SPATIAL AND DISEASE ECOLOGY OF THE PLAINS SPOTTED SKUNK

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by

Summer D. Higdon

Dr. Matthew Gompper, Thesis Supervisor

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

SPATIAL AND DISEASE ECOLOGY OF THE PLAINS SPOTTED SKUNK

Presented by Summer D. Higdon,

a candidate for the degree of Master of Science, and hereby certify that, in their opinion, it is worthy of acceptance.

____

Dr. Matthew Gompper, Thesis Supervisor,

School of Natural Resources

____

Dr. Frank Thompson, Committee Member,

U.S. Forest Service

____

Dr. Lori Eggert, Committee Member,

Division of Biological Sciences

____

Dr. Joshua Millspaugh, Committee Member,

Wildlife Biology Program, University of Montana

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ABSTRACT

The eastern spotted skunk (*Spilogale putorius*) is a species of conservation concern across much of its range in the eastern U.S. due to a range-wide population decline that began in the 1940s. The reason for the decline remains unknown; a combination of factors, including habitat loss, disease, and overharvest, may have collectively led to the population decline. As a result, a subspecies of eastern spotted skunk, the plains spotted skunk (*S. putorius interrupta*) has been petitioned for listing under the U.S. Endangered Species Act. Despite the petition for listing, habitat associations and other basic ecological assessments remain limited across the historic range of eastern spotted skunks, and especially within the range of the plains spotted skunk in the Midwestern U.S. To address current knowledge gaps, identify future research opportunities, and assess potential causes of the decline, we developed three studies on the plains spotted skunk.

We conducted a large-scale field research effort on a previously unstudied population of plains spotted skunks in the Ozark region of northern Arkansas. Our first objective was to identify whether there was a population persisting in the region using a camera trap grid in Ozark National Forest and Gene Rush Wildlife Management Area. We observed plains spotted skunks at four camera trap sites, but did not identify environmental characteristics associated with plains spotted skunk presence. Using information from the camera survey, we live-trapped and radio-collared one male and one female plains spotted skunk. We tracked the skunks to 12 rest sites and determined that groundcover was lower at used rest sites than paired, putatively available but

unused rest sites. We suspect the lower groundcover was a result of lower amounts of vegetation around the rocky outcrops most commonly selected by skunks as rest sites. The results of our study confirm the rarity of the species in the region.

Using data collected at camera trap sites during the field effort and combining it with data from several other studies, we built a MaxEnt species distribution model to predict areas of high probability of presence of plains spotted skunks in Missouri and Arkansas. We paired our camera trap data with known plains spotted skunk point location data collected as part of two additional, separate targeted research efforts in southern Missouri and western Arkansas. We identified nine environmental variables that were likely to influence plains spotted skunk presence on the landscape based on the existing literature. To validate the model, we used citizen science and incidental capture reports from the Missouri Department of Conservation and the Arkansas Game and Fish Commission. Of the nine environmental variables, eight were better than random at predicting plains spotted skunk probability of presence in a univariate model (test Area Under Curve > 0.5). Therefore, we included the eight variables in a multivariate model (test AUC = 0.835) and the strongest predictor of plains spotted skunk probability of presence was percent forest within 5 km (permutation importance = 87.8%). A predictive map of plains spotted skunk probability of presence across both states identified portions of southern Missouri and northern, western, and southern Arkansas as likely plains spotted skunk habitat, with >300,000 hectares with high probability of presence (> 0.75). Researchers seeking detailed information on plains spotted skunk microhabitat requirements should target hotspot areas identified by our predicted distribution map. Our results indicate that many high probability areas are already protected by federal public lands, but that populations may be isolated from one another in Missouri and Arkansas, and between these states and other range states.

Moving forward, an understanding of factors that may have caused the decline of eastern spotted skunks is important, as the same factors may continue to threaten populations today. The impacts of virulent parasites may have been, and perhaps continue to be, demographically important factors for spotted skunks. We used preserved spotted skunk skulls from six mammal collection in the Eastern and Midwestern U.S. to evaluate prevalence and severity of the impacts of skunk cranial worm (*Skrjabingylus chitwoodorum*) in two subspecies, or genetic clades, of eastern spotted skunk, including the plains spotted skunk, and two genetic clades of western spotted skunk (*S. gracilis*). Infection by skunk cranial worm can cause damage to the skull, and we identified and ranked damage on a scale of 1 to 3, with 3 being the most severe damage class. We used generalized linear models and multinomial log-linear regression models to evaluate prevalence and severity, respectively, of skunk cranial worm in response to host genetic clade, host sex, specimen collection year, and precipitation. For both metrics, host genetic clade, year prior to specimen collection, and precipitation in the year prior to specimen collection were present in the top models. Eastern spotted skunk genetic clades had lower infection prevalence and severity than western spotted skunk genetic clades. For all genetic clades, higher precipitation in the year prior to specimen collection resulted in higher rates of infection, but severity only worsened for western spotted skunk clades after high precipitation years. The behavioral effects of infection by skunk cranial worm remain unknown. We suspect that low prevalence and intensity in eastern spotted skunk clades could reflect a lower ability to survive with an infection than western spotted skunk clades.

This body of work collectively fills knowledge gaps in the literature on plains spotted skunks, while identifying areas of future research needs. Future efforts should seek to locate and identify habitat and resource requirements for additional populations of plains spotted skunk to get a more complete understanding of the ecology of the subspecies. Additional landscape-level questions, such as the extent to which populations are isolated, should be assessed. Further, recognition of the high prevalence of cranial damage cause by skunk cranial worm underpins how little we know about the disease ecology of the species and suggests a need for further research on the topic. Together, such landscape and disease ecology studies are a necessary area of research for supporting successful and informed management.

CHAPTER 1

INSIGHTS ON REST SITE USE AND APPARENT RARITY OF AN OZARK POPULATION OF PLAINS SPOTTED SKUNK

ABSTRACT

Eastern spotted skunks (*Spilogale putorius*) are a species of conservation concern due to a range-wide decline that began in the 1940s. As a result of the decline, a subspecies, the plains spotted skunk (*S. putorius interrupta*), is now petitioned for listing under the U.S. Endangered Species Act, but knowledge of basic habitat requirements of the taxon remains limited. Our objective was to determine whether a population of plains spotted skunk persisted in the Ozark region of north-central Arkansas, and to evaluate habitat requirements for the species in the region. We deployed camera traps for 8,119 trap nights at 73 sites in Ozark National Forest and Gene Rush Wildlife Management Area during January 2017 through March 2018. We recorded plains spotted skunks on six occasions at four sites (capture success rate = 0.074%), thus confirming the rarity of the species in the region. Environmental characteristics of sites where we recorded plains spotted skunks did not differ from those of unoccupied sites. Using information from the camera trap survey, we were able to capture and deploy radio-collars on one female and one male plains spotted skunks. We tracked the female individual to five rest sites and the male individual to seven rest sites. We collected environmental and rest site characteristics at the used sites and 12 putatively available, but unused rest sites. Both individuals used rocky outcrops more than other substrate types. There were no significant differences between characteristics of used and unused sites, except that

groundcover was lower at used sites, which may be associated with the selection of rocky outcrops.

INTRODUCTION

The eastern spotted skunk (*Spilogale putorius*) is an omnivorous mephitid with a wide geographic range throughout the Eastern and Midwestern U.S. (Gompper 2017). Historically, the species was common in many parts of its range, and several early twentieth century accounts of mammals in the Eastern and Midwestern U.S. include notes on eastern spotted skunks (Cory 1912, Howell 1921, Kellogg 1937, Kellogg 1939). The species often occurred on farms where it made use of drained prairie wetlands, agricultural sheds and other buildings, and the associated rodents (Spurrell 1917, Johnson 1921, Mohr 1931, Enders 1932, Van Gelder 1959). In the Midwestern U.S., trappers had success harvesting and selling eastern spotted skunk pelts in the early 1900s, with some years in excess of 100,000 pelts for states throughout the region (Gompper and Hackett 2005). However, beginning in the 1940s, eastern spotted skunk populations declined range-wide and did not recover (Gompper and Hackett 2005). As a result, there is increasing conservation concern for the species (Gompper and Jachowski 2016) and the plains spotted skunk (*S. putorius interrupta*), a subspecies of the eastern spotted skunk that ranges throughout the Midwestern U.S., is petitioned to be listed on the U.S. Endangered Species Act (Department of Interior, USFWS 2012).

Reasons for the decline in eastern spotted skunk populations are unclear (Gompper 2017). One potential explanation is that a decrease in suitable habitat availability and quality due to land-use change affected eastern spotted skunk populations. In the 1940s and 1950s, small-scale family farms were incorporated into large-scale, modernized agricultural units that may not have met the habitat requirements for eastern spotted skunks (Choate et al. 1973), in part because of the onset of widespread pesticide use affecting food resources (DeSanty 2001, Schwartz and Schwartz 2001). As a result, most known populations of eastern spotted skunks are now limited to forested habitats (Lesmeister et al. 2009, Thorne et al. 2017, but see Harris 2018).

Given the population decline of eastern spotted skunks, several states have prioritized research efforts for the species, primarily to determine where populations still occur (e.g., Wilson et al. 2016, Lombardi et al. 2016), and to identify habitat and resource requirements for the species (e.g., Lesmeister et al. 2009, Thorne et al. 2017). Findings to date suggest that eastern spotted skunks select dense, young forest within large forest stands, regardless of forest species composition, indicating that forest structure is important for the species (Hackett 2008, Lesmeister et al. 2009, Lesmeister et al. 2013, Thorne et al. 2017). In mountainous regions such as the middle and southern Appalachian Mountains, elevation may also play a role in habitat selection, with eastern spotted skunks more likely in lower elevations (Thorne et al. 2017, Eng 2018). However, the dense rhododendron patches present at high elevations may provide the same function as brushier, denser forests at lower elevations, and the species has been recorded at some high elevation locations (Diggins et al. 2015, Thorne et al. 2017). Den and rest site selection also appear to be influenced by forest structure, as studies from multiple states identified dense understory cover as being positively associated with den and rest site selection (Lesmeister et al. 2008, Sprayberry and Edelman 2018, Eng 2018). Eastern spotted skunks tend to select root burrows in overturned trees or ground burrows for denning and resting (Harris 2018, Eng 2018, Sprayberry and Edelman 2018). Selection for dense forest structure may offer eastern spotted skunks protection from avian predators (Lesmeister et al. 2010).

Despite the influx of new eastern spotted skunk research over the past two decades, research on the distribution and the habitat and resource requirements of the species remains limited. For example, intensive studies of the plains spotted skunk have focused on the Ouachita Mountains in Arkansas and the Ozark Mountains in Missouri (Lesmeister et al. 2008, 2009, 2010, 2013; Hackett et al. 2007; Hackett 2008). Detailed information on the status and ecology of the subspecies in other parts of its range are lacking. Given the need to assess whether current management schemes are meeting the needs of the plains spotted skunk, it is vital that additional population assessments occur.

Here, we report outcomes of an intensive effort to determine the relative abundance and the habitat and resource requirements of a previously unstudied population of plains spotted skunk in the Ozark Mountains of northern Arkansas. We established a large-scale camera trap survey with the objectives of confirming the presence of a plains spotted skunk population in northern Arkansas as well as identifying environmental factors important to the species at a microhabitat scale. We used records of plains spotted skunks from the camera trap survey to target locations for live-trapping and radio-tagging individuals with the objective of evaluating rest site selection. Here we use the resulting data to summarize trap success rates for the camera trap and live-trapping efforts and provide behavioral and morphometric details on captured individuals. We characterize locations where plains spotted skunks were captured on camera traps and where radio-collared animals rested. Finally, we suggest directions for future research on the Midwestern subspecies.

METHODS

Field-site description

We conducted our study in the Ozark Mountain region of north central Arkansas. The Ozarks span five states (MO, AR, OK, IL, and KS) and are the only highlands in the Midwestern U.S. (The Nature Conservancy, 2003). In Arkansas, the Ozarks are characterized by mixed oak-hickory and oak-pine forests interspersed with open glades and fields. Common under- and mid-story species include flowering dogwood (*Cornus florida*), wild grape (*Vitis spp.*), poison ivy (*Toxicodendron radicans*), blueberry (*Vaccinium spp*.), and blackberry (*Rubus spp.*). The terrain ranges from rolling hills to rugged and steep rocky bluffs. Historically, eastern spotted skunks were found throughout the region, although in numbers lower than were historically reported from more Northern states (Sasse and Gompper 2006). Other terrestrial mammalian carnivore species commonly occurring in the interior forests of the region include northern raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), bobcat (*Felis rufus*), grey fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), and black bear (*Ursus americanus*).

Our study area included the Bearcat Hollow Project Area in the northeast portion of Ozark National Forest and the Gene Rush Wildlife Management Area, which borders the National Forest to the north (24,611 ha; Figure 1.1). Both sites have been focal areas for extensive management that includes frequent dormant season prescribed burns and tree thinning. The primary management goal is to open the canopy and allow for herbaceous growth in the understory (D. Rambo, personal communication), thereby facilitating the expansion and reclamation of historic woodland and savannah conditions (Gene Rush WMA Master Plan 2015).

Camera trapping

We established a grid with 1.5 km^2 cells across the study site and placed a point in the center of each grid cell (Figure 1.1). We eliminated points that fell within private property. We attempted to visit all remaining sites but eliminated those that were inaccessible on foot due to dangerous terrain. We separated the grid into three approximately equal spatial sections of 25, 25, and 23 camera sites, respectively. We spent one week deploying cameras in each section, left the cameras for the following two-week interval, and shifted cameras to the next section every third week during "transition weeks." In this way, we sampled each camera site for six two-week periods between January 2017 and March 2018.

We deployed Bushnell Trophy Cam HD Agressor (Bushnell Outdoor Products, Overland Park, KS), and occasionally RECONYX Hyperfire (RECONYX, Inc., Homen, WI), cameras with 8 or 16 GB memory cards on the bole of a tree approximately 1.5 m above the ground, aimed at a bait tree between 5 and 10 m away. We baited camera sites with a 5 oz can of tuna in oil nailed to the bait tree approximately 1 to 1.5 m above the ground. Three holes were poked into the top of the can to allow the oil to drip down the tree, increasing the spread of bait scent. Additionally, we used approximately one teaspoon of Hawbaker's Skunk and Opossum Lure (Hawbaker & Sons, Fort Loudon, PA) spread on the bait tree above the tuna can. We programmed cameras to take a 3-photo burst at 30 s intervals when movement was detected.

We downloaded photos and cleared memory cards during every transition week. Using the eMammal Desktop Application, we entered the camera location, species present, and group size for all photos. When images are uploaded to eMammal, the program automatically records the date and time the photos were taken. After identifying animals in all photos, we used the eMammal Expert Review Tool to review and confirm animal identifications.

At each camera site, we established a 9-node 0.25 ha grid with the camera site at the central node. Nodes were spaced 25 m apart and the grid was oriented with the cardinal directions. At each camera site, we identified whether the surrounding forest was primarily deciduous, coniferous, or mixed. At each node excluding the camera site, we measured canopy density using a spherical densitometer, understory density using a checkered Nudd's board, and identified the ground cover as woody or herbaceous. We excluded the camera site to prevent bias from removing small amounts of vegetation for the camera line-of-sight. Along each north to south transect, we counted coarse woody debris (i.e., logs lying on the ground) larger than 10 cm in diameter at breast height.

Live-trapping and radio-telemetry

Live-trapping efforts occurred between November 2017 and February 2018. We identified sites for live-trapping efforts using the broad-scale camera trap survey described above. At three sites where plains spotted skunks were detected on cameras (see results), we placed 49 Tomahawk (#204; Hazelhurst, WI, USA) traps spaced 50 m apart within a 300 m^2 grid at the camera site. We also placed 54 traps along easily accessible and commonly used roads in the study area for opportunistic captures. We baited traps with 1 to 2 baits chosen from a variety of fish-flavored canned domestic cat foods, canned tuna, sardines, and mackerel, striped skunk and opossum lure, and striped skunk urine. Traps were opened the evening prior to the trapping session and remained open for the duration of the trapping session. Trapping sessions ranged from 5 to 15 days per site. We checked traps daily at sunrise.

We released non-target animals without handling. For plains spotted skunks, we removed the animal from the trap, immobilized the animal using a combination of ketamine and xylazine, and reversed the xylazine using yohimbine. After immobilization, we determined sex, ear-tagged (Monel ear tags, size 1005-1) and radio-collared the animal (Advanced Telemetry Systems, model m1545), and took several body measurements including weight, total body length, tail length, canine tooth width, length, and depth, and breadth between upper canines. We also examined the skunk for external parasites, collected fecal swabs and a blood sample (ca 3 ml from the jugular or femoral veins), and plucked hairs from the base of the tail for future genetic and pathogen work. We allowed the animal to recover in the trap and then released the plains spotted skunk at the site of capture.

We tracked collared plains spotted skunks during daylight hours using a 3-element Yagi antennae and receiver. We relocated skunks at rest sites using direct homing to the site. To home to the site, we followed the signal from the collar and identified the exact location where the skunk was resting. We marked the site using fluorescent flagging tape. We attempted to relocate plains spotted skunks at rest sites every 7 to 14 days from approximately one week after capture through mid-April, when collars were no longer emitting a signal.

In mid-May, we revisited each identified rest site and established two 50 m transects, one running north to south and one running east to west, with the rest site at the intersection of the two transects. The intersecting transects created five nodes: one in each cardinal direction at the end of each transect and one at the rest site. At the rest site, we recorded the type of rest site (i.e., burrow, root system, rocky outcrop, or hollow log/tree) and the rest site entrance size and orientation. In some cases, there appeared to be more than one entrance to the rest site. In these cases, we recorded the entrance size and orientation for all apparent entrances. At each node, we recorded percent canopy cover using a spherical densiometer, basal area using a glass prism, and percent ground cover by visually estimating the amount of vegetation covering the ground within a 1 m square. Along each transect, we counted the number of coarse woody debris (i.e., logs lying on the ground with diameter at breast height greater than 10 cm) and the number of rocks greater than 10 cm. We also identified a paired available but unused rest site for each identified rest site by randomly selecting a directional bearing, walking the direction of the bearing from the used rest site, and searching for a hollow log or burrow perceived as suitable for use as a rest site. We characterized the unused sites in the same manner as the used sites.

Data analyses

Photographs of the same species taken within a 1-minute window were considered a sequence. We calculated detection rates for all animal species for the entirety of the camera trap survey by dividing the total number of sequences of the species by the total number of trap nights. We also determined the proportion of all images of animals attributable to each species by dividing the number of sequences of each species by the total number of sequences with an animal. We calculated the mean and 95% confidence interval for the environmental factors we used to characterize each camera site (i.e., canopy cover, woody groundcover, understory at low, middle, and upper levels, and coarse woody debris). To test for potential selection for environmental variables, we compared camera site characteristics where plains spotted skunks were recorded to other sites using nonparametric Wilcoxon rank-sum tests. We compared the forest type (deciduous, coniferous, mixed) at camera sites with plains spotted skunk photographs to those without plains spotted skunk photographs using a Fisher's exact test.

