MULTISCALE HABITAT SELECTION OF A TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*) POPULATION IN MIDDLE TENNESSEE

by

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ABSTRACT

Habitat selection is an important aspect of a species' natural history, with applications specifically in the management of at-risk organisms. This study assesses habitat usage of the timber rattlesnake (*Crotalus horridus*) at three different spatial scales (landscape, home range, and microhabitat) and two separate temporal scales (entire active season, and core activity range) in Middle Tennessee. The study produced significant results at both the landscape and microhabitat scale when analyzed over the entire active season, and for landscape and home range scales during the core activity range. Rattlesnakes showed a preference for mixed-hardwood forests at the landscape scale and open-canopied habitats at the home range scale. Additionally, rock cover was a negative predictor of microhabitat selection. While statistically significant, the microhabitat models performed poorly when cross-validated. This suggests that timber rattlesnakes in Middle Tennessee are likely habitat generalists.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Habitat heterogeneity provides landscape complexity that offers niche diversity for numerous organisms (MacArthur and Macarthur, 1961). Such landscapes generally are composed of multiple habitat types, which facilitate species diversity (Kadmon and Allouche, 2007). Variation in habitat type and within-habitat preferences may allow organisms to choose features that best suit their needs based on seasonal events such as structures for optimizing thermoregulation (Peterson et al., 1993), predator avoidance (Gilliam and Fraser, 1987), rearing offspring (Kleinwachter and Burkel, 2008), and seasonal shelter requirements (Irschick and Losos, 1999; Sexton et al., 2009). Individuals will ideally select specific locations throughout the landscape to maximize resource acquisition, and relocate to obtain specific needs, with more mobile animals being exposed to a larger variety of habitat features (MacArthur and Pianka, 1966). Nevertheless, the distribution of resources will drive the use of space by animals, and this is particularly the case for species that exhibit preferences for specific habitats and resources. For example, two sympatric canids in Brazil varied in diet and habitat with *Lycalopex vetulus* having a more narrow dietary niche and hunting exclusively in grasslands while *Cerdocyon thous* exhibited a wider dietary niche and hunted equally in grassland and wooded savannahs (Juarez and Marinho-Filho, 2002).

Patterns in the use of space by animals are generally correlated with body size and activity levels. Larger-bodied species need more area of resources than smaller-bodies species because of their higher energy demands (McNab, 1963). As a result, for animals that establish home ranges, larger animals typically have larger home ranges than smaller animals (McNab, 1963). Therefore, species that occupy larger areas have a higher likelihood of exposure to a variety of habitats. Animal activity levels can influence habitat exposure as well (McNab, 1963). For example, some animals will make large migratory movements to reach specific habitats for needed resources (Fryxell, 1988; Rowe and Moll, 1991). Such activity levels of an individual can change seasonally, or based on reproductive status, prey availability, and weather variation (George et al., 2015; Tufto et al., 1996). As animals move and interact with their environment, they choose between available habitats providing information on preference to establish life history trends.

The assessment of usage in comparison to features available allows for analysis to elucidate selection patterns. Habitat selection is often considered best analyzed at multiple scales to maximize management efficiency, looking at what individuals are using from a landscape level down to what structural variables affect specific chosen sites (Johnson, 1980; Row and Blouin-Demers, 2006). In some cases it is possible to find populations in a macrohabitat that would be considered less than ideal due to specific microhabitat features (Hecker et al., 2020). One argument for this is that even if little variability in habitat types is considered, a species may require more specific needs within a habitat towards the edges of a range allowing for different variables to have significant role at different points in a geographic range (Oliver et al., 2009; Steen et al., 2010). Within snakes a major limiting factor of their geographic range is the need to thermoregulate (Blouin-Demers and Weatherhead, 2001a).

Movement of some temperate region snakes follow a semi-migratory pattern, in which snakes move to and from summer activity areas and over-wintering sites based on variables such as climate and food availability (Gregory, 1984; Heard et al., 2004; Reinert, 1993). The area these snakes utilize (i.e., home ranges) during their active season may necessitate travel of several kilometers to areas better suited for reproduction or foraging (Macartney et al., 1988; Ernst and Ernst, 2012; Waldon et al., 2006a,b). Snake movement, and ultimately use of space, not only varies by species but also by body size (Hyslop et al., 2014), sex (Delisle et al., 2019; Gerald et al., 2006; Hyslop et al., 2014), reproductive status (Reinert and Zappalorti, 1988), foraging behavior (Secor, 1995), weather (George et al., 2015), thermoregulatory needs (Blouin-Demers and Weatherhead, 2001b) and seasonality (Cobb and Peterson, 2008; Waldron et al., 2006b). Additionally, some species have different habitat usage and movement patterns from the center of their geographic range compared to the periphery (Constible et al., 2009; Kapfer et al., 2008), while habitat generalists seem to use similar microhabitat features throughout their ranges (Novak et al., 2020).

