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Florida spotted skunk ecology in a dry prairie ecosystem

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FLORIDA SPOTTED SKUNK ECOLOGY IN A DRY PRAIRIE ECOSYSTEM

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ABSTRACT

The eastern spotted skunk (*Spilogale putorius*) is a species of conservation concern in many portions of its range and has experienced a decline since the early to mid-1990s. However, little is known about the subspecies that inhabits peninsular Florida, the Florida spotted skunk (*S. p. ambarvalis*), which may still be abundant. To gather more information on this diminutive carnivore's ecology and to clarify its role as a predator of the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*), we conducted studies in 2016 and 2017 on the den site selection and diet of Florida spotted skunks in a dry prairie ecosystem in central Florida, where these 2 species co-occur. For the den site selection study, we tracked 36 individual skunks to 757 den sites and measured the habitat and den characteristics of these sites. Den sites were most often located in mammal burrows (61.6 %), followed by above-ground sites (35.5%), gopher tortoise (*Gopherus polyphemus*) burrows (1.5%), depressions (1.2%), and hollow logs (< 0.3%). Each of these used sites was compared to a paired, random available den site using discrete choice analysis. We found that male and female nonbreeding skunks at our study site were 5 times more likely to select a mammal burrow over a tortoise burrow and that the relative probability of selection of a den site increased by 45% for each 1-burrow increase in the number of nearby burrows. Similarly, breeding female skunks selected mammal burrows and shallow depressions over gopher tortoise burrows by 16- and 13-fold, respectively, and relative probability of selection of a den site increased by 75% for every 1-burrow increase in the number of nearby burrows. Our findings suggest that den

characteristics may be more important than habitat characteristics to Florida spotted skunk den site selection in dry prairie and that skunks in this ecosystem may be habitat generalists. For our diet study on this subspecies, we collected hair samples from skunks and tissue samples from potential food items across our study site. We subsequently conducted a stable isotope analysis to determine which food items comprised the Florida spotted skunk diet at our site. Our results suggested that skunks in the dry prairie have an omnivorous and generalist diet, as no food item groups composed a majority of their diet. The most prevalent food items in our study were millipedes (~27%) and insectivorous amphibians and reptiles (~25%). Insectivorous birds, like the grasshopper sparrow, comprised no more than approximately 15% of the skunk diet at our site. Overall, these studies contribute to the knowledge of Florida spotted skunk ecology and help elucidate the skunk's role as a predator of grassland birds in dry prairie ecosystems, while also providing some recommendations for land managers to consider for reducing predation pressure on grasshopper sparrows by these skunks. However, this research should be expanded upon to better discern Florida spotted skunk diet composition and selection, and to determine if the patterns observed in our studies on diet and denning ecology hold in other portions of this subspecies' range.

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INTRODUCTION

The eastern spotted skunk (*Spilogale putorius*) is a small-bodied skunk in the family Mephitidae that has undergone a precipitous decline since the early to mid-1900s across much of its range (Gompper and Hackett 2005). Historically, the species ranged east from the Continental Divide through much of the central and southeastern United States, southeastern Manitoba, southwestern Ontario and northeastern Mexico (Kinlaw 1995). The reasons for the species' decline are unknown, but hypotheses include the detrimental effects of habitat loss, agricultural industrialization (e.g., reduction in haystacks available for denning), pesticide use, overharvest, and disease (Choate et al. 1974, McCullough 1983, Schwartz and Schwartz 2001, Gompper and Hackett 2005). Most studies on the eastern spotted skunk have focused on forested habitats in mountainous regions (Lesmeister et al. 2008, Thorne et al. 2017, Eng 2018, Sprayberry and Edelman 2018), and there are still many knowledge gaps (e.g., current distribution, habitat preferences, evidence supporting reasons for decline) for the species across its range (Gompper and Jachowski 2016).

The Florida spotted skunk (*Spilogale putorius ambarvalis*; FSSK) is the least studied of the eastern spotted skunk's 3 subspecies (Gompper and Jachowski 2016). This subspecies occurs throughout peninsular Florida as far south as Lee County, is endemic

to the state, and was still thought to be relatively abundant in central and southern Florida in the 1990s (Hamilton 1941, Kaplan and Mead 1991, Kinlaw et al. 1995b). The only prior dedicated research on the subspecies was conducted on an Atlantic barrier island during the 1970s and 1980s (Kinlaw et al. 1995a, Kinlaw et al. 1995b), in a coastal strand habitat dominated by woody shrubs. However, the FSSK has also been reported using suburban and rural habitat types, such as improved pasture and native dry prairie (Gompper and Jachowski 2016; S. Glass and T. Hannon, Florida Fish and Wildlife Conservation Commission [FWC], pers. comm.).

The dry prairie, which differs greatly from the aforementioned coastal strand habitat, is a habitat that is restricted to south-central Florida and that has been reduced to less than 2% of its historic range (Noss et al. 1995, U. S. Fish and Wildlife Service 1999). The dry prairie contains an abundance of low shrubs and grasses and is maintained by frequent fire and seasonal flooding that keep vegetation heights low and prevent encroachment and establishment of tree species (Platt et al. 2006, Florida Natural Areas Inventory [FNAI] 2010).

Recent nest camera research has confirmed the FSSK as a nest predator of dry prairie ground-nesting birds, including the critically endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*; FGSP), an endemic of the dry prairie (Federal Register 1986, Pranty and Tucker 2006; E. L. Hewett Ragheb, FWC, pers. obs.). Additionally, nest success has been shown to be low in the FGSP (between 10% and 33%), and nest predation was identified as the primary cause of nest failures (Perkins et al. 2003). However, it is currently unknown how spotted skunks utilize dry prairie (as

they have never been studied in this ecosystem), or how habitat management for FGSP (including prescribed fire) influences FSSK behavior. Currently, FGSP nest predation by FSSKs and other mammalian predators is intensively managed at all known populations through the installation of predator deflection fencing around nests, but there is an urgent need to discover landscape-level predation management solutions.

Insights into FSSK den selection behavior could be used to inform management decisions that limit nest predation of ground-nesting birds. The den site is an important resource for the eastern spotted skunk, acting as refugia for male and female skunks during periods of inactivity (i.e., daylight hours) and as safe places for parturition and care of young by female skunks (Kinlaw 1995). Den site selection may be vital to the survival of individuals, as Crabb (1948) noted some eastern spotted skunk mortalities in Iowa which could be traced to poor selection of den sites. Accordingly, the objective of our study was to evaluate how habitat (e.g., vegetative cover, distance to landscape features) and den (e.g., den type) characteristics affecting den site selection of FSSKs at a site in south-central Florida dominated by dry prairie habitat.

MATERIALS AND METHODS

Study area—We conducted our study in the ‘Route 60 unit’ of Three Lakes Wildlife Management Area (TLWMA), located in Osceola County, Florida (Fig. 1.1). Dry prairie is the dominant natural community in this unit, covering approximately 3000 ha. This natural community is characterized by low shrubs and grasses. Common species

include wiregrass (*Aristida stricta* var. *beyrichiana*), shiny blueberry (*Vaccinium myrsinites*), gallberry (*Ilex glabra*), saw palmetto (*Serenoa repens*), and dwarf live oak (*Quercus minima*; Florida Natural Areas Inventory [FNAI] 2010). The dry prairie landscape is dotted with permanent depression marshes and is periodically wet or inundated after seasonal rain events (FNAI 2010). Other habitats interspersed with the dominant dry prairie habitat include wet prairie and various forest and scrub communities.

Our study area is divided into many smaller management subunits that generally receive prescribed fire treatments every 1 to 2 years, creating a landscape with a mosaic of fire return intervals (S. Glass and T. Hannon, FWC, pers. comm.). The application of prescribed fire is essential to maintaining the prairie in a primarily treeless state by limiting the recruitment of hardwood tree species and cabbage palmettos (*Sabal palmetto*). In addition, trees and palmettos are occasionally removed from the Route 60 unit mechanically to further prevent hardwood encroachment and to enlarge the size of the prairie (S. Glass and T. Hannon, FWC, pers. comm.).

Animal capture and processing—We captured FSSKs in the spring and summer of 2016 and 2017 in dry prairie habitat where FGSPs occurred or were known to occur in recent years. We also trapped skunks in different habitat types, along habitat ecotones (e.g., dry prairie–forest edge), in management subunits with differing intervals since the last prescribed fire application, and across a range of distances from roads, trails, firebreaks, and wetlands. We set and baited Havahart double-door live traps (Model #:1030-B; Havahart, Lititz, PA) with commercially-available wet cat food in the

afternoon and checked them the following morning. We weighed and marked with ear tags (Model #1005-1L1; National Band & Tag Company, Newport, KY) each individual that was captured. We also determined each individual's sex and whether it was an adult or a juvenile (based on body size and teeth wear; Lesmeister 2007).

We fit adult FSSKs with zip-tie collar-type very high frequency (VHF) radio-transmitters (Models M1525 [12 g] and M1545 [18 g]; Advanced Telemetry Systems, Inc., Isanti, MN) representing < 10% of each animal's mass (Wilson et al. 1996, Sikes et al. 2011). We attempted to recapture every collared skunk before their transmitter batteries died and once our study was complete, in order to remove the transmitters from the skunks. We followed American Society of Mammalogists guidelines and complied with Clemson University Animal Care and Use Committee protocol (permit # AUP2015-042) for all skunk trapping, processing, collaring, and radio-tracking (Sikes et al. 2011).

Tracking—We tracked collared skunks 1 to 3 times a week from February through July in 2016 and 2017. We divided the hours of daylight per day into 3 equal periods (e.g., 1: 0700–1100, 2: 1100–1500, 3: 1500–1900), alternated our tracking of each skunk between these periods, and waited > 24 hours between each attempt at tracking an individual to ensure that we were not biasing our tracking. We did not track skunks during evening hours (i.e., between 1900 and 0700) both because eastern spotted skunks are primarily nocturnal (Kinlaw 1995), meaning that the skunks were likely active during these hours and not utilizing den sites, and also because we wanted to avoid damaging ground-nesting bird nests.

When attempting to locate a skunk at a den site, we first moved towards its VHF signal until we believed that we were approximately 20 to 30 m from the skunk's location. We then walked to 2 or 3 other points at this same distance from the presumed location to help further pinpoint the skunk's exact location. After we felt confident in the general location of the skunk, we homed in to the skunk's exact site and recorded the coordinates of the site with a handheld Global Positioning System (GPS) unit. If we were unsure of the skunk's location at rest, or if the animal began to flee while we were tracking it, we would abandon the tracking of that skunk for the day and attempt to track it again on another day.

Den site habitat characterization—We returned to each identified den site within 15 days to measure the den and habitat characteristics at this used den site, as well as a paired available den site (Table 1.2). We located these paired available sites by walking along a randomly generated azimuth from the used den site until we found a potential available site where a skunk could den or rest, ≥ 50 m and ≤ 300 m from the chosen site (Lesmeister et al. 2008). If we did not locate an appropriate site ≤ 300 m from the den site along the azimuth, we would return to the den site location, choose another random azimuth, and repeat the procedure until we found an available site. We defined a site to be available if it met Crabb's (1948) 3 den requirements (i.e., provides darkness, shelter from weather, and protection from predators) or was a burrow with an entrance measuring at least 5 x 5 cm in size.