We calculated the live-trap success rate for plains spotted skunks by dividing the total number of captures by the total number of trap nights. For the used rest sites, we used nonparametric Wilcoxon rank-sum tests to test for differences among male and female rest site selection for each environmental characteristic (i.e., canopy cover, groundcover, coarse woody debris, rocks, and basal area) and for the rest site opening size. We used a Fisher's exact test to determine whether there were differences between male and female use for the categorical characteristics (i.e., rest site orientation and substrate). Finding no differences among male and female plains spotted skunk rest site selection, we pooled the data for analysis of used and unused sites. We calculated the mean and 95% confidence interval for environmental characteristics at used and unused rest sites separately. We compared environmental characteristics at the used and unused sites to evaluate potential selection using a nonparametric Wilcoxon signed rank test. We determined whether rest site opening orientation and substrate were different among used and unused sites using a Fisher's exact test.

RESULTS

Camera trapping

We deployed cameras for 8,119 trap nights during 428 camera trap deployments, collecting 5,603 sequences of animals excluding camera trappers. Ten deployments were excluded from the final dataset due to logistical constraints, or in one case, camera trap destruction by a black bear. We detected plains spotted skunks on six occasions at four camera trap locations for a detection rate of 0.074% (Figure 1.2). All images were collected between 19:00 and 5:00 during late summer or spring in the months of March 2017, September 2017, and March 2018 (Figure 1.3). Analysis of environmental characteristics (canopy cover, understory density, proportion of woody groundcover, coarse woody debris, and forest type) of camera sites with and without plains spotted skunk images did not reveal evidence of selection by plains spotted skunks (Figure 1.4).

In addition to plains spotted skunks, we detected 28 other taxa (Table 1.1). The most commonly recorded species were opossum (detection rate = 13.02%), black bear (detection rate = 11.15%), and white-tailed deer (*Odocoileus virginanus*; detection rate = 10.03%). Seven taxa were detected less frequently than plains spotted skunks, and with the exception of long-tailed weasel (*Mustela frenata*), these were principally non-native taxa or birds, which were not expected to be attracted to the camera (Figure 1.3; Table 1.1).

Live-trapping and rest sites

We captured two plains spotted skunks on three occasions for a trap success rate of <0.1%. We captured one male (captured on January 25 and February 9, 2018) and one female plains spotted skunk (captured on February 10, 2018). The male individual weighed 28.6% more than the female individual, but all other body measurements were greater for the female skunk (Table 1.2). The female skunk was not lactating and the male skunk had descended testes at the time of the second capture, but not during the first capture.

We tracked the female skunk to five rest sites and the male skunk to seven rest sites (Figure 1.5). There were few differences in characteristics of rest sites selected by each plains spotted skunk. On average, the animals used rest sites with lower proportions of canopy cover and groundcover, lower basal area, and higher counts of coarse woody debris and rocks compared to unused sites (Figure 1.6), but only groundcover differed significantly (p = 0.021). Rest sites used by plains spotted skunks were similar to unused but putatively available sites with regards to site opening size (p = 0.73) and orientation (p = 1; Figure 1.7). Both plains spotted skunks used burrows, root systems, and rocky outcrops as rest sites, with rocky outcrops being the most frequently used substrate for both individuals (Figure 1.8). The female skunk also used a hollow log and the male skunk used a root system that was entangled among a rocky outcrop as a rest site (combination in Figure 1.8), but there were not significant differences in rest site substrate selection among used and unused sites (p = 1).

DISCUSSION

Although capture success rates for camera trap surveys of eastern spotted skunks tend to be low (0.45% in Virginia [Lombardi et al. 2016], 0.38% in South Carolina [Wilson et al. 2016], and 2.8% in a survey that spanned North Carolina, South Carolina, and Georgia [Eng 2018]), our survey resulted in a comparatively lower rate (0.074%), despite more trap nights. This low capture success rate likely reflects the true rarity of the plains spotted skunks in the region. Yet despite this rarity, the insights gained from the image sequences that we did capture were generally in agreement with those gained from other studies in terms of seasonality and time of day. Our camera traps detected plains spotted skunk most commonly in early spring (March) and once during late summer (September), aligning with results from Hackett et al. (2007), who observed that late winter and early autumn had the highest probability of detection per survey for track plate and camera trap surveys, respectively in the Missouri Ozarks. And similar to other studies, plains spotted skunks at our site were captured during nocturnal hours (Lombardi et al. 2016, Wilson et al. 2016, Benson et al. 2019).

Analysis of environmental characteristics at camera trap sites that recorded plains spotted skunks and those that did not revealed no significant differences, and thus no evidence of selection by plains spotted skunk. Studies of eastern spotted skunk throughout the Eastern U.S. consistently reveal that the species selects for dense understory associated with young forests within large, forested regions (Hackett 2008, Lesmeister et al. 2009, Thorne et al. 2017). We may not have been able to discern selection for environmental variables due to our low sample size of sites that recorded plains spotted skunks.

Camera trap survey design may influence capture success rate for eastern spotted skunks. We transitioned camera traps frequently in an effort to capture seasonality differences at every site in our entire study area. However, probability of detecting eastern spotted skunks at a site, given occurrence, may increase with number of sites and deployment length (Shannon et al. 2014, Eng 2018). While our survey had a high number of deployments (n = 428), camera deployment length averaged 18.97 days (± 0.3 days). Future camera trap surveys in the Ozark region should consider increasing deployment length to improve capture success.

Reflecting the results of our camera survey, we had a low capture success rate (<0.1%) during our live-trapping efforts. Other studies with similar objectives successfully captured more individuals at higher capture success rates (0.38% in southern Missouri [Hackett et al. 2007], 0.88% in western Arkansas [Lesmeister et al.

2009], 4% in Alabama [Sprayberry and Edelman 2018]). Eastern spotted skunks captured during research efforts targeting other species, but using similar methods, also report higher capture success rates compared to our study. Diggins et al. (2015) incidentally captured six individual eastern spotted skunks at a success rate of 1.07% during trapping efforts for Carolina northern flying squirrels (*Glaucomys sabrinus coloratus*) in Virginia. The low trap success rate at our study site further emphasizes the rare nature of plains spotted skunks at the site.

We did not discern differences among environmental characteristics at used and unused rest sites, except that groundcover was lower at used sites than unused sites, a result that disagrees with much of the existing literature. Studies from Florida, the southern Appalachian Mountains, and western Arkansas report understory density or visual obstruction as important drivers of rest site selection (Lesmeister et al. 2008, Eng 2018, Harris 2018, Sprayberry 2016). Eastern spotted skunks in the southern Appalachian Mountains also selected for rest sites with greater snag density and coarse woody debris than was present at available, unused sites (Eng 2018, Sprayberry 2016). In the Ouachita region of western Arkansas, Lesmeister et al. (2008) identified greater canopy cover and more rocks as additional characteristics at rest sites selected by plains spotted skunks. We hypothesize that the higher amount of rocky outcrop use by plains spotted skunks in our study may have resulted in lower groundcover at used sites compared to unused sites, which were primarily hollow logs and root systems. Rocky outcrops are not commonly reported as the most frequently used substrate for rest sites (Eng 2018, Lesmeister 2008, Sprayberry 2016), but eastern spotted skunks may use them more frequently when available (Diggins et al. 2015). Although small rest site opening sizes were important for plains spotted skunks in western Arkansas (Lesmeister et al. 2008), we did not observe selection for smaller opening sizes at used rest sites compared to unused sites. The lack of apparent differences among characteristics of used and unused rest sites may also be an artifact of the small number of animals and the small number of rest sites examined.

The results from our study suggest that plains spotted skunk are present, but rare in the Ozark region of north-central Arkansas. Despite a large-scale effort, we were not able to determine major habitat requirements for the species. However, confirmation of a plains spotted skunk population in the region is promising and underpins the need for further research in the region. Indeed, given that population studies often occur in places where target species are relatively abundant (Smallwood and Schonewald 1996), the relatively low population density reflected in our study may reflect the norm for this taxon across much of its remaining occupied range.

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TABLES

Table 1.1. Number of sequences and detection rates of species captured during a camera trap survey in Ozark National Forest and Gene Rush Wildlife Management Area in Arkansas, U.S. during January 2017 through March 2018.

Species or Taxa Name	**Common Name**	**Number of Sequences**	**Detection Rate (Percent)**
Canis familiaris	Domestic Dog	21	0.259
Canis latrans	Coyote	194	2.389
Cervus elaphus	Elk	127	1.564
Corvus brachyrhynchos	Crow	2	0.025
Dasypus novemcinctus	Nine-Banded Armadillo	186	2.291
Didelphis virginiana	Opossum	1057	13.019
Equus caballus	Domestic Horse	1	0.012
Glaucomys volans	Southern Flying Squirrel	66	0.813
Homo sapiens	Humans	1	0.012
Lynx rufus	Bobcat	48	0.591
Meleagris gallopavo	Turkey	19	0.234
Mephitis mephitis	Striped Skunk	30	0.370
Mustela frenata	Long-Tailed Weasel	1	0.012
Odocoileus virginianus	White-Tailed Deer	814	10.026
Other Bird species	Birds	110	1.355
Procyon lotor	Raccoon	635	7.821
Raptor Species	Raptors	1	0.012
Sciurus carolinensis	Eastern Grey Squirrel	609	7.501
Sciurus niger	Fox Squirrel	7	0.086
Spilogale putorius	Eastern Spotted Skunk	6	0.074
Sus scrofa	Feral Hog	153	1.884
Sylvilagus floridanus	Cottontail	69	0.850
Tamias striatus	Chipmunk	16	0.197
Unknown Animal	Unknown Animal	342	4.212
Unknown Bird	Birds	4	0.049
Unknown Small Rodent	Small Rodents	161	1.983
Unknown Squirrel	Squirrels	3	0.037
Urocyon cinereoargenteus	Grey Fox	131	1.613
Ursus americanus	Black Bear	905	11.147

Table 1.2. Morphometrics of two plains spotted skunks captured during winter 2018 in Ozark National Forest.

Skunk Name	**F1**	**M1**
Sex	Female	Male
Weight (kg)	0.35	0.45
Tooth Width (mm)	3.1	3
Tooth Length (mm)	5.9	3.8
Tooth Depth (mm)	2	1.9
Canine Breadth (mm)	11.1	10.3
Body Length (cm)	31.5	29.9
Tail Length (cm)	20	18.1

FIGURES

Figure 1.1. Map of study area in Ozark National Forest and Gene Rush Wildlife Management Area with camera trap locations. Locations where plains spotted skunks were detected are denoted using a blue star. Camera traps were deployed six times (two weeks per deployment) at each site from January 2017 through March 2018.

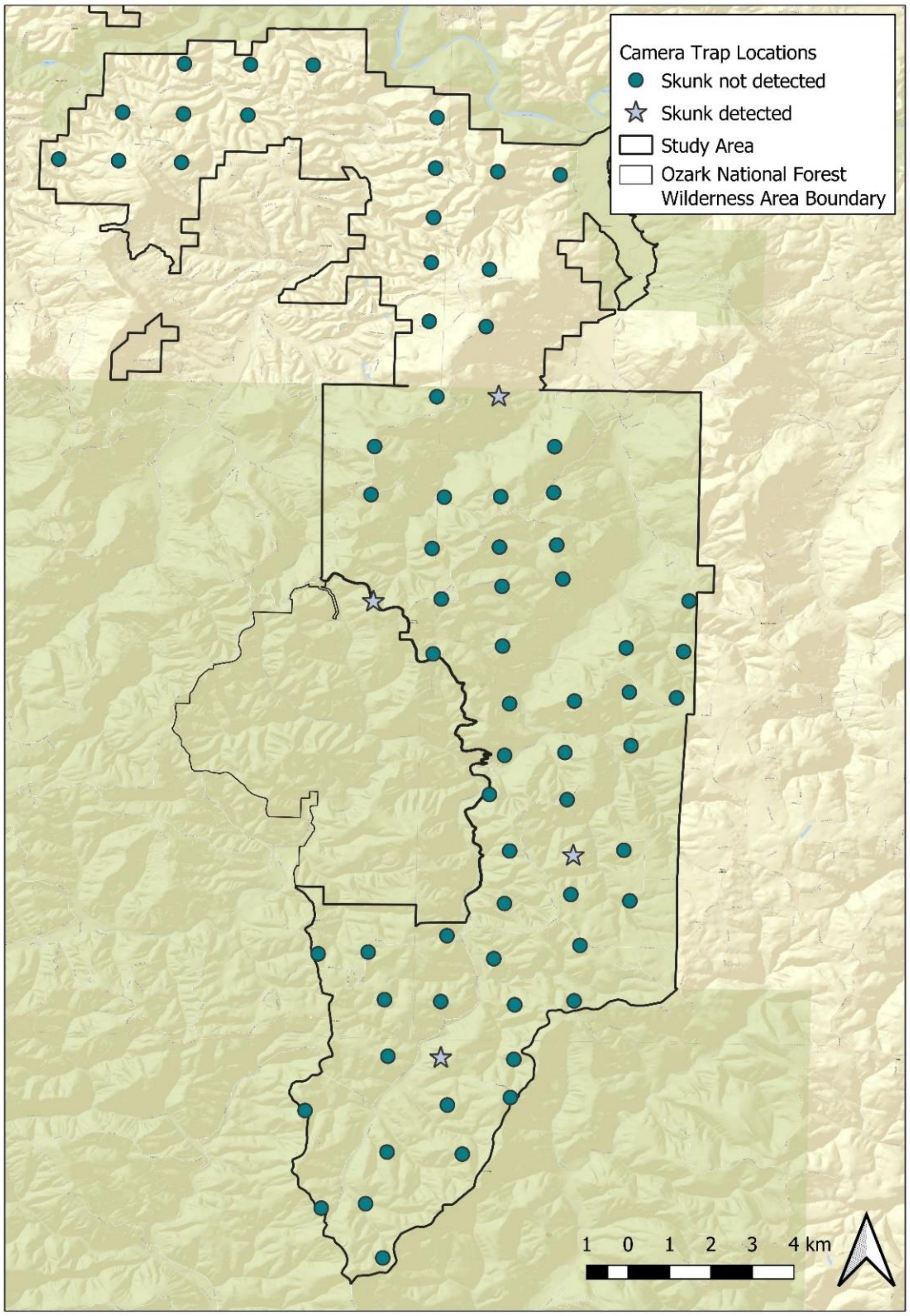


Figure 1.2. Images from sequences of plains spotted skunks taken as part of a large-scale camera trap survey in Ozark National Forest and Gene Rush Wildlife Management Area in Arkansas, U.S. during January 2017 through March 2018.

Figure 1.3. Counts of image sequences attributable to mammalian species or species groups captured during a camera trap survey in Ozark National Forest and Gene Rush Wildlife Management Area in Arkansas, U.S. during January 2017 through March 2018. Colors represent the proportion of sequences taken during each of the four seasons for that taxon.

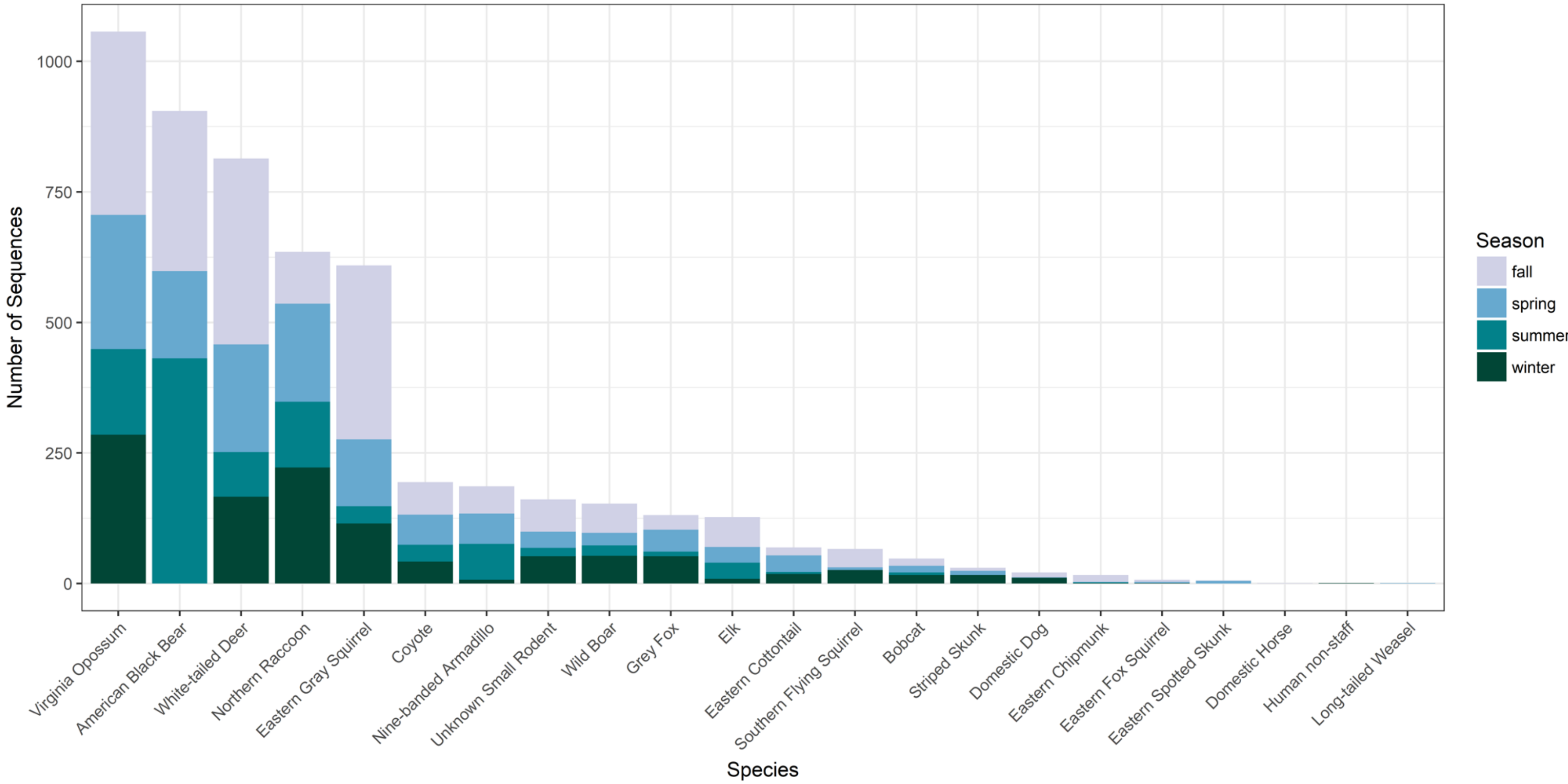


Figure 1.4. Scatterplots of environmental measures collected at each camera site. Each scatterplot includes a mean and 95% confidence interval inclusive of all camera sites in black. Camera sites that captured plains spotted skunks are identifiable with a dark green triangle.