One widely studied snake species in North America is the timber rattlesnake (*Crotalus horridus*). This wide-ranging pitviper is found throughout much of the Eastern United States, and historically in Ontario and Maine (Ernst and Ernst, 2012). However, many of the extant populations are considered endangered, threatened, or in need of management (Brown, 1993). Previous studies have found nonrandom habitat use in the northern part of their range (Reinert, 1984a,b; Reinert and Zappalorti, 1988) as well as in South Carolina (Waldron et al. 2006a,b). For instance, male and non-gravid female *C. horridus* were associated with more canopy cover and thick surface vegetation, while gravid snakes showed preference to less canopy cover and more fallen logs (Reinert and Zappalorti, 1988). As a semi-migratory species, fragmentation by road placement and genetic isolation can be contributing factor to population decline (Clark et al., 2010). Generally, timber rattlesnakes are associated with forested or woodland habitats throughout most of their range (Gibson et al., 2008; Reinert, 1984a,b; Reinert and Zappalorti, 1988; Waldron et al., 2006a,b), although specific habitat associations (e.g., prairies in Oklahoma) do occur is some parts of their range (Mohr and Duvall, 2017).

This study addresses the habitat use of a population of *C. horridus* in central Tennessee at three levels, based on scales set by Johnson (1980). First being second-order selection, an examination of the habitats available within individuals' home range compared to the available habitats throughout the surrounding area. Third-order selection assesses habitats used in relationship to what is available within an individual home range. Lastly, fourth-order selection or microhabitat is the measurement of specific structural variables at locations where individuals were found (Johnson, 1980). I utilize compositional analysis to compare larger scale habitat availability to snake usage, and logistic regression to examine the variables related to specific points being visited within individual home ranges. Based on observed habitat preferences in previously studied populations (Reinert, 1984a,b; Reinert and Zappalorti, 1988; Waldron et al., 2006a,b) I predict that this population of *C. horridus* would show similar trends of preference to mixed-hardwood forest on both macrohabitat scales however, because of the geographic differences from other sites, variation in ambient temperatures, and differences in the availability of anthropomorphic habitat I anticipate differences in microhabitat use to be possible.

METHODS

Study Site:

The study site is centralized on the Flat Rock Cedar Glades and Barrens State Natural Area in Rutherford County, Tennessee, as well as some of the adjacent privately-owned farmlands making the total used area of the study site approximately 541 ha (Figure 1). Defining the boundaries of the study site was accomplished by creating a minimum convex polygon that included the points of all individuals tracked and adding an outer buffer of 100 m (Constible et al., 2009). The buffer was included to compensate for the habitats outside of the furthest recorded points but that were still considered potentially available for selection by snakes. The study site is composed of a mosaic of different habitats including: (a) mixed-hardwood forests, predominantly covered with *Quercus* and *Acer* trees; (b) red cedar forests, dominated by thick stands of *Juniperus virginiana*; (c)cedar barrens and glades, an open canopy habitat with little to no ground vegetation, shallow soil, and exposed limestone; (d) fields, open-canopied habitats with perennial grass coverage that are further divided into old fields and agricultural fields based on whether or not they are anthropogenically maintained; and (e) additionally some paved roads, gravel roads, and lawns (frequently maintained monocultures of grass). Paved roads border the eastern portion of the study site and are incorporated into the study site as a result of the buffer around all snake locations. Although none of the rattlesnakes in this analysis crossed the paved roads, there are a few observations of nontelemetered snakes crossing these roads.

Telemetry:

Individual *C. horridus* (n = 25) were individually captured using both drift fences with traps and opportunistically during their active season from 2012 to 2015. Snakes were transported to Middle Tennessee State University and then surgically implanted with radio transmitters (models SI-2 or SB-2, Holohil Systems Ltd., Carp, Ontario). Following procedures similar to Reinert and Cundall (1982), surgical preparation included restraining individuals with appropriately sized clear acrylic tubes and anesthetizing individuals using isoflurane until the snake showed a loss of their righting response. After the surgical area was swabbed with betadine, a 2-cm incision was made laterally between the first and second dorsal scale rows on one body side and a radio transmitter was implanted into their body cavity at approximately 60% of their body length caudally with the antenna of the transmitter extended cranially. This location positioned the radio transmitter near the intestines, posterior to the stomach, as to not interfere with the consumption of large prey (e.g., *Sciurus carolinensis*). Postoperative snakes were maintained for 24 – 48 hours in an environmental chamber at 28 to 30°C to facilitate recovery from the surgery, before being released at their point of capture. Snakes were then radio tracked approximately three times per week throughout their active season with geographic locations determined using a 3-element Yagi antenna and a radio receiver (R-1000, Communications Specialist, Inc., CA), hand-held telemetry system. Locations were recorded with a hand-held GPS (GPSMAP 76CSx, Garmin International, Inc., KS).

Habitat Analysis:

At the landscape level, habitats were categorized into general types based on winter satellite images (high resolution orthoimagery courtesy of The U.S. Geological Survey) and in-field observations. Polygons were drawn to delineate distinct habitats in ArcGIS 10.3. Snake locations were used to generate active season home range estimates, as well as core activity areas. The snake active season was defined as the dates where the movement locations of an individual during egress from hibernation were greater than 50 m cumulatively from the overwintering site. Utilizing this definition of the active season prevented bias towards multiple smaller movements associated with activities near the overwintering sites, but assumes consistent habitat preferences outside of the overwintering sites. Core activity areas were established by removing points within the first and last 25% of each individual's total time above ground. The usage of core activity areas represented the half of their active season where the majority of foraging and mating occurs (Waldron et al., 2006b). Once polygons were defined, they were overlaid on the habitat map to calculate the proportions of available habitat types within each home range. By comparing the available habitat types within the home range of each individual to the habitat type at each snake location using a compositional analysis (Calenge, 2015), estimates of preference were predicted.