At each site, we used a 1 x 1 m modification of the Braun-Blanquet method and Daubenmire frame to measure the percentage (in 5% increments) of major vegetation

cover types and standing water (Bonham et al. 2004, Daubenmire 1959; Table 1.2). We recorded an index of visual obstruction by vegetation by placing a 1.5 m Robel pole (marked in 10 cm increments) at the identified den site, kneeling (at a height of 1 m) 4 m north of the den, and recording the lowest number visible on the pole from that location (Robel et al. 1970, Doty and Dowler 2006). We recorded Daubenmire plot and visual obstruction measurements at the den site center, 5 m to the east of the center, and 5 m to the west of the center. We then averaged each of these measures for a site prior to analysis. We categorized dens by type (above-ground, mammal burrow, gopher tortoise [*Gopherus polyphemus*] burrow, depression, woody debris or hollow log) and systematically searched within a 5 m radius from the chosen or random available den site for additional burrows that could act as den sites. Finally, we counted the number of woody shrub clumps ≥ 1.5 m tall within 50 m of the den site, the number of trees ≥ 3 m tall within 100 m of the den site, and the distance to the nearest woody shrub clump or tree.

We obtained Geographical Information Systems (GIS) layers of natural community types (Florida Cooperative Land Cover Map, FWC) and elevation (National Elevation Dataset, United States Geological Survey) at our study site for use with GIS software (ArcGIS 10.4, Environmental Systems Research Institute, Redlands, CA). We reclassified non-prairie natural communities into broader categories for a total of 5 community types (dry prairie, forest, scrub, wetland, and wet prairie) and subsequently determined distance to forest edge and distance to water feature for each den or available site. We obtained GIS layers containing primary roads and secondary roads (including

trails and firebreaks) from TLWMA staff and used these to determine the distance to primary road feature and secondary road feature for each den or available site. We also obtained prescribed fire dates from TLWMA staff and used these to calculate the time since last prescribed burn for each den and available site.

*Hypotheses—*We developed 6 *a priori* hypotheses on how habitat characteristics affect FSSK selection of den sites at our study site (Table 1.1). First, we hypothesized that spotted skunk den site selection at the study site would be positively associated with the amount of vegetative cover at a potential den site. Crabb (1948) stated that spotted skunk dens must provide protection from the elements, protection from predators, and darkness, all of which would be more likely to occur at a site with increased cover. Additionally, Lesmeister et al. (2008) found that site selection of spotted skunks in Arkansas was positively influenced by vegetative cover. Prescribed fire can completely eliminate vegetative cover in an area for 3 days or more, with vegetation not completely returning to pre-fire levels for 1 to 2 years, according to Abrahamson's (1984) findings in similarly poorly-drained plant communities of the nearby Lake Wales Ridge. This suggests that FSSKs should avoid these recently-burned areas due to the complete lack of cover. Second, we hypothesized that spotted skunks would select for gopher tortoise burrows on the landscape, as they have been documented occupying tortoise burrows in Florida previously (Manaro 1961, Toland 1991). We also hypothesized that the number of burrows (of any type) in an area would be positively associated with den site selection as this could indicate an area where soil characteristics were amenable to burrow excavation. Third, because Lesmeister et al. (2010) found that most spotted skunk

mortalities in Arkansas were likely due to predation by great horned owls (*Bubo virginianus*), we hypothesized that spotted skunks at our study site would avoid areas with tall shrubs or trees that could act as perches for raptors. Fourth, we hypothesized that skunks would select den sites farther from water (e.g., at higher elevations) because our study site can flood within hours of intense rain that often occurs in the summer months in Central Florida (SNH, pers. obs.). Fifth, we hypothesized that skunks would avoid recently burned areas due to decreases in potential prey (e.g., grassland bird nests and insects) available in areas soon after prescribed fire (Swengel 2001). Sixth, we hypothesized that road type would differentially impact den site selection depending on the intensity of vehicle use on these roads. We predicted that skunks would select den sites farther from gravel primary roads, which can represent a significant movement barrier to some small mammal species (Oxley et al. 1974, Swihart and Slade 1984, Merriam et al. 1989). By contrast, we predicted that dens would be found closer to unpaved, non-gravel secondary roads (including trails and firebreaks) that facilitate movement but more closely resemble natural, vegetated habitats.

Model development and validation—We used discrete choice modelling to fit and evaluate support for each *a priori* model developed for our hypotheses. Two major advantages of discrete choice analysis include the ability to determine the availability of resources separately for each selected site of interest, as each selected site is paired with one or more available sites, and the ability to accommodate changes in these resources temporally and spatially (Arthur et al. 1996, Cooper and Millspaugh 1999). Although there was some observed reuse of den sites, we treated located dens as independent sites

every time they were encountered. We did not include any nonlinear forms of covariates in our models because we visually plotted our raw data before fitting models and saw no evidence of nonlinear relationships in our covariates. We included a random effect in each model to account for variation in resource selection between individual skunks and added a constant of 0.01 to habitat covariates that were often measured as 0 (Bodinof et al. 2012).

In addition to fitting models based on our 6 *a priori* hypotheses, we also fit a global model including all covariates we measured, and 2 subglobal models to evaluate relative support for covariates within immediate proximity to the den sites. One subglobal model included only those covariates collected within 5 m of the used or available den site, and the other included all other covariates (Table 1.1). We used Pearson's correlation coefficients to determine that none of the covariates included in a model were collinear ($r \geq 0.7$ or ≤ -0.7; Bodinof Jachowski et al. 2016).

After visually plotting our raw data we observed some evidence to suggest that female spotted skunks at our study site were selecting den sites differently during the breeding season (June–July; hereafter referred to as 'breeding females') than male skunks overall and female skunks during the nonbreeding season (February–May; hereafter referred to as 'nonbreeding females'). Thus, we separated our den site data into 2 groups: 1) breeding females and 2) all males and nonbreeding females, and tested the same hypotheses on both groups. We defined our skunk breeding season as starting in June because parturition in the eastern spotted skunk is known to occur in late May and early

June, and because this corresponded to approximately 2 weeks prior to our 1st observations of females with blind, thinly furred kits (Mead 1968).

We fit our models using package ‘mlogit’ (Croissant 2015) in the software program R (R Core Team 2017). This package can be used for discrete choice analysis and allows for the consideration of data derived from individuals (Croissant 2015). We next calculated the log-likelihood ($\mathscr{L}$), number of parameters (K), Akaike’s Information Criterion with an adjustment for small sample sizes (AIC_c), rescaled AICc (ΔAICc), and Akaike weight (w_i) for each model (Burnham and Anderson 2002; Table 1.3).

To assess the performance of our resultant top models, we validated each top model using a 10-fold cross validation (Boyce et al. 2002). For each model, we randomly selected 80% (with 1:1 choice sets remaining intact) of our data to act as ‘training data’ for fitting and the remaining 20% of data as ‘test sets’ to test the newly ‘trained’ model (Bodinof et al. 2012). We repeated the random split of our data into training and testing sets 10 times. We subsequently used the trained model with our ‘test sets’ to estimate the relative probability of selection of each used or available point in our choice sets (Bodinof et al. 2012). We pooled across our test sets the number of occasions in which a used site was correctly predicted to determine the probability of our model correctly predicting selection of a used site (Bodinof et al. 2012). If the proportion of used sites correctly predicted from our pooled test sets was greater than 0.5, we determined that our model was a better fit for our data than what would be expected at random.

RESULTS

Den site habitat characterization—We tracked 19 male and 17 female (36 total) FSSKs to 757 used den sites at TLWMA in 2016 and 2017 (Table 1.4). Four additional skunks were fitted with VHF-transmitters, but these skunks were not successfully relocated, slipped their collars soon after being fitted with them, or died before data could be collected on them.

There were a total of 250 den site pairs located for breeding females and 507 den site pairs for males and non-breeding females (Table 1.4). The mean number of used den sites identified per individual skunk was 21 (range = 1–51). We observed FSSKs denning in a variety of den types, including mammal burrows (61.6%), above-ground sites (35.5%), gopher tortoise burrows (1.5%), depressions (1.2%), and hollow logs (< 0.3%; Table 1.4). Burrows were reused on 114 occasions (15.1%), sometimes by different individuals. Communal denning was rare, but we did observe 2 females in an above-ground mound on 1 occasion and a male and female in the same mammal burrow on another.

We could not confirm the origins of mammal burrows or depressions in our study, but based on our observations in the field, most burrows and depressions at our site were likely excavated by nine-banded armadillos (*Dasypus novemcinctus)*, rodents, or FSSKs themselves (Seton 1929). Some mammal burrows were likely abandoned, collapsed gopher tortoise burrows that were repurposed by mammals. Four of the sites that we classified as above-ground were large, bell-shaped mound-type structures that were

primarily comprised of small fragments of *S. repens* leaves. Fifteen other above-ground sites resembled the side-entrance nests of ground-nesting bird and were primarily composed of grasses, but did not contain a lining typical of actual birds' nests. All other above-ground sites (n = 250) had no discernible structures, and skunks using these locations appeared to simply be resting on the ground surface amongst vegetation. The mean visual obstruction index for all used sites in this study was approximately 40 cm (range = 0 – 150 cm; Table 1.2).

Male and nonbreeding female den site selection—For male and nonbreeding female skunks, we observed the most support for our subglobal model including all covariates measured within 5 m of the used or available den site (w_i = 1.0000; Table 1.3). Male and nonbreeding female FSSK den site selection at TLWMA was positively associated with den sites that were mammal burrows (Table 1.5). Relative to gopher tortoise burrows, the odds of a male or nonbreeding female skunk selecting a mammal burrow were approximately 5 times greater. Male and nonbreeding female skunks were approximately 45% more likely to select a site with every 1-burrow increase in the number of burrows within 5 m of the site. Across all den types, the relative probability of a male or nonbreeding female skunk selecting a den site increased as the number of nearby burrows increased (Fig. 1.2). The amount of visual obstruction measured at a den site also had a small positive effect on male and nonbreeding females' relative probability of selecting a den site, as the odds of a skunk selecting a den site increased by about 3% for every 10-cm increase in the visual obstruction index (Table 1.5). All other covariates in this top model had odds ratio 95% confidence intervals that overlapped 1.0, so we

were unable to determine if the effects from these covariates had a positive or negative influence on male and nonbreeding female spotted skunk den site selection.

Breeding female den site selection—For breeding females, we observed strong support for our burrow hypothesis ($Burr_{type + bur + I}$; $w_i = 0.9683$; Table 1.3). Breeding female spotted skunks at TLWMA were approximately 16 times more likely to select mammal burrows as den sites relative to gopher tortoise burrows (Table 1.5). Breeding female skunks were also 13 times more likely to select depressions as den sites over gopher tortoise burrows. Additionally, breeding female skunks' relative probability of selection of a site increased approximately 75% for every 1-burrow increase in the number of burrows within 5 m of the site. Relative probability of selection of a den site by these breeding female skunks increased, for all den types, as the number of nearby burrows increased (Fig. 1.2). Sites characterized as 'woody debris or hollow log' could not be included as a den site type in analysis for breeding females because they never used this den type during the course of our study. All other covariates in our top model had odds ratio 95% confidence intervals overlapping 1.0, indicating that we could not determine if the effects from these covariates had a positive or negative influence on breeding female spotted skunk den site selection (Table 1.5).

Model validation—Our 10-fold cross validation suggested that our top model for male and nonbreeding female skunks accurately predicted den site use by this group approximately 72% of the time. Our top model for breeding females performed slightly better, predicting den site use on approximately 74% of occasions.