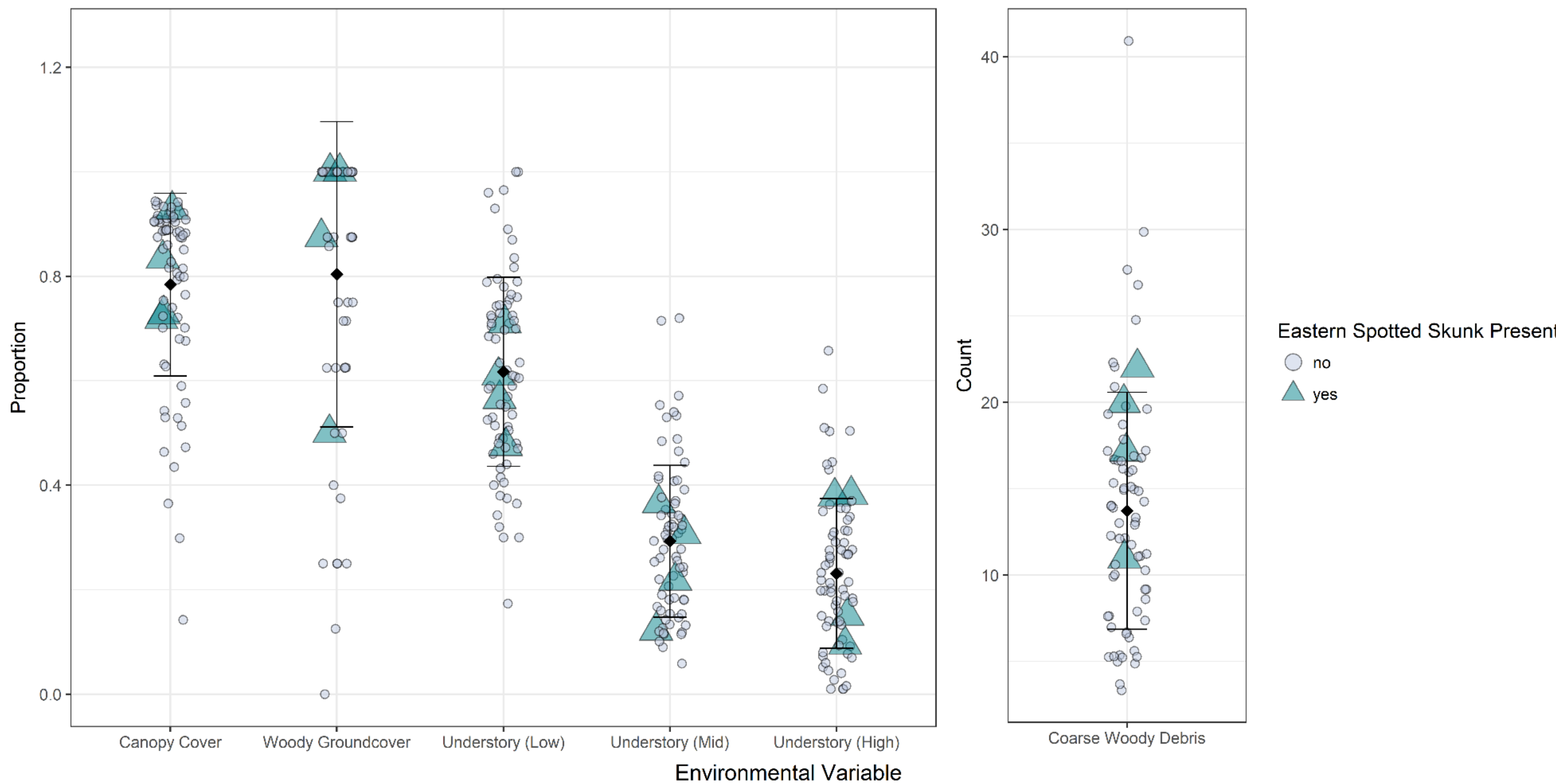


Figure 1.5. Location of collared female (blue diamonds) and male plains spotted skunk (green diamonds) rest sites in Ozark National Forest identified using radio telemetry during winter and spring 2018.

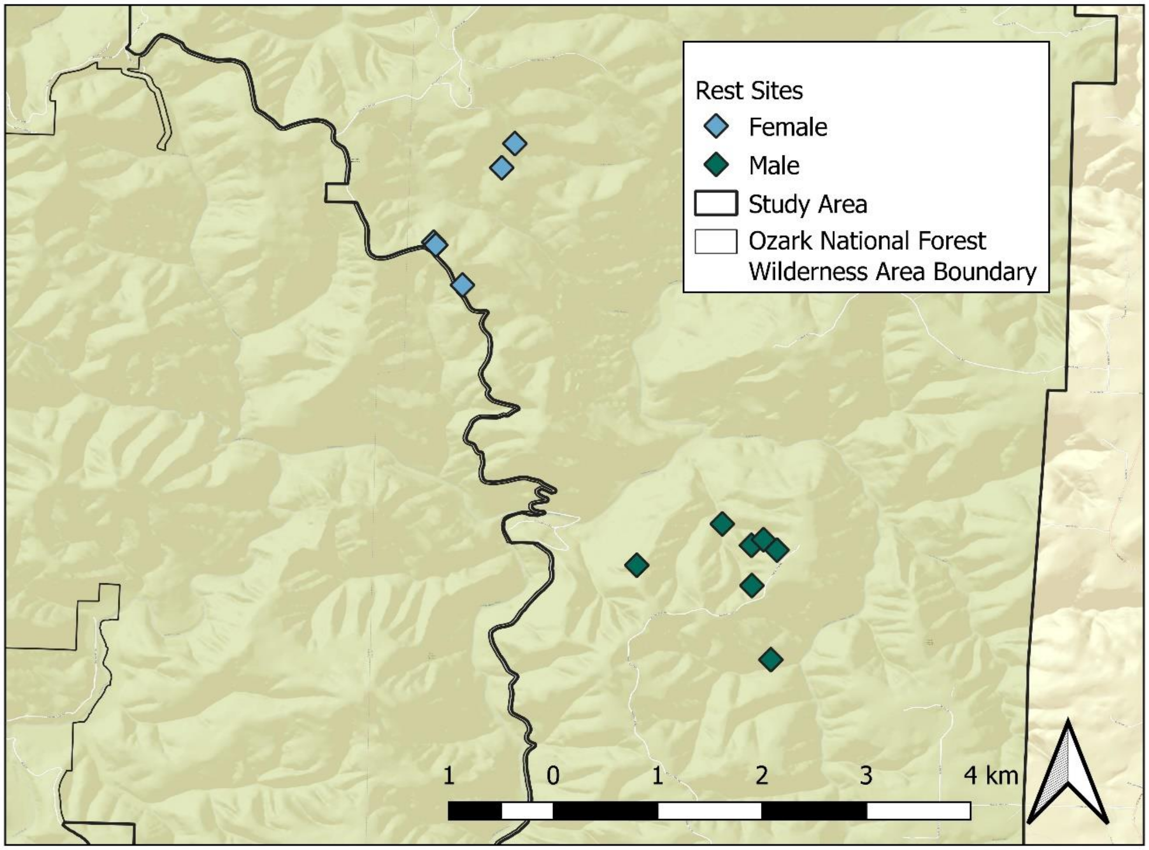


Figure 1.6. Environmental characteristics of used and paired unused rest sites for two eastern spotted skunks that were radio-tagged and tracked in Ozark National Forest during winter and spring 2018. Dark green markers represent used rest sites and light blue markers represent paired unused rest sites. Circular markers indicate an environmental characteristic value for used and paired unused rest sites of the male skunk, while triangular markers indicate female skunk sites. Diamond points and error bars represent means and 95% confidence intervals for environmental characteristics of used (dark green) and paired unused (light blue) rest sites for the male and female skunks.

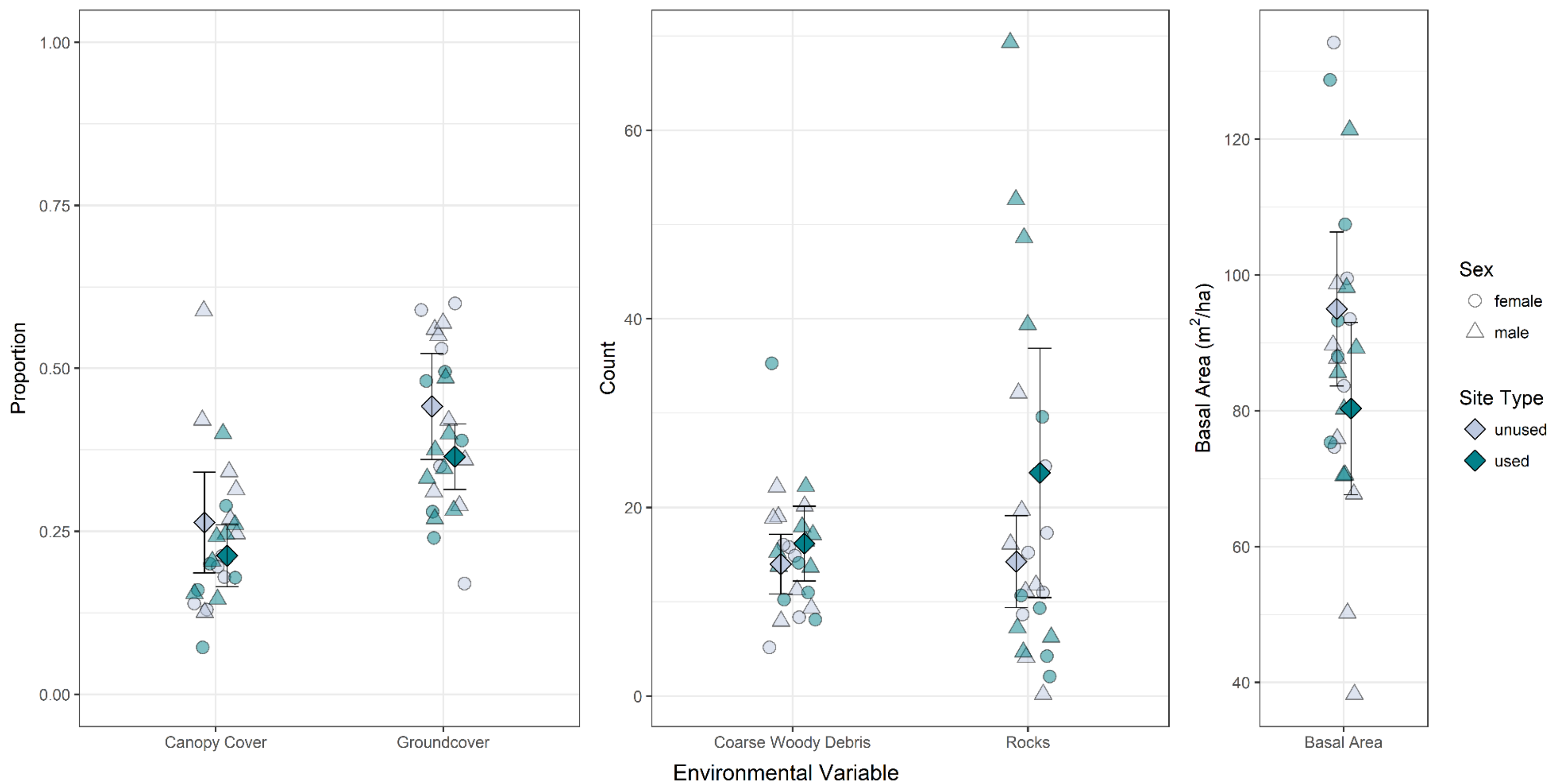


Figure 1.7. Site opening orientation and size of used and paired unused rest sites for two eastern spotted skunks that were radio-tagged and tracked during winter and spring 2018 in Ozark National Forest. Each line on the rest site opening orientation compass represents the direction in which the rest site opening was facing for used (dark green) or paired unused (light blue) male (triangle) and female (circle) skunk rest sites. In the rest site opening size graph, dark green markers represent used rest sites and light blue markers represent paired unused sites, with circular markers for female skunk sites and triangular markers for male skunk sites. The diamond markers and error bars represent the mean and 95% confidence intervals for used and paired unused rest site opening sizes.

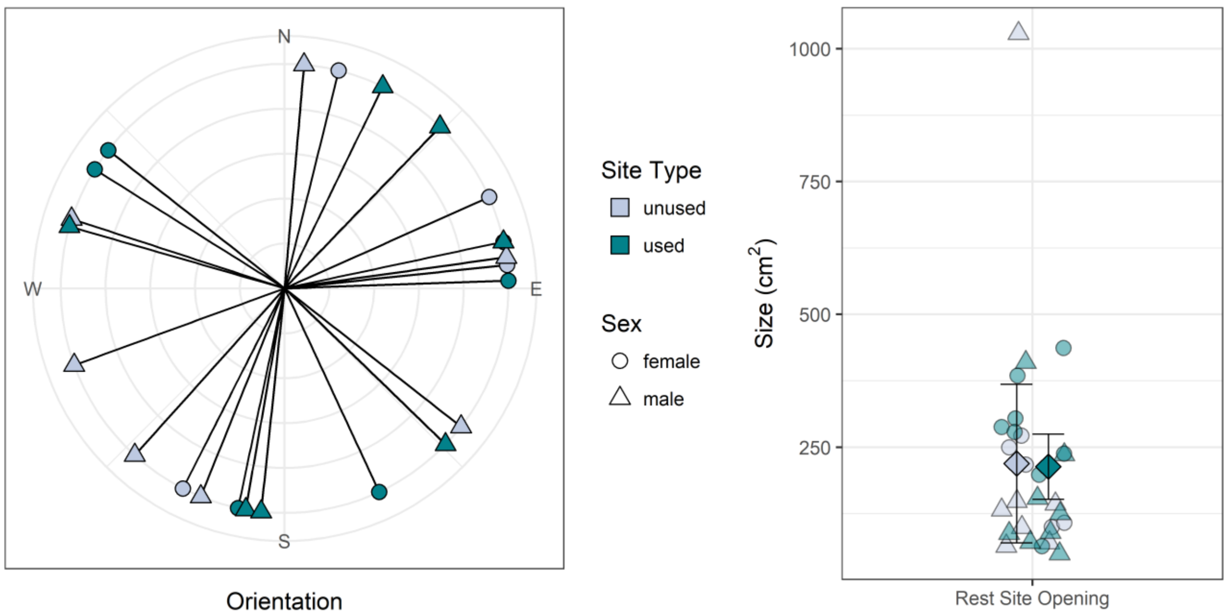
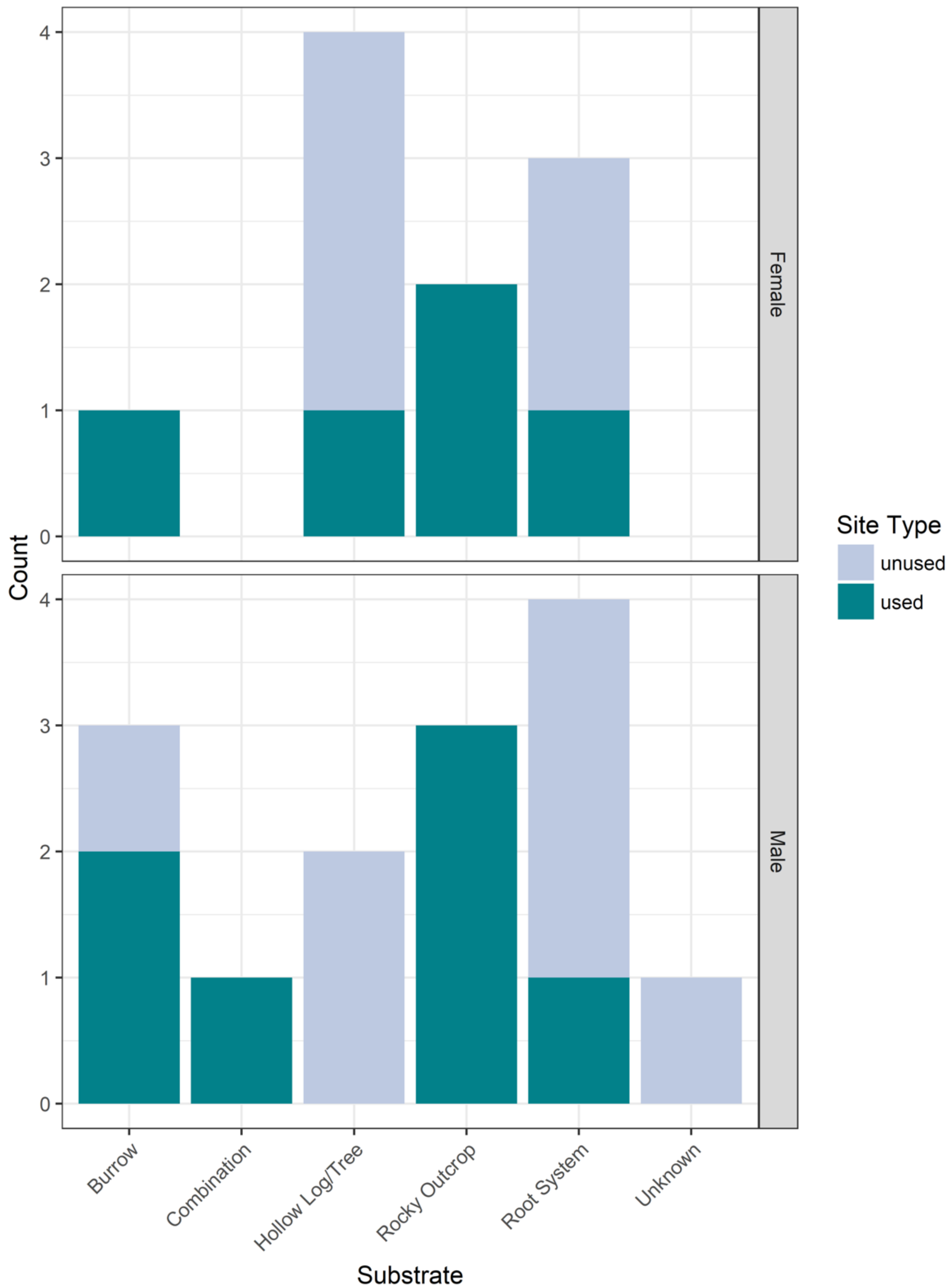


Figure 1.8. Substrate of used and paired unused rest sites for two eastern spotted skunks that were radio-tagged and tracked in Ozark National Forest. Dark green bars represent used sites and light blue bars represent unused sites, while the female skunk rest sites are in the top graph and the male skunk rest sites are in the bottom graph.



CHAPTER 2

A MAXENT PREDICTIVE DISTRIBUTION MODEL FOR THE PLAINS SPOTTED SKUNK (*SPILOGALE PUTORIUS INTERRUPTA*) CORE REMAINING GEOGRAPHIC RANGE IN MISSOURI AND ARKANSAS

ABSTRACT

The plains spotted skunk (*Spilogale putorius interrupta*), a subspecies of the eastern spotted skunk (*S. putorius*), is of conservation concern across its range due to widespread population declines and currently petitioned for listing under the U.S. Endangered Species Act. Information on habitat associations, resource requirements, and remaining distributions for the taxon are imperative. Although the relative rarity of plains spotted skunks make them difficult to study, recent research and reports has confirmed their presence in Missouri and Arkansas. We employed a MaxEnt species distribution model (SDM) to evaluate habitat associations and identify regions of high probability of plains spotted skunk presence. We selected percent forest, agriculture, and development within 5 km, basal area, land cover category, downed wood, distance to water, small stem density, and edge density as environmental variables based on the existing eastern spotted skunk literature. We used two presence-only datasets to inform and validate the MaxEnt model: a training dataset based on targeted research efforts and a testing dataset based on citizen science and other reports to state agencies. We removed variables that were worse than random at predicting species probability of presence (test AUC < 0.5) in a univariate model, and produced a multivariate model with the remaining variables. Our multivariate model (test AUC = 0.835) retained all variables

except edge density. However, percent forest within 5 km was the most important variable based on permutation importance (= 87.8%), indicating that plains spotted skunks select for contiguous forest at the landscape scale. Regions predicted to have high probability of presence occurred in southern Missouri and northern, western, and southern Arkansas, totaling >300,000 hectares. Our results provide direction for locating sites with a higher likelihood of occupation by plains spotted skunks and may be of value for application in other states and for other subspecies.