Structural habitat measurements were visually estimated at every snake relocation from 2012 - 2015. Descriptions and sampling techniques of these habitat variables are presented in Table 1. These structural measurements were chosen to allow comparison to of *C. horridus* (Reinert, 1984a,b; and Reinert and Zappalorti, 1988). Each snake location point was classified to a general habitat type; cedar forest, hardwood forest, field, maintained field, barren, glade, or other (habitat types are described in Table 2). The distance to edge was defined as the distance to the boundary of nearest open canopy habitat and closed canopy habitat and was measured as the straight-line distance in GIS when boundaries were not visible while standing at the snake location. The measurements of basal area, woody stem count, and ground vegetation count were not calculated at the time of snake relocation but rather were collected by revisiting GPS locations in the summer of 2016. The basal area (total area occupied by tree trunks greater than 7 cm in diameter) was calculated using the diameter at breast height (1.37m) of all trees in a 5 m^2 plot around the snake locations. The woody stem count is the total number of stems great than 1.5 m in height, but less than 7 cm in diameter in the 5 m^2 plot. To estimate ground vegetation, stemmed plants less than 1.5 m in height were counted in four -1 m^2 squares within the plot; this estimate included many first-year saplings, vined and herbaceous plants, but excluded grasses and mosses.

Data Analysis:

To maintain independence of habitat sampling sites during active season analysis, snake locations were counted once (first relocation), even if the snake remained at the site for multiple telemetry locations. Also, only the first complete field season of individual snakes, tracked multiple years was utilized for analysis. To compensate for GPS accuracy (± 5 m) and maintain spatial independence in locations being revisited, only one point was used if points were recorded within 10 m of each other. When two points occurred within 10 m of each other, the point with minimum overlap of surrounding 10 m buffers was chosen, or if two points were not in contact with any surrounding buffers, a point was randomly selected. If three or more points were within 10 m of each other, the point that was most central was used. Locations where the snake was actively traveling were excluded from analysis because these locations were considered points that the snake was not actually selecting to remain (Table 3).

Polytomous logistic regression, an extension of binary logistic regression that is used to predict the odds of an event occurring given a set of variables, was used to analyze microhabitat data. A grid with cell dimensions of 74.4 m^2, based on the average daily movement of snakes in this study (37.7 m), was centered over the study site. These dimensions would permit a snake at the center of a cell to have potentially been in an adjacent cell on any given day based on the average daily distance moved of all snakes. Each cell was classified as low use, medium use or high use based on the number of snake locations within the cell. A cell was considered low use if it was below the average number of relocations within a cell, excluding cells with no relocations. Medium use cells were defined as cells with greater than the average, but less than 1 SD greater than average relocations. High use cells were defined as cells which were greater than +1 SD of the average number of relocations.

Along with the structural measurements taken, each snake relocation was assigned a label based on the type of cell it was located in and used to build the regression. Polytomous logistic regression holds an advantage over discriminant function analysis, an alternative statistical technique used in previous studies of microhabitat selection (Reinert, 1984a,b; Reinert and Zappalorti, 1988; Harvey and Weatherhead, 2006), as it does not require the assumptions about multivariate distributions in the independent variables or the covariance matrix of independent variables being identical (North and Reynolds, 1996). This technique eliminated the need to collect data at random points and has been shown to generate similar results as a MANOVA procedure (Cross and Petersen, 2001). Variables were chosen based on significance in a forward selection, an alpha of 0.10 was used to mark significant contribution to the final model (North and Reynolds, 1996).

RESULTS

Between 2012 and 2015 GPS locations were recorded from 25 individuals *C. horridus* resulting in 1847 snake locations. Of their locations, 434 were used to measure microhabitat (see Table 3 for Point exclusion). The snakes sampled included 13 female snakes, 3 gravid snakes, and 10 male snakes. Snakes home range for the duration of the active season size varied from 0.75 to 84.75 ha (Table 4). The study site was composed of 72.69% mixed-hardwood forest, 12.36% fields, 5.79% cedar glades or barrens, 5.17% cedar forest, and 4.07% was classified as other. Home range size and habitat percentages selected by individual snakes are reported in Table 4.

Active Season Compositional Analysis:

Compositional analysis was run on two different scales to examine the different levels of habitat use described by Johnson (1980). The larger scale (Johnson's second-order selection) compared the proportions of habitat within an individuals' home range to the proportions of the habitat within the entire study site. The next smaller scale (Johnson's third-order selection) compared the proportion of habitat types for individual snakes in to the proportion of habitat types available within the home range of an individual. These analyses were run for all snakes combined, and then separately for male, female, and gravid snakes. The third-order selection was also repeated utilizing snake locations where the individual had been found at the same point multiple relocations as a procedure to include remaining at a point as a form of selection.

Due to the nature of a compositional analysis, and the effect of zero values in both available and used habitat types for many of the snakes, specifically at the third-order scale, 0.001 was substituted for zero values to allow the analysis to run. When 0.001 is substituted at the second-order scale which successfully ran with zeroes the overall results remained the same.

