SPATIAL ECOLOGY OF A VULNERABLE SPECIES: HOME RANGE DYNAMICS, RESOURCE USE, AND GENETIC DIFFERENTIATION OF EASTERN SPOTTED SKUNKS IN CENTRAL APPALACHIA

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Technical Abstract

The spatial distribution of a species is not random or uniform across all landscapes, nor is it independent of resource availability and risk of predation. A key aspect in the study of wildlife ecology is understanding how a species evaluates its surroundings and selects habitat that offers advantages to survival and reproductive success. In theory, an individual should select habitat that offers adequate resources to meet its biological requirements and allows it to adjust its use of resources based on a variety of abiotic and biotic habitat factors. Relationships between wildlife populations and habitat characteristics are difficult to assess, yet identification and characterization of these relationships can improve delineation of limiting habitat factors needed for effective conservation and management. The eastern spotted skunk (*Spilogale putorius*) is a small Mephitid (weasel family) that was once a fairly common furbearer throughout the central and southern United States, with annual range-wide harvests of over 100,000 individuals. In Virginia, the spotted skunk is classified as vulnerable, and anecdotal evidence suggests that this species has been rare or largely absent from the central and southern Appalachians over the last two decades. Limited knowledge of spotted skunk habitat associations in Virginia dictate the need for research on this species of concern. The aim of this study was to determine the associations of environmental characteristics ofwith spotted skunk habitat selection and genetic differentiation. I assessed first-order habitat selection by conducting an analysis of predicted occurrence at the landscape scale. Next, I assessed second- and third-order selection using resource utilization functions to determine habitat selection among, and within, home ranges.

Further, I assessed fourth-order selection by identifying microhabitat selection and resource use at spotted skunk den site. Finally, I evaluated genetic diversity and population structure of spotted skunks in the Appalachian Mountains. I found that spotted skunk habitat is distributed in small, spatially disjunct patches and that movement, home range size, and resource selection are impacted by availability of habitat and the isolation and/or fragmentation of this suitable habitat. I found overall low genetic diversity and evidence of inbreeding within populations and geographic patterns of genetic differentiation with distinct subpopulations isolated by unsuitable landscape characteristics. Application of these results will contribute to more effective conservation of eastern spotted skunks throughout the Appalachian Mountains.

SPATIAL ECOLOGY OF A VULNERABLE SPECIES: HOME RANGE DYNAMICS, RESOURCE USE, AND GENETIC DIFFERENTIATION OF EASTERN SPOTTED SKUNKS (*SPILOGALE PUTORIUS*) IN CENTRAL APPALACHIA

Emily D. Thorne

Popular Abstract

Wildlife species do not occur throughout the landscape randomly or evenly, but rather choose their locations based on the availability of food, cover, and water. A key aspect in the study of wildlife ecology is understanding how a species chooses its natural surroundings and selects habitat that allows it to survive and reproduce. In theory, an animal should choose a location that offers enough resources, such as food, cover, and water to meet its biological needs and allows it to adjust based on access to these resources. By understanding the how the availability of these resources influences an animal's choice of location, wildlife managers can better determine the need for protection or management of the species in a given area. The eastern spotted skunk (*Spilogale putorius*) is a small, weasel-like animal that was once fairly common throughout the central and southern United States. In Virginia, the spotted skunk is a protected species, and experts suggest that it has been rare in the central and southern Appalachians for over several decades. Because there is limited knowledge of spotted skunk habitat needs in Virginia, research on this species is necessary. The goals of this study were to determine the type of habitat in which spotted skunks are choosing and how it affects the species genetic makeup. First, I determined in which general areas spotted skunks exist in Virginia. Next, I determined that the habitat for spotted skunks includes forests with ample bushy plants and rocks to hide from predators. I then examined the genetics of spotted skunks to determine relatedness among individuals and whether inbreeding was occurring. Finally, I compared the relatedness of pairs of spotted skunks to the distance between their locations and found that

skunks that are located on the same mountain range are more closely related to each other than to skunks on different mountain ranges. These results will inform wildlife managers on how to provide spotted skunks with the habitat resources they need to survive and reproduce successfully, thus promoting skunk conservation.

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Introduction

Spatial distribution of a species is not random or uniform across landscapes, nor is it independent of resource availability and risk of predation (Brown 1988). Habitat selection is typically influenced in five ways by: (1) the need to consume food and water resources, (2) the amount of space required to live, i.e., access those resources, (3) the amount of interaction with conspecifics and sympatric species, (4) dynamic environments, and (5) the need to reproduce (Morris 2003). Additionally, the nature of habitat selection is hierarchical with four orders of selection. First-order selection describes a species' physical or geographical range. Within the geographic range, home range of an individual or social group is described by second-order selection. Third-order selection describes the use of habitat components within a home range and the procurement of resources from those habitat components is described by fourth-order selection (Johnson 1980). Understanding what factors influence habitat selection and use by a species is vital to its conservation and management across almost all taxa of interest (Scott et al. 1993).

Relative to this, body morphology has been shown to strongly influence resource selection patterns in mammals (Bolnick and Doebeli 2003). Differences in resource use within a species may be influenced by sexual dimorphism as a mechanism to avoid intraspecific competition between males and females with overlapping home ranges (Slatkin 1984, Bolnick and Doebeli 2003, Bolnick et al 2003, Meiri et al 2014). Mammals at higher latitudes display a general trend toward larger body size and greater sexual dimorphism (Ferguson and Lariviére 2008). For small- and medium-sized mammalian carnivores, a tubular-shaped body may enable a species to access small spaces such as rock crevices and tree cavities in search of prey or for protection from predators (Lariviére 2003). However, a high surface area to volume ratio may make small species vulnerable to extreme temperatures, especially in regions with dynamic seasonal weather such as in the central and southern Applachian Mountains. Small carnivores, such as mustelids and mephitids, may have limited foraging opportunities in severe weather conditions in the non-growing season. These species may partially compensate for elevated heat loss during cold seasons by modifying their habitat use and selecting areas closer to food sources to increase foraging efficiency, a tradeoff that could potentially increase risk of predation.

The eastern spotted skunk (*Spilogale putorius*) is a small mephitid that was once a fairly common furbearer throughout the central and southern United States, with annual range-wide harvests of over 100,000 individuals (Gompper and Hackett 2005). The eastern spotted skunk has a global rank of Vulnerable (Compper and Jachowski 2016) and is listed as Vulnerable, Imperiled, or Critically Imperiled in most of the eastern and southern states throughout its historic range (Chapman 2007). Perceived population declines since the 1980s have been attributed to the loss of oldfield habitats from more intensive agricultural practices (clean farming) or the maturation of early successional forests following land abandonment in the Midwest (Gomper and Hackett 2005, Lesmesiter et al. 2009). In addition to habitat change, competition with sympatric mesocarnivore species (i.e., striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and coyotes (*Canis latrans*)) has increased as a result of declining trapping pressures on those species and subsequent population increases. Lastly a series of rabies outbreaks occurred throughout its range beginning in the 1970s. All may have contributed to population declines (Chapman 2007, Lesmeister et al. 2008, Lesmeister et al. 2010).

In Virginia, the spotted skunk is classified as Vulnerable, and anecdotal evidence suggests that this species has been rare or largely absent from the central and southern

Appalachians over the last two decades (C. Waggy, West Virginia DNR, pers. comm.; N. Castleberry, University of Georgia MNH, pers. comm.). Very little is known about the spatial distribution or ecology of eastern spotted skunks in the region, though a loss of early-successional habitat, similar to that in the Midwest, may have potentially led to population declines in Virginia and West Virginia (Johnson 1992, Griffith and Widmann 2003). Recently, during an ongoing multi-state golden eagle (*Aquila chrysaetos*) wintering distribution study in the Appalachians using baited camera stations, spotted skunks have been recorded in West Virginia in the Peters-Potts Mountain, Shenandoah Mountain, and Great North Mountain areas along the Virginia border and within Shenandoah National Park in Virginia (J. Rodrigue, U.S. Forest Service, unpubl. data). These records, along with data from Virginia Department of Game and Inland Fisheries, suggest that eastern spotted skunk populations still persist over a large portion of the Blue Ridge and Ridge and Valley portions of the central Appalachians in Virginia and west into West Virginia to the Allegheny Mountains and Plateau.

Spotted skunk populations have been associated with early-successional shrub-scrub, oldfield, and young shortleaf pine (*Pinus echinata*) in Arkansas (Lesmeister et al. 2009) and with rosebay rhododendron (*Rhododendron maximum*) thickets near high-elevation emergent rock outcrops in the Blue Ridge region of Tennessee (Reed and Kennedy 2000). In the central and southern Appalachians, spotted skunks have been incidentally captured near emergent rock during Allegheny woodrat (*Neotoma magister*) monitoring (Webster et al. 1985; C. Stihler, West Virginia DNR, pers. comm.) and in a high-elevation red spruce (*Picea rubens*) forest during Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) trapping surveys (Diggins et al. 2015). It is currently unknown whether emergent rock and ericaceous shrub growth represent primary spotted skunk habitat in the Appalachians, whether these habitat features offer a structural surrogate for oldfield or clearcut brush or whether spotted skunks are selecting areas with reduced competition from competing species (Lesmeister et al. 2012). Thus, the goal of my first chapter was to assess the contempory spatial distribution of eastern spotted skunks throughout the George Washington and Jefferson National Forests using a site occupancy analsysis. Further, in chapter two, I assessed the spatial co-occurrence and temporal avoidance of eastern spotted skunks and sympatrically occurring mesopredator species.

Additionally, intraspecific variation in morphological, physiological, and behavioral adaptations allow some species to use a wide range of habitats and resources. Various combinations of these adaptations can constrain or liberate the extent of habitat that an individual within a species can use depending on population density, availability of limiting resources (e.g., food, water, shelter), and presence of predators and mates (Powell 2012). Physiological differences, such as reproductive condition, may further explain variation in habitat selection because pregnant or kit-rearing females likely require more food, are more vulnerable to predation, and may be restricted to smaller spatial movements.

These individual characteristics may enable or necessitate skunks to vary their movement patterns and home-range size based on body condition, seasonal weather changes, or presence of intra- or interspecific competition. Therefor I examined home range dynamics in chapter three and identified relationships between den use and intrinsic variation in chapter four. Examining habitat selection by a species in different environments and at different scales may reveal important habitat associations and adaptive constraints in resource use. These constraints could lead to potential population genetic consequences, as habitat fragmentation and loss can affect dispersal and gene flow, shaping the differentiation of spotted skunk populations (Lowe and Allendorf 2010). The disruption of dispersal movements due to habitat fragmentations decreases demographic and genetic connectivity, which in turn reduces genetic diversity and increases the risk of local extinction (Giplin and Soulé 1986, Lowe and Allendorf 2010). To develop effective management strategies for eastern spotted skunk conservation, it is vital to understand the associations between habitat selection by spotted skunks and the resulting genetic structuring and differentiation. Therefore, in chapter five, I evaluated the genetic diversity and population structure of eastern spotted skunks in Virginia to assess potential impacts of habitat fragmentation.

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Chapter One – Winter Habitat Associations of Eastern Spotted Skunks (*Spilogale putorius)* in Virginia[1]

Abstract

Eastern spotted skunk (*Spilogale putorius*) populations have declined throughout much of their range in the eastern United States over recent decades. Declines have been attributed to habitat loss or change, increased competition with sympatric meso-predator species, or disease. To better understand the contemporary distribution of spotted skunks in the Appalachian Mountains of western Virginia, USA, we used a detection-non-detection sampling approach using baited camera traps to evaluate the influence of landscape-level environmental covariates on spotted skunk detection probability and site occupancy. We conducted camera-trap surveys at 91 sites from January to May in 2014 and 2015. Spotted skunk occupancy was associated with young-aged forest stands at lower elevations and more mature forest stands at higher elevations. Both land-cover types in this region can be characterized as having complex forest structure, providing cover that varies with stand age, species composition, elevation, and management regime. Our results provide insight into factors that influence spotted skunk spatial distribution and habitat selection, information that can be used to generate conservation assessments and inform management decisions.

Introduction

The eastern spotted skunk (*Spilogale putorius*) is a small mephitid that was once a common furbearer throughout the central and southern United States, with annual range-wide harvests of over 100,000 individuals (Gompper and Hackett 2005). Although it has a federal

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status of Least Concern, the eastern spotted skunk is listed as Vulnerable, Imperiled, or Critically imperiled in most of the states throughout its historical range (Chapman 2007) and is listed as Vulnerable on the International Union for the Conservation of Nature Red List (Gompper and Jachowski 2016). Perceived population declines in the Midwest and upper South, USA, beginning in the 1940s, may be attributed to a decrease in available early-successional shrub-scrub, regenerating yellow pine (*Pinus* spp.), and oldfield habitat due to intensive agricultural practices (clean farming) or the maturation of early successional forests (Polder 1968, Gompper and Hackett 2005, Lesmeister et al. 2009). Declines also may have been influenced by increased competition with sympatric mesocarnivore species (e.g., striped skunks [*Mephitis mephitis*], raccoons [*Procyon lotor*], bobcats [*Lynx rufus*], and coyotes [*Canis latrans*]) as a result of declining trapping pressure or range expansions of such species (Chapman 2007, Lesmeister et al. 2010). A series of rabies outbreaks beginning in the 1970s, also may have contributed to Midwest population declines of spotted skunks potentially because of increased mortality and reduced productivity, an effect that has been observed in striped skunks (Greenwood et al. 1997).

In Virginia, parallel reduction of early- to mid-successional habitat associated with forest maturation occurred in the Appalachians in the later portion of the twentieth century (Yarnell 1998). Within the central and southern Appalachians, forest maturation occurred as a result of succession from earlier harvesting in the industrial logging period and decreased timber harvest on federally owned lands, which may have led to diminished habitat quality for spotted skunks. A raccoon rabies enzootic also spread throughout much of Virginia by the 1980s following a translocation of infected raccoons from Florida to the mid-Atlantic region to replenish hunting stock in the late 1970s (Torrence et al. 1992, Real et al. 2005). The combination of habitat change, increased competition with sympatric carnivores, and spread of the rabies virus, similar to the potential causes of spotted skunk declines in the Midwest, may have affected the current spatial distribution and habitat associations of spotted skunk populations in Virginia.

Little is known about the spatial distribution or ecology of eastern spotted skunks in the central and southern Appalachians. In the Blue Ridge portion of the southern Appalachians of Tennessee, spotted skunk populations have been associated with rhododendron (*Rhododendron maximum*) thickets near high elevation emergent rock outcrops (Reed and Kennedy 2000). Similarly, in South Carolina, spotted skunks were detected predominantly in areas of mixed softwood-hardwood forest containing dense understory (Wilson et al. 2016). In the central Appalachians of West Virginia and Virginia, spotted skunks have been incidentally captured during surveys of other rare Appalachian wildlife near emergent rock outcrops (Webster et al. 1985) and in a high elevation red spruce (*Picea rubens*) forest (Diggins et al. 2015). It is currently unknown whether emergent rock outcrops and ericaceous shrubs, such as rhododendron or mountain laurel (*Kalmia latifolia*), represent primary spotted skunk habitat in the Appalachians. These apparent associations may be an adaptive response to decreased early successional forest habitat, as seen in other areas where spotted skunks occur (Lesmeister et al. 2013), or may be random occurrences not reflective of spotted skunk habitat preferences.

Currently, the species is classified as Vulnerable in Virginia with anecdotal evidence suggesting that it has been rare or largely absent throughout the central and southern Appalachian portions of the state, where it formerly was believed abundant and widespread (M. L. Fies, Virginia Department of Game and Inland Fisheries, personal communication). However, anecdotal information from an ongoing multi-state, golden eagle (*Aquila chrysaetos*) wintering distribution study in the Appalachians using baited camera stations has recorded spotted skunks in West Virginia in the Peters-Potts Mountain, Shenandoah Mountain, and Great North Mountain areas along the Virginia-West Virginia border, and within Shenandoah National Park to the east (T. E. Katzner, U.S. Geological Survey, unpublished data). These recent records suggest that spotted skunks remain extant in areas throughout the Appalachians.

Our first objective was to identify landscape-level environmental characteristics associated with eastern spotted skunk habitat occupancy. Specifically, we intended to ascertain whether spotted skunk habitat associations in the central and southern Appalachians remain consistent with those reported in other parts of its range (Lesmeister et al. 2013). Our second objective was to create a predictive spotted skunk occurrence model for the Appalachian region of Virginia to determine eastern spotted skunk distribution across the Appalachians of Virginia. We hypothesized that spotted skunk predicted occupancy would be higher in forested areas of early to mid-successional growth with increased occupancy at high elevations as reported in other studies (Webster et al. 1985, Lesmeister et al. 2013, Diggins et al. 2015).

Methods

Study Area

We conducted our study in the central and southern Appalachian Mountains of western Virginia, in the Ridge and Valley and Blue Ridge physiographic subprovinces in 10 counties (Figure 1.1) extending from the North Carolina state line north to the eastern boundary of West Virginia, primarily on the George Washington and Jefferson National forests (GWJNF). The Valley and Ridge and the Blue Ridge regions are characterized by long mountain ridges with steep side slopes and narrow to moderately broad valleys. Elevations range from 350 to 1,460 meters. Including private land, the entire study area predominately is forested (67%), intermixed with pasture or hay production in the valleys (22%), and limited areas of row-crop agriculture, residential, and urban development (Homer et al., 2015). The climate is cool-temperate with a mean annual temperature of 6.1°C, annual mean minimum temperature of -10.6°C in January, and mean maximum of 27.5°C in June (National Oceanic Atmospheric Administration, public data 2015; www.noaa.gov). With the exception of the southernmost portion of the Blue Ridge, annual precipitation is approximately 110 cm, as much of the area is in the rain shadow created by the Allegheny Mountains along the West Virginia border (Ford et al. 2006). Because of this, the dominant forest types over much of the area on ridges and side slopes are relatively xeric hardwood oak-hickory or oak-pine associations dominated by white oak (*Quercus alba*), chestnut oak (*Q. prinus*), northern red oak (*Q. rubra*), black oak (*Q. velutina*), red maple (*Acer rubrum*), pitch pine (*Pinus rigida*), mountain pine (*Pinus pungens*), and mountain laurel. Hardwood non-oak forests occurred on north-facing, sheltered landforms and were dominated by yellow poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), and sugar maple (*A. saccharum*). The montane riparian areas commonly were composed of white pine (*Pinus strobus*)-eastern hemlock (*Tsuga canadensis*) associations, often with dense rhododendron understories. At the highest elevations, small amounts of northern hardwood-montane boreal forest dominated by yellow birch (*Betula alleghaniensis*), sugar maple, American beech, and red spruce were present (Braun 1950, Simon 2011, 2013). Common mammalian fauna in the study area include American black bear (*Ursus americanus*), bobcat, coyote, gray fox (*Urocyon cinereoargenteus*), raccoon, red fox (*Vulpes vulpes*), striped skunks, Virginia opossum (*Didelphis virginiana*), and white-tailed deer (*Odocoileus virginianus*, Morin et al. 2016).

Sampling Methods

We conducted baited camera trapping surveys from January through April in 2014 and 2015, deploying 91 camera stations spatially grouped into 9 clusters throughout the study area (Figure 1.1). We used a stratified random sampling approach to sample 3 broadly characterized forest types (i.e., oak-dominated, non-oak dominated, and mixed oak-pine) in proportion to their availability on the landscape (Gasaway et al. 1986). To minimize false absences, we concentrated our surveys in the dormant season because Hackett et al. (2007) reported highest detection rates using camera traps from October through May, with little to no trap success during summer in Arkansas. Moreover, our use of bait also constrained our effort at that time to avoid interference to cameras and bait by American black bears.

Our camera-trap stations consisted of 1 remote-triggered, motion-activated camera with light-emitting diode (LED) flash. We mounted HyperFire HC500 infrared (Reconyx, Holeman, WI, USA), Bushnell Trophy Cam Model 119436, and Bushnell Trophy Cam HD Model 119739 (Bushnell, Overland Park, KS, USA) cameras to trees approximately 1 m above ground level following methods of Jachowski et al. (2015). We secured road-killed, white-tailed deer carcasses to the ground with rebar approximately 3–4 m in front of the camera (Jachowski et al. 2015). We spaced camera stations within study sites >1.5 km apart to exceed the diameter of the average male spotted skunk winter home range (Lesmeister et al. 2009) to limit the possibility of detecting a single skunk at multiple camera stations, to promote independence of camera stations, and to maximize forest type coverage. Hackett et al. (2007) observed a 7.2-day latency to initial detection using baited camera traps; therefore, we deployed each camera for ≥8 uninterrupted days to increase probability of detecting skunks in truly occupied habitat patches. We checked cameras weekly to ensure proper function and bait availability. We defined sampling occasions as 24-hour intervals and a detection as any number of spotted skunks photo-captured at an individual camera (Fig. 2). This research was approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee (protocol number 13-119-FIW).

To assess habitat associations, we related probability of occurrence to landscape-level attributes (based on remotely-sensed geographic information system layers) derived using ArcMap 10.2.2 (ESRI, Redlands, CA, USA). We derived elevation (meters above sea level), slope (degrees), aspect (sine-transformed) using 30-m digital elevation models (DEM; U. S. Geological Survey 2000). We calculated a topographic exposure index (TEI), where higher TEI values indicate greater topographic exposure, using the zonal statistics tools in ArcMap, by subtracting the average elevation of a 1.75-km^2 circular area around each camera (Evans et al. 2014, Ford et al. 2015). We buffered each camera location based on average male winter home range estimate for the species (1.75 km^2; Lesmeister et al. 2009). Within each of these buffers, we classified land cover type as oak-dominated, non-oak, and mixed oak-pine forests using the Northeast Terrestrial Wildlife Habitat Classification System (Gawler 2008). We used the 2011 National Land Cover Database (NLCD) classification system (Homer et al. 2011) and ArcMap 10.2.2 to measure distance to nearest primary road, water source, and agricultural or pastoral area. We used 2011 NLCD Tree Canopy analytical data to estimate mean percent canopy cover within the 1.75-km^2 buffer (U. S. Forest Service 2011). We also used the United States Forest Service landclass inventory data for the GWJNF to determine stand age ($\bar{x}$ age of stand since last harvest) and stand size (area of distinct stand type) for each camera station.

Data Analysis

We used a likelihood-based, information-theoretical modeling framework to estimate detection probability (p) and probability of site occupancy (Ψ) to compare models that included site and survey covariates (Mackenzie et al. 2006). We standardized all continuous site covariates to a mean of 0 and standard deviation of 1 to compare influence of covariates on Ψ and p. Camera locations differed between 2014 and 2015 samples, so we included an effect of year in our candidate model set. We combined all data and used a single-species, single-season modeling framework using Program R (R Development Core Team 2014) with package unmarked (Fiske and Chandler 2011). We compared models using Akaike's Information Criterion (*AIC*) corrected for small sample sizes (AIC_c) to rank all p and Ψ models to determine the best-fit models for detection-non-detection data. We considered models competing if ΔAIC_c < 2.0.

We first estimated p (holding Ψ constant) to determine which environmental factors most influenced detection. These factors included average daily temperature, rainfall, snowfall, snow depth, and percent moon illumination (moon surface fraction illuminated) at each camera site. We acquired daily climate data from the National Weather Service (www.weather.gov, accessed 25 May 2015) and percent moon illumination data from the United States Naval Observatory (http://aa.usno.navy.mil/data/docs/MoonFraction.php, accessed 25 May 2015). Because occasionally deep snow at the higher elevations prevented access to many camera stations throughout the study and occasional camera malfunctions, lengths of time that camera stations remained operational at study sites varied from 8 to 62 days. To account for a large range of survey length on detectability of spotted skunks, we included survey effort (number of days camera station was operational) as a detection covariate. Additionally, we included ordinal date to account for temporal variability throughout the study period for each sample year, and daily capture rate (number of photographs captured/day) of bobcats, coyotes, raccoons, striped skunks, and Virginia opossums to account for effects of other carnivore species' presence on spotted skunk detection. We ranked p models based on AIC_c, the difference between the model with the lowest AIC_c and the AIC_c of the ith model (Δ_i), and Akaike weights (w_i), to select the most supported p model (Burnham and Anderson 2002, MacKenzie et al. 2006). We selected the model with the lowest AIC_c value and highest w_i as most supported (Burnham and Anderson 2002). After identifying the most-supported p model, we examined relationships between Ψ and environmental factors using a suite of 17 *a priori* models. We examined all covariates for multicollinearity using Spearman's rank correlation with an exclusion criterion of $|r_s| > 0.7$. Additionally, we conducted a parametric bootstrap analysis using 10,000 iterations to assess goodness of fit of the most parameterized model and to ensure that the underlying model fit the data (MacKenzie et al. 2002). Using our best model, we created a map of eastern spotted skunk predicted Ψ in the GWJNF.

Results

We detected eastern spotted skunks at 19 of 91 camera stations, 13 out of 50 in 2014 and 6 out of 41 in 2015, resulting in a naïve occupancy (survey sites with skunk detection/no. survey sites) of 0.21 (Figure 1.1). Mean survey period length was 38.7 days (± 1.4 days [SE]). Eighty percent of camera stations were located in oak-dominated forest stands, 14% in non-oak cove, northern hardwood or riparian stands, and 6% in mixed oak-pine stands. Percent canopy cover at camera stations ranged from 81.7% to 98.9% ($\bar{x}$ = 92.1 ± 0.04%). Elevation at our stations ranged from 349.0 m to 1,468.1 m ($\bar{x}$ = 942.7 ± 22.3 m). Topographic exposure index (TEI) values varied from −75.1 to 107.3 ($\bar{x}$ = 8.2 ± 4.6). Mean distances from camera stations to nearest primary road, water source, and agricultural or pastoral area were 5.4 ± 0.3 km, 2.1 ± 0.1 km, and 2.4 ± 0.2 km, respectively. Forest stand age varied from 26 years to 164 years ($\bar{x}$ = 91.9 ± 3.1 yr) and stand size varied from 2.4 ha to 231.0 ha ($\bar{x}$ = 49.4 ± 5.1 ha), though in most cases absolute forest patch size was large (>1,000 ha).

No significant multicollinearity occurred between covariates and we retained all covariates for Ψ and p analysis. Additionally, we found no effect of sample year on Ψ for the combined 2014 and 2015 data. Our top detection model, indicated a negative influence of percent moon illumination on spotted skunk detection (β = -0.7, 95% CI = -0.5 – -0.1)) with no competing models based on AIC_c score and Akaike weight **(**Table 1.1). Detection probability generally was low and was estimated to decline from 0.14 on nights with 0% moon illumination (new moon) to 0.04 on nights with 100% moon illumination (full moon; Figure 1.2).

The stand age and elevation interaction best predicted occupancy based on AIC_c score and w_i (Table 1.2, Figure 1.4). The evidence ratio of w_i between our 2 highest ranking models indicated 3.4 times more support for the top model over the second-highest ranking model. Additionally, the difference between AIC_c scores of the 2 highest ranking models exceeded 2. Therefore, we did not average occupancy models. Occupancy decreased as stand age and elevation at camera stations increased (Figure 1.4). The 95% confidence intervals on the beta for the interaction of stand age and elevation (β = 0.6; 95% CI = 0.1–1.1) did not contain 0, which suggested an important effect of both on Ψ. The map of spotted skunk predicted Ψ revealed small, spatially disjunct patches of high predicted occupancy surrounded by large patches of moderate to low predicted occupancy (Figure 1.5). Approximately 2.7% of the study area consisted of high predicted occupancy habitat (predicted $\Psi \geq 0.90$) and 3.1% of the study area consisted of moderate predicted occupancy habitat (predicted Ψ = 0.75–0.89).

Discussion

Detection probability for eastern spotted skunks was generally negatively influenced by percent moon illumination. Detection probability peaked at the new moon (0–25% illuminated), which agreed with many studies of nocturnal small-mammal response to moon phase (Wolfe and Summerlin 1989, Orrock et al. 2004, Kotler et al. 2010, Read et al. 2015). Moonlight increases the conspicuousness of small mammals to nocturnal predators such as owls, bobcats, and coyotes, which in turn leads to greater predation risk (Wolfe and Summerlin 1989, Orrock et al. 2004, Kotler et al. 2010). Lesmeister et al. (2010) reported aerial predators, most likely the nocturnal great horned owl (*Bubo virginianus*), and mammalian predators, to be the greatest cause of eastern spotted skunk mortality in the Ouachita Mountains of western Arkansas. We suspect the negative association of spotted skunk detection probability with percent moon illumination in our study is suggestive of predator avoidance and risk-reducing behaviors.

The indices for nocturnal illumination may not be accurate because we did not measure light intensity directly in our study. However, average daily temperature, rainfall, snowfall and percent canopy cover had no effect on spotted skunk detection probability. Therefore, overcast skies from inclement weather or shade from canopy cover may not be affecting the response of spotted skunks to moon illumination. In areas of variable percent canopy or forest type, activity patterns and detectability of nocturnal animals may be influenced by light intensity irrespective of moon phase (Vignoli et al. 2014); however, our study was unable to distinguish between the effects of light intensity and moon phase. We found no strong effect of survey effort on detectability, which suggests that the variation in survey length among sites did not bias spotted skunk detection probability. Ordinal date also showed no effect, which suggests no temporal variation in detection probability throughout the study period. Additionally, capture rates of other carnivore species had little effect on spotted skunk detection probability.

An interaction between forest age and elevation best predicted spotted skunk winter habitat occupancy throughout the study area. Probability of occupancy was highest in early to mid-successional forest stands (<50 yr old) at elevations <525 m and tended to decrease as forest age and elevation increased. Forest age is likely not the ultimate factor influencing spotted skunk winter habitat selection *per se,* but rather the structural characteristics of forests associated with age. In eastern North America, forest structure tends to be more important for many wildlife species than plant species composition for a variety of taxa (Greenburg et al. 2011). Newly regenerated Appalachian forests often are characterized by high woody stem density prior to the stem exclusion phase of succession (Swanson et al. 2011, Wilson et al. 2014). In the central and southern Appalachians, a variety of species benefit from such complex forest structure, such as ruffed grouse (*Bonasa umbellus*), where populations in habitats with greater forest cover and high understory cover reach higher densities than populations in mature forests with lower understory cover (Thompson and Dessecker 1997, Devers et al. 2007). Similarly, Lesmeister et al. (2010) reported higher spotted skunk mortality from avian predators in lower-quality spotted skunk habitat consisting of mature shortleaf pine (*Pinus echinata*) stands with open canopy and little understory cover. Relationships between spotted skunk occupancy and forest age have been observed in the Ouachita Mountains (Lesmeister et al. 2008, 2009, 2010, 2013) and in the Missouri Ozarks (Hackett 2007). Furthermore, for many species in the Appalachian region - including migrating songbirds, rodents, white-tailed deer, and American black bears - young forests provide increased foraging opportunities through greater quantities of fungi, hard and soft mast, and invertebrate food sources (Harlow 1984, Loeb 1996, Mitchell and Powell 2003, McDermott and Wood 2010, Wilson et al. 2014).

Although our top model suggests that spotted skunks are occupying habitat associated with young forests at low elevations, we detected eastern spotted skunks in a high elevation virgin red spruce forest in the Whitetop Mountain area of Grayson County, VA (second, smaller peak in Figure 1.4). The year-round, closed canopy and dense rhododendron thickets characteristic of old-growth red spruce forests may provide thermal cover and protection from aerial predators in a way that is functionally similar to younger hardwood forests. Whereas this forest type is limited to isolated patches in the southwestern-most region of the Appalachians in Virginia, relatively large and continuous patches persist in the central Appalachians of West

Virginia and the southern Appalachians of Tennessee and North Carolina (Nowacki et al. 2010). These larger forest patches may provide habitat for persistence of spotted skunk populations and travel corridors that allow movement between selected habitat patches separated by mountain ridgetops. This dichotomy of habitat selection suggests that, given enough structural cover, spotted skunks may show plasticity in elevational and forest type use when selecting for habitat.

Our predictive map of spotted skunk occupancy shows a fragmented distribution of small, high occupancy areas surrounded by a matrix of moderate to low occupancy areas (Figure 1.5). Loss of early and mid-successional habitat to forest maturation and reduced harvesting, and declines in natural and anthropogenic disturbance regimes in forests of eastern North America (i.e., fire; Brose et al. 2001, King and Schlossburg 2013) may explain the patchy distribution of occupied habitat in the central and southern region of the Appalachians. In the GWJNF, annual timber harvest objectives total approximately 2,100 ha, whereas annual prescribed burn objectives total approximately 17,000 ha, of which selected areas of repeated treatment account for only 13% of the 287,691 ha of forest designated for active management (U.S. Forest Service 2004, 2014). Currently, the majority of timber stands in the GWJNF are in the 90–130-year-old age class, which is either at, or exceeding, specified rotation ages of 80–100 years, in comparison to 11% of forest in the ≤ 50-year-old age classes (U.S. Forest Service 2004, 2014). Eastern spotted skunk populations may experience reduced winter habitat availability as production and maintenance of early- to mid-successional habitat remain low in much of the National Forest land in the central and southern Appalachians.

Habitat loss and fragmentation have been reported to cause population declines and reduced habitat occupancy for various sensitive mesocarnivore species across a variety of habitats throughout North America, including western spotted skunks (*Spilogale gracilis*) and long-tailed weasels (*Mustela frenata*) in the Southwest (Crooks 2002) and fishers (*Martes pennanti*) in the Pacific Northwest (Marshall 1992, Zielinski et al. 1995) and the Northeast (Brander and Books 1973, Powell 1993). Loss of young forest habitat in the GWJNF over time, due to maturation of forests on public lands, possibly has reduced winter habitat availability for eastern spotted skunks. If vegetative cover as a means of predator avoidance and thermal protection is influencing spotted skunk winter habitat selection, this species may be vulnerable to environmental changes that reduce the structural complexity of forests. Populations experiencing declining winter habitat availability, reduced habitat patch size, and increased patch isolation are highly susceptible to the negative consequences of demographic, environmental, and genetic stochasticity (Lawton and May 1995, Boyce et al. 2005, Frankham 2006). As a result, there is potential for eastern spotted skunk populations to remain relatively rare or experience further declines in Virginia, and other parts of the central and southern Appalachians, as forest fragmentation increases and availability of young forest declines regionally.

Although the use of bait at camera sites may potentially bias occupancy results by increasing detection rates for some species, unbaited camera surveys may fail to detect small, rare, and elusive carnivores in occupied habitat patches. For example, within the same region, Kelly and Holub (2008) were unable to detect eastern spotted skunks using unbaited sampling, whereas we detected spotted skunks at 4 baited camera stations from 2014–2015 where they had previously sampled. Moreover, fewer than 6 spotted skunks were detected throughout all the Appalachian region of Virginia throughout >10 years of unbaited camera surveys (M. J. Kelly, Virginia Tech Department of Fish and Wildlife Conservation, unpublished data). Baited camera sampling methods may increase precision of population estimates of small carnivores by increasing recapture rate, particularly when population sizes are small (Gerber et al. 2012, Gerber et al. 2014). Therefore, we did not suspect the use of bait in our study to have influenced spotted skunk detection probability or patch occupancy estimates. Lastly, limiting our work to the dormant season may also have introduced another source of bias. Causes of seasonal variation in detectability of eastern spotted skunks are unclear. However, patterns of detectibly and capture success of many carnivore species, including other species of skunk, may be attributed to seasonal shifts in resource availability, home range size, habitat use, and foraging ability (Bailey 1971, Bixler and Gittleman 2000, Zapata et al. 2001, Cantú-Salazar et al. 2005, Doty and Dowler 2006).

Future research on eastern spotted skunks should assess the effects of creating additional early- to mid-successional habitat through forest management (e.g., stand thinning, prescribed burning) with the goal of increasing patch connectivity that may reduce or prevent further population decline and aid in recovery. Maintaining habitat for spotted skunks will require more detailed knowledge of spotted skunk ecology, micro-habitat selection, movement patterns, and genetic health of the population. Although occupancy analysis is a suitablemethod for predicting habitat associations at the landscape level, detection-non-detection methods are limited in scope because of the inability to identify individual spotted skunks for abundance or density estimates, home range analysis, sex segregation, or detailed movement patterns. Intensive capture-recapture efforts combined with the use of radio telemetry are necessary to further understand eastern spotted skunk habitat requirements. In addition, effective population monitoring and sampling methods are necessary for implementing successful management practices.

Management Implications

The overall low availability of habitat that was highly selected by spotted skunks (i.e., predicted $\Psi \geq 0.90$) throughout the GWJNF indicates a potential need to consider this species when generating conservation and management assessments in forested areas. The baited remote-sensing camera methods used in this study are an effective technique for detecting spotted skunks and may provide a relatively low-cost method for long term, large-scale monitoring. Managers should also consider the dichotomous forest types that spotted skunks use throughout their range, which may require separate assessments of habitat associations and adaptiveadapt management practices at local scales. Additionally, non-habitat factors may influence spotted skunk spatial distribution and habitat use. Further research is needed to investigate the effects of sympatric carnivore interactions, including competition, predation, and potential zoonotic disease transfer on eastern spotted skunk population level, habitat use, and spatial distribution at both the local and regional scales

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Table 1.1 Model selection results for detection probability (*p*) of eastern spotted skunks (*Spilogale putorius*) in the George Washington and Jefferson National Forests, Virginia, USA, January–May, 2014–2015. We held occupancy constant and fit survey data from 91 camera stations to the candidate model set to estimate *p*. Models ranked based on Akaike's Information Criterion corrected for small sample sizes (AIC_c).

Model	AIC_c	Δ_i[a]	w_i[b]	K[c]
p(moon illumination)	454.68	0.00	0.8751	3
p(Virginia opossum)	459.82	5.14	0.0668	3
p(snow depth)	463.08	8.40	0.0131	3
p(temp)	464.12	9.44	0.0078	3
p(bobcat)	464.55	9.87	0.0063	3
p(.)	464.76	10.08	0.0057	2
p(rainfall)	464.98	10.30	0.0051	3
p(coyote)	465.14	10.46	0.0047	3
p(raccoon)	466.13	11.45	0.0029	3
p(survey effort)	466.16	11.48	0.0028	3
p(accumulated snowfall)	466.19	11.51	0.0028	3
p(striped skunk)	466.22	11.54	0.0027	3
p(canopy cover)	466.68	12.00	0.0022	3
P(Ordinal date)	466.76	12.08	0.0021	3

[a]Difference in AIC_c of given model and top model.
[b]Akaike weight.
[c]Number of parameters.

Table 1.2 Model selection results for occupancy probability (Ψ) of eastern spotted skunks (*Spilogale putorius*) in the George Washington and Jefferson National Forests, Virginia, USA, January–May 2014–2015. All models included percent moon illumination as a detection (p) covariate based on the best-supported detection model. We fit survey data from 91 camera stations to the candidate model set to estimate Ψ. Models ranked based on Akaike's Information Criterion corrected for small sample sizes (AIC_c).