DISCUSSION

Den type and burrow presence (i.e., number of burrows within 5 m of a site) were the most important factors affecting den site selection of male and female FSSKs at our site. However, FSSKs did not select for gopher tortoise burrows over the other den types observed at the study site, as we had hypothesized. These results suggest that den type and prevalence of nearby burrows may be more important in spotted skunk den site selection at our site than either covariates collected within 5 m of a used den site (i.e., microhabitat characteristics) or those covariates collected greater than 5 m from a used den site (i.e., coarser-scale habitat characteristics). Mammal burrows were the most selected den type at our site, echoing findings by Lesmeister et al. (2008) and Sprayberry and Edelman (2018), who observed that eastern spotted skunks in the Ouachita Mountains of Arkansas and the Appalachian Mountains of Alabama, respectively, selected mammal-derived burrows most often. The mammal burrows at our site meet Crabb's (1948) 3 den site requirements, and these burrows intrinsically provide much more protection than above-ground den sites, which were the 2nd most commonly used den type at our site.

Our study suggests that den site selection differs to some degree between breeding female spotted skunks and male and nonbreeding female spotted skunks at our site. While burrow type and nearby burrow prevalence were important for both groups of skunks, our top model for female skunks during the breeding season only included these covariates. This outcome suggests that den site selection by these female skunks during parturition

and care of young may be solely driven by finding a den site type suitable for them to raise young in. While female spotted skunks at our site most often used mammal-excavated burrows, they also appeared to select for depressions. The greater odds of selection for mammal burrows and depressions by breeding females than for tortoise burrows available on the landscape may potentially be explained in a few ways. For one, tortoise burrows may be more likely to be inhabited or explored by other animals, including potential spotted skunk predators, than den sites with smaller entrances (Jackson and Milstrey 1989, Lips 1991, Lesmeister et al. 2008). Second, breeding females may simply prefer those dens they excavate themselves, though the origins of each mammal burrow or depression in our study could not be confirmed. As mammal burrows are clearly important den site locations for female spotted skunks at our study site, removing these burrows in a specific area could be a potential management strategy for reducing predation, as has been similarly suggested for other mammal species that predate ground-nesting birds like the FGSP (Herkert 1994, Henner et al. 2004).

The appearance of vegetative cover covariates in our top model for male and nonbreeding female den site selection, along with the slight positive effect of the visual obstruction index on this group, lends some support to our cover hypothesis. Increased vegetative cover was also found to be a driver of eastern spotted skunk den site selection in other parts of its range (Lesmeister et al. 2008, Sprayberry and Edelman 2018). However, the positive effect at our site was very small, and all other habitat or vegetative covariates we included in our study had undiscernible impacts on spotted skunk den site selection. This suggests that spotted skunks at our site may be habitat generalists,

different in their ecology from eastern spotted skunks in other regions where aspects of habitat appear to be more important to den site selection. Specifically, we suggest that 2 factors, the wide dietary breadth of eastern spotted skunks, and the lack of competitors and predation risk at our study site, are influencing this generalist behavior. First, the omnivory of the species may allow them to utilize different parts of their home range without needing to return to areas containing specific food resources, as eastern spotted skunks have been documented consuming a variety of food items including mammals, birds, reptiles, amphibians, arthropods, fungi, and plant material (Crabb 1941, Kinlaw 1995, Sprayberry and Edelman 2016, Thorne and Waggy 2017), all of which are present and would be readily accessible to a spotted skunk at our study site. Second, spotted skunks in the Route 60 unit likely do not have to cope with the food competitors or predators that they must contend with in other regions, which may provide some explanation as to why our predator avoidance hypothesis was unsupported. In particular, Kinlaw (1995) lists striped skunks (*Mephitis mephitis)* and weasel species (Mustelidae) as sympatric food competitors, but we did not observe any of these species during our 2 years of field work at the study site. Spotted skunks may also be less wary, and thus they may be more willing to use areas with less cover because of the lack of predators in the Route 60 unit. Great horned owls are known to be major predators of the eastern spotted skunk elsewhere, but this species was never heard or seen at our site (Lesmeister et al. 2010). Barred owls (*Strix varia*) are common in the forests surrounding the Route 60 unit but are rarely seen in the prairie habitats themselves. In fact, of 7 known mortalities of spotted skunks in the Route 60 unit in 2016 and 2017, only 2 were likely due to

predation, and neither were likely killed by an avian predator (SNH, pers. obs.). Because the dry prairie ecosystem at our study site does not seem to have many of these mammalian competitors or avian predators, we believe that future research on the FSSK should be conducted at sites where striped skunks and owls occur in adequate numbers to assess how predators and competitors may influence spotted skunk den site selection.

Further supporting our hypothesis that FSSKs are habitat generalists within dry prairie habitat, we failed to find support for our water avoidance, recent burn avoidance, and road avoidance hypotheses. Though it happened on too few occasions (n = 19) to likely influence support for our water avoidance hypothesis, the use, and possible construction, of above-ground structures by spotted skunks at our site may have been a response to occasional flooding at our study site. Dry prairie habitats can flood seasonally (FNAI 2010), which did occur at our study site during the 2 years of our study, but it was difficult to determine how long portions of our study site and the burrows in these areas were affected by high water levels. Also, the small prescribed fire management subunits at our study site (generally 400 m wide) may have dampened any effects of different fire return intervals we may have observed, as skunks in many cases may not have had to move far to escape a subunit being burned and to take refuge in an adjacent unburned unit. Additionally, while the roads at TLWMA were open to public travel, and multiple roads run through the Route 60 unit, the remote location of our site meant that vehicle travel was not high.

Overall, the tracking methodology we used in our study (locating skunks every 1 to 3 days) prevented us from studying how spotted skunks handle recent prescribed fires

and floods in the hours and days immediately following these events (e.g., the average time for a skunk to recolonize an affected subunit after a fire). Further research on the fine-scale movements of this species may be revealing. More generally, given the unique attributes of our study area, we suggest that future research on FSSKs is needed in other portions of Florida to more broadly assess the effects of fire, water, and roads on spotted skunk distribution and space use. It is possible that some of the covariates we measured for these hypotheses were too coarse-scale to have much impact on the spotted skunks we were studying at a single site, and that these covariates could reveal other patterns in spotted skunk den site selection if studied at a larger spatial scale (Wiens 1989).

The few habitat associations we did find regarding FSSK den site selection may inform biologists tasked with conserving the FGSP and managing its habitat. Although making management decisions based on the importance of burrows to skunks would be exceedingly difficult, our study's conclusion that vegetative cover (e.g., visual obstruction) is at least marginally important to male and nonbreeding female skunk den site selection suggests that reduction of vegetation heights in a particular section of the Route 60 unit, perhaps below the 40-cm average observed for used den sites with our visual obstruction index, may reduce the odds that a spotted skunk would select a den site in that section. Though the amount of time since a prescribed fire treatment was not itself an important covariate in our study, prescribed fire as a management technique could still be utilized as a means to reduce vegetative cover in priority sparrow breeding habitat. Reduction of vegetative cover by mechanical means (e.g., roller-chopping) is another potential management technique that could be strategically used to decrease vegetative

cover in dry prairie habitat (FNAI 2010). However, the presence and prevalence of vegetative cover may be important to FGSP resource selection at the site as well, so any decisions to reduce cover at a site inhabited by the FGSP would have to be carefully weighed (Delany et al. 1998). Ultimately, while our study suggests that FSSKs are opportunistic in their selection of den sites in the dry prairie ecosystem, this does not mean that habitat characteristics are not important to other aspects of the skunks' ecology. In particular, some habitat characteristics may be important to the feeding ecology of this species (an aspect critical to limiting nest predation on FGSP), so more research on this species fine-scale foraging movements and patterns is necessary to elucidate any positive or negative associations with habitat attributes in the dry prairie ecosystem.

Apart from providing novel information on this subspecies' ecology in peninsular Florida, our study also shows some stark differences in the ecology of the eastern spotted skunk in Florida versus other parts of its range. For one, previous studies on eastern spotted skunk den site selection have shown that cover is an important factor in the selection of skunks' den sites, which our study did not find support for in the dry prairie ecosystem (Lesmeister et al. 2008, Sprayberry and Edelman 2018). Similarly, these studies found spotted skunk den sites in forested environments, which differs from the results of our study, in which most dens were found in dry prairie (i.e., grassland) habitat (Lesmeister et al. 2008, Sprayberry and Edelman 2018). It is possible that FSSKs at our study site do not need to select for den sites in areas with an abundance of cover because, as previously discussed, they are not subject to the predation risks eastern spotted skunks

in forested ecosystems face.

While our study focused on FSSKs in a dry prairie ecosystem of south-central Florida, there is still little known about this subspecies across its range. Research in northern Florida, where the Florida subspecies' range meets the larger Appalachian subspecies' range, would be useful in determining if the denning ecology we observed in our study is particular to the Florida subspecies, or if it occurs in multiple subspecies and is more dependent on external environmental factors affecting skunks at a given location. Reports of FSSKs in the backyards of suburban homes have been confirmed (D. S. Jachowski, pers. comm.), and it would be informative to know if the Florida subspecies is more tolerant of human disturbance than other subspecies. The abundance of spotted skunks at our site (> 100 unique individuals caught over a 2-y period), coupled with a report from the 1990s of high densities in coastal Florida (40 skunks per km^2; Kinlaw et al. 1995b) and documentation of spotted skunks inhabiting several other native prairie and agricultural (e.g., cow pasture) sites in south-central Florida (E. L. Hewett Ragheb, FWC, pers. comm.), foster questions about whether spotted skunks in peninsular Florida could have been insulated from, are in the midst of recovering, or have recovered from the range-wide decline documented for the species in the early to mid-1900s (Gompper and Hackett 2005). Regardless, the rarity or cryptic nature often attached to eastern spotted skunks in other parts of their range may not apply to FSSKs in peninsular Florida. More research on this subspecies is necessary, both to determine how it may have been differentially affected by stressors that contributed to the decline seen in the other subspecies, as well as to establish how in a broader sense this knowledge may be used to

aid conservation and management of the eastern spotted skunk in other portions of its range.

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TABLES AND FIGURES

Table 1.1. *A priori* models developed for hypotheses of Florida spotted skunk (*Spilogale putorius ambarvalis*) den site selection at Three Lakes Wildlife Management Area, Osceola County, Florida in 2016 and 2017. *I* represents a random effect for individual skunks.