The second-order scale, male ($\lambda = 0.167$, $p = 0.003$), female ($\lambda = 0.366$, $p = 0.007$), and all snakes grouped ($\lambda = 0.312$, $p > 0.001$) showed habitat preferences differing from the available habitat (Figure 2). All snakes combined showed a significant preference to mixed-hardwood forests over all other categories except the barren-glade habitat type, where the preference was not significant. Female snakes showed preference to mixed-hardwood forests over all other habitat types, but the preference was only significant when compared to the “other” habitat type. Male snakes showed preference to the barren-glade habitat type, with the preference being significant over all types except mixed-hardwood forest. In all cases the anthropogenic habitat type was ranked the lowest for preference. For the Johnson’s third-order selection, no groups were found to show significant preference of used habitat compared to available habitat within home ranges (Table 5).

Active Season Microhabitat Analysis

Microhabitat analysis was performed on the entire sample of snakes, and separately for male, female, and gravid snakes, using both spatially-independent locations and repeat locations. None of the models were significant when using spatially-independent points (all p-values > 0.1 for forward selection) . However, when the total number of relocations at a single GPS location with all snakes were used, rock cover had a negative relationship with use classification (Table 6). When cross-validated, the model predicted 49.8% of the points accurately. The final model of female snakes show a preference to more ground vegetation and less rock cover, with the model predicting 53.3% of points correctly (Table 6). Male snakes showed a negative relationship with distance to cover and rock cover, with the model predicting 60.9% of points correctly when cross-validated (Table 6).

Core Activity Area Analysis:

Habitat preferences were analyzed for Johnson's second-order and third-order selection and only with snakes in which data were collected for an entire active season. At the second level when male and female snakes were grouped, snakes exhibited significant selection behavior with preference to mixed-hardwood forests over all other habitat types ($n = 14$, $\lambda = 0.341$, $p = 0.026$). Female snakes ($n = 9$, $\lambda = 0.272$, $p = 0.020$) maintained a preference for mixed-hardwood forests while male snakes ($n = 5$, $\lambda = 0.199$, $p = 0.089$) showed no significant habitat selection (Figure 3). At the third-order, male and female snakes collectively showed habitat specificity with an aversion to cedar forests (n = 14, λ = 0.021, p = 0.014, Figure 4). To compensate for analytical issues associated with zeroes in both used and available habitats I substituted .001 for all zeroes to run the analysis for males and females separately. Female snakes showed no habitat preferences within their core activity areas (n = 9, λ = 0.572, p = 0.285,). Male snakes showed the strongest preference to fields, and least preference to cedar forests (n = 5, λ = 0.037, p = 0.002).

DISCUSSION

Timber rattlesnakes in this study exhibited some selection behavior but not at all of Johnson's orders. In regards to Johnson's second-order of selection (i.e., macrohabitat), The average values of *C. horridus* showed differences in the percentages of habitat used within a home range in comparison to the percentages of habitats available within the study site. Regardless of category (all snakes, female, or male), snakes consistently showed the least preference for the anthropogenic habitats, a classification that was largely characterized by features such as roads, houses, and lawns. While roads have been shown to disrupt seasonal migration in *C. horridus* (Clark, 2010), all major roads and most anthropogenic features at this site were on the periphery of the study area making it difficult to imply avoidance or a lack of preference. Although none of the snakes included in the analyses crossed roads one telemetered individual was killed crossing the road and occasional non-telemetered *C. horridus* were observed dead on the road.

The habitat *C. horridus* utilized the most was the mixed-hardwood forest which was also the most abundant composing approximately 70% of the study site. Snakes were rarely observed in the open-canopied areas of cedar barrens and glades or within fields. Observations of snakes within these open habitats revealed that they chose to be in vegetation cover or along the edges. Avoidance of open areas is likely a behavior to avoid exposure and increased vulnerability to predation but could be influenced by thermoregulatory behavior as well (Huey, 1991; Herr et al., 2020). At least for fields, most of the use of this habitat occurred on the periphery of the study site, after snakes had traveled hundreds of meters away from their overwintering sites. Cedar forest sites were not used by snakes often. Although cedar forests can provide dense cover and rodent activity was observed, the primary prey of *C. horridus*, *S. carolinensis* (Ernst and Ernst, 2012), was not observed in cedar forest habitats. However, it was commonly observed in the mixed hardwood forest.

The habitat preferences of snakes within their core activity areas, the median 50% of their active season date range, did not differ from the entire active season at the second-order level. When viewed at the third level, snakes showed the strongest preference to field and anthropogenic habitats. As stated above, snake encounters with field habitats were mostly after significant distances had been traveled. Although fields had an open canopy, vegetational islands within them did occur and vegetation was abundant along the edges of the habitats. Snakes observed moving in these open habitats, commonly utilized fencerows or vegetational islands for cover and were rarely observed out in the open.

