as diurnal resting sites in locations that provided overhead cover and were slightly higher in elevation than the surroundings. We were unable to monitor three of the females during selection of a burrow-based diurnal rest site, parturition, and pup rearing while the fourth died during the season.

Chapter 4: Plains spotted skunk's response to changes in land cover and presence of cattle

Introduction

The popularity of camera traps has resulted in increased research designed to inform many common ecological aspects of species. Perhaps no species grouping has benefited more from the advancement in camera trap technology and use than mesocarnivores (i.e., small, and mid-sized carnivores). In the United States, uncommon mesocarnivores have been traditionally understudied because of their apparent rareness as well as their colloquial and managerial designation as predators, pests, or unregulated species. Assemblages containing more common species (e.g., striped skunk–*Mephitis mephitis*, northern raccoon–*Procyon lotor*, coyote–*Canis latrans*) have been well studied. While this group of mesocarnivores includes species adapted to anthropogenic changes, it also contains some of the least successful, many of which are of considerable conservation concern.

With the increase of camera trap usage, occupancy modeling (MacKenzie et al. 2002) has emerged as a common analysis framework (MacKenkie et al. 2018). Occupancy analysis allows the inclusion of explanatory variables and, most importantly, by calculating detection probabilities when detection rates are less than 1, allows the use of missing observations and missing survey periods. This functionality assists with distinguishing between sites where the focal species is not present and sites where the focal species is present, but not detected. Repeated sampling of individual sites helps disentangle parameters of interest from potentially biased survey design (Mackenzie et al. 2003). Site occupancy can change through time and repeated sampling can inform extinction and colonization of discrete patches (MacKenzie et al. 2003). Dynamic occupancy models use repeated sampling to determine the initial occupancy of a site and assess the probability of an initially unoccupied site to become occupied through time and the probability of an initially occupied site to become extinct through time. One important assumption of dynamic occupancy is that occupancy does not change within a primary survey period but can change among primary survey periods. With this framework, a dynamic occupancy model can assess the effects of land cover alteration on focal species.

Spotted skunks, especially plains and eastern spotted skunks (*Spilogale interrupta* and *S. putorius*, respectively) are understudied mesocarnivores with uncertain conservation status that have received significant research attention in the 21st century (Jachowski and Edelman, 2021), while being bereft of quantifiable research attention in the 20th century. However, these works have primarily focused on distribution, conservation status, taxonomy, and denning ecology. Recently, the logistical benefits and noninvasive nature of camera traps have made this technique the dominant method in spotted skunk research (Dukes et al. 2022). Use of an appropriate bait or lure has improved detection efficacy in North Carolina and Texas (Eng and Jachowski, 2019; Avrin et al. 2021). Additionally, significantly increased detection rates were observed at cameras lured with inaccessible sardines compared to cameras without lure (Avrin et al. 2021). Station set up, defined as configuration and distance from bait or lure to camera, affected detection probability in the Appalachians Mountains (Eng and Jachowski 2019) and it has been suggested that brand and type of camera can have an impact on detectability (Dukes et al. 2022).

Prior to population decline in the mid-20th century (Gompper and Hackett 2005), the ecology and management of plains spotted skunks was examined in southeastern Iowa (Crabb 1948). While this work is considered the seminal examination of the plains spotted skunk, researchers struggle to compare results to contemporary examinations. During the study, only relic portions of native prairie remained within the study unit and much of the study unit consisted of agriculture fields, grazed wood lots, pastures, and locations and structures associated with small-scale agriculture and livestock production. Plains spotted skunks were heavily associated with anthropogenic structures including barns, houses, other outbuildings, and locations used for seasonal hay and crop storage. This association seems two-fold as skunks selected diurnal rest sites within these structures and preyed upon commensal rodents, consumed cottontail rabbits lethally removed and left by farmers, and occasionally preyed on chickens and chicken eggs (Crabb 1941, Crabb 1948). While there is anecdotal evidence supporting the hypothesis that a commensal relationship between plains spotted skunks and traditional farms may exist in locations where the species persists (Fino et al. 2019, Perkins et al. 2022), most of the results from Crabb (1948) have never been replicated. Moreover, the plains spotted skunk may be extirpated in Iowa and throughout much of the Great Plains region (Perry et al. 2021).

Our objectives were to 1) assess plains spotted skunk occurrence within varying land cover types within a coastal prairie rangeland and 2) assess changes in plains spotted skunk occupancy due to land cover management and presence of cattle.

Materials and Methods

To identify camera trap deployment locations, we first quantified habitat type at the survey sites using the 2011 National Landcover Database (Homer et al. 2015). Land cover types were reclassified at 100-m resolution in ArcGIS (ArcGIS 10.7.1, Environmental Systems Research Institute, Redlands, CA). Reclassification resulted in four land cover types that comprised 97% of all cover types at the survey locations: cultivated crops, pasture/hay/grassland, forest, and scrub/shrub. The number of stations deployed per survey location (Warren Ranch and Coastal Prairie Conservancy) was stratified based upon total hectares of the location and the percentage of the four land cover classes present. At each station, the first camera location was randomly chosen from within the available cover type and the second was randomly chosen from the adjacent cardinal cells such that both stations were 100 m apart. Locations within permanent wetlands, roadways, or anthropogenic locations were removed and reassigned. If randomly assigned stations were within 500 m of each other, these stations were not concurrently operated during the same season.

Prior to winter 2020, our camera trap array was redesigned to incorporate results from the 2019 survey period (hereafter survey 1), to incorporate observations from plains spotted skunks monitored with GPS transmitters, and to ground truth camera location within land cover type. Camera stations deployed in 2019 within 50 meters of a road, in forest land cover, or in an ephemeral wetland basin were removed. If two stations occupied the same pasture, one station was randomly removed. Fifty-seven percent of camera stations (20 of 35) at Warren Ranch were moved or removed. Five of these stations were located on a portion of the ranch where accessibility necessitated removal of all stations. Two stations where a spotted skunk was detected in 2019 were moved; both stations were in a pasture with an additional camera station.

For new camera stations, all cells less than 100 m from a road, less than 100 m from a cattle supplemental feed location, or within an ephemeral wetland basis were removed. We then randomly chose new camera stations using the same techniques as in 2019. As in 2019, stratification of land cover type was conducted to inform the number of stations per land cover and only one station was deployed per pasture. From 2020 through spring 2021 (hereafter survey 2), we surveyed plains spotted skunks using 27 unique camera stations, 15 of which were retained from the 2019 survey and twelve new stations.

We surveyed plains spotted skunk occupancy with four camera models to assess detectability of plains spotted skunk based upon camera type. In 2019, Reconyx PC800 Hyperfire Professional IR cameras (Reconyx, Holmen, WI, USA), Reconyx Hyperfire 2 Professional HP2X cameras, and Bushnell Trophy Cameras (hereafter B1; Bushnell Outdoor Products, Overland Park, KS, USA) were deployed. During survey two, the use of Reconyx PC800 cameras was reduced to less than 5% of all survey nights. In 2021, Bushnell Core DS No Glow cameras (hereafter B2; Model 119977C) were added to the camera array. Cameras were set to use the closest focal area, take the maximum number of images per trigger event (5; 3 – B1 only), and reload as quickly as possible between successive trigger events.

After locating the pre-plotted camera trap survey location, we used a string trimmer to cut all standing vegetation in an area approximately 3.5 m X 1.5 m. To reduce false triggers, all emergent vegetation above the mean height was removed on the end of the station opposite of the camera in an arc that was approximately 5 m X 5 m. The stations were randomly oriented either north or south to reduce interference from the rising and setting sun. Next, a 6’ t-post was pounded into the ground up to the spade, a 2” x 6” piece of untreated lumber was attached to the t-post via a 4 7/8" pipe clamp, and cameras were strapped to the t-post/lumber. Next, a 24” or

36” wooden survey stake was placed exactly 3-m in front of the pole centered on the camera. The clamp allowed the movement of the lumber and camera up or down depending on the elevational differences at each location. During survey 1, camera lenses were deployed 350 – 380 mm above the ground. During survey 2, camera lenses were deployed approximately 330 mm above the ground. The station was then lured by affixing unopened, canned sardines (Season Brand, distributed by The Manischewitz Company, Newark, NJ, USA) to the survey stake by placing two pieces of tie-wire through the can and tightly affixing it to the post.

Camera deployments were scheduled for 21 consecutive days and maintenance was performed every seventh day (change SD card, check battery status, cut growing vegetation, and replace sardines). After camera maintenance, we checked SD cards for plains spotted skunk presence, deleted false triggers, and saved all remaining images to long-term storage. The presence of plains spotted skunks informed live trapping efforts (Perkins et al. 2021), but live trap and camera trap surveys were not conducted concurrently within individual pastures. Next, data quality control was conducted to ensure all images were assigned to the correct camera and station, all date and time metadata was correct, all false triggers were removed, and all extraneous images of cattle (*Bos taurus*), turkey vultures (*Cathartes aura*), black vultures (*Coragyps atratus*), crested caracaras (*Caracarus plancus*) were deleted. All images of wild mammalian species were retained in entirety. In the event a camera malfunction resulted in incorrect date and time metadata within the 7-day maintenance period, we manually corrected the date and image time.

We reviewed all remaining images for species identification and tagged individual images by writing metadata using the program digiKam (https://www.digikam.org/). In addition to species identification, all images were tagged with site and station information, individual who cataloged the image, camera operation and bait status, and whether both skunk species were marked. After image cataloging was complete, all image tags were reviewed to ensure proper species identification was correct by using the filter function in digiKam. Finally, all associated image metadata were read into the program camtrapR (Niedballa et al. 2016). Plains spotted skunk detections were aggregated by defining the minimum time between independent records as 12 hours between the two cameras per station. As we recorded only one plain spotted skunk during diurnal hours, this method partitioned detections to ensure a maximum of one observation per night per camera station. For analysis, we partitioned surveys across years and seasons, with a maximum of four total surveys per year and six total. We categorized the primary survey periods as our seasonal surveys (n = 6) and secondary surveys (repeat visits to account for imperfect detection) as the three successive 7-day sampling occasions within each primary survey period or the planned period between successive rebaiting of the station. We defined a successful secondary survey as any 7-day period in which there were at least 5 operable camera trap nights between the 2 cameras. Per primary survey, we retained all stations that had at least 2 successful secondary survey periods and capped the maximum number of secondary survey periods at 4. Contextually, a “perfectly” deployed station would have been operational for 21 successive nights with maintenance performed on the 7th and 14th day. Our minimum secondary survey period included for analysis was 10 days and our maximum survey period was 28 days. Any data from cameras operational for less than 10 days or more than 28 days per primary survey period were removed.

To aggregate environmental covariates associated with each camera trap station, we applied a 600 m buffer around a center point between each camera at a single station. The resultant buffer was 24.59 ha and approximated the observed cumulative mean home range of female spotted skunks (26.15 ha; Chapter 3). As a result of this buffer size, we had a group of four camera stations with partially overlapping buffers. To maintain spatial independence among stations, we randomly selected one camera station from each group and removed it from analysis. Results from these stations are reported below but are not included in the analysis.

Landcover classification

Based upon localized land management practices and partitioning, we define "**p**asture" as a discrete land unit defined by perimeter-based fences with land management activities applied entirely within the unit. The proportion of individual pastures that underwent management during a particular year varied from ~0 to 100%. Additionally, each pasture had a small portion that was either mowed annually (e.g., roads or roadsides) or plowed (firebreaks). Below, we define a land cover classification as **P**asture and make the distinction here between the two.

We assigned land cover classes to the study site by performing a supervised classification using Google Earth Engine (Gorelick et al. 2017). We selected Copernicus Sentinel Data (Harmonized Sentinel-2 Multispectral Instrument, Level 2A) and filtered the aerial imagery into two time periods. The first (December 2019 - March 2020) was used to classify land cover types and locations that were manipulated by either mechanical cutting or plowing in summer and early fall 2019. The second (December 2020 - March 2021) similarly was used to confirm earlier land cover classifications and assess total acreage manipulated during summer and early fall 2020. This method allowed us to incorporate a time period when land cover manipulation was not occurring while simultaneously providing the best period to identify mowed areas (during early spring green up) and Macartney rose which retained leaves during the winter season in contrast with native vegetation.

First a cloud cover mask was applied to the time series to accommodate individual dates when cloud coverage was over the study site. Second, we created 3 band analysis functions (normalized difference vegetation index - NDVI, enhanced vegetation index - EVI, and normalized difference water index - NDWI) and retained these bands along with bands B2 - B8, B11, and B12 for classification. A single composite image was then created of the median values for all bands during each time period. We then performed a supervised land cover classification by assigning spatial designations to 9 land cover types (natural, Pasture, water, forest, winter forage (agriculture), impervious surface, ephemeral wetlands and rushes/sedges, and fallow) as well as Macartney rose clumps and mowed locations. We applied a minimum of 50 points to each of these land covers and randomly assigned 80% of points to a training dataset and 20% to a testing dataset. Next, we used a random forest decision tree with 500 iterations to classify land cover at Warren Ranch. Our final step was to create a confusion matrix to assess accuracy within both our training dataset and our testing dataset. This process was used for both time periods with different spatial points each year to account for annual differences in the location of manipulated land cover. The result was a land cover classification map of our study site at 10-m resolution that incorporated land cover type, land cover manipulation, and Macartney rose presence during the 18-months of our camera trap survey.

All points used in the land cover classification were applied solely within discreet land cover types that could positively be identified during the period. Pasture land cover types included hay pastures, were primarily composed of sod-forming or other non-native grasses and underwent yearly manipulation including mechanical cutting and fertilizer application. Natural land cover was either relic or restored tallgrass prairie that was primarily composed of native bunch grasses. Water (open), forests, and winter forage (agriculture) classifications were applied similar to the National Landcover Database (Dewitz and U.S. Geological Survey, 2021). To assess anthropogenic footprint at the macro level, we classified impervious surfaces using points placed along local roads, internal gravel roads, housing and barn units, gravel parking lots, oil and gas pads, and plowed firebreaks.

We identified locations that were mechanically cut (hereafter mowed). To identify this land cover type, we identified locations that were not classified as Pasture that had been mowed during summer or early fall of the previous year. Contextually, these were locations that were either natural land cover that was mowed for management purposes or locations that underwent mowing more than once per year (e.g., near ranch headquarters or adjacent roadsides).

Additionally, we applied two other land cover classifications within discrete boundaries that identified unexpected vegetative cover types. We *a priori* identified discrete ephemeral wetlands and placed all classification points within the boundaries of these wetlands. Our supervised classification indicated, unsurprisingly, that these wetlands were composed of emergent vegetation, primarily sedges and rushes. Deep-rooted sedge (*Cyperus entrerianus*) is an aggressive sedge native to South America, that if left unmanaged, can entirely dominate native vegetation especially those found within coastal prairies (Carter and Bryson 1996, King et al. 2015). On Warren Ranch, at the pasture level, we did not record any locations entirely composed of deep-rooted sedge. However, within individual pastures, we noted many infestations that were greater than 90% deep-rooted sedge. At 10-m spatial resolution, we were unable to partition deep-rooted sedge clumps from clumps of native sedges and rushes. As our supervised classification indicated that this vegetative community also occurred outside of ephemeral wetland basins (e.g., within wet prairie landscapes) we classified this community as ephemeral wetland vegetation (ephemeral) to incorporate the idiosyncratic changes in vegetative communities across the study site.