Hypothesis	Model
Cover (Cov)	
1. Positive effect of visual obstruction index, leaves %, forb %, grass %, oak %, woody shrub %, litter %; negative effect of trunk %, bare ground %	$Cov_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + I}$
2. Positive effect of leaves %, grass %, forb %, oak %, woody shrub %	$Cov_{leav + grass + forb + oak + shrub + I}$
3. Positive effect of visual obstruction index	$Cov_{vobs + I}$
4. Positive effect of litter %	$Cov_{litt + I}$
5. Negative effect of trunk %	$Cov_{trunk + I}$
6. Negative effect of bare ground %	$Cov_{bare + I}$
Burrow (Burr)	
7. Positive effect of den type (gopher tortoise burrow)	$Burr_{type + I}$
8. Positive effect of number of burrows	$Burr_{bur + I}$
9. Positive effect of den type (gopher tortoise burrow), positive effect of number of burrows	$Burr_{type + bur + I}$
Predator avoidance (Pred)	
10. Positive effect of community type (dry prairie), distance to forest edge, distance to nearest shrub clump/tree; negative effect of number of trees, number of shrub clumps	$Pred_{comm + for\_dist + clump\_dist + tree + clump + I}$
11. Positive effect of community type (dry prairie)	$Pred_{comm + I}$
12. Positive effect of community type (prairie), distance to nearest forest edge	$Pred_{comm + for\_dist + I}$
13. Positive effect of distance to forest edge, positive effect of distance to nearest shrub clump/tree	$Pred_{for\_dist + clump\_dist + I}$
14. Positive effect of distance to nearest shrub clump/tree; negative effect of number of trees,	$Pred_{clump\_dist + tree + clump + I}$

number of woody shrub clumps	
Water avoidance (Water)	
15. Negative effect of water %, positive effect of distance to water feature; positive effect of elevation	$Water_{wat + wat\_dist + elev + I}$
16. Negative effect of water %; positive effect of distance to water feature	$Water_{wat + wat\_dist + I}$
17. Negative effect of water %; positive effect of elevation	$Water_{wat + elev + I}$
Recent burn avoidance (Burn)	
18. Positive effect of time since burn	$Burn_{time + I}$
Road avoidance (Road)	
19. Positive effect of distance to primary road; negative effect of distance to secondary road, trail or firebreak	$Road_{road\_dist + trail\_dist + I}$
20. *Global model (Glb)*	$Glb_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + comm + for\_dist + tree + clump + clump\_dist + wat + wat\_dist + elev + time + road\_dist + trail\_dist + I}$
Subglobal models (Sub)	
21. Covariates collected within 5 m of the den site	$Sub_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + wat + I}$
22. Covariates generally collected farther than 5 m from the den site	$Sub_{comm + for\_dist + tree + clump + clump\_dist + wat\_dist + elev + time + road\_dist + trail\_dist + I}$

Table 1.2. Habitat covariates measured for each identified Florida spotted skunk (*Spilogale putorius ambarvalis*) den site and random available site during 2016 and 2017 at Three Lakes Wildlife Management Area, Osceola County, Florida. Covariates with an asterisk (*) often had values of 0 for a used or available site and thus have had a constant of 0.01 added to each value.

Variable	Description	Range/categories
time	Time since the most recent prescribed fire (in days)	0 – 6321
clump*	Number of woody shrub clumps ≥ 1.5 m tall within 50 m	0 – 70
tree*	Number of trees ≥ 3m tall within 100 m	0 – 225
clump_dist*	Distance to the nearest woody shrub clump or tree ≤ 1.5 m tall (in m)	0 – 381
type	Classification of the den site	Above-ground, depression, mammal burrow, gopher tortoise burrow, woody debris/log
bur*	Number of burrows within 5 m of the den/random site	0 – 10
for_dist*	Distance to nearest forest edge (in m)	0 – 1131
road_dist*	Distance to nearest primary road (in m)	6 – 1240
trail_dist*	Distance to nearest secondary road, trail, or firebreak (in m)	0 – 325
wat_dist*	Distance to nearest water feature (in m)	0 – 489
elev	Elevation at the site (in m)	16 – 19
comm	Natural community type reclassified from Florida Cooperative Land Cover Map	Dry prairie, forest, scrub, wet prairie, wetland
The following covariates were measured at each den site, a point 5 m east, and a point 5 m west, and then averaged		Range
vobs*	Visual obstruction index using a Robel pole (in 10-cm increments)	0 – 150

bare*	Percent of bare soil in plot	0 – 88
litt*	Percent of leaf litter in plot	0 – 97
trunk*	Percent of palmetto trunk in plot	0 – 62
leav*	Percent of palmetto leaves in plot	0 – 82
grass*	Percent of graminoids in plot	0 – 100
forb*	Percent of non-woody, herbaceous plants in plot	0 – 82
oak*	Percent of oak species in plot	0 – 72
shrub*	Percent of non-oak woody shrub plants in plot	0 – 100
wat*	Percent of standing, reflective water in plot	0 – 33

Table 1.3. Output of top 5 discrete choice models for hypotheses of den site selection for male and nonbreeding female, and breeding female, Florida spotted skunks (*Spilogale putorius ambarvalis*) at Three Lakes Wildlife Management Area, Osceola County, Florida in 2016 and 2017.

Model	log($\mathscr{L}$)	K	$AICc$	$\Delta\ AICc$	w_i
Male and nonbreeding females					
$Sub_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + wat + I}$	-233.82	17	502.46	0.00	1.0000
$Glb_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + comm + for\_dist + tree + clump + clump\_dist + wat + wat\_dist + elev + time + road\_dist + trail\_dist + I}$	-229.73	31	524.19	21.72	< 0.0001
$Cov_{vobs + bare + litt + trunk + leav + grass + forb + oak + shrub + I}$	-283.12	10	586.53	84.07	< 0.0001
$Cov_{vobs + I}$	-299.24	2	602.50	100.03	< 0.0001
$Cov_{leav + grass + forb + oak + shrub + I}$	-297.93	6	607.97	105.51	< 0.0001
Breeding females					
$Burr_{type + bur + I}$	-116.72	6	245.55	0.00	0.9683
$Sub_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + wat + I}$	-109.83	16	252.39	6.84	0.0317
$Glb_{vobs + leav + forb + grass + oak + shrub + trunk + bare + litt + type + bur + comm + for\_dist + tree + clump + clump\_dist + wat + wat\_dist + elev + time + road\_dist + trail\_dist + I}$	-104.47	30	271.49	25.94	< 0.0001
$Burr_{type + I}$	-136.54	5	283.16	37.61	< 0.0001
$Burr_{bur + I}$	-141.52	2	287.06	41.50	< 0.0001

Table 1.4. Counts of used and available den types examined for discrete choice analysis of male and nonbreeding female, and breeding female, Florida spotted skunk (*Spilogale putorius ambarvalis*) den site selection at Three Lakes Wildlife Management Area, Osceola County, Florida in 2016 and 2017.

	Used			Available		
Den type	Male and non-breeding female	Breeding female	Total	Male and non-breeding female	Breeding female	Total
Above-ground	230	39	269	276	89	365
Depression	3	6	9	16	7	23
Mammal burrow	263	203	466	177	128	305
Gopher tortoise burrow	9	2	11	36	26	62
Woody debris/log	2	0	2	2	0	2
Total	507	250	757	507	250	757

Table 1.5. Parameter estimates for each covariate in the top models for den site selection of male and nonbreeding female, and breeding female, Florida spotted skunks (*Spilogale putorius ambarvalis*) at Three Lakes Wildlife Management Area, Osceola County, Florida in 2016 and 2017. *SE* = standard error. *OR* = odds ratio. *CI* = 95% confidence interval. Selection of den types is relative to the reference category, gopher tortoise (*Gopherus polyphemus*) burrows. * denotes a covariate with a *CI* that does not overlap 1.0.

Parameter	Estimate	*SE*	*OR*	OR Lower *CI*	OR Upper *CI*
Males and nonbreeding females					
typeABOVE GROUND	-0.357	0.523	0.700	0.251	1.952
typeDEPRESSION	-0.776	0.884	0.460	0.081	2.599
typeMAMMAL BURROW	1.656*	0.443	5.241	2.197	12.499
typeWOODY DEBRIS/LOG	0.565	1.229	1.760	0.158	19.585
bur	0.372*	0.096	1.450	1.201	1.751
vobs	0.032*	0.006	1.032	1.020	1.045
bare	0.006	0.010	1.006	0.987	1.025
litt	0.008	0.007	1.008	0.994	1.022
trunk	0.013	0.015	1.013	0.985	1.042
leav	0.014	0.007	1.014	1.000	1.029
grass	0.007	0.008	1.007	0.992	1.023
forb	-0.003	0.010	0.997	0.976	1.017
oak	< 0.001	0.010	1.000	0.980	1.021
shrub	0.004	0.010	1.004	0.984	1.025
wat	-0.036	0.022	0.965	0.924	1.007
Breeding females					
typeABOVE GROUND	0.858	0.864	2.359	0.433	12.839
typeDEPRESSION	2.572*	1.022	13.098	1.766	97.166
typeMAMMAL BURROW	2.771*	0.764	15.975	3.575	71.385
bur	0.561*	0.122	1.753	1.381	2.225

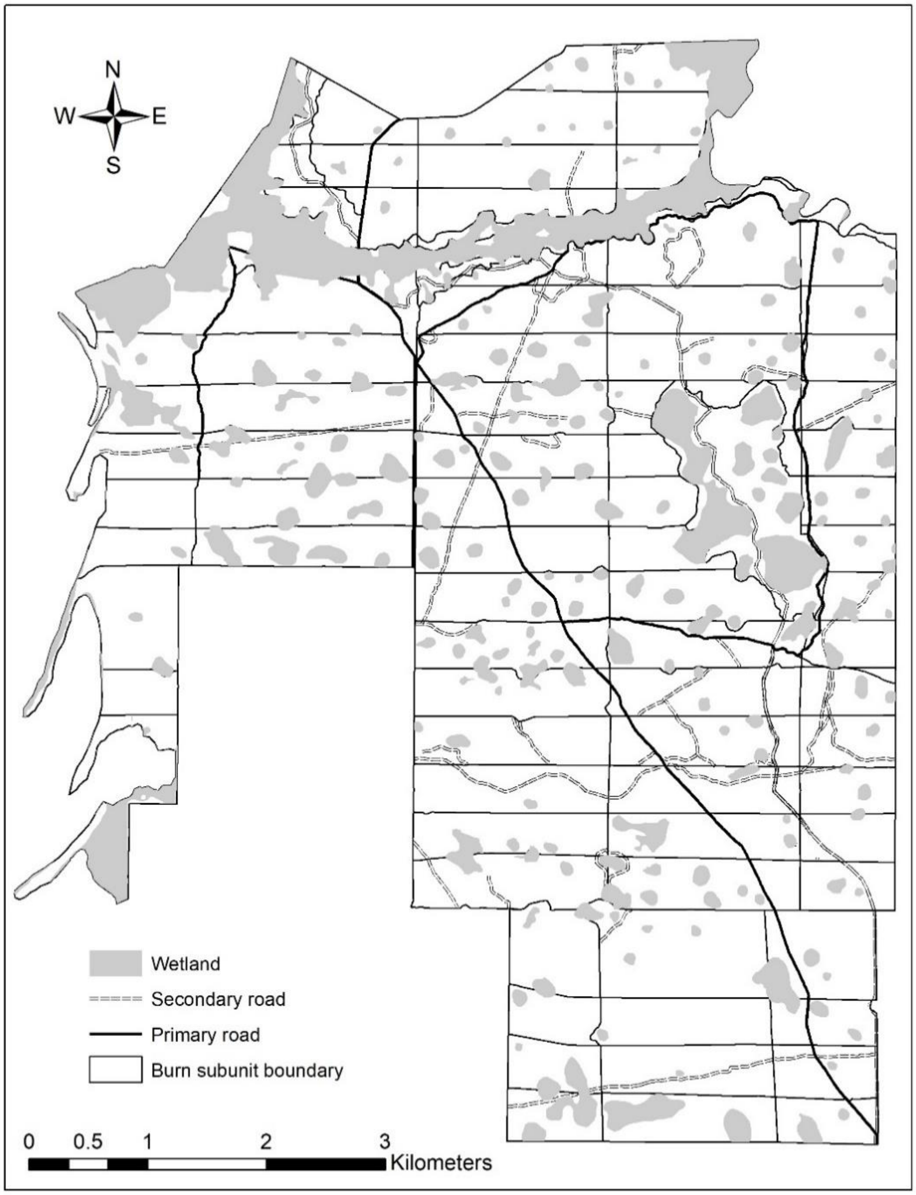

Figure 1.1. The Route 60 unit of Three Lakes Wildlife Management Area, Osceola County, Florida where Florida spotted skunk (*Spilogale putorius ambarvalis*) trapping and radio-tracking occurred between the months of February and July in 2016 and 2017.