Model	AIC_c	Δ_i[a]	w_i[b]	K[c]
Ψ(stand age × elevation)	451.61	0.00	0.3681	6
Ψ(stand size)	454.07	2.46	0.1076	4
Ψ(.)	454.96	3.35	0.0690	3
Ψ(slope)	455.16	3.55	0.0624	4
Ψ(stand age)	455.31	3.70	0.0579	4
Ψ(yr)	455.51	3.90	0.0524	4
Ψ(elevation)	455.7	4.09	0.0476	4
Ψ(area × elevation)	456.63	5.02	0.0299	6
Ψ(canopy cover)	456.11	4.50	0.0388	4
Ψ(aspect)	456.2	4.59	0.0371	4
Ψ(age × area)	456.78	5.17	0.0278	6
Ψ(TEI*)	456.99	5.38	0.0250	4
Ψ(distance to agriculture)	457.08	5.47	0.0239	4
Ψ(land cover†)	457.09	5.48	0.0238	6
Ψ(distance to roads)	457.37	5.76	0.0207	5
Ψ(.)P(.)	459.22	7.61	0.0082	2
Global	474.26	22.65	0.0000	17

[a]Difference in AIC_c of given model and top model.
[b]Akaike weight.
[c]Number of parameters.
*Topographic exposure index was calculated by subtracting the average elevation of a 1.75-km^2 circular area around each camera.
†Land cover type was classified as oak-dominated, non-oak, and mixed oak-pine forests using the Northeast Terrestrial Wildlife Habitat Classification System

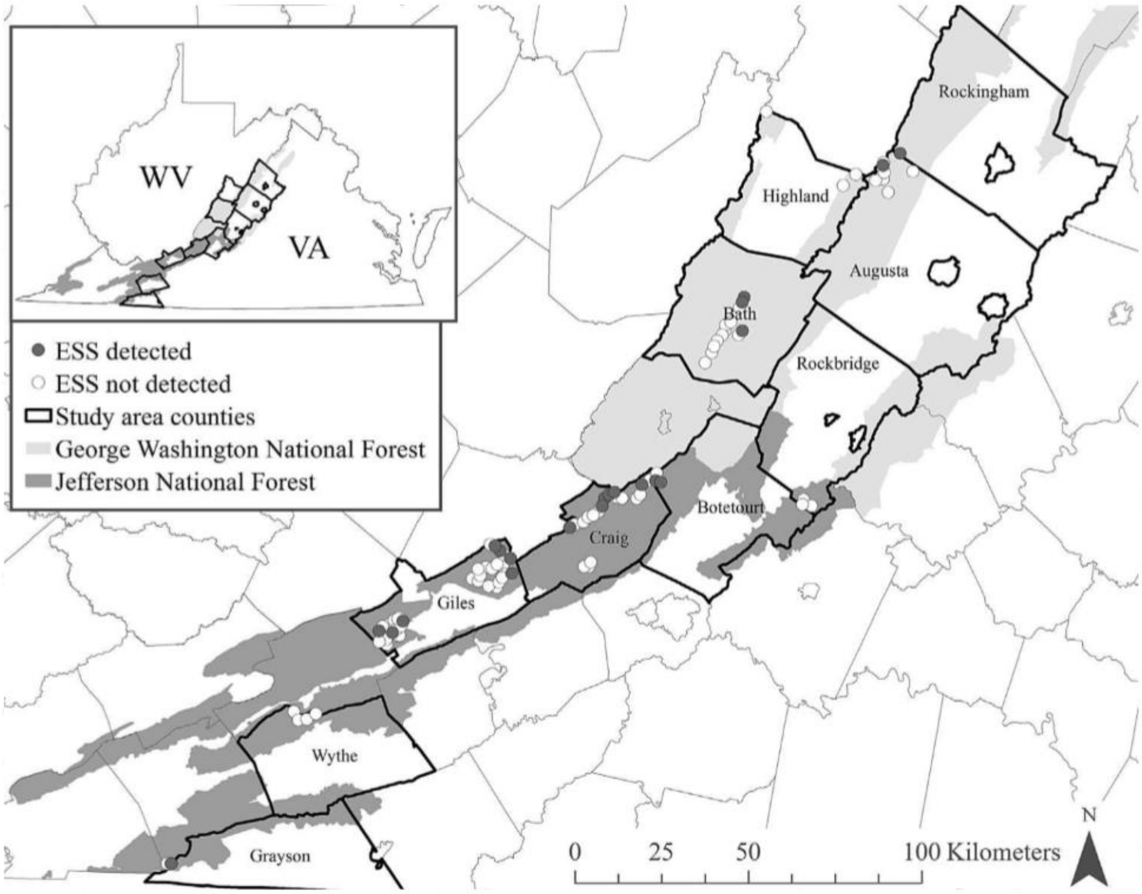

Figure 1.1 Study area across the Appalachian Mountain region of western Virginia, USA, showing the George Washington and Jefferson National Forests. Study area includes Augusta, Bath, Botetourt, Craig, Giles, Grayson, Highland, Rockbridge, Rockingham, and Wythe counties. We detected eastern spotted skunks (ESS; *Spilogale putorius*) at 19 of 91 camera trap sites from January to April in 2014 and 2015.

Figure 1.2 Example of baited camera station with photo-captured eastern spotted skunk (*Spilogale putorius*) in the George Washington and Jefferson National Forests, Virginia, USA, January–April 2014–2015.

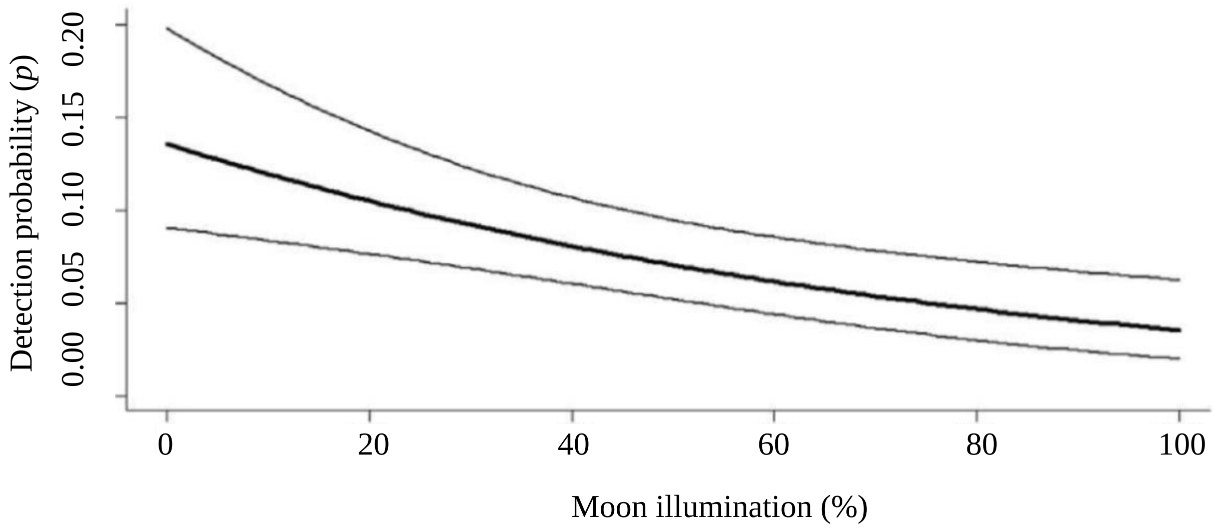

Figure 1.3 Eastern spotted skunk (*Spilogale putorius*) probability of detection (± SE) as a function of percent of moon surface illuminated in the George Washington and Jefferson National Forests, Virginia, USA, January–April 2014–2015. We estimated probability from the best-supported occupancy model.

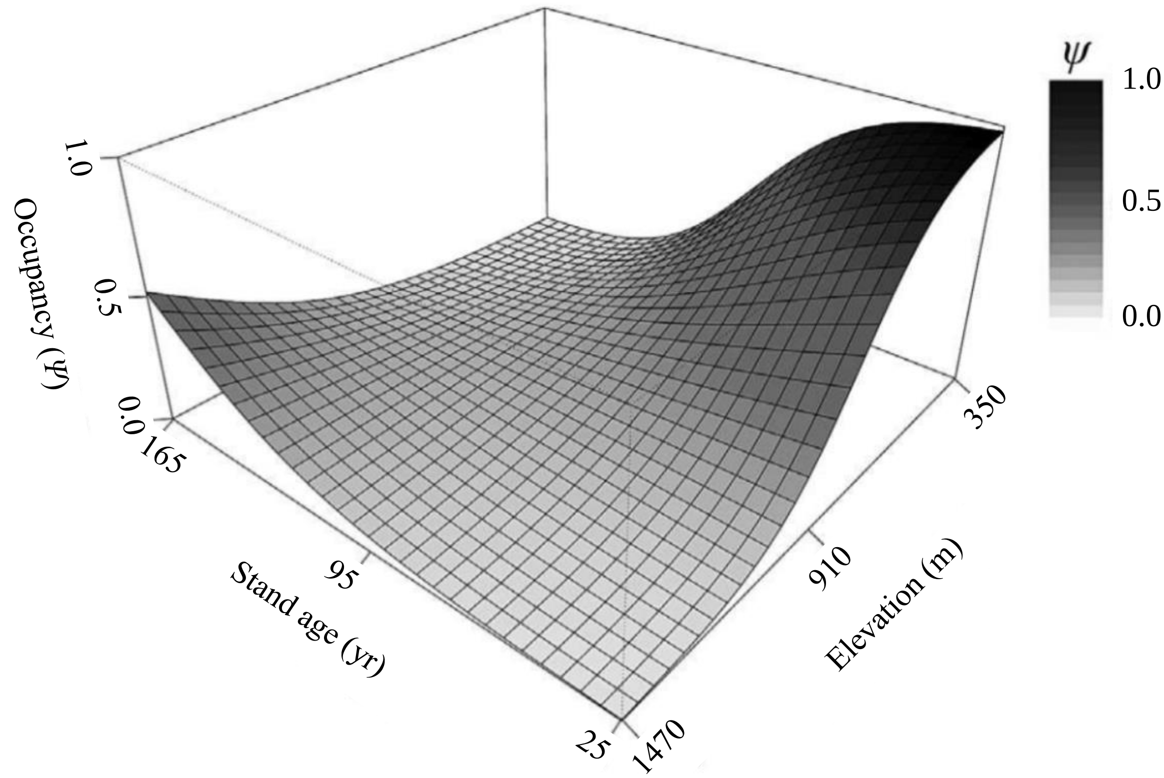

Figure 1.4 Eastern spotted skunk (*Spilogale putorius*) probability of winter occupancy (*Ψ*) as a function of forest stand age and elevation in the George Washington and Jefferson National Forests, Virginia, USA, January–May 2014–2015. We estimated probability from the best-supported occupancy model.

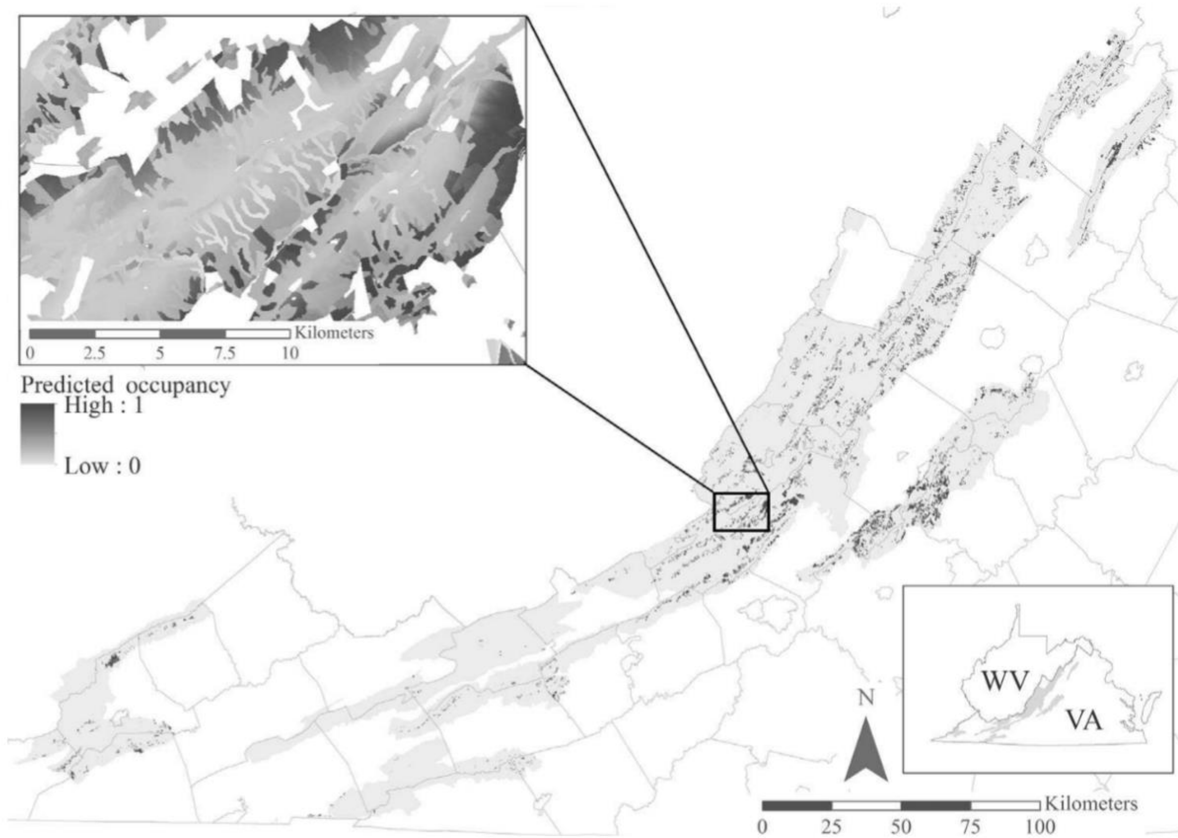

Figure 1.5. Map of predicted eastern spotted skunk (*Spilogale putorius*) winter occupancy (Ψ) across the landscape in the George Washington and Jefferson National forests, Virginia, USA, January–May 2014–2015. Areas of > 50% predicted occupancy are shown in dark gray and areas of < 50% occupancy are shown in light gray. Inset demonstrates patchy distribution of small, spatially disjunct areas of high predicted occupancy (> 90%) surrounded by large areas of moderate to low predicted occupancy. We developed the occupancy map by applying our best-supported occupancy and detection (p) model, Ψ(stand age × elevation) p(moon illumination), to each pixel.

Chapter Two – Spatial Co-occurrence and Temporal Activity Within the Mesocarnivore Guild in Central Appalachia

Abstract

Overlap in resource use by sympatric predators can lead to direct or indirect competition. Within the predator guild, direct competition can be manifested by aggressive interactions, and occasionally intraguild predation or killing of competitors. Meso-predators in the Appalachian region of Virginia – bobcats, coyotes, raccoons, striped skunks, spotted skunks, and Virgnia opossums – occur sympatrically and use similar resources. We utilized remotely-triggered cameras baited with road-killed deer to monitor site use and activity patterns of these meso-predator species in the George Washington and Jefferson National Forests from January – April, 2014 and 2015. Our objectives were to identify habitat associations of each species, assess probability of species co-occurrence, and identify potential shifts in acdiel patterns of co-occurring speices. We found no evidence of spatial avoidance by sympatric species; however, we found strong evidence of temporal avoidance. Co-occurring species are likely adjusting the timing of their activity patterns when competing species are present. This finding may indicate a behavioral mechanism to avoid direct competition and reduce antagonistic interactions.

Introduction

Exploitation of limiting resources by coexisting species within the same locality often results in interspecific competition (Chesson 2000; Creel et al. 2001; Amarasekare 2009). Within a carnivore community, this direct or indirect competition can be manifested by aggressive interactions, and occasionally intraguild predation or killing of competitors (Rosenweig 1966; Polis et al. 1989; Palomares and Caro 1999; Creel et al. 2001; Amarasekare 2009). Large carnivores, such as wolves (*Canis lupis*) and cougars (*Puma concolor*), are known to influence the population dynamics, distribution, and behavior of sympatric small to medium-sized predators or "meso-predators", and therefore play an important role in some ecosystems (Paine 1980; Alexander et al. 2006; Hayward and Slotow 2009; Roemer et al. 2009; Levi and Wilmers 2012). However, meso-predator release resulting from large predator declines across the landscape has changed the composition of predator communities throughout North America (Prugh et al. 2009; Ritchie and Johnson 2009; Newsome et al. 2015; Wang et al. 2015).

Meso-predators are more numerous and diverse than large predators, yet little is known about the mechanisms of competitive coexistence of sympatric meso-predator species (Roemer et al. 2009). Meso-predators can range from solitary to highly social, specialist to generalist, and omnivorous to entirely carnivorous (Roemer et al. 2009). Unlike large predators that often require large or abundant prey items to meet their caloric needs, the diets of many smaller predator species are more varied, often consisting of small vertebrates, invertebrates, and soft mast (Carbone et al. 1999; Roemer et al. 2009). Athough diverse in resource requirements, competition between two similar Meso-predators for shared resources can lead to agonistic interactions (Rosenzweig 1966; Polis et al. 1989; Palomares and Caro 1999). Nonetheless, multiple mechanisms of avoidance that can allow competitive coexistence among sympatric Meso-predators species (Amarasekare 1966; Chesson 2000; Creel et al. 2001; Ritchie and Johnson 2009). For example, spatial and temporal avoidance enable predator species to vary the place and time that a given common resource is used, thereby limiting costly agonistic encounters (Carothers and Jaksić 1984; Kronfeld-Schor and Dayan 2003; Hayward and Slotow 2009; Bischof et al. 2014). Community structure and competitive coexistence also may be influenced by body size, whereby larger, more dominant species may impose top-down dominance on smaller species and where morphologically similar species are mutually competitive (McDonald 2002; Grassel et al. 2015). Understanding how these mechanisms influence the spatial distribution and temporal activity patterns of Meso-predator assemblages in the absence of apex predators is important for wildlife conservation and management (Johnson et al. 2001; Woodruffe 2001). Further, these mechanisms dictate the consideration of at least four dimensions associated with the ecological niche: resource requirements, competing species, space, and time (Chesson 2000).

North America hosts a diverse assortment of mammalian predatore species. In the Appalachian Mountains of Virginia, USA, these species vary in morphology, diet, habitat associations, and resource requirements (Chapman et al. 2007; Collins et al. 2007). Common mammalian preditors in this region include American black bear (*Ursus americanus*), bobcat (*Lynx rufus*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), raccoon (*Procyon lotor*), and striped skunk (*Mephitis mephitis*), and the Virgnia opossum (*Didelphis virginia*), a marsupial omnivore (Kelly and Holub 2008; Morin et al. 2016; Chapman et al. 2007; Pagels and Moncrief 2015). In addition, less common members of this predator community, regionally, include American mink (*Mustela vison*), eastern spotted skunk (*Spilogale putorius*), fisher (*Martes pennanti*), least weasel (*Mustela nivalis*), long-tailed weasel (*M. frenata*), northern river otter (*Lonta canadensis*), and red fox (*Vulpes Vulpes*, Chapman et al. 2007; Collins et al. 2007; Campbell et al. 2010; Pagels and Moncrief 2015). Of these species, bobcats, coyotes, gray foxes, raccoons, red foxes, spotted skunks, striped skunks, and Virginia opossums (hereafter, opossum) constitute the meso-predator guild.

In the Appalachians, bobcats and coyotes are the largest of the meso-predator guild, commonly weighing up to 20 kg each (Chapman 2007a; Trani and Chapman 2007). White-tailed deer (*Odocoileus virginianus*), voles (*Microtus* spp., *Myodes gapperi*), squirrels (*Sciurus* spp., *Glaucomys* spp.) and other rodents, and lagamorphs (*Sylvilagus* spp.) comprise the majority of bobcat and coyote diets though coyotes also consume soft mast (Chapman 2007; Trani and Chapman 2007; Morin et al. 2016). Both will occasionally prey on or kill, often without consuming, other meso-predator species, including raccoons, skunks, and opossums (Gehrt and Clark 2003; Kamler and Gipson 2004; Trani et al. 2007; Melville et al. 2015; Rose and Prange 2015; Cherry et al. 2016b). The remaining species in the meso-predator guild are generally smaller and more omnivorous or insectivorous. Body size varies among species, with raccoons ranging from 5 – 15 kg, red foxes from 2.7 – 7.8 kg, gray fox ranging from 2.5 – 5.7 kg, striped skunks from 1.2 – 5.5 kg, opossums from 0.9 – 5.9 kg, and spotted skunks up to 1 kg (Trani et al. 2007). In the Appalachians, the typical diet of these species overlaps greatly and consists mainly of small mammals, birds, insects and plant matter during most of the year, with a higher consumption of small mammals and carrion during winter months (Trani et al. 2007).

Meso-predators in this region use a similar variety of resources. Emergent rock, brush piles, hollow logs, and anthropogenic structures are common resting and den resources for each of these species (Berner and Gysel 1967; Kitchings and Story 1984; Crimmins et al. 2012). Additionally, foxes and skunks use underground burrows that have been excavated either by themselves or by other animals (Sullivan 1956; Houseknecht and Tester 1978; Samuel and Nelson 1982; Nicholson et al. 1985; Weller and Pelton 1987; Lesmeister et al. 2013).

Bobcats and gray foxes are associated with many forest types regionally, athough mature hardwood forests and early pine (*Pinus* Spp.) regeneration sites, and dense understory with high prey density sites tend to be preferred (Rolley and Warde 1985; Conner and Leopold 1993, 1996), particularly along forest edges for foxes (Golley 1966; Carey et al. 1978; Chamberlain and Leopold 2000). Coyotes, raccoons, opossums, red foxes, and striped skunks inhabit a diverse array of land-cover types, including forests, grasslands, swamps, rural, and urban environments

(Hamilton 1943; Verts 1967; Andelt 1977; Gardner et al. 1982; Gese and Beckoff 2004). In the Appalachian region, eastern spotted skunks are associated with rocky talus slopes and young forested areas with dense vegetative cover (Webster et al. 1985; Reed and Kennedy 2000; Thorne et al. 2017).

Overlap in resource and space use among meso-predators in Virginia is suspected, and may be possible through mechanisms of spatial and temporal avoidance, which enable coexistence between these similar, competitive species. Shared resource use may be particularly strong during winter when species' interactions concentrate around fewer seasonally available resources (Schoener 1974; Torretta et al. 2016; Humphries et al. 2017). Alternatively, if shared resources are not a limiting factor, or if species can vary resource and space use when shared resources are limited, similar species may co-occur more often than expected by random chance (Case and Gilpin 1974; Monterroso et al. 2016). Unfortuntely, snow cover and low air temperatures make winter research difficult, resulting in relatively few studies of winter activity and spatial occurrence patterns of small to medium-sized mammals (Schwingel and Norment 2010; Torretta et al. 2016; Shamoon et al. 2017). In this study, we sought to investigate whether, and to what extent, competitive coexistence and potential avoidance mechanisms may influence winter spatial distribution and temporal activity among meso-predators in the Appalachian Mountains of Virginia. As a byproduct of baiting for an eastern spotted skunk survey (Thorne et al. 2017), we found that winter baiting remotely-triggered cameras with road-killed deer carcasses enabled us to examine scavanging activity at an artificially provided, shared food resources. This approach allowed us an opportunity to study meso-predator competition and resource partitioning in space and through time.

Our objectives were to: 1) identify the general winter landscape-level habitat associations influencing probability of detection and site occupancy for each meso-predator species, 2) assess the effects of spatial co-occurrence on the probabilities of site occupancy and detection in pairwise combinations of sympatric species, 3) identify temporal shifts in species' diel activity patterns as a response to the presence of a potentially competing species at a scavenging site, and 4) estimate temporal overlap of diel activity periods by co-occurring species. We predicted that subordinate (usually smaller or more generalized) species would exhibit reduced probability of site occupancy and altered temporal activity at sites co-occupied by a dominant (usually larger or more specialized) species in order to minimize direct encounters between dominant and subordinate species (Glazier and Eckert 2002). Additionally, we predicted that because generalist predators are capable of exploitative competition (e.g., scavenging, prey-switching), that the probability of site occupancy by generalist meso-predators would increase at sites where highly carnivorous species co-occur, but that temporal activity patterns would be altered to avoid direct competition and aggressive interactions.

Methods

Study Area

We conducted our study on the George Washington and Jefferson National Forests (GWJNF) and neighboring private lands of cooperators in the central and southern Appalachian Mountains of Virginia, USA. This area lies within the Valleyand Ridge and Blue Ridge physiographic subprovinces and is characterized by long parallel mountain ridges of low to moderate elevations (< 326 – 1066 m) running from southwest to northeast, with some peaks reaching elevations from 1200 – 1436 m, and valleys of various widths. The area is mostly forested (67%), with interspersed pastoral, agricultural, residential, and urban areas; however, much of the public land consisted of large acreages of relatively unfragmented core forest

(Homer et al. 2015). A cool-temperate climate predominates, with an annual mean temperature of -10.6 to 27.5°C and annual rainfall of approximately 110 cm and occasional large snow events at higher elevations (National Oceanic Atmospheric Administration, public data 2015; www.noaa.gov).

Forest type varies with latitude, elevation, and slope exposure. Dominant forest types include oak (*Quercus* spp)-hickory (*Carya* spp), or mixed oak-pine (Pinus spp) on intermediate and xeric sites, whereas cove hardwoods at lower to mid-elevatons and northern hardwoods at higher elevations occur on mesic aspects. Scattered areas of red spruce (*Picea rubens*)-Fraser fir (*Abies fraseri*) communities occur at the highest elevations (Collins et al. 2007). White pine (*Pinus strobus*) and eastern hemlock (*Tsuga Canadensis*) overstories and dense rhododendron (*Rhododendron maximum*) shrub understories occur throughout riparian areas. Forest management activites in the George Washington and Jefferson national forests (GWJNF) include approximately 2,100 ha of timber harvest and 17,000 ha of prescribed burns. These activities account for approximately 13% of the 287,691 ha of forest designated for active management (U. S. Forest Service 2004, 2014). Throughout the GWJNF, approximately 11% of timber stands are aged < 50 years, while the majority for stands are aged 90 – 130 years (U.S. Forest Service 2004, 2014).

In addition to predatory species, depending on habitat type and condition, other common mammalian fauna include eastern cottontail (*Sylvilagus floridanus*), Appalachian cottontail (*Sylvilagus obscurus*), eastern chipmunk (*Tamias stiatus*), eastern gray squirrel (*Sciurus carolinensis*), eastern fox squirrel (*Sciurus niger*), red squirrel (*Tamisciurus hudsonicus*),southern flying squirrel (*G. volans*), Allegheny woodrat (*Neotoma magister*), white-tailed deer, and numerous species of Arvicolinae, Cricetidae, and insectivorous rodents, (Pagels and Moncrief 2015).

Sampling Methods

From January through April, 2014 and 2015, we conducted surveys using remotely-triggered, motion-activated cameras with infra-red light-emitting diode (LED) flash. We deployed 91 camera traps throughout the GWJNF and adjacent private lands (Figure 2.1). Each camera station consisted of one tree-mounted infra-red flash Bushnell Trophy Cam, Model 119436 (Bushnell, Overland Park, KS, USA), or Reconyx HyperFire HC500 (Reconyx, Holeman, WI, USA), camera baited with a single white-tailed deer carcass following the methods of Jachowski et al. (2015). Cameras were set to remotely trigger upon sensing movement with one-minute delay between photos. We used road-killed deer carcasses to simulate a natural ungulate death or a kill-site to attract scavenging carnivores, allowing for observations of interspecific use of a shared food source. Bait was staked to the ground using rebar approximately 3 – 4 meters infront of the camera and replenished at one-week intervals or as needed. We checked cameras at these times to ensure proper function and to change memory cards.

We used a random stratification design to distribute camera traps throughout three broadly characterized forest types proportionate to each type's occurrence within the study area (Gasaway et al. 1986). We characterized forest type at each camera trap as oak-dominated, non-oak hardwood, or mixed oak-pine forests using classification methods adapted from the Northeast Terrestrial Wildlife Habitat Classification System (Gawler 2008). Because this study was originally designed to estimate eastern spotted skunk occupancy (see Thorne et al. 2017), cameras traps were spaced at distances > 1.5 km apart, the diameter of the mean home range size of male eastern spotted skunks found by Lesmeister et al. (2013). To maximaze sample effort of each forest cover type, cameras were moved to new locations > 1.5 km away after a maximum of 2 months. Survey efforts were conducted from winter into early spring, when bears are not on the landscape due to hibernation, and when meso-predator detection rates are typically highest (Hackett et al. 2007; Crowley et al. 2014; Monterroso et al. 2014) and alternative food availability is low, to maximize detection probability. We assumed geographic closure (i.e., no migration) during the entire sample period for all species for each year (MacKenzie et al. 2002). We defined a sample occasion as a one-week (7-day) interval beginning from the day of deployment of each camera. We defined a species detection event as at least one photo-capture of the given species at an individual camera within a 7-day sampling occasion. Field survey work was approved by the Virginia Polytechnic Institute and State University Animal Care and Use Committee (protocol number 13-119-FIW).

We sampled several landscape-level habitat attributes that might influence site use by meso-predators. These attributes were based on remotely-sensed geographic information system (GIS) layers that we derived using ArcMap 10.2.2 (ESRI, Redlands, CA, USA). We used 30-m digital elevation models (DEM, U. S. Geological Survey 2000) to determine elevation in meters above sea level, degree of slope, and sine-transformed aspect at each camera trap. We created a 1.75-km^2 buffer around each camera trap based on the average male winter home range of the smallest species in our study, the eastern spotted skunk (Lesmeister et al. 2009). We used the zonal statistics tool in ArcMap to calculate a topographic exposure index (TEI) by subtracting average elevation within the buffer around each camera, with exposed TEI being indicated by higher TEI values (Evans et al. 2014; Ford et al. 2015). We calculated distance from camera traps to nearest primary or secondary road, permanent water source, and agricultural or pastoral area in ArcMap 10.2.2 using the 2011 National Land Cover Database (NLCD) classification system (Homer et al. 2015). We estimated percent tree canopy cover with each camera trap buffer using the 2011 NLCD Tree Canopy analytical data (U.S. Forest Service 2011). To determine stand age ($\bar{x}$ age of stand since last harvest or stand-replacing wildfire or prescribed fire) and stand size (area of distinct stand type) for each camera trap, we used the U. S. Forest Service landclass inventory data from the GWJNF (Simon 2013). We included sample year as a categorical variable to account for between-year differences in site occupancy resulting from unknown environmental factors or random effects that were not measured.

We acquired data on environmental factors, including minimum weekly temperature, total weekly rainfall, total weekly snowfall and maximum weekly snow depth, from the National Weather Service (www.weather.gov, accessed 25 May 2015) and percent moon illumination (proportion of moon surface illuminated) data from the United States Naval Observatory (http://aa.usno.navy.mil/data/docs/MoonFraction/php, accessed 25 May 2015). To account for equipment and battery failures, we included sampling effort, defined as the number of days that a camera trap was operational. Additionally, we included ordinal date as a covariate to account for within-year temporal variability. All environmental and habitat covariates were normalized and examined for multicollinearity using Spearman's rank correlation with an exclusion criterion of $|r_S| > 0.7$.

Single-species Occupancy

We identified the general landscape-level habitat associations and probability of site use for bobcats, coyotes, raccoons, striped skunks, spotted skunks, and opossums using a likelihood-based, information-theoretical modeling framework (MacKenzie 2006) to estimate detection probability (p) and probability of site occupancy (Ψ) for each species. We recorded 9-week detection histories (i.e., 9 encounter occasions) based on weekly detection or non-detection data from camera traps. We built single-season occupancy models using Program R (R Development Core Team 2014) with package *unmarked* (Fiske and Chandler 2011) to assess the influence of landscape-level habitat attributes as well as weather and other environmental factors on p and on Ψ for each species. We evaluated models using Akaike's Information Criterion corrected for small sample sizes (AIC_c) to determine the best-fit models for detection-non-detection data (Burnham and Anderson 1998). Models were considered to be competing if ΔAIC_c (difference between the AIC_c of the top model and the AIC_c of the ith model) was < 2.0 for this and all subsequent analyses.

To determine the effect size of environmental covariates on the detection probability of each species, we then constructed sets of 12 univariate *a priori* detection models while holding Ψ constant. We combined covariates from competing models to create multivariable *post-hoc* models for each species to further improve model fit. Next, we used the covariates identified in the best-supported p models to build a similar suite of *a priori* and *post-hoc* Ψ models to identify the important landscape-level habitat attributes that influence site occupancy for each species. Additionally, we assessed goodness-of-fit of the most parameterized (global) model using a 10,000-iteration parametric bootstrap analysis to ensure that the underlying model fit the data (MacKenzie et al. 2002).

Two-species Occupancy

To assess the effect of presence of one species on probability of detection and site use of a second species, we also conducted two-species co-occurrence analyses for each species pair. We used body size classes presented by Brown and Nicoletto (1991) to categorize similar-sized and different-sized species pairs. Our similar-sized pairs included bobcat–coyote, bobcat–

raccoon, coyote–raccoon, striped skunk–spotted skunk, striped skunk–opossum, and spotted skunk–opossum. Different-sized pairs included bobcat–striped skunk, bobcat–spotted skunk, bobcat–opossum, coyote–striped skunk, coyote–spotted skunk, coyote–opossum, raccoon–striped skunk, raccoon–spotted skunk, and raccoon–opossum. This two-species occupancy approach models the probability of detection and occupancy status of one species as a function of the detection and occupancy status of the other in the species pair (Table 2.1), while also incorporating the effects of habitat attributes and environmental factors found to be important in single species modeling (MacKenzie 2006). We used the parameterization method of Richmond et al. (2010) which incoproates the relationship between subordinate and dominant species on the probability of co-occurance. When the species interaction factor (ϕ) of a species pair < 1, the two species are considered to co-occur less often than by random chance (spatial avoidance). Whereas when $\phi > 1$, two species are considered to co-occur more often than by random chance. If $\phi = 1$ or if the Confidence Intervals (CIs) of ϕ overlap 1.0, the two species are considered to occur independently of each other. Likewise, when the species co-detection factor (δ) < 1, both species will be detected at the same site less often than by random chance given that both species are present. If $\delta > 1$, both species will be detected at the same site more often than by random chance. If $\delta = 1$ or if the confidence interval overlapped 1.0, the detection probabilities of each species are independent of the other.

We constructed a set of 12, two-species occupancy models (Table 2.2) and carried over the best detection and occupancy covariates for each species in the single season models to test several hypotheses about pair-wise meso-predator spatial avoidance. Competing models were averaged to produce estimates of detection probability (p), site-use probability (Ψ), and species interaction factor (ϕ). For differently-sized pairs, we predicted that detection probability of smaller (subordinate) species (denoted B) would be lower at co-occupied sites ($p^{B} > r^{Ba} > r^{BA}$) and $\delta < 1$ resulting from modified activity patterns of the subordinate species in the presence of a dominant species, whereas detection probability of larger (dominant) species (denoted A) would be unaffected by the presence of a subordinate species ($p^{A} = r^{A}$) (Hayward and Slotow 2009). It should be noted that capital A or B denotes sites with the species present, while lower case a or b indicates sites without the species present. We also predicted that probability of species A is present ($\Psi^{BA} < \Psi^{Ba}$) and ϕ <1) for differently-sized species pairs, as subordinate species would spatially avoid dominant species in order to reduce the risk of intraguild predation or agonistic interactions (Dröge et al. 2017). For like-sized pairs, we predicted that detection probability will decrease for both species in each pair ($p^{A} > r^{A}$ and $p^{B} > r^{BA}$) and $\delta < 1$. Additionally, we expected $\Psi^{BA} < \Psi^{Ba}$ for the more generalist (subordinant) species of the pair and ϕ <1 because generalist carnivores are more capable of altering resource use to avoid competition and agonistic interactions than more specialized carnivores (Sollmann et al. 2012). Our analyses enabled us to determine whether species interactions alone, habitat alone, or combinations of both, better predicted site use and detection within species pairs.

Temporal Overlap and Activity Changes

We investigated the potential for temporal avoidance among species pairs using the timestamp recorded on remote camera images and Program R package *overlap* version 0.3.0 (Ridout 2017). To evaluate potential changes in activity patterns of a given species when occurring with another competing species, we divided data into two categories for each species within a species pair: 1) sites where dominant species A and subordinant species B were co-detected; and 2) sites where subordinate species B was detected but where dominant species A was not detected. We followed the approach of Ridout and Linkie (2009) to fit non-parametric kernel density curves to compare activity patterns of each species at sites where a second species was present versus sites where the given second species was not present. We classified activity patterns based on percentage of detection that occurred at night: diurnal (< 10%), mostly diurnal (10 – 30%), nocturnal (> 90 %), mostly nocturnal (70 – 90%), crepuscular (50% during crepuscular period), and cathemeral (active both day and night) following van Schaik and Griffiths (1996) and Gomez et al. (2005). We used the non-parametric Mardia-Watson-Wheeler test (Wheeler and Watson 1964) in Program R package *circular* version 0.4-93 (Lund et al. 2017) to test for homogeneity in the activity curves of each species occurring both with and without the other competing species. We used a Bonferroni-corrected alpha value with a $p \leq 0.003$ critical significance level to account for multiple test comparisons and to avoid Type I errors. To assess potential reduction of temporal overlap when occurring together, we calculated and compared the coefficient of overlap for small sample sizes ($\hat{\Delta}_1$) of activity curves for each species pair when they occurred together versus when they occurred separately. We obtained 95% confidence intervals for $\hat{\Delta}_1$ using 1000 bootstrap samples (Linkie and Ridout 2011). We considered overlap estimates to be significantly different when confidence intervals surrounding $\hat{\Delta}_1$ were non-overlapping.

Results

Landscape-level and Environmental Covariates

Over our survey effort, we detected 14 mammalian carnivore species, 10 other mammal species, and 16 avian species during 5,642 camera-trap nights. We collected 6,524 photos of carnivores, including bobcats (2,413), raccoons (1,245), coyotes (1,089), striped skunks (682), and spotted skunks (438). Additionally, we collectged 657 photos of opossums. Other carnivores detected at low frequencies included American black bears, gray foxes, red foxes, long-tailed weasels, American minks, domestic dogs (*Canis lupis familiaris*), and domestic cats (*Felis catus*). Survey period length varied among sites and ranged from 2 – 9 weeks ($\bar{x}$ = 5.84 ± 0.21 weeks) per site due to inclement weather preventing access to some sites for rebaiting and occasional equipment malfunctions. Oak-dominated forest was the primary forest type at 80% of the camera trap locations, followed by non-oak hardwood at 14%, and mixed oak-pine at 6%. Canopy cover was relatively similar across the study area, ranging from 81.7% – 98.9% ($\bar{x}$ = 92.1 ± 0.04%). Elevation ranged from 349.0 m – 1,468.1 m ($\bar{x}$ = 942.7 ± 22.3 m). Topographic exposure index (TEI) ranged from -75.1 – 107.3 ($\bar{x}$ = 8.2 ± 4.6). Forest stand age ranged from 26 – 164 years ($\bar{x}$ = 91.9 ± 3.1 yr) and delineated stand size ranged from 2.4 ha – 231.0 ha ($\bar{x}$ = 49.4 ± 5.1 ha), although the continuous forest patch size was > 1,000 ha in most areas throughout the GWJNF. Camera traps were located a mean distance of $\bar{x}$ = 5.4 ± 0.3 km from the nearest primary road, located a mean distance of $\bar{x}$ = 2.1 ± 0.1 km from the nearest permanent water source, and located a mean distance of $\bar{x}$ = 2.4 ± 0.2 km from the nearest agricultural area. Temperatures throughout the study ranged from -20.83 °C to 23.33 °C ($\bar{x}$ = 4.36 ± 0.004 °C), with a mean weeky total precipitation of $\bar{x}$ = 7.23 ± 2.1 cm, 75% of which was snowfall, and mean maximum weekly snow depth of 4.67 ± 0.92 cm. Average weekly percent moon illumination ranged from 5.71 – 96.43% ($\bar{x}$ = 52.98 ± 3.20%).

Single-species Occupancy

We estimated probabilities of detection and site occupancy for 6 of the mesopredator species: bobcats, coyotes, raccoons, striped skunks, spotted skunks, and opossums. Gray and red foxes were detected too infrequently to generate robust detection or occupancy estimates. Coyotes occurred at 57.1% of camera traps, bobcats at 45.1% of camera traps, raccoons at 39.6% of cameras traps, spotted skunks at 19.8% of camera traps, and striped skunks and opossums each at 17.6% of camera traps. Detection probability (*p*) estimates for all species was generally low and ranged from 0.16 for spotted skunks to 0.37 for opossums (Table 2.3). Bobcat and coyote detection both decreased over time with survey effort. However, coyote detection probability increased with total weekly rainfall. Raccoon detection probability decreased with total weekly snowfall and minimum weekly temperature. Striped skunk and spotted skunk detection probabilities both increased with decreasing percent moon illumination, and increased with total rainfall and total snowfall, respectively. There was no evidence to support an effect of any measured environmental covariate on opossum detection probability (Supplementary Table 2.1).

Estimates of site occupancy (Ψ) were greater than naïve occupancy (proportion of camera traps where a given species was detected in the field) for all species (Table 2.3). Occupancy estimates were similar among the three larger species (i.e., bobcat, coyote, and raccoon), ranging from 0.51 for raccoons to 0.68 for coyotes, and were more than twice that of the smaller species (i.e., striped skunk, spotted skunk, and opossum). Occupancy estimates for smaller species ranged from 0.21 for spotted skunks and opossums to 0.24 for striped skunks.

The null occupancy and detection model, in which Ψ and *p* were held constant with respect to landscape-level habitat attributes and atmospheric covariates, was a competing model for Virginia opossums only. For all other species, the null model was either the lowest-ranked model or produced a $\Delta AIC_c > 2.0$ (Supplementary Table 2.2). The top-ranked Ψ model for bobcats contained no landscape-level covariates but indicated a difference in Ψ between sample years, with higher Ψ in 2015 (β = 1.24, SE = 0.61). Three coyote Ψ models were competing with $\Delta AIC_c < 2.0$. We averaged the two highest-ranked of these models (cumulative $w_i = 59\%$) to produce Ψ with a weighted effect of distance to agricultural land (β = -0.48, SE = 0.17) and percent canopy cover (β = 0.37, SE = 0.13) on Ψ. Raccoon Ψ was best predicted by the top detection model, with no landscape-level covariates influencing Ψ. Striped skunk Ψ increased with slope steepness (β = 1.15, SE = 0.375). Three spotted skunk Ψ models produced ΔAIC_c < 2.0, one of which was a nested model and not considered a competing model. We averaged the two top-ranked models to estimate spotted skunk Ψ with a weighted effect of forest stand age (β = -0.32, SE = 0.09) and elevation (β = -0.33, SE = 0.13).