Similarly, we identified 3 discrete pastures at our study site that were classified as agriculture during the most recent NLCD classification (Dewitz and U.S. Geological Survey, 2021). These locations were last farmed in 2016. From 2017 through 2020, these pastures were fallow with minimal management. In 2020, two of these pastures underwent mechanical manipulation and one was put back into agriculture (winter forage). Ground cover within these pastures was visually assessed as less than 10 % grasses (native or nonnative), with the majority composed of either deep-rooted sedge or forbs and a striking overstory component of seasonal forbs. Our supervised classification also identified locations across the study site composed of overstory forbs. However, these locations were not randomly located; they were primarily located where management activities had occurred in early summer allowing the forbs to bolt and emerge above the grasses. As such, we classified this land cover type as forbs rather than fallow.

Occupancy Analysis

Using *a priori* hypothesized models, we conducted hierarchical multi-phase occupancy analyses designed to assess factors that influence our ability to detect plains spotted skunks, factors that influenced plains spotted skunk occupancy (Ψ; winter 2020), and factors that influenced spotted skunk colonization (Υ) and extinction (Φ) within unique patches. This analysis was conducted on the dataset from Warren Ranch during survey 2 only (winter 2020 through spring 2021). We evaluated *a priori* factors known to influence both plain spotted skunk probability of detection (p) and occupancy (Eng and Jachowski 2019, Marneweck et al. 2022, Thorne et al 2017). Model evaluation and ranking was performed using Akaike's Information

Criterion and we retained models with a ΔAICc less than 2 (Akaike 1974). We conducted a multi-season, single species occupancy analysis (MacKenzie et al. 2003) using the R package unmarked (Fisk and Chandler 2011) and function colext.

To assess detection probability (*p*) of plain spotted skunks, we evaluated effects of six *a priori* models: weather, distance, effort, season, a null model, and global model with all covariates. Previous efforts suggest that weather or moonphase may either decrease spotted skunk nightly movement distances or detectability (Arts et al. 2022; Marneweck et al. 2022) We defined our weather model as the cumulative influences of precipitation, temperature, and moon phase averaged across the 7 days of each secondary survey. Moon phase data was downloaded from the “suncalc” package in program R (Thieurmel, 2022) while temperature and precipitation data were downloaded from NOAA (https://www.ncei.noaa.gov/cdo-web/). We defined our distance model as the station level effects of distance to the nearest road and distance to the nearest ephemeral wetland. We defined roads as local farm to market highways, internal gravel roads, internal grass pathways mowed at least once per year - and continuously traveled, and firebreaks created in late summer or early fall and then continuously traveled. In concert with our method of deploying 1 camera station per pasture, distance to nearest road assessed both antagonistic effects of travel while also assessing edge effects. We used the best supported detection probability model for all subsequent stages of analysis.

Table 1. Environmental variables used to assess plains spotted skunk occupancy from a camera trap survey in Harris County, Texas; 2020-2021.

Variable	Description	Hypothesized influence
D2E	Distance from station to nearest wetland	-
D2MR	Distance from station to nearest MR clump	+
D2R	Distance from station to nearest road	-
Emergent	Proportion of emergent wetland vegtation (sedges, rushes)	-
Forb	Proportion of forbs	-
Imperv	Proportion of impervious surfaces	-
Mowed	Proportion of mowed land cover	-
MR	Proportion of Macartney rose	+
Pasture	Proportion of Pasture	-
Water	Proportion of standing water	-

We evaluated 5 models to assess plains spotted skunk initial occupancy. Variables contained within these models were either unchanging throughout the duration of the survey or a mixture of variables *a priori* deemed to influence plains spotted skunk occupancy. We assessed the effects of water on spotted skunks including the proportion of standing water, proportion of emergent vegetation, distance to wetland as well as all iterations of these variables within individual models. We also assigned an anthropogenic model that included proportion of mowed, proportion of Pasture, and proportion of impervious surfaces within the patch as well as every iteration of these variables. Our final *a priori* model included the proportion of natural land cover, proportion of Macartney rose, and distance to the nearest Macartney rose cluster. Additionally, we assessed a null model and a global model with all variables.

We evaluated 3 *a priori* hypothesized models to assess plains spotted skunk colonization and extinction within discrete patches associated with our camera traps. To reduce influences associated with idiosyncratic differences between our two land cover classifications, we held proportion of and distance to nearest Macartney rose clump as a constant. Larger sized Macartney rose clumps (> 2 m in height) were unable to be mowed and smaller sized Macartney rose clumps were less likely to be identified by supervised classification at our 10-m resolution.

To further reduce influences, we held the proportion of natural, Pasture, mowed, ephemeral, and forb land cover as a constant at 4 stations where management activities did not occur during our survey. At all other stations, proportion of natural, Pasture, mowed, ephemeral, and forbs varied among the primary survey periods based upon the results of our supervise classifications. The effects of cattle presence were assessed with the percentage of days during each 7-day survey with cattle present.

To assess both probability of patch extinction through time(Φ) and probability of patch colonization(Υ), we separately evaluated 3 *a priori* models, with all iterations of individual variables within each model, a null model, and a global model with all variables. We assessed a cattle model that included cattle presence and proportion of Pasture, natural, and mowed land covers. We assessed a natural model which included proportion of natural and Macartney rose and distance to the nearest Macartney rose clump. We assessed an anthropogenic model which included proportion mowed and proportion of Pasture and ephemeral.

Results

From 2019 – 2021, plains spotted skunks were detected with all camera types and in all 4 seasons (Table 2). Cumulatively, we recorded 35 unique spotted skunk detections at Warren Ranch in three seasonal surveys during 2019. These observations originated from 14 total camera stations with an average of 1.93 detections per station that detected a spotted skunk (range: 1 -7). During survey two we recorded 154 unique detections at 17 stations (63% naive occupancy). We observed an average of 9.06 unique detections at the 17 stations (range: 1-39); however, 2 stations accounted for 48% of all observations (n = 74).

During survey 1, 17% of plains spotted skunks detections included an individual with a visible GPS transmitter (n = 6), 54% of observations were without a visible GPS transmitter (n =

19), and 29% of the images were not clear enough to ascertain whether the skunk was transmittered (*n* = 10). During survey 2, 23% of plains spotted skunks detections included an individual with a visible GPS transmitter (*n* = 35), 50% of observations were without a visible GPS transmitter (*n* = 77), and 27% of the images were not clear enough to ascertain whether the skunk was transmittered (*n* = 42). During our final season, spring 2021, we recorded 33 unique plains spotted skunk detections of which 33% were transmittered (*n* = 11), 30% were not transmittered (*n* = 10), and 36% (*n* = 12) were not clear enough.

During survey 2 at Warren Ranch, 87% of plains spotted skunk detections originated from 11 camera trap stations deployed within natural habitat pastures (*n* = 134). We recorded nine detections from two camera stations deployed in pastures last burned prior to 2016 (6% of detections) and 6 detections from 2 camera stations deployed in locations classified as pasture habitat (4% of detections). Finally, we recorded four detections at a single camera station deployed within Cultivated habitat (3% of detections) and a single detection at a camera station located within pastures most recently burned after 2016.

During 2019, we recorded four unique plains spotted skunk detections at our CPC site. During survey 2, we recorded 15 unique spotted skunk detections at 6 individual camera trap stations. We recorded a 197% increase in detections from 2019 to 2020 at both sites, a 188% increase at Warren Ranch, and a 275% increase at CPC. It is likely that these increases were a result of refinement of survey techniques and location rather than an increase in the population.

Table 2. Descriptive results from a plains spotted skunk (PSS) camera trap survey conducted at Warren Ranch in Harris County, Texas from 2019 through 2021. Survey 1 was conducted in three seasons during 2019 across 35 unique survey stations (defined as two non-independent cameras placed 100 m apart). Survey 2 was conducted over six seasons across 2020 – 2021 utilizing 27 unique camera stations. Survey 2 utilized 15 unique camera stations that were used in 2019 and 12 new stations. Station (+) refers to the number of stations with at least 1 PSS detection while Station (+) % is the percentage of stations with a PSS detection / the number of stations surveyed. Nights is the cumulative amount of station level survey nights. Detections are the cumulative independent PSS detections per season. Temporal independence is defined as at least 12 hours since the last independent PSS detection per station.

Survey	Season		Stations	Stations (+)	Station (+) %	Nights	Detections	Detection rate (%)
1	Spring		34	6	17.6	496	6	1.2
	Summer		33	8	24.2	576.5	10	1.7
	Fall		33	8	24.2	619	19	3.1
		Total	35	14	40.0	1691.5	35	2.1
2	Winter		26	9	34.6	489	23	4.7
	Spring		26	10	38.5	582	30	5.2
	Summer		26	5	19.2	575.5	7	1.2
	Fall		24	8	33.3	490.5	26	5.3
	Winter		26	7	26.9	525.5	35	6.7
	Spring		26	8	30.8	537.5	33	6.1
		Total	27	17	63.0	3200	154	4.8

Occupancy analysis

For occupancy analysis, we retained 23 camera stations at Warren Ranch during survey 2 and detected a plain spotted skunk at least once at 14 unique camera stations (61% naive occupancy). Cumulatively, we recorded 66 total detections (defined as 1 observation per 7-day secondary survey period). Temporally, we accumulated 79 total secondary surveys over 6 successive seasons at these 14 stations and spotted skunks were detected in 48% of these surveys (n = 38). Of the 14, nine stations were operational for 6 primary surveys while 5 were operational for 5 primary surveys. We observed continuous occupancy - defined as spotted skunk detections in greater than 80% of all primary surveys at 3 stations and singleton occupancy (detection in only 1 primary survey) at 4 stations. At the remaining 7 stations, our observed survey occupancy ranged from 33% to 50%. At the 3 continuously occupied stations, we accumulated 33 total secondary survey detections that accounted for 50% of all secondary detections and recorded an observation in 15 of the 16 secondary survey periods that these cameras were operational.

Our detection probability (*p*) analysis suggested that the distance to the nearest road affected our ability to detect plain spotted skunks. A single variable model, distance to nearest road, was our top-ranking model, more than two ΔAICc from the next most supported model and the null model. As the distance from the camera station to the nearest road increased, plains spotted skunk detection probability also increased (Figure 1).

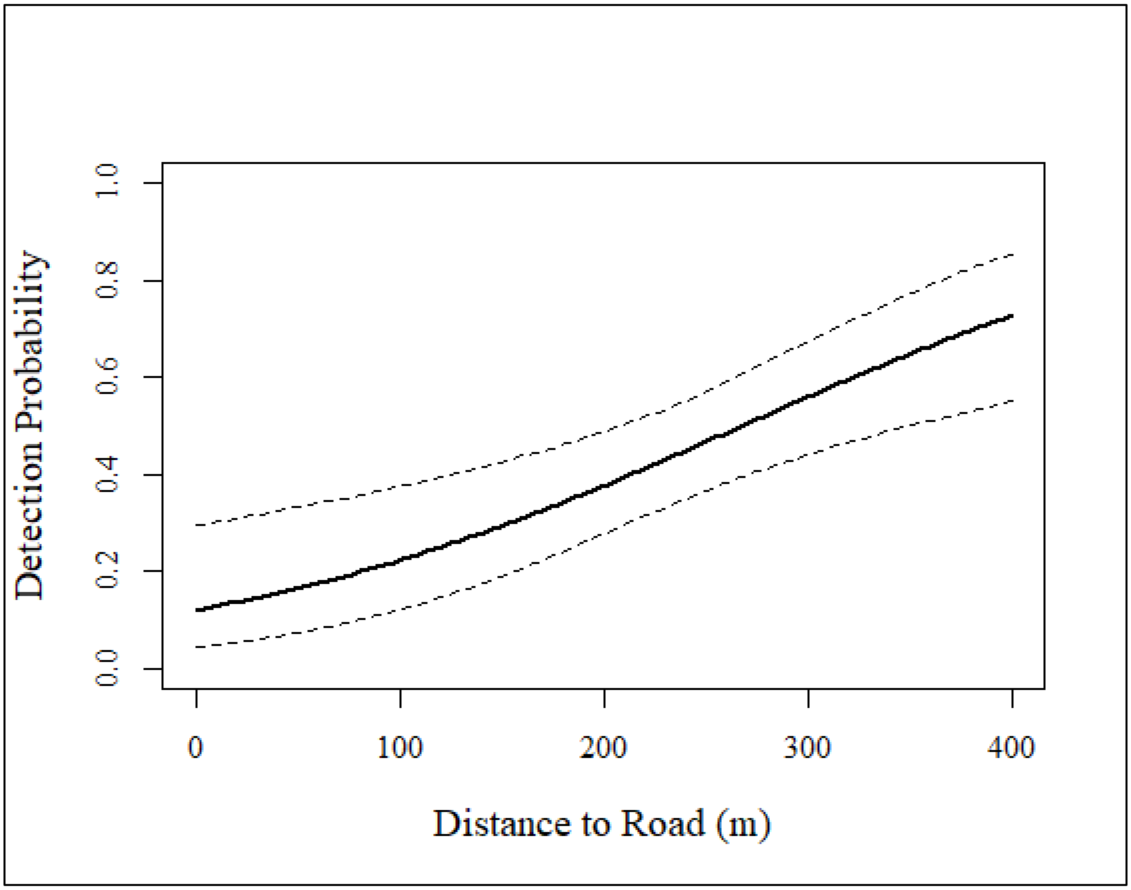

Figure 1. Predicted probability of detection of plains spotted skunks as a function of distance to nearest road (in meters) with 95% confidence intervals from a camera trap survey in Harris County, Texas; 2020 - 2021.

During our initial survey of winter 2020, we detected spotted skunks at 7 of 23 spatially unique camera stations. Analysis suggested that our null model was the most supported model, however, a second, single variable model (proportion of impervious surfaces) had a ΔAICc of less than 2 (1.40). It is likely that potentially spurious observations from this survey period (e.g., detection of spotted skunks in a Pasture land cover type) along with a low number of detections negatively influenced our ability to assess environmental factors that influence spotted skunk occupancy. In the future, conducting a single season analysis may allow us to identify these factors.

Our assessment of the probability of plains spotted skunks to colonize discrete patches indicated that the distance from the camera station to the nearest Macartney rose cluster was our most supported model. This single variable model, however, was not more than 2 ΔAICc from the null model (ΔAICc: 1.61). These were the only two models under 2 ΔAICc. As distance to the nearest Macartney rose decreased, probability of spotted skunk colonization increased; however, these results were no better supported than our null model which was a more parsimonious model.

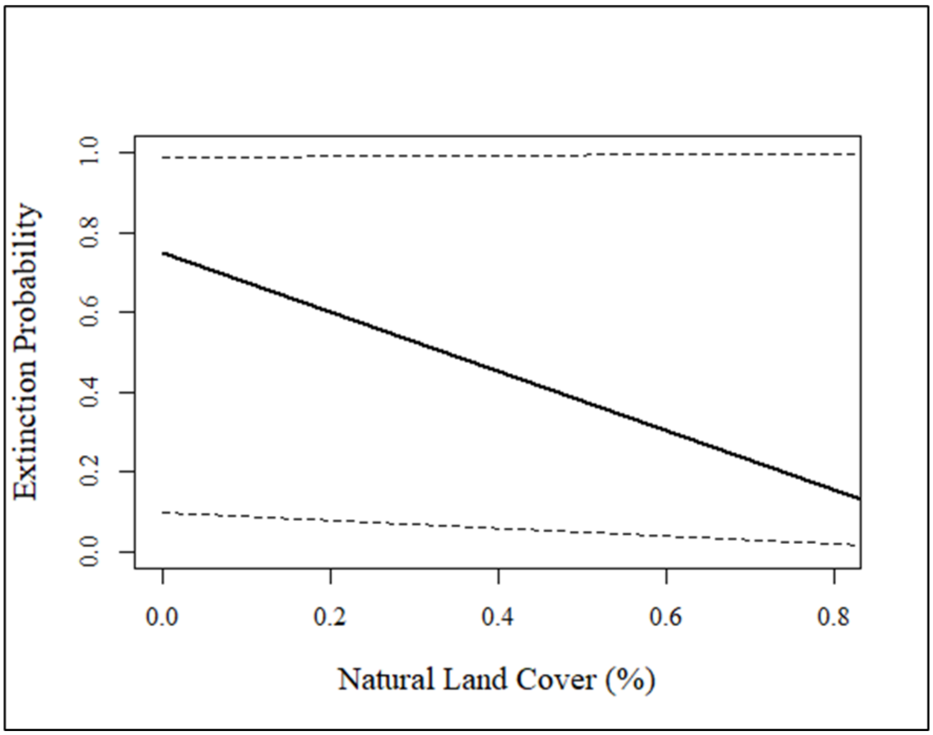

Figure 2. Predicted plains spotted skunk extinction probability as a function of proportion of natural land cover with 95% confidence intervals from a camera trap survey in Harris County, Texas; 2020 - 2021.