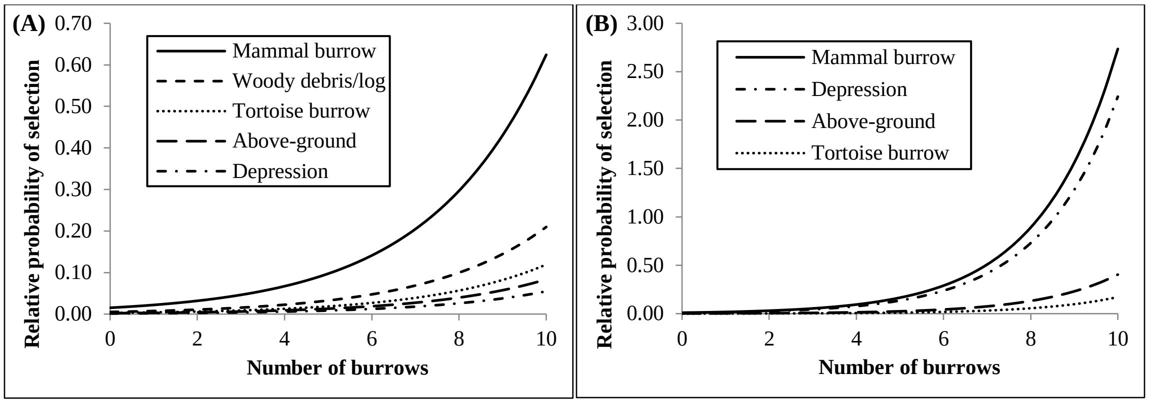

Figure 1.2. Relative probability of (A) male and nonbreeding female and (B) breeding female Florida spotted skunks (*Spilogale putorius ambarvalis*) at Three Lakes Wildlife Management Area, Osceola County, Florida (2016 – 2017) selecting a den site as the number of burrows of any type (within 5 m of the den site) increases. 'Woody debris/log' is not included as a den site type in (B) because breeding females never used this den type in our study.

Chapter 2: Dietary composition of the Florida spotted skunk (*Spilogale putorius ambarvalis*) in a dry prairie ecosystem using stable isotope analysis

INTRODUCTION

The eastern spotted skunk (*Spilogale putorius;* Mephitidae) is a small-bodied skunk native to eastern North America that is known to have undergone a "biologically real" decline since the early to mid-1900s across much of its distribution (Gompper and Hackett 2005; p. 199). Historically, it is known to have ranged east from the Continental Divide through much of the central and southeastern United States, southeastern Manitoba, southwestern Ontario and northeastern Mexico (Kinlaw 1995). The exact causes that led to this species' decline are currently unknown, but are thought to be linked to habitat loss, effects of agricultural industrialization (e.g., reduction in haystacks available for denning, reduced food availability), widespread use of pesticides, overharvest, or disease (Choate et al. 1974, McCullough 1983, Schwartz and Schwartz 2001, Gompper and Hackett 2005). Currently, the eastern spotted skunk is a species of conservation concern in several states, and it is listed as vulnerable by the International Union for Conservation of Nature (Gompper and Jachowski 2016). Additionally, the plains subspecies (*S. p. interrupta*) is currently under review for listing under the Endangered Species Act by the U.S. Fish and Wildlife Service (Federal Register 2012).

The Florida spotted skunk (*S. p. ambarvalis*; FSSK) is the smallest and least known of the eastern spotted skunk subspecies and is endemic to peninsular Florida

(Kinlaw 1995, Gompper and Jachowski 2016). Only 2 dedicated studies have been conducted on the subspecies previously, with both occurring on a barrier island along the Atlantic Coast of Florida (Fig. 2.1; Kinlaw et al. 1995*a*, Kinlaw et al. 1995*b*). The Florida subspecies was still thought to be relatively abundant in central and southern Florida in the 1990s, with a density of 40 skunks/km^2 reported on a barrier island in central Florida, much higher than the only previous published density estimate for eastern spotted skunks, 5 skunks/km^2 in Iowa in the mid-1900s (Crabb 1948, Kaplan and Mead 1991, Kinlaw et al. 1995b). Recent mammal live-trapping in the central part of the state suggests that FSSK may still be in high abundances or densities at some sites (S. L. Glass and C. L. Hannon, Florida Fish and Wildlife Conservation Commission [FWC], unpubl. data). It is unknown if FSSKs may have been insulated from the decline experienced by the other eastern spotted skunk subspecies or if they may have recovered more quickly from it, and any mechanisms behind this potential recovery are also unknown.

The eastern spotted skunk is an omnivorous species that can have a varied diet (Crabb 1941). Insects are believed to be an important food source for the species, with beetles (Coleoptera) and grasshoppers (Orthoptera) being major components of their diet (Howell 1906, Crabb 1941, Kinlaw 1995). Crabb (1941) showed that while insects were an important food source in summer and fall, importance of small mammals increased in the winter and spring, when insects were less available (Crabb 1941). In addition to these food items, eastern spotted skunks are likely opportunistic in their foraging and have been documented consuming birds (including eggs), lizards, snakes, Anurans (frogs or toads), salamanders, crayfish, fungi, carrion, and plant material (Howell 1906, Pellett 1913,

Selko 1937, Crabb 1941, Crabb 1948, McCullough and Fritzell 1984, Sprayberry and Edelman 2016, Thorne and Waggy 2017). However, the diet of the FSSK has not been explicitly studied. All previous literature on the eastern spotted skunk diet is focused on the Appalachian (*S. p. putorius*) or plains subspecies. The only mention of the FSSK diet is in Manaro (1961), where it is noted that FSSKs consumed live snakes, frogs, fruit, and milk-soaked bread when presented with them in captivity.

A greater understanding of the FSSK diet would provide insight into how the ecology of this subspecies may compare to the other eastern spotted skunk subspecies that have declined. While a reduction in food availability has been suggested as a possible factor in the observed decline of the eastern spotted skunk (Gompper and Hackett 2005) and other skunk species (*Conepatus leuconotus*; Dragoo and Sheffield 2009), the Florida subspecies may have been insulated from this decline.

Gaining more knowledge about the diet of the FSSK is also a conservation priority because it has been confirmed as a nest predator of imperiled grassland, ground-nesting birds in a recent nest-camera study (E. L. Hewett Ragheb, FWC, unpubl. data). Specifically, this includes the Florida grasshopper sparrow (*Ammodramus savannarum floridanus*; FGSP), an endemic of the dry prairie ecosystem of south-central Florida that is critically endangered and federally listed (Federal Register 1986, Pranty and Tucker 2006). Perkins et al. (2003) documented high rates of nest failure in the FGSP, with nest predation as the major cause of these failures. In fact, less than 100 male FGSPs were documented across all known populations in 2015, and FSSKs were the most common mammalian nest predator of grassland bird nests at 1 site, responsible for 29% of all nest

predation events (E. L. Hewett Ragheb, unpubl. data).

The objective of this study was to gather baseline information on the diet of a population of FSSKs in the dry prairie ecosystem of south-central Florida, at a site where the skunk co-occurs with the FGSP and other ground-nesting birds. We used stable isotope analysis to determine the main food items that comprised the skunk diet at our site and the relative proportions of these food items, with a special emphasis on determining the importance of ground-nesting birds in this diet. The results of this study will be useful in evaluating whether FSSKs are a consistent nest predator of grassland birds in Florida. This study also represents the first on FSSK diet and may help land managers at known FGSP sites determine if land management or predator control practices intended to reduce skunk abundances or densities will benefit the sparrow.

MATERIALS AND METHODS

Study area.—Our study occurred in the ‘Route 60’ unit of the Three Lakes Wildlife Management Area (TLWMA), located in Osceola County, Florida, approximately 76 km south-southeast of Orlando, Florida (Fig. 2.1). Our study focused on the approximately 3000 ha of dry prairie habitat present in the unit, where FSSKs have been documented in recent years (S. L. Glass and C. L. Hannon, FWC, pers. comm.; Fletcher et al. 2010). Florida spotted skunks have been readily trapped in this unit, and they have been the most abundant mesopredator captured during an ongoing mark-recapture study in the unit that began in 2016 (S. L. Glass and C. L. Hannon, unpubl.

data).

The dry prairie is a natural community that contains an abundance of low shrubs and grasses and is maintained by frequent fire and seasonal flooding that keep vegetation heights low and prevent encroachment and establishment of tree species (Platt et al. 2006, Florida Natural Areas Inventory 2010; Fig. 2.2). This natural community is restricted to south-central Florida and Noss et al. (1995) determined that more than 98% of historical dry prairie in Florida has disappeared (U.S. Fish and Wildlife Service 1999). However, this is still the dominant natural community in the Route 60 unit. The dry prairie frequently grades into another natural community on the site, wet prairie, which, while also flat and treeless with an abundance of grasses, contains more herbaceous vegetation, mostly lacks for shrubs, and is often wet or inundated (Florida Natural Areas Inventory 2010). Small, ephemeral depression wetlands are common throughout these prairie habitats (Fig. 2.3). The prairie is primarily treeless, only sparsely occupied by cabbage palms (*Sabal palmetto*) and hardwood tree species. Some common plant species on the prairie include wiregrass (*Aristida stricta* var. *beyrichiana*), shiny blueberry (*Vaccinium myrsinites*), gallberry (*Ilex glabra*), saw palmetto (*Serenoa repens*) and dwarf live oak (*Quercus minima*; Florida Natural Areas Inventory 2010).

Three Lakes Wildlife Management Area is 1 of only 3 public properties where the FGSP continues to persist. Besides the FGSP, several species of ground-nesting grassland bird regularly breed at TLWMA. These include the northern bobwhite (*Colinus virginianus*), common ground-dove (*Columbina passerine*), mourning dove (*Zenaida macroura*), common nighthawk (*Chordeiles minor*), eastern towhee (*Piplio*

erythrophthalmus), Bachman's sparrow (*Peucaea aestivalis*), and eastern meadowlark (*Sturnella magna*).

Animal capture. —We trapped FSSKs as part of concurrent studies in which skunks were captured and fitted with very high frequency (VHF) radio-transmitters and Global Positioning System (GPS)-enabled collars. We captured the skunks in Havahart double-door live traps (Model #:1030-B; Havahart, Lititz, PA) baited with commercially-available wet cat food. We placed traps primarily in dry prairie habitat where FGSPs occurred or were known to occur in recent years, but we also trapped in different habitat types, along habitat ecotones (e.g., dry prairie–forest edge), and in management subunits with differing times since prescribed fire application. We set each trap in the afternoon and checked each trap the following morning. Each skunk we captured was marked with ear tags (Model #1005-1L1; National Band & Tag Company, Newport, KY), sexed, and aged (i.e., adult or juvenile) based on body size and teeth wear (Lesmeister 2007). We followed American Society of Mammalogists guidelines and complied with Clemson University Animal Care and Use Committee protocol (permit # AUP2015-042) for all skunk trapping and processing (Sikes et al. 2011).