Two-species Occupancy

Number of co-detections varied by species pair throughout the survey period (Table 2.4). Bobcats were co-detected with coyotes at 23.1% of locations, with raccoons at 14.3% of locaitons, and with striped skunks, spotted skunks, and opossums at 7.7% of locations each. Coyotes were co-detected with raccoons at 24.6% of camera traps, with striped skunks at 9.9% of traps, with spotted skunks and opossums at 11% of traps each. Raccoons were co-detected with striped skunks and opossums at 7.7% of camera traps and with spotted skunks at 5.5% camera traps. Striped skunks were co-detected with spotted skunks at 2.2% of camera traps and with opossums at 5.5% of camera traps. Spotted skunks and opossums were co-detected at only one camera trap (1.1%).

We averaged all competing models within a model set (Supplementary Table 2.3) to produce estimates of p and Ψ for all species pairs, except for the bobcat–opossum model set for which there were no competing models (Table 2.5). Of the different-sized pairs, only the bobcat–opossum pair showed significant decrease in opossum detection probability from p^{B} = 0.70 (SE=0.17) where not co-occurring to r^{BA} = 0.28 (SE=0.06) and r^{Ba} = 0.28 (SE=0.06). Striped skunks showed a decreasing trend where bobcats were present however, 95% confidence intervals surrounding the occupancy estimates overlapped. Opossum detection probability showed an increased trend in the presence of both coyotes and raccoons; however, confidence intervals of p^B and r^B overlapped in both cases. There were no significant differences between p^A and r^A for any of the different sized-species pairs, except for raccoon–striped skunk with a decrease from $p^A = 0.27$ (0SE = 0.05) where striped skunks were not detected to $r^A = 0.12$ (SE = 0.05) when co-detected. For most different-sized species pairs, δ was either equal to 1 or the 95% confidence intervals contained 1. However, the co-detection factor for the bobcat–striped skunk pair was $\delta = 1.39$ (SE = 0.03) and for the raccoon–opossum pair was $\delta = 0.79$ (SE = 0.06). The co-detection factor for like-sized pairs was greater than1 for the opossum-striped skunk pair (δ = 1.13, SE 0.06), whereas all other did not differ from 1. Of the like-sized species pairs, only the spotted skunk–opossum pair showed a significant decrease in opossum detection probability from $p^B = 0.41$ (SE = 0.06) when not co-occurring with spotted skunks to $r^{BA} = 0.12$ (SE = 0.04) and $r^{Ba} = 0.12$ (SE = 0.04) where co-occurring with spotted skunks. Probability of occurrence of opossums decreased significantly from 0.33 (SE = 0.09) where bobcats were not detected to 0.09 (SE = 0.02) where the two species co-occurred, whereas there was no significant decrease in probability of occurrence for any other different-sized or like-sized pair. The estimated ϕ was less than 1 for the raccoon–spotted skunk pair only ($\phi = 0.86$, SE = 0.07), while $\phi > 1$ for the bobcat–opossum, coyote–striped skunk, and striped skunk–spotted skunk pairs. The estimate of ϕ for all other pairs was either equal to 1 or the 95% confidence interval contained 1 (Table 2.5).

Temporal Avoidance: Shifts in Activity Patterns

We found significant shifts in activity periods where large-bodied, like-sized species co-occurred with other species (Figure 2.2). Where coyotes were not detected, bobcats were most active during crepuscular periods and when co-detected, bobcats showed a more sporadic, cathemeral activity pattern ($U^2 = 1.44$, $p < 0.001$). Activity patterns of coyotes changed from crepuscular at traps where bobcats were not detected to nocturnal when co-detected (U^2 = 2.26, p < 0.001). Bobcats showed a mostly crepuscular activity pattern when raccoons were not detected, in contrast to a mostly nocturnal pattern with a high activity peak in early evening when raccoons were present (U^2 = 6.39, p < 0.001). When not co-detected with bobcats, raccoons showed a mostly nocturnal activity pattern, with the greatest peak in activity around sunset and changed to nocturnal with greatest activity peak around sunrise when co-detected (U^2 =3.81, p < 0.001).

Where raccoons were not detected, coyotes exhibited a crepuscular activity pattern which shifted to nocturnal where raccoons were present (U^2 = 3.81, p < 0.001). The activity pattern of raccoons changed from nocturnal with a peak around sunrise where coyotes were not detected to nocturnal with a peak around sunset where coyotes were present (U^2 = 1.05, p < 0.001).

Significant shifts in activity periods also were found for most small like-sized species pairs (Figure 2.3). In the absence of opossum detections, striped skunks were nocturnal, with peak activity occurring around sunset (U^2 = 2.02, p < 0.001). When opossums were present, peak striped skunk activity shifted to around midnight. Opossum activity changed from a mostly diurnal pattern where striped skunks were not detected, to a cathemeral pattern where striped skunks were detected (U^2 = 2.64, p < 0.001). Opossums shifted from a cathemeral pattern to a diurnal pattern when co-detected with spotted skunks (U^2 = 6.07, p < 0.001). However, spotted skunks showed no change in activity when co-detected with opossums.

Almost all species in the large-bodied pairs shifted their activity periods when co-detected with a smaller species (Figure 2.4); however, not all small-bodied species shifted activity when co-detected with a larger species (Figure 2.5). Bobcats showed a cathemeral activity pattern in the absence of striped skunk detections, but shifted to mostly nocturnal with peaks in the crepuscular periods when co-detected with striped skunks (U^2 = 4.59, p < 0.001).

When occurring without spotted skunks, bobcats were mostly nocturnal, with some activity during midday, and when occurring together bobcats were mostly cathemeral ($U^2 = 0.89$, $p < 0.001$). Neither striped skunks nor spotted skunks exhibited significant shifts in activity patterns when co-detected with bobcats. Bobcats were mostly nocturnal with some daytime activity at camera traps where opossums were not detected, but showed a strong nocturnal activity pattern with peak activity around midnight at camera traps where opossums were co-detected ($U^2 = 4.91$, $p < 0.001$). Conversely, opossums detected at camera traps without bobcats and shifted from a cathemeral pattern to strongly diurnal when co-detected with bobcats ($U^2 = 3.57$, $p < 0.001$).

Coyotes shifted from crepuscular to nocturnal when co-detected with striped skunks ($U^2 = 3.28$, $p < 0.001$), and striped skunks shifted from nocturnal to a peak activity period between late afternoon and midnight ($U^2 = 2.94$, $p < 0.001$). When co-detected with spotted skunks, coyote activity did not significantly change ($U^2 = 0.16$, $p > 0.003$). When co-detected with coyotes, spotted skunks exhibited a crepuscular pattern. There were too few observations of spotted skunks detected at camera traps without coyotes determine a shift in activity patterns; however, the activity pattern observed at the one camera trap where there was no co-detection showed greater activity from midnight to sunrise with reduced activity from sunset to midnight. When not co-detected, coyote activity was nocturnal and shifted slightly to mostly nocturnal with a small peak in activity around midday when co-detected with opossums ($U^2 =0.66$, $p <0.001$). Opossum activity shifted from cathemeral to diurnal when co-detected with coyotes ($U^2 = 2.73$, $p < 0.001$). Where striped skunks were not detected, raccoons were mostly nocturnal. This changed to nocturnal with the greatest peak in activity around sunset when striped skunks were co-detected ($U^2 = 4.16$, $p < 0.001$). Striped skunks exhibited a moderately crepuscular activity pattern when not co-detected with raccoons; however, the pattern shifted slightly to more nocturnal when raccoons were detected ($U^2 = 0.45$, $p < 0.001$). Raccoons detected without spotted skunks exhibited a mostly nocturnal activity pattern. Spotted skunks occurring at traps where raccoons were not detected exhibited a moderately crepuscular pattern with a greater peak of activity between midnight and sunrise. Raccoons shifted to peak activity from early evening to midnight ($U^2 = 3.87$, $p < 0.001$), and spotted skunks exhibited no significant change in activity. When co-detected, raccoons shifted from a mostly nocturnal activity pattern with greater activity before sunrise to a nocturnal activity pattern with greater activity around sunset when opossums were present ($U^2 = 1.79$, $p < 0.001$). Opossums shifted from a strongly diurnal to a cathemeral pattern when co-detected with raccoons ($U^2 = 3.67$, $p < 0.001$).

Temporal Avoidance: Overlap of Activity Patterns

The activity periods of different-sized species pairs generally overlapped less when the two species in the pair were co-detected than when they were not co-detected. Bobcats and opossums exhibited a high degree of overlap in activity periods when occurring separately ($\hat{\Delta}_1 =$ 0.63, 95% CI: 0.56, 0.72), yet the overlap decreased significantly when the two species occurred together ($\hat{\Delta}_1 = 0.17$, 95% CI: 0.16, 0.21). Similarly, overlap decreased between opossums and coyotes from $\hat{\Delta}_1 = 0.39$ (95% CI: 0.34, 0.58) when occurring apart to $\hat{\Delta}_1 = 0.18$ (95% CI: 0.15, 0.23) when occurring together, and between opossums and raccoons from $\hat{\Delta}_1 = 0.70$ (95% CI: 0.49, 0.87) when occurring apart to $\hat{\Delta}_1 = 0.90$ (95% CI: 0.07, 0.11) when occurring together. Raccoons and spotted skunks exhibited the highest overlap in activity periods of all species pairs ($\hat{\Delta}_1 = 0.81$, 95% CI: 0.75, 0.86) when occurring separately. However, when occurring together, overlap between raccoon and spotted skunk activity periods decreased to $\hat{\Delta}_1 = 0.44$ (95% CI: 0.36, 0.53). Overlap between coyotes and striped skunks decreased from $\hat{\Delta}_1 = 0.67$ (95% CI: 0.64, 0.74) when the two species occurred separately, to $\hat{\Delta}_1 = 0.59$ (95% CI: 0.505, 0.53) when they occurred together. Small differences were observed between bobcats and spotted skunks when separate $\hat{\Delta}_1$ = 0.59, 95% CI: 0.55, 0.64) and together ($\hat{\Delta}_1$ = 0.49, 95% CI: 0.44, 0.56) and between coyotes and bobcats when separate ($\hat{\Delta}_1$ = 0.71, 95% CI: 0.59, 0.81) and together ($\hat{\Delta}_1$ = 0.666, 95% CI: 0.60, 0.73), although confidence intervals overlapped in each case. No differences in overlap of activity periods were found for bobcats and striped skunks or raccoons and striped skunks when occurring separately or together. Additionally, no differences were found for the bobcat-coyote and bobcat-raccoon pairs. However, overlap of activity periods between the striped skunk-spotted skunk pair decreased from $\hat{\Delta}_1$ = 0.71 (95% CI: 0.66, 0.77) to $\hat{\Delta}_1$ = 0.51 (95% CI: 0.45, 0.63) when co-detected and overlap estimates of spotted skunks and opossums decreased from $\hat{\Delta}_1$ = 0.36 (95% CI: 0.36, 0.49) to $\hat{\Delta}_1$ = 0.06 (95% CI: 0.04, 0.08). Overlap estimates for the coyote-raccoon and striped skunk-opossum pairs increased from $\hat{\Delta}$ = 0.60 (95% CI: 0.56, 0.67) to $\hat{\Delta}_1$ = 0.74 (95% CI: 0.71, 0.79) and from $\hat{\Delta}_1$ = 0.17 (95% CI: 0.14, 0.20) to $\hat{\Delta}_1$ = 0.81 (95% CI: 0.70, 0.91), respectively.

Discussion

When occurring sympatrically, predatory species may experience higher levels of resource competition, creating a need for mechanisms that promote coexistence through minimization of competition (Amarasekare 1966; Chesson 2000; Creel et al. 2001; Ritchie and Johnson 2009). Our findings support our predictions that sympatric meso-predators would modify activity periods when occurring together. Although our results did not suggest that meso-predators in the Appalachian region are spatially segregated, we observed adaptability in activity patterns at scavenging sites that may enable coexistence and minimize some aspects of competition among meso-predators despite overlap in resource use.

Single-species Occupancy

The negative association of survey effort with bobcat and coyote detection probability may represent a behavioral response, i.e. trap shyness, i.e., aversion to the infrared flash or noise produced by the camera when a picture is taken, as seen other camera studies (Per Wegge et al. 2004; Lesmeister et al. 2015) or decreasing availability of bait over time. The increased detection of coyotes during periods of greater rainfall may be in response to increased prey availability, as rodent and amphibian activity has been found to increase with precipitation (Stokes et al. 2001; Otto and Roloff 2011). Conversely, snow and cold temperatures have been shown to influence the behavior and activity patterns of many mammalian species (Martin 1983; Pierce and Vogt 1993; Merritt and Zegers 2002). Some small-bodied mammal species experience difficulty traversing deep substrates such as snow (Shepard et al. 2013). Additionally, many species reduce activity periods (Martin 1983) or enter prolonged periods of torpor (Pierce and Vogt 1993) to conserve energy. Although rainfall may lead to increased detection of carnivores, snowfall and cold temperatures may have contrasting effects. Difficulty moving through snow and limited access to prey species likely explain the observed variation in raccoon detectability during periods of greater snowfall and why raccoon detection probability was lower during periods of lower ambient temperatures. Sharp and Sharp (1956) attributed similar reduction in activity of raccoons at winter feeding stations on nights with ambient temperature lower than 5°C and following snow events to periods of semi-hibernation. Similarly, Johnson (1970) observed semi-hibernation denning behavior by raccoons in Alabama during periods of unusally harsh weather. Along with the effect of temperature on small mammal activity, reduced activity on bright nights is typical of small-bodied mammals, particularly those susceptible to nocturnal predators (Wolfe and Tan Summerlin 1989; Reed and Kennedy 2000; Orrock et al. 2004; Kotler et al. 2010) and more so under snowy conditions (Griffin et al. 2005), which may account for decreased striped and spotted skunk detection with increased percent moon illumination, as spotted and striped skunks are common prey items of nocturnal avian predators (Lesmeister et al. 2010; Errington et al. 2017). although energy expenditure is typically higher during inclement winter conditions, predation risk may be lower on cloudy nights and during bad weather. This may further explain the observed variation in detection probability of striped skunks and spotted skunks in relation to rainfall and snowfall, respectively. Constant detection probability for Virginia opossums was consistent with the findings of Cove et al. (2012) and was not surprising considering their behavioral plasticity and adaptability relative to other meso-predators (McManus 1974) and ability to withstand cold ambient temperatures and heavy snowfall (Kanda 2005; Kanda et al. 2005).

Bobcat, coyote, and raccoon occupancy estimates were relatively high compared to other species in the study. These are the largest species in the study area and typically maintain large home ranges (Morin 2015; Owen et al. 2015; McNitt et al. 2019). Therefore coyotes, bobcats, and raccoons may be generally move more across the landscape and thus are easier to detect. Additionally, camera spacing was originally set with the intention of collecting independent eastern spotted skunk detection. As larger-bodied species typically move larger distances, detections of obcats, coyotes, and raccoons across multiple camera stations may be more likely and may not be independent. Estimates of predicted site use for bobcats in GWJNF were were higher than in the northeastern United States. (Long et al. 2011), however they were similar to occupancy estimates in the midwestern and western portions of the United States (Clare et al. 2015; Lesmeister et al. 2015, Lewis et al. 2015). Similar to our study in a comparable forested landscape, Lesmeister et al. (2015) found that bobcat occupancy was not influenced by habitat attributes, but was strongly influenced by anthropogenic landscape features. Locally, in our study areas, bobcats also avoid anthropogenic influences on the landscape (Kelly and Holub 2008). Although our models containing anthropogenic covariates (i.e., distance to roads and agricultural areas) were not competing, these models were among the best, lowest ranked, with $\Delta AIC_c < 4$, and therefore had some empirical support (Supplemarty Table 2.2). Sample year was the greatest predictor of bobcat occupancy, with greater probability of occupancy in 2015. We attributed this difference to random effects between years or unmeasured environmental variables that may have influenced bobcat survival and abundance, such as prey abundance (Litvaitis et al. 2006).

As a generalist species, coyotes have been associated with various habitat types throughout North America, including agricultural, pastoral, forested and urban habitats, with variable and occasionally contradictory patterns (Gese and Beckoff 2004; Crimmins et al. 2012; Lesmeister et al. 2015; Mitchell et al. 2015; Cherry et al. 2016a). AltThough our study occurred on predominately forested areas, the higher predicted site use of coyotes within areas of increased canopy covered located nearer to agricultural and pastoral areas and primary or secondary roads was consistent with studies of forest edge use in the eastern United States (Lesmeister et al. 2015; Mitchell et al. 2015; Cherry et al. 2016a).

Neither raccoons nor opossums were associated with any of the measured occupancy covariates. Raccoons and opossums are highly adaptive generalist species, and the lack of association of any specific site use covariates with these two species is likely reflective of their use of a variety of habitat and resource types throughout the Appalachian Mountains (Kissell and Kennedy 1992). Striped skunk occupancy was associated with increasing slope steepness, and striped skunks are known to use a variety of habitat types throughout the Appalachian region (Chapman 2007b). Regionally, emergent rock outcrops found along steep slopes at high elevations typically lack dense overstory canopy cover, but often are associated with dense woody shrub understory that may offer a potential refuge from avian and terrestrial predators, such as owls, bobcats, coyotes, and domestic dogs. Similarly, predator avoidance also may explain the association of eastern spotted skunk occupancy with forest stand age and elevation, as timber harvest and prescribed burning forest management practices alter forest structure by promoting thick woody regeneration that provides dense cover and abundant soft mast production (Signell 2005, Small and McCarthy 2002). Complex forest structure in the form of dense understory species and tree saplings are characteristic of these younger forests (approximately 20 to 50 years old) and offer protection from avian and large terrestrial predators that have difficulty negotiating the thick vegetation (Lesmeister et al. 2013, Miller et al 2009).

Spatial Co-occurrence

The results of our spatial co-occurrence analysis supported our prediction that detection probability of a larger species within a pair would remain unchanged; however, a reduction in detection probability of a smaller species was found only for the bobcat-opossum pair. This finding may be attributed to the propensity of bobcats to prey on opossums more often than on other small carnivores (Fritts and Sealander 1978; Story et al. 1982). Additionally, the co-detection factor was less than one for only the raccoon-opossum pair and was greater than one for the bobcat-striped skunk pair, although this may be an artifact of a small number of co-detections between raccoons and opossums and bobcats and striped skunks.

Similarly, our prediction of a change in probability of occurrence of the small species in a pair was supported by the large reduction in probability of site occupancy by opossums when co-occurring with bobcats and a general trend of reduced probability of occupancy by smaller species co-occurring with coyotes. The spatial avoidance of the larger meso-predators by opossums, striped skunks, and potentially spotted skunks supports the mechanism of intraguild predation or killing of competitors, as bobcats and coyotes are known predators of these species (Fritts and Sealander 1978; Story et al. 1982; Swingen et al. 2015). Contrary to our predictions, the species co-detection factor was either equal to 1 or greater to 1 for like-sized species pairs, only opossum detection probability decreased when in the presence of spotted skunks, and no differences in probability of occurrence were detected for either species of the all like-sized pairs. The overall lack of spatial avoidance among meso-predators in this study was not wholly unexpected. These species often are considered to be generalists and may be variable enough in their resource use to limit competition with other species occupying the same area. Simlilar lack of avoidance was observed in the southeastern (Ladine 1998) and midwestern (Lesmeister et al. 2015) United States.

Temporal Avoidance

We correctly predicted that the more omnivorous (resource generalist) species of the pair would alter its temporal activity period when co-occurring with a more dominant species. However, for most like-sized pairs, both species in the pair exhibited significant shifts in activity patterns. Our observations suggest that rather than a more generalist species altering its behavior to avoid a more specialized species, both will employ temporal avoidance as a mechanism to limit direct competition and agonistic interactions over a shared resource. Similarly, for most different-sized pairs, both species in each pair exhibited shifts in activity periods when co-detected with another species. Striped and spotted skunks exhibited the lowest temporal avoidance (highest delta) among species. In the case of mephitids, the pressure to avoid agonistic interactions with other species may be reduced by anti-predator chemical defense (Lartviere and Messier 1996).

The general observed reduction of overlap in the temporal activity periods of different-sized pairs when co-occurring supports our prediction that temporal avoidance may be a mechanism for smaller meso-predators to avoid agonistic interactions with, and possible intraguild killing by, larger competing species. This pattern has been well documented in many systems (Ramesh et al. 2017; Davis et al. 2018; Haidir et al. 2018). However, the lack of reduced overlap of activity periods of species in like-sized pairs was surprising given that most species significantly shifted their activity patterns when co-occurring with another like-sized species. In this case, we must consider the nocturnal nature of these species. Each of these species is generally active between sunset and sunrise, which limits the extent that an individual or species may alter its behavior without critically reducing its ability to efficiently acquire resources (Cozzi et al. 2012; Mugerwa et al. 2017). Opossums, however, are the most generalist species of the meso-predator guild in the Appalachian region and they exhibited the most drastic change in temporal activity (nocturnal to diurnal) when co-occurring with nearly every other meso-predator species. Although mostly nocturnal, opossums can be active in the daytime, especially during winter when night temperature limit mobility (Gardner et al. 1982). Additionally, opossums acquire food resources through both hunting, scavenging, and foraging (Gardner et al. 1982). This behavioral plasticity may enable opossums to alter their activity patterns to avoid intraspecific interactions without reducing foraging efficiency.

Phylogenetic similarities may also play a role in shaping meso-predator communities, where species within the same family may experience greater levels of resource overlap and competition than more distantly related species (Humphreys and Barraclough 2014). Striped skunks and spotted skunks are closely related species and use similar resources, therefore it is likely that the more direct resource competition between striped and spotted skunks may be an explanation for why reductions in activity period overlap were found between this species pair and not between other pairs, such as bobcat-coyote or coyote-raccoon. Temporal avoidance may likely accommodate the competitive coexistence of the two species of skunks to reduce direct and indirect competition.

The competitive relationships between sympatric species are complex and variable. Although our results revealed little evidence of spatial avoidance among our study species in relation to habitat characteristics, we found complex diel activity pattern relationships suggestive of temporal avoidance. We hypothesize that the large home ranges of some species, such as bobcats and coyotes, in relation to the spacing of our cameras coupled with the attrivness of bait may artificially reduce spatial avaoidance. However, we postulate that temporal avoidance is used instead as a mechanism for competitive coexistence within the meso-predator guild in the Appalachian region of Virginia. We advise that future studies expand on conventional camera-trapping methods and include video and audio recording to assess rates of agonistic behavior during direct intraspecific interactions at the bait source to further understand competitive interactions among carnivore species and the effect of a bait source on behavior and activity. It is also important to note that detection probability is typically higher for larger, wider-ranging species (e.g., bobcat, coyote) and lower for small, less mobile or more cryptic species (e.g., striped skunks, spotted skunk) when using remote-triggered cameras in carnivore surveys (Gompper et al. 2006; Reed 2011). Increased observation time or number of camera traps may be necessary in future studies to adequately estimate temporal activity patterns of small and difficult-to-detect species to better understand the competitive relationships between rare and common, or small and large, meso-predators.

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Table 2.1. Notation and definition of parameters used in the single-season, two-species co-occurrence models, adapted from MacKenzie (2006).

Parameter	Definition
Ψ^{A}	Probability that site is occupied by species A[1]
Ψ^{BA}	Probability that site is occupied by species B, given species A is present
Ψ^{Ba}	Probability that site is occupied by species B, given species A is absent
ϕ	Species interaction factor (SIF)
p^{Ab}	Probability of detecting species A, given species B is absent
r^{AB}	Probability of detecting species A, given species B is present
p^{Ba}	Probability of detecting species B, given species A is absent
r^{BA}	Probability of detecting species B, given species A is present and detected
r^{Ba}	Probability of detecting species B, given species A is present but not detected
δ	Species co-detection factor

[1] Note: Species A is assumed to be dominant and species B subordinate. Capitalized letters refer to the species being present or detected at a site, while lower case indicate not present or not detected.

Table 2.2 Set of 12 single-season, two-species co-occurrence models developed to investigate the spatial relationship between 5 mesocarnivore species in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.

Model		Definition
Ψ^A, Ψ^B	p^A, p^B	Ψ and p are independent.
$\Psi^A, \Psi^{B,}$	p^A, p^B, r^A	Ψ is independent, p of species A is dependent on presence of species B.
Ψ^A, Ψ^B	$p^{A,} p^B, r^A, r^B$	Ψ is independent, p of both species is dependent on presence, but not detection of the other species.
Ψ^A, Ψ^B	$p^A, p^B, r^A, r^{BA}, r^{Ba}$	Ψ is independent, p of species A is dependent on presence of species B, p of species B is dependent on presence and detection of species A.
$\Psi^A, \Psi^{B,}$	p^A, p^B, r^B	Ψ is independent, p of species B is dependent on presence but not detection of species A.
Ψ^A, Ψ^B	p^A, p^B, r^{BA}, r^{Ba}	Ψ is independent, p of species B is dependent on presence and detection of species A.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	p^A, p^B	Ψ of species B is dependent on presence of species A, p of both species is independent.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	p^A, p^B, r^A	Ψ of species B is dependent on presence of species A, p of species A is dependent on detection of species B.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	p^A, p^B, r^A, r^B	Ψ of species B is dependent on presence of species A, p of both species is dependent on the detection of the other species.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	$p^A, p^B, r^A, r^{BA}, r^{Ba}$	Ψ of species B is dependent on presence of species A, p of species A is dependent on the detection of species B, p of species B is dependent on the presence and detection of species A.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	p^A, p^B, r^B	Ψ of species B is dependent on presence of species A, p of species B is dependent on the presence of species A.
$\Psi^A, \Psi^{BA}, \Psi^{Ba}$	p^A, p^B, r^{BA}, r^{Ba}	Ψ of species B is dependent on presence of species A, p of species B is dependent on the presence and detection of species A.

Note: Parameter definitions are listed in Table2.1.

Table 2.3 Estimates for single-season, single-species probability of detection *p* and standard error (SE) and site occupancy *Ψ* (SE) for 6 species of meso-predators in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.

Species	***p* (SE)**	***Naïve Ψ***	***Ψ* (SE)**
Bobcat *Lynx rufus*	0.33 (0.03)	0.46	0.66 (0.07)
Coyote Canis *latrans*	0.31 (0.03)	0.58	0.68 (0.07)
Raccoon *Procyon lotor*	0.23 (0.04)	0.41	0.51 (0.07)
Striped skunk *Mephitis mephitis*	0.24 (0.05)	0.22	0.24 (0.06)
Sptoted skunk *Spilogale putorius*	0.16 (0.05)	0.20	0.22 (0.06)
Virginia opossum *Didelphis virginiana*	0.37 (0.05)	0.19	0.21 (0.05)

Table 2.4 Number of camera traps where Species A and B were or were not co-detected in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.

	Species B detected						Species B not detected					
Species A	**Bobcat**	**Coyote**	**Raccoon**	**Striped Skunk**	**Spotted Skunk**	**Opossum**	**Bobcat**	**Coyote**	**Raccoon**	**Striped Skunk**	**Spotted Skunk**	**Opossum**
Bobcat	–	44	54	19	21	13	–	13	27	59	57	65
Coyote		–	24	19	26	10	28	–	15	69	62	47
Raccoon			–	7	15	7	19	30	–	51	31	29
Striped Skunk				–	8	5	8	8	8	–	14	5
Spotted Skunk					–	7	6	1	12	14	–	16
Opossum						–	11	10	9	12	20	–

Table 2.5 Estimates for single-season, two-species co-occurrence parameters and standard errors (SE) for 15 pair-wise species comparisons of meso-predators in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.

Species A	**Species B**	Ψ^{A}	Ψ^{BA}	Ψ^{Ba}	ϕ	p^{A}	r^{A}	p^{B}	r^{BA}	r^{Ba}	δ
Coyote	Bobcat	0.61 (0.08)	0.75 (0.08)	0.75 (0.09)	1.00	0.26 (0.05)	0.27 (0.04)	0.33 (0.08)	0.33 (0.05)	0.33 (0.05)	1.00
Bobcat	Raccoon	0.54 (0.09)	0.65 (0.08)	0.67 (0.8)	0.99	0.29 (0.05)	0.30 (0.05)	0.28 (0.05)	0.33 (0.04)	0.33 (0.04)	1.00
Bobcat	Striped skunk	0.45 (0.08)	0.47 (0.10)	0.48 (0.10)	0.99	0.28 (0.05)	0.28 (0.06)	0.29 (0.08)	0.18 (0.09)	0.11 (0.05)	1.39
Bobcat	Spotted skunk	0.56 (0.09)	0.34 (0.09)	0.33 (0.10)	1.01	0.26 (0.05)	0.27 (0.05)	0.16 (0.07)	0.16 (0.07)	0.16 (0.04)	1.00
Bobcat	Va. opossum	0.58 (0.09)	0.33 (0.09)	0.09 (0.02)	1.44	0.26 (0.05)	0.30 (0.06)	0.70 (0.17)	0.28 (0.06)	0.28 (0.06)	1.00
Coyote	Raccoon	0.66 (0.08)	0.54 (0.08)	0.53 (0.09)	1.01	0.31 (0.07)	0.26 (0.07)	0.22 (0.05)	0.25 (0.06)	0.24 (0.5)	1.03
Coyote	Striped skunk	0.54 (0.08)	0.52 (0.15)	0.28 (0.11)	1.27	0.26 (0.06)	0.29 (0.05)	0.17 (0.06)	0.17 (0.05)	0.17 (0.05)	1.00
Coyote	Spotted skunk	0.67 (0.07)	0.35 (0.09)	0.28 (0.10)	1.07	0.26 (0.04)	0.26 (0.04)	0.15 (0.04)	0.15 (0.04)	0.15 (0.04)	1.00
Coyote	Va. opossum	0.68 (0.08)	0.24 (0.06)	0.19 (0.09)	1.07	0.26 (0.05)	0.26 (0.05)	0.25 (0.15)	0.39 (0.06)	0.39 (0.06)	1.00
Raccoon	Striped skunk	0.53 (0.07)	0.29 (0.08)	0.23 (0.09)	1.11	0.27 (0.05)	0.12 (0.05)	0.27 (0.06)	0.27 (0.06)	0.27 (0.06)	1.00
Raccoon	Spotted skunk	0.55 (0.08)	0.27 (0.09)	0.37 (0.14)	0.86	0.19 (0.05)	0.39 (0.14)	0.19 (0.05)	0.19 (0.06)	0.19 (0.06)	1.00
Raccoon	Va. opossum	0.50 (0.07)	0.22 (0.05)	0.22 (0.05)	1.00	0.23 (0.05)	0.26 (0.05)	0.27 (0.07)	0.36 (0.12)	0.49 (0.08)	0.79
Striped skunk	Va. opossum	0.27 (0.07)	0.23 (0.06)	0.23 (0.06)	1.00	0.23 (0.07)	0.24 (0.07)	0.36 (0.06)	0.45 (0.16)	0.38 (0.07)	1.13
Spotted skunk	Striped skunk	0.21 (0.02)	0.26 (0.02)	0.27 (0.02)	1.28	0.33 (0.01)	0.37 (0.01)	0.25 (0.10)	0.23 (0.01)	0.23 (0.01)	1.15
Spotted skunk	Va. opossum	0.32 (0.10)	0.28 (0.07)	0.28 (0.07)	1.00	0.15 (0.06)	0.15 (0.05)	0.41 (0.06)	0.12 (0.04)	0.12 (0.04)	1.00

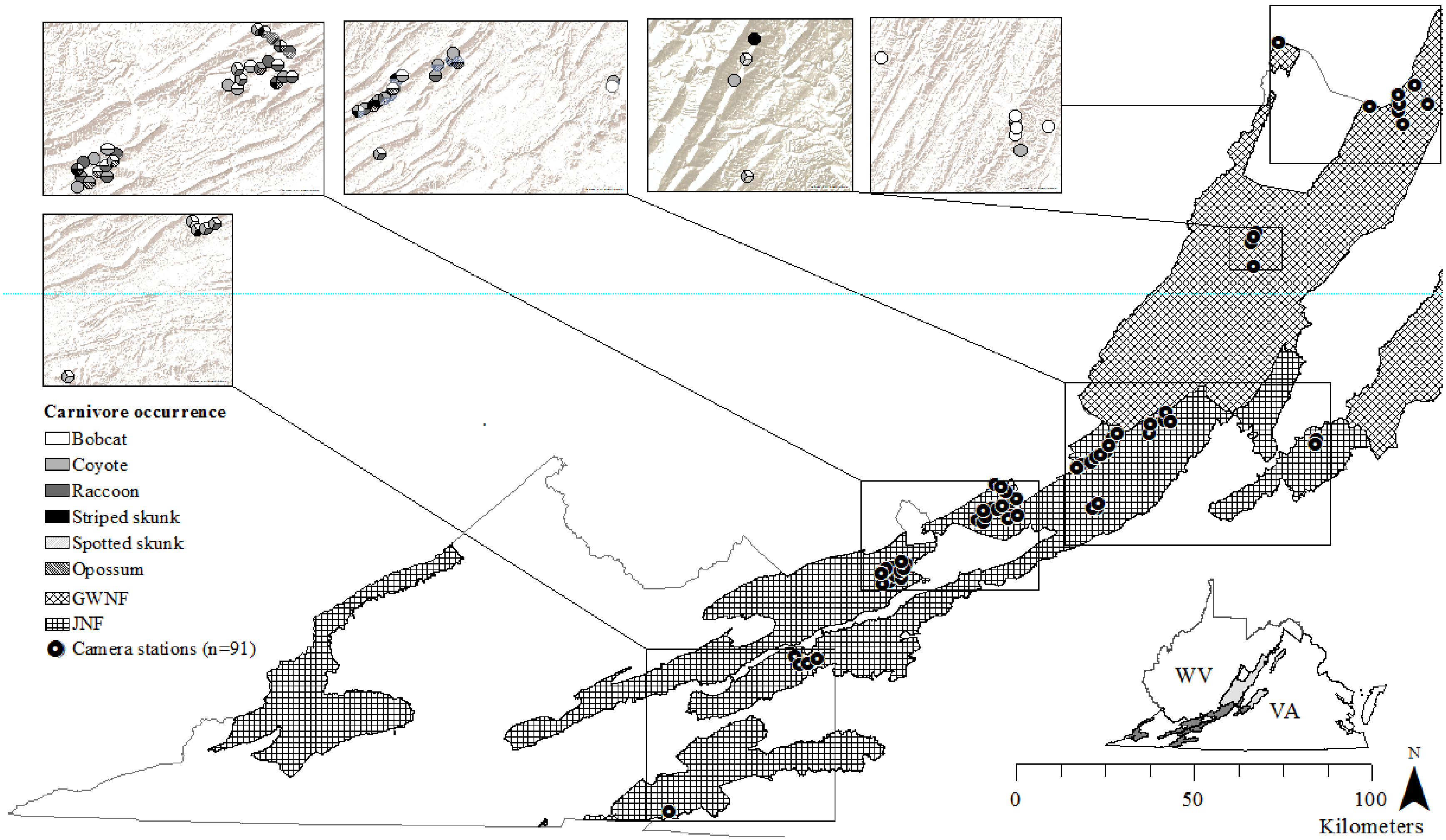

Figure 2.1 Map of 91 remotely-triggered camera stations deployed throughout the George Washington (GWNF) and Jefferson National Forests (JNF) and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015. Pie charts depict mesocarnivore species detected at each camera station.

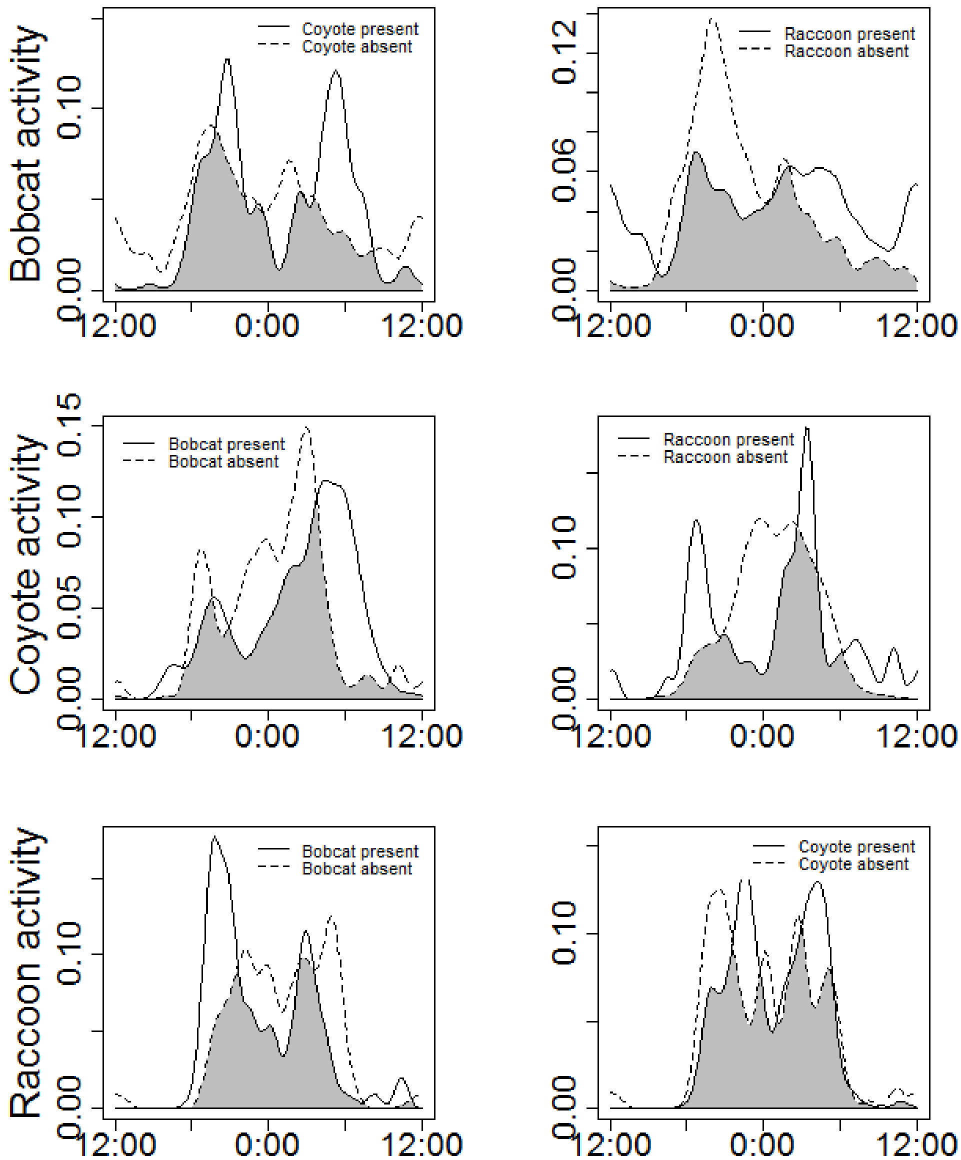

Figure 2.2 Estimated diel activity overlap of large-bodied mesocarnivores when co-detected and not co-detected with a competing large-bodied mesocarnivore in the George Washington and Jefferson National Forest and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.and 2015. Solid lines represent kernel density estimates of bobcat (*Lynx rufus*), coyote (*Canis latrans*), or raccoon (*Procyon lotor*) when another species was not co-detected. Dashed lines represent kernel density estimates of bobcat, coyote, or raccoon at sites were another species was detected.