From a microhabitat perspective, this population of *C. horridus* appear to be habitat generalists. The use of repeated locations revealed significant results in the microhabitat analysis, but when cross-validated all models placed the points in the most common usage category (low, medium or high), leaving the model used as questionable. The negative relationship of *C. horridus* with rock cover may stem from the difficulty to remain hidden on a rocky substrate as opposed to leaf litter. For example, mammals utilizing cedar glade habitats have been shown to avoid rocky substrate in preference of habitat with a thicker organic substrate (Seagle, 1985). This may also affect the distribution of timber rattlesnakes which are known to select ambush sites which possess profitable food items (Clark, 2004). Prior studies have shown the strongest habitat preferences of *C. horridus* to be within gravid snakes (Reinert, 1984a,b), particularly as it relates to thermoregulatory opportunities with embryonic development (Peterson et al., 1993). While three gravid snakes were tracked during this study, the sample size was too small to elucidate any specific trends. However, anecdotal observations suggested gravid snakes used more open-canopied areas for gestation, which is a common thermoregulatory strategy in snakes (Peterson et al., 1993 Herr et al., 2020). Differences between male and female snakes' use of microhabitat existed with females having a negative relationship with rock cover as their strongest predictor while males appeared to have distance to cover as their strongest predictor.

Timber rattlesnakes have previously shown sex differences in habitat preferences when behaviorally-based seasons were considered (Waldron et al., 2006b). Waldron's study showed that male *C. horridus* showed no habitat preference when no seasonality was considered, but different preferences in foraging and breeding seasons (Waldron et al., 2006b). Furthermore, male and female snakes were associated with similar habitats during the breeding season and had different preferences during the foraging season. For this study, the differences between the entire active season and the core activity areas at the second-order selection level support the use of behaviorally-based seasons. This Tennessee population did not indicate habitat selection was occurring when the entire active season was analyzed. However, a preference of open-canopied habitats and avoidance of cedar forests occurred when analysis was restricted to the core activity areas (mid 50th percentile of active season). Further investigation into more specific behavior and habitat usage relationships could potentially show clearer patterns. Additionally, comparing usage and availability of additional study sites would be beneficial for addressing variability in habitat specificity. Snakes have been shown to utilize available habitat differently depending on environmental or biological conditions giving reason to suspect different habitat usage from site to site, or year to year (Blouin-Demers and Weatherhead, 2001b; Cobb and Peterson, 2008; Constible et al., 2009; George et al., 2015; Gerald et al., 2006; Hyslop et al., 2014; Kapfer et al., 2008; Reinert and Zappalorti, 1988; Secor, 1995). If neonates use conspecific scent trails to locate initial dens (Cobb et al., 2005), adult movement behavior may influence how young snakes establish their own home ranges by affecting which habitats individuals were exposed to.

Habitat specificity can vary for a species depending on the location within a geographic range (Fermon et al., 2000; Oliver et al., 2009). This change in specificity can be attributed to abiotic or biotic factors, with the former being more relevant at higher latitudes or elevations (Brown et al., 1996). With that in mind, this study of *C. horridus* predicted less habitat specificity compared to previous research partially as an effect of the higher ambient temperature in central Tennessee allowing for reliance on thermoconforming behavior (Hoekstra, 2015). Future research could potentially show gravid females to demonstrate more specificity compared to males and non-gravid females, particularly a preference for more open habitats. Additionally, the utilization of behaviorally-based seasons (i.e., taking into account specific behaviors such as foraging and mate seeking), as done by Waldron et al. (2006b), may show more pronounced differences between the sexes.

The perceived evasion of areas with the most human development also implies a need to manage human activity in areas with known populations of timber rattlesnakes, especially in extreme parts of the *C. horridus* range where populations are most at risk (Brown, 1993; Clark et al., 2011). Habitat modification is a leading cause of population decline among many taxa (Fischer and Lendenmayer, 2007), and specifically has been identified as a primary cause of decline in reptiles and amphibians (Gibbons et al., 2000). Therefore, a clear understanding of requisite habitat variables is crucial for proper management and conservation for specific organisms (Falconi et al., 2015). Simplified models of habitat selection illustrate the relationship of available resources to potential risks (Gilliam and Fraser, 1987). Ideally, an organism will choose habitats that contribute to their success, yet resource availability may vary seasonally, not all habitats are ideal for every circumstance or condition. The presence of sympatric species may require more specific resource usage (Goodyear and Pianka, 2008), and some tradeoffs, good foraging sites may not be optimal for gestation (Orians and Wittenberger, 1991). Northern Pacific rattlesnakes showed the ability to utilize specific microhabitat features to overcome perceptively undesirable macrohabitats (Hecker et al., 2020) giving more credence to the need to examine habitat usage at all scales. The variation of habitat usage in different parts of a species geographic range (Steen et al., 2010), as well as shifting suitable habitat as a result of anthropogenic climate change (Archis et al., 2018), provide reason to monitor habitat usage as an important aspect of proper management of a species.

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Table 1. Structural variables used in microhabitat analysis for *Crotalus horridus.*

Variable	Sampling Method
Basal area	Total area within a 5 m^2 quadrant occupied by tree trunks
Canopy cover	Visual estimate at snake location
Distance to cover	Visual estimate from snake location
Distance to edge	Distance to nearest non-forested habitat
Vegetation cover	Visual estimate at snake location of percentage of ground covered in vegetation
Rock cover	Visual estimate at snake location of percentage of ground covered in rocky substrate
Snake substrate	Noted at time of relocation
Woody stem count	Total number of woody stems within plot
Non-woody stem count	Estimate of number of stems less than 1.5 m tall within plot

Table 2: Descriptions of macrohabitat classifications available at the study site and used for compositional analysis.