Hair sample collection.—Hair samples provide a relatively noninvasive way to obtain stable isotope ratios for dietary analysis from living mammals (Schoeninger et al. 1998). Hair typically mirrors diet across a longer period of time than other sample types such as muscle or feces, reflecting food items consumed since an animal's last molt (Tieszen et al.1983, Sponheimer et al. 2003, Dalerum and Angerbjörn 2005, Ben-David and Flaherty 2012). Using pliers, we collected a hair sample from a portion of the flank

(that was not visibly soiled) of each adult spotted skunk. We did not collect hair samples from individuals during each subsequent capture of that individual. We then placed each sample into a dry coin envelope, sealed it, and placed it in a freezer for future analysis.

Food item sample collection.—We opportunistically collected samples of potential spotted skunk food items in the study area, with collection informed by previous research on the spotted skunk diet. We collected fungi and fruiting bodies of plants with clean, zipped plastic bags. We collected arthropod samples using dip-nets, insect nets, and pitfall traps. We collected grassland bird eggs from dry prairie habitat that had either been carried from nests by predators or ejected from nests by adult birds. We only collected eggs that had clearly been abandoned and never collected eggs that were in the vicinity of a nest. Eggs were never collected from FGSP nests, with the other insectivorous grassland bird samples serving as surrogates for this species in the stable isotope analysis. In most other cases, we collected samples from fresh roadkill discovered on the roads of the Route 60 unit during almost daily drives through the unit. In the case of some large prey items, such as snakes and rabbits, we simply collected smaller tissue samples from the prey items. We placed all food item samples into zipped plastic bags and froze them for future analysis.

Analysis preparation.—We processed all hair and food item samples at the Wildlife Physiology Lab in the Department of Forestry and Natural Resources at Purdue University. We initially cleaned hair samples with a 2:1 chloroform:methanol solution and vortexed samples to remove any surface contaminants. We then dried all hair and food item samples for 48 hours in a drying oven at a temperature of 60°C (Ben-David

and Flaherty 2012). Afterwards, we cut the hair samples into small pieces with scissors and ground all food item samples into a powder using a mixer mill (Retsch MM 200; Glen Mills Inc., Clinton, New Jersey). We used a Sartorius microbalance (model CPA2P; Arvada, Colorado) to weigh smaller subsamples of each of these hair and food item samples into a 3 x 5 mm tin weighing capsule (Costech Analytical Tech Inc., Valencia, California). When possible, we prepared a duplicate subsample to pair with the original subsample. We sent the subsamples and their duplicates to the University of Wyoming's Stable Isotope Facility for stable isotope analysis of each hair and food item sample.

Stable isotope analysis.— In a stable isotope analysis, the ratios of naturally occurring, but rare, stable isotopes, such as Carbon-13 (^{13}C), Nitrogen-15 (^{15}N), and deuterium (^{2}H), can be compared between a consumer of interest and potential dietary sources (i.e., food items). A food item that is eaten by the consumer would be partially assimilated into the tissues of the organism, along with its unique ratio of stable isotopes. Subsequent analysis of tissue samples from the consumer and the potential food items can discern what food items are and are not present in the consumer's diet based on the overlap in these various isotopic signatures. Stable isotope analysis has multiple advantages over other types of dietary sample analyses, like those involving stomach contents and feces, including the ability to obtain samples from living animals in a less invasive manner (e.g., hair samples), the lack of need to obtain fecal samples from uncooperative animals that are tracked or trapped, the absence of limitations on the inferences made from fecal samples due to differences in food item digestibility, and the ability to study diets over differing or multiple periods of time based on what type of

tissue samples are used in the stable isotope analysis (Tieszen et al. 1983, Schoeninger et al. 1998, McFadden et al. 2006).

At the University of Wyoming's Stable Isotope Facility, $\delta^{13}C$ and $\delta^{15}N$ values for each subsample and duplicate we submitted for analysis were determined using a Carlo Erba 1110 Elemental Analyzer (Carlo Erba Reagents S.A.S., Val-de-Reuil, France) coupled to a Thermo Delta Plus XP IRMS mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). The standard used in the analysis for $\delta^{13}C$ was Vienna PeeDee Belemnite, and that used for $\delta^{15}N$ was atmospheric nitrogen (air). After the stable isotope analysis, we used samples in our subsequent mixing model analysis if the variance between duplicate subsamples did not exceed the variance of the standards used in the stable isotope analysis for $\delta^{13}C$ and $\delta^{15}N$, 0.10 and 0.15, respectively (Ben-David and Flaherty 2012). We included additional samples in our analyses if both the subsample and the duplicate subsample fell within the variance range of the other food items of the same type. We conducted a multivariate analysis of variance (MANOVA) to determine if male and female skunk hair samples differed significantly in their $\delta^{13}C$ and $\delta^{15}N$ values. We then used a MANOVA with post hoc Tukey's multiple comparison test and a k-nearest neighbors randomization test, to determine appropriate groupings for food items (Rosing et al. 1998). We grouped items together that did not differ significantly in both $\delta^{13}C$ and $\delta^{15}N$ values.

We then incorporated these food groups into the mixing model 'MixSIAR' (Stock and Semmens 2016) in software program R (R Core Team 2017) and ran a Bayesian-based, dual-isotope mixing model to calculate the proportion of each food item group in

the diet of our sample of FSSKs. We predicted that arthropods would comprise the majority of the skunk diet in our study, owing to the skunks' ability to dig, the abundance of depression ponds at the study site that harbor crayfish, and the known importance of arthropods as a food source for eastern spotted skunks in other parts of their range (Seton 1929, Crabb 1941, Crabb 1948). We used an uninformative prior in our mixing model, allowing all source contributions to be equally likely *a priori* (Moore and Semmens 2008). Because different organisms assimilate tissues differently (Ben-David and Flaherty 2012), we used a diet-tissue isotopic discrimination factor of 1.6‰ for $\delta^{13}C$ and 3.8‰ for $\delta^{15}N$ for our skunk hair samples, based on Hobson and Quirk's (2014) study on discrimination factors of the striped skunks (*Mephitis mephitis*), a related species in the same family as the eastern spotted skunk. Discrimination factors can be estimated through controlled studies on a consumer's diet-related physiology. They are used to offset isotopic signatures of food items so that there is correct overlap with the consumer's isotopic signatures in an isotopic mixing space to allow for accurate estimation of dietary proportions (Martínez del Rio et al. 2009, Hopkins and Ferguson 2012).

RESULTS

Hair and food item sample collection.—We captured and collected hair samples from 101 unique (63 females, 36 males) adult FSSKs between March 2016 and June 2017 in the Route 60 unit of TLWMA. We selected a random subsample of 20 male and

20 female hair samples from this total for the stable isotope analysis. One female hair sample was subsequently found to be too small to make an adequate subsample for analysis, leaving a total of 39 hair samples (20 males, 19 females) used in the stable isotope analysis (Table 2.1). We collected a total of 135 samples of potential food items for inclusion in our stable isotope analysis (Table 2.2).

Stable isotope analysis.— Hair samples of male and female FSSKs were grouped together for the dual-isotope mixing model as a MANOVA showed that these 2 groups did not differ significantly in their isotopic signatures ($p = 0.59$, $\alpha = 0.05$). The mean $\delta^{13}C$ and $\delta^{15}N$ (± SD) values for all hair samples were -21.81 (± 1.39) and 5.85 (± 0.62), respectively. Potential food items were similarly placed into 6 groups based on the results of a MANOVA with a post hoc Tukey's multiple comparison ($p < 0.001$, $\alpha = 0.05$) and k-nearest neighbor randomization test ($p < 0.01$, $\alpha = 0.05$; Table 2.3, Fig. 2.4).

The results of the dual-isotope mixing model show that millipedes contributed the most to diet in 2016 and 2017 at TLWMA, with a mean relative contribution of 0.27, followed closely by amphibians, lizards, and the rough green snake (*Opheodrys aestivus*) at 0.25 (Table 2.4). Rough green snakes were a separate food item from other snake species due to their primarily insectivorous diet. Coleoptera, earthworms, insectivorous birds, omnivorous mammals, and other snake species were the 3rd most prevalent food item group in the skunk diet (0.154; Table 2.4). The 'insectivorous birds' group included 4 species: common nighthawk (*Chordeiles minor*), eastern towhee (*Piplio erythrophthalmus*), Bachman's sparrow (*Peucaea aestivalis*), and eastern meadowlark (*Sturnella magna*). Fungi, granivorous or herbivorous birds, other arthropods, and rabbits

constituted the 4th most prevalent group with a mean relative proportion of 0.135 (Table 2.4). The ‘granivorous or herbivorous birds’ group included 3 species: northern bobwhite (*Colinus virginianus*), common ground-dove (*Columbina passerine*), and mourning dove (*Zenaida macroura*). Plants were even less prevalent in the skunk diet with a mean relative contribution 0.124 (Table 2.4). All samples in the plant group were from the fruiting bodies of the respective species, such as oak (*Quercus* sp.) acorns or saw palmetto (*Serenoa repens*) berries. Lepidoptera contributed the least with a mean relative contribution of 0.07 (Table 2.4). Lepidoptera collected during this study consisted solely of caterpillars.

DISCUSSION

Our stable isotope analysis suggests that FSSKs in the Route 60 unit at TLWMA have a varied, generalist diet, as has been documented in the species previously outside of Florida (Crabb 1941). This is supported by our finding that no single diet item composed more than approximately 27% of the skunk diet at the site. The skunks also appear to be omnivorous, as approximately 12% of the diet of the sampled skunks was derived from plant materials. Unfortunately, we were unable to determine whether arthropods comprised a majority of the skunk diet at the study site, as we had initially expected. This was due to our necessary grouping of food items with similar isotopic signatures prior to fitting our data with a mixing model. We were also unable to determine what relative proportion insects (Class: Insecta), a food source known to be important to eastern

spotted skunks (Crabb 1941), comprised specifically in the skunk diet at our site. At a minimum, arthropods (millipedes and caterpillars) comprised approximately 34% of the skunk diet, but possibly up to about 63% of the diet, indicating that arthropods may be a large component of the FSSK diet relative to other potential food items. We had expected that arthropods would be important in the skunk diet due to the high availability of some arthropods (e.g., crayfish) in the many depression wetlands that dot the landscape of the Route 60 unit. However, crayfish could have only contributed 14% or less to the skunk diet at the site.

The generalist nature of the spotted skunk diet at our site is supported by our findings that, at the least, skunks at our site consume arthropods, herpetofauna (amphibians or reptiles), and plant materials. Primarily insectivorous herpetofauna were the 2nd most prevalent food item in our dietary analysis. Snakes and Anurans were only recently documented as food items of the eastern spotted skunk (Sprayberry and Edelman 2016), and it is possible that they are more important in the diet of the species, or at least the Florida subspecies, than previously thought. This generalist diet suggests that food availability is not a limiting factor for the FSSK population at our study site, which has likewise been suggested by Sprayberry and Edelman (2016) regarding the decline of eastern spotted skunks range-wide. It is also possible that spotted skunks at our site benefit from a lack of competitors for food and thus have a wider variety of food available to them in the dry prairie habitat. Kinlaw (1995) listed striped skunks (*Mephitis mephitis)* and weasels (Mustelidae) as sympatric food competitors of the eastern spotted skunk, but neither striped skunks nor long-tailed weasels (*Mustela frenata*) were

observed during 2 years of research in the Route 60 unit or captured in recent mammal live-trapping efforts in the unit (S. L. Glass and C. L. Hannon, FWC, pers. comm.).