Our assessment of the extinction probability of plains spotted skunk within discrete patches suggested that natural land cover affected plain spotted skunk's persistence. A single variable model, proportion of natural land cover, was our top-ranking model, and was more than two ΔAICc from the next most supported model and the null model (Table 3). We observed an inverse relationship between the proportion of natural land cover and spotted skunk occupancy. As the proportion of natural land cover within a patch decreased, the probability of patch extinction increased (Figure 2).

Table 3. Ranked *a priori* models for assessing probability of plains spotted skunk extinction within discrete patches in Harris County, Texas; 2020-2021. All models include distance to the nearest road (*p*) as this was determined to be the factor that most influenced the detection probability of plain spotted skunks.

Model	df	LL	AIC	ΔAIC	w_i^c
p + natural	6	-127.693	272.637	0.00	0.396
p + mowed	6	-128.825	274.899	2.26	0.128
p + null	5	-130.902	275.333	2.70	0.103
p + MR + natural	7	-127.219	275.904	3.27	0.077
p + cattle + natural	7	-127.670	276.807	4.17	0.049
p + Pasture + ephemeral + mowed	8	-125.285	276.856	4.22	0.048
p + Pasture	6	-129.824	276.898	4.26	0.047
p + Pasture + mowed	7	-128.075	277.617	4.98	0.033
p + MR + D2MR	7	-128.197	277.860	5.22	0.029
p + cattle + mowed	7	-128.246	277.959	5.32	0.028
p + cattle	6	-130.604	278.458	5.82	0.022
p + ephemeral	6	-130.799	278.847	6.21	0.018
p + cattle + Pasture	7	-129.093	279.652	7.02	0.012
p + cattle + Pasture + mowed	8	-126.862	280.010	7.37	0.010
p + Global	10	-122.454	283.242	10.61	0.0020

df: Number of model parameters
LL: Log likelihood
AIC: Akaike's Information Criteria
w_i^c: Model weight

Discussion

At Warren Ranch, 87% of all plains spotted skunk observations (from surveys 1 and 2) originated from camera stations deployed where the dominant land cover was classified as natural land cover. During survey 2, forty one percent of camera stations deployed at Warren Ranch were deployed in this land cover type while natural land cover constituted 48% of the total land cover at the site.

Our results suggest that as the total proportion of natural land cover decreased spotted skunk occupancy also decreased. During our survey, we observed 3 patches that were initially occupied that became extinct. Cumulatively, plains spotted skunks were detected during 50% of all secondary survey periods in winter and spring 2020 (9/18). Management of land cover occurred in summer 2020, resulting in a reduction in natural land cover (two pastures were partially mowed; 1 pasture was converted to agriculture). Afterwards, a single collared, spotted skunk was detected twice during 34 secondary survey periods (5.8%) over the next 4 primary survey periods in these 3 patches. Based upon spatial data, we can confirm that the spotted skunk evacuated the patch near the camera station after it had been mowed, remained in a portion of the pasture that was less intensely mowed for 3-months and then returned to an area near the camera traps in late fall 2020. Within 2 weeks of returning to the area near the camera traps, the skunk had been predated by an avian predator. We additionally observed similar patterns from 2019 through 2020 that were not incorporated into our occupancy analysis.

Of the three camera stations in patches with continuous known occupancy, all three patches were composed of more than 50% natural land cover. Two patches were not managed during the survey period, one was partially mowed, but both cameras at the station remained in the unmowed portion of the patch. While frequency of skunk detections decreased after the patch was mowed, this patch did remain occupied during the entire survey. These 3 stations contributed 40.5% of all secondary surveys with a positive skunk detection and two stations attributed 48% of all detections recorded at Warren Ranch during survey 2. Moreover, during survey 2, by either capture or spatial relocations (Chapter 3), we recorded more than 25 unique plains spotted skunks using these 3 pastures.

To our knowledge, it has never been hypothesized that plains spotted skunks are internal, obligate species. However, the results of our detection probability analysis suggested that distance to the nearest road affected our ability to detect spotted skunks. As previously noted, our roads category included local farm to market roads, internal gravel roads, and firebreaks applied along the external peripheries of individual pastures. We fully acknowledge that as presented here, we cannot disentangle the effects from daily traffic on these roads from edge effects created by the installment and maintenance of the roads. Previous works have suggested that road-based mortality can provide information sufficient to identify remaining populations of spotted skunks (Dowler et al. 2007, Perry et al. 2021, Perkins et al. 2022), but local analyses have either failed to incorporate this metric or provide mixed results as to the influence of roads upon spotted skunks (Eng and Jachowski 2019, Harris et al. 2020, Jefferson 2021).

We *a priori* hypothesized that management strategies implemented on Warren Ranch resulted in a dynamic system whereby occupied pastures continually went extinct and were subsequently recolonized. While we were unable to find support for our pasture recolonization hypothesis (likely because our survey period was too short), we did find support for our extinction hypothesis. Patch extinctions were tied to a decrease in proportion of natural land cover within the patch. Moreover, changes that decreased the proportion of natural land cover within a patch could be directly tied to management activities within individual pastures. While habitat loss and alteration have long been the leading hypothesized cause of plains spotted skunk decline (Choate et al. 1973, Gompper 2017), this is to our knowledge, the first time a quantifiable analysis has shown that localized land cover change negatively affects spotted skunk occupancy. Moreover, we only detected spotted skunks twice at three camera stations where the majority land cover type was classified as Pasture even though these three stations were operational for 9 continuous seasons, spring 2019 through spring 2021. We believe that the combined results of low spotted skunk occurrence in Pasture land cover locations combined with our results suggesting that a decrease in natural land cover decreases plains spotted skunk occupancy within an individual patch validate previous hypotheses detailing species decline. Moreover, our results, at a localized level, confirm that the loss of natural land cover in a short time, will cause either abandonment or mortality of spotted skunks.

Perhaps the most encouraging takeaway from this chapter is this: plains spotted skunks can persist and seemingly thrive in locations that include a diverse management strategy, a landscape that includes both natural and invasive species, and, in our case, a location that includes a diverse array of anthropogenic uses. It came as no surprise to us, that the presence of cattle had little effect on spotted skunk occupancy. While the presence of cattle was higher in Pasture land covers, we recorded few overall spotted skunk detections in Pastures. Within natural land cover, we recorded fewer cattle, but routinely recorded spotted skunks and cattle at individual camera traps during individual nights. While we recorded more than 30 unique events that included both striped skunks and cattle in a single image, we recorded only a single event that included a spotted skunk and cattle.

Our classification of impervious surfaces, that included both permanent land covers (e.g., county roads, oil/gas pads) as well as manipulation to land cover (e.g., fire breaks) suggested that proportion of impervious surfaces within a patch was not relevant to spotted skunk occupancy. We note in chapter 6 that we cannot attribute a single spotted skunk mortality event to an anthropogenic cause. While only tangentially established here, we hypothesize that conserved locations, such as Warren Ranch, with management plans that simultaneously conserve natural land cover and incorporate a wide variety of anthropogenic land uses are important to the future conservation of this species. As noted throughout this report, anthropogenic activities at Warren Ranch included oil and gas extraction, salt extraction, cattle production, as many as 5 discrete housing units, wetland restoration and conservation, and prairie restoration and conservation. Moreover, an examination of historical aerial imagery combined with conversations with landowners (see Warren 2020) indicates that prior to the initiation of CPC's conservation efforts in 2003, the majority of Warren Ranch was not conducive to spotted skunk persistence. We propose that in the coastal prairie ecoregion, multiple land use practices combined with a robust prairie management plan will allow plains spotted skunks to persist and potentially thrive.

Chapter 5: Rest site selection of plains spotted skunks (*Spilogale interrupta*) in a Texas coastal prairie

Introduction

Wildlife habitat is species specific, and contains essential resources including space, cover, food, and water (Leopold 1933, Hall et al. 1997). Cover is often required for foraging, resting, loafing, and rearing of young and can further be partitioned based on circadian rhythms of species. Nocturnal species require diurnal resting locations that provide protection from the elements and predation (Crabb 1948) and specific requirements of a rest site can vary seasonally, annually, or based upon the age, sex, or reproductive status of the individual (Thorne and Forde 2022).

Diurnal rest sites are essential resources for many carnivores (Rabinowitz and Pelton 1986; Doty and Dowler 2006; Gess et al. 2013) and availability of rest sites potentially limits the abundance and distribution of spotted skunks (Crooks 1994; Thorne and Ford, 2022). Plains spotted skunks (*Spilogale interrupta)* exhibited variation in den site selection in Iowa using anthropogenic sources such as crop storage locations, dens commandeered from local conspecific species, logs or hollow trees, and ground dens composed of grass or hay (Crabb 1948). In forested land cover types, both plains and eastern spotted skunks (*Spilogale putorius*) are known to utilize diurnal resting locations that are both above ground and sub-terrestrial burrows (Hassler et al. 2021, Lesmeister et al. 2008, Sprayberry and Edleman 2018). Desert spotted skunks (*S. leucoparia*) in Texas mesquite scrub selected sites with dense cover (Doty and Dowler 2006). Harris et al. (2020) examined den site selection of Florida spotted skunks in a dry prairie vegetative community and reported differences in den site selection from studies in more mountainous regions. These results suggest that diurnal resting site selection for spotted skunks varies between species and regions.

This is the first comprehensive examination of rest site selection of plains spotted skunks in a rangeland setting and is based on research first reported by Jefferson (2021). Historical descriptions from coastal Texas noted opportunistic use of diurnal resting cover in bushes, tall grass, stream banks, and old buildings by plains spotted skunks (Bailey 1905). Our objectives were to investigate rest site selection of plains spotted skunks in a rangeland coastal prairie and assess sex or seasonal differences in rest site selection.

Materials and Methods

We conducted our study within the Katy Prairie of Texas, a coastal prairie within the northern portion of the Western Gulf Coastal Plain (WGCP) ecoregion (Griffith et al. 2007). The Katy Prairie is bounded by pine forests of the Post Oak Savannah ecoregion on the north, greater Houston on the east, and the Brazos River on the west. The WGCP ecoregion extends from central Louisiana to southern Texas and lies immediately adjacent to the Gulf of Mexico. This ecoregion is characterized by its flat topography, grassland type vegetation, slow drainage, and wet soils (Griffith et al. 2007). The WGCP ecoregion was historically a fire maintained coastal prairie with a grassland community dominated by little bluestem (*Schizachyrium scoparium*), yellow indiangrass (*Sorghastrum nutans*), brownseed paspalum (*Paspalum plicatulum*), gulf muhly (*Muhlenbergia capilaris*), and switchgrass (*Panicum virgatum*; Griffith et al. 2007). At our site, Warren Ranch, shrubs, brambles, and woody saplings were prevalent in pastures last burned prior to 2016 and pastures with sporadic mechanical manipulation. Brambles included the invasive Macartney rose (*Rosa bracteata)* in association with southern dewberry (*Rubus trivialis*) and pepper vine (*Ampelopsis arborea*), while the deciduous shrubs yaupon holly (*Ilex vomitoria*) and eastern baccharis (*Baccharis halimifolia*) were also present.

Live trapping and processing

Live capture and processing methods were previously reported (Perkins et al. 2021; Chapter 3). All handling and immobilization procedures were in accordance with the guidelines of the American Society of Mammalogists for the use of wild animals in research (Sikes et al. 2016). Methodology was conducted under Angelo State University IACUC numbers 18-208 and 19-202, Texas Tech University IACUC number 18103-12, and Texas Parks and Wildlife Department Scientific Research Permit number SPR-0390-029.

Tracking

We used a portable telemetry receiver (R-1000 Receiver, Communication Specialists Inc., Orange, CA, USA) and a 2-element H antenna to track skunks weekly (range 1-5 days per week) to a diurnal rest site until mortality, transmitter failure, or the individual traveled outside of our study site. During each tracking event, we recorded the type of overhead cover directly above the skunk (e.g., Macartney rose). We classified all located sites as rest sites, although locations where females were rearing young that may have best been classified as den sites.

Rest site habitat characterization

Within 15 days of a tracking event, we measured the habitat characteristics at each rest site. To evaluate den site selection relative to what was available (Hall et al. 1997), we surveyed a random, paired site (henceforth “paired site”) for each rest site. We determined paired sites by using a handheld GPS unit (Garmin, Kansas City, KA, USA) to mark a location 100 m from the rest site on a randomly generated azimuth. One-hundred m was previously reported to be within the range of minimum nightly-traversable distances for eastern spotted skunks (Eng and Jachowski 2019). If a paired site was within a wetland, outside the study site, or inaccessible due to management activities, we repeated the process until an appropriate paired site was chosen. If a skunk re-used a rest site after 30 days, we treated it as an independently selected location. We did not record habitat characteristics at a rest site when it was reused by a skunk within 30 days of its last recorded use, the rest site was inaccessible, the location was significantly altered after the tracking event (e.g., mowed or plowed), or we were unable to survey within 15 days.

At each site, we measured habitat characteristics within a 10 m radius plot centered at the site (Appendix 5.1). We deployed four transects leading from the center of the plot to a terminal node in each cardinal direction. Measurements recorded at the nodes were averaged across all four nodes and reported as the plot average. Relative saturation of the soil was measured at the center of each plot using a Kelway Soil Acidity and Moisture Tester (Model HB-2, Teaneck, NJ, USA). When a site was flooded, we recorded relative saturation as 100%. Canopy cover measurements were taken using a densitometer (Convex Model A, Forestry Suppliers, Jackson, MS, USA) held level in each cardinal direction then averaged for the center and for the plot. The depth of grassland litter was measured at the center and at each node using a 305-mm ruler (Bakker et al. 2002).

Visual obstruction within each plot was recorded with a 2-m Robel pole (marked in 10 cm increments) at the plot center and each terminal node (Robel et al. 1970; Toledo et al. 2008). Visual obstruction measurements were averaged across to obtain both center and plot means. The tallest vegetative heights both within and at the plot center were measured. We recorded the dominant ground cover type along each transect by belt-line survey using 0.5 m increments (Grant et al. 2004). To record prevalence and relative height of non-grass vegetation within each plot, we recorded the total number of shrubs, trees and discreet brambles including southern dewberry, vines, and Macartney rose. Chinese tallow, eastern baccharis, and Macartney rose within each plot were further classified by height – small (< 1 m, present but species was a part of the overall matrix), medium (1 m – 2.5 m, moderately prevalent), large (>2.5 m, species was dominant within clumps; Appendix 5.1).