INTRODUCTION

Species distribution models (hereafter SDMs) are widely used to forecast probable distributions outside of smaller sampling areas. These models are used extensively to first identify relationships between species presence and environmental conditions, then are applied to a broader landscape to predict species probability of presence across a larger area or another area of interest (Phillips et al. 2006). SDMs have been used to predict future species distributions with effects of climate and land-use change (Hijmans and Graham 2006, Soultan et al. 2019), to evaluate the efficacy of reserve systems for protected species (Cooper-Bohannon et al. 2016, Smith et al. 2016), to predict susceptibility of landscapes to invasive species colonization (Akin-Fajiye and Gurevitch 2018, Ward 2007), and to target potential sites for research, reintroduction, or habitat restoration for a particular species or suite of species (Picanço et al. 2017). An abundance of literature on best practices and tools for building SDMs make them an accessible, replicable, and widely-accepted method for making predictive species distribution maps.

Maximum entropy models (generally abbreviated as MaxEnt and conducted using the open source software MaxEnt; see Methods) are often employed to build SDMs for rare or cryptic species due to their high performance with minimal data requirements (Aubry et al. 2017, Pearson et al. 2007), including outperformance of other types of SDMs with 10 or fewer presence points (Wisz et al. 2008). MaxEnt modeling is a widely used method for predicting or inferring species distributions with incomplete information (Phillips et al. 2006). As true absence data is difficult to obtain and choosing a wrong absence when modeling rare species could significantly reduce the quality of the SDM (Engler et al. 2004), MaxEnt was designed to correlate environmental predictors (e.g., precipitation, temperature, elevation, etc.) with presence-only point locations.

Although MaxEnt modeling relies on strong and critical assumptions (e.g., Royle et al. 2012), there have been multiple studies that detail various approaches to minimize model overfitting. For example, gathering presence data from a random sample (e.g., point data from transects or camera traps placed in a grid) produces more reliable predictions of relative occurrence (Fithian and Hastie 2012, Merow et al. 2013). To reduce bias from occurrence data (e.g., observations closer to roads, trails, or rivers), MaxEnt produces pseudo-absence points, or background points where presence or absence of the target species is unknown (Phillips and Dudík 2008, Phillips et al. 2009). MaxEnt SDMs can be further strengthened by comparing the predictive SDM built with presence-only and pseudo-absence data against a second, independent dataset of test points (Araujo and Rahbek 2006, Phillips et al. 2009). The contemporary availability of presence data collected through citizen science initiatives lends itself well as a source for test data in MaxEnt modeling (e.g., MacPherson et al. 2018). Thus, building a MaxEnt SDM is an ideal starting point for identifying correlations between species presence and environmental variables for rare or cryptic species so as to identify areas of high-suspected relative occurrence rates.

One such rare and cryptic species is the eastern spotted skunk (*Spilogale putorius*). The eastern spotted skunk is a species of conservation concern (Gompper and Jachowski 2016), with one subspecies – the plains spotted skunk (*S. putorius interrupta*) – currently petitioned for listing under the U.S. Endangered Species Act (Department of Interior, USFWS 2012). Problematically, the exact cause of the decline remains unknown (Gompper and Hackett 2005, Gompper 2017), and as such, most research efforts have focused simply on attempting to identify populations and their resource needs (see e.g., chapter 1, Lesmeister et al. 2009, Harris 2018, Thorne et al. 2017). Urgently needed local habitat information has led several range states to reach out to citizens for information on eastern spotted skunks, asking to report sightings to the state wildlife agency (Dowler et al. 2017, Eastern Spotted Skunk Cooperative Study Group 2018). Combining data from field research with data from citizen reports in a MaxEnt modeling approach provides an excellent opportunity to identify a refined predicted distribution for the species.

Here we focus on the distribution of the plains spotted skunk subspecies within Missouri and Arkansas. These two states appear to represent core remaining range of the subspecies (although it is known to persist in other states), and also represent the two states where intensive studies of the subspecies have occurred (see chapter 1; Lesmeister et al. 2009, 2010, 2013; Hackett et al. 2007, Hackett 2008). To evaluate plains spotted skunk habitat preferences and identify regions of high occurrence probability for targeted research efforts, we designed a study to combine the results of field surveys, citizen science datasets of sightings of plains spotted skunks, and environmental variables predicted to play roles in habitat preference for plains spotted skunk across both range states using a MaxEnt SDM. We used the existing literature on eastern spotted skunks (including studies of multiple subspecies) to identify a suite of environmental variables likely to influence plains spotted skunk presence in Missouri and Arkansas (Table 2.1). We employed a two-dimensional kernel density method to restrict the training model to the range of conditions experienced nearby known presence locations and predicted presence across the entirety of Arkansas and Missouri. We collected training data through three large-scale, targeted research efforts between 2005-2018 in three National Forests, one Wildlife Management Area, and private lands. Independent testing point data was gathered from incidental observation and non-random trapping from the Arkansas Game & Fish Commission and the Missouri Department of Conservation.

We predicted that availability of forests within 5 km would be positively correlated with training and testing point datasets, as associations between eastern spotted skunks and forested regions have routinely appeared in habitat selection studies (Lesmeister et al. 2009, Hackett 2008, Thorne et al. 2017). Conversely, we expected a negative relationship between availability of agricultural land within 5 km and plains spotted skunk presence, as modern agricultural practices are considered a contributing factor to the decline of eastern spotted skunks (Choate et al. 1973, DeSanty 2001, Schwartz and Schwartz 2001; Gompper 2017), and evidence suggests plains spotted skunks avoid open pasture (Lesmeister et al. 2009). We expected variables associated with brushy forest understory cover to correlate positively with plains spotted skunk presence because home range, rest site selection, and occupancy research confirm associations between eastern spotted skunks and dense, brushy understory (Lesmeister et al. 2008, Lesmeister et al. 2009, Thorne et al. 2017, Eng 2018, Harris 2018, Sprayberry and Edelman 2018). Distance to water was expected to correlate negatively with plains spotted skunk presence because areas closer to water can offer better foraging quality (Eng 2018). We expected amount of developed land within a 5 km radius to negatively influence plains spotted skunk presence because previous research has shown avoidance of developed areas in the Ozark region (Hackett 2008).

METHODS

Study area

Our study focused on the central U.S. states of Missouri and Arkansas. In northern and southeastern Missouri and eastern Arkansas, row crop agriculture dominates the landscape, while pastured livestock agriculture is interspersed in the mountainous portions of both states. Southern Missouri and northern Arkansas hold the majority of the Ozark Mountains, a region characterized by oak and mixed oak-pine forests interspersed with open glades and rocky, steep terrain. In the Ouachita Mountain region of western Arkansas, open pine forests dominate, but hardwood forests are also present.

Prior to the plains spotted skunk population decline in the mid-1900s (Gompper and Hackett 2005, Sasse and Gompper 2006), the plains spotted skunk occurred across the majority of both states. More recent occurrences of plains spotted skunks have been reported from the Missouri and Arkansas Ozarks (Hackett et al. 2007, chapter 1) and Arkansas Ouachitas (Lesmesiter et al. 2009), as well as from southern and eastern Arkansas (Sasse and Gompper 2006, Sasse 2018).

Training datasets

We developed a MaxEnt SDM for eastern spotted skunks in Missouri and Arkansas using training data collected from three large-scale surveys targeting the plains spotted skunk subspecies found there. We used data collected from one Missouri and two Arkansas sites as part of larger efforts to understand the ecology of plains spotted skunks in the Ouachita and Ozark regions. For the Missouri survey, which occurred in 2005-2006, Hackett (2008) used camera traps and track plate surveys across southern Missouri to evaluate mesocarnivore occupancy and interactions in the region, recording plains spotted skunks at 15 sites. The first Arkansas survey occurred in 2006-2007 and used track plate surveys to evaluate eastern spotted skunk habitat selection (Lesmeister et al. 2009); it resulted in 57 records of eastern spotted skunks in Ouachita National Forest. The second Arkansas survey, which took place in 2017-2018, consisted of a large-scale camera trap grid with the intention of identifying eastern spotted skunk locations for future live-trapping efforts within Ozark National Forest and Gene Rush Wildlife

Management Area (see chapter 1). The second Arkansas survey contributed four eastern spotted skunk locations to the SDM.

Testing datasets

We incorporated incidental captures and citizen science reports of plains spotted skunks from the past 20 years (since 1999) in Missouri and Arkansas into the model. Incorporating a second, independently collected dataset for model testing purposes reduces bias in that it measures model ability to predict species presence without depending on a subset of the same dataset that was used to build the model (Araújo and Rahbek 2006, Phillips et al. 2009). Citizen science reports are expected to produce point locations that differ in bias from standardized sampling (described above for the training datasets) in fundamental ways such as being closer to more disturbed areas, including urban centers, agricultural lands, and lands subject to greater hunting or trapping pressures (and thus subject to the use of traplines and game cameras for other species). A model more reflective of a species' true distribution is produced by including a second dataset with different biases.

We solicited 49 testing data points from the Missouri Department of Conservation Natural Heritage Database (Missouri Natural Heritage Program 2019) and the Arkansas Game and Fish Commission (B. Sasse, pers comm). These included incidental captures of plains spotted skunks that occurred during routine trapping efforts for other state agency research projects (Arkansas: n = 15). Additionally, two observations were reported by university researchers who were conducting camera trap surveys (Arkansas = 1; Missouri = 1), while one observation was made in an Arkansas cave by state agency biologists during a bat survey. Citizen scientists reported eastern spotted skunk observations to state biologists as game camera photos (Arkansas: n = 5), observations at home or while spending time outdoors (Arkansas: n = 2; Missouri: n = 13), and observations of a spotted skunk crossing the road or observed as road kill (Arkansas: n = 2; Missouri: n = 4). Citizen trappers incidentally captured four individuals in Missouri while trapping for other species. We also included records reported on iNaturalist (inaturalist.org) when the observation included a photo verification (Arkansas: n = 1). All occurrences used in our model were confirmed by a researcher or biologist familiar with spotted skunk identification, as data quality and misidentification of target species can affect the accuracy of the SDM (Aubry et al. 2017).

Environmental variables

We identified nine environmental variables that may be important for plains spotted skunk distribution based on the existing literature (Table 2.1). We used QGIS version 3.2.2 to project environmental raster layers in the NAD83 / Conus Albers (EPSG: 5070) projection. We aligned raster layers to a size of 250 x 250 m pixels, using the bilinear resampling method for continuous variables and the nearest neighbor method for categorical variables. We also clipped the geographic range of the environmental raster layers to the extent of our study area, Missouri and Arkansas. We converted the raster layers from GeoTIFF files to ASCII files for use in MaxEnt software using packages ‘raster’ and ‘rgdal’ in R version 3.4.1.

MaxEnt species distribution model

We used Maximum Entropy Species Distribution Modeling software, version 3.4.1 (https://biodiversityinformatics.amnh.org/open_source/maxent/) to evaluate the environmental data layers as predictors of plains spotted skunk distribution in Missouri and Arkansas to identify potential regions for research and management priority throughout these states. We used known locations of plains spotted skunk (presence-only data from our training dataset) paired with environmental variables to predict the distribution of plains spotted skunks across the region of interest. We set MaxEnt to randomly select a set of 10,000 background, or pseudo-absence locations across the landscape with the objective of sampling the full range of environmental conditions available to plains spotted skunk (Elith et al. 2011, Phillips and Dudík 2008, Phillips et al. 2009). To ensure a conservative estimate, pseudo-absence locations are randomly selected without replacement and may include some points where presence was recorded, and including a bias file that defines sampling area further prevents bias toward sampled areas (Merow et al. 2013). MaxEnt compares the environmental conditions at presence locations in the training dataset to the conditions at pseudo-absence locations to correlate the strength of environmental conditions at presence locations. This information is used to build a predictive distribution across all grid cells in a broader region of interest. The resulting output identifies the most important predictors describing plains spotted skunk locations from among the environmental variables and projects the predictive species distribution map as a gradient of relative occurrence probabilities.

We used a multi-step approach to modeling plains spotted skunk distribution. We began by using 46 training points and nine environmental predictors (Table 2.1) to build nine univariate MaxEnt SDMs and added the 49 test points to test each model. As SDMs can be spatially biased to predict presence in regions where heavy sampling occurred (Phillips et al. 2009, Syfert et al. 2013, Beck et al. 2014), we created a bias file using a two-dimensional kernel density estimator developed in R version 3.4.1 and packages 'raster,' 'MASS,' 'magrittr,' and 'maptools.' We selected the automatic features in MaxEnt (linear, quadratic, product, and hinge), selected the logistic output format, defined the training and test datasets as explained above, and used the bias file to limit spatial area to the vicinity surrounding the training and test points. We used the test Area Under Curve (AUC) metric to determine whether each univariate model was able to predict plains spotted skunk presence probability better than random (i.e., AUC > 0.5).

Then, we built a multivariate MaxEnt SDM using only those environmental variables that were better than random at predicting plains spotted skunk distribution in a univariate model. We used the same settings described above and evaluated the model using test AUC. Finally, we used the multivariate model to predict the probable distribution of plains spotted skunks across Missouri and Arkansas. We used clamping to produce the predictive distribution outside of the area of our bias file, which limits environmental conditions in the prediction region to values encountered in the training region. Clamping holds presence probability predictions constant at environmental condition values in the prediction region that fall beyond the range encountered during training (Phillips 2017). We calculated the area in hectares of predicted probability of presence at four levels (low: 0-0.24, medium low: 0.25-0.49, medium high: 0.5-0.74, and high: 0.75-1) for the final model using R version 3.4.1 and packages 'raster,' 'rgdal,' and 'measurements.'

RESULTS

Of the nine environmental variables, eight were better than random at predicting plains spotted skunk distribution in a univariate model. We removed edge density because the test AUC was <0.5 (Table 2.1). The multivariate model had a test AUC of 0.835, indicating that it was able to correctly predict probability of occurrence of plains spotted skunks at test data sites 83.5% of the time. The most important variable was percent forest within a 5 km radius as it had the highest regularized training gain of any single-variable model based on the jackknife test of variable importance (Figure 2.1). Percent forest within 5 km also had the highest permutation importance (87.8%; Table 2.2), indicating that it contributed more than any other variable to the model. Percent agriculture within a 5 km radius decreased the regularized training gain the most when excluded from the model, indicating that it contained the most unique information of the variables present (Figure 2.1). Percent agriculture within 5 km was the second most important variable behind percent forest within 5 km based on permutation importance (7.2%, Table 2.2). All other variables contributed 5.1% to the model combined based on permutation importance, and were thus unimportant compared to percent forest and agriculture within 5 km.

Across the entirety of the Missouri and Arkansas landscape, the average probability of presence was 8.58%. MaxEnt calculated that 315,518.8 hectares of land reflected a high probability of presence and the majority of hectarage (>48 million hectares) fell below a probability of presence estimate of 50% (Table 2.3). Response curves for the variables indicated that locations of plains spotted skunks were positively correlated with percent forest within a 5 km radius and negatively correlated with percent agriculture within a 5 km radius (Figure 2.2). Response curves of downed wood, distance to water, small stem density, and basal area revealed positive relationships with plains spotted skunk probability of occurrence, while there was a negative relationship between percent development within 5 km and predicted occurrence probability (Figure 2.2). Responses to the land cover categories reflected relatively low probability of plains spotted skunk occurrence for most categories, but predicted occurrence was higher than other categories for forested land cover types and the shrub/scrub land cover type. The developed, open space land cover category also had a slightly higher probability of occurrence for plains spotted skunk compared to other land cover types, but was lower than the forested and shrub/scrub categories (Figure 2.2).

According to the predictive map, plains spotted skunk probability of presence was heavily concentrated within large blocks of forest, including Mark Twain National Forest, Ozark National Forest, and Ouachita National Forest, with additional high probability regions in portions of southern Missouri, north-central Arkansas, and southern Arkansas (Figure 2.3). The Mississippi River Alluvial Plain region of eastern

Arkansas and southeastern Missouri, as well as nearly all of northern Missouri were areas of particularly low probability of presence.

DISCUSSION

Our MaxEnt SDM revealed that large, primarily forested blocks (i.e., sites with higher proportions of forest within a 5 km radius) are important for plains spotted skunk presence, which is in agreement with findings from recent studies of habitat associations of eastern spotted skunks (both the plains and Appalachian [i.e., *S. putorius putorius*] subspecies). Recent research has shown that eastern spotted skunks select for denser forests within large forest stands at the microhabitat scale (Hackett 2008, Lesmeister et al. 2009, Lesmeister et al. 2013, Thorne et al. 2017). Further, plains spotted skunk probability of presence response to the land cover category variable revealed that forested land cover categories resulted in higher probability of plains spotted skunk presence than other category types (Figure 2.2), although this variable was far less important in the multivariate model than percent forest within a 5 km radius.

Our hypotheses on the responses of plains spotted skunk probability of occurrence to six of the other variables present in the multivariate model were supported (Table 2.1; Figure 2.2). However, while our anticipated responses were supported, the importance of those responses was overshadowed by the importance of forest at the landscape scale. For example, our hypothesis that small stem density and downed wood were important factors was not supported by our analysis based on permutation importance (Table 2.2). Several studies have identified dense, brushy understory cover as factors contributing to eastern spotted skunk use of rest and den sites (Lesmeister et al. 2008, Sprayberry and Edelman 2018, Eng 2018) as well as home ranges (Hackett 2008, Lesmeister et al. 2009, Lesmeister et al. 2013, Thorne et al. 2017). We suspect that while those factors may play a large role in home range dynamics and rest or den site selection at the local scale (i.e. microhabitat scale), our results indicated that at the landscape scale (i.e., 250 m resolution), plains spotted skunks may require contiguous forest. Our model also indicates that large forest blocks are a more important factor at the landscape level than basal area, even though certain basal area values may promote characteristics suitable for plains spotted skunks, such as understory brushiness. Evaluating the probable distribution of plains spotted skunks within a contiguous forest block, rather than across the entirety of two states, may illuminate finer-scale forest characteristics that are important for skunks to fulfill their life-history needs. Although research suggests plains spotted skunks may avoid human settlements (Hackett 2008), development was not a major predictor of probability of presence in our analysis. Lack of large cities within our sampled area and a corresponding low level of variability in the development environmental layer at our sampled sites could explain why development was not an important factor in our analysis.

Two environmental variables that we identified as potentially important predictors for plains spotted skunk distribution performed differently than we expected during our analysis. Distance to the nearest drainage channel has been identified as a predictor of eastern spotted skunk presence due to the higher availability of food resources and cover closer to channels (Eng 2018) and we therefore expected to observe a negative response of plains spotted skunk probability of occurrence in association with distance to water. Instead, we observed a positive response such that plains spotted skunks were more likely to be present with increasing distance to water (Figure 2.2). We also predicted that edge density would be a factor contributing to plains spotted skunk distribution because brushy cover common along edges is similar to dense forest cover selected by plains spotted skunks (Lesmeister et al. 2009, Thorne et al. 2017). However, edge density performed worse than random (test AUC = 0.487) in a univariate model and we excluded it from the multivariate model. We suspect that selection for forest cover and general avoidance of open areas (Lesmeister et al. 2009) may preclude use of edge adjacent to open areas.