The most prevalent diet item of spotted skunks in our study was millipedes, likely comprising more than a quarter of the skunk diet at our study site. In a review of the literature available on eastern spotted skunk diet, we could find no explicit mention of millipedes or Myriapoda (centipedes and millipedes) more generally as prey items consumed by the species. There also exists the possibility that these prey items were lumped with other arthropods or had their exoskeletons misidentified as those of insects in analysis of fecal samples. However, there is some precedence for the apparent importance of millipedes to spotted skunk diet at our site. In a study on the diet of the congener pygmy spotted skunk (*S. pygmaea*) in Mexico, Cantú-Salazar et al. (2005) found that Myriapoda were positively selected for when compared to their availability. Similarly, centipedes were documented in some scats of *S. gracilis leucoparia* collected from a montane location in Durango, Mexico (Baker and Baker 1975). Cantú-Salazar et al. (2005) suggested that Myriapoda are more easily digested than some other arthropods and therefore may represent a highly nutritious food item for a spotted skunk.

Unfortunately, we were unable to resolve exactly how important grassland, ground-nesting birds were to the diet of FSSKs at our study site. This was due to the similar isotopic signatures between certain food items, which resulted in insectivorous birds and primarily granivorous or herbivorous birds being placed into separate groups; these 2 groups each also contained other unrelated food items such as arthropods or mammals. However, insectivorous birds likely composed no more than 15.4% of the

FSSK diet at TLWMA, and likely less because of the other diet items grouped with them, such as Coleoptera, a known important prey source of the eastern spotted skunk (Crabb 1941, Kinlaw 1995). Consumption of insectivorous, ground-nesting birds and their eggs by FSSKs at TLWMA may only be incidental as they forage for other food items (Vickery et al. 1992). The high predation rates on grassland bird nests documented at the site by FSSKs may partially be a byproduct of their seemingly high abundance and wide distribution there. However, further research on food resource availability at the site would be needed to determine whether this is indeed the case. The grouping of insectivorous birds with omnivorous mammals and all snake species (other than the rough green snake) prevented us from discerning whether spotted skunks at the study site regularly consume other documented grassland bird nest predators in the dry prairie ecosystem, like the eastern corn snake (*Pantherophis guttatus*), eastern ratsnake (*Pantherophis alleghaniensis*), and cotton rat (*Sigmodon hispidus*; E. L. Hewett Ragheb, unpubl. data). Therefore, it is unknown if spotted skunks at TLWMA consume these other threats to grassland ground-nesting birds in greater or lesser proportions than they consume the birds themselves.

There are a number of aspects of our study that could be improved upon in the future to gain a clearer understanding of the FSSK diet in the dry prairie ecosystem of Florida. For one, the results and conclusions of our study were less clear because of the groups that food items were placed into for analysis based on their similar isotopic signatures, as 4 of 6 groups contained multiple food items, some of which were quite dissimilar to each other from an ecological standpoint. An increased number of samples

would help remedy this situation by reducing the variance in the isotopic signatures of each food item type. Second, at TLWMA in 2016 and 2017, we pooled both hair samples and food item samples from across years. It is possible that there was interannual variation in the isotopic signatures of both skunks and some of the collected food items based on environmental variables outside of the scope of this research. Additionally, we were unable to clearly determine how long a period of time the skunk hair samples used in our analysis represented. While hair samples are known to reflect diet since a mammal's last molt, it is currently unknown when spotted skunks (*Spilogale* sp.) undergo molt (Van Gelder 1959). Finally, our study only investigated the diet of FSSKs at TLWMA without determining the availability of these food resources on the landscape. Therefore, we have no insight into what food items skunks at our study site may be selecting or purposefully searching out. Including this resource selection component into our study would have helped us resolve the predator-prey relationship between spotted skunks and ground-nesting birds, like the FGSP.

Overall, the results of this study provide new information on the diet and general ecology of the FSSK. These results also demonstrate some potential dietary differences between this subspecies and the other subspecies of the eastern spotted skunk, as Myriapoda and herpetofauna may be more important food sources for the FSSK than for the other subspecies. This study suggests that the imperiled grassland birds that FSSKs are known to consume may only make up a small proportion of the skunks' varied diet. However, more research is needed to clarify the importance of birds in the FSSK diet and whether the relationship between these two groups is predicated upon chance encounters

between the two.

We suggest that more research on the diet of the FSSK in other habitats and parts of its range would be beneficial in understanding the ecology of the subspecies and would be helpful in determining whether the results of our study in the dry prairie ecosystem are representative of the subspecies' diet elsewhere. Similarly, more studies on the diet of the other eastern spotted skunk subspecies would be helpful in investigating dietary differences between the subspecies and how food availability may have been a factor in the decline of the species. For example, food availability may be more important for larger-bodied skunks in the northern part of the species' range, where they experience seasonal inactivity during the harsh winter months in which they must partially rely on fat stores to survive (Van Gelder 1959, Kinlaw 1995).

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TABLES AND FIGURES

Table 2.1. Mean $\delta^{13}C$ and $\delta^{15}N$ values of hair samples collected from adult male and female Florida spotted skunks (*Spilogale putorius ambarvalis)* in 2016 and 2017 at Three Lakes Wildlife Management Area, Osceola County, Florida.

Sample	Sex	δ13C	δ15N
23	Female	-21.6	5.7
25	Female	-21.7	5.8
27	Female	-22.2	5.5
30	Female	-21.7	6.1
31	Female	-21.5	5.7
35	Female	-21.4	5.3
38	Female	-20.4	6.8
39	Female	-21.8	6.7
40	Female	-21	6
61	Female	-22.7	5.9
62	Female	-22.5	6.3
66	Female	-22.6	5
69	Female	-24.1	5.4
70	Female	-22.4	6.5
71	Female	-23.2	5.6
73	Female	-22	6.6
74	Female	-21.2	5.9
79	Female	-21.2	5.9
80	Female	-20.4	6.5
1	Male	-21.8	5.7
3	Male	-22.1	6.1
4	Male	-22.4	5.7
5	Male	-22.8	6
6	Male	-22.4	5.1
10	Male	-16.7	6.8
11	Male	-22.4	6.2
14	Male	-22.4	6.9
18	Male	-22.7	5.3
20	Male	-21.3	5.3
41	Male	-20.8	5.3
42	Male	-17.3	4.7
45	Male	-23.1	5.5
46	Male	-21.1	4.2

47	Male	-22.4	5.9
50	Male	-22.1	5.8
52	Male	-22.7	5.6
54	Male	-23.2	5.7
55	Male	-22.8	6.2
60	Male	-22.4	7.2

Table 2.2. Mean $\delta^{13}C$ and $\delta^{15}N$ (±SD) values, sample sizes (*n*), and groupings of potential Florida spotted skunk (*Spilogale putorius ambarvalis*) food items collected in 2016 and 2017 at Three Lakes Wildlife Management Area, Osceola County, Florida. Food items were grouped based on the significant differences found between food items in a multivariate analysis of variance (MANOVA) with a post hoc Tukey's multiple comparison test and a k-nearest neighbor analysis.

Item	*n*	$\delta^{13}C$	$\delta^{15}N$	Group
Lepidoptera (caterpillars)	5	-28.93 ± 0.72	5.26 ± 2.46	A
Blattodea	1	-23.32	7.09	B
Common ground-dove (*Columbina passerine*)	2	-26.22 ± 1.76	10.62 ± 1.09	B
Crayfish	10	-24.32 ± 1.88	6.99 ± 0.82	B
Rabbits	3	-25.63 ± 0.22	7.34 ± 1.60	B
Epigeous fungi	5	-24.44 ± 1.34	10.46 ± 2.23	B
Hemiptera	2	-25.03 + 0.04	6.69 ± 0.51	B
Mourning dove (*Zenaida macroura*)	1	-26.56	11.76	B
Northern bobwhite (*Colinus virginianus*)	1	-25.80	9.26	B
Odonata	7	-23.78 ± 2.96	8.79 ± 1.13	B
Orthoptera	9	-23.26 ± 1.62	5.91 ± 1.39	B
Spiders	7	-23.87 ± 3.03	8.48 ± 1.40	B
Amphibians	9	-19.63 ± 1.01	9.67 ± 0.96	C
Lizards	6	-19.90 ± 1.21	8.42 ± 1.08	C
Rough green snake (*Opheodrys aestivus*)	2	-17.98 ± 0.49	7.91 ± 0.10	C
Bachman's sparrow (*Peucaea aestivalis*)	5	-20.57 ± 1.23	8.33 ± 0.40	D
Coleoptera	5	-21.36 ± 0.89	8.50 ± 2.58	D
Common nighthawk (*Chordeiles minor*)	1	-22.00	9.68	D
Cotton rat (*Sigmodon hispidus*)	5	-22.17 ± 1.16	9.63 ± 1.34	D
Earthworms	11	-21.32 ± 1.67	9.65 ± 0.70	D
Eastern meadowlark (*Sturnella magna*)	5	-22.90 ± 1.26	9.37 ± 0.69	D
Eastern towhee (*Piplio erythrophthalmus*)	2	-21.65 ± 0.92	9.38 ± 0.05	D

Other snakes	8	-23.25 ± 2.40	10.51 ± 0.72	D
Raccoon (*Procyon lotor*)	1	-21.37	10.49	D
Millipedes	5	-22.25 ± 0.89	3.39 ± 0.41	E
Ilex glabra fruit	1	-26.34	1.02	F
Vitis rotundifolia fruit	7	-25.34 ± 0.55	2.60 ± 0.99	F
Quercus sp. acorns	2	-26.35 ± 0.79	1.10 ± 0.82	F
Serenoa repens fruit	5	-26.95 ± 0.29	2.18 ± 1.10	F
Vaccinium myrsinites fruit	3	-24.64 ± 0.47	0.17 ± 0.80	F

Table 2.3. Mean $\delta^{13}C$ and $\delta^{15}N$ (± SD) values and sample sizes (*n*) of food item groups of potential Florida spotted skunk (*Spilogale putorius ambarvalis*) food items collected in 2016 and 2017 at Three Lakes Wildlife Management Area, Osceola County, Florida.

Group	*n*	$\delta^{13}C$	$\delta^{15}N$
Lepidoptera (caterpillars)	5	-28.93 ± 0.72	5.26 ± 2.46
Amphibians, lizards, rough green snake (*Opheodrys aestivus*)	16	-19.53 ± 1.17	8.98 ± 1.16
Coleoptera, earthworms, insectivorous birds, omnivorous mammals, other snakes	43	-21.91 ± 1.72	9.49 ± 1.26
Millipedes	5	-22.25 ± 0.89	3.39 ± 0.41
Fungi, granivorous or herbivorous birds, other arthropods, rabbits	48	-24.24 ± 2.09	7.94 ± 2.00
Plants (fruits)	18	-25.84 ± 0.98	1.82 ± 1.27

Table 2.4. Relative mean proportions (± SD) and 95% Bayesian credible intervals of food item groups in the Florida spotted skunk (*Spilogale putorius ambarvalis*) diet determined through stable isotope analysis. All hair and food item samples were collected in 2016 and 2017 at Three Lakes Wildlife Management Area, Osceola County, Florida.