To develop explanatory variables, we used Geographical Information Systems (GIS) software (ArcGIS 10.7.1, Environmental Systems Research Institute, Redlands, CA) to georeference water and road features and measure distance to nearest feature class for both rest and paired sites. We categorized water features into three classes: riparian corridors, permanent wetlands, and ephemeral wetlands. We defined riparian corridors as the areas encompassing a stream channel and the immediate proximate banks of the stream. We distinguished wetlands based on water permanence (degree of water retention) and basin size, such that permanent wetlands were identified as basins that sustained water year-round and were more than 0.3 ha in size; conversely, ephemeral wetlands were identified as basins that remained flooded for short periods of time during the year and were 0.3 ha or less in size (Kantrud and Stewart 1977; Dahl 2014). Road features were separated into two classes, primary – gravel, traveled daily; secondary grass paths, traveled less than daily.

We hypothesized that four biological factors would affect diurnal rest site selection of plains spotted skunks at our study site. First, we hypothesized that rest site selection would be positively associated with the type and amount of cover at a potential rest site. Crabb (1948) established that an appropriate spotted skunk site must exclude sunlight, provide protection and insulation from weather conditions, and provide protection from potential predators. Studies conducted in other regions of the range of spotted skunks have also found cover to be an important influence in site selection (Lesmeister et al. 2008; Sprayberry and Edelman 2018). Second, we hypothesized that protection from predators would have a positive influence on rest site selection. Previous studies have indicated that spotted skunks selected sites that reduce the visibility of skunks to predators and the maneuverability of predators, especially larger mesocarnivores and owls (Lesmeister et al. 2008; Eng and Jachowski 2019; Sprayberry and Edelman 2018).

Third, we hypothesized that rest site selection was negatively associated with relative saturation of the soil. The WGCP ecoregion has poor drainage due to the clay subsoil and low relief, which can result in heavy flooding for extended durations of the year (Griffith et al. 2007). Alternatively, Eng and Jachowski (2019) indicated that proximity to a water source may serve as an indicator of higher quality forage availability and cover, suggesting that plains spotted skunks would select sites closer to water.

Fourth, we hypothesized that rest site selection would be negatively associated with heavily trafficked, caliche primary roads. Previous studies have indicated that roads may serve as a barrier to movement between habitats for wildlife (Oxley et al. 1974; Richardson et al. 1997; Rico et al. 2007; Benítez-López 2010). In contrast, secondary roads that were only maintained annually and potentially provided a transition for skunks moving between adjacent pastures. Thus, we expected rest sites to be located closer to secondary roads.

Model development and validation

We used a discrete choice modeling approach to fit and evaluate support for each *a priori* hypothesis. Discrete choice analysis allows for associations of relevant variables and availability of resources at each rest site to be evaluated separately, as each rest site is compared to a paired site, while still accounting for temporal and spatial variations in resources (Arthur et al. 1996, Cooper and Millspaugh 1999). When fitting our discrete choice models, each sample consisted of a ‘choice set’ that included data from a rest site and its corresponding paired site (Cooper and Millspaugh 1999). We included a random effect (‘id’) in each model to account for variation in resource selection among individuals (Bodinof et al. 2012).

We developed 22 models to evaluate support for the four factors hypothesized to influence rest site selection of plains spotted skunks (Table 2). We used multinomial logistic regression through the package 'mlogit' (Croissant 2020) in the Program R version 4.0.4 (R Development Core Team 2021) to implement our discrete choice modeling. In addition to the models created for each *a prior* hypothesis, we also fit a global model incorporating all recorded covariables and 3 sub-global models to evaluate relative support for variables measured within 5 m of the rest site, variables measured more than 5 m from the center of the rest site, and variables representing percentage of ground cover classes (Appendix 5.1). We used Kendall's correlation coefficients to determine that none of the variables included in a model were colinear ($|r| \geq 0.7$). To increase parsimony in our models we simplified overhead cover to Macartney rose, southern dewberry, grass, forbs, or other (cover types recorded ≤ 1%of all tracking events).

To rank competing models, we used Akaike's Information Criterion with an adjustment for small sample sizes (AICc) and calculated distance from lowest AICc ($\Delta AICc$), log-likelihood (L), number of variables (K), and model weights (w_i) to compare model support (Burnham and Anderson 2002). We assessed the performance of our top model using ten iterations of a 10-fold cross validation (Boyce et al. 2002). For each iteration we created a random subset of 80% of our data (maintaining 1:1 choice sets) to function as 'training sets' to fit our model and the remaining 20% to function as "test sets" to provide an unbiased evaluation of the trained model. We assessed our trained model with the test sets and for each iteration we calculated the relative probability of selection for each rest site and paired site in our choice sets (Bodinof et al. 2012). Across our test sets we pooled the proportion of rest sites that were correctly predicted as being selected by our model. If the relative probability of selection across our pooled test sets was greater than 0.5, we determined that the performance of our top model was better than what would be expected at random (Bodinof et al. 2012).

Results

From May 2019 through February 2021, we monitored rest site selection of 30 plains spotted skunks (13 females and 17 males). We recorded 652 total tracking events at 426 unique rest sites (212 female and 214 male; Fig. 1, Table 1). Monitored skunks reused rest sites 22% of the time (n = 143); however, 57 reuse events were attributed to 2 skunks that exhibited high fidelity to 2 anthropogenic debris piles. We collected data at an average number of 14 rest sites (range 1-40) per skunk. No individual female contributed more than 10% of unique rest sites and no individual male contributed more than 8% of unique rest sites overall. Overall, we recorded an average of 106 rest sites per season with an average of 6.7 rest sites per female per season and an average of 5.5 rest sites per male per season (Table 1).

Table 1. Number of unique plains spotted skunks with monitored diurnal rest site usage per season (n^a), total number of plains spotted skunk diurnal rest sites per season (n^b), and mean number of rest sites used per individual ($\bar{x}$) plains spotted skunk in Harris County, Texas, 2019 - 2021.

		Season				
Sex		Winter	Spring	Summer	Fall	Total
Female						
	n^a	11	6	7	8	13
	n^b	70	45	45	52	212
	$\bar{x}$	6.36	7.50	6.43	6.5	6.70
Male						
	n^a	10	9	10	10	17
	n^b	57	54	59	44	214
	$\bar{x}$	5.70	6.00	5.90	4.4	5.50
Total		127	99	104	96	426

Rest site habitat characterization

While we recorded skunks utilizing a variety of structures as rest site overhead cover, most rest sites were located under Macartney rose brambles (75.4%, n = 321; 162 small, 134 medium, and 25 large; Figure 1). Secondarily, we recorded rest sites in southern dewberry brambles (14.6%, n = 62) and bunch grass (6.1%, n = 26). Other cover types included pepper vine (0.7%, n = 3), forbs (0.7%, n = 3), deep-rooted sedge (0.7%, n=3), yaupon holly (0.7%, n=3), and grassland litter (0.5%, n=2).

Model selection and validation

Our cover hypothesis received the most support with the $Cover_1$ model ranking as the top model (Table 2). Our $Cover_1$ model included southern dewberry as overhead cover, height of visual obstruction, depth of grassland litter at the center of the rest site, total number of small Macartney rose brambles, and percentage of Macartney rose as ground cover (w_i = 1.000). Other variables in our top model had odds ratio 95% confidence intervals that overlapped 1.0, thus we were unable to determine the influence of the effects of these variables on plains spotted skunk rest site selection.

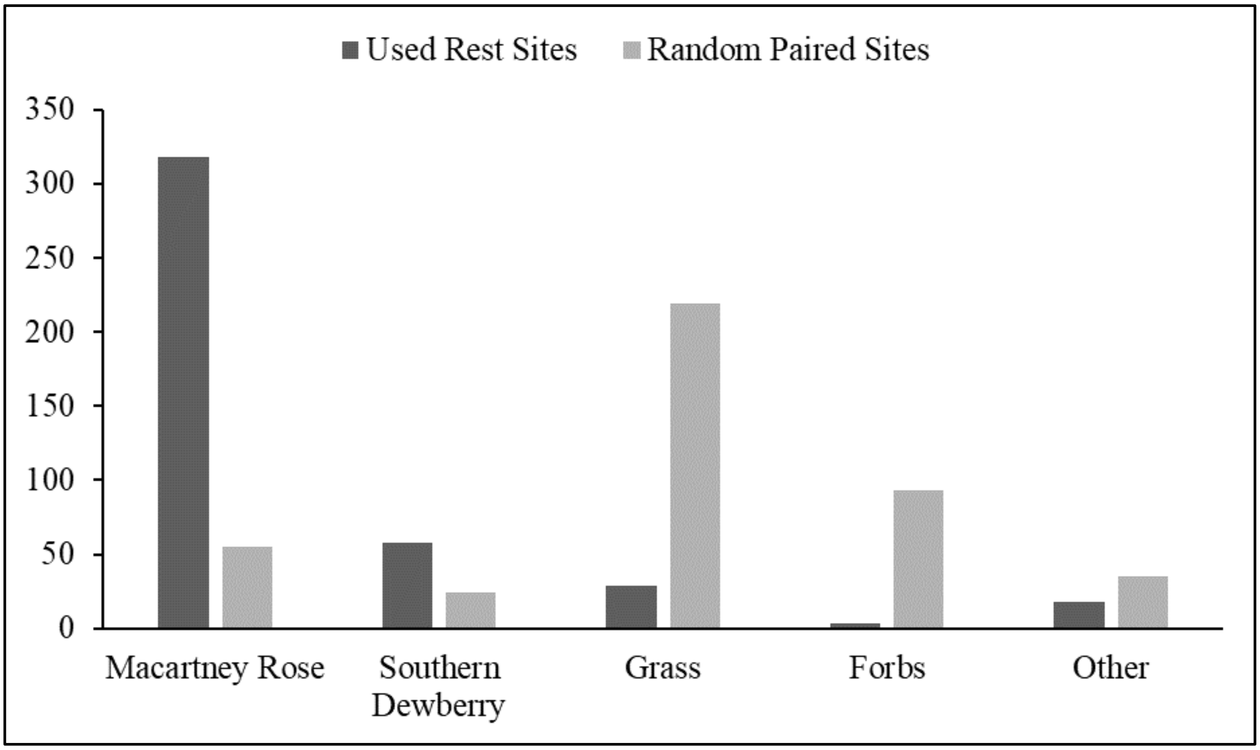

Figure 1. Overhead rest site cover selected by plains spotted skunks at Warren Ranch from 2019-2021, compared with paired sites surveyed.

Table 2. Output of the five top-ranking *a priori* models developed to predict plains spotted skunk rest site selection at Warren Ranch from 2019 - 2021.

Model	Log(L)	K	AICc	ΔAICc	wi
Cover1: overhead + center.rob + center.lit + node.lit + node.rob + mr.sm + mr.med + mr.lg + mr + db + litter + drs + grass + forb + bare + id	-55.66	20	148.13	0	1
Subglobal1: overhead + center.ht + center.rob + center.can + center.lit + id	-77.41	10	171	22.86	0
Global: overhead + center.ht + plot.ht + moisture + center.rob + node.rob + center.can + node.can + center.lit + node.lit + mr.sm + mr.med + mr.lg + eb.sm + eb.med + eb.lg + ct.sm + ct.med + ct.lg + db.ct + vine.ct + shrub.ct + tree.ct + grass + forb + shrub + tree + litter + bare + msv + drs + mr + db + vine + debris + hay + cwd + rock + water + primroad.dist + secroad.dist + riparian.dist + ephemeral.dist + permanent.dist + id	-42.5	48	171.26	23.13	<0.001
Subglobal3: grass + forb + shrub + tree + litter + bare + msv + drs + mr + db + vine + debris + hay + cwd + rock + water + id	-104.18	17	230.67	82.53	<0.001
Cover5: mr + db + drs + id	-112.79	4	231.6	83.47	<0.001

Plains spotted skunk rest site selection was positively associated with southern dewberry as overhead cover. Relative to Macartney rose, the odds of a skunk selecting for southern dewberry as the overhead cover was approximately 5-fold greater, contrary to the predicted positive influence of Macartney rose overhead cover in our cover hypothesis. Our results showed a positive relationship with the height of visual obstruction at the center of the rest site. Relative probability of a skunk selecting a rest site increased by 6% for every 10-cm increase in the height of visual obstruction at the center of the rest site, providing support to our Subglobal_1 model of all variables measured within 5 m of the center of the rest site (Figure 2). Contrary to our model, there was a negative relationship with the height of visual obstruction for the plot average. In such cases the relative probability of selection decreased by about 5% for every 10-cm increase in the height of visual obstruction of the plot, lending support to our $Subglobal_2$ model of all variables measured more than 5 m from the center of the rest site. For every 10% increase in the percentage of Macartney rose ground cover, the relative probability of selection increased by 7%, thus adding support for our $Subglobal_3$ model for ground cover classes. We also observed a slight positive influence of depth of grassland litter at the center of the rest site and the total amount of small Macartney rose brambles, providing limited support to our $Subglobal_1$ model and our predator avoidance hypothesis, respectively. For every 1-unit increase in grassland litter depth or amount of small Macartney rose brambles, there was an equal 1% increase in the relative probability of selection. We found no support for our hypotheses for road avoidance or water avoidance.

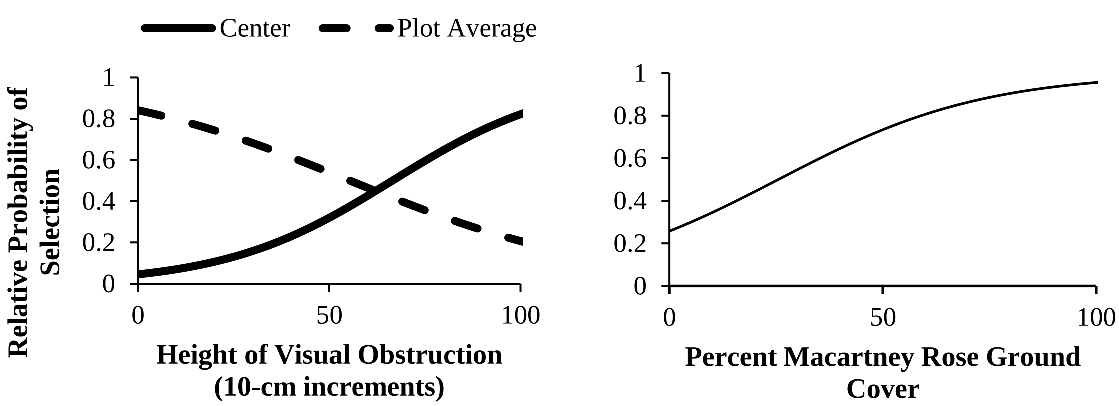

Figure 2. Predictive plots illustrating the change in relative probability of plains spotted skunks at Warren Ranch, Harris County, Texas (2019-2021) selecting a rest site with changes in the height of visual obstruction at the center and within a 10 m radius of the center (i.e., plot average) and the percentage of Macartney rose ground cover.

When we separated our data by sex, we found that rest site selection of female skunks was best explained by our Global model (w_i = 0.9971). For males, we found there was no change in the resulting $Cover_1$ model as the top model (w_i = 1.0000). The influence of variables from the

Global model and the $Cover_1$ model on sex differences in rest site selection were indiscernible due to the variables all having odds ratio 95% confidence intervals overlapping with 1.0.