Habitat classification	Description
Cedar forest	Plot dominated by red cedar
Mixed-hardwood forest	Plot dominated by deciduous trees
Field	Large open canopy, thick ground vegetation
Maintained field	Field habitat that receives semi-regular anthropogenic maintenance
Barren	Open canopy, some ground vegetation, shallow ground soil
Glade	Open canopy, little to no ground vegetation and exposed limestone
Anthropogenic	Area covered by human made structures

Table 3: Number of telemetry locations for *Crotalus horridus*. Not all snake locations were used for statistical analysis. Some points were omitted because they were: 1) outside of the individual's active season (date restrictions), 2) the individual was found at the same location for multiple tracking events (repeat locations), 3) the recorded GPS point was within 10 m of a previously recorded point (10 m buffer), or 4) the snake was actively traveling at the time of location and therefore no selection had occurred.

Reason	Points removed	Points remaining
Total locations	-	1,847
Date restrictions[a]	820	1,027
Repeat locations	431	596
10 m buffer	129	467
Traveling snakes	33	434

[a.] Date restrictions include hibernation locations, and snakes tracked multiple years.

Table 4: Movement descriptions and habitat usage (%) of all telemetered *Crotalus horridus.*

Snake	Maximum distance(m)	Cumulative distance(m)	Home range(ha)	Barren/ glade	Cedar forest	Field	Mixed forest	Other
Female								
3	1,468	4,625	51.5	10.3	15.4	5.6	68.6	0.2
4	1,835	5,710	41.9	7.7	0.9	5.3	85.1	1.0
6	399	1,838	4.5	0.4	0.0	0.0	99.6	0.0
7	1,323	3,785	17.3	11.4	11.6	11.2	63.7	2.1
8	1,827	2,914	30.7	6.5	10.9	55.7	26.8	0.0
10	1,207	1,461	12.4	0.0	0.0	96.8	1.4	1.9
11	911	3,100	34.6	3.3	4.6	0.0	92.2	0.0
13	884	2,396	17.9	0.4	0.0	4.4	94.8	0.5
15	664	2,009	4.3	9.3	0.0	1.8	88.9	0.0
16	338	1,208	3.7	0.0	0.0	0.0	99.2	0.7
24	404	1,358	26.1	0.0	0.0	0.0	99.0	1.0
25	644	1,885	14.5	6.8	0.0	13.6	79.6	0.0
27	595	1,903	12.1	1.6	10.2	0.0	88.2	0.0
$\bar{x}$	961	2,630	20.9	4.4	4.1	15.0	75.9	0.6
SE	146	378	4.2	1.2	1.6	8.0	8.3	0.2
Gravid								
9	1,524	3,057	10.1	17.3	7.4	2.0	72.4	0.8
19	664	4,663	23.3	35.3	0.0	64.7	0.0	0.0
11	1,220	381	14.2	0.0	0.0	8.1	91.2	0.7
$\bar{x}$	1,136	2,700	15.9	17.5	2.5	24.9	54.5	0.5
SE	252	1,249	3.9	10.2	2.5	20.0	27.8	0.3
Male								
1	1555	6,430	84.7	7.7	2.0	7.5	79.1	3.7
5	686	2,713	9.2	20.7	22.5	0.0	56.9	0.0
14	626	821	9.2	1.5	42.9	6.8	48.8	0.0
17	1,288	2,926	36.5	2.0	0.0	6.9	89.8	1.4
18	566	3,191	20.6	23.5	3.3	10.7	62.5	0.0
20	1,356	2,389	56.2	7.9	0.8	13.7	77.6	0.0
23	395	1,107	8.3	3.6	0.9	0.0	95.6	0.0
26	486	642	0.7	30.1	0.0	0.0	69.9	0.0
28	296	805	2.5	0.9	0.0	0.0	99.1	0.0
29	811	2,689	15.6	5.3	0.0	25.4	69.3	0.0
$\bar{x}$	807	2,371	24.4	10.3	7.2	7.1	74.9	0.5
SE	139	549	8.6	3.3	4.5	2.6	5.2	0.4
Total $\bar{x}$	**922**	**2,539**	**21.6**	**8.2**	**5.1**	**13.1**	**73.1**	**0.5**

Table 5: Third-order habitat selection for *Crotalus horridus*. Snakes showed no difference in proportion of habitats utilized within a home range compared to the proportions of the habitats available within the study site.