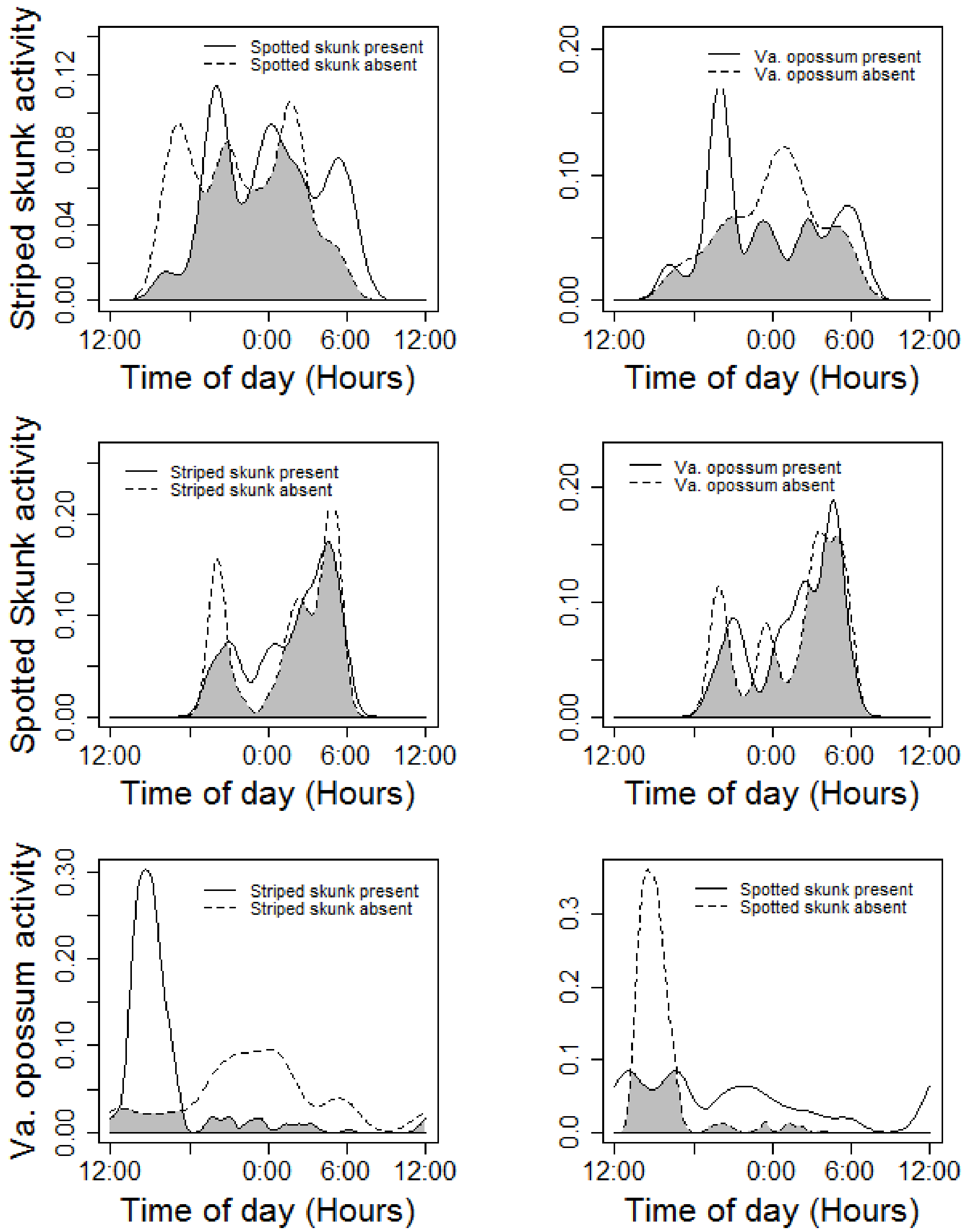

Figure 2.3 Estimated diel activity overlap of small-bodied mesocarnivores when co-detected and not co-detected with a competing small-bodied mesocarnivore in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.and 2015. Solid lines represent kernel density estimates of striped skunk (*Mephitis mephitis*), eastern spotted skunk (*Spilogale putorius*), or Virginia opossum (*Didelphis virginianus*) when another species was or not co-detected. Dashed lines represent kernel density estimates of striped skunk, eastern spotted skunk, or Virginia opossum at sites were another species was co-detected. The shaded area under the curve denotes overlap of activty periods of striped skunks, eastern spotted skunks, or Virginia opossums at sites where a competing species was and was not co-detected.

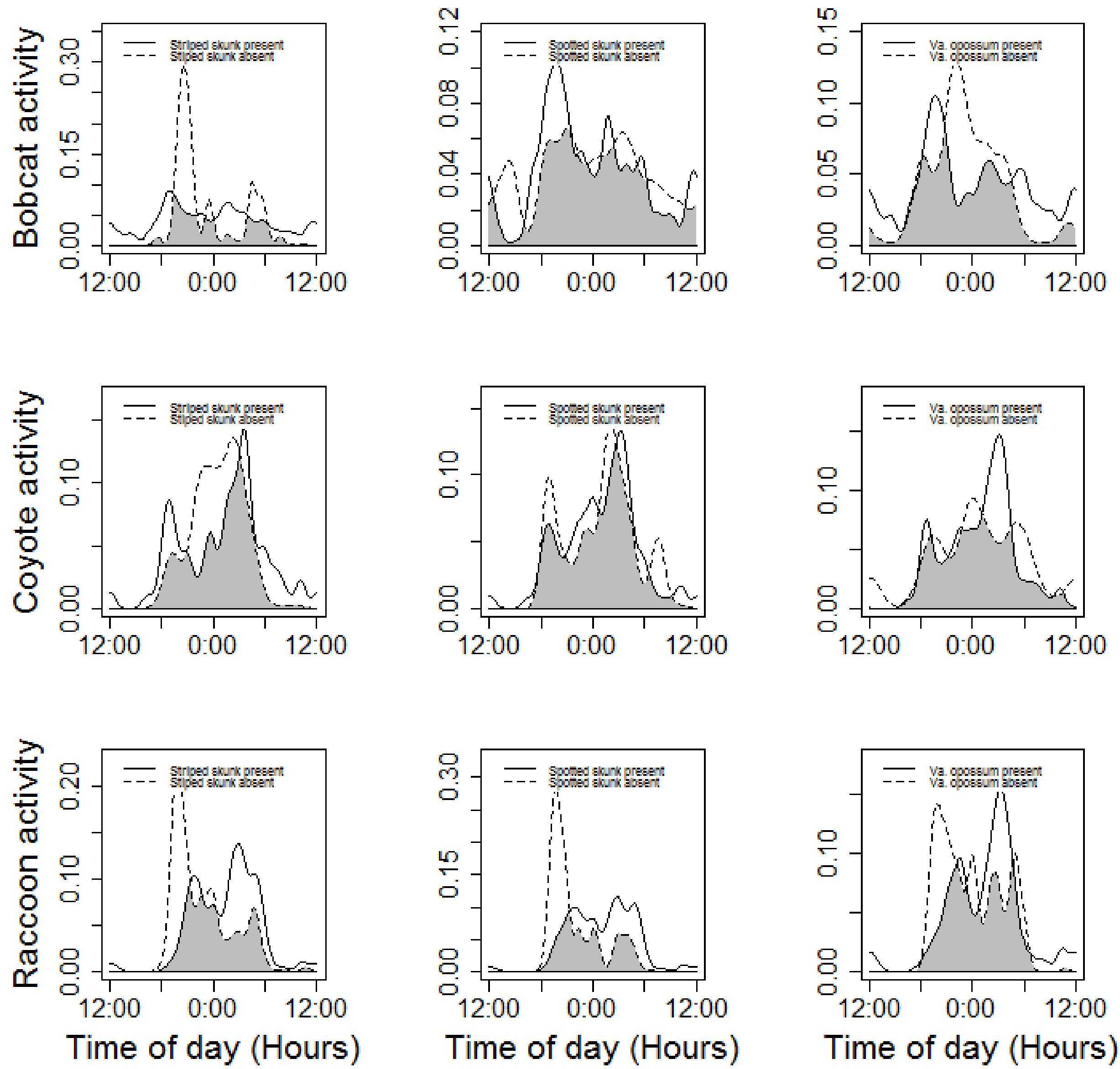

Figure 2.4. Estimated diel activity overlap of large-bodied mesocarnivores when co-detected and not co-detected with a competing small-bodied mesocarnivore in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.and 2015. Solid lines represent kernel density estimates of bobcat (*Lynx rufus*), coyote (*Canis latrans*), or raccoon (*Procyon lotor*) when striped skunk (*mephitis mephitis*), eastern spotted skunk (*Spilogale putorius*), or Virginia opossum (*Didelphis virginianus*) was not co-detected. Dashed lines represent kernel density estimates of bobcat, coyote, or raccoon at sites were a striped skunk, spotted skunk, or Virginia opossum was co-detected. The shaded area under the curve denotes overlap of activty periods of bobcats, coyotes, or raccoons at sites where a competing species was and was not co-detected.

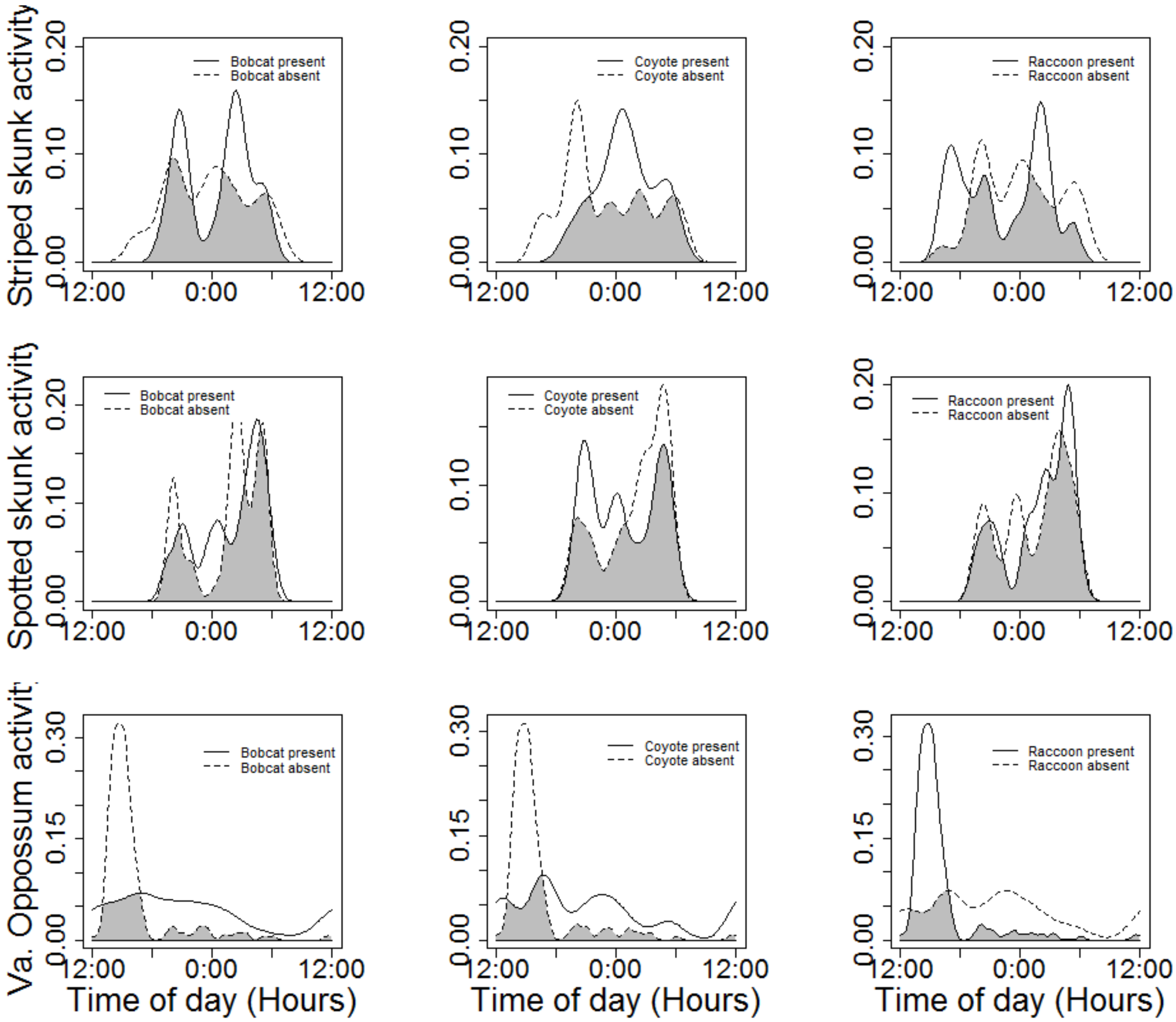

Figure 2.5. Estimated diel activity overlap of small-bodied mesocarnivores when co-detected and not co-detected with a competing large-bodied mesocarnivore in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015.and 2015. Solid lines represent kernel density estimates of striped skunk (*mephitis mephitis*), eastern spotted skunk (*Spilogale putorius*), or Virginia opossum (*Didelphis virginianus*) when bobcat (*Lynx rufus*), coyote (*Canis latrans*), or raccoon (*Procyon lotor*) was not co-detected. Dashed lines represent kernel density estimates of striped skunk, spotted skunk, or Virginia opossum at sites were a bobcat, coyote, or raccoon was co-detected. The shaded area under the curve denotes overlap of activty periods of striped skunks, eastern spotted skunks, and Virginia opossums at sites where a competing species was and was not co-detected.

Supplementary Table 2.1 Single-season, single-species detection probability model sets for 6 meso-predator species in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015. Bold font designates best-performing model(s) of each model set.

Models	***k***	***AIC*$_c$**	**Δ*AIC*$_c$**	***w*$_i$**
Bobcat *Lynx rufus*				
***p*(effort)**	**3**	**399.54**	**0.00**	**0.33**
p(effort+rain)	4	400.72	1.18	0.18
p(effort*avgmoon)	4	400.88	1.34	0.17
p(effort+mintemp)	4	401.42	1.88	0.13
p(.)	2	403.58	4.04	0.04
p(avgmoon)	3	404.38	4.84	0.03
p(totalrain)	3	404.89	5.35	0.02
p(mintemp)	3	405.41	5.87	0.02
p(avgtemp)	3	405.45	5.91	0.02
p(maxdepth)	3	405.63	6.09	0.02
p(date)	3	405.67	6.13	0.02
p(totalsnow)	3	405.69	6.15	0.02
p(rain+mintemp)	4	406.66	7.12	0.01
p(moon)	5	409.13	9.59	0.00
Coyote *Canis latrans*				
***p*(effort+rain)**	**4**	**488.25**	**0.00**	**0.83**
p(effort)	3	492.22	3.98	0.11
p(effort+mintemp)	4	494.35	6.11	0.04
p(totalrain)	3	498.35	10.10	0.01
p(rain+mintemp)	4	498.64	10.40	0.00
p(.)	2	501.06	12.81	0.00
p(mintemp)	3	501.59	13.35	0.00
p(avgmoon)	3	501.68	13.43	0.00
p(totalsnow)	3	502.17	13.92	0.00
p(date)	3	502.24	13.99	0.00
p(avgtemp)	3	502.83	14.59	0.00
p(maxdepth)	3	502.87	14.63	0.00
Raccoon *Procyon lotor*				
***p*(snow+mintemp)**	**4**	**376.79**	**0.00**	**0.74**
p(effort+snow)	4	381.20	4.41	0.08
p(totalsnow)	3	382.19	5.41	0.05
p(effort)	3	382.68	5.89	0.04
p(effort+mintemp)	4	383.96	7.18	0.02
p(.)	2	384.15	7.36	0.02
p(mintemp)	3	384.55	7.77	0.02
P(date)	3	385.23	8.44	0.01
p(maxdepth)	3	385.43	8.64	0.01

p(avgtemp)	3	385.96	9.19	0.01
p(totalrain)	3	386.28	9.50	0.01
p(moon)	5	390.51	13.73	0.01
Striped skunk *Mephitis mephitis*				
***p*(moon+rain)**	**4**	**232.12**	**0.00**	**0.40**
p(moon+date)	4	233.35	1.23	0.21
p(avgmoon)	3	233.90	1.78	0.16
p(date+rain)	4	235.15	3.03	0.09
p(totalrain)	3	236.15	4.03	0.05
p(date)	3	238.00	5.88	0.02
p(.)	2	238.26	6.14	0.02
p(mintemp)	3	238.57	6.45	0.02
p(effort)	3	238.87	6.74	0.01
p(maxdepth)	3	239.88	7.76	0.01
p(totalsnow)	3	240.29	8.17	0.01
p(moon)	5	244.80	12.68	0.00
Spotted skunk *Spilogale putorius*				
p(avgmoon+totalsnow)	4	196.36	0.00	0.19
***p*(avgmoon)**	**3**	**196.80**	**0.44**	**0.15**
***p*(totalsnow)**	**3**	**197.05**	**0.69**	**0.13**
p(avgmoon+date)	4	197.42	1.05	0.11
p(date+snow)	4	198.07	1.70	0.08
p(.)	2	198.13	1.77	0.08
P(date)	3	198.66	2.29	0.06
p(mintemp)	3	198.84	2.48	0.05
p(totalrain)	3	198.88	2.51	0.05
p(effort)	3	199.24	2.87	0.04
p(maxdepth)	3	199.50	3.14	0.04
p(moon)	5	202.78	6.41	0.01
Virginia opossum *Didelphis virginiana*				
p(maxdepth)	3	243.58	0.00	0.19
***p*(.)**	**2**	**243.68**	**0.10**	**0.18**
p(depth+avgmoon)	4	245.01	1.43	0.09
p(avgmoon)	3	245.23	1.65	0.08
p(effort)	3	245.36	1.78	0.08
p(depth+effort)	4	245.62	2.02	0.07
p(mintemp)	3	245.43	1.83	0.0
p(totalsnow)	3	245.42	1.84	0.07
p(totalrain)	3	245.77	2.19	0.06
P(date)	3	245.82	2.24	0.06
p(avgmoon+effort)	4	246.82	3.235	0.04
p(moon)	5	250.24	6.65	0.01

Supplemental Table 2.2 Single-season, single-species site occupancy probability model sets for 6 meso-predator species in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Viginia, USA from January – April 2014 and 2015. Bold font font designates best-performing model(s) of each model set.

Models	***k***	***AIC_c***	**Δ*AIC_c***	***w_i***
Bobcat *Lynx rufus*				
***Ψ*(year) *p*(effort)**	**4**	**396.8**	**0.00**	**0.31**
Ψ(year+canopy) *p*(effort)	5	399.04	2.24	0.11
Ψ(year+roads) *p*(effort)	5	399.04	2.24	0.11
Ψ(year+agpast) *p*(effort)	5	399.05	2.25	0.11
Ψ(null)P(effort) *p*(effort)	3	399.54	2.74	0.07
Ψ(AgPast) *p*(effort)	4	401.38	4.58	0.03
Ψ(Road) *p*(effort)	4	401.40	4.60	0.03
Ψ(canopy) *p*(effort)	4	401.42	4.62	0.03
Ψ(Aspect) *p*(effort)	4	401.48	4.66	0.03
Ψ(Age) *p*(effort)	4	401.48	4.66	0.03
Ψ(TEI) *p*(effort)	4	401.50	4.70	0.03
Ψ(Slope) *p*(effort)	4	401.70	4.90	0.03
Ψ(Elev) *p*(effort)	4	401.71	4.91	0.03
Ψ(Area) *p*(effort)	4	401.73	4.93	0.03
Ψ(HAB) *p*(effort)	5	402.24	5.44	0.02
Ψ(.) *p*(.)	2	403.58	6.78	0.01
Coyote *Canis latrans*				
***Ψ*(AgPast) *p*(effort+rain)**	**5**	**481.31**	**0.00**	**0.33**
***Ψ*(canopy) *p*(effort+rain)**	**5**	**481.78**	**0.48**	**0.26**
***Ψ*(Road) *p*(effort+rain)**	**5**	**482.78**	**1.48**	**0.16**
Ψ(Aspect) *p*(effort+rain)	5	484.07	2.80	0.08
Ψ(agpast+roads) *p*(effort+rain)	7	485.72	4.42	0.06
Ψ(canopy+agpast) *p*(effort+rain)	7	485.84	4.54	0.03
Ψ(TEI) *p*(effort+rain)	5	485.84	4.54	0.03
Ψ(roads+canopy) *p*(effort+rain)	7	486.13	4.83	0.03
Ψ(Slope) *p*(effort+rain)	5	486.16	4.86	0.03
Ψ(Area) *p*(effort+rain)	5	489.48	8.17	0.01
Ψ(year) *p*(effort+rain)	5	490.12	8.81	0.00
Ψ(Age) *p*(effort+rain)	5	490.18	8.87	0.00
Ψ(Elev) *p*(effort+rain)	5	490.23	8.93	0.00
Ψ(HAB) *p*(effort+rain)	6	491.62	10.32	0.00
Ψ(.) *p*(effort+rain)	3	492.22	10.92	0.00
Ψ(.) *p*(.)	2	501.06	19.75	0.00
Raccoon *Procyon lotor*				
***Ψ*(.)(totalsnow+mintemp)**	**4**	**376.79**	**0**	**0.50**

Ψ(Elev) *p*(snow+mintemp)	3	379.29	2.50	0.14
Ψ(Area) *p*(snow+mintemp)	3	379.94	3.15	0.10
Ψ(elev+area) *p*(snow+mintemp)	4	381.03	4.25	0.06
Ψ(elev+TEI) *p*(snow+mintemp)	4	381.08	4.30	0.06
Ψ(area+TEI) *p*(snow+mintemp)	4	381.78	4.99	0.04
Ψ(TEI) *p*(snow+mintemp)	3	382.83	6.04	0.02
Ψ(.) *p*(.)	2	384.15	7.36	0.01
Ψ(Aspect) *p*(snow+mintemp)	3	384.52	7.73	0.01
Ψ(Slope) *p*(snow+mintemp)	3	384.70	7.92	0.01
Ψ(canopy) *p*(snow+mintemp)	3	384.95	8.17	0.01
Ψ(year) *p*(snow+mintemp)	3	385.64	8.86	0.01
Ψ(Road) *p*(snow+mintemp)	3	386.05	9.27	0.00
Ψ(AgPast) *p*(snow+mintemp)	3	386.19	9.41	0.00
Ψ(Age) *p*(snow+mintemp)	3	386.28	9.50	0.00
Ψ(HAB) *p*(snow+mintemp)	4	388.32	11.53	0.00
Striped skunk *Mephitis mephitis*				
Ψ(Slope) *p*(moon+rain)	**4**	**222.04**	**0.00**	**0.47**
Ψ(slope+elev) *p*(moon+rain)	6	223.74	1.70	0.20
Ψ(slope+agpast) *p*(moon+rain)	6	224.19	2.15	0.16
Ψ(SLOPE+roads) *p*(moon+rain)	6	224.34	2.30	0.15
Ψ(Elev) *p*(moon+rain)	4	230.52	8.48	0.01
Ψ(.) *p*(moon+rain)	3	232.12	10.08	0.00
Ψ(Road) *p*(moon+rain)	4	232.33	10.29	0.00
Ψ(AgPast) *p*(moon+rain)	4	232.40	10.36	0.00
Ψ(TEI) *p*(moon+rain)	4	233.73	11.69	0.00
Ψ(canopy) *p*(moon+rain)	4	233.77	11.73	0.00
Ψ(HAB) *p*(moon+rain)	5	233.96	11.92	0.00
Ψ(Aspect) *p*(moon+rain)	4	234.30	12.26	0.00
Ψ(year) *p*(moon+rain)	4	234.33	12.29	0.00
Ψ(Area) *p*(moon+rain)	4	234.36	12.33	0.00
Ψ(Age) *p*(moon+rain)	4	234.37	12.33	0.00
Ψ(.) p(.)	2	238.26	16.23	0.00
Spotted skunk *Spilogale putorius*				
Ψ(Age) *p*(avgmoon+totalsnow)	**5**	**196.17**	**0.00**	**0.21**
Ψ(Elev) *p*(avgmoon+totalsnow)	**5**	**196.85**	**0.69**	**0.15**
Ψ(age+elev) *p*(avgmoon+totalsnow)	6	197.56	1.39	0.10
Ψ(.) *p*(.)	2	198.13	2.01	0.08
Ψ(Age+slope) *p*(avgmoon+totalsnow)	4	198.29	2.12	0.07
Ψ(Slope) *p*(avgmoon+totalsnow)	3	198.68	2.51	0.06
Ψ(elev+slope) *p*(avgmoon+totalsnow)	4	198.99	2.82	0.05

Ψ(.) *p*(avgmoon+totalsnow) *p*(avgmoon+totalsnow)	4	199.24	3.07	0.05
Ψ(year) *p*(avgmoon+totalsnow)	3	199.29	3.13	0.04
Ψ(canopy) *p*(avgmoon+totalsnow)	3	199.36	3.20	0.04
Ψ(Aspect) *p*(avgmoon+totalsnow)	3	199.41	3.24	0.04
Ψ(Road) *p*(avgmoon+totalsnow)	3	199.42	3.25	0.04
Ψ(TEI) *p*(avgmoon+totalsnow)	3	199.76	3.59	0.03
Ψ(AgPast) *p*(avgmoon+totalsnow)	3	201.06	4.90	0.02
Ψ(HAB) *p*(avgmoon+totalsnow)	4	202.12	5.95	0.01
Ψ(Area) *p*(avgmoon+totalsnow)	3	264.25	68.08	0.00
Virginia opossum *Didelphis virginiana*				
Ψ(canopy) *p*(.)	3	242.79	0.00	0.13
Ψ(canopy+AgPast) *p*(.)	5	243.14	0.35	0.11
Ψ(AgPast) *p*(.)	3	243.25	0.46	0.1
Ψ(year) *p*(.)	3	243.59	0.80	0.08
***Ψ*(.) *p*(.)**	**2**	**243.68**	**0.89**	**0.08**
Ψ(year+canopy) *p*(.)	4	243.71	0.92	0.08
Ψ(Area) *p*(.)	3	244.13	1.34	0.06
Ψ(Aspect) *p*(.)	3	244.20	1.41	0.06
Ψ(year*AgPast) *p*(.)	4	244.22	1.43	0.06
Ψ(TEI) *p*(.)	3	244.36	1.57	0.06
Ψ(Road) *p*(.)	3	244.47	1.68	0.05
Ψ(Slope) *p*(.)	3	244.61	1.81	0.05
Ψ(Elev) *p*(.)	3	245.72	2.93	0.03
Ψ(Age) *p*(.)	3	245.80	3.01	0.03
Ψ(HAB) *p*(.)	4	246.86	4.07	0.02

Supplemental Table 2.3 Single-season, two-species site co-occurrence model sets for 15 meso-predator species pairs in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian region of Virginia, USA from January – April 2014 and 2015. Bold font designates best-performing model(s) of each model set.

Model	***k***	AIC_c	ΔAIC	w_i
Bobcat - coyote				
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**868.70**	**0.00**	**0.34**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**11**	**869.50**	**0.80**	**0.23**
Ψ^{A} $\Psi^{B}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**870.56**	**1.86**	**0.14**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	9	871.91	3.21	0.07
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	872.11	3.41	0.06
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	13	872.86	4.16	0.04
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	7	873.10	4.40	0.04
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	9	873.23	4.53	0.04
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	10	874.78	6.08	0.02
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	8	874.93	6.23	0.02
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	11	876.28	7.58	0.01
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	877.85	9.15	0.00
Bobcat - raccoon				
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**7**	**900.35**	**0.00**	**0.20**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**9**	**900.63**	**0.28**	**0.17**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=p^{BA}=r^{Ba}$	**8**	**901.06**	**0.71**	**0.14**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**901.8**	**1.45**	**0.10**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**901.98**	**1.63**	**0.09**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**902.21**	**1.86**	**0.08**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	11	902.90	2.55	0.06
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	9	902.93	2.58	0.05
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	903.14	2.79	0.05
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	904.13	3.78	0.03
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	904.13	3.78	0.03
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	13	905.38	5.03	0.02
Bobcat - striped skunk				
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**9**	**627.49**	**0.00**	**0.20**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**11**	**627.57**	**0.08**	**0.19**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**12**	**628.21**	**0.72**	**0.14**
Ψ^{A} $\Psi^{Ba}=\Psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**628.21**	**0.72**	**0.14**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**12**	**629.45**	**1.96**	**0.07**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**629.46**	**1.97**	**0.07**
Ψ^{A} $\Psi^{Ba}\neq\Psi^{BA}$ $p^{A}\neq r^{A}$ p^{B} r^{A} r^{BA} r^{Ba}	13	630.03	2.54	0.06

$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	11	630.11	2.62	0.05
$\psi^{A}\ \ \psi^{B}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	7	630.98	3.49	0.03
$\psi^{A}\ \ \psi^{B}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	8	632.19	4.70	0.02
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	8	632.59	5.10	0.02
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	9	633.79	6.30	0.01
Bobcat - spotted skunk				
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**7**	**588.66**	**0.00**	**0.23**
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**9**	**589.04**	**0.38**	**0.20**
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**11**	**589.79**	**1.13**	**0.13**
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**8**	**590.25**	**1.59**	**0.10**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**8**	**590.37**	**1.71**	**0.01**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{B}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{B}=r^{BA}$	10	591.02	2.36	0.07
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	12	591.66	3.00	0.05
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	12	591.77	3.11	0.05
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	9	591.94	3.28	0.04
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	13	593.51	4.85	0.02
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	12	594.85	6.19	0.01
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	13	596.88	8.22	0.00
Bobcat - opossum				
$\psi^{A}\ \ \psi^{B}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**9**	**634.32**	**0.00**	**0.35**
$\psi^{A}\ \ \psi^{B}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**10**	**636.18**	**1.86**	**0.14**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	8	636.32	2.00	0.13
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	8	636.67	2.35	0.11
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	7	637.57	3.25	0.07
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	9	637.95	3.63	0.06
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	9	638.20	3.88	0.05
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	8	638.95	4.63	0.03
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	8	639.55	5.23	0.03
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	7	639.71	5.39	0.02
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	6	639.88	5.56	0.02
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	7	640.50	6.18	0.02
Coyote - raccoon				
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**10**	**861.38**	**0.00**	**0.22**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**8**	**861.63**	**0.25**	**0.19**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**12**	**862.94**	**1.56**	**0.10**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	**11**	**863.13**	**1.75**	**0.10**
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}\neq r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	**14**	**863.20**	**1.82**	**0.09**
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}=r^{Ba}$	**10**	**863.25**	**1.87**	**0.08**
$\psi^{A}\ \ \psi^{Ba}\neq\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}=r^{BA}=r^{Ba}$	9	863.63	2.25	0.07
$\psi^{A}\ \ \psi^{Ba}=\psi^{BA}\ \ p^{A}=r^{A}\ \ p^{B}\neq r^{BA}\neq r^{Ba}$	12	864.67	3.29	0.04

ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	864.97	3.59	0.04
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	13	865.01	3.63	0.04
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	15	865.20	3.82	0.03
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	13	866.37	4.99	0.02

Coyote - striped skunk

ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**9**	**717.46**	**0.00**	**0.20**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**717.62**	**0.16**	**0.18**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**10**	**717.73**	**0.27**	**0.17**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**718.52**	**1.06**	**0.12**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**11**	**718.81**	**1.35**	**0.10**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	11	719.81	2.35	0.06
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	14	720.09	2.63	0.05
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	12	720.33	2.87	0.05
ψ^{A} ψ^{Ba} ψ^{BA} $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	13	721.39	3.93	0.03
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	721.97	4.51	0.02
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	13	722.30	4.84	0.02
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	15	725.35	7.89	0.00

Coyote - spotted skunk

ψ^{A} $\psi^{Ba}=\psi^{BA}$ $P^{a}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**670.91**	**0.00**	**0.30**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{a}$ $p^{B}=r^{BA}=r^{Ba}$	**9**	**671.63**	**0.72**	**0.21**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**15**	**672.67**	**1.76**	**0.12**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**13**	**672.86**	**1.95**	**0.11**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	673.98	3.07	0.06
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	674.54	3.63	0.05
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	10	674.93	4.02	0.04
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	674.97	4.06	0.04
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	14	675.40	4.49	0.03
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	10	675.68	4.77	0.03
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	12	677.40	6.49	0.01
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	13	681.33	10.42	0.00

Coyote - opossum

ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**8**	**723.98**	**0.00**	**0.24**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**7**	**724.13**	**0.15**	**0.22**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**724.84**	**0.86**	**0.16**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**9**	**725.55**	**1.57**	**0.11**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**9**	**725.84**	**1.86**	**0.10**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	10	727.43	3.45	0.04
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	10	727.88	3.90	0.03
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	9	727.89	3.91	0.03
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	10	728.27	4.29	0.03

$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	11	729.34	5.36	0.02
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	11	729.73	5.75	0.01
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	12	731.23	7.25	0.01
Rccoon - striped skunk				
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**11**	**593.51**	**0.00**	**0.49**
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**10**	**593.99**	**0.48**	**0.39**
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}$	8	597.67	4.16	0.06
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}$	9	599.18	5.67	0.03
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	10	600.18	6.67	0.02
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	11	601.85	8.34	0.01
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	12	603.90	10.39	0.00
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\ r^{Ba}$	13	605.56	12.05	0.00
Raccoon - spotted skunk				
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**10**	**573.35**	**0.00**	**0.30**
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**8**	**574.54**	**1.19**	**0.16**
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**9**	**574.68**	**1.33**	**0.15**
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}=r^{Ba}}$	**13**	**574.83**	**1.48**	**0.14**
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	15	575.83	2.48	0.09
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	12	576.76	3.41	0.05
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	11	576.87	3.52	0.05
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	14	578.35	5.00	0.02
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	10	578.50	5.15	0.02
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	13	580.29	6.94	0.01
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	12	581.88	8.53	0.00
Raccoon - opossum				
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}}$	**8**	**618.33**	**0.00**	**0.26**
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}}$	**7**	**618.58**	**0.25**	**0.23**
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	9	620.33	2.00	0.09
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	8	620.57	2.24	0.08
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}$	6	620.99	2.66	0.07
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	9	621.02	2.69	0.07
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	10	621.19	2.86	0.06
$\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}=r^{BA}=r^{Ba}$	8	622.30	3.97	0.04
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}=r^{Ba}$	10	622.74	4.41	0.03
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}$	7	622.85	4.52	0.03
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}=r^{BA}=r^{Ba}$	9	623.02	4.69	0.02
$\Psi^{A}\ \Psi^{Ba}\neq\Psi^{BA}\ p^{A}\neq r^{A}\ p^{B}\neq r^{BA}\neq r^{Ba}$	11	623.03	4.70	0.02
Striped skunk - opossum				
$\boldsymbol{\Psi^{A}\ \Psi^{Ba}=\Psi^{BA}\ p^{A}=r^{A}\ p^{B}=r^{BA}=r^{Ba}}$	**7**	**464.85**	**0.00**	**0.20**

ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**9**	**465.62**	**0.77**	**0.14**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	**8**	**466.00**	**1.15**	**0.11**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**466.22**	**1.37**	**0.10**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**9**	**466.48**	**1.63**	**0.09**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	10	467.14	2.29	0.06
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	11	467.19	2.34	0.06
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	10	467.27	2.42	0.06
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	9	467.59	2.74	0.05
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	10	467.72	2.87	0.05
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	12	468.24	3.39	0.04
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	468.82	3.97	0.03
Spotted skunk - striped skunk				
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**8**	**466.01**	0.00	**0.31**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	**9**	**466.34**	**1.33**	**0.16**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**467.64**	**1.66**	**0.13**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	10	467.67	2.44	0.09
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}r\neq^{A}$ $p^{B}=r^{BA}=r^{Ba}$	12	468.84	2.83	0.07
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	11	469.30	3.29	0.06
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	11	469.64	3.63	0.05
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	12	470.11	4.10	0.04
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	13	470.34	4.33	0.04
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	14	471.18	5.17	0.02
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	13	471.58	5.57	0.02
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}\neq r^{Ba}$	15	472.64	6.63	0.01
Spotted skunk - opossum				
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**8**	**433.47**	**0.00**	**0.60**
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	**10**	**435.12**	**1.65**	**0.25**
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}\neq r^{A}$ $p^{B}\neq r^{BA}=r^{Ba}$	11	436.83	3.36	0.11
ψ^{A} $\psi^{Ba}\neq\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	8	438.34	4.87	0.05
ψ^{A} $\psi^{Ba}=\psi^{BA}$ $p^{A}=r^{A}$ $p^{B}=r^{BA}=r^{Ba}$	7	439.71	6.24	0.03

Chapter 3 – Home-Range Size and Resource Use by Eastern Spotted Skunks (*Spilogale putorius*) in the Central and Southern Appalachians

Abstract

Throughout much of western Virginia, forested ecosystems have experienced high reduction of core forest areas into smaller fragments as a result of land-use change and urbanization. Extant populations of rare wildlife species may be restricted to forest fragments and isolation from neighboring populations by a surrounding matrix of suboptimal ecological conditions. The eastern spotted skunk (*Spilogale putorius*) is classified as Vulnerable on the IUCN Red List and is considered a species of concern in many states. This includes Virginia, where populations are believed to have declined and the overall distribution has constricted. Nonetheless, our previous research defined small and spatially disjunct areas of ecologically suitable conditions that contained spotted skunks in the Appalachian Mountains of western Virginia. Accordingly, we caught and radio-tagged spotted skunks to assess whether current habitat fragmentation is restricting skunk movements and hence distribution, potentially leading to isolation within suitable habitat patches. Home range size (~ 1-1.5 km^2) in our study was smaller than those in other studies in the Southern and Midwestern USA, and excursive movements were limited to core forested areas. Additionally, core forested areas larger than 2 km^2 were strongly selected for by skunks, whereas non-forested and forest-edge areas were avoided. We conclude that reduction in core forest areas and increases in the unsuitable surrounding matrix areas, i.e., pastoral land and development, limit spotted skunk movement between mountain ridges. Management actions that increase forest patch connectivity may help prevent further genetic and demographic isolation of extant populations, reduce the likelihood of local extinctions, and facilitate colonization of suitable unoccupied areas.

Introduction

Habitat fragmentation resulting from anthropogenic landscape change has led to high losses in biodiversity and has threatened ecosystems globally over the last 200 years (Haddad et al. 2015). While most ecosystems have experienced negative consequences of habitat loss and fragmentation to some degree, many forest ecosystems have experienced high loss as a consequence of modern agricultural practices and urban and suburban expansion (Bogaert et al. 2015., Wilcox and Murphy 1985, Czech and Krausman 1997, Lawler et al. 2002). The size, shape, quality, and degree of isolation of habitat fragments influence the spatial distribution, population demography, and genetic diversity of species across taxa (Didham et al. 1998, Schneider 2001, Cushman 2006, Devictor et al. 2008, Prugh et al. 2009). Species occurrence may be limited to areas of environmental conditions suitble for that species (i.e., to habitat patches) that are surrounded by a matrix of environmental conditions that are unsuitable for that species (Kozakiewicz 1993), for example, a forest fragment surrounded by an urban area. The matrix surrounding the habitat patch may behave as a movement barrier and impede dispersal of individuals between neighboring habitat patches (Kozakiewicz 1993, Szacki 1987).

In these instances, individuals' movements may be strongly limited to the area within their home range or they may make an exploratory excursion, a temporary long-distance movement outside the home range, but ultimately return to their home range area (Lidicker and Stenseth 1992). Understanding an animal's ability to traverse extra-home range environments is a key component to research involving animal movement patterns, migration rates, and resource utilization upon which management decisions are based. Whereas true dispersal events are difficult to monitor, excursions may be pre-dispersal movements, which can bring light to the degree of connectivity versus patch isolation and to the availability of mates and resources outside of a familiar home range (Lidicker and Stenseth 1992). However, if not identified as excursive, these movements can contribute to overestimations of home range size and lead to erroneous interpretations of resource utilization functions (Burt 1943, Hodder et al. 1998). Despite the importance of dispersal and excursive events on population connectivity, it remains difficult to document these movements on the landscape.

Further, dispersal distance of mammals is positively correlated with home-range size (Bowman et al. 2002). However, due to resource limitation and intra-specific competition, home-range size and degree of overlap vary inversely with habitat patch size and population density for many species (Sanderson 1966, Kozakiewicz 1985, Efford et al. 2016, Crooks et al. 2017). Compared to a larger continuous forested area, the use of smaller home ranges within a forest fragment may reduce the degree of range overlap and provide a mechanism for avoiding antagonistic interactions between mutually intolerant individuals (Kozakiewicz 1985). Conversely, home ranges may not be restricted in high density areas, but rather the degree of overlap of home ranges may increase and increased aggression among individuals may be observed (Wolff 1985).

The eastern spotted skunk (*Spilogale putorius*) is a small mephitid that was once a fairly common furbearer throughout the central and southern United States, with annual range-wide harvests estimated to be greater than 100,000 individuals (Gompper and Hackett 2005). The eastern spotted skunk has a global rank of Vulnerable (Gompper and Jachowski 2016) and is listed as Vulnerable, Imperiled, or Critically Imperiled in most of the states throughout its historic range (Chapman 2007). Perceived population declines since the 1940s have been attributed to the loss of oldfield habitats, more intensive agricultural practices (clean farming), and the maturation of early successional forests established following land abandonment in the Midwest that are now in older age-classes (Gompper and Hackett 2005, Lesmeister et al. 2009).