When we separated data by season, we found differences in the top models to explain plains spotted skunk rest site selection. Rest site selection in the spring was best explained by our $Subglobal_1$ model (w_i = 0.989), which included all variables measured at the center of the rest site. Height of visual obstruction had a positive influence on spring rest site selection and was the only variable with odds ratio 95% confidence intervals not overlapping 1.0, providing support to our cover hypothesis. Rest site selection in the summer was best explained by our $Cover_5$ model (w_i = 0.608) with the variables for percent of ground cover of Macartney rose, dewberry, and deep-rooted sedge. However, only the percent ground cover of Macartney rose and dewberry had a positive influence that could be interpreted, lending further strength to our cover hypothesis as well as our forage availability hypothesis and $Subglobal_3$ model. For the summer season, the second-ranking model, $Cover_1$, was within 2 AIC scores of our top model (w_i = 0.3094, ΔAIC = 1.4). The influence of variables in the $Cover_1$ model on the summer rest site selection of plains spotted skunks could not be determined because of 95% confidence intervals overlapping 1.0. The top-ranking model to explain rest site selection in the fall and winter was the $Cover_1$ model (w_i = 0.9800 for fall; w_i = 1.000 for winter), however the influence of the variables in this model could not be determined due to 95% confidence intervals overlapping 1.0. Our 10-fold cross validation suggested that our top-ranking $Cover_1$ model to explain plains spotted skunk rest site selection was accurate in predicting use of a rest site approximately 80% of the time.

Discussion

Our results suggest that plains spotted skunk rest site usage was primarily dictated by availability of cover and most importantly by overhead cover type. Although spotted skunks overwhelmingly selected Macartney rose as the overhead cover type at rest sites, discrete choice analysis suggested they selected southern dewberry over Macartney rose when available. Macartney rose, a native species in China and Taiwan, was introduced into the United States in the 1800s as hedge rows for fencing purposes but has since rapidly spread in the southern United States and become difficult to control (Scifres 1975). In southeast Texas, Macartney rose reduces livestock's foraging ability by obstructing the establishment of forbs and grasses and limiting livestock to the areas where the species is not present. If left untreated, individual Macartney rose brambles will eventually merge into a single, large thicket (Scifres 1975; Gordon and Scifres 1977; Enloe et al. 2013). Although there is a limit to livestock foraging, Macartney rose provided cover for plains spotted skunks at our study site.

One explanation for the modeled importance of southern dewberry over Macartney rose may be that the shape and growth of southern dewberry is more effective at concealing plains spotted skunks from predators and resource competitors. Southern dewberry is a low-growing, trailing shrub native to Texas (Blanchard 1911; Oefinger and Halls 1974). At our site, southern dewberry clumps were primarily under 1 m in height, allowing the dense foliage to effectively blanket the area under the bramble (KPJ pers. obs.). Macartney rose, conversely, is a climbing and trailing shrub with thorny, arching canes that can extend greater than 1 m from the parent bramble to attach to nearby vegetation or back onto itself to increase in size (Scifres 1975; Gordon and Scifres 1977). At our study site, plains spotted skunks used rest sites with southern dewberry as overhead cover had an average height of 80 cm at its center compared to 120 cm at rest sites with Macartney rose as overhead cover. As Macartney rose grows into tall, dense thickets, the lower portion of the bramble receives less sunlight than the central or upper parts of the bramble, thus the foliage in these areas is less dense. Eng et al. (2018) suggested that vegetative cover may increase the efficacy of the cryptic appearance of eastern spotted skunks to avoid detection by predators. Of the 11 known mortalities of spotted skunks from 2019-2021 at our study site, 7 (64%) were categorized as predation based. The contrasting pattern of white spots and stripes on the black body of a spotted skunk enhances pattern blending against patches of leafy shade and moonlight shadows (Howell 1906; Kinlaw 1995; Caro 2009; Caro et al. 2013). Spotted skunks may have stronger selection for southern dewberry because it enhances their cryptic appearance and for the denser cover it provides to reduce detection by predators.

During our study, we recorded the diameter and width of the entrance width of plains spotted skunk burrows; however, most burrow entrances could not be accessed without major disruption of the overhead cover. We never tracked a plains spotted skunk to burrow with an entrance large enough to allow entry of sympatric species (striped skunk, raccoon, opossum, or nine banded armadillo). Similar to findings reported by Lesmeister et al. (2008) and Harris et al. (2020) regarding the size of burrow entrances, it may be more difficult for animals larger than plains spotted skunks to enter brambles with small openings. As the lower portion of tall (> 1 m) Macartney rose brambles loses foliage, openings into the bramble become more accessible to entry by wildlife (Dickinson and Arnold 1996; KPJ, pers. obs.). At our study site, we observed coyotes (*Canis lupus*) and great horned owls (*Bubo virginianus*), known predators of spotted skunks, (Kinlaw 1995; Lesmeister et al. 2010), striped skunks (*Mephitis mephitis*) and Virginia opossums (*Didelphis virginiana*), potential resource competitors of spotted skunks (Kinlaw 1995; Doty and Dowler 2006), and other animals (small mammals and raptors) occupying Macartney rose brambles (KPJ, pers. obs.; M. H. Hamilton, pers. comm.). The larger size of these animals suggests that Macartney rose is more likely to be inhabited or explored than southern dewberry, thus limiting their use by plains spotted skunks.

Crabb (1948) indicated that the three site requirements for spotted skunks are cover from predators, exclusion of sunlight, and protection from weather events, all of which the dense cover of southern dewberry can potentially provide. Lesmeister et al. (2008) indicated that thermoregulation influences rest site selection of eastern spotted skunks; grassland litter at the center of the rest site increased the odds of selection of a used rest site, indicating that it may be useful in thermoregulation. Moreover, an examination of two plains spotted skunk burrow systems revealed both systems contained a central cavity with fine plant litter for thermoregulation (Benson et al. 2023).

Another explanation for selection of southern dewberry as overhead cover at their rest sites by plains spotted skunks is the historical component. Southern dewberry is native to our study site whereas Macartney rose is an introduced species (Hart et al. 2008). Neither plant species was specifically mentioned in previous studies conducted in the range of the plains spotted skunk (Bailey 1905; Crabb 1948; Choate et al. 1974). Crabb (1948) mentioned that rose (*Rosa* sp.) and raspberry (*Rubus* sp.) species of shrubs were characteristic of the woodland portion of his study area in Iowa, though they were not considered as habitat for spotted skunks compared to the more abundant tall grass prairie and farmland surrounding the woodland area. Crabb's (1948) findings were based on incidental observations of skunks rather than tracking radio-collared individuals. Our findings indicated that Macartney rose ground cover had a positive influence on rest site selection, suggesting that the plant species is valued more in the surrounding 10 m of the rest site than directly at its center. Combined with the slight positive effects of small Macartney rose brambles and a decreasing height of visual obstruction in the plot, we suggest that plains spotted skunks utilize Macartney rose for cover when its height is low enough to conceal plains spotted skunks and limit the entry of larger wildlife. More research into the habitat selection of the species is needed, especially in parts of Texas where Macartney rose is not present, to further understand the importance of southern dewberry to plains spotted skunk rest site selection.

Arthropods, specifically beetles (family Coleoptera), grasshoppers (family Orthoptera), and millipedes (class Diplopoda), have been reported as dietary components of eastern spotted skunks (Howell 1906; Crabb 1941; McCullough and Fritzell 1984; Kinlaw 1995; Harris et al. 2020). Previous studies suggested that ground litter or leaf litter may provide habitat for potential arthropod prey items (McCullough and Fritzell 1984; Eng and Jachowski 2019; Harris et al. 2020). For our study, grassland litter at the center of the rest site increased the odds of selection of a used rest site, although contrary to our cover and forage hypotheses, the percent of litter as ground cover and the average depth of grassland litter at the 4 terminal nodes of the plot had no discernible impact on plains spotted skunk rest site selection. No other variables included under our forage hypothesis, except for the percent of southern dewberry ground cover in the summer had any discernible effects on rest site selection. Southern dewberry flowers in the spring and the fruit matures by early summer (Oefinger and Halls 1974; KPJ, pers. obs.), potentially providing an abundant source of food to spotted skunks, either directly for berries or indirectly for insect foraging, for a limited time in the late spring and summer.

Similar to Harris et al. (2020), our findings indicated that the height of visual obstruction had a small influence on rest site selection overall, and more specifically, in the spring. Visual obstruction at plot center had a positive influence on rest site selection, while visual obstruction at plot nodes had a comparable negative influence on rest site selection. From these findings, in addition to the high importance of dewberry as overhead cover at the center of the rest site, we suggest that plains spotted skunks are valuing cover at the fine-scale (within 5 m of the rest site) over cover at a coarser-scale (more than 5 m from the rest site). In conjunction with our findings of selection for southern dewberry brambles and the high frequency of use of small Macartney rose brambles, we suggest that plains spotted skunks value a heterogenous habitat that provides sources of cover with adjacent areas for foraging.

The top-ranking model for female rest site selection was our global model that included all variables measured. This suggests that the models developed from our hypotheses are absent of the habitat characteristics or combinations of habitat characteristics that females are selecting, females are acting more as habitat generalists than males when it comes to their rest site selection, or we lacked sufficient sample sizes of female skunks and their rest sites to determine influences on their rest site selection.

Plains spotted skunks exhibited a seasonal variation in rest site selection, but there were also shared variables among all three models highlighted from our discrete choice analyses. During the spring season, variables measured at the center of the rest site, specifically height of visual obstruction, were important to rest site selection. During the summer, we found that the percent ground cover of Macartney rose and southern dewberry had positive influences on rest site selection. Lastly, the fall and winter seasons both shared the Cover1 model as the top-ranking model, same as for the overall dataset. Across all four seasons, variables related to the amount of cover at the rest site were included in the top-ranking model.

Consistent with previous studies of plains and eastern spotted skunks (Lesmeister et al. 2008; Sprayberry and Edelman 2018; Eng and Jachowski 2019; Harris et al. 2020), our results indicated that plains spotted skunks selected rest sites with greater cover availability. Particularly, we found that small-sized brambles of southern dewberry and Macartney rose were important in the rest site selection of plains spotted skunks. Notably, our study was conducted in a coastal prairie rangeland in contrast to other studies conducted in forested or mountainous regions (Lesmeister et al. 2008; Sprayberry and Edelman 2018; Eng and Jachowski 2019). Harris et al. (2020) reported that den type and burrow presence were the most important factors to rest site selection of Florida spotted skunks in a dry prairie habitat and we similarly found that visual obstruction plays a role in rest site selection and a lack of support of distance to a water feature or road feature to rest site selection.

The strong preference for southern dewberry by plains spotted skunks suggests the potential to use management of southern dewberry to improve spotted skunk rest site habitat on the Katy Prairie. Chance et al. (2019) found that after an intermediate disturbance event (e.g., prescribed fire), a high abundance of established native plant species reduced the ability of non-native plant species, even if already established, to invade habitats and displace native species. Although our study was focused on a single site, the similarities of our findings with studies in other parts of the range of the eastern spotted skunk indicate the generality of our findings. The amount and type of cover available to eastern spotted skunks is important in their rest site selection. Because of the unique management of our study site, selection for southern dewberry at the center and Macartney rose in the surrounding area, and the contrasting influence of visual obstruction, we suggest further research into the habitat selection of plains spotted skunks on other portions of the Katy Prairie to determine the area- and site-specificity of our results.

Appendix 5.1. Variable names, abbreviations, and descriptions of habitat parameters measured at each plains spotted skunk rest site and random paired site to evaluate plains spotted skunk rest site selection. Plains spotted skunk rest site selection was evaluated from 2019 – 2021 in Harris County, Texas.

Variable	Abbreviation	Description
Overhead cover type	overhead (MR = Macartney rose; DB = southern dewberry; GRASS; FORBS; OTHER)	Classification of the overhead cover at the center of a plot
Tallest height at center	center.ht	Height of tallest vegetation at center of plot (in m)
Tallest height in plot	plot.ht	Height of tallest vegetation in plot (in m)
Soil moisture	moisture	Percent relative saturation of soil at center of plot
Visual obstruction at center of plot	center.rob	Height of visual obstruction at center of plot (in 10-cm increments)
Visual obstruction plot average	plot.rob	Height of visual obstruction averaged in plot (in 10-cm increments)
Canopy cover at center of plot	center.can	Percent cover from canopy vegetation at center of plot
Canopy cover plot average	plot.can	Percent cover from canopy vegetation averaged in plot
Depth of grassland litter at center of plot	center.lit	Depth of grassland litter at center of plot (in cm)
Depth of grassland litter plot average	plot.lit	Depth of grassland litter averaged in plot (in cm)
Number of small Macartney rose	mr.sm	Number of Macartney rose brambles < 1 m tall
Number of medium Macartney rose	mr.med	Number of Macartney rose brambles 1-2.5 m tall

Number of large Macartney rose	mr.lg	Number of Macartney rose brambles > 2.5 m tall
Number of small eastern baccharis	eb.sm	Number of eastern baccharis shrubs < 1 m tall
Number of medium eastern baccharis	eb.med	Number of eastern baccharis shrubs 1-2 m tall

Variable	Abbreviation	Description
Number of large eastern baccharis	eb.lg	Number of eastern baccharis shrubs > 2 m tall
Number of small Chinese tallow	ct.sm	Number of Chinese tallow trees < 2 m tall
Number of medium Chinese tallow	ct.med	Number of Chinese tallow trees 2-4 m tall
Number of large Chinese tallow	ct.lg	Number of Chinese tallow > 4 m tall
Number of southern dewberry	db.ct	Number of southern dewberry brambles
Number of vines	vine.ct	Number of vine clumps
Number of shrubs	shrub.ct	Number of shrubs
Number of trees	tree.ct	Number of trees
Percent grass	grass	Percent of grass ground cover along transect lines
Percent forbs	forb	Percent of forbs ground cover along transect lines
Percent shrubs	shrub	Percent of shrubs ground cover along transect lines
Percent trees	tree	Percent of trees ground cover along transect lines
Percent grassland litter	litter	Percent of grassland litter ground cover along transect lines
Percent bare ground	bare	Percent of bare ground along transect lines

Percent moist soil vegetation	msv	Percent of moist soil vegetation (rushes and sedges) ground cover along transect lines
Percent deep-rooted sedge	drs	Percent of deep-rooted sedge ground cover along transect lines

Variable	Abbreviation	Description
Percent Macartney rose	mr	Percent of Macartney rose ground cover along transect lines
Percent southern dewberry	db	Percent of southern dewberry ground cover along transect lines
Percent vines	vine	Percent of vine ground cover along transect lines
Percent anthropogenic debris	debris	Percent of anthropogenic debris ground cover along transect lines
Percent hay	hay	Percent of hay ground cover along transect lines
Percent coarse woody debris	cwd	Percent of coarse woody debris ground cover along transect lines
Percent rocks	rock	Percent of rock ground cover in debris
Percent water	water	Percent of standing, reflective water along transect lines
Distance to riparian corridor	riparian.dist	Distance to nearest riparian corridor (in m)
Distance to ephemeral wetland	ephemeral.dist	Distance to nearest ephemeral wetland (in m)
Distance to permanent wetland	permanent.dist	Distance to nearest permanent wetland (in m)
Distance to primary road	primary.dist	Distance to nearest primary road (in m)
Distance to secondary road	secondary.dist	Distance to nearest secondary road (in m)

Appendix 5.2. Hypotheses and *a priori* models developed to evaluate plains spotted skunk rest site selection in Harris County, Texas; 2019 - 2021. A random effect ('id') was added to each model to account for resource selection amongst individual skunks. MR = Macartney rose. DB = southern dewberry. DRS = deep-rooted sedge. EB = eastern baccharis. CT = Chinese tallow.