Analysis	Lambda	P
All points		
Female+male+gravid snakes	0.918	0.886
Female snakes	0.530	0.126
Male snakes	0.792	0.736
Gravid snakes	0.370	1.000
Nonrepeating points		
Female+male+gravid snakes	0.963	0.848
Female snakes	0.809	0.420
Male snakes	0.384	0.414
Gravid snakes	0.037	0.484

Table 6: Forward selection results of microhabitat for *Crotalus horridus* based on total sample as well as divided by sex. Underlined variables have a significant contribution to model and variable strength increases from left to right across the table. Abbreviations are: (B = intercept only model, RC = rock cover, DC = distance to cover, BA = basal area, CAN = canopy cover, NWS = non-woody stems, VEG = ground vegetation cover, DE = distance to edge, WSC = woody stem count, and parentheses represent a negative relationship)

Current model	Habitat variable (Highest selection factor → Lowest)							
All snakes								
B +	(RC)	DC	BA	(CAN)	(NWS)	VEG	DE	WSC
RC+	---	DC	WSC	(CAN)	VEG	BA	DE	(NWS)
Female								
B+	(RC)	VEG	(CAN)	NWS	WSC	(BA)	(DC)	DE
RC+	---	VEG	(CAN)	NWS	WSC	DE	(DC)	BA
Male								
B+	(DC)	(RC)	DE	VEG	BA	(CAN)	WSC	NWS
DC+	---	(RC)	VEG	DE	BA	(CAN)	WSC	(NWS)
RC+	---	---	DE	VEG	BA	(CAN)	WSC	NWS
Gravid								
B+	DC	(BA)	RC	VEG	CAN	DE	(NWS)	WSC

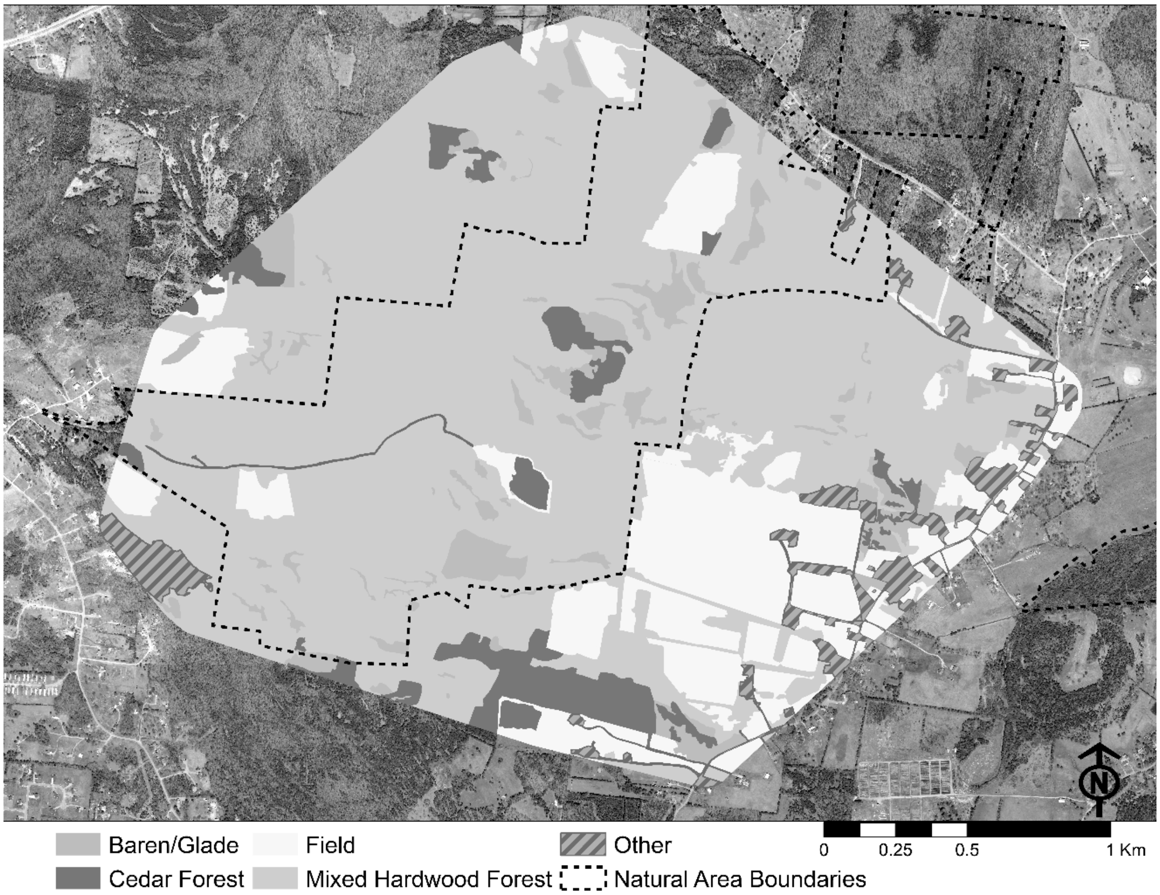

Figure 1: Habitat map of Flat Rock Cedar Glades and Barrens Natural Area. Dashed lines indicate boundary of natural area. Private land holdings are outside the dashed lines