In Virginia, the spotted skunk is classified as Vulnerable, and a recent study of eastern spotted skunk site occupancy in the central Appalachians suggests a wide extent of small, disjunct forest patches of high predicted occurrence throughout its historic range (Thorne et al. 2017). Eastern spotted skunks demonstrate a striking degree of sexual dimorphism in body size (Crabb 1944). Average mass of male and female spotted skunks is approximately 723 g and 482 g, respectively (Crabb 1944). Accordingly, the size difference between male and female spotted skunks contributes to variation in home-range size and resource utilization, a pattern often observed in other mammalian species, especially carnivores (Lindstedt et al. 1986). These morphological characteristics may enable or necessitate skunks to vary their movement patterns and home-range size based on body condition, seasonal weather changes, or presence of intra- or interspecific competition (McNab 1963, Powell 1979, Moors 1980, Lindstedt et al. 1986). Physiological differences, such as reproductive condition, may further explain variation in movement patterns because pregnant or kit-rearing females likely require more food, are more vulnerable to predation, and may be restricted to smaller daily movements (Powell 1979, Mysterud et al. 2001). If current habitat fragmentation or lack of suitable habitat is restricting spotted skunk movements, these constraints could lead to potential demographic and genetic consequences upon spotted skunk populations generally in their distributions (Templeton et al. 1990, Lowe and Allendorf 2010), an effect that has been documented in other species throughout the central Appalachian Mountains (Francl et al. 2008, Kanine 2013).

The objectives of our study were to: 1) identify patterns in extra-home range exploratory excursions, 2) estimate site- and sex-specific differences in home-range size of eastern spotted skunks, 3) estimate percent home-range overlap between male-male, male-female, and female-female dyads with study sites, and 4) assess within-study site (2^{nd} order) and within-home range

(3rd order) resource availability and use. First, we predicted that male spotted skunks would make more frequent and longer distance extra-home range exploratory excursions than females, potentially in search of additional mating opportunities from mid-February – mid-April. Second, we predicted that due to larger body size, potential territoriality, and mate-seeking behavior, male spotted skunks would occupy larger home ranges than female spotted skunks and that home-range size would be greater at sites with larger patches of core forest areas. Third, we predicted that as a solitary carnivore species, spotted skunks would display little home-range overlap overall with conspecifics, though more so between male-female dyads (Powell 1979). Finally, we predicted that resource use would not be uniform across an individual's home range, but rather that it would be a function of environmental conditions and availability of resources within and outside the home range. We predicted greater use would be associated with large core forests areas at lower elevations where complex forest structure may provide foraging and escape cover.

Methods

Study Area

We conducted this study within the central and upper southern Appalachian Mountains, from the Monongahela National Forest in eastern West Virginia, and the George Washington and Jefferson National Forests south of Sugar Run Mountain, across the Ridge and Valley and Appalachian Plateau along and into the Blue Ridge Mountains below Mt. Rogers along the Virginia-North Carolina state line (Figure 3.1). This included areas of Botetourt, Craig, Grayson, and Nelson counties in Virginia and Pendleton county in West Virginia. Elevation ranges from 350 to 1460 m throughout the study area. Forest composition in these regions varies by elevation and aspect, but consists predominately of white oak (*Quercus alba*), chestnut oak *(Q. prinus*), northern red oak (*Q. rubra*), black oak (*Quercus velutina*), red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), yellow-poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), pitch pine (*Pinus rigida*), mountain pine (*Pinus pungens*), mountain laurel (*Kalmia latifolia*), with white pine (*pinus strobus*), eastern hemlock (*Tsuga canadensis*), and rosebay rhododendron (*Rhododendron maximum*) concentrated along riparian zones. High- elevation forest of the Mt. Rogers National Recreation Area is dominated by northern hardwood and red spruce (*Picea rubens*) communities (Yarnell 1998, Kniowski and Ford 2018). Annual precipitation is approximately 110 cm throughout most of the region, but varies from north (lower precipitation) to south (higher precipitation, Ford et al. 2006). Annual temperature ranges from -10.6°C in winter to 27.5°C in summer (National Oceanic Atmospheric Administration, public data 2019; www.noaa.gov). Contemporary forest management throughout the George Washington and Jefferson National Forests consists of timber harvest and prescribed burns on approximately 13% of lands designated for active management (U.S. Forest Service 2004, 2014).

Sampling Methods

To assess the potential relationships between spotted skunk movement patterns, home-range size, and resource use, we initiated a radio telemetry study using very high frequency (VHF) radio collars. During the winter seasons of 2015 – 2019, we deployed live traps (model # 103, Tomahawk Live-Trap Co., Wisconsin, USA) in five areas of high predicted spotted skunk occupancy (Figure 3.1, Thorne et al. 2017): North Tract Mountain (TM), Bald Mountain (BM), Wintergreen Mountain (WG), and Whitetip Mountain (WT). To increase trapping efficiency, we targeted areas with observed spotted skunk presence using baited camera traps following the methods of Thorne et al. (2017) prior to live trap deployment. Traps were baited with a mixture of peanut butter, bacon grease, and oats. Captured spotted skunks were removed from the traps and physically immobilized using a cloth handling bag. We sexed all captured individuals, collected measurements of body length, head length, head width at zygomatic arch, tail length, right hind foot length, testis length, and paired testes width of males, right thoracic nipple length and width of females, and body mass. We assessed age class (juvenile, subadult, adult) based on weight and tooth wear (Crabb 1944) and categorized individuals as juvenile (< 350 g) and adult.

We affixed a uniquely numbered ear tag to each captured individual and deployed 16-g VHF radio collars (model # M1740, Advanced Telemetry Systems Inc., Insanti, MN, USA) to adult individuals with a mass > 350 g. These collars comprised approximately 2.3 – 4.6% of body weight of individuals with a mass of 350 – 700 g. Throughout the study, we attempted to recapture all collared individuals to replace collars prior to battery failure or remove collars at the conclusion of our fieldwork. We tracked spotted skunks using TRX-1000S receivers and folding 3-element Yagi antennas (Wildlife Materials Inc., Carbondale, IL, USA) and attempted to locate each animal at least once per week through all seasons for up to 2 years or until the individual was lost due to collar failure or mortality.

We recorded daytime den location points using ground-based tracking and homing techniques when field conditions permitted. When homing, skunk locations were recorded with precision, as the animal was tracked directly to its physical den location during daylight hours. We recorded GPS locations of dens using a hand-held GPS unit (model eTrex 20x, Garmin, Ltd., Olathe, KS). When field conditions, i.e., cliff lines, prevented us from traversing safely to physically locate an individual's den, we used standard triangulation and homing radio-tracking techniques to determine den location (Gorman *et al.* 2006). We recorded three observer GPS coordinates and respective azimuth direction and used Program LOAS (Ecological Software Solutions, LLC, Sacramento, CA, USA) to triangulate an individual's estimated daytime location. We estimated triangulation error by using ground-based tracking and triangulation methods on den locations by comparing the difference in distance between the actual and estimated location pairs following methods of Lesmeister et al. (2009).

Data Analysis

We identified potential exploratory excursions using the objective exclusion Incremental Cluster Polygon method (Hodder et al. 1998). We compared number of excursions and excursion distance (m) between sexes using Mann-Whitney *U* tests. We pooled all non-excursive den locations and triangulated locations of an individual to calculate home-range size using three methods: minimum convex hull (Worton 1995), minimum convex polygon (Mohr 1947), and 95% and 50% fixed-kernel density estimates with reference bandwidth (Worton 1989). We compared estimated home-range areas among our various study sites using a Kruskal-Wallis test, and between sexes using a Mann-Whitney *U* test. Additionally, we calculated the volume of intersection (VI) index to assess the degree of overlap (0 = no overlap, 1 = complete overlap) of the 95% kernel density home range and 50% kernel density core area estimates for male-male, male-female, and female-female dyads across and within study sites. We then used a Kruskal-Wallis test with a Bonferroni correction for multiple comparisons with α =0.003 to test for sex- and site-specific differences in VI of home range and core areas (Kruskal 1952, Holm 1979).

We examined predictors of habitat use within the home range of collared individuals using a weighted compositional analysis (RUF, Millspaugh et al. 2006). We used the weighted compositional analysis approach to characterize the relationship between the utilization distribution (UD) of an animal and a set of spatially-explicit landscape-level attributes, including land- cover class, forest fragmentation, and elevation. We used the 2016 National Land Cover Database (NLCD) classification system (Yang et al. 2018) to categorize land-cover types at our study sites as hardwood forest, conifer forest, mixed hardwood-conifer forest, shrub-scrub, and non-forested. Non-forested areas in our study included wildlife openings, oldfields, pasture and non-woody wetland areas. We derived a forest fragmentation map in ArcMap 10.2.2 (ESRI, Redlands, CA, USA) following the methods of Vogt et al. (2007) to classify areas within study sites as patch, edge, perforated, and core (> 250 acres). To calculate elevation categories in these mountainous areas, we reclassified the 30-m National Elevation Dataset (NED, U.S. Geological Survey 2002) into ten categories using Jenks natural breaks optimization in ArcMap (Jenks 1967).

We estimated resource selection at two levels according to the Johnson (1980) order of selection criteria following reccomendations of Aebische (1993). At the second order of selection, i.e. location of a home range within the study area, we compared the proportion of desicrete land-cover types (e.g. hardwood, mixed-conifer-hardwood, conifer) present within all homeranges within a given study site to the proportion available within the study site. At the third order of selection, i.e. selection of relocations within the home range, we calculated the proportion of each used resource type within each individual's home range by summing the UD value for each individual within each discrete resource category available within a home range and dividing by the total UD value (0.95). We defined the study area at each site by calculating a buffer equal to the mean radius of all home ranges at that site using the Buffer tool in ArcMap. We then compared pooled UD values for all individuals within a study site and used the weighted compositional analysis to compare within-study site resource use to availability. We used the Wilk's lambda statistic to test for significant selection of each habitat characteristic and perform a rank analysis on each resource class. All analyses were performed using R statistical software (R. Development Core Team[2], 2014) using package *adehabitatHS* (Calenge 2011) and R base package *stats* (R Core team 2014).

Results

We captured, radio-collared, and tracked 16 male and 10 female spotted skunks and estimated home-range size for 10 males and 8 females (Table 3.1). Three males and two females died, presumably from predation, before a sufficient number of locations (≥ 30) was collected to produce reliable denning and home-range estimates. Additionally, we lost signal of three males likely due to broken collar antennae. Body mass of adult males ranged from 390 – 755 g ($\bar{X}$ = 566.14 ± 15.4 g) and adult females from 401 – 655 g ($\bar{X}$ = 470.41 ± 25.8 g). We tracked individuals for an average of 295.8 ± 42.3 days and collected an average of 47 ± 6.7 locations per individual. Of the 846 total locations collected, 57.1% were known den locations tracked using homing techniques, whereas 43.9% were estimated using triangulation techniques. Triangulation estimate error was 0.94 ± 2.11° azumith and 83.24 ±15.08 m distance. Triangulated points with an error polygon diameter greater than 115.5 meters were removed from the dataset.

Extra-Home Range Excursions and Home Range Size

We observed a total of 22 exploratory excursions made by male spotted skunks: 10 during mating season, 12 during summer, and 0 during the non-mating winter months. Additionally, we observed a total of 23 exploratory excursions by female spotted skunks: 3 during mating season, 7 by pregnant females, 10 by females with ambulatory kits, and 1 during non-mating winter months. No females made excursive movements with non-ambulatory kits. Number of excursions varied among individuals and ranged from 0 – 5 ($\bar{X}$ = 2, SE =0.49) and 1 - 4 ($\bar{X}$ = 2.3, SE = 0.30) for males and females, respectively, which did not differ significantly between sexes. Average excursion distance was 1.07 (± 0.82) km and 0.59 (± 0.33) km for males and females, respectively, and were nearly significantly different at a 95% confidence level (U =

307, p = 0.08). Eleven of the 45 excursions occurred within 200 m of forest edge and two occurred within forest edge conditions. All other excursions occurred within forest fragments that were greater than 2 km^2. Four excursive events were associated with mortality of the individual, two males during mating season (15 Feb 2016 and 21 Mar 2017), one summering male (25 Jul 2016), and one female with non-ambulatory kits (11 Jun 2016). All three male mortalities occurred within a forest patch of less than 2 km^2, and the female mortality occurred within 200 m of the forest edge and were attributed to aerial predators, likely great horned owls (*Bubo virginianus*).

In general, male home-range estimates tended to be larger than female home-range estimates, although not significantly for any of the four estimators (Table 3.2). Unexpectedly, home-range sizes were not significantly different among study sites; however, TM home-range size estimates were always largest for both males and females, and WG home-range estimates were always smallest.

Home Range Overlap

Among all study sites, the volume of intersection (VI) of 95% KDE home ranges was not significantly influenced by sex (X^2 = 4.09, *df* = 2, *p* = 0.13), but did differ by study-site location (X^2 = 27.22, *df* = 3, *p* = <0.001, Table **Error! Reference source not found.**, Figure 3.2). Mean h ome range VI was largest at WT ($\overline{VI}$ = 0.48, SE = 0.05), followed by BM ($\overline{VI}$ = 0.39, SE = 0.04), TM ($\overline{VI}$ = 0.11, SE = 0.03), and WG where the two home ranges never overlapped. Similarly, mean 50% KDE (core area) also was not influenced by sex (X^2 =2.97, *df* = 2, *p* = 0.23), but differed among study sites (X^2 = 21.85, *df* = 3, *p* < 0.001). The largest mean core area VI occurred at the WT site ($\overline{VI}$ = 0.18, SE = 0.04), followed by BM ($\overline{VI}$ = 0.11, SE = 0.02) and TM (X^2 = 0.02, SE = 0.04). The WG core areas did not overlap.

Within study sites, VI of 95% KDE home range estimates were not influenced by sex for WT (X^2 = 0.05, df =1, p = 0.83) or BM (X^2 = 4.09, df =2, p = 0.13). No females were tracked at WG. Mean home range VI did differ significantly by sex-pair within the TM study site (X^2 = 7.15, df = 2, p = 0.03). Within TM, male-female dyads shared a greater mean VI than male-male dyads; however, there was no difference in mean home range VI between female-female and male-male dyads or between female-female and female-male dyads. Additionally, mean VI of core areas was not influenced by sex within study sites.

Resource Utilization Function

The mean home-range radius for each study site was: TM = 2.15 km, BM =1.53 km, WT = 1.09 km, and WG = 0.73 km. Total area of each study site defined by the buffer was: TM = 271.98 km^2, PT = 32.02 km^2, WT = 20.67 km^2, and WG = 9.34 km^2. When all sites were assessed together, the weighted compositional analysis showed that selection of discrete land-cover types available within study sites (second-order selection) differed from random (λ = 0.37 p = 0.0001), with an overall greater selection for hardwood forest, followed by evergreen and mixed hardwood-conifer forest cover types (Table 3.4-A). Selection of cover types at den site relocations within home ranges (third-order selection) also differed from random (λ = 0.33, p = 0.0001, Table 3.4-B). Eastern spotted skunks selected mixed deciduous-evergreen forest cover most and avoided non-forested areas within their home ranges. Selection of forest fragmentation type differed from random expectation at both the second order (λ = 0.05, p = 0.001, Table 3.5-A) and third order (λ = 0.00, p = 0.001, Table 3.5-B) with greatest selection of large core areas (> 250 acres) and lowest of non-forested areas. Core areas and perforated forest were generally selected more often within home ranges than other fragment types, whereas small patches, forest edge, and non-forested areas were strongly selected against. Elevation at home ranges located within study sites differed significantly from random ($\lambda = 0.00$, $p = 0.001$, Table 3.6-A). Elevations below 650 m were selected most, followed by elevations above 1050 m, whereas elevations in the middle of the range were selected against. Selection of elevation sites within home ranges varied significantly from random ($\lambda = 0.17$, $p = 0.0001$, Table 3.6-B) and followed patterns similar to home-range selection.

Discussion

In this study, we assessed patterns of excursive movements, home-range size and overlap, and resource use by eastern spotted skunks to ascertain the influence of environmental variation on animal movement. Our first objective was to detect whether eastern spotted skunks conducted extra-home range exploratory excursions that may lead to overestimation of home-range size and to identify movement barriers. Both sexes made excursive movements; however, we did not detect excursions for all individuals. In general, our findings did not support our prediction that male spotted skunks would make more frequent excursions than females; however, we observed patterns of seasonality that differed between sexes. Nearly half of male excursive movements occurred during mating season (mid-February – mid-April) compared to less than 14% of female excursions. In contrast, the majority of female excursions occurred during summer months after kits became ambulatory (mid-July – October). Male spotted skunk exploratory movements were, however, approximately twice as large as female movements, which did support our prediction.

Typically, animals whose movements are fixed within their home range are likely exploiting known and reliable resources, whereas individuals who leave their home range into novel landscapes risk the possibility of higher energetic expenses with no guarantee of resource availability or increased fitness (Nunes 2007). In our study, male spotted skunks were potentially risking the benefits of reliable winter resources (e.g., familiar food locations, thermal cover) for the benefits of additional mating opportunities or to avoid mating with related individuals (Nunes

2007). Potentially, winter excursive movements may act as a form of breeding dispersal driven by an individual's reproductive behavior where the benefits gained by additional reproductive opportunities may outweigh the risks associated with unreliability of resources (Salsbury and Armitage 1994, Debeffe et al. 2014).

Breeding excursions have been observed in males of various small carnivores, including European polecat (*Mustela putorius*, Lodé 2001), gray fox (*Urocyon cinereoargenteus*, Deuel et al. 2017), and red fox (*Vulpes vulpes*, (Cavallini 1996). The more frequent excursions of females with ambulatory kits may be a response by nursing females toto seek food resources due to a greater energy demand associated with lactation and kit-rearing. Larger home-range sizes and greater movement distances by females travelling with offspring have been observed in black bears (*Ursus americanus*, Alt et al. 1980), Florida panthers (*Felis concolor coryi,* Maehr et al. 1989), and honey badgers (*Mellivora capensis*, Begg et al. 2005). Animals often show behavioral plasticity related to environmental conditions (Komers 1997, Miner et al. 2005); however, in this study, we observed behavioral plasticity potentially related to intrinsic condition (i.e., reproductive status), which is particularly important to understand when designing and implementing management plans for a species of conservation or management concern (Becker and Bradley 2007).

We observed few excursive movements near or within forest edge areas and no excursions or intra-home range movements outside of forested areas. This suggests that spotted skunks in our study region may be limited to movements within core forest patches, with non-forested areas in the surrounding matrix serving as barriers to movement between populations, a pattern observed in many other small carnivore forest obligates (Hargis et al. 1999, Gehring and Swihart 2003, 2004). Movement barriers may explain the similarity in home-range size between sexes in our study areas. In a previous study in the Quachita Mountains of Arkansas, Lesmeister et al. (2013) found male eastern spotted skunk home ranges to be significantly larger than female home ranges. Although female home ranges sizes in our study were similar to those found by Lesmeister et al. (2013), male home ranges in our study were generally smaller. This difference may be attributed to land-cover type available for use by spotted skunks within each study. Land-cover types present in the previous study consisted entirely of forested areas, whereas non-forested areas accounted for approximately 12% of the cover types at our study sites. Further, home-range size was largest and inter-sex differences in home range size were more pronounced at the TM site, where the amount of non-forested area was lowest. Home range overlap was also lowest at the TM site (11%) and greater at the BM (39%) and WT (48%) sites. However, it should be noted that nocturnal movements were not included in our home-size estimates which could potentially contribute to apparent size differences between our home-range estimates and those found by Lestmeister at el. (2013).

Variable estimates of home-range overlap were consistent with those of other species within Mephitidae, suggesting a similar solitary behavior with flexible territorial strategies (Bixler 2000, Kasper et al. 2012, Jones et al. 2013). Further, we conducted intense trapping efforts and found high recapture rates of marked individuals at each site. Thus, we are condifent that nearly all animals were captured within site-years and our estimates of home range distributions and overlap are representative of the population.

. Eastern spotted skunks in this study did not use discrete cover types in proportion to their availability, either within study sites or within individual home ranges. Hardwood forests were selected more often than conifer or mixed-hardwood-conifer dominated stands. In the Appalachian region, hardwood forests often are generally associated with greater understory cover, i.e., herbaceous ground-cover and woody midstory tree cover, than conifer or mixed-hardwood-conifer forests, and may provide greater cover from predation and a greater variety of den resources (Kalisz 1986, McComb et al. 1986, Paragi et al. 1996, Taulman 1999, Menzel et al. 2002, 2004, see Chapter 3). Hardwood trees, particularly oaks (*Quescus* spp.), provide tree cavities used by spotted skunks as den and resting sites (Lesmeister et al. 2008, Sprayberry and Edelman 2018, Eng and Jachowski 2019, see Chapter 4). However, evergreen species (e.g., conifer trees, ericaceous shrubs) provide year-round canopy cover, which may still provide some proctection value from aerial predators, primarily great horned owls (*Bubo virginianus,* Lesmeister et al. 2010) despite more limited understory or ground cover.

Mixed forests may afford optimal heterogeneity for spotted skunks, with an ideal mix of canopy cover and understory cover while providing ample den resources, which may explain the greater selection of mixed cover type by individuals within home ranges.

Elevations below 650 m and above 1050 m were strongly selected over mid-range elevations. These findings are consistent with those of previous research on eastern spotted skunk habitat associations in the central and southern Appalachian Mountains (Thorne et al. 2017, Eng and Jachowski 2019). Lower elevations in the central and southern Appalachian Mountains typically are associated with cove hardwood forests which are characterized by high species diversity and complex vegetative structure (Bolstad et al. 1998, Ford et al. 2002, Turner et al. 2003, Warren 2008), which may offer a greater variety of resources for eastern spotted skunks. Alternatively, patches of old growth evergreen cover or emergent rocky outcrops present at high elevation also may provide the necessary vegetation structure and ample cover from predation required by eastern spotted skunks (Lesmeister et al. 2008, Thorne et al. 2017, Sprayberry and Edelman 2018, Eng and Jachowski 2019). Much of the mid-elevation forests in this region are comparatively homogenous with little to no understory cover and lower biodiversity (Yarnell 1998), and thus may be lower in habitat value to eastern spotted skunks.

As we observed no use of non-forested areas together with restricted home range size and increased home range overlap at sites with smaller core forest areas, we surmise that home ranges and movement of spotted skunks are limited by forest patch size. Consequences of movement barriers, such as vulnerability to demographic stochasticity and loss of genetic variation, can strongly reduce the potential for demographic recovery of a vulnerable species. Following population declines beginning in the 1940s, spotted skunk abundance was reduced by over 90% (Gompper and Hackett 2005), likely resulting in numerous local population extinctions. Reduction in core forest areas throughout the Appalachian Mountains may impede spotted skunk extra-home range movement, dispersal among extant populations, and recolonization of unoccupied areas. Additionally, limited geneflow among spotted skunk populations (Chapter 5) may result in inbreeding depression, genetic drift, and reduced effective population size, further reducing the potential for recovery of this vulnerable species. Assessments of spotted skunk population dynamics (vital rates) and genetic diversity should be conducted to determine current baselines and monitor future trends. Wildlife managers should seek to include these assessments in management planning and actions plans and plan to increase connectivity among current occupied and potentially available forest patches.

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Table 3.1 Distribution of number of occasions individual skunks were radio-tracked and number of unique den locations located for 10 male (M) and 8 female (F) eastern spotted skunks (*Spilogale putorius*) at all sites and for all seasons in the George Washington and Jefferson National Forests in the central and southern Appalachians of Virginia and West Virginia, USA, March 2015 – February 2019.

Site	Skunk ID	Occassions tracked	Den locations
North Tract Mountain	F004	107	38
	F026	133	56
	F028	35	22
	M001	49	34
	M024	68	45
	M027	36	16
Bald Mountain	F009	50	39
	F012	37	25
	F018	42	29
	F029	30	22
	M008	30	26
	M010	32	31
	M011	31	27
Wintergreen Mountain	M013	31	28
	M014	30	26
Whitetop Mountain	F015	35	33
	F006	33	25
	M007	34	23
	M016	36	12

Table 3.2 Various home-range size estimates (SE) of 10 male and 8 female eastern spotted skunks (*Spilogale putorius*) across all sites and within sites for all seasons in the central and southern Appalachians of Virginia and West Virginia, USA, March 2015 – February 2019. Home-range estimators include minimum convex hull (MCH), minimum convex polygon (MCP), 50 % fixed kernel density estimator (KDE 50%), and 95 % fixed kernel density estimator (KDE 95%). Estimates are displayed in km^2.

Group	**MCH**	**MCP**	**KDE 50%**	**KDE 95%**
All sites				
Males (*n* = 10)	0.90 (0.80)	1.07 (0.80)	1.38 (.73)	5.72 (3.37)
Females (*n* = 8)	0.62 (0.32)	1.10 (0.57)	1.19 (0.63)	3.61 (0.87)
Bald Mountain				
Males (*n* = 4)	0.60 (0.19)	1.13 (0.13)	0.85 (0.17)	3.32 (0.68)
Females (*n* =3)	0.66 (0.17)	1.08 (0.47)	1.07 (0.26)	4.04 (0.95)
White Top Mountain				
Males (*n* = 3)	0.91 (0.36)	1.13 (0.55	1.12 (0.32)	4.08 (1.12)
Females (*n* =1)	0.80	0.79	0.75	3.40
Wintergreen Mountain				
Males (*n* = 2)	0.36 (0.29)	0.68 (0.03)	0.46 (0.23)	1.70 (0.83)
North Tract Mountain				
Males (*n* = 3)	0.99 (0.70)	5.06 (3.09)	5.00 (3.59)	8.72 (3.22)
Females (*n* =3)	0.52 (0.18)	2.40 (0.72)	1.43 (0.37)	5.87 (1.62)

Table 3.3 Results of Tukey's Honest Significant Difference test of differences in mean volume of intersection (VI) of 95% kernel density estimate (KDE) home-ranges 50% KDE (core area) of eastern spotted skunks (*Spilogale putorius*) among study sites (North Tract Mountain = TM; Bald Mountain = BM; Wintergreen Mountain = WG; Whitetop Mountain = WT) in the central and southern Appalachians of Virginia and West Virginia, USA, March 2015 – February 2019.

	95 % KDE				**50 % KDE (core area)**			
Site pair	**VI difference**	**Lower 95% CI**	**Upper 95% CI**	***p-adj***	**VI difference**	**Lower 95% CI**	**Upper 95% CI**	***p-adj***
WG - BM	-0.39	-0.84	0.06	0.1	-0.11	-0.32	0.11	0.57
WT - BM	0.09	-0.11	0.29	0.63	0.07	-0.27	0.17	0.23
TM - BM	-0.29	-0.47	-0.15	< 0.001	-0.08	-0.15	-0.02	0.007
WT - WG	0.48	0.01	0.96	0.04	0.18	-0.05	0.4	0.19
TM - WG	0.11	-0.34	0.56	0.92	0.02	-0.19	0.24	0.99
TM - WT	-0.38	-0.58	-0.17	< 0.001	-0.15	-0.25	-0.05	0.001

Table 3.4 Comparison of within study-site home range (second-order) and within home-range relocation (third-order) selection and ranking of cover types based on eastern spotted skunk (*Spilogale putorius*) home-range estimates in the central and southern Appalachians of Virginia and West Virginia, USA, March 2015 – February 2019. Log ratios indicate whether land cover type in row is selected more (+) or less (-) than land cover type in column. Significant divergence from random (p < 0.05) represented by bold font. Lower ranks indicated higher level of selection.

	Land cover type					
Land cover type	Non-forested	Hardwood	Evergreen	Mixed	Shrub-scrub	Rank
A) Second order						
Non-forested	.	**-1.75**	**-1.65**	**-1.62**	-0.285	5
Hardwood	**1.75**	.	0.11	0.14	**1.48**	1
Evergreen	**1.65**	-0.11	.	0.03	**1.37**	2
Mixed	**1.62**	-0.14	-0.03	.	**1.34**	3
Shrub-Scrub	0.28	**-1.48**	**-1.37**	**-1.34**	.	4
B) First order						
Non-forested	.	**-1.40**	-0.79	**-1.27**	-0.68	5
Hardwood	**1.40**	.	0.44	-0.03	0.63	2
Evergreen	0.79	-0.44	.	-0.12	-0.11	4
Mixed	**1.27**	0.03	0.12	.	0.51	1
Shrub-scrub	0.68	-0.63	0.11	-0.51	.	3

Table 3.5 Comparison of within study-site home range (second-order) and within home-range relocation (third-order) selection and ranking of forest fragment types based on eastern spotted skunk (*Spilogale putorius*) home-range estimates in Virginia and West Virginia, USA, March 2015 – February 2019. Log ratios indicate whether forest fragment type in row is selected more (+) or less (-) than forest fragment typoe in column. Significant divergence from random expaction ($p < 0.05$) represented by bold font. Lower ranks indicated higher level of selection.

	Forest fragment type					
Forest fragment type	Non-forested	Patch	Edge	Perforated	Core > 250 acres	rank
A) Second order						
Non-Forested	.	**-0.88**	**-2.18**	**-2.28**	**-4.10**	5
Patch	**0.88**	.	**-1.30**	**-1.40**	**-3.22**	4
Edge	**2.18**	**1.30**	.	-0.10	**-1.92**	3
Perforated	**2.28**	**1.40**	0.10	.	**-1.82**	2
Core > 250 acres	**4.10**	**3.22**	**1.92**	**1.82**	.	1
B) Third order						
Non-Forested	.	**1.13**	-0.03	**-0.64**	**-1.04**	3
Patch	**-1.13**	.	**-0.85**	**-1.63**	**-2.41**	5
Edge	0.03	**0.85**	.	-0.61	**-0.83**	4
Perforated	**0.64**	**1.63**	0.61	.	-0.26	2
Core > 250 acres	**1.04**	**2.41**	**0.83**	0.26	.	1

Table 3.6. Weighted comparison of within study-site home range (second-order) and within-home range relocation (third-order) selection and ranking of cover types based on eastern spotted skunk (*Spilogale putorius*) home-range estimates in Virginia and West Virginia, USA, March 2015 – February 2019. Log ratios indicate whether elevation class in row is selected more (+) or less (-) than elevation class in column. Significant divergence from random ($P < 0.05$) represented by bold font. Lower ranks indicated higher level of selection.

	Elevation class (m)										
Elevation class (m)	< 450	450 – 550	550 – 650	650 – 750	750 – 850	850 – 950	950 – 1050	1050 – 1150	1150 – 1250	1250 – 1450	Rank
A) *Second order*											
< 450	.	**-0.51**	**-0.78**	0.59	**1.01**	**2.47**	**2.10**	0.22	0.55	0.51	3
450 – 550	**0.51**	.	-0.28	**1.10**	**1.52**	**2.98**	**2.60**	0.73	1.06	1.01	2
550 – 650	**0.78**	0.28	.	**1.38**	**1.80**	**3.26**	**2.88**	**1.01**	1.34	1.29	1
650 – 750	-0.59	**-1.10**	**-1.38**	.	**0.42**	**1.88**	**1.50**	-0.37	-0.04	-0.90	7
750 – 850	**-1.01**	**-1.52**	**-1.80**	**-0.42**	.	**1.46**	**1.08**	-0.79	-0.46	-0.51	8
850 – 950	**-2.47**	**-2.98**	**-3.26**	**-1.88**	**-1.46**	.	**-0.38**	**-2.25**	**-1.92**	**-1.97**	9
950 – 1050	**-2.10**	**-2.60**	**-2.88**	**-1.50**	**-1.08**	**0.38**	.	**-1.87**	**-1.54**	**-1.59**	10
1050 – 1150	-0.22	-0.73	**-1.01**	0.37	0.79	**2.25**	**1.87**	.	0.33	0.28	4
1150 – 1250	-0.55	-1.06	-1.34	0.04	0.46	**1.92**	**1.54**	-0.33	.	-0.04	6
1250 – 1450	-0.51	-1.01	-1.29	0.09	0.51	**1.97**	**1.59**	-0.28	0.04	.	5
B) *Third order*											
< 450	.	**0.80**	**0.82**	**1.52**	**1.91**	**2.12**	**1.82**	**1.46**	**0.98**	0.54	1
450 – 550	**-0.80**	.	**0.01**	**0.72**	**1.11**	**1.32**	**1.02**	0.66	0.18	-0.28	3
550 – 650	**-0.82**	-0.01	**.**	**0.71**	**1.09**	**1.30**	**1.00**	0.65	0.17	-0.30	4
650 – 750	**-1.52**	**-0.72**	**-0.80**	.	**0.39**	0.60	0.30	-0.06	-0.54	**-1.02**	7
750 – 850	**-1.91**	**-1.11**	**-0.82**	-.39	.	0.21	-0.09	-0.45	-0.93	**-1.39**	9
850 – 950	**-2.12**	**-1.32**	**-1.52**	**-.60**	-.21	.	-0.30	-0.65	**-1.14**	**-1.61**	10
950 – 1050	**-1.82**	**-1.02**	**-1.91**	-.30	0.09	.30	.	-0.36	**-0.84**	**-1.61**	8
1050 – 1150	**-1.46**	**-0.66**	**-2.12**	0.06	0.45	.65	0.36	.	-0.48	**-1.29**	6
1150 – 1250	**-0.981**	-0.18	**-1.7**	0.54	0.93	**1.14**	**0.84**	0.48	.	**-0.91**	5
1250 – 1450	-.54	0.28	**0.30**	**1.02**	**1.39**	**1.61**	**1.28**	**0.91**	0.40	.	2

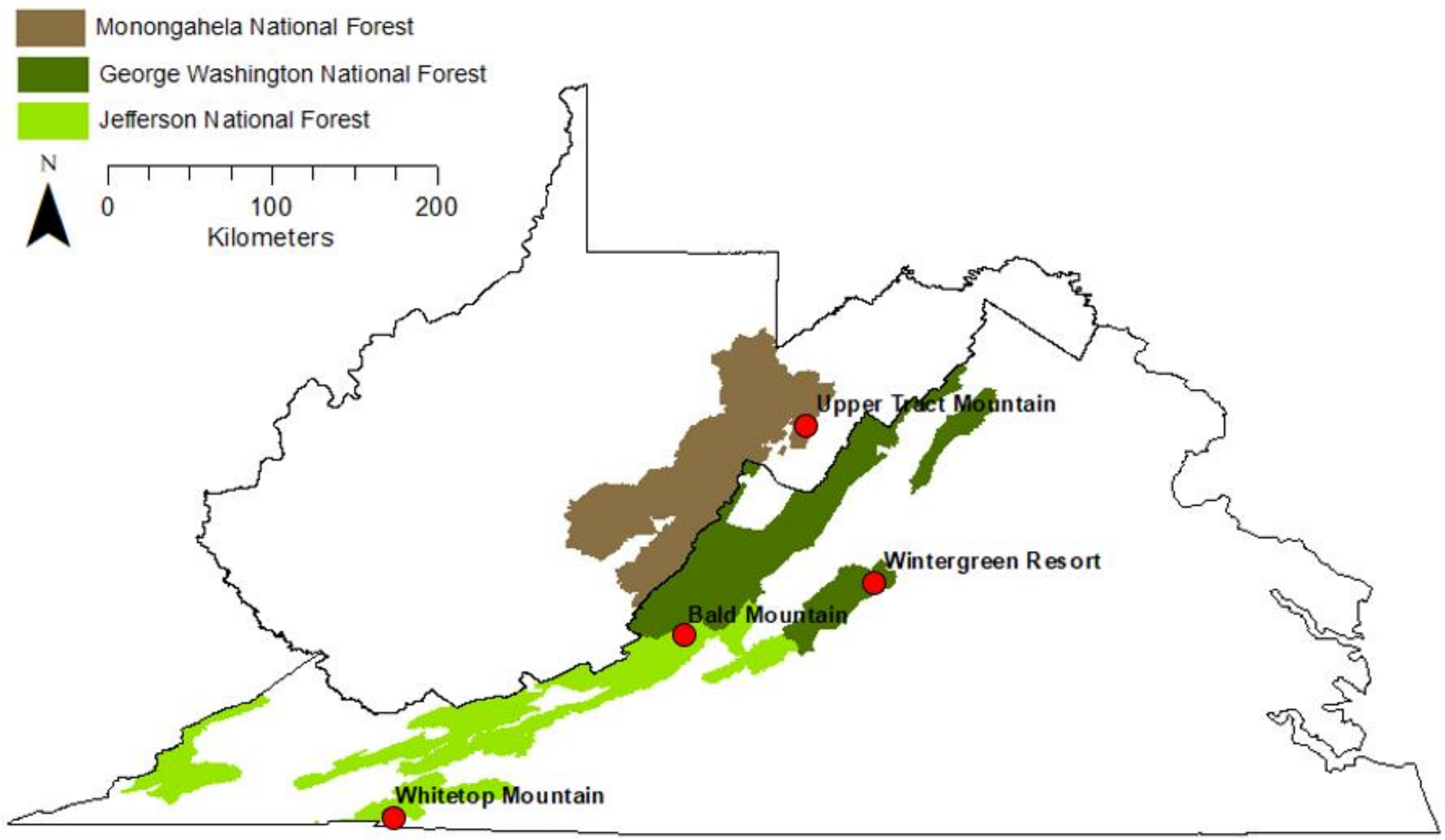

Figure 3.1 Eastern spotted skunk (*Spilogale putorius*) home-range study sites throughout the Monongahela, George Washington, and Jefferson national forests in in the central and southern Appalachian Mountains of Virginia and West Virginia, USA, March 2015 – February 2019.

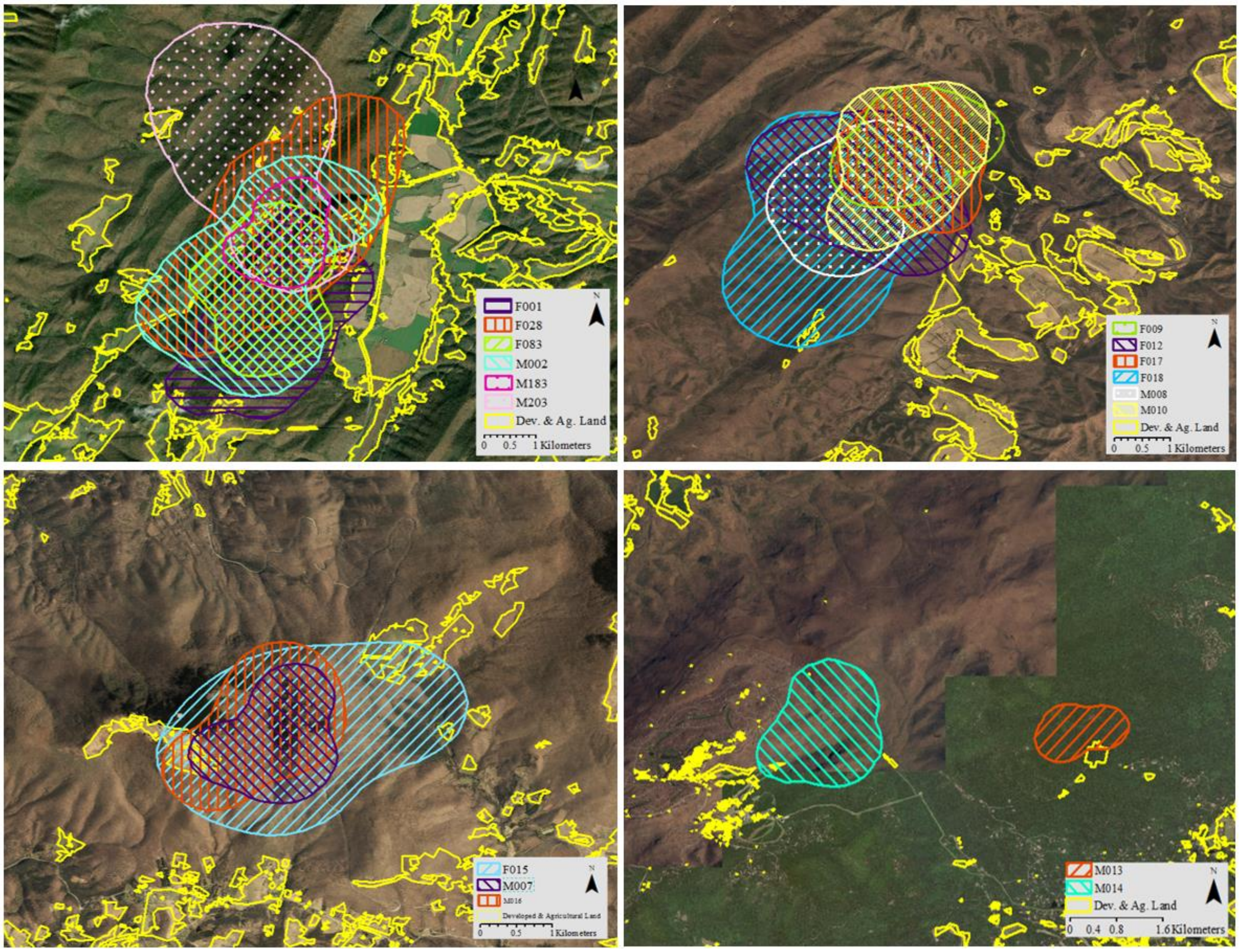

Figure 3.2 Example of eastern spotted skunk (*Spilogale putorius*) home-ranges at four study sites: North Tract Mountain (top left), Bald Mountain (top right), Whitetop Mountain (bottom left), and Wintergreen Mountain (bottom right) in the Monongahela, George Washington, and Jefferson national forests in in the central and southern Appalachian Mountains of Virginia and West Virginia, USA, March 2015 – February 2019.