One of the leading explanations for the decline of eastern spotted skunk populations in the 1940s and 1950s suggests that modernized agriculture and the onset of pesticide use contributed to decreased habitat and food availability (Choate et al. 1973, DeSanty 2001, Schwartz and Schwartz 2001). In our multivariate model, agriculture was the second most important variable predicting plains spotted skunk probability of presence. Our response curve indicated that plains spotted skunks can tolerate agriculture at low levels or nearby, but not high levels (Figure 2.2). It is likely that the type of agriculture also plays a role in whether plains spotted skunks will be present, and future research may improve SDM estimates for the plains spotted skunk by incorporating more detailed layers of agricultural land use. In the Ozark region, pasture lands are more prevalent than in the Mississippi River Alluvial Valley and northern Missouri, where row crops dominate. However, recent research suggests that plains spotted skunks do not use pasture typical of the Ouachita Mountain region, likely due to enhanced risk of predation (Lesmeister et al. 2009). As such, agricultural practices that promote brushy understory growth, canopy cover, tree planting and growth, and farm diversity, such as silvopasture, alley cropping, forest farming, and cover crops may facilitate plains spotted skunk use of farmland (Crabb 1948, Choate et al. 1973). Although the impacts of these and similar practices are well studied for some taxa (e.g., Millspaugh et al. 2009), an understanding of whether plains spotted skunks would use such agricultural systems remains unclear and warrants further research.

Historically, plains spotted skunks were widespread across much of Arkansas and Missouri, but our results suggest their distribution is presently more limited. In Arkansas, the species was present during the early twentieth century in the northwestern and north central portions of the state, and there is evidence that the species ranged into small portions of eastern Arkansas (Sasse 2017). Since the early 1900s, plains spotted skunks were harvested broadly across the state in the 1940s and 1970s-1980s (Sasse and Gompper 2006). Presently, most incidental harvests of plains spotted skunks in Arkansas occur in the Ozark and Ouachita regions in the northwestern portion of the state, but a small portion, including two recent (2012-2017) captures, have occurred in the southern and eastern portions of the state (Sasse and Gompper 2006, Sasse 2018). These recent harvest records in the southern region of Arkansas align with our predicted distribution, as sections of south-central Arkansas fell within the high probability of presence category (Figure 2.3). Although harvest records from the early

2000s indicate there was still a population of plains spotted skunks in east-central Arkansas (Sasse and Gompper 2006), more recent records, as well as our results, suggest the population may no longer exist, or may be largely reduced (Sasse 2018; Figure 2.3).

In Missouri in the early part of the 1900s, plains spotted skunks ranged throughout most of the state, especially the western regions (Sasse 2017, Schwartz and Schwartz 2001). According to Missouri Natural Heritage Database accounts, plains spotted skunks were broadly distributed in the state throughout the 1900s, with records as recent as the 1990s in northern Missouri (Missouri Natural Heritage Program 2019). However, since the 2000s, no new records have entered the Missouri Natural Heritage Database from northern Missouri (Missouri Natural Heritage Program 2019), and our model indicates that plains spotted skunks may no longer occur in appreciable numbers in that region (Figure 2.3). Further, our model suggests that plains spotted skunks could potentially be extirpated from the southwestern corner of Missouri, a region that was historically occupied (Sasse 2017).

With the potential for the plains spotted skunk listing under the U.S. Endangered Species Act, an understanding of where the species is distributed and threats to its persistence are valuable. Our species distribution model indicated a strong correlation between species presence and percent forest cover within a 5 km radius. However, we do not have a clear understanding of how habitat fragmentation at the landscape level could affect metapopulation dynamics and population persistence across the range. Despite the high to medium high probability of presence of the species in large portions of southern Missouri and northern, western, and southern Arkansas predicted by our model, there are significant spatial gaps that could lead to isolation of populations if they exist. Genetic isolation among plains spotted skunk populations could contribute to a decrease in the already low genetic variability within populations, an issue that has been discussed in the literature (Shaffer et al. 2018), but warrants further research. Literature suggests that plains spotted skunks select for areas within forest stands (Lesmeister et al. 2009) and avoid human disturbance (Hackett 2008); therefore, obstacles like roads, human settlements, and even water bodies could hinder plains spotted skunk movement to maintain populations or reoccupy lands across Arkansas and Missouri.

Our species distribution model supports that plains spotted skunks could potentially persist in regions with contiguous forest in southern Missouri and northern, western, and southern Arkansas as those areas have a similar environmental landscape as where our presence data from training and testing datasets originated. Several such forested blocks are already protected and managed federally, and these National Forests could serve as ideal locations for further examinations of plains spotted skunk population persistence. Further, researchers seeking to gather microhabitat-level information on the species may consider focusing efforts on areas of predicted high probability of presence.

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TABLES

Table 2.1. List of environmental variables used to predict plains spotted skunk distribution, their description and data source, research justifying their selection, our hypothesized response, and the test AUC associated with a univariate model.

Environmental Variable	Description	Source	Justification	Expected Response	Test AUC
Downed wood	Dead/downed wood (6' 8" dbh) (tons carbon/ac)	Derived from FIA/MODIS imputation methods (Wilson et al. 2012)	Eng 2018 Sprayberry and Edelman 2018	Positive	0.764
Small stem density	Stems/ha	Derived from FIA/MODIS imputation methods (Wilson et al. 2012)	Lesmeister et al. 2008 Sprayberry and Edelman 2018 Eng 2018	Positive	0.786
Basal area	Basal area (m^2/ha)	Derived from FIA/MODIS imputation methods (Wilson et al. 2012)	Lesmeister et al. 2009	Positive	0.783
Distance to water	Distance (m) to water, includes streams and waterbodies	Based on USGS National Hydrography Data	Eng 2018	Negative	0.709
Agriculture	Percent of agriculture land within a 5 km radius, includes all agricultural types	Derived from National Land Cover Data (Homer et al. 2015)	Choate et al. 1973 DeSanty 2001 Schwartz and Schwartz 2001 Lesmeister et al. 2009	Negative	0.83
Forest	Percent of forested land within a 5 km radius	Derived from National Land Cover Data (Homer et al. 2015)	Hackett 2008 Lesmeister et al. 2009 Lesmeister et al. 2013 Thorne et al. 2017	Positive	0.85
Development	Percent of developed land within a 5 km radius	Derived from National Land Cover Data (Homer et al. 2015)	Hackett 2008	Negative	0.6
Edge density	Proportion of forested versus non-forested land	Derived from National Land Cover Data (Homer et al. 2015)	Lesmeister et al. 2009 Thorne et al. 2017	Positive	0.487
Land cover class	Land cover classification	National Land Cover Data	Lesmeister et al. 2009 Hackett 2008	Positive with forested land classes	0.685

Table 2.2. Permutation importance of environmental variables that remained in the multivariate model. The test Area Under Curve (AUC) of the model represents the ability of the model to predict plains spotted skunk probability of occurrence correctly on the test data set. Mean, minimum, and maximum columns represent the mean and range variable values in the background, or pseudo-absence, and training data points used to build the model.

Environmental Variable	Permutation Importance (Percent)	Test AUC	Mean	Minimum	Maximum
Forest	87.8	0.848	0.84	0.21	0.96
Agriculture	7.2		0.1	0	0.59
Downed Wood	2.4		2.07	0.06	2.81
Distance to Water	1.6		3411.58	221.22	12294.29
Land Cover	0.8		NA	NA	NA
Development	0.2		0.04	0.01	0.29
Small Stem Density	0.1		6136.48	110.18	14791.51
Basal Area	0		18.1	0.6	25.11

Table 2.3. Hectares of land that fall within four categories of probability of presence for eastern spotted skunks in Missouri and Arkansas.

Category	Probability of Presence	Hectares of Land
High	0.75-1	315,518.8
Medium high	0.5-0.74	1,923,362.5
Medium low	0.25-0.49	3,896,593.8
Low	0-0.24	44,369,937.5

FIGURES

Figure 2.1. Test (top) and regularized training (bottom) gain for variables that remained in the multivariate model.

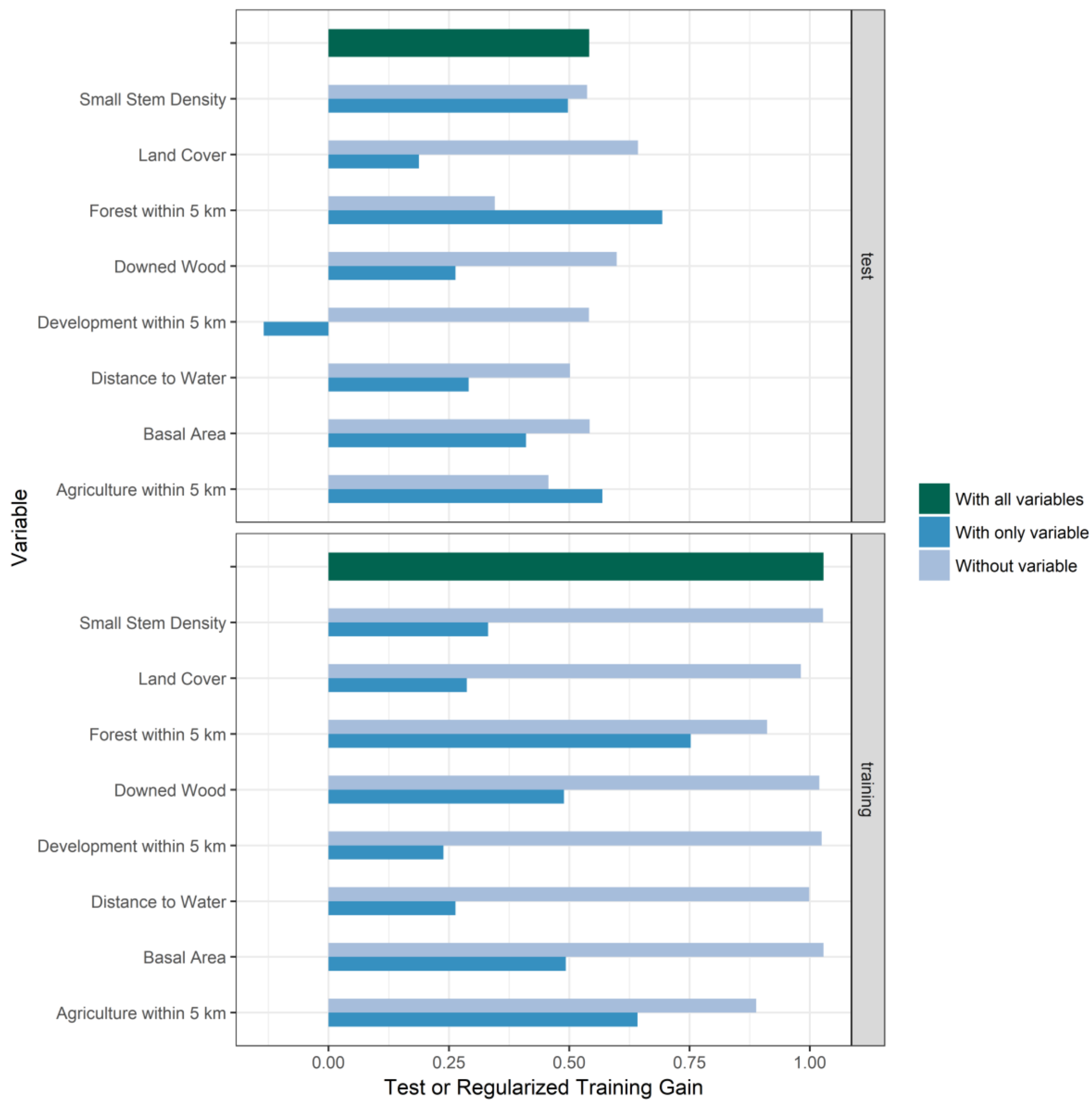


Figure 2.2. Response curves for variables that remained in the multivariate model. Response curves are based on univariate models to prevent collinearity among variables from affecting the relationships shown.

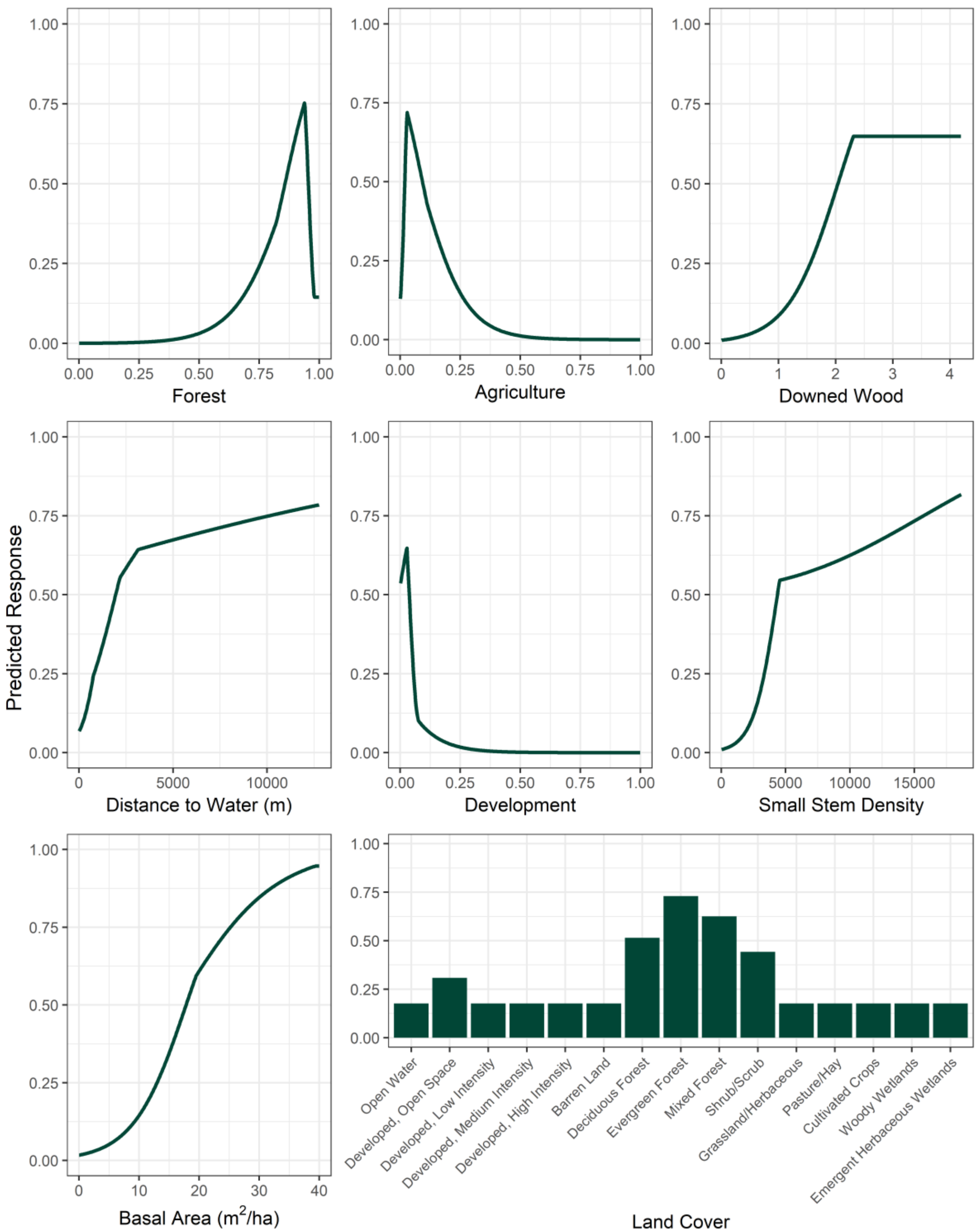
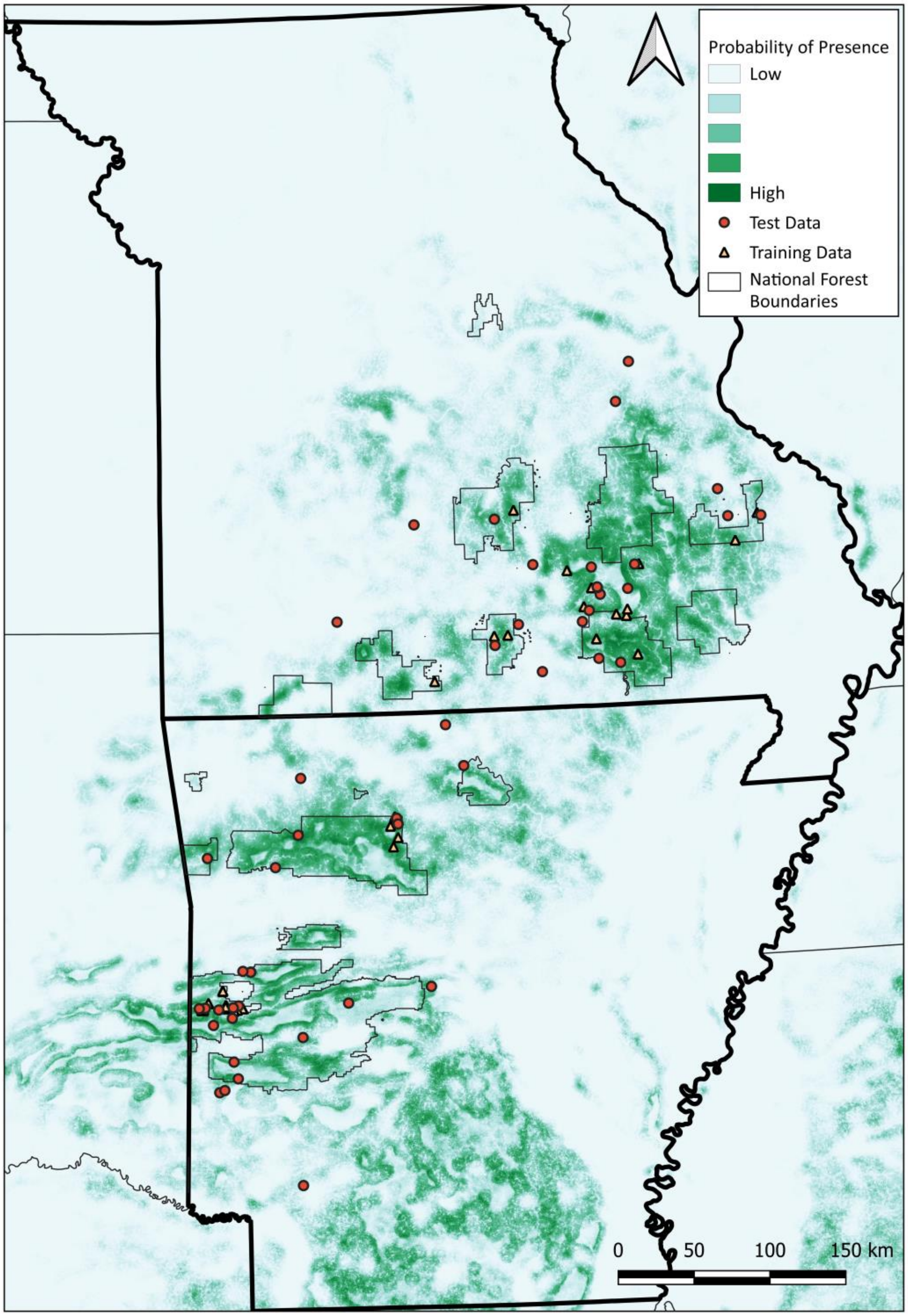


Figure 2.3. Predicted probability of presence of plains spotted skunks in Missouri and Arkansas based on the multivariate species distribution model. Dark green regions represent areas of high probability of presence, while light blue represents areas of low probability of presence.