Hypothesis	Model
Cover	
1. Positive effect of overhead cover type (MR), height of visual obstruction, depth of grassland litter, number of MR brambles, MR %, DB %, grassland litter %, and DRS %; negative effect of grass %, forbs %, bare ground %	$Cover_1$: overhead + center.rob + node.rob + center.lit + node.lit + mr.sm + mr.med + mr.lg + mr + db + litter + drs + grass + forb + bare + id
2. Positive effect of overhead cover	$Cover_2$: overhead + id
3. Positive effect of height of visual obstruction	$Cover_3$: center.rob + node.rob + id
4. Positive effect of MR brambles	$Cover_4$: mr.sm + mr.med + mr.lg + id
5. Positive effect of MR %, DB %, and DRS %	$Cover_5$: mr + db + drs + id
6. Positive effect of grassland litter %	$Cover_6$: litter + id
7. Positive effect of depth of grassland litter	$Cover_7$: center.lit + node.lit + id
8. Negative effect of forbs %, grass %, and bare %	$Cover_8$: forb + grass + bare + id
Predator Avoidance	
9. Positive effect of number of MR small brambles, MR medium brambles, and DB brambles; negative effect of number of MR large brambles, EB large shrubs, CT medium trees, CT large trees, shrubs, and trees	$Predator_1$: mr.sm + mr.med + db.ct + mr.lg + eb.lg + ct.med + ct.lg + shrub.ct + tree.ct + id

10. Positive effect of number of MR small brambles, MR medium brambles, and DB brambles	$Predator_2$: mr.sm + mr.med + db.ct + id
11. Negative effect of MR large brambles	$Predator_3$: mr.lg + id
12. Negative effect of number of EB large brambles, CT medium trees, CT large trees, shrubs, and trees	$Predator_4$: eb.lg + ct.med + ct.lg + shrub.ct + tree.ct + id

Hypothesis	Model
Forage Availability	
13. Positive effect of grass %, forb %, grassland litter %, DB %, and distance to water; negative effect of DRS % and distance to water	$Forage_1$: grass + forb + litter + db + riparian.dist + ephemeral.dist + permanent.dist + drs + id
14. Positive effect of grass %, forb %, litter %, and DB %	$Forage_2$: grass + forb + litter + db + id
15. Positive effect of distance to water	$Forage_3$: riparian.dist + ephemeral.dist + permanent.dist + id
16. Negative effect of DRS %	$Forage_4$: drs + id
Water Avoidance	
17. Positive effect of distance to water; negative effect of soil moisture content	$Water_1$: riparian.dist + ephemeral.dist + permanent.dist + moisture + id
Road Avoidance	
18. Positive effect of distance to primary road; negative effect of distance to secondary road	$Road_1$: primary.dist + secondary.dist + id
Sub-Global Models	
19. Variables measured within 5 m of the center of the rest site	$Subglobal_1$: overhead + center.ht + center.rob + center.can + center.lit + id
20. Variables measured more than 5 m from the center of the rest site	$Subglobal_2$: plot.ht + node.rob + node.can + node.lit + riparian.dist + ephemeral.dist + permanent.dist + primary.dist + secondary.dist + id
21. Variables measured as percent ground cover	$Subglobal_3$: grass + forb + shrub + tree + litter + bare + msv + drs + mr + db + vine + hay + debris + cwd + rock + water + id
Global Model	
22. All variables measured	Global: overhead + center.ht + plot.ht + moisture + center.rob + node.rob + center.can + node.can + center.lit + node.lit + mr.sm +

	mr.med + mr.lg + eb.sm + eb.med + eb.lg + ct.sm + ct.med + ct.lg + db.ct + vine.ct + shrub.ct + tree.ct + grass + forb + shrub + tree + litter + bare + msv + drs + mr + db + vine + debris + hay + cwd + rock + water + primary.dist + secondary.dist + riparian.dist + ephemeral.dist + permanent.dist + id

Chapter 6: Evaluation of plains spotted skunk cause-specific mortality with an analysis of environmental toxicants in deceased skunks

Introduction

An accurate assessment of cause-specific mortality is often an essential component of understanding population dynamics and conservation of wildlife species. Documentation of causes of mortality, including those determined to be anthropogenic, is especially important for species for which there are conservation concerns (Collins and Kays 2011). Few studies have addressed cause-specific mortality in plains spotted skunks (*Spilogale interrupta*), primarily because of the difficulty in locating these uncommon and elusive animals. As a subset of mortality concerns, environmental contaminants, including pesticides, have been implicated in the decline of biodiversity (Gibbs et al. 2009). Herein we review previous research on cause-specific mortality and environmental toxicants as they relate to plains spotted skunks and present data from our research.

Crabb (1948) was first to address the topic of spotted skunk mortality and reported mostly human-caused deaths for the skunk population on farmland in southeastern Iowa. Of 77 reported deaths in a 2-year span, 25 (32.4%) were trapped for furs, although most were bycatch of traps set for mink or striped skunks. Twenty-one were killed by farmers when spotted skunks preyed on chicken or because skunks were a nuisance with their odor around farm buildings. The third highest cause of mortality was from attacks by dogs with 20 (25.9%). The remainder were small numbers killed by traffic, cats, malice, or unknown. Interestingly, none were known to be taken by natural predators.

More recently, survival and cause-specific mortality was addressed for an Arkansas plains spotted skunk population (Lesmeister et al. 2010). Of 19 mortality events, 12 (63%) were attributed to avian predators, presumed to be great horned owls (*Bubo virginianus*) and 5 (26%)

were by mammalian predators. The remaining 11% were from unknown causes. In South Dakota, a report (White et al. 2023) suggested the potential predation of a plains spotted skunk by a long-tailed weasel (*Neogale frenata*). In other studies, predation of both eastern (*S. putorius*) and western spotted skunks (*S. gracilis*) by barred owls (*Strix varia*) has been reported (Hassler et al. 2021; Tosa et al. 2022). Of four mortalities of eastern spotted skunks in Alabama, two were determined to be mammalian predators and two by avian predators (Arts et al. 2021). A canine distemper outbreak in North Carolina caused the death of five eastern spotted skunks recovered over a period of 15 days (Harris et al. 2021). One Florida spotted skunk was found in the stomach of an American alligator (*Alligator mississippiensis* - Harris et al. 2019).

Toxicology Introduction

Persistent organic pollutants (POPs) are organic chemicals that persist in the environment and may accumulate in living organisms. Most familiar POPs, including dichlorodiphenyltrichloroethane (DDT) and its metabolites and polychlorinated biphenyls (PCBs), are considered legacy contaminants because they are no longer commercially produced and have not been for decades (de Solla 2016). Still, they continue to be present in our environment and have an impact on wildlife. The bioaccumulation of POPs has had devastating effects on many species including terrestrial wildlife (de Solla 2016). Most POPs accumulate in animal tissue from their diet and are most damaging in species near the top of food webs (Carson 1962; Mitra et al., 2011). In addition to diet, animals can acquire POPs through the atmosphere and young mammals can absorb POPs across the placenta during development or in milk from the mother during nursing (de Solla 2016, Capella et al. 2023).

Our objectives were to 1) monitor causal mortality of sympatric plains spotted skunks and striped skunks in southeastern Texas; 2) report necropsy and histopathology results from two deceased plains spotted skunks; 3) analyze presence of polychlorinated biphenyls and organochlorinated pesticides from plains spotted skunks (South Dakota and Texas) and striped skunks (Texas).

Materials and Methods

This chapter is a collaborative effort with researchers from South Dakota State University (SDSU). We first outline the collaborative efforts of the chapter before outlining the traditional Methodology section. From 2019 through 2022, 2 separate groups monitored populations of plains spotted skunks in the United States, our own group in the Katy Prairie region of southeastern Texas and a second group in central and eastern South Dakota. Both locations are known to hold remnant, robust populations of plains spotted skunks (Perry et al. 2020). Our SDSU colleagues were supported by a South Dakota Department of Game, Fish, and Parks State Wildlife Grant and tasked with assessing plain spotted skunk distribution, habitat selection, survivability, and cause specific mortality within the state. They collaborated with personnel from South Dakota Game, Fish, and Parks to salvage plains spotted skunk carcasses statewide from hunters, trappers, and road-based mortalities. Additionally, they salvaged any individuals from their monitored population including deceased individuals that were opportunistically located (White et al. 2023). Cumulatively, we report on eight individuals from South Dakota; seven of which were included in a toxicological analysis and 1 for which a necropsy was performed. To allow our colleagues the opportunity to first report their results and to provide anonymity to the locations these skunks originated from, we provide only information essential to understanding the analyses and results.

From 2019 through 2021, we monitored plains spotted and striped skunks in the Katy Prairie region of southeastern Texas. Plains spotted skunk live capture and processing methods were previously reported (Perkins et al. 2021; Chapter 3). All handling and immobilization procedures were in accordance with the guidelines of the American Society of Mammalogists for the use of wild animals in research (Sikes et al. 2016). Methodology was conducted under Angelo State University IACUC numbers 18-208 and 19-202, Texas Tech University IACUC number 18103-12, and Texas Parks and Wildlife Department Scientific Research Permit number SPR-0390-029.

From September 2019 through August 2021, striped skunks were captured using species specific trapping surveys and by nocturnal spotlight survey (Hamilton 2022). For striped skunk specific live trap surveys, we set Tomahawk live traps arrayed as a transect along pasture edges, spaced 100 m apart, and baited with chub mackerel or sweet bait. Secondarily, striped skunks were manually captured during nocturnal hours by locating a foraging individual via spotlight, approaching the skunk, and placing a transparent plastic tub over the skunk, a modification of the bagging method described in Adams et al. (1964). Striped skunks were monitored with both VHF (Lotek, Model V6C 163 VHF; Newmarket, Ontario, Canada) and GPS (Lotek, Model Series Litetrack 40; Newmarket, Ontario, Canada) transmitters and recaptured every season, primarily to exchange VHF transmitters with GPS transmitters and vice versa.

Tracking and salvage

We used a portable telemetry receiver (R-1000 Receiver, Communication Specialists Inc., Orange, CA, USA) and a 2-element H antenna to track skunks weekly (range 1-5 days per week) until mortality, transmitter failure, or the individual traveled outside of our study site. Both GPS and VHF collars were programmed to record a mortality signal 8-hours after the transmitter ceased moving. Attempts were made to monitor every used frequency for a mortality signal daily; however, the spatial configuration of the study site, antenna attachment issues associated with GPS transmitters, and the distributed nature of individual skunks hampered these attempts. As such, the period from skunk mortality to recovery ranged from 12-hours to over 1-month. Additionally, a plains spotted skunk skull and attached transmitter was opportunistically found 17 months after the individual went missing and at least a year postmortem.

Upon recovery of skunk remains, we categorized causal mortality as the best of our ability. Based upon disposition of remains including mammalian tooth marks, proximity to potential avian perches, remains within an avian perch, or observation of the event, we categorized these mortality types as predation-based events (Lesmeister et al. 2010). As three diurnal avian scavengers were present at our site, when possible, we used both antemortem and postmortem GPS data to further identify mortality events that were mammalian based, but for which the remains had subsequently been scavenged. During our study we recorded two researched-based mortalities of plains spotted skunks and collected 4 striped skunks and 1 plains spotted skunk as research vouchers. While reported here, these seven individuals were not retained for causal mortality analysis.

To identify potential causal differences, we partitioned mortalities between species among five types (predation, unknown, natural (see below), road-based, and disease). While predator type was well known, for analysis, we elected to not partition predation events by either mammal or avian predator. Striped skunks were monitored with GPS and VHF transmitters along with individuals that were captured, and ear tagged but not tracked weekly; these individuals were not used in analysis, only those individuals with active transmitters at the time of mortality. Analysis was performed with a chi-squared test of independence between the two species and among the causal mortality types.

Description of unknown, natural mortality type

During our study we recorded 4 plains spotted skunk mortalities in which individuals exhibited similar antemortem behavior and causal mortality could not be classified as predation, direct anthropogenic causes, vehicle-based mortality, or disease. Prior to death all four individuals (3♂: 1♀) exhibited decreased nocturnal movements and continued use of a single diurnal rest site where mortality eventually occurred. Disposition of the remains indicated that mortality occurred when the animal was asleep, as all four individuals were recovered within the diurnal rest location in a known sleeping posture (Manaro 1960). One skunk was fully skeletonized when recovered, two were recovered with severely degraded tissues, and one was salvaged immediately postmortem. All four were recovered in an above ground diurnal rest site, with extensive overhead cover (3 were recovered underneath large Macartney rose brambles), and none of the individuals had been scavenged upon postmortem. Our SDSU colleagues confirmed observation of similar mortalities (e.g., recovery of deceased individuals, both transmittered and non-transmittered, with no signs of external trauma, and the deceased in the sleeping pose).

We were unable to categorize these mortalities as anything other than unknown; however, in Texas, we were able to potentially eliminate 2 mortality types. Temporally, none of the mortalities were related, with one occurring in 2019, 2 in 2020 and 1 in 2021. If mortalities were disease based, we may have observed temporally grouped mortalities, as observed in eastern spotted skunks with canine distemper in North Carolina (Harris et al. 2021). Additionally, we did not observe unknown causal mortalities in our monitored striped skunks or opportunistically observe an increase in unexplained mesopredator mortalities at the site indicative of disease. Second, we have eliminated research-induced mortality from improper attachment of transmitters. If collars were too tightly attached, the skunk's ability to eat and drink could have been curtailed; however, during our study we never observed signs of an improperly attached collar on spotted skunks (neck abrasions or lacerations) or on skunks postmortem. Due to known localized rodenticide usage and disposition of recovered remains, it was hypothesized that the South Dakota skunks either ingested rodenticides or had secondary rodenticide ingestion. In Texas, based upon known spatial history prior to death and distance to the nearest location where rodenticide use was possible, it is unlikely that mortalities were due to direct rodenticide ingestion. Moreover, even though we monitored a similar number of striped skunks (*n* = 25) during our survey and these individuals more frequently used anthropogenic locations where rodenticide use could be expected, we did not observe any unknown, natural mortalities in striped skunks.

Herein, we provide results of necropsy and histopathological examination from two plains spotted skunk with an unknown, natural mortality type. We report on a spotted skunk from South Dakota necropsied by the Texas A&M Veterinary Medical Diagnostic Laboratory and one from Texas that was necropsied by Houston VetPath Services, LLC. Unfortunately, these were the only two individuals from both states recovered quickly enough postmortem to allow examination.

Toxicological Screening

Toxicological screening requires a tissue sample, with adipose or liver often providing the best assays depending on the organochlorine compounds (Rattner et al. 2011). As there were not any skunk adipose tissues available within existing natural history collections, we collected and submitted liver samples for toxicological screening. In Texas, plains spotted skunk samples were collected from the Katy Prairie from deceased individuals within our study population.

These samples were stored at the Angelo State Natural History Collection. Striped skunk samples were also collected from our study population (within 5-km of Warren Ranch boundary) and originated from either monitored individuals or were salvaged from road-based mortalities that could be collected less than 12-hours postmortem. In South Dakota, spotted skunk samples were collected from multiple counties northeast of the Missouri River and originated from the SDSU monitoring project, salvaged from trappers, or from road-based mortalities.

Liver samples were submitted to B & B Laboratories, Inc. (www.tdi-bi.com/analytical-services/) for preparation, extraction, and analysis using gas chromatography mass spectrophotometry to identify presence of polychlorinated biphenyls and organochlorinated pesticides. Organic compounds were isolated from wet tissue samples using 100% dichloromethane inside stainless-steel extraction cells held at elevated temperature and solvent pressure. Percent moisture was determined by measuring the loss in mass of the sample due to drying at 105 degrees Celsius to a constant weight and a gravimetric determination of lipid concentration was performed for samples. Finally, a determination of organochlorines was performed by capillary gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring mode (SIM). The gas chromatograph was temperature-programmed and operated in splitless mode. The capillary column was an Agilent Technologies DB-5MS (60 m long by 0.25 mm ID and 0.25 µm film thickness), or equivalent and carrier flow was by electronic pressure control. Submitted samples were evaluated for 31 organochlorinated pesticides and 209 PCB congeners.