Chapter 4 – Multivariate Analysis of Den Selection by Eastern Spotted Skunks (*Spilogale putorius*) Reveals Complex Habitat Relationships of a conditional generalist

Abstract

Wildlife managers tasked with understanding habitat and resource selection at the population level attempt to characterize patterns in nature that aid and inform management and conservation action plans. Resource selection functions, such as discrete choice analyses, are conventionally used to characterize the effects of habitat attributes on resource selection patterns. However, not accounting for complex sources of variation among habitat attributes and physical factors such as weather on target species' response may produce resource selection models with poor predictive ability, yield inappropriate interpretations, and ultimately lead to ineffective management actions. We compared the application of generalized linear mixed modeling (GLMM) and redundancy analysis (RDA) on eastern spotted skunk (*Spilogale putorius*) den selection data at four study sites in the George Washington and Jefferson National Forests and surrounding private lands in the Appalachian Mountains of western Virginia and northeastern West Virginia. We assessed the need for the inclusion of alternative sources of variation (i.e., weather conditions and individual intrinsic variation (e.g., age, sex, reproductive status) in addition to habitat attributes to better identify sources of variation in den selection. The RDA elucidated complex and opposing relationships, e.g., different use of den type based on reproductive status or weather condition, between characteristics at used den sites and habitat, weather, and intrinsic variation that were not evident in the GLMM model. Our results demonstrate the importance of examining resource selection data using multivariate techniques in addition to conventional discrete choice analyses to better understand intricate habitat–species relationships needed for effective management.

Introduction

A key aspect in the study of wildlife ecology is understanding how a species evaluates its natural surroundings and selects habitat that is advantageous to survival and reproductive success. In theory, an individual should select habitat that offers adequate resources to meet its biological requirements and that enables it to adjust its use of resources based on a variety of abiotic and biotic factors (Hutto 1985, Block and Brennan 1993). Relationships between wildlife populations and habitat characteristics are difficult to assess, yet identification and characterization of these relationships can improve delineation of limiting habitat factors needed for informing effective conservation and management planning and action (Rosenzweig 1981). Wildlife researchers often rely on multiple regression analyses to reveal the existence and direction of general trends and resource selection patterns. However, models of general habitat selection patterns may yield inappropriate conclusions for a species if key ecological factors are unaccounted for or if multiple dependent variables are interrelated (Andrén 1994, Jones 2001, Ahmadi-Nedushan et al. 2006). Additionally, ecological noise, such as confounding variables, uncontrolled for characteristics, and inter-individual variation, can increase the difficulty of identifying patterns; often it cannot easily be accounted for or can only beconsidered a random effect (Duchesne et al. 2010, Lesmerises and St. Laurent 2017). Without accounting for these complex sources of variation, models may show poor predictive ability or produce uninformative or errant results leading to inappropriate management actions (Rosenzweig 1981, Duchesne et al. 2010, Lesmerises and St-Laurent 2017). This issue may be more problematic when modeling habitat selection of species that exhibit behavioral plasticity and generalist or opportunistic characteristics where opposite responses among individuals may be cancelled out at the population level (Franklin et al. 2009, Lesmerises and St. Laurent 2017). The ability of a species to modify its habitat use when faced with variable resources and environmental conditions can increase the difficulty in identifying habitat selection patterns. Further, if research includes individuals that exhibit opposing behaviors due to various intrinsic characteristics such as different sex, age, reproductive, or hierarchical status, predictive models may produce misleading, equivocal, or incomplete results (Lesmerises and St. Laurent 2017). Unfortunately, this has been common in studies of rare or endangered species when sample sizes are small and data are pooled across all individuals or study sites (Bissonette 1999, Engler et al. 2004).

Discrete choice analysis is a modelling technique widely used in ecology to quantify habitat selection along linear trends (Cooper and Millspaugh 1999, 2001). The objective of this approach is to model the probability of a single response variable such as habitat selection as a function of measured habitat attributes present at matched pairs of used and random (potentially available for use, but not selected) sites (Hastie 1983, Cooper and Millspaugh 1999, Duchesne et al. 2010). The resulting model then can be used to predict future habitat selection in areas with similar habitat attributes for which the probability of habitat selection is unknown (Hastie 1983). However, when assessing habitat use across homogenous landscapes or when species detection is low, such as with rare species, discrete choice analysis may show little or no significant differences between predictor variables at used and unused sites and thereby produce models with poor predictive ability (Boyce et al. 2002). Another drawback is the assumption of uniform selection among individuals in a populationwithin which intrinsic characteristics may lead to variation in habitat selection among individuals that obfuscate patterns of habitat selection in the population (Boyce et al. 2002, Duchesne et al. 2010, Lesmerises and St. Laurent 2017).

General linear mixed models (GLMM) attempt to account for these differences by including a random regression coefficient to represent inter-individual variation as a random effect (Bolker et al. 2009, Duchesne et al. 2010). The inclusion of a random effect is intended to relax the need to sample individuals from a single group, such as same sex or age group, if the sample size is large enough to adequately represent each group in the dataset (Gillies et al. 2006, Dingemanse and Dochtermann 2013). A large sample size might be achievable for abundant species with large distributions and high population densities (Dolton and Kendall 1993, Peterjohn et al. 1996); however, this becomes increasingly difficult for rare species or small populations (Kunin and Gaston 1993). Additionally, discrete choice analysis typically requires a large sample size to prevent sparse-data bias where all or nearly all observations fall into one data group and few or none in another. GLMMs were originally developed to account for small sample size to prevent sparse-data bias, but may still result in bias or uninterpretable confidence intervals when too few matched pairs or too many covariates are used (Greenland et al. 2000). Often, GLMMs are limited to assessing one response variable such as "used" and "random" locations in discrete choice analysis. This limits a researcher to assessing differences between predictor variables at paired sites. However, this technique does not allow for the inclusion of covariates that are the same at paired sites but that may also influence site selection, such as weather and intrinsic variables. For example, a specific den site for a species may be selected or avoided as a strategy of protection against inclement weather, to prevent predation upon offspring, or for access to temporally variable resources (Aquino and Encarnación 1986, Smith et al. 2007, Koeler Lira et al. 2018).

The application of multivariate statistical methods can be an effective alternative to conventional resource selection functions, such as discrete choice analysis, as they can incorporate complex relationships among multiple sources of explanatory and response variables (Connolly et al. 2002, Ahmadi-Nedushan et al. 2006, Zhang et al. 2007, Basille et al. 2008, Clavero et al. 2009). Although commonly used in community ecology to assess the relationship between environmental variation and community composition at various sites, these methods can be used to assess the relationships among variables such as weather, other intrinsic variables (e.g. sex, age), and the microhabitat characteristics present at each selected site (Maki-Petäys et al. 1997, Basille et al. 2008). Redundancy analysis (RDA) is an ordination techniques that can be used to identify and condense the variation within a set of response variables (e.g., site characteristics) that can be explained by a set of predictor variables (e.g., weather, intrinsic differences, environmental variation) by expanding multiple linear regression to include multiple response variables (van den Wollenberg 1977). Rather than providing an estimate of probability of use, the result of RDA quantifies the amount of variance present among response variables that is accounted for by variation among predictor variables (James and McCulloch 1990), thereby allowing researchers to identify complex resource selection patterns that otherwise may be masked when comparing only site use to non-use. However, because this method is descriptive rather than analytical, the addition of multivariate analysis of variance (MANOVA) permits hypothesis testing on effects of independent variables on more than one dependent variable (James and McCulloch 1990).

In our study, we aimed to investigate the effects of multiple sources of variation on resource selection of a rare but generalist carnivore species, the eastern spotted skunk (*Spilogale putorius*) in a homogenous landscape by examining microhabitat characteristics, landscape-level characteristics, weather conditions, and inter-individual intrinsic variation linked to den selection. Although generally rare, eastern spotted skunks typically show generalist and opportunistic characteristics where present in the central and southern Appalachian Mountains (Thorne et al. 2014, Sprayberry and Edelman 2018, Eng and Jachowski 2019). Recent research of eastern spotted skunk den site selection in the Appalachian region has used discrete choice analysis to characterize resource selection, with consistent results indicatingshowing increased probability of site use with increased forest canopy and understory cover and amounts of coarse, downed woody debris (Eng and Jachowski 2019). However, in Alabama due to small sample size, Sprayberry and Edelman (2018) were unable to examine male and female den site selection separately. Eng and Jachowski (2019) achieved an adequate sample size to examine site selection by sex and found significant differences in variables that influenced males versus females in South Carolina. However, their research was conducted during winter and spring only. Neither project examined effects of intrinsic characteristics, such as reproductive status, or variation in weather conditions on den site selection by eastern spotted skunks. Additionally, in using discrete choice analysis, both examinations were limited to a single response variable to compare habitat characteristics between used and unused den sites. Accordingly, we sought to expand on the conventional method of prediction of site use (discrete choice analysis) to include multiple response variables (multivariate analysis) in order to elucidate and interpret the complex relationships among multiple sources of variation and site selection. Therefore, the goals of our study were to: 1) determine if variation in den selection by eastern spotted skunks can be explained by factors including weather conditions and inter-individual variation in addition to habitat attributes; and 2) assess whether the incorporation of a multivariate analysis can better explain complex resource selection patterns potentially masked or ignored by discrete choice analysis. To meet these goals, we compared results of two analyses, the conventional discrete choice method of resource selection (GLMM) and a less-conventional multivariate regression method (RDA) on the same eastern spotted skunk den selection data set.

Methods

Study Area

We conducted our study in the Blue Ridge and Valley and Ridge physiographic provinces of the central Appalachian Mountains in western Virginia and northeastern West Virginia, specifically on or near the George Washington, Jefferson, and Monongahela National Forests and on adjacent private lands (Thorne et al. 2014, Thorne and Waggy 2017) . The study area is characterized by a cool-temperate climate with annual temperatures ranging from -10.6° C - 27.5° C, and a mean annual temperature of 6.1° C (National Oceanic Atmospheric Administration, public data 2019; www.noaa.gov). Annual precipitation is approximately 110 cm throughout most of the range (Ford et al. 2015) We captured and radio-collared eastern spotted skunks at four focal study sites: Mount Rogers National Recreation Area, Jefferson National Forest, Grayson County, VA (MR); Bald Mountain, George Washington National Forest, Botetourt County, VA (BM); The Nature Foundation at Wintergreen Resort, Nelson County, VA (WG); and Upper Tract Mountain, Monongahela National Forest, Pendleton County, WV (TM). All study sites were rugged mountainous areas with fairly continuous, unfragmented core forest landscapes. Elevation varied among study sites, with the highest elevation ranging from 1300 – 1700 m at MR, from 370 – 1200 m at WG, from 300 – 1100 m at BM and WG. Principal forest cover types at BM, WG and TM included mixed hardwood forests dominated by Appalachian oak (*Quercus* spp.) and oak-pine (*Pinus* spp.) forest types, whereas MR was dominated by a high elevation red spruce (*Picea rubens*)-northern hardwood community (Braun 1950, Simon 2011, 2013). Whereas much of MR was old-growth or had not been overly disturbed since the turn of the 20th century, the other sites were an admixture of older, mature forest, mid-aged stands and some early successional forests.

Capture and Handling

From March 2015 through February 2019, we conducted a telemetry-based study to assess microhabitat selection of eastern spotted skunks. To increase trapping efficiency, we selected four capture sites where spotted skunks were known to be locally present from use of established baited camera sites following the methods of Thorne et al. (2014). When spotted skunks were detected on camera, we deployed 8 Tomahawk # 103.5F0 box traps (Tomahawk Live-Trap Co., Tomahawk, Wisconsin, USA) in an array of 4 traps spaced 3 m and 4 traps spaced 10 m from the baited camera. We baited traps with a mixture of peanut butter, bacon grease, and rolled oats. Traps were set between 12:00 and 16:00 hrs and were checked 30 minutes after sunrise daily. We covered the back two-thirds of the traps with a layer of plastic and duct tape to protect any captured animals from wind, precipitation, and low temperatures.

We removed captured individuals, placed them in a cloth handling bag, and collected measurements of sex, mass to the nearest 5 g, total body length, tail length, head length, head width at zygomatic arch, right hind foot length, testis length and paired width of testes for males, and thoracic teat length and width of females. We classified individuals as juvenile or adult by body size, tooth wear, and capture history (Crabb 1944). We affixed a uniquely numbered ear tag to each captured individual and outfitted each individual with a 14-g VHF (very high frequency) radio collar (ATS #M1740) to adult individuals with a mass greater than 350 g. Collared individuals were tracked for up to two years from date of initial capture, at which time we recaptured the individual and removed the collar, or until the individual perished or the collar malfunctioned. When possible, individuals were recaptured to replace malfunctioning collars.

When female individuals were observed to have ambulatory kits, we deployed traps surrounding the den entrance females following the trapping methods above for a minimum of four nights or until all kits were captured. All measurements listed above were taken of kits and adult females, kits were sexed and ear-tagged, females were examined for evidence of lactation, and all individuals were released simultaneously at the den entrance. All trapping and handling procedures were approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee (protocol number 13-119-FIW) and permitted by Virginia Department of Game and Inland Fisheries and West Virginia Division of Natural Resources.

Tracking and Data Collection

We physically located den sites of collared individuals 1-3 days per week and mounted Bushnell Trophy Cam HD (Model 119739, Overland Park, KS) or Browning Strike Force (Model BTC-5HD, Morgan, UT) remote-triggered infrared cameras on a tree trunk approximately 3 – 4 m from the den entrance to record times and frequency of entrance and exit of den and duration of use. Additionally, we recorded GPS location and physical characteristics of the den microhabitat variables within 10 m of the den entrance. We classified primary den type as: 1) underground burrow, 2) cavity in a live tree, 3) cavity in a snag, 4) rock crevice, or 5) downed log or stump. Secondary characteristics at the den entrance were classified as: 1) base of tree, 2) under vegetation, 3) emergent rock outcrop, 4) small rock pile, 5) in root wad, 6) none, and 7) other/anthropogenic (Figure **Error! Reference source not found.**). Other measured den c haracteristics included height and width of den entrance, and distance to nearest shrub. To measure microhabitat characteristics at each den sites, we modified the methods of Dueser and Shugart (1978) and Urban and Swihart (2011). We established a grid centered on the den entrance with 10-m transects extending in the four cardinal directions to measure microhabitat variables. At the den entrance and each corner, we calculated percent canopy cover using a spherical densitometer, visually estimated percent understory cover, and recorded dominant canopy and understory species. We classified dominant canopy species into: 1) hardwood, 2) coniferous, and 3) mixed-hardwood-coniferous (mixed). Dominant understory species were classified into six groups: 1) predominantly mountain laurel, 2) predominantly rosebay rhododendron, 3) predominantly saplings, 4) mixed understory, 5) other (e.g., huckleberry (*Gaylussacia* spp.), blueberry (*Vaccinium* spp.), greenbrier (*Smilax* spp.), ferns, and 6) no understory. Within a randomly chosen quadrant of the grid, we recorded the number of downed woody debris > 10 cm in diameter along each transect and counted the total number of exposed rocks > 10 cm, number of snags, and number of vines. For every physically located spotted skunk den, we used a random number generator to choose an azimuth and distance between 50 and 500 m from the "used" den. We then searched for a similar random den at the random location that was potentially available for selection by the individual but not chosen by a radio-tagged individual and repeated den characteristics and microhabitat assessment for unused dens. An unused den was deemed potentially available for use only if the opening was ≥ 10 cm in diameter, seemingly excluded sunlight during daytime, and likely provided protection from weather and predators (Crabb 1948, Lesmeister et al. 2008a).

To assess the relationship of den selection to landscape-level attributes, we used remotely-sense geographic information system layers in ArcMap 10.2.2 (ESRI, Redlands, CA, USA). Using a 10-m, 3D Elevation Model (U.S. Geological Survey, 2017), we extracted elevation (m) and derived slope (degrees) and sine-transformed aspect (Evans et al. 2014) for every used den and unused den location using the surface analysis tools in ArcMap. Additionally, we used the Zonal Statistics tool in ArcMap to calculate 10-m and 1-km topographic exposure indices (TEI, Evans et al. 2014) surrounding the den entrance by subtracting the average elevation within a 10-m and 1-km radius of the den entrance from the elevation at the den entrance. Higher TEI values indicate greater topographic exposure. We then used the Near tool in ArcMap to extract distances to nearest permanent stream or river, permanent water body, and road.

In addition to habitat characteristics, we collected daily weather data recorded by the nearest land-based weather observation station (National Oceanic and Atmospheric Administration, www.noaa.gov) for each den. We extracted weather variables for the date a given den was physically located and for the date prior to physical location to assess whether den selection by spotted skunks was driven by prevailing or imminent weather conditions. Weather variables included maximum, minimum and average daily temperature; maximum, minimum and average daily humidity; daily rainfall, snowfall, total precipitation; atmospheric pressure, percent moon illumination, maximum wind speed, and wind direction. We also included the ordinal date that each den was physically located to account for temporal variation throughout the study period. We tested our data to ensure compliance with the assumptions of linear regression, transformed non-linear covariates using arcsine, log or square-root transformation as appropriate, removed highly correlated covariates ($r^2 > 0.7$), and tested for spatial autocorrelation.

Generalized linear mixed modeling (GLMM)

To identify key habitat characteristics that influence den selection by eastern spotted skunks, we used a discrete choice analysis to compare microhabitat features between used and unused den sites (Cooper and Millspaugh 1999, 2001). We developed three *a priori* models sets representing different categories of den site features: 1) primary den type and secondary den characteristics, 2) microhabitat characteristics, and 3) landscape-level characteristics. We created generalized linear mixed models to compare environmental factors present at used and unused dens, with study site included as a random effect to account for within-site spatial autocorrelation. To account for the effect of inter-individual variability on den selection, we examined null models that included individual identification and reproductive status (i.e., reproductive male, reproductive female, summering male, wintering male, wintering female, pregnant female, female with non-ambulatory kits, and female with ambulatory kits) as a random effect.

We classified males as summering from mid-April through October and males and females as wintering from November through mid-February. Males and females were classified as reproductive from March through mid-April, females were classified as pregnant from the mid-April through the second-to-last week of May, females were classified as with non-ambulatory kits from the last week of May through the first week of July, and with ambulatory kits from the second week of July through October (Crabb 1944, Kinlaw 1995). We used additional opportunistic evidence to confirm reproductive status throughout the study including: 1) locating a male-female pair occupying a single den simultaneously from mid-February through the first week of April; 2) using remote sensing cameras at den sites to note increased abdominal size of females during pregnancy, decreased abdominal size of females post-parturition, increased female daytime activity, and females transferring kits from dens; 3) observations of kit vocalizations when a natal den was located; 4) presence of ambulatory kits in videos and photos, and 5) cessation of kit detections at the den as a result of either dispersal or unknown mortality events. We carried forward the best selected random effect variable in all fixed-effect models and used Akaike's Information Criterion (*AIC*) and Akaike weights (w_i) to determine the best and most parsimonious model for each model set (Burnham and Anderson 2002). Additionally, we created *post hoc* models containing additive and interactive combinations of covariates from all competing models of each model set. Because environmental factors may influence den selection at multiple scales simultaneously, we created a *post hoc* model set by combining top covariates from all competing models within the original three model sets. We used Akaike's Information Criterion (*AIC*) and Akaike weights (w_i) to determine the single best model to estimate probability of den use based on amalgamation of den, microhabitat, and landscape-level characteristics. All GLMM analyses were conducted using package *lme4* (Bates et al. 2019) in Program R (R Core team 2014).

Redundancy analysis (RDA)

To assess whether complex relationships among multiple categories of environmental variation influence selection of den sites by eastern spotted skunks, we conducted a two-part redundancy analysis (RDA) using data from used den sites. First, to reduce the number of redundant variables, we identified the most relevant variables in each variable category (i.e., microhabitat attributes, landscape-level attributes, weather condition, and intrinsic variation) using principle feature analysis (PFA) following the methods of Lu et al. (2007). A PFA is used to select a subset of predictor covariates that to explain the most variation in a set of response variables (Lu et al. 2007). This is done by conducting a principal components analysis on the covariance matrix of the original predictor covariates to identify clusters of correlated covariates using a *K*-means algorithm (Lu et al. 2007). For each cluster, the predictor covariate closest to the cluster mean is selected as a principal feature (Lu et al. 2007).

Next, we conducted RDA using the variables selected from the PFA as constraining (predictor) variables and the den type and secondary den characteristics as unconstrained (response) variables. Additionally, we included individual ID and study site as conditional variables to account for inter-individual variation and within-site spatial autocorrelation. We then produced an RDA ordination plot to visually describe relationships between environmental variables and den characteristics. On an RDA plot, arrows represent explanatory covariates and arrow length indicates the amount of variance explained by that covariate (ter Braak 1990, Oksanen 2007). Distance of den characteristic points from an environmental covariate arrow indicates the strength of the relationship between the den characteristic and the environmental covariate (ter Braak 1990, Oksanen 2007). Finally, we conducted a multivariate analysis of variance (MANOVA) to estimate the significance of the proportion of variance in the response variables explained by each covariate. All ordination analyses were conducted using package *vegan* (Oksanen et al. 2019) in Program R.

Results

Capture and Handling

From March 2015 through February 2018, we captured a combined total of 35 eastern spotted skunks across all 4 study sites: 16 adult males, 10 adult females, 7 juvenile males, and 2 juvenile females. Of the captured adults, we radio-collared and collected a sufficient number of data points for 10 males and 8 females. Juvenile skunks were not collared due to small body size. From March 2015 to April 2016, three adult males could notbe tracked within one to two weeks of collar deployment due to collar failure. We successfully re-trapped all three individuals and determined collar failure was a result of antenna damage, removed the damaged collar, and released the individuals. Three adult males and 6 adult females were found deceased within one-to-four weeks of collar deployment. We attributed the source of mortality of all 9 adult skunks to be an aerial predator, most likely great horned owls (*Bubo virginianus*) or barred owls (*Strix varia*). Eight of the mortality events occurred between the months of March and May, and one mortality occurred in December. Collars of deceased individuals were typically found on the ground surrounded by tufts of spotted skunk fur and lacked teeth marks and the general vicinity smelled of skunk odor. On one occasion the head of a skunk was found with the collar attached, another collar was found near an owl pellet, and a third collar was detected in a bird nest approximately 9-m high in a live pitch pine approximately 36 cm in diameter and could not be recovered. Additionally, observers flushed unidentified owls from the site where a collar was found on two occasions. We tracked individual spotted skunks for a mean of 273.3 (± 51.7) days and located and assessed a total of 364 used and 208 unused skunk dens. To maintain independence of used dens, each den location was included in the dataset once regardless of length of use or number of times reused.

Minimum duration of den occupancy ranged from 1 to 37 nights with females typically remaining in one den longer than males (Figure 4.2).

. Duration of den occupancy ranged from 1 to 37 days though Additionally, females with non-ambulatory kits and wintering females spent the greatest duration of time occupying a den. During our study, male-female pairs were detected sharing a den on four occasions. At the MR site, one pair was tracked to a shared burrow at the base of a tree on 9 March 2016 and to a similar burrow at the base of a tree on 10 May 2016. On 1 March 2016, a pair was tracked to a live tree cavity at the PM site. In 2017, the same pair was tracked to a live tree cavity at the PM site and on 29 March, and a second pair was tracked to a snag cavity on 5 April. No same-sex pairs, other than a female with kits, were found to use a den simultaneously, although dens were infrequently reused by the same and different individuals within and across years at the four study sites.

Environmental Covariates

Few covariates differed significantly between used and unused sites (Table **Error! Reference source not found.**). Of the 364 used dens located among all sites, we classified 62.1 %

as underground burrows, 26.1% as rock crevice, 9.3% as live tree cavities, and 2.5% as snag cavities. The most common secondary den characteristics selected were none (29.0%), in emergent rock outcrop (24.0%), and under vegetation (20.0%) followed by in small rock pile (9.2%), in root system (7.3%), at base of tree (6.5 %), in log or stump (3.3 %), and other or anthropogenic (0.7%). Thirty-nine percent of selected burrows had no secondary characteristic present, 14% of burrows were in or under vegetation, and 14% were located in root systems. Rock crevices were mostly associated with emergent rock outcrops (77%) and small rock piles (16%) and were rarely associated with vegetation (4%). Live tree and snag cavities typically had no secondary characteristic present (56% and 89%, respectively). Forty-one percent of live tree cavity entrances were located at the tree base; however, tree height ranged from 3 m to 18.3 m and entrances to cavities ranged from ground level to approximately 16.8 m, with a mean entrance height of 3.5 ± 0.1 m. Of the tree dens, 88.5% were located in oak: white oak (*Q. alba*), 44.2%, chestnut oak (*Q. montana*) 25.6 %, red oak (*Q. rubra*) 14%, and black oak (*Q. velutina*) 4.7%. Additionally, 7% of dens were located in black gum (*Nyssa sylvatica*) and 2.3% were located in red spruce and white pine (*P. strubus*) each.

Despite the observed differences between proportions of den types used, the best selected den characteristic model did not contain primary or secondary den type. Rather, the top model showed a positive relationship with den entrance size (Table 4.2). However, mean den entrance size was smaller at used sites than at unused sites, but confidence intervals overlapped. This discrepancy is a result of opposing differences in entrance sizes at different den types that was not evident GLMM analysis. Whereas entrance size of rock crevices ($\bar{X}$ = 167.9 ± 17.3 cm) and live tree cavities ($\bar{X}$ = 65.5 ±18.1 cm) were smaller at used sites, burrows were used far more often than other den types and used burrow entrances were larger at used sites ($\bar{X}$ = 121.8 ± 8.8

cm) than at unused sites ($\bar{X}$=51.2 ± 12.1 cm). The majority of used dens (46.2%) were located in hardwood stands, whereas 31.0% were located in mixed stands, 17.9% were in coniferous stands, 2.8% were located in mixed hardwood-spruce stands, and 2.9% were in red spruce stands.

Generalized Linear Mixed Modeling (GLMM)

There were multiple competing microhabitat models, with no strong evidence of support for any one model or microhabitat attribute effect (Table 4.2). Similarly, all landscape level models produced were competing with ΔAIC <2 with low model weights (Table 4.3). However, only hardwood, coniferous, and mixed cover types produced 95% confidence intervals that did not overlap zero. Standard errors were large for most effect size estimates for all covariates for both the microhabitat and landscape model sets, and most 95% confidence intervals overlapped zero. Additionally, the den characteristic model set contained large confidence intervals that overlapped zero. The top den characteristic model contained entrance size only, with a model weight over three times greater than the next- best model and ΔAIC > 2.0 (Table 4.4).

Our *post hoc* models containing interactive combinations of covariates failed to converge and were not included in the final model set (Supplementary Table 4.1). *Post hoc* models containing both microhabitat and landscape-level covariates did not perform better than the top models containing only microhabitat or landscape level covariates (Supplementary Table 4.1). There was no strong support for any one best *post hoc* model and the addition of landscape-level covariates to the top den characteristic model did not improve model *AIC*.

Redundancy Analysis (RDA)

The PFA reduced the number of microhabitat, landscape-level, weather, and intrinsic variables to 11 highly influential variables. Retained variables included previous-day atmospheric pressure, minimum daily temperature, average daily humidity, distance to nearest shrub, percent canopy cover, percent understory cover, dominant canopy cover type, dominant understory species, number of coarse woody debris items, number of exposed rocks, distance to nearest road, distance to closest permanent water body, and female reproductive status (i.e., breeding female, pregnant, female with non-ambulatory kits, female with ambulatory kits). Together, the retained covariates, individual ID, and study site explained approximately 42.7% of the variation in den selection. The variation in the constraining covariates accounted for approximately 36.9% of the variation in response variables and conditional covariates accounted for the remaining 5.8%. Approximately 79.7% of the total variation in den selection was accounted for by RDA axes 1 and 2 (Figure 4.3). The RDA plot provides a qualitative interpretation of the relationships between den characteristics and predictor covariates.

Selection of den characteristics varied by weather conditions, microhabitat characteristics, landscape-level characteristics, and intrinsic conditions. Burrows and tree cavities were positively associated with atmospheric pressure and humidity. Conversely, rock crevices were negatively associated with atmospheric pressure and positively associated with minimum daily temperature. Burrows and tree cavities also were associated with greater canopy cover and mixed hardwood-pine mixed hardwood-red spruce, and red spruce cover types. Burrows also were associated with greater understory cover and mountain laurel understory species. Live tree cavities were associated with greater downed woody debris, sapling understory type, and no secondary den characteristics. Alternatively, rock crevices were strongly associated with number of exposed rocks and emergent rock outcrops, no understory, and hardwood or pine canopy cover types. Burrows were strongly associated with greater distance from roads, and rock crevices were associated with greater distance from permanent water bodies. Den selection also varied by female reproductive status, with a strong association of breeding females with tree crevices and a weaker association with burrows. Females with non-ambulatory kits were strongly associated with underground burrows, while pregnant females and females with ambulatory kits were associated with rock crevices. Males and wintering females showed no strong associations with any specific den type or secondary den characteristic. Our MANOVA results showed that nearly all retained covariates explained a significant proportion of variation in den selection by spotted skunks at the 95% confidence level (Table 4.5). Although previous-day minimum humidity and atmospheric pressure were significant only at the 90% confidence level, these covariates may still be biologically important.

Discussion

Our study is the first to directly assess the effects of intrinsic variation and weather conditions on den selection of eastern spotted skunks using a multivariate approach. Similar to our predictions, including a multivariate approach in our analysies enabled us to detect important relationships that were not evident when using discrete choice analysis alone. When comparing used sites to unused sites with GLMM, we found little evidence to support any one top model. The lack of sufficient support for any one model may result from opportunistic selection of available dens by spotted skunks coupled with an abundance of available den resources or due to the homogenous landscape across our studies sites, as demonstrated by the lack of significant differences in environmental covariates. Few covariates differed significantly between used and unused sites, which led to low model predictive ability. Additionally, univariate modeling techniques, such as the one used in this study, consider the effects of individual covariates on the response variable separately, whereas multivariate techniques determine the response to the cumulative effect of multiple interacting covariates (van den Wollenberg 1977, James and McCulloch 1990). For example, using a multivariate analysis, Wilbur (1987) found that predation on eastern newts (*Notophthalamus viridescens*) led to an increase in American toad (*Anaxyrus americanus*) survival by reducing competition in desiccating ponds, a relationship they were unable to detect with additive or interactive univariate models. Our attempt to examine a more complex GLMMs by introducing covariate interactions produced models that failed to converge. However, competing models included microhabitat covariates related to canopy and understory cover, which concurs with previous findings (Eng and Jachowski 2019, Sprayberry and Edelman 2018).

In general, used den sites had greater understory cover. Spotted skunks den sites were strongly associated with rhododendron, saplings, and mixed understory types over mountain laurel or other species. Spotted skunks may be using areas with thick rhododendron cover or high sapling density as a mechanism to avoid predation by aerial predators, such as owls, which are likely unable to navigate flight through areas of complex forest structure (DeGraaf et al. 1992, Lesmeister et al. 2008b). The association of eastern spotted skunk with greater understory cover has been demonstrated and the predator avoidance hypothesis has been proposed by several studies of eastern spotted skunk habitat selection (Lesmeister et al. 2008a, Thorne et al. 2014, Sprayberry and Edelman 2018, Eng and Jachowski 2019). In addition to predation cover, thick rhododendron patches may provide areas of greater prey abundance. As a riparian species, rhododendron is often located in drainages near ephemeral or permanent water sources (Ford et al. 2006) where prey species such as insectivores, rodents, amphibians, and invertebrates typically occur at greater densities (McComb et al. 1986, Doyle 1990, Tockner and Ward 1999, Malo et al. 2013, Hamilton et al. 2015). Despite the apparent benefits of dense understory cover to eastern spotted skunks, we found a more complex relationship than expected between den characteristics and understory cover when examining the effect of microhabitat attributes, weather, and reproductive status through RDA.

Understory cover was strongly positively associated with underground burrows and negatively associated with rock crevices, which instead were associated with a greater number of exposed rocks. Moreover, tree cavity sites were strongly associated with no understory cover and a greater distance to nearest shrub, but contained higher amounts of downed woody debris items. This result suggests that the secondary den characteristics and microhabitat attributes present at a den site may be influencing the selection of each den type differently, yet cover of some sort is always important. The opposing effects of percent understory at different den types likely was masked when examining these variables in the discrete choice analysis, leading to poor predictive ability by our models.

This relationship become more apparent when examining the effect of female reproductive status. Although breeding females appear to select tree cavities, pregnant females and females with ambulatory kits show stronger associations with rock crevices, and females with non-ambulatory kits showed a preference for underground burrows. Coupled with greater rocky cover available to spotted skunk in rock piles and emergent rock outcrops, rock crevice den sites may offer better protection from predators for many smaller carnivore species (Burbidge and Manly 2002, Hwang et al. 2007, Ross et al. 2010). Female spotted skunks are likely more vulnerable to predation when pregnant or with kits. Pregnant females are burdened with larger body mass, females with non-ambulatory kits occupy a single den for a greater duration of time, and females with ambulatory kits must select den sites that provide adequate cover for naïve offspring. Also, predation risk increases as time spent outside the den to meet foraging needs increases for pregnant, nursing, and food-provisioning females (Kaczmarski 1966, Dias et al. 2011, Larimer et al. 2011, Rödel et al. 2016, Dudeck et al. 2018). For these reasons, females with ambulatory kits may select areas with greater rocky cover to maximize opportunities for themselves and offspring to escape from predators (Holmes 1991, Dudeck et al. 2018), whereas females with newborn kits may exploitsuitable moisture and temperature levels in underground burrows (Kumar and Pasahan 1993, Shenbrot et al. 2002, Moore and Roper 2003). Similar to our findings, Hwang et al. (2007) observed maternal female striped skunks (*Mephitis mephitis*) using underground dens and rocky cover, whereas males used above-ground refuges. Disparity in den selection by different sexes also has been observed in other small mammal species, particularly during parturition and rearing of offspring (Endres and Smith 1993, Hamilton and Barclay 1994, Hwang et al. 2007). In our study, male spotted skunk reproductive status was not as strongly related to den type as it was forfemale reproductive status. This may have obfuscated the GLMM results and further dictates the need for investigation beyond conventional discrete choice analysis.

There was also an unexpected association of breeding females with the use of tree cavities as mating season occurs in winter when temperatures are lowest and underground den temperature are likely more suitable (Shenbrot et al. 2002, Moore and Roper 2003). We hypothesize that the use of above-ground dens during mating season may aid in mate attraction or detection. Similar to other mephitids and mustelids, spotted skunks possess anal scent glands. While these glands are most commonly known as a defensive mechanism in skunks, they may also serve the purpose of chemical signaling similar to that observed in mustelids (Zhang et al. 2005, Davies 2009). Scent-marking conspicuous objects or structures in an animal's environment with anal gland secretions and urine is commonly used to delineate territories and notify potential mates of receptiveness to reproductive advances (Gorman and Mills 1984, Gorman and

Trowbridge 1989). Eastern spotted skunks are a solitary carnivore species that shows a moderate level of territoriality and exists at low population densities throughout most of their range (Reed and Kennedy 2000, Gompper and Hackett 2005, Lesmeister et al. 2009). Elevated den sites, such as tree cavities, may potentially enhance the conveyance of scent over greater distances than underground dens such as burrows and rock. Further, tree cavities were associated with higher atmospheric pressure and humidity, and rock crevices with higher minimum temperature. We found that eastern spotted skunks in our study changed den sites frequently; therefore, female skunks may be using tree cavities during periods of mild weather and moving to underground dens during periods of high precipitation or very dry weather, when underground dens would maintain a more suitable environment (Shenbrot et al. 2002, Moore and Roper 2003).

Contrary to observations of eastern spotted skunk habitat selection in South Carolina (Eng and Jachowski 2019) and Alabama (Sprayberry and Edelman 2018), our GLMM analysis revealed a negative relationship with percent canopy cover. Although confidence intervals overlapped zero, this trend may suggest that spotted skunks in our study area are selecting den sites with more open forest overstories. Canopy openings provide more light to the forest floor, which typically permits increased understory growth as opposed to mature forests with closed canopies which typicaly lackprotective understory cover (Anderson et al. 1969, Dharmadi et al. 2019). Our RDA analysis revealed an important relationship between percent canopy cover and canopy cover type that was not evident from the GLMM alone. Greater percent canopy cover was associated with areas of mixed hardwood-pine, mixed hardwood-red spruce, and red spruce cover types. Lower canopy cover was associated with predominantly hardwood or pine stands. In some areas, canopy cover and understory cover may be complementary to each other, offering spotted skunks protection from one cover source in the absence of the other.

However, understory was generally low or absent from white pine stands in our study area and the absence of both dense canopy and understory cover may increase predation risk by aerial predators, which may explain why only 17.3% of dens were located in pine-dominated areas. Tree cavity dens were not strongly associated with any one canopy cover type, which was interesting yet unexpected considering that all but two tree cavity dens were located in hardwood trees. Cavity quality and abundance may be lower in pines than hardwoods, which may have led to lower use of pine cavities than hardwood cavities (McComb et al. 1986, Menzel et al. 2004). Greater use of hardwood tree cavities than coniferous tree cavities has been documented for other North American tree-roosting mammals in hardwood-dominated systems with some coniferous component (Paragi et al. 1996, Taulman 1999, Menzel et al. 2002, 2004). Lesmeister et al. (2008a) found spotted skunks used underground dens more than tree cavities in short-leaf pine (*Pinus enchinata*) and mixed pine-hardwood (*Quercus* and *Carya*) stands in the Ouachita National Forest, Arkansas, where prescribed fire was used to manage for young pine stands in an effort to restore red-cockaded woodpecker (*Leuconotopicus borealis*) habitat. Sprayberry and Edelman (2018) found similar results in the southern-most extent of the Appalachian Mountains where forest lands are also being managed for red-cockaded woodpecker habitat using prescribed fire. The lower abundance of fire-intolerant hardwood tree species and higher abundance of young pine trees may have resulted in increased use of emergent rock outcrop dens by eastern spotted skunks in these regions.

Moreover, Eng and Jachowski (2019) noted a greater use of tree cavities than underground dens in the Sumter National Forest in South Carolina, a mesic area with a higher abundance of hardwood species. In our study, both underground burrows and rock crevices were used with greater frequency than either live or snag cavities. Additionally, hardwood and pine stands were also strongly associated with emergent rock outcrops and rock crevice dens on steeper slopes, where soil erosion resulting from historic farming practices may have altered substrate structure and chemistry (Kalisz 1986). Thus, burrows and rock crevices may be generally used by eastern spotted skunks in the central Appalachians where available whereas tree cavities may be sought in areas lacking suitable substrate for underground dens.

The variety of habitat attributes used by eastern spotted skunks has led researchers to describe eastern spotted skunks as habitat generalists on many occasions (Eng and Jachowski 2019a, Lesmeister et al. 2008, Thorne et al. 2014, Sprayberry and Edelman 2018) . However, our study has brought to light the complicated relationships between spotted skunk den use, habitat attributes, weather conditions, and intrinsic variables. Whereas a true generalist species is able to use a variety of habitat types with equal survival and fecundity, eastern spotted skunks appear to select habitat attributes conditionally, based on variation in habitat attributes, weather condition, and intrinsic variation, rather than at random (e.g., rocky outcrops with ambulatory kits). We propose that eastern spotted skunks are in effect a 'conditional generalist' species. We define the term 'conditional generalist' to represent a species that is not limited to a narrow range of environmental conditions (i.e., specialist species), and that can make use of a wide variety of resources, but is conditionally selective of a given resource in response to one or more current environmental or intrinsic conditions.