CHAPTER 3

INFECTION PREVALENCE AND SEVERITY OF SKUNK CRANIAL WORM (*SKRJABINGYLUS CHITWOODORUM*) IN SPOTTED SKUNKS (*SPILOGALE* SPP.)

ABSTRACT

Eastern and western spotted skunks (*Spilogale putorius* and *S. gracilis*) serve as definitive hosts for skunk cranial worm (*Skrjabingylus chitwoodorum*), a metastrongylid nematode that spends its adult stage inhabiting the host cranium. Skunk cranial worm can cause severe damage to the skull in spotted skunks, and this damage is identifiable in preserved specimens. We used 578 spotted skunk skull specimens from six mammal collections to identify patterns in prevalence and severity of skunk cranial worm in spotted skunks across the U.S. and since the late 1800s. We tested for effects of specimen genetic clade, collection year, precipitation, and sex on infection and severity rates. We hypothesized that the midwestern genetic clade (*S. putorius interrupta*), which experienced a range-wide population decline in the mid-1900s, would experience the highest infection and severity rates. We expected precipitation, but not sex to influence infection and severity. Our top models indicated that specimen genetic clade, precipitation in the year prior to specimen collection, and the year prior to specimen collection best predicted prevalence and severity of skunk cranial worm. We suggest the positive association with precipitation is a result of local availability of gastropods, the obligate intermediate host for skunk cranial worm. Our results indicate that skunk cranial worm infects spotted skunks throughout the U.S., but future research on the

impacts of infection on behavior and survival would clarify the extent to which skunk cranial worm affects populations.

INTRODUCTION

Spotted skunks (*Spilogale* spp.) are small, omnivorous mesocarnivores from the Mephitidae family that range broadly across North America. In the 1940s and 1950s, the eastern spotted skunks (*S. putorius*) experienced a range-wide population decline, from which it has yet to recover (Gompper and Hackett 2005). Although it is still present across most of its historical range in the eastern U.S., the species is considered rare, threatened, endangered, or of conservation concern by many states. Further, the plains spotted skunk (*S. putorius interrupta*), a subspecies of the eastern spotted skunk found in the Midwestern U.S., is petitioned to be listed on the U.S. Endangered Species Act (Department of Interior, USFWS 2012). Several factors have been implicated in the decline of eastern spotted skunks, but no clear definitive causes have been confirmed. Potential causes include habitat loss, the onset of intensified agriculture and pesticide use, overharvest, and diseases such as those caused by a variety of viruses and nematodes (Gompper 2017). These factors may continue to threaten the species, but research on the decline remains limited. Additionally, studies have not evaluated patterns in potential threats across eastern and western spotted skunks (*S. gracilis*), despite the utility of understanding how similar species remained resilient in the face of potential threats.

Skunk cranial worm (*Skrjabingylus chitwoodorum*) is a metastrongylid worm found in several skunk species (Hill 1939, Goble and Cook 1942). Larval stages of skunk cranial worm pass in skunk feces where they later encounter gastropods, an obligate intermediate host, and grow into third stage larvae (Lankester and Anderson 1971). Skunks become infected by consuming infected gastropods, such as snails or slugs, or paratenic hosts including mice, shrews, snakes, and frogs (Gamble and Riewe 1982, Hobmaier 1941). Spotted skunks then serve as definitive hosts, where this nematode spends its final developmental stage. Infection by skunk cranial worm can cause damage to the skunk cranium ranging in severity from slight bulging in the frontal cranium to lesions and holes (Kirkland and Kirkland 1983; Figure 3.1). The effect of skunk cranial worm on skunk behavior is poorly understood, but infection may lead to abnormal behavior, resulting in increased likelihood of death either directly caused by the nematode or indirectly as a function of increased predation risk (Goble 1942, Lankester and Anderson 1971, Hughes et al. 2018).

Due to the damage caused by skunk cranial worm, impacts of infection are observable in preserved skulls. Infections by skunk cranial worm in spotted skunks have been reported in Arkansas (Lesmeister et al. 2008), Kansas (Ewing and Hibbs 1966), Oklahoma (Hill 1939), Texas (Tiner 1946), and California (Mead 1963). Two large-scale studies, collectively spanning North America, have assessed patterns of skunk cranial worm infection in spotted skunks (Kirkland and Kirkland 1983, Kirkland and Maldonado 1988). Kirkland and Kirkland (1983) evaluated prevalence of damage by skunk cranial worm in striped skunks (*Mephitis mephitis*), hooded skunks (*M. macroura*), spotted skunks, and hog-nosed skunks (*Conepatus* spp.) in the U.S. and Canada using preserved specimen skulls. Kirkland and Maldonado (1988) used the same method to conduct a similar study on striped skunks, hooded skunks, spotted skunks, and pygmy spotted skunks (*S. pygmaea*) in Mexico. Both studies reported an age-dependent pattern of prevalence wherein adult skunks were primarily infected. Neither study found differences in prevalence based on sex. Kirkland and Kirkland (1983) identified a regional trend wherein skunks from regions with higher precipitation were more likely to be infected by skunk cranial worm. While both studies provide insights on skunk cranial worm ecology, unfortunately in the context of eastern spotted skunk declines, neither study attempted to assess changes in prevalence over time. Further, these studies combine data from eastern and western spotted skunks, which represents a taxonomic and phylogeographic discrepancy.

Beginning in the 1990s (Dragoo et al. 1993), it was increasingly accepted that at least two species of spotted skunks occur in the U.S. (western spotted skunk and eastern spotted skunk). Further, recent genetic analyses (Shaffer et al. 2018; Ferguson et al. 2017; A. Ferguson, personal communication) suggest there are at least four distinct genetic clades of spotted skunk: two which divide the currently recognized eastern spotted skunk and two which divide the currently recognized western spotted skunk (Figure 3.2). Of the eastern spotted skunk clades, the eastern clade includes populations found east of the Mississippi River, while the midwestern clade, which corresponds with the currently recognized plains spotted skunk subspecies, extends west of the Mississippi River to approximately central Texas and the Rocky Mountains. Within the western spotted skunk clades, the western clade occurs throughout the western U.S., excepting southern New Mexico and Arizona, where a southern clade occurs. These unique genetic clades are reflective of regional isolation and adaptations (Shaffer et al. 2018; Ferguson et al. 2017) and are therefore important factors for evaluating spatial components of the disease ecology of spotted skunks.

The objective of this study was to evaluate the infection prevalence and severity of skunk cranial worm in spotted skunks across their North American ranges since the late 1800s. We sought to determine factors influencing skunk cranial worm prevalence and measures of the severity of infection, hypothesizing that both may be influenced by precipitation and the timing of specimen collection (that is, prevalence rates may have increased over time). We hypothesized that different spotted skunk clades would experience different levels of skunk cranial worm infection and severity (the extent of cranial damage, possibly attributed to increased nematode intensity) due to the varying climates in which they live. We expected higher prevalence and severity of skunk cranial worm to be associated with higher average annual precipitation in the collection year or in the previous year, based on the assumption that high precipitation in the year prior to specimen collection might affect the availability of gastropods, and in turn the availability of intermediate stage skunk cranial worms on the landscape (Fuller and Kuehn 1984). Given the lack of sex-dependence in previous studies of skunk cranial worm in striped and spotted skunks, we did not anticipate sex-dependent infections (Gehrt et al. 2010, Kirkland and Kirkland 1983, Kirkland and Maldonado 1988). Finally, we tested support for the hypothesis that skunk cranial worm might have contributed to the population decline of eastern spotted skunks. We anticipated the midwestern and eastern clades would experience overall higher prevalence and severity of skunk cranial worm compared to other clades.

METHODS

Data collection

We examined spotted skunk skulls from six mammal collections housed in the Eastern and Midwestern U.S. (Table 3.1) to determine prevalence and severity of skunk cranial worm. For each specimen, we recorded the collection locality, collection date, and specimen sex using mammal collection records. Given the recent research on spotted skunk taxonomy described above, we used the collection locality to classify each specimen into one of the four spotted skunk genetic clades (i.e., eastern, midwestern, western, or southern; Figure 3.2). Specimens were classified into one of two age classes: juvenile (cranial sutures were present and unfused) and adult (cranial sutures were almost or completely fused). Although age is recognized as an important predictor of the presence of cranial damage (Kirkland and Kirkland 1983, Kirkland and Maldonado 1988), we excluded age from our analyses because it was not possible to explain whether juveniles were uninfected or simply died before exhibiting symptoms (that is, cranial lesions) of infection. Therefore, we used the age classification to remove juvenile specimens from the analysis.

We classified specimens as infected based on the presence of cranial lesions and holes caused by skunk cranial worm infection (Figure 3.1). We ranked damage on a scale of 1 to 3, with 1 being the least damaged and 3 representing the most severe damage to the cranium, following Kirkland and Kirkland (1983) and Kirkland and Maldonado (1988).

Specimens ranked as 1 had minimal damage in the form of small holes on one or both sides of the cranium, while specimens ranked as 3 had extensive damage, characterized by large holes, on one or both sides of the cranium. We photographed all examined skulls and referred to photographs from earlier collection visits to maintain consistency in damage ranking among mammal collections.

We used the Climate at a Glance tool from the National Centers for Environmental Information to download annual precipitation data (NOAA 2018) for the U.S. states represented by the observed specimens. Data were the annual average for each state for the year in which each specimen was collected and the year prior to which each specimen was collected. Precipitation data were available from 1895 through 1981 (the final year represented by the observed specimens); therefore, specimens that were collected prior to 1895 (in years 1885-1894, n = 167) were excluded from the analysis.

Data analysis

We used 578 specimens (Table 3.1) to evaluate prevalence of infection by skunk cranial worm. From the raw data, we calculated a naïve estimate of infection prevalence, or proportion of the observed specimens that were infected, by dividing the number of infected individuals by the total number of individuals for all specimens, by sex, and by clade. We then modeled infection prevalence using a candidate set of generalized linear models using a binomial distribution (Table 3.2). We used specimen clade, sex, and collection year, as well as average annual precipitation in the year and state in which the specimen was collected as explanatory variables (Figure 3.3). In addition, we examined the same set of models with year prior to specimen collection and average annual precipitation from the state in which the specimen was collected in the year prior to the collection year to test the hypothesis that precipitation had a delayed effect on skunk cranial worm prevalence. Finally, we compared candidate models to the null model. We used Akaike's Information Criterion (AIC) to identify the best model from the candidate set. When models were competing (i.e., $\Delta AIC \leq 2$), we explored variables present and used the model with the best predictive variables to predict prevalence of damage from skunk cranial worm for the four spotted skunk genetic clades. Analyses were conducted in R version 3.5.1 using package 'emmeans' to evaluate prevalence, predict prevalence estimates and 95% confidence intervals for each spotted skunk clade, and to test for differences in predicted prevalence among clades using the Tukey method for comparing a family of four estimates. We used packages 'ggplot2' and 'gridExtra' to visualize results.

For the severity of infection analysis, we included only those specimens that showed evidence of infection (n = 277). We calculated naïve estimates of severity, or proportions of individuals that fell within each damage class, from the raw data by dividing the number of individuals at each severity level by the total number of individuals for all specimens, by sex, and by clade. We examined patterns in severity of skunk cranial worm infection using a candidate set of multinomial log-linear regression models because there were three possible severity of infection ranking outcomes (i.e., damage class 1, 2, or 3; Table 3.3). We used the same sets of explanatory variables for the severity models as we used in the prevalence models described above (Figure 3.3).

We used the best model to predict skunk cranial worm severity among the four spotted skunk clades. We used R version 3.5.1 to conduct analyses, using package 'nnet' to test the candidate multinomial log-linear regression models, 'emmeans' to test for differences among variables of interest, and 'ggplot2' and 'gridExtra' to visualize results.

RESULTS

Prevalence

Prevalence of damage caused by infection was 0.48 (95%CI = 0.44-0.52) across all specimens based on the raw data. Naïve calculations revealed that the midwestern clade experienced the lowest infection prevalence (0.25, 95%CI = 0.19-0.3) and the southern and western clades had the highest infection prevalence (southern: 0.73, 95%CI = 0.58-0.89; western: 0.68, 95%CI = 0.62-0.75). The eastern clade had an intermediate infection prevalence (0.48, 95%CI = 0.4-0.56) compared to the other three clades (Figure 3.4). Male and female spotted skunks had similar rates of infection based on naïve estimates (males = 0.5, 95%CI = 0.44-0.55; females = 0.54, 95%CI = 0.47-0.62).

The generalized linear model including the variables specimen genetic clade, precipitation in the year prior to specimen collection, and year prior to specimen collection best predicted skunk cranial worm infection prevalence in spotted skunks (Table 3.2). There were no other competitive models. Based on top model predictions, the midwestern clade had significantly lower infection prevalence than the western clade ($p < 0.0001$) and the southern clade ($p < 0.0001$). The eastern clade also exhibited lower infection prevalence than the western clade ($p = 0.0857$) and the southern clade ($p = 0.0501$). There was no difference in infection prevalence between the two western spotted skunk clades ($p = 0.4399$), or between the two eastern spotted skunk clades ($p = 0.4521$). At 0.28 (95%CI = 0.22-0.36) infection prevalence, the midwestern clade had the lowest predicted prevalence (Figure 3.4). Precipitation in the year prior to specimen collection had a positive effect on infection prevalence ($ß_{prior\_precipitation} = 0.023$; $p = 0.045$), such that years with high average annual precipitation resulted in higher infection prevalence the following year (Figure 3.5). This pattern was observed in each of the spotted skunk clades.

Severity

Among those specimens that were infected with skunk cranial worm, the two western spotted skunk clades experienced more severe infections than the two eastern spotted skunk clades based on naïve calculations. Eastern spotted skunk clades had a higher proportion of individuals within the lowest damage class (eastern clade: 0.64, 95%CI = 0.53-0.76; midwestern clade: 0.57, 95%CI = 0.43-0.7). In contrast, the majority of infections for western spotted skunk clades fell within the highest damage classes (i.e., damage classes 2 and 3; Figure 3.6).

The multinomial log-linear regression model that included specimen genetic clade, precipitation in the year prior to specimen collection, and year prior to specimen collection best predicted severity of the infection. A model which contained the same variables with the addition of specimen sex was also competitive ($\Delta AIC < 2$; Table 3.3), however there were no significant differences among the sexes and damage classes within each clade (Figure 3.7). Thus, we used the model without specimen sex for subsequent predictive analyses. Generally, both western spotted skunk clades experienced more severe infections than both eastern spotted skunk clades based on model predictions (Figure 3.6). Infections in the eastern and midwestern clades were ranked in damage class 1 significantly more often and damage class 3 significantly less often than infections in the western and southern clades. Among the two western spotted skunk clades, the southern clade contained significantly fewer specimens classified as damage class 3 than did the western clade ($p = 0.0051$). Among the two eastern spotted skunk clades, there were no differences in severity of infection (damage class 1: $p = 0.61$; damage class 2: $p = 0.93$; damage class 3: $p = 0.99$). For all clades, more precipitation in the year prior to specimen collection resulted in an increase in infections ranked as damage class 3, a decrease in infections ranked as damage class 2, and no change in infections ranked as damage class 1 (Figure 3.8). The increase in infections ranked as damage class 3 was especially prominent for western spotted skunk clades.

DISCUSSION

We evaluated infection prevalence and severity of skunk cranial worm across the four spotted skunk genetic clades present in the U.S. and determined that genetic clade, precipitation in the year prior to specimen collection, and year prior to specimen collection were the most important drivers of both metrics. Our results support the hypothesis that infection prevalence and severity vary among genetic clades. However, across all specimens, infection mean prevalence was lower (0.48) than other reports (0.838 for male and 0.807 for female spotted skunks throughout the U.S. and Canada [Kirkland and Kirkland 1983]; 0.849 for spotted skunks in Mexico [Kirkland and Maldonado 1988]). Rather, our prevalence estimates were more similar to regionally focused reports from eastern spotted skunks (0.34 in the present study; 0.21 in Arkansas [Lesmeister et al. 2008]; 0.32 in Kansas [Ewing and Hibbs 1966, but note this study combined data for eastern spotted skunks and striped skunks]) and higher compared to another report from western spotted skunks (0.69 in the present study; 0.429 in California [Mead 1963]).

Contrary to our hypothesis, the midwestern and eastern clades experienced the lowest levels of infection prevalence and infections were less severe than in western spotted skunk clades. At the surface, our results suggest skunk cranial worm was likely not a major contributing factor to the range-wide population decline of eastern spotted skunks. However, the effects of infection on morbidity and survival in skunks remains unknown, and low infection prevalence and severity may actually be associated with higher levels of mortality if infected midwestern and eastern hosts are more likely to die prior to reaching damage class 3. Differing hypothetical outcomes of *Skrjabingylus*-*Spilogale* interactions might be caused by a variety of processes that differ in their strengths among the clades, including spatially distinct age-intensity relationships, difference in host body condition among clades, and spatial variance in the virulence of *Skrjabingylus* in spotted skunks (Wilson et al. 2002). Metrics of *Skrjabingylus* infection may appear low, when in reality, skunks did not survive long enough to develop classic signs of infection (i.e., damage to the skull). Research targeting survival in relation to skunk cranial worm infection would shed more light onto the potential contribution the disease had on the decline of eastern spotted skunks.

Our findings on the important role of precipitation as a driving factor in infection prevalence and severity of skunk cranial worm build on the findings of earlier studies. Kirkland and Kirkland (1983) illuminated a positive association between skunk cranial worm severity and regional precipitation. The relationship between precipitation and infection by *Skrjabingylus* is likely a result of improved environmental conditions for gastropods, which are obligate intermediate hosts (Dubay et al. 2014, Fuller and Kuehn 1984, Kirkland 1975). Transmission of *Skrjabingylus* may also be a function of first stage larval survival outside of a host in feces, which is likely improved with increased environmental moisture (Hansson 1974). In contrast, studies from Mexico and Texas did not observe a relationship between spotted skunk skull damage and precipitation, but suggested that milder winters may facilitate larval survival in feces, resulting in sustained or higher levels of infection prevalence, despite the drier climate (Kirkland and Maldonado 1988, Hughes et al. 2018).