Results

From 2019-2021, we recorded 12 plains spotted skunk mortalities (6 ♂: 6♀) in southeastern Texas. These events were categorized as predation (7), unknown natural mortality

(4), and one unknown mortality. We classified predation events as avian (4), mammalian (2), and unknown (1). The unknown predation event was from a skunk that was scavenged postmortem by an avian species, and we could not identify the original predator type from the remains. We did not record any vehicle collision mortalities for plains spotted skunks although one was opportunistically salvaged ~ 10 km from the study site in spring of 2019.

We recorded at least 22 unique striped skunk vehicle collision mortalities from state highways within five kilometers of our study site. One of these skunks was actively being monitored by a GPS transmitter and two were previously marked with ear tags. From our monitored population, we recorded seven mortality events categorized as predation (4), unknown (1), vehicle collision (1), and disease (1). We did not observe a single natural mortality type like those observed in spotted skunks in our monitored striped skunk population. A chi-squared goodness of fit analysis indicated no difference in frequency of causal mortality types between the species (x^2: 5.811, *df* (5), *p*: 0.325).

Unknown, natural mortalities and necropsies

Of the 4 plains spotted skunks from Texas classified as natural mortality, 1 was recovered in time to conduct a necropsy. At first capture, S031 was a male skunk, aged as an adult with parturition occurring prior to 2020 and likely in 2019. At first capture, 20 November 2020, S031 weighed 530 grams and we observed no external features indicative of poor health. S031 was recovered on 16 December 2020; collar data indicated that the mortality signal was activated 4 hours prior, and mortality occurred within the prior 12 hours. At recovery, the skunk was in rigor mortis; however, we recorded little evidence of evacuation of the carcass by external parasites. Internally, we first examined the carcass 3 hours post recovery, recorded external measurements, and collected external parasites (Arszulowicz 2022). During this examination, we found no external contusions including abrasions around the neck indicative of an improperly attached transmitter.

On 17 December 2020, a necropsy and pathological examination was performed on S031 by a consultant pathologist from Houston VetPath Services LLC. The skunk weighed 415 grams, a 22% decrease in weight from its initial capture weight on 20 November 2020. S031 exhibited heart mineralization, cardiomyocyte degeneration, pneumonia, and amyloidosis of the kidney, pancreas, liver, and spleen. Additionally, the skunk displayed lung lesions due to verminous pneumonia and filariid nematodes in the lung parenchyma. Examination of the gastro-intestinal tract revealed that the stomach was empty with loosely formed feces in the colon. Within the feces, we collected partially digested sections of keeled snake scales.

The necropsy and histopathological examination suggested the probable cause of death was a combination of hepatorenal insufficiency due to amyloidosis and cardiac mineralization with the latter secondary to the former. It is possible that cardiac disease was a causal factor in the observed lung lesions. While gross observations suggested that hypertrophic cardiomyopathy may have contributed to mortality, histopathological results did not support this hypothesis.

From South Dakota, a male adult plains spotted skunk was recovered postmortem on 08 July 2021. A postmortem necropsy, performed in June 2023, indicated abundant fly eggs on the individual along with maggots (suspected first to second instar) in the oral cavity. No external lacerations, abrasions, or lesions were observed on the skunk, although postmortem autolysis and freeze artifacts could have obscured these. Unfortunately, these same artifacts prevented a completed histopathological analysis. There were no obvious lesions on any organ and the brain showed no evidence of encephalitis. An anticoagulant screen was negative for targeted coumarins and indandiones, common components of rodenticides. Postmortem cleaning of the skull revealed a fracture in the left mandible near the mandibular symphysis with an expanded, cavitated mass and associated loss of teeth. It was suspected that an osteomyelitis occurred in this area, maybe associated with a tooth decay abscess or other form of oral trauma. While the timing of the fracture (e.g., antemortem or postmortem) could not be confirmed, an antemortem fracture could have led to infection which may have been a source of septicemia as the cause of death. An empty stomach with no feces in the colon lends support to the fracture occurring antemortem, but the cause of death for this individual is listed as undetermined.

Toxicological Screening

Toxicological screening indicated detectable presence of two organochlorinated pesticide groups and 25 unique polychlorinated biphenyl congeners. No animals had detectable levels of dieldrin, aldrine, or endrine. Two of three Texas plains spotted skunks had hexachlorobenzene and pentachlorobenzene, compounds used as fungicides, but neither were detected from South Dakota samples or in striped skunks. Similarly, Mirex–a pesticide used for control of fire ants but discontinued in 1977–was found in 2 of 3 Texas spotted skunks but none of the South Dakota samples or Texas striped skunks (Appendix 6.1).

Chlordane was used in the United States from 1948 to 1988 as an agricultural or residential pesticide. We detected two chlordane congeners (Heptachlor-Epoxide and Oxychlordane) in 4 of 7 plains spotted skunks from South Dakota (Table 1; Appendix 6.1). Only oxychlordane was detected in two striped skunks (76.7 and 184.2 ng/g dry weight). There were no detectable chlordane congeners present in the three plains spotted skunks from Texas.

Dichloro-diphenyl-trichloroethane (DDT) was widely used in the United States from the 1940s through 1972 as an insecticide. We detected 3 DDT congeners (2, 4' - DDD; 4,4' - DDD; and 4,4' - DDE) in striped and plains spotted skunks. We detected 4,4'-DDE presence in 3 of 9 striped skunks from Texas with amounts detected at 0.9, 1.4, and 3.8 ng/g dry weight. We detected 4,4'-DDE in six plains spotted skunks from South Dakota (86%), and 1 plains spotted skunk from Texas (33%). Additionally, 2,4'-DDE was detected from two plains spotted skunks from South Dakota (29%) and 4,4'-DDD was detected from two additional plains spotted skunks from South Dakota (29%). Table 1 and Appendix 6.1 summarize amounts and totals for DDT and metabolites detected in spotted skunks evaluated.

Polychlorinated biphenyls (PCBs) were produced for industrial and commercial uses such as insulating fluids and heat resistant media (Alaee, 2016). PCB congeners were detected in every sample analyzed. Fourteen unique PCBs were detected from striped skunks in Texas, with an average of 7.9 detectable PCBs per skunk. Detectable levels varied from a low of 0.373 ng/dry g to a high 0f 1.360 ng/dry g for PCBs in skunks. Nineteen unique PCBs were detected from plains spotted skunks from South Dakota, with an average of 9.57 PCBs per skunk. Twenty-three unique PCBs were detected from plains spotted skunks from Texas with an average of 15 PCBs per skunk. Table 1 and Appendices 6.1 and 6.2 summarize amounts and totals for PCBs and detected in the striped and spotted skunks evaluated.

Table 1. Descriptive presence statistics of grouped polychlorinated biphenyls (PCBs) and organochlorinated pesticides from striped skunk (*Mephitis mephitis*) samples originating from southeastern Texas (2019 – 2021) and plains spotted skunks (*Spilogale interrupta*) originating from southeastern Texas (2019 – 2021) and central South Dakota (2018 – 2022). Target compound Chlordane refers to 7 Chlordane congeners with presence detected in 2 (Heptachlor-Epoxide and Oxychlordane); DDT refers to 7 DDT congeners with presence detected in 3 (2, 4' DDD, 4, 4' DDD, and 4,4' DDE). Detected amounts of chlordane and DDT congeners are presented in Appendix 6.1. Target compound PCB refers to (~150) congeners with presence detected in 25 (Appendix 6.2, 6.3). Individual parameters are listed below the table.

Species	State	Parameter	Chlordane	DDT	PCB
M. mephitis (9)	Texas	n^a	2	3	9
		n^b	1	1	14
		$\bar{x}^a$	0.22	0.11	7.9
		$\bar{x}^b(w)$	130.47	2.06	362.27
		Range	76.2 - 184.2	0.9 - 3.8	8.2 - 1253.6
		Total	260.93	6.18	3260.4
S. interrupta (7)	South Dakota	n^a	4	6	7
		n^b	2	3	19
		$\bar{x}^a$	0.57	1.43	9.57
		$\bar{x}^b(w)$	90.91	57.59	648.94
		Range (w)	2.6 - 190.1	4.8 - 86.2	182.2 - 2351.7
		Total	363.62	345.53	4542.56
S. interrupta (3)	Texas	n^a	0	1	3
		n^b	0	1	23
		$\bar{x}^a$	0	0.33	15
		$\bar{x}^b(w)$	0	0.33	704.77
		Range	0	0-3	229.3 - 1479.5
		Total	0	3	2114.77

n^a Per species and state, total number of individuals with detectable limits of target compound
n^b Per species and state, total number of detectable congeners per target compound
$\bar{x}^a$ Per species and state, mean number of congeners per target compound
$\bar{x}^b(w)$ Weighted mean of congeners present per sampled individual (ng/g dry weight)
Range Per species and state, weighted range of total congeners present (ng/g dry weight)
Total Per species and state, total amount of target compound (ng/g dry weight)

Discussion

During monitoring, we observed no difference in causal mortality types between plains spotted and striped skunks. Moreover, we observed no anthropogenic-based mortalities in spotted skunks. While Lesmeister et al. (2010) also reported no anthropogenic-based plains spotted skunk mortalities, Crabb (1948) reported that humans were a significant form of plains spotted skunk mortality in an agricultural landscape, including fur trapping, killing nuisance animals, and domestic dog kills. However, as Crabb noted, other skunks undoubtedly died from natural causes in places where they were never found. Without the ability to monitor the population with VHF or GPS transmitters, recording of a predation-based mortality would have been incumbent upon opportunistically locating the kill site postmortem. As such, while the information reported in Crabb (1948) provides valuable insight into anthropogenic mortality of plains spotted skunks at the time, little can be extrapolated to causal mortality of plains spotted skunks in a contemporary setting. Lesmeister et al. (2010), reported on 19 mortality events of plains spotted skunks from the Ouachita Mountains of Arkansas. Like our observations, predation was the highest cause of mortality with 12 individuals (63%) succumbing to avian predators, five to mammalian predators (26%), and 2 deaths were categorized as unknown (11%). Predation accounted for seven of the 12 Texas plains spotted skunk mortalities not categorized as research induced, with four individuals succumbing to avian predators, 2 to mammalian predators, and 1 unknown. The percentage of known avian mortalities compared to total known mortalities, for both sites (AR: 71%; TX: 67%), suggests avian predators may be the dominant plains spotted skunk predator. A collective demographic analysis of Appalachian spotted skunks similarly reported avian predation as the highest percentage (43%), with canine distemper virus and unknown each attributing 17%, mammalian mortality attributing 13% and research induced contributing 9% (Butler et al. 2021). While the species of avian predator was not confirmed in Arkansas (great horned owl was suspected), recent analysis has confirmed predation of both eastern and western spotted skunk by barred owls (Hassler et al. 2021, Tosa et al. 2022). During the 3 years of our study, we did not observe or record (via camera trap) a single barred owl at our site, although at least one barred owl was confirmed previously (Perkins et al. 2022). Both great horned owls (*Bubo virginianus*) and barn owls (*Tyto alba*) were present at the site with a higher frequency of barn owls occurring via camera trap survey. To our knowledge, no mammalian predator has been confirmed genetically as a predator of any spotted skunk species.

A meta-analysis of causal mortality in North American mammals suggested anthropogenic mortality types (including legal and illegal harvest and vehicle collision) surpass natural causes, however, protection status of individual species significantly reduced anthropogenic mortality rates (Collins and Kays 2011). Additionally, there was a positive correlation between human footprint and vehicle collision but a nonsignificant correlation between human footprint and overall anthropogenic mortality. *A priori*, we expected higher rates of plains spotted skunk anthropogenic mortality because our study site was adjacent to residential areas, bisected and bordered by county roads, and there was moderate to heavy hunting activity at the site. Both hunters and ranch staff removed an unknown number of mesopredators (primarily northern raccoon, *Procyon lotor*, and coyote, *Canis latrans*) during the study; however, all individuals were requested not to take either species of skunk. To our knowledge, this request was adhered to during our survey. We recorded no road-based mortalities in plains spotted skunks even though spatial relocations from GPS transmitters indicated that male plains spotted skunks frequently crossed the local roads in spring while searching for breeding opportunities. Outside of the breeding season (mid-February - mid June) we observed a single road crossing by a plains spotted skunk. Comparatively, we recorded vehicle collision mortalities for two ear tagged striped skunks, 1 GPS transmittered striped skunk, and at least 19 additional untagged striped skunks within 5-km of Warren Ranch. In rural northeastern Illinois, vehicle collision was the highest striped skunk causal mortality type followed closely by disease or poor body condition with predation noted as being a rare occurrence (Gehrt et al. 2005). In an urban park, striped skunks less frequently crossed roads with elevated levels of traffic and the roadways subsequently formed home range boundaries (Gehrt et al. 2005). Outside of breeding season, every monitored plains spotted skunk with an established home range near a county road also used the road or associated edge as a boundary.

Initially, we hypothesized that mortalities were at least partially influenced by localized management activities. CPC conducts yearly management activities including full or partial mowing of pastures from late spring through early fall. Pastures with management have less standing vegetation to function as plains spotted skunk cover during the fall and winter months. However, plains spotted skunk mortalities occurred only slightly more in fall and winter (55%) compared to spring and summer (45%). Moreover, only three plains spotted skunk mortalities (all predation based) occurred within areas that had been mowed. These management activities maintain a prairie-based seral stage and retard the proliferation of invasive species and shrubs. It is likely that the benefit to the species is outweighed by the potential of increased predation to the individual.

Toxicological Screening

To our knowledge, this is the first report of persistent organic pollutants occurring in spotted skunks of any species and only the second report in striped skunks. A single study in southern Ontario, Canada (Frank et al., 1979) reported that skunk liver samples had a mean level of 6 ppb DDE and 28 ppb PCBs in samples taken within 10 years of the phase-out of organochlorine insecticides and PCBs. Our samples were taken 5 decades after the phase-out of these legacy POPs. The detection of measurable amounts in liver tissue of both *Spilogale interrupta* and *Mephitis mephitis* is noteworthy.

DDT and its metabolites, congeners of DDE and DDD, are among the most recognized organochlorine pesticides because of the attention drawn by Rachel Carson in her 1962 book *Silent Spring*, which led to a ban of DDT for agricultural uses in the United States and Canada in 1972. Its use in other countries continued but declined in the following decades (de Solla 2016). Currently only India is still producing DDT commercially, primarily for mosquito eradication associated with malaria (Van den Berg et al. 2017). The potential implication of DDT and other organochlorine pesticides in the decline of plains spotted skunks has been proposed for over 2 decades (Landholt and Genoways 2000, Gompper and Hackett 2005; Gompper 2017). The beginning of the decline in some spotted skunk populations preceded the widespread use of DDT beginning in the late 1940s (Gompper 2017; Sasse 2021); however, the potential for a negative effect on spotted skunk populations after widespread use of organochlorine pesticide use increased through the 1950s and 1960s still may be a contributing factor to the broadscale spotted skunk decline.

We were unable to locate details on organochlorine pesticides use in Texas, in general, or at Katy Prairie, in particular. We do know that as early as 1947, a large-scale field trial of DDT for control of ticks was conducted at Camp Bullis NW of San Antonio (George and Stickel 1949). In that study of a 206.6-acre plot, birds and mammals were sampled before and after DDT dusting with approximately 900 lbs. of 10% DDT dust. Ground and bush-feeding birds were severely affected with some species eliminated from the area and 15 found dead but medium-sized mammals, including striped skunks, were not obviously impacted (George and Stickel 1949).