Food item group	Mean proportion (± SD)	95% credible interval
Millipedes	0.271 (± 0.153)	0.031 – 0.521
Amphibians, lizards, rough green snake (*Opheodrys aestivus*)	0.252 (± 0.146)	0.028 – 0.494
Coleoptera, earthworms, insectivorous birds, omnivorous mammals, other snakes	0.154 (± 0.124)	0.011 – 0.401
Fungi, granivorous or herbivorous birds, other arthropods, rabbits	0.135 (± 0.127)	0.007 – 0.401
Plants (fruits)	0.124 (± 0.095)	0.008 – 0.307
Lepidoptera (caterpillars)	0.065 (± 0.055)	0.004 – 0.173

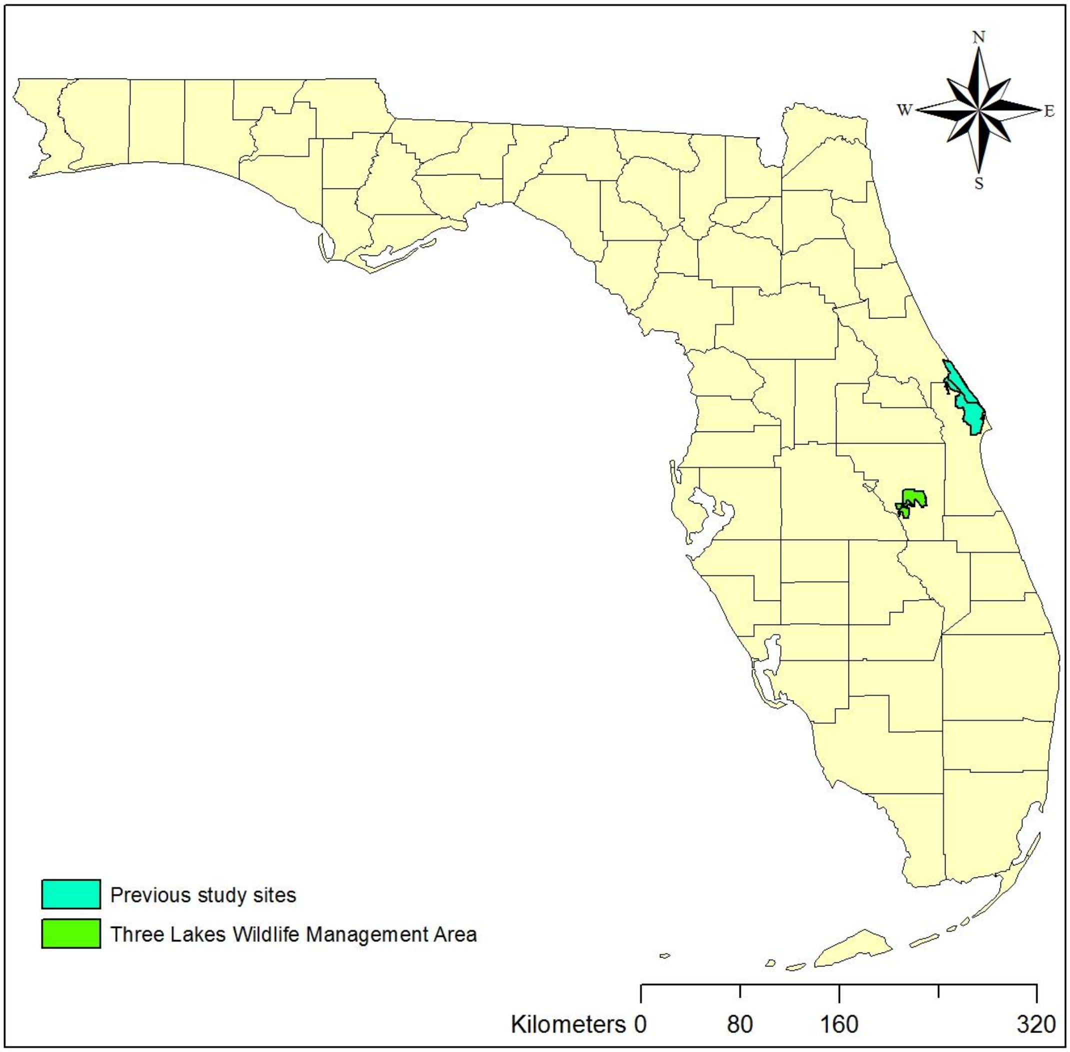

Figure 2.1. Locations of previous studies on Florida spotted skunks (*Spilogale putorius ambarvalis*) and this study at Three Lakes Wildlife Management Area, Osceola County, Florida. Previous studies occurred at Canaveral National Seashore and Merritt Island National Wildlife Refuge

Figure 2.2. Dry prairie ecosystem in the Route 60 unit at Three Lakes Wildlife Management Area, Osceola County, Florida.

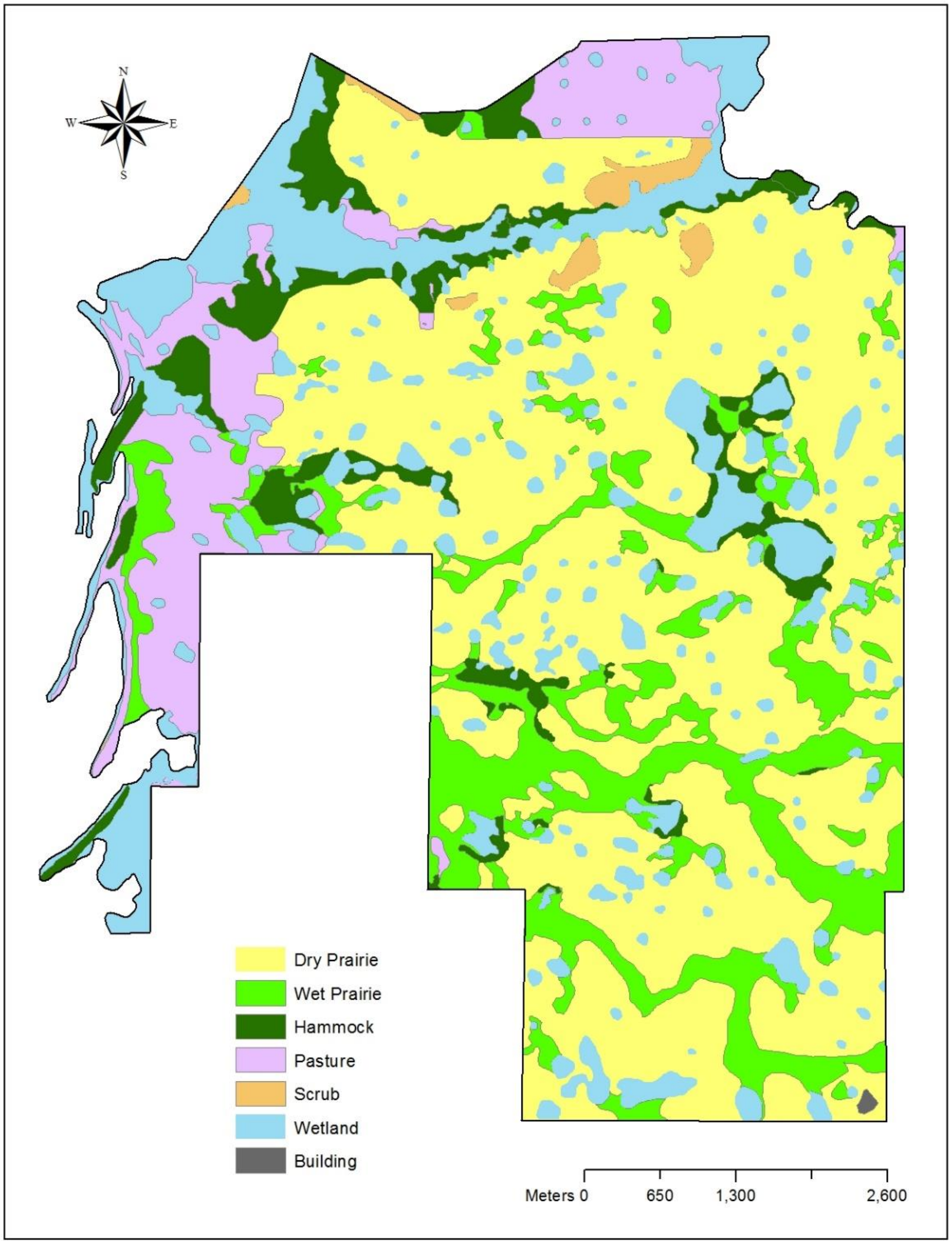

Figure 2.3. Habitats occurring in the Route 60 unit of Three Lakes Wildlife Management Area, Osceola County, Florida. Habitats simplified from data obtained from the Florida Cooperative Land Cover Map (Florida Fish and Wildlife Conservation Commission).

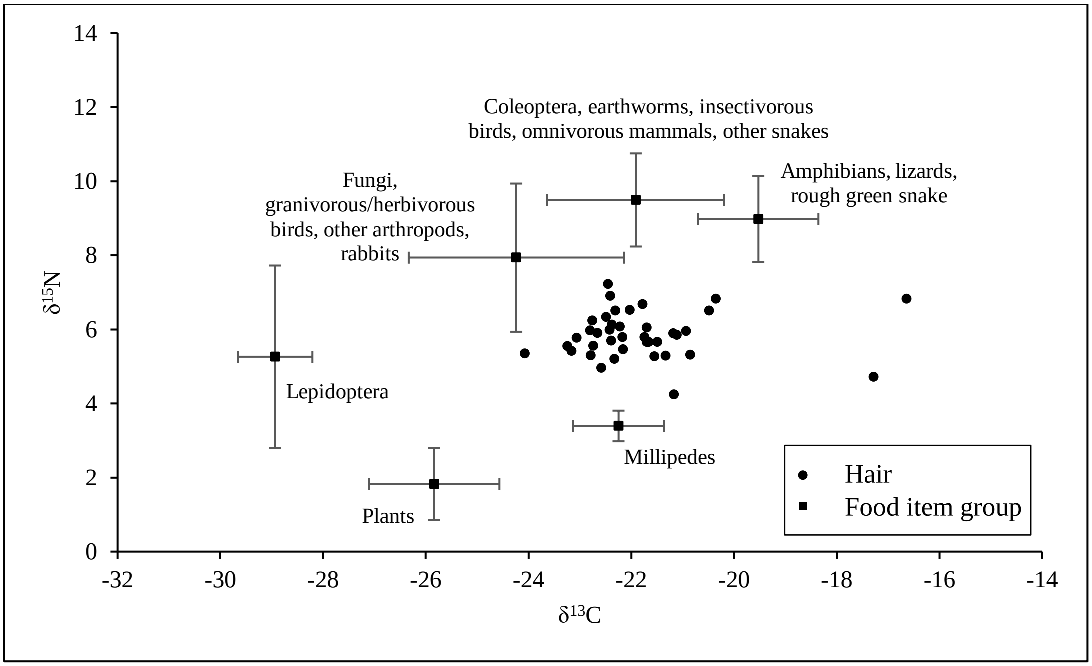

Figure 2.4. Mean $\delta^{13}C$ and $\delta^{15}N$ values (± SD) of food item groups representing potential dietary components of Florida spotted skunks (*Spilogale putorius ambarvalis*), and mean $\delta^{13}C$ and $\delta^{15}N$ values of individual skunk hair samples from Three Lakes Wildlife Management Area, Osceola County, Florida, in 2016 and 2017.