Although our GLMM results were mostly consistent with those of other studies of eastern spotted skunk den site selection in the southern Appalachian region (Eng and Jachowski 2019b, Sprayberry and Edelman 2018), our RDA results have elucidated many complex relationships between den characteristics associated with use by spotted skunks and multiple sources of variation in the environment. Our results demonstrate the importance of examining resource selection data using multivariate techniques in addition to conventional discrete choice analyses to better understand intricate habitat – species relationships and to subsequently take effective management actions. We advise future studies regarding resource selection by conditional generalist species incorporate the use of multivariate approaches to fully understand the influence of variables other than physical habitat attributes and to reveal opposing correlations masked by the more conventional discrete choice analyses. By incorporating multivariate techniques, researchers and managers are not limited to simply predicting site use, but are able to make interpretations about resource selection that are impossible using only conventional multiple regression techniques, thereby increasing effectiveness of management action plans. Additionally, given the conditional generalist nature and observed rarity of the species, we recommend that studies be conducted over all seasons and through multiple years to prevent seasonal bias of interpretation of habitat attribute selection. To further understand the importance of resource selection by eastern spotted skunks, future research should include assessments of survival and reproduction rates in relation to habitat attributes.

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Table 4.1 Mean ± SE for used (*n*=364) and unused (*n* = 151) site microhabitat and landscape level characteristics used in mixed effects logistic regression models of eastern spotted skunk (*Spilogale putorius*) den-site selection in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Values in bold font reflect significant difference.

	Used		Unused			
Variable	ℜ	*SE*	ℜ	*SE*	*W*	*p*
Microhabitat						
Den entrance size (cm^2)	**130.02**	**4.89**	**142.99**	**39.15**	**12518**	**<0.001**
Distance to nearest shrub	11.04	1.2	13.65	3.27	9043	0.6
Canopy cover (%)	**74.9**	**1.3**	**88.9**	**195**	**6041**	**<0.001**
Understory cover (%)	34.35	1.9	23.93	3.15	10169	0.38
Coarse woody debris	2.66	0.25	2.77	0.47	8948.5	0.51
Vines	13.83	5.93	10.9	4.63	7945.5	0.006
Snags	0.66	0.17	0.48	0.1	8800	0.31
Rocks	67.07	6.4	57.9	12.09	8716	0.34
TEI (10m)	-0.28	0.68	-8.04	10.65	9673	0.8
Landscape-level						
Slope (°)	**19.84**	**0.61**	**14.64**	**1.34**	**11823**	**0.003**
Aspect	**208.29**	**4.81**	**156.861**	**11.46**	**12393**	**<0.001**
Elevation (m)	657.06	12.01	688.31	48.49	11020	0.06
TEI (1km)	11.89	2.46	8.13	14.23	8839	0.44
Distance to permanent creek/river (m)	2590.51	145.0919	3394.32	573.32	10477	0.21
Distance to permanent water body (m)	**1446.92**	**28.4**	**1869.65**	**64.94**	**5050**	**<0.001**
Distance to road (m)	**231.85**	**9.06**	**123.57**	**15.86**	**13369**	**<0.001**

Table 4.2 Model results and effect size estimates (SE) of 15 competing (ΔAIC < 2.0) *a priori* microhabitat models for eastern spotted skunks (*Spilogale putorius*) den selection in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Models are ranked by Akaike's Information Criteria (*AIC*), ΔAIC, model weight (w_i), and number of parameters (*K*). Microhabitat covariate fixed effects comprise percent canopy cover (CC), percent understory cover (US), distance to nearest shrub (DS), dominant understory species (DUS): mountain laurel (ML), rhododendron (RO), saplings (SAP), mixed species (MXD, other species (Other), and no understory (None), number of coarse woody debris >10c m in diameter (CWD), number of exposed rocks > 10cm in diameter (RCK), and topographic exposure index within 10m (TEI10). Study site was included as a random variable for all models. Values in bold represent significant effects (p < 0.05).

Model	***CC***	***US***	***DS***	***CWD***	***RKS***	***TEI10***	**Dominant understory species**						***AIC***	***ΔAIC***	***w_i***	**K**
							ML	**RO**	**SAP**	**MXD**	**Other**	**None**				
CC	-1.90 (1.2)												244.6	0.0	0.10	4
US							2.4 (1.4)	**4.9 (1.8)**	**3.79 (1.57)**	**3.5 (1.5)**	2.6 (1.5)	2.6 (1.4)	245.5	0.9	0.06	9
CWD +CC	-2.01 (1.3)			2.1 (2.3)									245.6	1.0	0.06	5
RKS + CC	-1.9 (1.2)				0.6 (0.6)								245.8	1.2	0.06	5
CC + TEI10	-2.1 (1.3)					-6.6 (10.7)							245.8	1.2	0.05	5
CWD				1.9 (2.2)									246.0	1.3	0.05	4
US		0.5 (0.5)											246.0	1.4	0.05	4
CC + US	-1.8 (1.2)	0.4 (0.5)											246.0	1.4	0.05	5
DS			-0.5 (0.6)										246.1	1.5	0.05	4
RKS					0.50(0.6)								246.1	1.5	0.05	4
CC + DS	-1.8 (1.2)		-0.4 (0.6)										246.1	1.5	0.05	5
CC + DUS	-1.1 (1.3)						-1.1 (0.5)	1.3 (1.3)	0.28 (0.88)	**4.2 (1.6)**	-1.0 (0.8)	-1.0 (0.6)	246.1	1.5	0.05	10
TEI10						-4.3 (8.7)							246.4	1.7	0.04	4
DS + DUS			-0.7 (0.7)				2.7 (1.5)	**5.13 (1.8)**	**4.2 (1.4)**	**4.0 (1.5)**	23.0 (1.6)	**3.0 (1.5)**	246.6	1.9	0.04	10

Table 4.3 Model results and effects size estimates (SE) of 10 competing (ΔAIC < 2.0) *a priori* landscape characteristic selection models for eastern spotted skunks (*Spilogale putorius*) in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Models are ranked by Akaike's Information Criteria (*AIC*), ΔAIC, model weight (w_i), and number of parameters (*K*). Landscape-level covariate fixed effects comprise distance to river (RVR), distanced to nearest permanent water body (WB), distance to nearest drainage (DD), distance to nearest primary road (PR), distance to nearest secondary road (SR), distance to nearest dirt road (RD), elevation (ELV), slope (SLP), aspect (ASP), and topographic exposure index within 1 km (TEI1k). All distances measure in meters. Study site was included as a random variable for all models. For all variables, confidence intervals overlapped 0.

Model	***RVR***	***WB***	***DD***	***PR***	***SR***	***RD***	***ELV***	***SLP***	***ASP***	***TEI1k***	***AIC***	***ΔAIC***	***w_i***	**K**
TEI1k										-2.25 (2.31)	245.8	0.000	0.13	4
PR				-0.40 (.39)							245.8	0.003	0.13	4
SLP								0.56 (0.67)			246.1	0.33	0.11	4
DD			0.76 (1.01)								246.3	0.45	0.10	4
WB		0.65 (0.86)									246.3	0.46	0.10	4
RVR	0.60 (0.92)										246.4	0.60	0.10	4
RD						0.68 (1.24)					246.5	0.72	0.09	4
SR					-0.23 (0.55)						246.6	0.84	0.08	4
ASP									-0.16 (0.66)		246.7	0.96	0.08	4
ELV							-0.03 (0.96)				246.8	1.02	0.08	4

Table 4.4 Model results and effect-size estimates (SE) of *a priori* den characteristic models for eastern spotted skunks (*Spilogale putorius*) in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Models are ranked by Akaike's Information Criteria (*AIC*), Δ*AIC*, model weight (w_i), and number of parameters (*K*). Den characteristics include primary den type (Prim): underground burrow (UB), live tree cavity (LC), snag cavity (SC), and rock crevice (RC); secondary den characteristics (Sec) include at treebase (TB), in emergent rock outcrop (RO), in small rock pile (RP), in or under log or stump (LS), in or under vegetation (Veg), in root system (RS), no secondary characteristic (None), other (Other); and entrance size in cm^2 (Ent). Effect size estimates in bold indicate significant effect ($p < 0.05$).

Model	*UB*	*LC*	*SC*	*RC*	*TB*	*RO*	*RP*	*LS*	*Veg*	*RS*	*None*	*Other*	*Ent*	*AIC*	*ΔAIC*	w_i	K
Ent													1.66 (1.05)	244.31	0.00	0.65	4
Prim + Ent	2.41 (1.30)	1.62 (1.34)	2.55 (1.53)	2.52 (1.34)									1.62 (1.07)	246.80	2.49	0.19	8
Prim	**3.01 (1.27)**	2.34 (1.32)	**3.09 (1.50)**	**3.19 (1.29)**										247.13	2.82	0.16	7
Sec					**2.89 (1.44)**	**3.11 (1.35)**	**3.72 (1.41)**	**3.78 (1.53)**	2.73 (1.48)	2.71 (1.46)	**2.89 (1.37)**	1.58 (1.52)		257.85	13.55	0.00	11

Table 4.5 Multivariate Analysis of Variance (MANOVA) results showing the effects of various sources of environmental variation on den use by eastern spotted skunks (*Spilogale putorius*) in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019.

Covariate	***p***	***F***
Microhabitat		
Distance to shrub	0.001	70.65
Percent canopy cover	0.001	10.34
Percent understory cover	0.001	10.63
Hardwood cover type	0.001	5.93
Pine cover type	0.001	8.76
Spruce cover type	0.012	2.91
Mixed hardwood-spruce cover type	0.024	2.39
Mixed hardwood-pine cover type	0.001	5.15
Mountain laurel understory	0.001	10.07
Sapling understory	0.005	3.78
No understory	0.023	2.59
Number of coarse woody debris	0.001	4.85
Number of exposed rocks	0.001	55.46
landscape-level		
Distance to road	0.046	2.26
Distance to permanent water body	0.036	2.41
Weather		
Minimum humidity	0.088	1.79
Minimum Temperature	0.043	2.14
Daily atmospheric pressure	0.095	1.80
Reproductive status		
Breeding Female	0.028	2.38
Pregnant female	0.001	9.29
Ambulatory kits	0.051	2.18
Non-ambulatory kits	0.002	4.64

Figure 4.1 Examples of types of eastern spotted skunk (*Spilogale putorius*) dens sites in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Top left: underground burrow at base of tree; Top right: rock crevice in emergent rock outcrop; Bottom left: tree cavity; Bottom right: burrow under small rock pile.

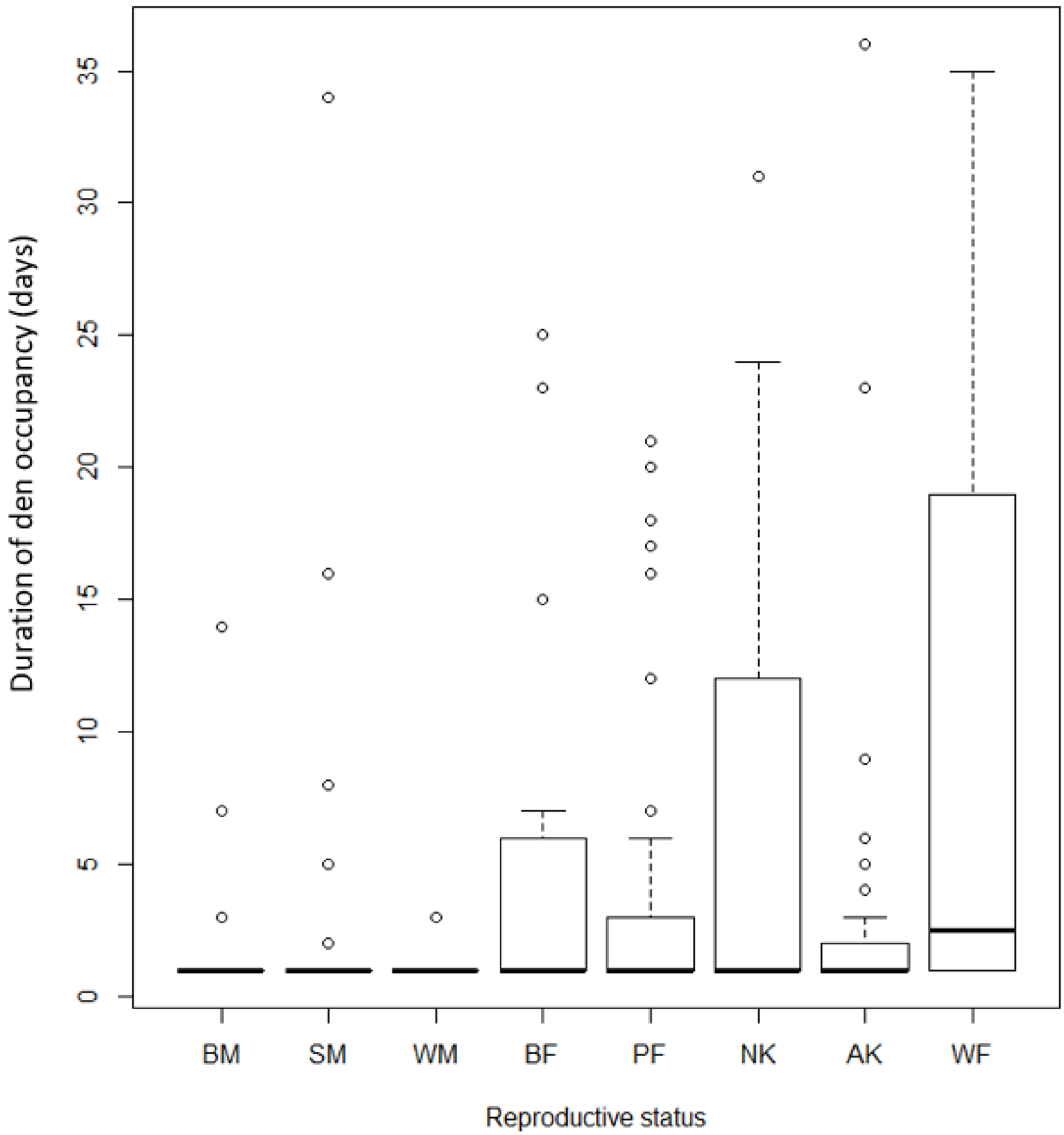

Figure 4.2 Box plots illustrating the number of continuous days that a single den was occupied by an eastern spotted skunk (*Spilogale putorius*) at four study sites in the George Washington and Jefferson National Forests and surrounding private lands in the central Appalachian Mountains of western Virginia and northeastern West Virginia from March 2015 – February 2019. Reproductive status includes breeding male (BM), summering male (SM), wintering male (WM), breeding female (BF), pregnant female (PF), female with non-ambulatory kits (NK), females with ambulatory kits (AK), and wintering female (WF).

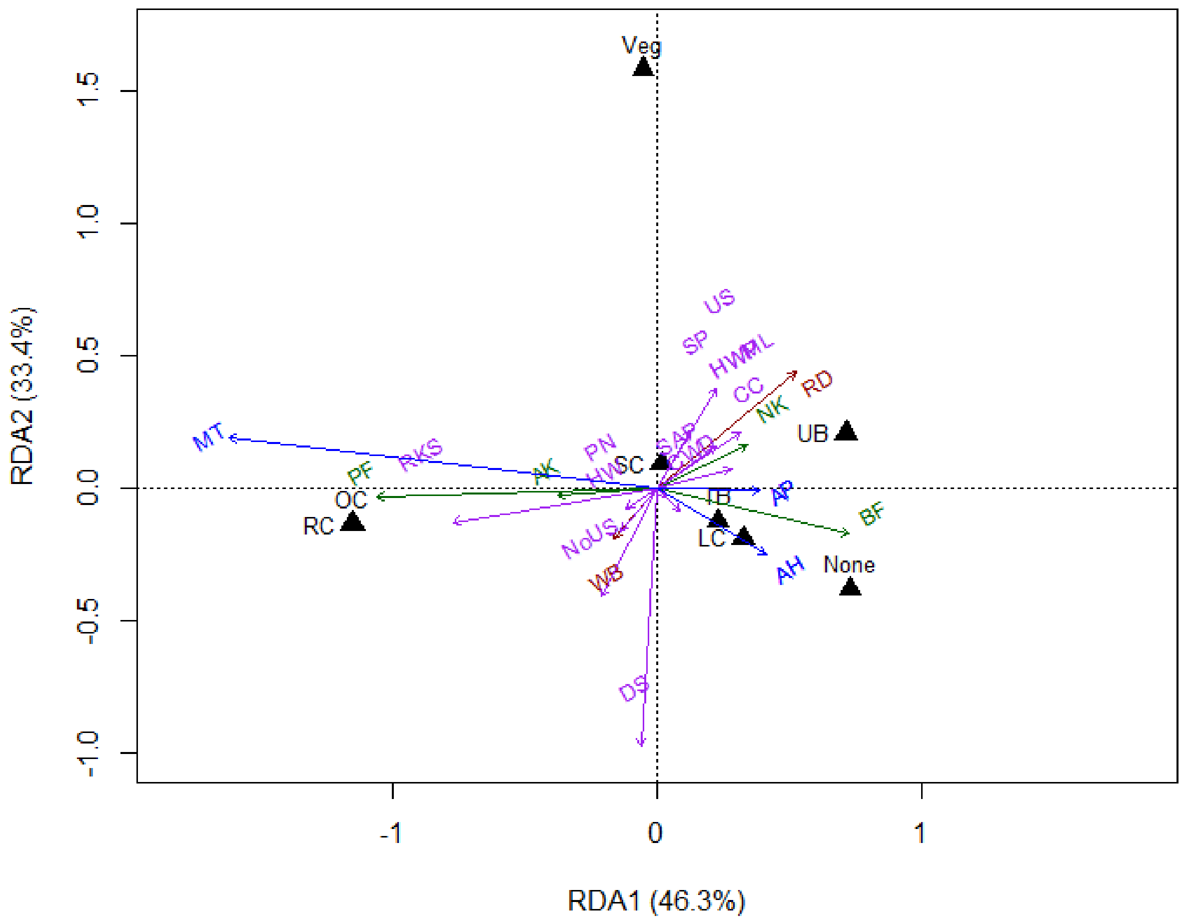

Figure 4.3. Redundancy analysis (RDA) ordination plot showing similarity between eastern spotted skunk (*Spilogale putorius*) den site characteristics and microhabitat (purple), landscape (dark red), weather (blue), and intrinsic (green) variation at sites in the George Washington and Jefferson National Forests and surrounding private lands in in the central Appalachian Mountains of western Virginia and northeastern West Virginia March 2015 – February 2019. Microhabitat covariates include distance to nearest shrub (DS), percent canopy cover (CC), Hardwood cover (HW), pine cover (PN), red spruce cover (SP), mixed hardwood-pine cover (HWP), mixed hardwood-spruce cover (HWSP), percent understory cover (US), mountain laurel understory (ML), sapling understory (SAP), no understory (NoUS), coarse downed woody debris (CWD), and number of exposed rocks (RKS). Landscape level covariates include distance to nearest road (RD), and distance to nearest water body (WB). Weather covariates include previous day atmospheric pressure (AP), minimum temperature (MT), and average humidity (AH). Intrinsic covariates include breeding female (BF), pregnant female (PF), female with non-ambulatory kits (MK), and female with ambulatory kits (AK). Den characteristics include primary den type: underground burrow (UB), rock crevice (RC), live tree cavity (LC), snag cavity (SC), and secondary den characteristic: emergent rock outcrop (OC), tree base (TB), under vegetation (Veg), and none secondary characteristic (None).

Chapter 5 – Genetic Differentiation and Population Structure of Eastern Spotted Skunks (*Spilogale putorius*) in Central Appalachia

Abstract

The adaptive potential of a species relies on genetically effective migration among populations. Habitat fragmentation causing barriers to migration can reduce or prevent geneflow between neighboring populations. Negative effects of genetic isolation can include reduced effective population size, loss of genetic diversity, and inbreeding depression. Following large declines in the 1940s, eastern spotted skunk populations were reduced by over 90% range-wide. In Virginia and West Virginia, reduction of core forested areas due to agricultural practices and urbanization may have contributed to a spatially disjunct distribution of small patches of spotted skunk habitat. Home-range selection and movement by spotted skunks in Virginia and West Virginia appear to be limited by availability of forested areas and restricted by unsuitable environmental conditions surrounding these areas. Using tissue collected from spotted skunks in Virginia and West Virginia, we assessed genetic diversity and population structure of eastern spotted skunks in the central Appalachians. Our goals were to assess whether reduction of core forest area createed barriers to gene flow among neighboring populations and to identify signals of decreased genetic diversity that may affect spotted skunk populations in Virginia. Our results suggest that population genetic structure of spotted skunks corresponds broadly to the Blue Ridge and Valley and Ridge sub-provinces, and that within the Valley and Ridge, between ridges separated by human-dominated valleys.

Introduction

The adaptive potential of a species relies in part on genetically effective migration among populations (Fisher 1930, Wright 1978). That is, large effective population sizes and high subpopulation connectivity contribute to exchange of genetic material, countering random genetic drift and reducing the likelihood of inbreeding (Dasmann 1980, Lande 1995, Garant et al. 2007). However, barriers to migration can reduce or prevent geneflow between neighboring subpopulations, shaping the genetic structure of populations (Templeton et al. 1990, Andrén 1994, Lowe and Allendorf 2010).

Consequences of genetic isolation can include reduced effective population size, loss of genetic diversity through random genetic drift, inbreeding depression, and consequently reduction of adaptive potential and vulnerability to environmental stochasticity (Soulé 1985, Templeton et al. 1990, Andrén 1994, Franklin et al. 2009, Méndez et al. 2014). Barriers to dispersal may include simple geographic distance among individuals or subpopulations (Johnson and Black 1995) or, depending on the species in question, natural landscape features such as unsuitable vegetation types, mountains and rivers (Peres et al. 1996, Cushman et al. 2006, Zalewski et al. 2009). In human-modified systems, anthropogenic landscape features such as developed areas and highways often are prove barriers to migration (Forman and Alexander 1998, Wilson et al. 2016). Lastly, in all landscape types, behavioral barriers such as mating systems, limited dispersal ability, and social organization patterns can serve as barriers to geneflow (Singleton and Hay 1983).

In conservation biology, it is vital to understand the relationships between the genetic structure of a species and the effects of landscape variation on its spatial distribution (Soulé 1985). Because habitat fragmentation, whether natural or anthropogenically-induced, can reduce the ability of individuals to disperse among subpopulations, the number of individuals that contribute to the genetic variability within a population, or effective population size (N_e), can be reduced and is often lower than the census population size (Lande 1995, Barton and Whitlock

1997). Thus, it is essential to understand the degree to which habitat fragmentation limits a species' ability to disperse and ultimately its effect on genetically effective migration.

Following large population declines in the 1940s, eastern spotted skunk (*Spilogale putorius*) populations were reduced by over 90% range-wide (Gompper and Hackett 2005). The species recently was listed as Vulnerable by the International Union for Conservation of Nature (Gompper and Joachowski 2015). In Virginia, changes in forest condition, agricultural land use and increased urbanization may have contributed to a spatially disjunct distribution of small patches of suitable spotted skunk habitat (Thorne et al. 2017). Home-range selection and movement patterns by spotted skunks in Virginia appear to be limited by availability of forested areas and are restricted by unsuitable environmental conditions surrounding these areas (see Chapter 3). To develop effective management strategies for eastern spotted skunk conservation, it is vital to understand the associations between habitat selection by, and distribution of, spotted skunks and the resulting genetic structuring and differentiation. Thus, the aim of our study was to investigate the genetic differentiation of eastern spotted skunk subpopulations across a wide portion of the southern and central Appalachian Mountain region of Virginia and West Virginia as it relates to the species' landscape ecology. Beginning at the landscape scale and working toward finer-scale genetic relationships, our objectives were to: 1) assess standard metrics of genetic diversity of spotted skunks; 2) identify contemporary population structure; 3) evaluate relationships between landscape variation and population structure; and 4) estimate effective population size and potential inbreeding within subpopulations. Within this study, we tested three hypotheses. First, we hypothesized that genetic diversity is lower for spotted skunks in our study area than for other, more generalist mesocarnivore species. Second, we hypothesized that population structure is influenced by natural (mountain ridges and river valleys) and anthropogenic (non-forested land-cover type) barriers to dispersal. Third, we hypothesized that effective population size is lower than what is required to meet the criteria of Franklin et al. (1980) for population persistence. Lastly, we hypothesized that there is genetic evidence of inbreeding within subpopulations. To address these hypotheses, we conducted a baseline population genetic assessment using microsatellite markers on DNA isolated from tissue collected from eastern spotted skunks at five study sites in Virginia and east-central West Virginia.

Methods

Study Area

We conducted our study in the southern and central Appalachian Mountains of western Virginia and eastern West Virginia (Figure 5.1). The study area was within the Valley and Ridge and Blue Ridge physiographic subprovinces ranging from Pendleton County, West Virginia south to the North Carolina-Virginia state line, primarily within the Monongahela, George Washington, and Thomas Jefferson national forests. Generally, these portions of the Appalachian Mountains are characterized by long, linear southwest-to-northeast-running ridge lines (Johnson and Fecko 2008). Within this landscape, mountain ranges are separated by narrow to broad valleys, and elevations range from 350 m to 1460 m. The entire study area consists of approximately 67% forested area, including private and public land, with intermixed areas of privately owned arable land (Homer et al. 2015). Annual temperature in this region ranges from -10.6° C in winter to 27.5° C in summer (National Oceanic Atmospheric Administration, public data 2019; www.noaa.gov), and annual precipitation for most of the region is about 110 cm (Ford et al. 2006). Forested areas are predominantly xeric hardwood or mixed pine-hardwood stands composed of oak species (e.g., *Quercus alba*, *Q. mantana*, *Q. rubra*, *Q. velutina*), red maple (*Acer rubrum*), pine species (e.g., *Pinus rigida*, *Pinus pungens*). Mesic forests on north-facing slopes and sheltered coves consist of yellow poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), and sugar maple (*A. saccharum*). At high elevations, forests consist of white pine (*Pinus strobus*) – eastern hemlock (*Tsuga canadensis*) associations with small patches of northern red spruce along the highest peaks (*Picea rubens*). The vegetative understory is dominated by large patches of mountain laurel (*Kalmia latifolia*) on drier sites and patches of rosebay rhododendron (*Rhododendron maximum*) on mesic sites and along montane riparian areas. Non-forest areas are dominated primarily by cool-season grasses for livestock grazing and haying, although some valley land is devoted to row-crop production (Kniowski and Ford 2018).

Capture and Handling

We sampled at five locations throughout the study area: Tract Mountain (TM) in Pendleton County, WV; Bald Mountain (BM) in Botetourt County, VA; Sugar Run Mountain (SR) in Giles County, VA; Wintergreen Mountain (WG) in Nelson County, VA; and Whitetop Mountain (WT) in Grayson County, VA (Figure 5.1). Using results of Thorne et al. (2017), we focused our spotted skunk collection efforts in areas of high predicted spotted skunk occurrence from March 2015 – April 2019. Prior to live trapping, we deployed baited trail cameras to determine spotted skunk presence. Upon confirmation of presence, we deployed 4 to 8 #103.5FO Tomahawk box traps (Tomahawk Live-Trap Co., Tomahawk, Wisconsin, USA) baited with a mixture of peanut butter, bacon grease, and rolled oats within a 10-m radius of the baited camera station. We removed captured spotted skunks from traps and physically immobilized them using a cloth handling bag. We collected standard morphometric measurements, determined sex, assessed age, and affixed a uniquely numbered ear tag to each individual following the methods of Chapter 4. We collected ≥ 20 hair follicles from each captured skunk before releasing the animal at the capture site. Hair samples were stored in dry envelopes at room temperature until the time of DNA extraction. All trapping and handling procedures were conducted in concordance with Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee protocol number 13-119-FIW and permitted by the Virginia Department of Game and Inland Fisheries and West Virginia Division of Natural Resources.

Molecular Methods

We isolated DNA from hair samples of 59 individuals captured across our five trapping areas. Additionally, we included tissue samples from three salvaged road-killed specimens and three incidental captures donated by fur trappers. We isolated DNA using the DNEasy Blood and Tissue Kit (Qiagen, Germantown, MD) and attempted to amplify 12 microsatellite loci using published primer sets (Table 5.1). PCR reactions were performed in a T-100 thermal cycler (Bio-Rad Laboratories, Hercules, CA) in 11-µl reactions volumes using solutions containing 1.1µl 5X Promega reaction buffer, 0.7µl forward dye-labeled primer, 0.7µl reverse primer, 0.5µl of 25mM $MgCl_2$, 0.5µl 8mM dNTP mix, 1µl of 6.5 U/µl *GoTaq* polymerase (Promega Corporation, Madison, WI), 1 µl of DNA ranging from 20 ng/µl - 300 ng/µl concentrations, and 5.6 µl deionized water, for all primer pairs except *Mel 1, nRio-08, rLut818,* and *Meph 22-89*. $MgCl_2$ volume was decreased to 0.3µl for *Mel 1, nRio-08* and *rLut818*, increased to 0.7 for *Meph 22-89*, and water volume was adjusted accordingly.

The PCR protocol had an initial denaturation at 94ºC for 4 minutes; 40 cycles of: 94° C for 40 seconds, 50-56° C for 40 seconds, and 72° C for 60 seconds; and a final extension stage at 72° C for 5 minutes (Floyd et al. 2011). We determined successful amplification by subjecting amplification products to electrophoresis through a 2% TBE agarose gel and observation of ethidium bromide-stained DNA fragments of appropriate sizes under UV illumination.

Amplified DNA fragment size was estimated using an ABI 3730x1 DNA sequencing instrument at the Cornell University Institute of Biotechnology (Ithaca, NY). We scored fragment size using GeneMarker (SoftGenetics, College Park, PA). All data were scored a second time for accuracy. To ensure as large and reliable a dataset as possible, samples that did not amplify or that were unclear were amplified and analyzed a second time. Inconsistent results were removed from the dataset.

Data Analysis

We first conducted preliminary analyses on the genetic data set to test for linkage disequilibrium and departures from Hardy-Weinberg Equilibrium (HWE) using Arlequin v3.5.2.2 (Excoffier et al. 2005) and tested for the segregation of null alleles using MicroChecker (Oosterhout et al. 2004). We then used Arlequin to examine multiple indices of genetic variation. We identified the number of alleles (A) and calculated allelic richness (A_R) for each locus and determined expected (H_e) and observed (H_o) heterozygosities among and within subpopulations (Nei 1978). To assess the level of inbreeding within subpopulations, we calculated F_{IS}, the proportion of departure from HWE within a subpopulation(Wright 1922). We estimated genetic distance between subpopulation pairs using G_{ST}, which compares heterozygosities within and among subpopulations (Nei 1972), using GenAlex (Peakall and Smouse 2006, 2012) and F_{ST}, which quantifed departures from HWE among subpopulations (Wright 1951). Additionally, we used GenAlEx to examine population differentiation by calculating Jost's D, or between-subpopulation pairwise comparisons of the proportion of unique alleles within subpopulations (Jost 2008).

To identify clusters of multi-locus genotypes and to identify likely cluster of origin for all individuals, we applied the Markov chain Monte Carlo Bayesian algorithm in STRUCTURE v2.3.4 (Pritchard et al. 2000). We set STRUCTURE analyses with a burn-in period of 100,000 iterations with 1,000,000 MCMC iterations and 10 replicates for $K = 1 – 10$ clusters. We assumed admixture with correlated allele frequencies to estimate the best-supported number of clusters (K) using the Ln$P(K)$ metric reported by STRUCTURE. We also used the Evanno et al. (2005) criterion calculated in Structure Harvester (Dent and von Holdt 2012) to identify the optimal K and to produce visual representations of K. We then visualized STRUCTURE plots using CLUMPAK (Kopelman et al. 2015). We then conducted assignment tests of individuals to each subpopulation and to genetic clusters identified by STRUCTURE. GeneClass2 (Piry et al. 2004) also was used to assess probability of assignment of individuals to subpopulation of origin using our five study sites as predefined subpopulations following the partial Bayesian approach of Rannala and Mountain (1997).

We conducted an analysis of molecular variance (AMOVA) and quantified pair-wise population differentiation (F_{ST}) among sampled subpopulations and clusters identified in STRUCTURE. Using AMOVA, we identified the proportion of genetic variation explained by: (1) within-individual variation, or heterozygosity, (2) variation among individuals within a population or cluster, or heterozygote departure from Hardy-Weinberg expectations, and (3) variation among populations or clusters, or non-local departure from Hardy-Weinberg expectations. To avoid potential Type I error, we used a Bonferroni-corrected significance value of $\alpha = 0.004$ for all pairwise comparisons. We used the linkage equilibrium approach in NeEstimator v2 (Do et al. 2014) to estimate N_e for each subpopulation.

We also assessed relatedness among individuals using ML Relate (Excoffier et al. 1992, Kalinowski et al. 2006). For all pairwise significance tests we used a Bonferroni-corrected α value of 0.004 to reduce the probability of Type I errors (Holm 1979). We conducted all analyses either including and excluding loci containing null alleles with frequencies ≥ 0.1 to evaluate the effect of these data on the interpretation of results. We detected no differences between approaches; therefore, we retained data for all loci to analyze the largest dataset possible.

We used redundancy analysis (RDA) to assess the relationships between genetic structure of subpopulations and landscape variation. We assessed Euclidean distance to evaluate the fit of the isolation-by-distance model (IBD, Wright 1943). Additionally, we evaluated the fit of the isolation-by-barrier model (IBB) using accumulated cost distances (least-cost paths) for three potential barriers to gene flow identified in Chapter 4 – elevation, land-cover category, and forest fragmentation – using the Spatial Analyst Toolset in ArcMap 10.2.2. (ESRI, Redlands, CA, USA). We first used ArcMap to create cost distance raster layers using each individual landscape covariate and an inclusive cost layer that represented the sum cost of all three landscape covariates in ArcMap. Because the assignment of the most appropriate cost to a covariate is crucial to a cost distance analysis, we calculated various cost values for each covariate to determine the best magnitude of cost for each landscape covariate. We calculated cost by using selection ratios for each class of elevation, land cover, and forest fragment type presented in Chapter 4 rounded to the nearest whole number, as the cost distance tool requires integer values. We then multiplied each value > 1 by 1, 10, 100, and 1000 (Table 5.2, Breyne et al. 2014) using the Raster Calculator tool. This method holds the cost of landscape covariate classes with the highest selection ratios equal to 1 and assumes that an area with cost = 1 would be the easiest to traverse, whereas cost > 1 would be increasingly difficult to traverse. We then identified the pairwise least-cost paths for all study sites for each of the three covariates at each order of magnitude using the Cost Distance and Cost Path tools in ArcMap. To assess the effect of valleys and ridges as genetic barriers, we created binary dummy variables representing mountain range (collection site) of origin for each individual, where 1 = captured on that ridge and 0 = not captured on that ridge.

We calculated the Queller and Goodnight (1989) measure of genetic relatedness among subpopulations in GENALEX v.6.4.1 and conducted an RDA using Euclidean distance, accumulated cost distance from each cost path, and mountain range as predictor variables. In total, we assessed 12 *a priori* models, including a global model, for each magnitude of cost. Model performance was assessed by comparing Akaike's Information Criterion (*AIC*) and Akaike weights (w_i) to identify which model best explained the relationship between genetic relatedness and landscape variation. To determine the significance of correlation of each landscape covariate with genetic relatedness, we conducted Mantel tests using 9999 permutations. We conducted RDA using package vegan (Oksanen et al. 2019) and Mantel tests using package ade4 (Dray and Dufour 2007) in Program RStudio v.1.1453.

Results

From March 2015 through April 2019, we collected samples from a total of 65 individual eastern spotted skunks across five potential subpopulations in the central Appalachian Mountains. Sample size varied across sites, with the greatest number of samples collected from TM (n = 24) and BM (n = 21) and the least collected from SR (n = 4). All 65 individuals were genotyped successfully. Eight microsatellite loci amplified successfully; however, *Meme* 77 was monomorphic, thus data only from the seven polymorphic loci were used for analysis. We observed a total of 32 different alleles across all 7 polymorphic loci, with a mean of 4.6 (± 0.6) alleles per locus. There was no strong evidence of departure from HWE or of linkage disequilibrium among loci (Supplementary Table 5.1).

Indices of genetic diversity were suggestive of genetic drift within subpopulations (Table). Mean number of alleles per locus and mean allelic richness were comparably low across all sites and were not correlated with sample size (p = 0.20 and p = 0.47, respectively). Mean observed heterozygosity was lower than expected on te basis of HWE for all sites except SR. All M-ratios were below the Garza and Williamson (2001) criterion of 0.68, suggesting that these subpopulations have undergone a recent genetic bottleneck.

Analysis of the STRUCTURE results produced an estimate of the best-supported value of K = 3 clusters using both the Ln$P(D|K)$ and Evanno et al. (2005) δK methods (Figure 5.2, Supplementary Table 5.2), though varying levels of admixture were evident within all three clusters. At K = 3 clusters, differentiation is apparent between the TM subpopulation in the Allegheny Plateau region, the BM and SR subpopulations in the Valley and Ridge region, and the WG and WT subpopulations in the Blue Ridge region (Figure 5.3). Results of the individual assignment test conducted in GeneClass2 successfully placed 58% of individuals into their subpopulation of capture. Assignment to STRUCTURE-based cluster of capture was successful for 72% of individuals, although success varied by site (Table 5.4).

The AMOVA analysis partitioned the greatest amount of genetic variation (75%) to within-individual and 14% to amongindividual variance when conducted using K = 2 and K = 3 (Table 5.5). With K = 2, 10% of genetic variance was explained by among-population variance and only 1% by among-region (i.e., Valley and Ridge region and Blue Ridge region) variation and. However, at K = 3, only 3% of genetic variance was explained by among-population variance and 8% within-region (i.e., Tract Mountain region, Valley and Ridge region, and Blue Ridge region).

Estimates of pair-wise Jost's D, F_{ST}, and G_{ST} metrics were indicative of genetic differentiation among some, but not all sampled subpopulations (Table 5.6). The TM subpopulation was moderately differentiated from the BM and WT (Jost's D = 0.09 and 0.095, respectively) and was strongly differentiated from SR (Jost's D = 0.204); however, we found no significant differentiation between TM and WG (Jost's D = 0.000). The BM subpopulation was moderately differentiated from WG (Jost's D = 0.091) and strongly differentiated from WT (Jost's D = 0.152), but not from SR (Jost's D = .060). We found low-to-moderate, but not significant, differentiation between SR and and the WG and WT subpopulations (Jost's D = 0.054 – 0.123). The WG and WT subpopulations consistently had the lowest estimates of differentiation (Jost's D = 0.000) despite being separated by the second-to-highest geographic distance (274 km). The estimated number of migrants per generation varied from 0.95 between TM and SR to 57.8 between WG and WT (Table 5.6).

Inbreeding was apparent as departure from HWE-expected genotype frequencies within three of the five sampled subpopulations, with F_{IS} values significantly greater than zero (Table 5.3). F_{IS} was not correlated with sample size (p = 0.66), as the largest sampled subpopulation had an F_{IS} value significantly greater than zero and the smallest sampled subpopulation had nearly the lowest.

Estimates of effective population size varied among subpopulations (Table 5.7). The subpopulation with the largest N_e was WG, with an estimated effective population size of 26.1 individuals. The BM and TM subpopulations followed, with estimated N_e values of 17.4 and 16, respectively. Estimates of N_e were undefined for the WT and SR subpopulations, likely due to small initial sample sizes and low genetic diversity. None of the five subpopulations met the criterion of N_e = 50 for short-term persistence or N_e = 500 for long-term adaptive potential (Franklin et al. 1980).

Proportions of half-siblings or other relations ranged from 0.14 to 0.29, except for the SR subpopulation where only males were captured and all four were unrelated (Figure 5.4). High F_{IS} and low N_e may be related to our observations of high proportions of first-degree relatives within subpopulations.

All measures of geographic distance explained a significant proportion of genetic variation and outperformed the null model (Table 5.8). Of all the magnitudes of cost of movement, we found that the 1000x cost values performed best and explained the greatest percentage of variation in the genetic dataset. The cost distance measure including the summed cost of elevation, land-cover type, and forest fragmentation produced the lowest *AIC* and w_i; however, all models containing explanatory variables were found to be competing models. Results of Mantel tests demonstrated significant inverse correlations of all distance measures with genetic distance, with forest fragmentation being the most negatively correlated (Table 5.8). Although the cost distance of fragmentation was most highly correlated with genetic distance, none of the landscape covariates greatly outperformed Euclidean distance, suggesting that the more parsimonious model of isolation-by-distance may best represent genetic differences among subpopulations at the geographic scale measured in this study.