Host sex was not an influencing variable in skunk cranial worm infection prevalence or severity, and while this finding is in agreement with other skunk studies, it does not agree with the literature on *Skrjabingylus* patterns of infection in mustelids. Previous research on spotted, striped, hog-nosed, and hooded skunks has repeatedly identified no differences in infection prevalence, severity, or intensity among the sexes (Kirkland and Kirkland 1983, Kirkland and Maldonado 1988, Fuller and Kuehn 1984, Gehrt et al. 2010), with the exception of the findings of Kirkland (1975) who reported female striped skunks exhibited higher severity in the form of more extreme skull damage than male striped skunks. In contrast, mustelids infected by *S. nasicola* tend to show higher prevalence and intensity in males (Hansson 1968, Santi et al. 2006, Dubay et al. 2014, but see Hawkins et al. 2010). Collectively this body of work suggests that *Skrjabingylus*-host interactions for mustelids and mephitids may differ fundamentally. Nonetheless, with respect to the possible impacts of *Skrjabingylus* on various skunk species, it is worth recognizing that similar infection and severity rates among males and females may mask a potentially more important question: whether infection results in differing consequences among the sexes (i.e., reproductive and survival impacts).

Age apparently plays a role in infection prevalence, intensity, and severity for mephitids and mustelids, but we did not include it as an explanatory variable in any of our models because we could not discern whether juveniles were uninfected or died (i.e., were collected for museum collections) before exhibiting signs of infection. Using fresh specimens may be a more accurate method for evaluating the effects of age on skunk cranial worm infection. Gehrt et al. (2010) used necropsy to assess macroparasite intensity in striped skunks and found no relationship between intensity and age. In contrast, Hughes et al. (2018) used necropsy to determine that striped skunk age significantly and positively influenced the chances of an infection by skunk cranial worm. Kirkland and Maldonado (1988) did not use necropsy to evaluate infection, but posit that the higher infection rates they observed in older skunks could result from higher amounts of paratenic hosts in the diet of adult skunks. Their hypothesis was supported by a mustelid study that found no infections in juveniles that still had milk teeth (Hansson 1968). Future research on *Skrjabingylus* infections should seek to incorporate age classes where possible, especially when whole carcasses are available such that presence of cranial worms can be confirmed independently of skull damage. As with the question of whether hosts of different sexes respond to infection in the same way, the question of whether age classes are differently affected by infection remains unanswered.

Each of the spotted skunk clades had relatively high prevalence of skunk cranial worm. Given that this nematode causes significant osteologic damage to the cranium, managers should recognize the contributory potential for population-scale effects of *Skrjabingylus* infection on spotted skunks. Few studies have examined how infection by skunk cranial worm influences skunk behavior and vital rates. Several studies have suggested infection could result in neurological changes that alter behavior in striped skunks (Goble 1942, Lankester and Anderson 1971, Hughes et al. 2018). In spotted skunks, which are relatively short-lived (Lesmeister et al. 2010), such behavioral changes may reduce skunk ability to reproduce, rear young, and survive, which could result in population-level effects, especially in regions where infection rates are high. Coupled with other sources of mortality, skunk cranial worm could additively and negatively affect populations.

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TABLES

Table 3.1. Sample size of eastern spotted skunk (*Spilogale putorius*) and western spotted skunk (*S. gracilis*) specimens examined. Genetic clades for each of the two currently recognized species are identified below the currently recognized species name. Count of specimens is broken down by sex (F represents female, M represents male, and UN represents unknown sex). Specimen catalog numbers are available in Appendix 3.1.

Mammal Collection	**Sample Size**			
	S. putorius		***S. gracilis***	
	Eastern Clade	**Midwestern Clade**	**Western Clade**	**Southern Clade**
University of Missouri	F: 0 M: 0 UN: 0	F: 2 M: 3 UN: 0	F: 0 M: 0 UN: 0	F: 0 M: 0 UN: 0
University of Kansas	F: 6 M: 10 UN: 1	F: 19 M: 39 UN: 71	F: 0 M: 0 UN: 0	F: 0 M: 0 UN: 0
Field Museum of Natural History	F: 4 M: 11 UN: 6	F: 3 M: 5 UN: 1	F: 0 M: 0 UN: 0	F: 0 M: 0 UN: 0
Illinois Natural History Survey	F: 0 M: 0 UN: 0	F: 1 M: 0 UN: 0	F: 0 M: 0 UN: 0	F: 0 M: 0 UN: 0
American Museum of Natural History	F: 10 M: 10 UN: 0	F: 13 M: 13 UN: 0	F: 3 M: 7 UN: 3	F: 1 M: 5 UN: 1
National Museum of Natural History	F: 18 M: 52 UN: 8	F: 7 M: 27 UN: 4	F: 85 M: 94 UN: 12	F: 5 M: 16 UN: 2
TOTAL	**F: 38 M: 83 UN: 15**	**F: 45 M: 87 UN: 76**	**F: 88 M: 101 UN: 15**	**F: 6 M: 21 UN: 3**
	136	**208**	**204**	**30**
	578			

Table 3.2. Candidate generalized linear models for skunk cranial worm infection prevalence, where *k* is the number of predicted parameters in the model. The response variable, infection, is listed on the left side of the '~'and the explanatory variables used in the model are listed on the right side of the '~'.

Model	**k**	**AIC**	**ΔAIC**
infection~prior_year+clade+prior_precipitation	6	683.31	0
infection~prior_year+clade+prior_precipitation+sex	8	687.2	3.9
infection~clade+prior_precipitation	5	687.48	4.2
infection~year+clade+precipitation	6	712.5	29.2
infection~clade+precipitation	5	716.18	32.9
infection~year+clade+precipitation+sex	8	716.45	33.14
infection~1	1	802.28	118.9

Table 3.3. Candidate multinomial log-linear regression models for skunk cranial worm infection severity, where *k* is the number of predicted parameters in the model. The response variable, severity, is listed on the left side of the '~' and the explanatory variables used in the model are listed on the right side of the '~'.

Model	k	AIC	ΔAIC
severity~prior_year+clade+prior_precipitation	12	529.51	0
severity~prior_year+clade+prior_precipitation+sex	16	531.46	1.95
severity~clade+prior_precipitation	10	535.44	5.93
severity~year+clade+precipitation	12	557.51	28
severity~year+clade+precipitation+sex	16	560.57	31.06
severity~clade+precipitation	10	564.13	34.62
severity~1	2	594.94	65.43

FIGURES

Figure 3.1. Skull damage in spotted skunks caused by an infection of *Skrjabingylus chitwoodorum*. Damage classes are 0 (a; no damage), 1 (b; minimal damage), 2 (c; intermediate damage), and 3 (d; severe damage) based on guidance from Kirkland and Kirkland (1983).

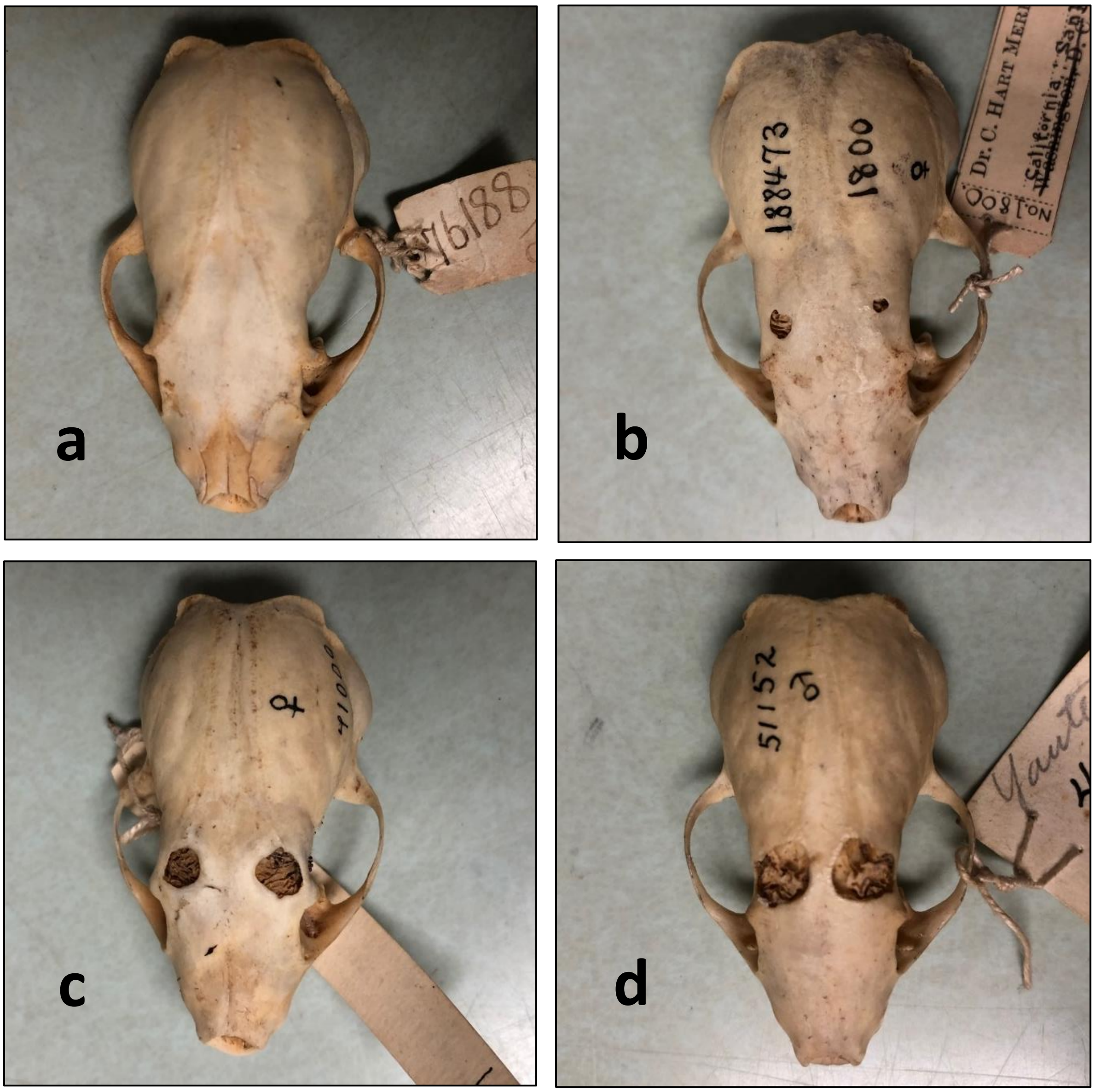


Figure 3.2. Relative sample size of adult specimens from four spotted skunk genetic clades within their states of origin.

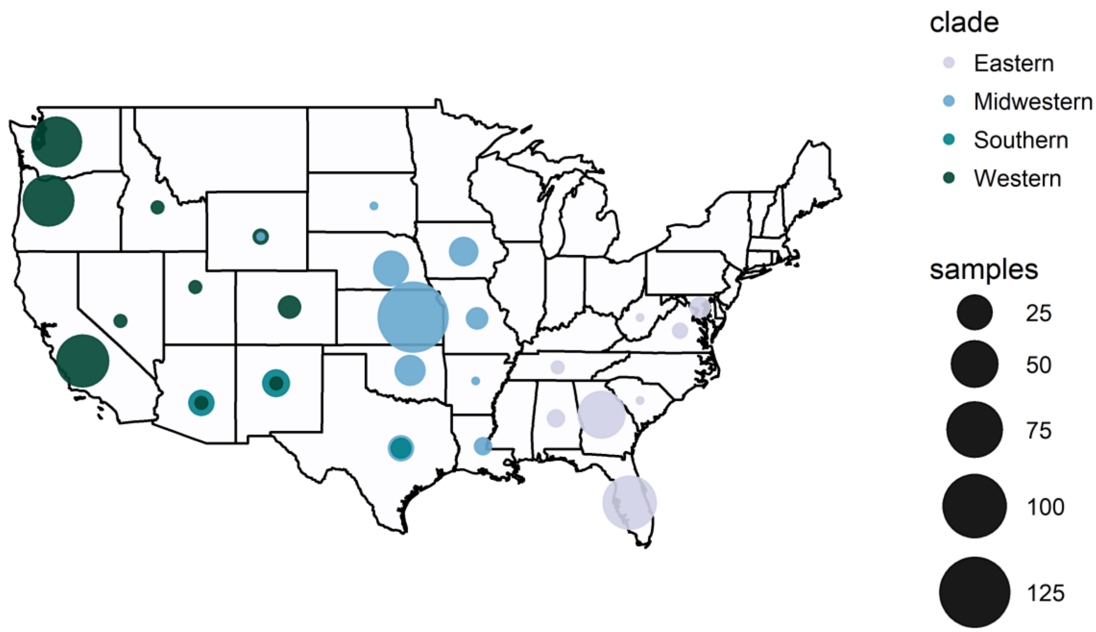


Figure 3.3. Histograms of explanatory variables precipitation, precipitation in the year prior to specimen collection, and year of specimen collection. Colors represent genetic clade.

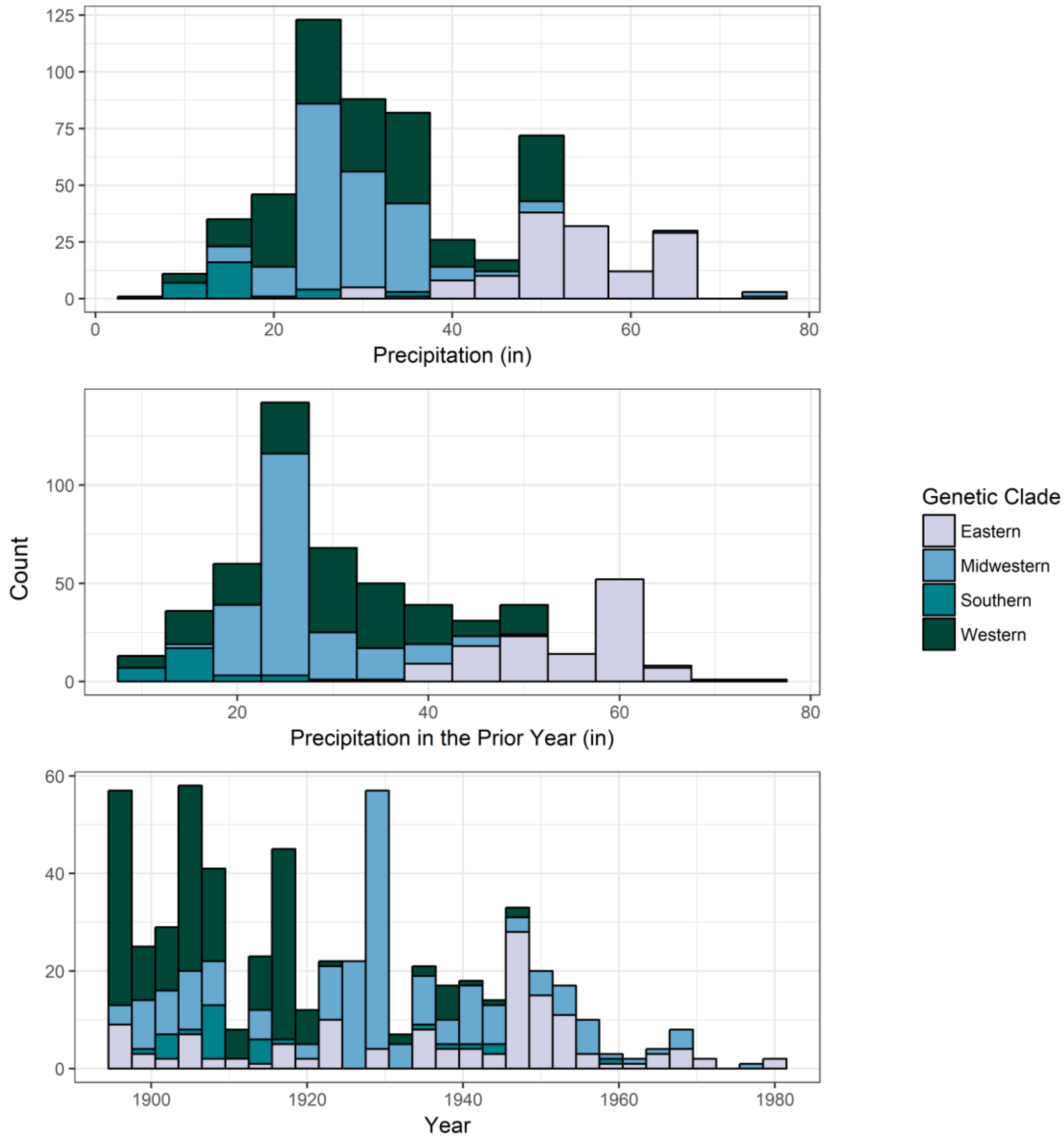


Figure 3.4. Naïve (light blue) and top model (dark green) predictions for skunk cranial worm prevalence in four spotted skunk genetic clades. Error bars represent 95% confidence intervals.

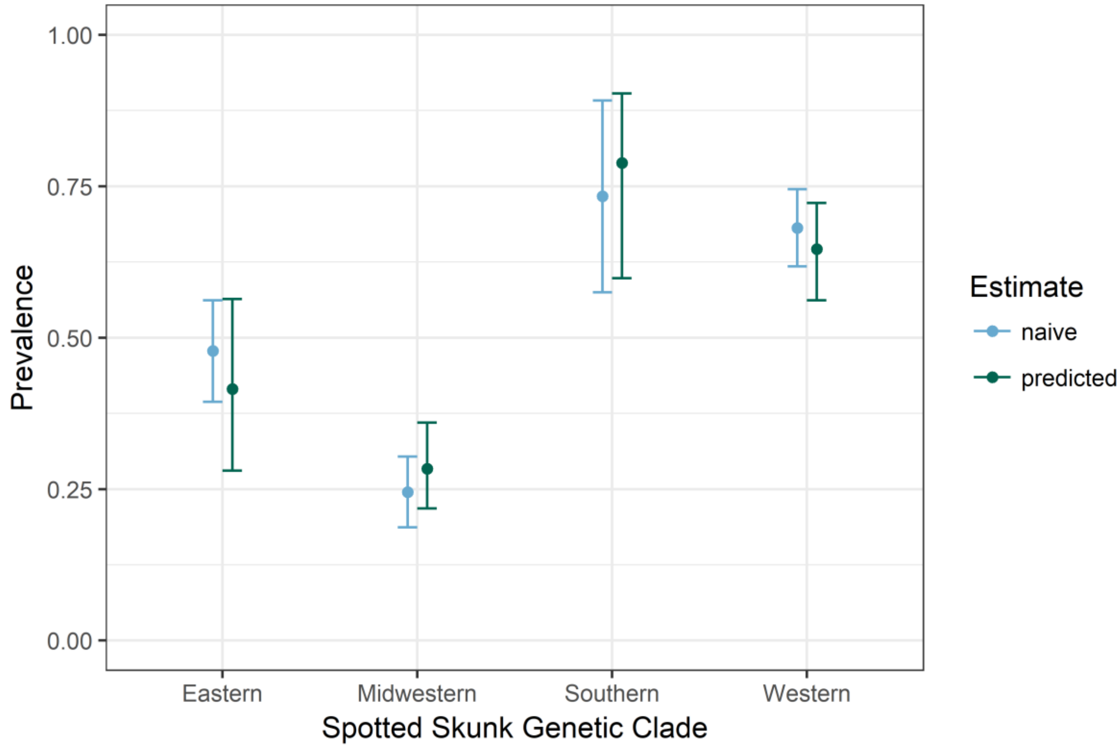


Figure 3.5. Top model predictions for skunk cranial worm prevalence across precipitation levels in four spotted skunk genetic clades. Error ranges represent 95% confidence intervals.