Most data on levels of DDT and metabolites in mammals suggest that aquatic species or those with aquatic-based diets accumulate these organochlorine pesticides to a greater degree than terrestrial mammals (Larsson et al. 1990). Despite this, terrestrial species not associated with bodies of water are known to have considerable levels of DDT metabolites and other organochlorine pesticides (Dip et al. 2003, Mateo et al. 2012). For example, mean concentrations (ng g-1 wet weight) of DDE detected in Iberian lynx (*Lynx pardinus*), red fox (*Vulpes vulpes*), Egyptian mongoose (*Herpestes ichneumon*) and common genet (*Genetta genetta*) were 55.1, 4.1, 19.3, and 754, respectively. Our detection of DDT congeners in 7 of 10 plains spotted skunks suggests that organochlorine pesticides in their diet or through other environmental exposures are still a potential health risk almost 50 years after the ban of this agricultural pesticide. Without further research it is difficult to ascertain whether the levels we detected are sufficient to cause problems with populations of spotted skunks.

We observed two chlordane-related compounds in striped and plains spotted skunk liver tissues. Oxychlordane was detected in two striped skunks from Texas while Oxychlordane and Heptachlor-epoxide were detected in four of the six adult spotted skunks submitted for analysis from South Dakota. Neither of these compounds were present in the Texas spotted skunks. Oxychlordane is a primary breakdown product of chlordane and is more toxic and bioaccumulative than the parent compound (Bondy et al. 2003) Heptachlor-epoxide has been detected in liver tissue of Iberian lynx, common genets, and Eurasian otters in Spain (Mateo et al. 2012). Chlordanes have also been reported from liver samples of arctic foxes (Hoekstra et al. 2003) as well as rodents in Brazil (Capella et al. 2023).

Polychlorinated biphenyl production in the United States ceased in 1977 but continued in Europe until 1985. PCBs continue to be released into the environment and estimates are 0.2 - 0.4 million tons are currently bioavailable (de Solla 2016). Although there are 209 PCB congeners, the majority found in terrestrial wildlife tissues are a small subset of the congeners (Eisler and Belisle 1996, de Solla 2015). Of the 25 PCB's detected in our samples, three congeners (187, 180, and 138) occurred in every skunk submitted; moreover, these three congeners accounted for 66% of the cumulative amount of all PCBs from the striped skunks, 47% of the cumulative amount for South Dakota spotted skunks, and 50% of Texas spotted skunks. The dominant congener (180) accounted for 44% of the cumulative amount for striped skunks, 34% for South Dakota spotted skunks, and 43% of Texas spotted skunks. Previously, PCB-180 was noted as the dominant congener in Spanish red foxes (Mateo et al., 2011), second most dominant congener in arctic foxes (Hoekstra et al. 2003), and fourth most dominant in Illinois bobcats (Boyles and Nielsen 2017).

There is little doubt now about the harmful effects that POPs have on both humans and wildlife species. One of the most well-documented forms of health hazards is the role these substances play as endocrine disrupting chemicals (Encarnação et al. 2019). All classes of compounds (organochlorine pesticides and PCBs) found in our toxicological screening of skunks have been implicated in developmental abnormalities, sex ratio of offspring, and higher mortality rates and even exceedingly small doses have been shown to have effects in animal-based studies (Encarnação et al. 2019). The other striking finding over the past 30 years is the degree to which these compounds can travel from where they were deposited via water and air. Atmospheric levels of most of these compounds are now being studied in remote parts of the planet that demonstrate how easily they are transported (Montone et al. 2003). Although toxicological substances are likely not the primary cause of the decline in populations of plains spotted skunks, our evidence of detectable levels in liver samples from two states confirms that persistent organic pollutants likely have played a contributing role. The combined effects of agricultural shifts to large-scale farming and broad use of pesticides (Gibbs et al. 2009) may well have increased the likelihood of loss of plains spotted skunks over at least parts of their former range.

Appendix 6.1. Organochlorine pesticides (chlordane, DDT, and chlorobenzene congeners and Mirex) detected in liver samples from plains spotted skunks (*Spilogale interrupta*) from South Dakota (SD) and Texas (TX). Values are ng/g dry weight.

	SD	SD	SD	SD	SD	SD	SD	TX	TX	TX
Heptachlor-Epoxide		33.8	2.6							
Oxychlordane	111.2	156.4			59.7					
2,4'-DDD		1.3		0.8						
4,4'-DDD	4.6						4.4			
4,4'-DDE	81.6	7.4	20.7	40.6	4.8		6.6	3		
Hexachlorobenzene								44.5	67.2	
Pentachlorobenzene								11	15.9	
Mirex								24.2	8.2	

Appendix 6.2. Polychlorinated biphenyls (PCBs) detected in liver samples of spotted skunks (*Spilogale interrupta*) from South Dakota (SD) and Texas (TX). Values are ng/g dry weight.

State	SD	SD	SD	SD	SD	SD	SD	TX	TX	TX
Sample ID	ASK 15245	ASK 15246	ASK 15248	ASK 15249	ASK 15626	ASK 15627	ASK 15628	ASK 15023	ASK 15159	ASK 15218
PCB 118/108				6						
PCB 165/131	10.5	3.3		4		11.4				
PCB 142/146/161								2.1	11.8	
PCB 153/168	27	9.7	1.8	36.8	32.3	33.8	7.1	5.6	60.6	3.1
PCB 137									3.1	
PCB 138/164/163	35.2	9.3	2.3	23.4	30	57.9	10.8	17.9	30.5	12.4
PCB 166						11.6			2.7	
PCB 128/167									1.3	
PCB 156						10.1		2.2	14.8	
PCB 157									2	
PCB 186/178									1.3	
PCB 187/182	59.9	13.8	5.8	10.8	28.4	295	15.7	9.7	66.3	21
PCB 183	6.5			2.6		9			3.2	
PCB 192/172		53				761.2				
PCB 180/193	173.6	59.5	36.8	173.3	63	787.6	242.5	166.6	633.1	109.8
PCB 170/190	35.7	17.3		59.9	17.9	101.6	62.2	60.8	235	31.6
PCB 189								1.4	7.9	
PCB 198								3.8	22.8	
PCB 199										5.7
PCB 203/196		10.1	16.7	18.6				15.8	67.9	14
PCB 195						31.3		1.5	16.2	
PCB 194							72.7	68.1	180.9	
PCB 205							3.4	8	14.5	
PCB 206	29.7	15.7	96.7	88.8	18.2	225.3	255.3	36.7	81.2	26.5
PCB 209	9.1	5.7	22.1	21.3	9.6	15.8	102.5	5.7	22.5	5.2
Total	387.2	197.3	182.2	445.5	199.4	2351.7	772.2	405.9	1479.5	229.3

Appendix 6.3. Polychlorinated biphenyls (PCBs) detected in liver samples of striped skunks (*Mephitis mephitis*) in Texas. Values are ng/g dry weight.

Sample ID	ASK 15252	ASK 15253	TK 212505	TK 212506	TK 212507	ASK 15251	TK 212511	TK 212509	TK 212510
PCB 165/131	10							4.4	
PCB 42/146/161									6.4
PCB 153/168	0	42.9	3.2		35.7	1.2	8.9	11.4	
PCB 138/164/163	15.9	79.3	11.3	1.6	44.7	10.3	24.3	48.9	14.7
PCB 187/182	46	212	15.6	0.8	26.8	20	39.5	65.6	19.9
PCB 180/193	39.9	535	86.6	4.5	345.9	31.3	241.4	119.4	45.6
PCB 170/190	10.1	127.2	25.7	1.2	120.6	5.8	59.7	39.5	
PCB 199						6.4			
PCB 203/196	153.6	32.9		24.5		33			
PCB 195	4.8					9.6		29.8	
PCB 194					35.1		58.3		
PCB 205					4.2				
PCB 206	8.8	53.3	26.7		9.8	17.4	38.1	12.6	6.4
PCB 209	1.8	10.3	3.9		1.6	4.9	6.6	2.5	2.7
Total	122.5	1253.6	173.1	8.2	664.8	90.9	514.6	332.8	100

Chapter 7: Notes on plains spotted skunk population structure and breeding ecology

Aging of plains spotted skunks based on body morphology and tooth wear was difficult but made easier by assigning relative age. We aged skunks to 4 categories: juvenile (young of the year, pre-dispersal), sub-adult (young of the year, post dispersal and pre-reproductive condition), adult (young of previous year, in or post-reproductive condition), and older adult (at least 1.5 years old, males with a relatively high weight, females with signs of previous reproductive success, and both sexes with worn and discolored teeth). We note here that this aging classification was only applied to the information presented within this chapter.

As opposed to recent and concurrent spotted skunk research projects, our efforts yielded the capture of only a single pre-dispersal juvenile and fewer sub-adults. At first capture, we categorized 22 individuals as adults, 17 individuals as old adults, 10 as sub-adults and one as a juvenile. Cumulatively we monitored plains spotted skunks an average of 4.5 months, with this period defined as the period when skunks were collared with working collars and, if the skunk was recaptured and recollared, the period when the skunk was collared with an inactive (battery depleted) collar. By removing the individuals with no spatial data from the dataset, the average number of months as a study unit increases to 5.5 for females, 6 for males, and 5.8 overall (n = 38; ♀ = 15; ♂ = 23).

Relative age, relative age at time of mortality, and time in study are needed when calculating the number of breeding opportunities (defined as an adult being capable of breeding during the appropriate season). We were unable to identify the number of successful breeding events and the number of offspring recruited into the population. However, we can provide general information on the number of potential opportunities for each sex. For all older adult skunks, we assume two potential opportunities at time of first capture regardless of whether the actual number is two or higher. Through the 2021 breeding season, we recorded 49 individuals with an average of 1.49 breeding opportunities per skunk (1 individual was removed from this dataset due to research induced mortality at time of first capture). Females (n = 18) averaged 1.27 opportunities per individual, while males (n = 31) averaged 1.61 opportunities per individual with 6 skunks having 2 opportunities. Moreover, we recorded 37% of females with multiple breeding opportunities and 55% of males; however, this number may be influenced by the fact that males were actively monitored longer than females.

We were broadly unable to record data on kit rearing and nursing ecology. In 2019, females were not first captured until mid-summer. In 2020, disruption of our supply chain due to COVID-19 resulted in females with battery-depleted collars during pup rearing season. In late spring 2021, we recorded two females confirmed to have neonates and in summer 2021 we recorded a third female that was lactating with scarred nipples, however, due to cessation of field data collection in summer 2021, we could not monitor these individuals. Of the 19 females monitored, we can provide notes on breeding ecology for 11. Four females were confirmed to have birthed at least once by visual observation of young, one female showed evidence of nursing, but kits were never observed, and four individuals showed signs of either being pregnant or having copulated, but kits were never observed. Three individuals were non-breeding at the time of capture. Of these, one was an old female captured at the same time as three others that were nursing and 2 were previous young of the year. One female, first captured in June 2021 and classified as being born in summer, showed signs of attempted, but unsuccessful copulation.

In concert with our inability to monitor female plains skunks during the kit rearing season, we must acknowledge that this survey was intentionally scrapped due to potential negative effects to females and kits. In 2019, we observed both defensive behavior from females with kits as well as an immediate relocation of diurnal rest sites after a tracking event. Initially, we attempted to monitor the ecology and behavior of females and kits by placing camera traps adjacent to the denning sites. However, by the time we first collared females in 2019 (29 July – 02 August), all kits were capable of nocturnal ventures with the adult female. As such, we could not ascertain whether it was our presence during tracking events, our camera trap monitoring efforts, or the natural inclination of the adult female that caused her to move the kits nightly to a different denning site.

Our aging of the skunks was difficult, especially identifying skunks between 1.5 and 2 years old. Genomic analysis of relatedness should increase accuracy of our relative aging classification. If what we have recorded is representative of the population at Warren Ranch, females may be being removed from the population prior to successive breeding opportunities. Additionally, if some females are not breeding their first year and some are not breeding as older adults, this may shift population recruitment to a small subset of females. While little is known about breeding and pup rearing ecology for this species, it has been suggested in other species that older females with accumulated experience are more successful at rearing kits to dispersal. While only hypothesized at this point, the inability of females to have multiple breeding opportunities or successfully rear multiple litters to dispersal may be an additive factor in species decline.

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Appendix 1: List of publications generated by funding provided by Texas Comptroller of Public Accounts for plains spotted skunk research

The following is a list of publications (theses, manuscripts, and popular articles) published with plains spotted skunk data collected by TCPA funded projects. As of report finalization, this list is comprehensive and includes authors identified within TCPA funded projects. The list additionally includes two theses whose datasets included significant contributions from TCPA funded projects. This list does not include publications that used data collected under TCPA funded projects that were released into the public domain.

Theses:

Shaffer, A.A. 2017. Genetic structure and differentiation within the eastern spotted skunk (*Spilogale putorius*): a microsatellite analysis. M.Sc. Thesis. Angelo State University, San Angelo, TX

Perkins J.C. 2017. Conservation status of the plains spotted skunk in Texas. M.Sc. Thesis. Angelo State University, San Angelo, TX.

Bell, Z.H. 2020. Genomic markers recognition of at least four forms of spotted skunks in the United States. M.Sc. Thesis. University of Wyoming, Laramie, WY.

Jefferson, K.P. 2021. Rest site selection of plains spotted skunks (*Spilogale putorius interrupta*) in a Texas coastal prairie. M.Sc. Thesis. Angelo State University, San Angelo, TX.

Hamilton, M.M. 2022. Home range analysis of sympatric striped skunks and plains spotted skunks in southeastern Texas. M.Sc. Thesis. Angelo State University, San Angelo, TX.

Arszułowicz, M.N. 2022. A study of the parasitic fauna of North American spotted skunks (genus *Spilogale*) across the eastern and central United States. M.Sc. Thesis. Angelo State University, San Angelo, TX.

Published manuscripts:

Shaffer, A.A., R.C. Dowler, J.C. Perkins, A.W. Ferguson, M.M. McDonough, and L.K. Ammerman. 2018. Genetic variation in the eastern spotted skunk (*Spilogale putorius*) with emphasis on the plains spotted skunk (*S. p. interrupta*). Journal of Mammalogy 99:1237-1248.

Gulas-Wroblewski, B.E., M.D. Luper, A.A. Gibson, J.C. Perkins, and R.C. Dowler. 2021. Itching for recognition: fungal dermatophytosis identified in an eastern spotted skunk (*Spilogale putorius*) population in Texas. Southeastern Naturalist Special Issue 11:191-198.

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Additional species diversity and Pleistocene climate change as a major driver of diversification. Molecular Phylogenetics and Evolution
Jefferson, K.P., S.L.A. Garcia, D.M. Krejsa, J.C. Perkins, S. Stevens, R.S. Matlack, and R.C. Dowler. 2022. Noteworthy records, range extensions, and conservation status of skunk species in Texas. Occasional Papers, The Museum, Texas Tech University 384:1-16.
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Gulas-Wroblewski, B.E., R. Gorchakov, R.B. Kairis, R.C. Dowler, and K.O. Murray. 2023. Prevalence of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, infection in Texas skunks (Mammalia: Mephitidae). Vector-borne and Zoonotic Diseases 23:18-28. DOI: 10.1089/vbz.2022.0056.
Benson, D.J., J.C. Perkins, K.P. Jefferson, R.C. Dowler, R.D. Stevens, and C.C. Rega-Brodsky. 2023. Examination of Plains Spotted Skunk (*Spilogale interrupta*) burrow systems. Southeastern Naturalist 22:352-363.

Popular articles

Perkins, J.C. 2022. A spotty history. The Wildlife Professional 5:26-30.
Ferguson, A.W., M.M. McDonough, and R.C. Dowler. 2022. A skunk by any other name. The Wildlife Professional 5:32-35.