Discussion

Results of our study demonstrate that spotted skunks in the southern and central Appalachian Mountains of western Virginia and West Virginia exhibit relatively low genetic diversity. Mean numbers of alleles and allelic richness were lower than those found for the plains spotted skunks (*Spilogale putorius interrupta*), Florida spotted skunks (*Spilogale putorius ambarvalis*), and eastern spotted skunks in the southern Appalachians south of Virginia (Shaffer et al. 2018). Additionally, our estimates were lower than those of mainland populations of western spotted skunks (*Spilogale gracilis*), yet were similar to those found for island-dwelling populations of the western spotted skunk subspecies (*Spilogale gracilis amphilia*, Floyd et al. 2011). Estimates of observed heterozygosity were also slightly lower than those for other populations of eastern and western spotted skunks (Floyd et al. 2011, Shaffer et al. 2018). The low *M*-ratios that we observed are indicative of a recent genetic bottleneck in many estern spotted skunk populations, which suggests loss of genetic diversity following early 20th-century population declines (Gompper and Hackett 2005). Genetic drift may be exacerbated by limited gene flow among isolated subpopulations. Fragmentation-driven population differentiation has been observed in North America, including fishers (*Martes pennati*, Kyle et al. 2001) and in Europe, including polecats (*Mustella putorius*, Moller et al. 2004, Pertoldi et al. 2006). Further, similar levels of genetic diversity have been found in other forest-obligate species experiencing forest fragmentation (e.g., mouse lemurs [*Microcebus* spp.], Olivieri et al. 2008; tropical bats [Chiroptera], Reis et al. 2012; pied tamarin [*Saguinus bicolor*], Farias et al. 2015).

AMOVA results revealed that differentiation among groups of collections from different mountain ranges was greater than among subpopulations, which indicates genetic clustering of subpopulations within the respective mountain ranges. This finding was supported by results of STRUCTURE analysis, which revealed three (North Tract Mountain region, Valley and Ridge region and Blue Ridge region) well-supported genetic clusters. We observed moderate-to-high levels of genetic differentiation among subpopulations, with estimates of Jost's D, G_{ST}, and F_{ST} values over 0.05 for most subpopulation comparisons, and over 0.10 for subpopulations located in different genetic clusters (Hartl and Clark 2006). Within the Valley and Ridge region cluster, the Bald Mountain and Sugar Run Mountain collections showed lower levels of differentiation from one another than from North Tract Mountain or the Blue Ridge sites. Similarly, the Blue

Ridge collections showed low genetic differentiation from one another, but were moderately to highly differentiated from collections from other sites. The overall along-mountain connectivity along the Blue Ridge physiographic subprovince may facilitate spotted skunk movement along the ridge line (Johnson and Fecko 2008). In constrast, the Valley and Ridge subprovince, characterized by long parallel ridges alternating with linear valleys (Johnson and Fecko 2008) may inhibit large movements, especially across valleys. Analogous to our results, Kanine (2013) found that subpopulations of Allegheny woodrats in the Blue Ridge region were genetically differentiated from more westerly subpopulations.

Our results suggest that the isolation-by-distance model of migration explained variation in the genetic dataset better than did the isolation-by-barrier model. This outcome is not surprising given the large distances between study sites (86 – 310 km) in comparison to mean spotted skunk home-range size < 5 km^2 (see Chapter 3). However, we inferred that the greatest number of migrants per generation were between our two Blue Ridge subpopulations, WG and WT. Although these two populations are separated by a large geographic distance, they are more genetically similar to each other than to subpopulations within closer spatial proximity that were located within the Valley and Ridge region. Conversely, subpopulations within the Valley and Ridge region share far fewer apparent migrants per generation among themselves or with Blue Ridge subpopulations. Thus, the significant correlations of landscape variation with genetic distance may suggest a limitation by cost-of-travel across various landscapes on genetically effective migration of spotted skunks in our study area, similar to results found by Kanine (2013) for Allegheny woodrats. However, genetically effective migration may be higher among populations located in closer spatial proximity, and future studies should seek to sample multiple subpopulations within various mountain ranges. Further, patterns of apparent connectivity may reflect historical as opposed to current patterns of genetically effective migration. Historically, valley bottoms throughout the Appalachian region were forested pre-European settlement (Yarnell 1998). However extensive timber harvest, surface mining, and expanding agriculture throughout the 1800 – 1900's severly altered the forest structure and compostion in this region (Yarnell 1998, Schuler and Gillespie 2000). Although contemporary landscape changes may be limiting genetically effective migration, migration-drift equilibrium may not yet have been established.

Overall, low genetic diversity throughout our study region is suggestive of subpopulation isolation for eastern spotted skunks, despite the perceived homogeneity and contiguous distribution of forested areas throughout the Appalachian Mountains regionally. Other mesocarnivore species common to the area, such as raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*), exploit various habitats, including arable and developed areas, that may be unsuitable for use by eastern spotted skunks (Trani et al. 2007). Unlike eastern spotted skunks in our study, these generalist species display relatively large effective population sizes and high genetic variability, with little to no evidence of population structure (Bixler 2000, Hennessy et al. 2015). Spotted skunks in our study region appear to be forest obligates and show strong avoidance of the non-forested areas within and surrounding our study sites (see Chapters 3, 4). The strong association with forested areas coupled with high levels of forest fragmentation (relative to pre-settlement conditions; Yarnell 1998) in western Virginia and West Virginia are likely negatively influencing spotted skunk dispersal, genetic diversity, and ultimately the adaptive potential of local populations of this vulnerable species. Forest management actions should seek to increase connectivity among habitat patches (e.g., increase understory cover using forest thinning or prescribed fire) to increase the potential for genetically effective migration among subpopulations of eastern spotted skunk, negating the effects of genetic drift and inbreeding and preventing further loss of genetic diversity in extant populations.

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Table 5.1 Summary information for 8 microsatellite loci used to assess genetic diversity and population structure of eastern spotted skunks (*Spilogale putorius*) in the Monongahela, George Washington, and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019.

Locus	Size Range (bp)	Number of Alleles	T_m (°C)	Source
Meph 22-16	185-197	3	55	Dragoo et al. 2009
Meph 22-89	185-195	6	55	Dragoo et al. 2009
Meph 42-25	215-223	5	55	Dragoo et al. 2009
Meph 42-73	150-162	7	55	Dragoo et al. 2009
Meme 20	128-154	4	55	Munguia-Vega et al. 2009
Meme 77	127	1	54	Munguia-Vega et al. 2009
Meme 82	125-131	4	55	Munguia-Vega et al. 2009
Meme 84	130-140	5	56	Munguia-Vega et al. 2009

Table 5.2 Cost surface values for land cover, forest fragmentation, and elevation weighted at different magnitudes multiplied by selection ratios from chapter 4. Higher values represent higher cost of travel through a given landscape feature.

		Cost Value		
Landscape feature	**SR**	**x10**	**x100**	**x1000**
Land cover				
Deciduous forest	1	1	1	1
Evergreen Forest	1	1	1	1
Mixed forest	1	1	1	1
Shrub-scrub	2	20	200	2000
Non-forested	2	20	200	2000
Fragmentation				
Core	1	1	1	1
Perforated	2	20	200	2000
Edge	3	30	300	3000
Patch	3	30	300	3000
Non-forested	4	40	400	4000
Elevation (m)				
< 450	1	1	1	1
450 – 550	1	1	1	1
550 – 650	1	1	1	1
650 – 750	2	20	200	2000
750 – 850	2	20	200	2000
850 – 950	3	30	300	3000
950 – 1050	3	30	300	3000
1050 – 1150	1	1	1	1
1150 – 1250	1	1	1	1
1250 – 1450	1	1	1	1

Table 5.3 Genetic diversity of five subpopulations of eastern spotted skunks (*Spilogale putorius*) in the Monongahela, George Washington and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019. N = number of individuals sampled, A = mean number of alleles per locus, Ar = allelic richness, H_o= mean observed heterozygosity, H_e = mean expected heterozygosity, allelic range = size difference between largest and smallest alleles at a given locus, M = ratio of number of observed to possible alleles at a given locus (Garza and Williamson 2001), F_{IS} = inbreeding coefficient, and associated p-value < 0.05 indicates F_{IS} significantly greater than zero.

Subpopulation	N	A	A_r	Allelic range	H_o	H_e	M	F_{IS}	p
North Tract Mtn.	24	3.25	2.36	6.67	0.46	0.57	0.41	0.19	0.001
Bald Mtn.	21	3.13	2.15	5.71	0.35	0.44	0.38	0.01	0.44
Sugar Run Mtn.	4	2.25	2.25	4.67	0.53	0.40	0.31	0.03	0.51
Wintergreen Mtn.	8	3.25	2.84	10.00	0.52	0.69	0.40	0.43	<0.001
Whitetop Mt.	8	2.75	2.58	5.49	0.42	0.61	0.36	0.29	0.004

Table 5.4 Results of assignment tests conducted in GeneClass2 (Piry et al. 2004) for eastern spotted skunks (*Spilogale putorius*) sampled from five origin subpopulations in the Monongahela, George Washington and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019. Proportion of individuals correctly assigned to subpopulation of origin is shown in bold.

Origin subpopulation	**Assigned subpopulation**				
	TM	**BM**	**SR**	**WG**	**WT**
Upper Tract Mtn.	**0.63**	0.10	0.00	0.38	0.13
Bald Mtn.	0.17	**0.76**	0.25	0.25	0.00
Sugar Run Mtn.	0.00	0.05	**0.25**	0.00	0.25
Wintergreen Mtn.	0.17	0.05	0.00	**0.25**	0.13
Whitetop Mtn.	0.04	0.05	0.50	0.13	**0.50**

Table 5.5 Analysis of molecular variance results for genetic variation of five subpopulations (with *K* = 2 and *K* = 3) of eastern spotted skunks (*Spilogale putorius*) in the Monongahela, George Washington and Jefferson national forests of western Virginia and West Virginia from March

Source of variation	***d.f.***	**Sum of squares**	**Variance components**	**Percent variation explained**
Among regions (*K* = 2)	1	7.13	0.02 V_a	1.10
Among subpopulations within regions	3	19.31	0.19 V_b	10.29
Among individuals within sub populations	60	113.32	0.25 V_c	13.64
Within individuals	65	90.00	1.38 V_d	74.97
Among regions (*K* = 3)	2	20.89	0.15 V_a	8.37
Among subpopulations within regions	2	5.56	0.06 V_b	3.26
Among individuals within sub populations	60	113.15	0.25 V_c	13.61
Within individuals	65	90.00	1.38 V_d	74.77

Table 5.6 Measures of pairwise genetic differentiation (Jost's D, G_{ST}, and F_{ST} values, below diagonal) and associated p-values (above diagonals) and estimated number of pairwise migrants per generation (nM) for four subpopulations of eastern spotted skunk (*Spilogale putorius*) at Upper Tract Mountain (TM), Bald Mountain (BM), Sugar Run Mountain (SR), Wintergreen Mountain (WG), and Whitetop Mountain (WG) in the Monongahela, George Washington and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019. p-values in bold indicate significant difference from zero.

		Site				
Index of differentiation	**Site**	**TM**	**BM**	**SR**	**WG**	**WT**
Jost's D	Upper Tract Mtn	–	**0.001**	**0.001**	0.405	**0.004**
	Bald Mtn.	0.099	–	0.011	**0.002**	**0.001**
	Sugar Run Mtn.	0.204	0.060	–	0.041	0.186
	Wintergreen Mtn.	0.000	0.091	0.123	–	0.699
	Whitetop Mtn.	0.095	0.152	0.054	0.000	–
G_{ST}	Upper Tract Mtn	–	0.001	0.001	0.404	0.006
	Bald Mtn.	0.223	–	–	0.002	0.001
	Sugar Run Mtn.	0.377	0.145	0.000	–	0.181
	Wintergreen Mtn.	0.000	0.179	0.216	0.000	–
	Whitetop Mtn.	0.174	0.284	0.103	0.039	0.000
F_{ST}	Upper Tract Mtn	–	**0.001**	**0.001**	0.331	**0.009**
	Bald Mtn.	0.086	–	0.021	**0.002**	**0.001**
	Sugar Run Mtn.	0.163	0.086	–	0.038	0.184
	Wintergreen Mtn.	0.030	0.080	0.120	–	0.694
	Whitetop Mtn.	0.073	0.111	0.086	0.039	–
nM						
	Upper Tract Mtn	–				
	Bald Mtn.	1.60	–			
	Sugar Run Mtn.	0.95	2.20	–		
	Wintergreen Mtn.	17.17	1.62	1.61	–	
	Whitetop Mtn.	2.34	1.11	3.45	57.82	–

Table 5.7 Estimated effective population size (N_e) of five eastern spotted skunk (*Spilogale putorius*) subpopulations in the Monongahela, George Washington and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019, with 95% confidence intervals. Undefined bounds may be attributed to small sample size. *N* = sample size, CI = confidence interval.

Subpopulation	N	N_e	95% CI	Jackknife estimates
Upper Tract Mtn.	24	16	10.7 – 26.2	3.0 – undefined
Bald Mtn.	21	17.4	8.3 – 60.7	4.6 – undefined
Sugar Run Mtn.	4	Undefined	1.6 - undefined	5.1 – undefined
Wintergreen Mtn.	8	26.1	3.3 - undefined	1.3 – undefined
Whitetop Mtn.	8	Undefined	4.5 – undefined	5.7 - undefined

Table 5.8 Model selection results of redundancy analysis and Mantel tests on pairwise comparisons of genetic distance to accumulated travel cost through various landscape features for five eastern spotted skunk (*Spilogale putorius*) subpopulations in the Monongahela, George Washington, and Jefferson national forests in Virginia and West Virginia from March 2015 – April 2019.

Model	*AIC*	Δ*AIC*	w_i	R^2_{adj}	*p*
Forest fragmentation + land cover +elevation	123.83	0	0.18	0.25	0.0001
Forest fragmentation	124.91	1.08	0.108	0.28	0.001
Elevation	124.91	1.08	0.108	0.24	0.001
Land cover	125.06	1.23	0.10	0.25	0.011
Euclidean distance	125.06	1.23	0.10	0.25	0.0004
Null	125.12	1.29	0.10	0.24	0.0001
Mountain range	125.34	1.51	0.088	0.26	0.0001
Mountain range + Euclidean distance	125.76	1.93	0.07	0.25	0.001
Mountain range + forest fragmentation	125.76	1.93	0.07	0.25	0.001
Mountain range + elevation	125.81	1.98	0.073	0.25	0.001
Mountain range + land cover	127.21	3.38	0.03	0.26	0.001
Global	137.45	13.62	0.00	0.27	0.001

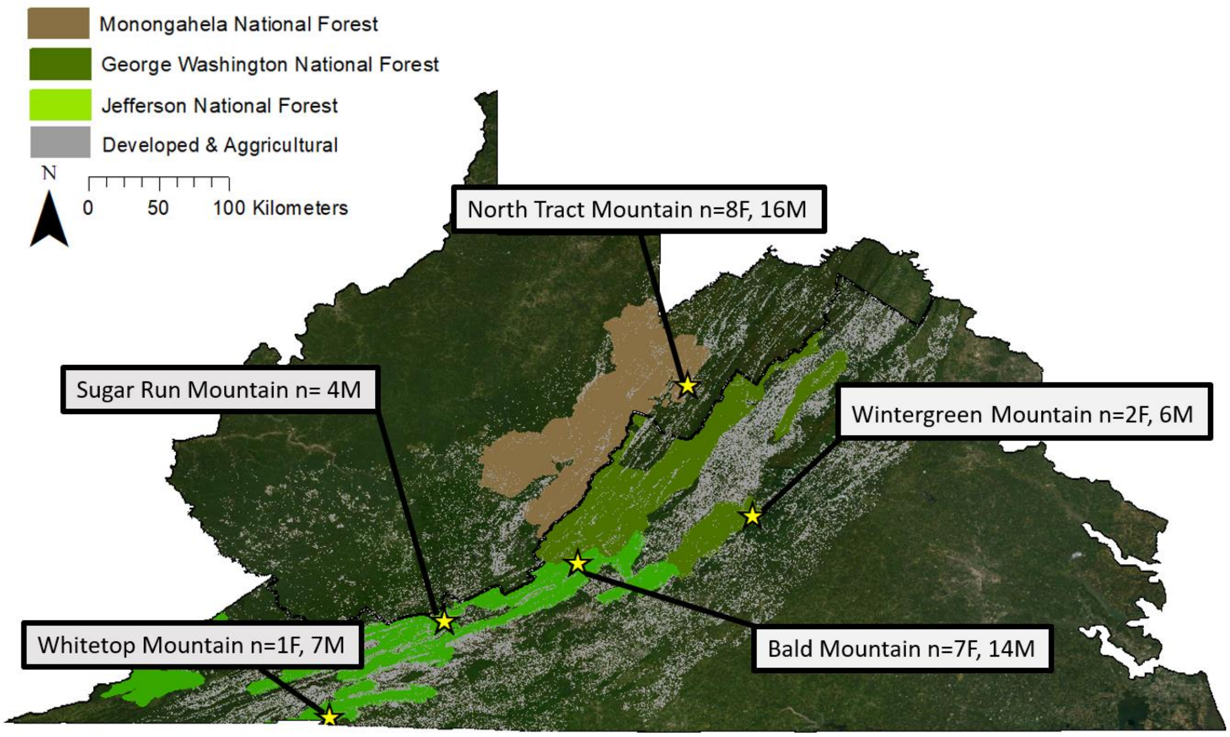

Figure 5.1 Eastern spotted skunk (*Spilogale putorius*) sampling sites in the Monongahela, George Washington, and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019.

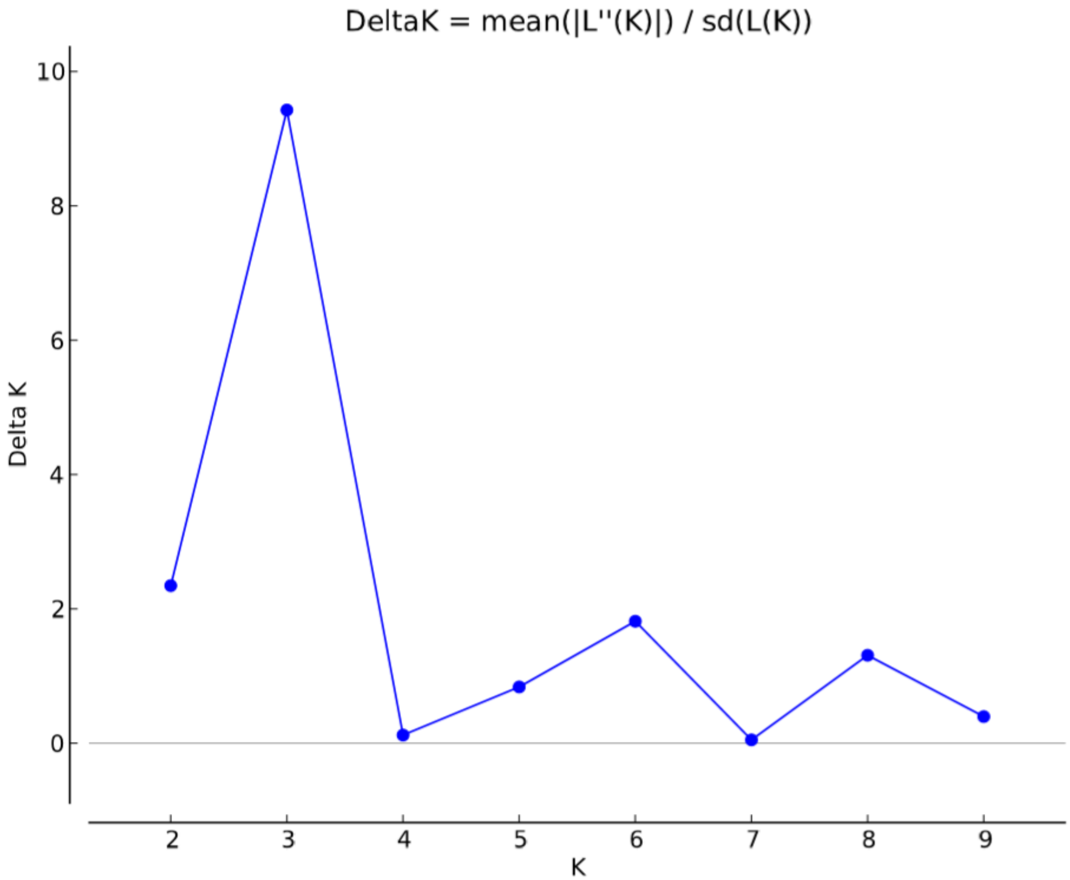

Figure 5.1. Results of the Evanno et al. (2010) method of determining the best-supported number of multilocus genetic clusters, *K*, showing *K* = 3 as the best-supported number clusters for eastern spotted skunk (*Spilogale putorius*) samples collected at five study sites in the Monongahela, George Washington, and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019.

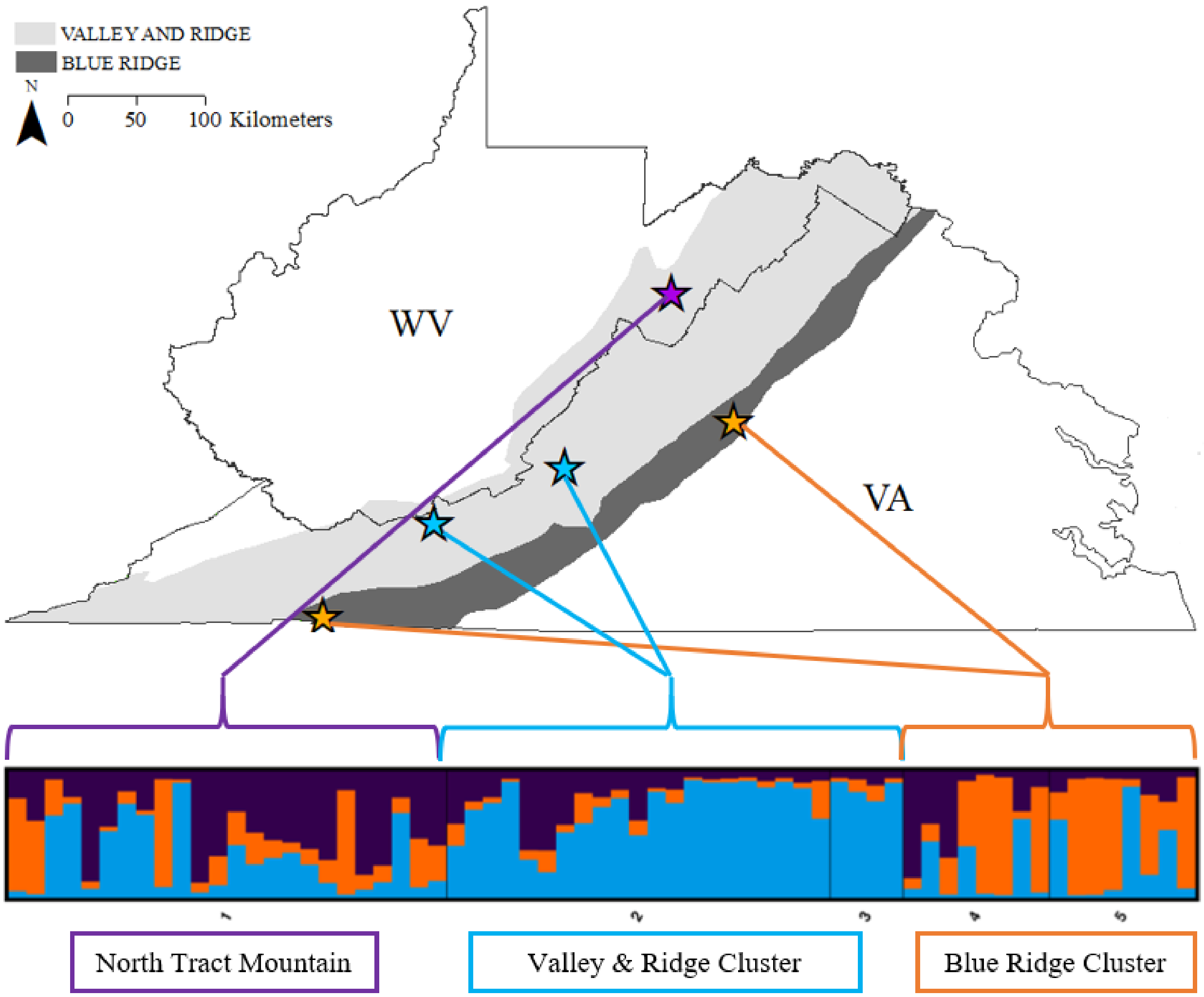

Figure 5.3 STRUCTURE plots depicting population structuring of eastern spotted skunks (*Spilogale putorius*) captured at: North Tract Mountain, Bald Mountain, Sugar Run Mountain, Wintergreen Mountain, and Whitetop Mountain in the Monongahela, George Washington, and Jefferson national forests from March 2015 – April 2019.

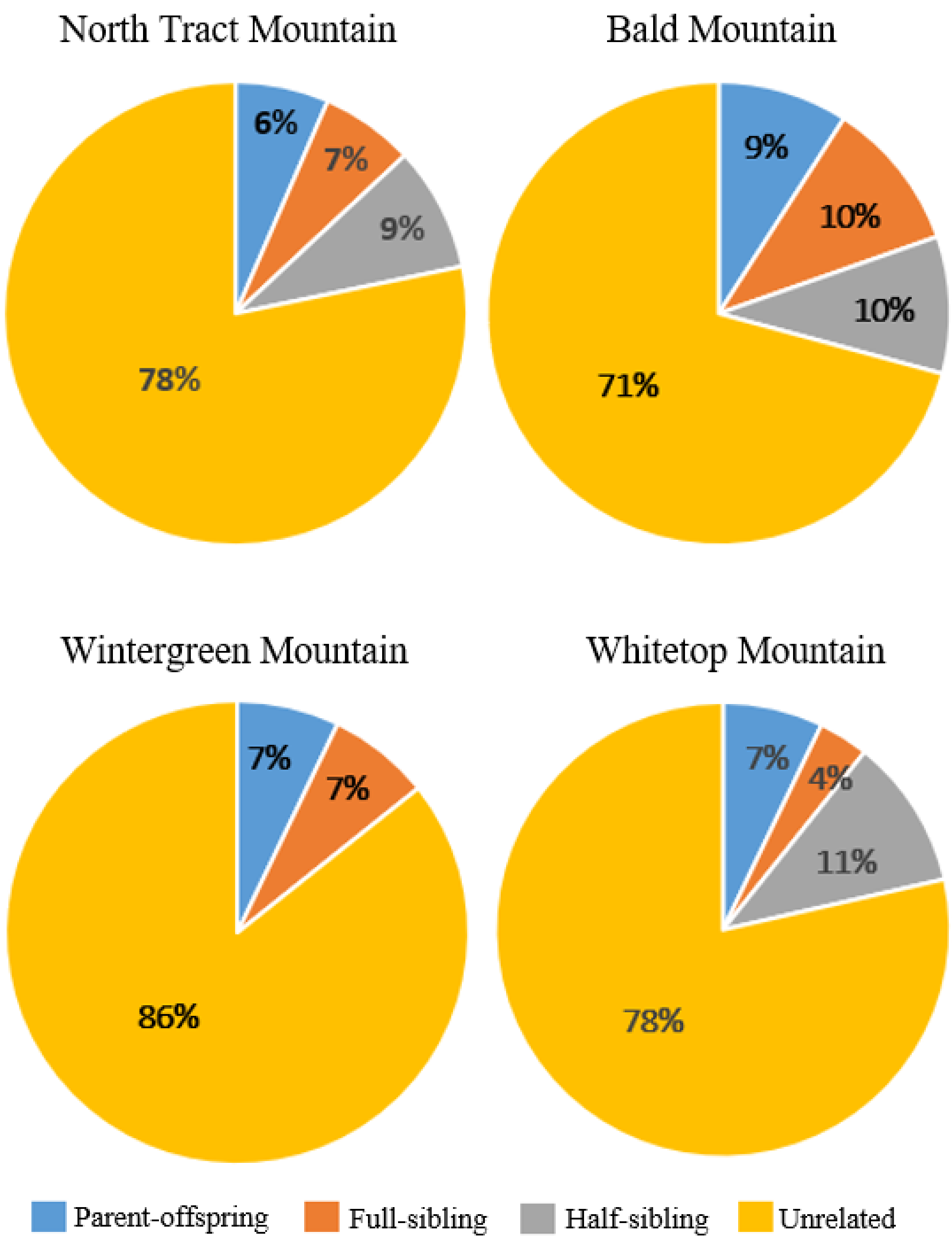

Figure 5.2 Results of maximum likelihood analysis of relatedness among individual eastern spotted skunks (*Spilogale putorius*) within four subpopulations in the Monongahela, George Washington, andJefferson national forests of western Virginia and West Virginia from March 2015 – April 2019. All individuals sampled at Sugar Run Mountain were unrelated, not shown.

Supplementary Table 5.1. Locus-by-locus genetic diversity indices for five subpopulations of eastern spotted skunk (*Spilogale putorius*) in the Monongahela, George Washington, and Jefferson national forests of western Virginia and West Virginia from March 2015 – April 2019. Indices include number of gene copies, number of alleles (N_A), allelic richness (A_R), observed (H_o) and expected heterozygosity (H_e), significance of divergence from Hardy-Weinberg Equilibrium (HWE-*p*), and *M*-ratio. Only polymorphic alleles shown.

Site	Locus	Gene copies	N_A	A_R	H_o	H_e	HWE-*p*	*M*-ratio
Upper Tract Mountain	*Meph 22-16*	44	2	1.97	0.45	0.44	1	0.67
	Meph 22-89	48	6	3.18	0.38	0.56	0.02	0.55
	Meph 42-25	48	4	1.00	0.25	0.64	<0.001	0.44
	Meph 42-73	48	5	3.49	0.83	0.72	0.67	0.56
	Meme 20	48	3	2.49	0.38	0.54	0.05	0.43
	Meme 82	48	4		0.50	0.50	0.20	0.57
Bald Mountain	*Meph 22-16*	42	2	2.00	0.29	0.37	0.54	0.07
	Meph 22-89	42	4	2.97	0.62	0.49	0.79	0.44
	Meph 42-25	40	4	3.50	0.20	0.19	1.00	0.60
	Meph 42-73	42	4	3.50	0.90	0.73	0.11	0.55
	Meme 20	38	3	2.75	0.26	0.49	0.01	0.43
	Meme 82	42	2	2.90	0.14	0.14	1.00	0.67
	Meme 84	42	4	2.00	0.29	0.30	0.45	0.44
Sugar Run Mountain	*Meph 22-16*	8	3	2.50	0.5	0.43	1.00	0.67
	Meph 22-89	8	3	2.75	0.00	0.71	0.03	0.33
	Meph 42-73	8	5	4.22	1.00	0.82	0.77	0.57
	Meme 20	8	4	3.7	0.25	0.25	1.00	0.29
	Meme 82	8	3	2.95	0.5	0.43	1.00	0.67
	Meme84	8	3	2.76	1.00	0.68	0.31	0.60
Wintergreen Mountain	*Meph 22-16*	16	2	2.00	0.63	0.59	1.00	0.23
	Meph 42-25	14	3	3.00	0.43	0.48	0.44	0.57
	Meph 42-73	14	4	4.00	0.43	0.80	0.06	0.56
	Meme 20	12	2	2.00	0.33	0.74	0.01	0.15
	Meme 82	16	2	2.00	0.50	0.69	0.04	0.60
	Meme 84	16	2	2.00	0.00	0.63	0.001	0.23
Whitetop Mountain	*Meph 22-16*	14	2	1.91	0.29	0.53	0.44	0.67
	Meph 22-89	16	4	2.68	0.5	0.71	0.24	0.33
	Meph 42-25	14	3	1.73	0.57	0.74	0.58	0.57
	Meph 42-73	14	6	3.71	0.43	0.74	0.05	0.44
	Meme 20	16	3	2.56	0.38	0.61	0.11	0.43
	Meme 82	16	2	1.48	0.75	0.68	1.00	0.60
	Meme 84	16	4	1.25	0.38	0.53	0.53	0.40

Supplementary Table 5.2 Results of STRUCTURE Bayesian cluster analysis of multilocus genotypes at 7 microsatellite loci among eastern spotted skunk (*Spilogale putorius*) from five sample sites in thethe Monongahela, George Washington, and Jefferson national forests in Virginia and West Virginia from March 2015 – April 2019. Ln$P(D|K)$ = log probability of the data given K, where K = a given number of clusters. Results presented are the mean Ln$P(D|K)$ from ten iterations. Each run was performed with a burn-in of 100,000 and 100,000 MCMC cycles.

K	**Mean Ln*P*(*K*)**	**Stdev Ln*P*(*K*)**	**Ln'(*K*)**	**|Ln''(*K*)|**	**Delta *K***
1	-1010.10	0.23	—	—	—
2	-982.82	8.05	27.28	18.88	2.35
3	-974.42	3.66	5.40	34.48	9.43
4	-1000.50	19.63	-26.08	2.40	0.12
5	-1028.98	10.02	-28.48	8.40	0.84
6	-1049.06	19.67	-20.08	35.74	1.82
7	-1104.88	37.90	-55.82	1.92	0.05
8	-1158.78	56.58	-53.90	74.12	10.31
9	-1138.56	69.94	-20.22	27.82	0.40
10	-1146.16	46.21	-7.60	—	—

Synthesis and Management Recommendations

The following is a synthesis of eastern spotted skunk (*Spilogale putorius*) habitat associations, home range dynamics, resource use and population structure presented in the previous five Chapters. Furthermore, I present my recommendations for directed management of this species in the Central Appalachian Mountains of Western Virginia and eastern West Virginia.

Site occupancy and landscape-level habitat associations

This research is the first of its kind for eastern spotted skunks in Virginia and West Virginia. Notable declines in lack of detections on the landscape suggest local population declines throughout the species' distribution (Gompper and Hackett 2005). My results suggest that, at the first order level of habitat selection, eastern spotted skunk site occupancy is strongly associated with forested areas < 50 years in age at elevation < 650 m in elevation. Additionally, there was a moderate association of mature to old growth forest at elevation > 1050 m in elevation. These forest types are typically associated with complex forest structure (Swanson et al. 2011, Wilson et al. 2014) that may provide necessary escape cover from aerial predators such as great horned owls (*Bubo virginianus;* Lesmeister et al. 2010). These areas occur in small, disjunct patches throughout the George Washington and Jefferson National Forests which raises concerns related to patch isolation such as limited dispersal ability and genetic isolation.

Spatial co-occurrence and temporal activity

Overlap in resource use by sympatric predators can lead to direct or indirect competition Creel et al. 2001). Within the predator guild, direct competition can be manifested by aggressive interactions, and occasionally intraguild predation or killing of competitors (Rosenweig 1966). I found little evidence of spatial avoidance of sympatric meso-predators by eastern spotted skunks. However, spotted skunks appeared to alter their activity patterns at baited camera sites where other meso-predators were detected. Spotted skunks may avoid sympatric species scavenging at a food resource to reduce the likelihood of antagonistic interactions and therefore reduce risk of injury. This pattern was particularly strong between spotted skunks and striped skunks (*Mephitis mephitis*), two closely related species. It is likely that resource competition is strongest between two species with similar niches, therefore a stronger pattern of avoidance may accommodate the competitive coexistence of the two species of skunks to reduce direct and indirect competition (Humphreys and Barraclough 2014).

Home-range size and resource use

Throughout much of western Virginia and to a lesser degree in eastern West Virginia, forested ecosystems have experienced high reduction of core forest areas into smaller fragments as a result of land-use change and urbanization (Yarnell 1998, Schuler and Gillespie 2000). Extant populations of rare wildlife species may be restricted to forest fragments and isolation from neighboring populations by a surrounding matrix of suboptimal ecological conditions (Kozakiewicz 1993). My results suggest that spotted skunks are using smaller home ranges (<1-1.5km^2) than those found in other studies. Additionally, spotted skunks strongly selected for core forested areas greater than 1 km^2 whereas non-forested areas and forest edge were avoided, both at the second and thirds order levels of habitat selection. Based on these results, I conclude that spotted skunk movement and dispersal may be limited by large areas of unsuitable land, i.e., developed and arable areas, surrounding small patches of spotted skunk habitat. These findings support the findings of Chapter 1 and further suggest the occurrence of patch isolation.

Den selection

Understanding the relationships between landscape variation and resource selection is a central tenet in wildlife management. However, it is equally important to consider the influence of individual variation and intrinsic characteristics (Hutto 1985, Block and Brennan 1993). Although generally rare, eastern spotted skunks have typically shown generalist and opportunistic characteristics throughout the length of the Southern and Central Appalachian Mountains (Thorne et al. 2014, Sprayberry and Edelman 2018, Eng and Jachowski 2019). However, my findings have shown that selection of den types and microhabitat surrounding den entrances it strongly related to multiple sources of variation including reproductive condition and weather. This suggests that management actions considering only landscape characteristics may be less impactful for spotted skunks under varying reproductive condition or experiencing various types of weather conditions. Based on the complex relationships between den use, habitat attributes, weather conditions, and intrinsic variables, I propose that eastern spotted skunks are in effect a 'conditional generalist' species. Moreover, I defined the term 'conditional generalist' to represent a species that is not limited to a narrow range of environmental conditions (i.e., specialist species), and that can make use of a wide variety of resources, but is conditionally selective of a given resource in response to one or more current environmental or intrinsic conditions.

Genetic differentiation and population structure

The adaptive potential of a species relies on genetically effective migration among populations (Fisher 1930, Wright 1978). Habitat fragmentation causing barriers to migration can reduce or prevent geneflow between neighboring populations (Forman and Alexander 1998, Wilson et al. 2016). Negative effects of genetic isolation can include reduced effective population size, loss of genetic diversity, and inbreeding depression (Soulé 1985, Templeton et al. 1990, Andrén 1994, Franklin et al. 2009, Méndez et al. 2014). In previous chapters I presented evidence that forest fragmentation has led to isolation of suitable spotted skunk habitat patches and may limit movement and dispersal among patches. In my fifth chapter, I examined the genetic diversity and population structure of eastern spotted skunks in western Virginia and eastern West Virginia. My findings suggest low genetic diversity, discernable populations differentiation, and evidence of inbreeding. Sampled individuals fell into three genetic clusters: North Tract Mountain cluster, Valley and Ridge cluster, and Blue Ridge cluster. Genetic distance among these clusters was positively associated with increased cost of travel across non-forests areas, predominantly within valleys separating ridgelines where samples were collected. The apparent reduced effective genetic migration through the human-modified valley landscapes may be the cause of low genetic diversity and inbreeding within clusters.

Habitat management recommendations

The findings of this study led me to frame recommendations for enhancing eastern spotted skunk management in the Central Appalachian Mountains. I have provided evidence of eastern spotted skunk habitat fragmentation and patch isolation. Therefore, forest management actions should seek to increase connectivity among habitat patches (e.g., increase understory cover using forest thinning or prescribed fire) to increase the potential for genetically effective migration among subpopulations of eastern spotted skunk. These actions could help negate the effects of genetic drift and inbreeding and preventing further loss of genetic diversity in extant populations.

Loss of early- and mid-successional habitat to forest maturation and reduced harvesting, and declines in natural and anthropogenic disturbance regimes in forests of eastern North America (i.e., fire; Brose et al. 2001, King and Schlossburg 2013) may explain the patchy distribution of occupied habitat in the central and southern region of the Appalachians. Currently, the majority of timber stands in the George Washington and Jefferson National Forest are in the 90–130-year-old age class, which is either at, or exceeding, specified rotation ages of 80–100 years, in comparison to 11% of forest in the ≤50-year-old age classes (U.S. Forest Service 2004, 2014). Eastern spotted skunk populations may experience reduced habitat availability as production and maintenance of early- to mid-successional habitat remain low in much of the National Forest land in the central and southern Appalachians. Some spotted skunk habitat management should focus on creating additional early – to mid-successional forests through stand thinning and prescribed burning whereas opportunities to use passive or active management to increase stand complexity and decadence in mature forests to accelerate old growth characteristics could also be considered. Ideally, management plans would seek to increase patch connectivity of occupiable habitat to reduce or prevent further population decline and aid in recovery of the spotted skunk population.

Increased patch connectivity should result in the colonization of previously unoccupied areas and increase effective genetic migration among occupied patches. Further, increased genetic migration among occupied patches can provide genetic rescue to subpopulations experiencing low genetic diversity and inbreeding. Potential genetic enhancement of subpopulations may further be facilitated by translocating individual spotted skunks from to sites with lower effective population sizes and evidence of inbreeding. However, without management for patch connectivity, subpopulations will remain isolated post-translocation and long-term benefits of human-facilitated genetic exchange will be minimal. Therefore, to ensure the long-term persistence of eastern spotted skunks in the Central Appalachian region it is vital to design management action plans that seek to restore large core forested areas of early- to mid-successional forest.

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