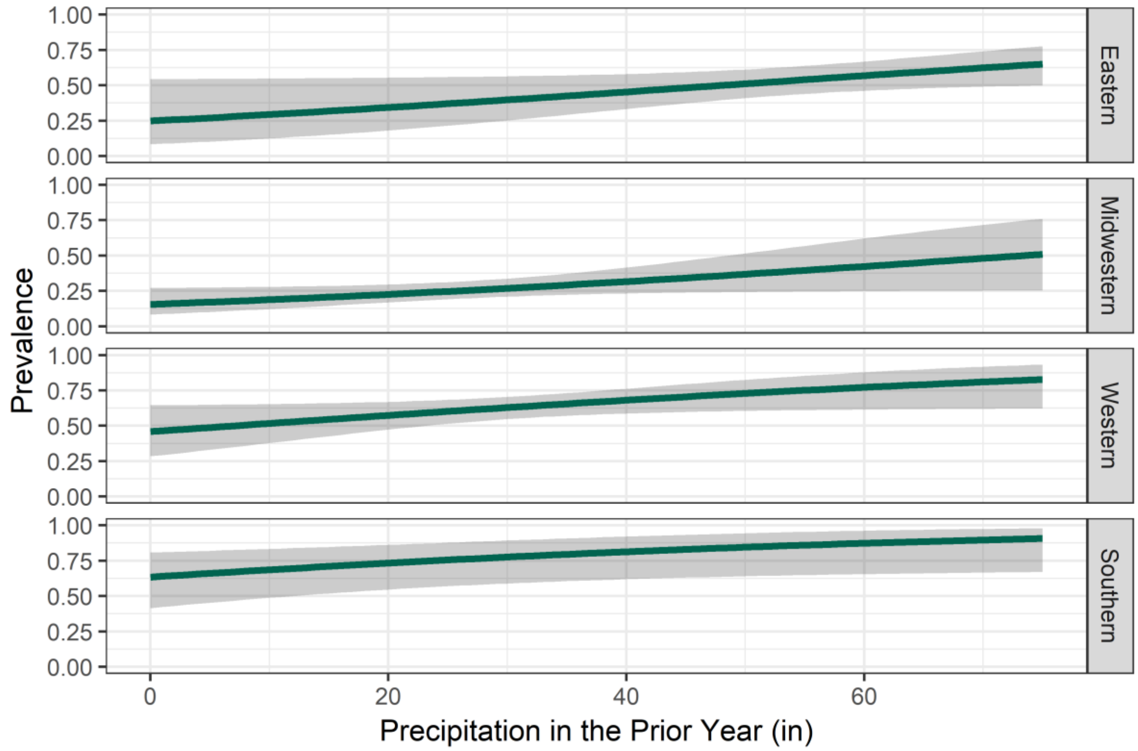


Figure 3.6. Top model predicted (blue) and naïve estimates (green) for skunk cranial worm severity in four spotted skunk genetic clades. A severity ranking of 1 represents low severity, while a ranking of 3 represents high severity.

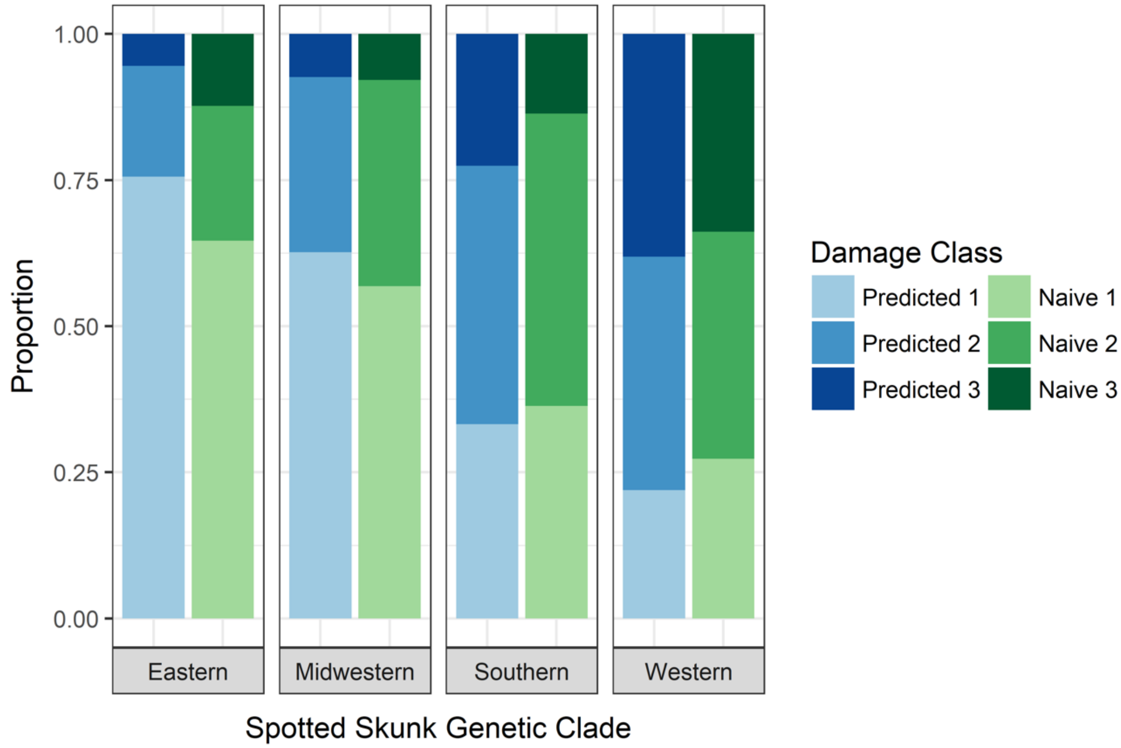


Figure 3.7. Competing model predictions for skunk cranial worm severity among female, male, and combined (female, male, and unknown) sex classes in four spotted skunk genetic clades. Damage class is listed on the right side of the graph. A severity ranking of 1 represents low severity, while a ranking of 3 represents high severity. Error bars represent 95% confidence intervals.

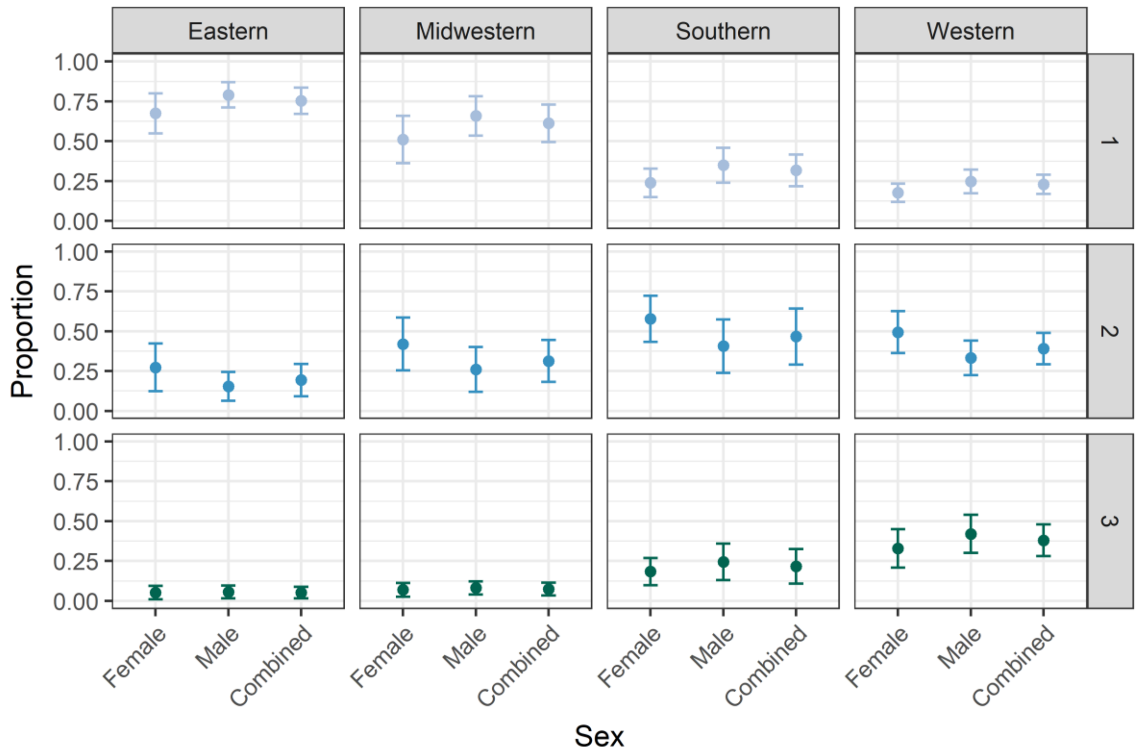
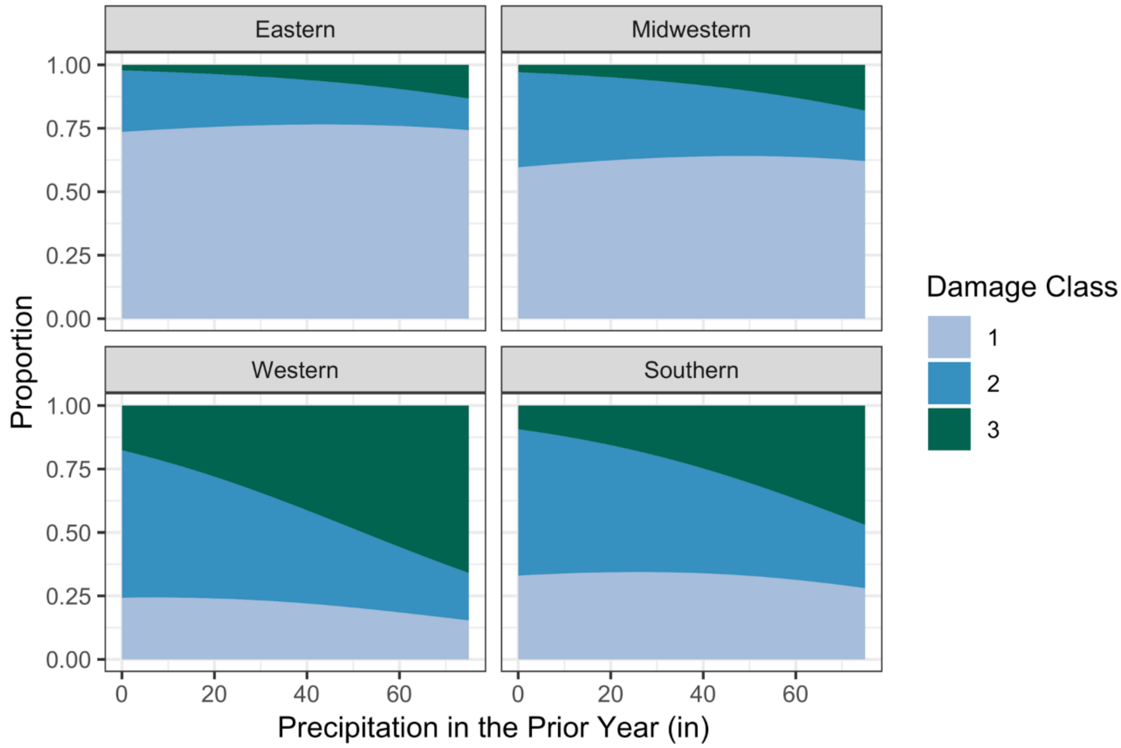


Figure 3.8. Top model predictions for skunk cranial worm severity across precipitation levels in four spotted skunk genetic clades.



SUPPLEMENTAL INFORMATION

Appendix 3.1. Collection and specimen identification number for 578 specimens used to evaluate prevalence and severity of skunk cranial worm in spotted skunks. Collection codes are as follows: AMNH (American Museum of Natural History, New York City, NY), FMNH (Field Museum of Natural History, Chicago, IL), INHS (Illinois Natural History Survey, Urbana-Champagne, IL), KU (University of Kansas at Lawrence, Lawrence, KS), MU (University of Missouri, Columbia, MO), and USNM (United States National Museum of Natural History, Washington, D.C.).

AMNH: M-100347 , M-120923 , M-120924 , M-120925 , M-120926 , M-121109 , M-121586 , M-121587 , M-121588 , M-123037 , M-123217 , M-123218 , M-123219 , M-123221 , M-123796 , M-123924 , M-135061 , M-135570 , M-135961 , M-135962 , M-136421 , M-137373 , M-137374 , M-138391 , M-138392 , M-142073 , M-142737 , M-143824 , M-144012 , M-144127 , M-144923 , M-144924 , M-148592 , M-149851 , M-15722 , M-164710 , M-164855 , M-166422 , M-166423 , M-166424 , M-166425 , M-166426 , M-166427 , M-169983 , M-169985 , M-171369 , M-174312 , M-185351 , M-188955 , M-21537 , M-243440 , M-243441 , M-243442 , M-243443 , M-255656 , M-255657 , M-30215 , M-31770 , M-35205 , M-35206 , M-35207 , M-35208 , M-35209 , M-35210 , M-40853 , MO-8334

FMNH: 6875 , 6876 , 6877 , 6878 , 7734 , 7735 , 10959 , 10960 , 10961 , 15046 , 15050 , 15052 , 15053 , 21920 , 51373 , 199837 , 199838 , 199839 , 199840 , 199841 , 199842 , 199843 , 199844 , 199845 , 199846 , 199847 , 199848 , 199849 , 199850 , 199852

INHS: 3635

KU: 1306 , 1307 , 2040 , 2517 , 2631 , 2635 , 3100 , 3556 , 3578 , 3579 , 3580 , 3581 , 3583 , 3835 , 3966 , 4103 , 4130 , 4131 , 4132 , 4680 , 4757 , 4820 , 4976 , 5581 , 6284 , 6295 , 6296 , 6298 , 6299 , 6303 , 6305 , 6306 , 6308 , 6309 , 6311 , 6317 , 6321 , 6323 , 6327 , 6328 , 6331 , 6333 , 6335 , 6338 , 6347 , 6353 , 6355 , 6357 , 6359 , 8405 , 9020 , 9021 , 9022 , 9023 , 9024 , 9027 , 9030 , 9031 , 9032 , 9033 , 9035 , 9037 , 9039 , 9040 , 9041 , 9043 , 9044 , 9045 , 9046 , 9047 , 9049 , 9050 , 9051 , 9053 , 9054 , 9055 , 9058 , 9059 , 9062 , 9063 , 9064 , 9065 , 9067 , 9068 , 9069 , 9070 , 9073 , 9074 , 9076 , 9077 , 9080 , 9081 , 9085 , 9086 , 9087 , 9088 , 9089 , 9090 , 10332 , 12120 , 13003 , 13460 , 13545 , 14236 , 14237 , 14605 , 14606 , 14736 , 27069 , 29340 , 39186 , 41553 , 54326 , 54330 , 54331 , 54334 , 54335 , 54336 , 54339 , 54340 , 54342 , 64559 , 74492 , 79425 , 109832 , 112840 , 119637 , 134413 , 151899 , 151900 , 151901 , 151902 , 151903 , 151904 , 151905 , 151906 , 151908 , 151909 , 151911 , 151912 , 151913 , 154221 , 168980 , 168981 , 168983 , Zool. 342

MU: 530 , 982 , 2961 , 4355 , 4356

USNM: 32809 , 70304 , 70305 , 70306 , 70307 , 70308 , 70309 , 70310 , 70311 , 71294 , 75643 , 75644 , 75645 , 76232 , 76450 , 76451 , 76452 , 76453 , 76454 , 76455 , 76456 , 76475 , 76476 , 76477 , 76478 , 76479 , 76480 , 77120 , 77121 , 77122 , 77126 , 77127 , 77128 , 77129 , 77130 , 77131 , 79378 , 80757 , 83861 , 83862 , 83863 , 86429 , 87051 , 87052 , 87159 , 87320 , 87321 , 87936 , 89551 , 91430 , 91590 , 93283 , 93295 , 93296 , 93297 , 93298 , 93299 , 93300 , 93301 , 97032 , 97033 , 98561 , 99580 , 99892 , 99910 , 100099 , 100100 , 100101 , 101332 , 101988 , 102562 , 107222 , 108759 , 108784 , 109074 , 115947 , 116348 , 117208 , 118427 , 118428 , 119860 , 119963 , 119964 , 120201 , 126326 , 126327 , 126339 , 126340 , 126370 , 126372 , 126643 , 126644 , 126726 , 126727 , 130942 , 130978 , 132434 , 132466 , 132806 , 133021 , 133095 , 135090 , 135091 , 135474 , 135475 , 135476 , 135477 , 135478 , 135479 , 135488 , 135489 , 135490 , 135904 , 135906 , 140205 , 140206 , 140207 , 140208 , 140435 , 140559 , 142014 , 142015 , 142664 , 142665 , 142666 , 142668 , 146266 , 146267 , 146268 , 146269 , 146270 , 146290 , 146291 , 146375 , 146376 , 146377 , 146378 , 147252 , 147427 , 147428 , 147434 , 147629 , 147751 , 148716 , 148717 , 148718 , 150539 , 150540 , 151412 , 151413 , 151416 , 151417 , 151418 , 156619 , 156638 , 156639 , 156640 , 157043 , 157065 , 157117 , 157118 , 157119 , 157120 , 157121 , 157122 , 157505 , 157857 , 157858 , 157859 , 158875 , 159234 , 159426 , 159525 , 160115 , 160662 , 161111 , 161628 , 166812 , 167702 , 167940 , 171336 , 171338 , 177423 , 177852 , 178478 , 179167 , 181311 , 188461 , 202935 , 203904 , 204293 , 205236 , 205237 , 205831 , 206001 , 206365 , 207155 , 207156 , 207188 , 207382 , 207675 , 208089 , 208090 , 210534 , 211225 , 213137 , 214348 , 215139 , 215140 , 215372 , 216129 , 221695 , 221696 , 222229 , 222342 , 222343 , 222720 , 223013 , 223100 , 223584 , 223585 , 223802 , 224031 , 224032 , 224033 , 224097 , 224557 , 225091 , 225134 , 228059 , 228060 , 228061 , 228062 , 228063 , 228085 , 228086 , 228254 , 228255 , 228372 , 228382 , 228514 , 228801 , 228822 , 230109 , 230110 , 230638 , 231137 , 231607 , 231608 , 231609 , 233627 , 233628 , 233720 , 241805 , 241952 , 243510 , 243511 , 244349 , 244691 , 245463 , 245507 , 253844 , 255388 , 261650 , 261651 , 261841 , 261842 , 262214 , 263375 , 263620 , 263681 , 264619 , 264642 , 265298 , 271982 , 273393 , 273394 , 273968 , 273969 , 274325 , 274327 , 274328 , 274329 , 274330 , 274331 , 274931 , 286676 , 287708 , 287709 , 287710 , 287711 , 287712 , 287713 , 287714 , 287715 , 287716 , 287717 , 287718 , 287719 , 287720 , 287721 , 287722 , 287723 , 287725 , 287726 , 287727 , 287728 , 287729 , 287730 , 287732 , 287733 , 287734 , 287735 , 287737 , 287738 , 287740 , 348089 , 348090 , 392846 , 392848 , 392849 , 392850 , 392851 , 392852 , 503395 , 530289 , 564280 , 564281 , 589250 , 589252