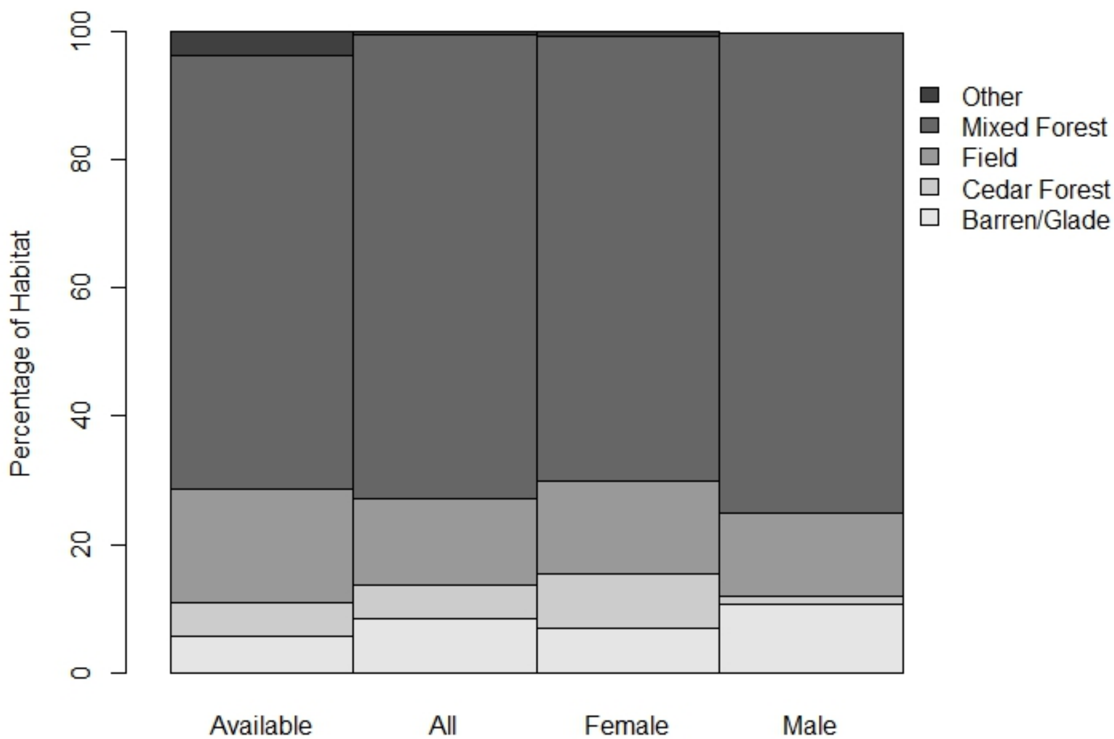

Figure 2: Percentage of habitat types used by *Crotalus horridus* during the active season. The (Available) category refers to the entire study site and the category (All) refers to the average percentages of each habitat type within home ranges based on female, male, and gravid snakes combined. The sample of gravid females was too small for compositional analysis. Each snake category was significantly different from the available habitat.

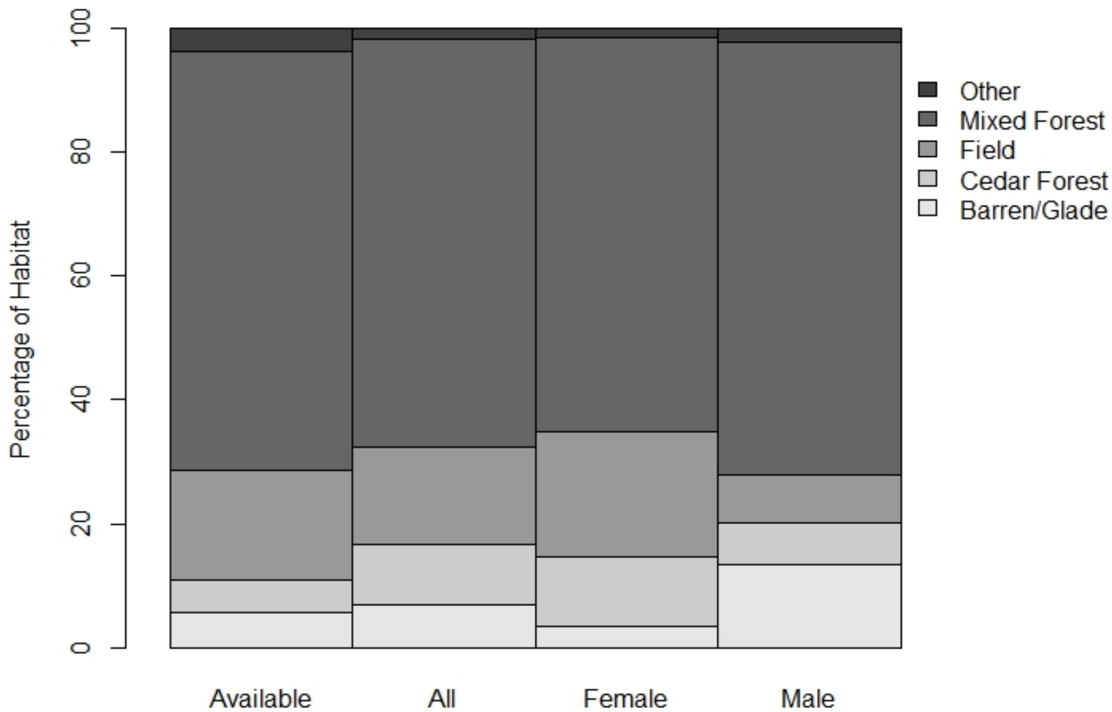

Figure 3: Percentage of habitat types used by *Crotalus horridus* within the core activity ranges. The (Available) category refers to the entire study site and the category (All) refers to the average percentages of each habitat type within home ranges based on female, and male snakes combined. The sample size of gravid females was too small for compositional analysis. All snakes and the female snakes were showed significant differences from available habitat.

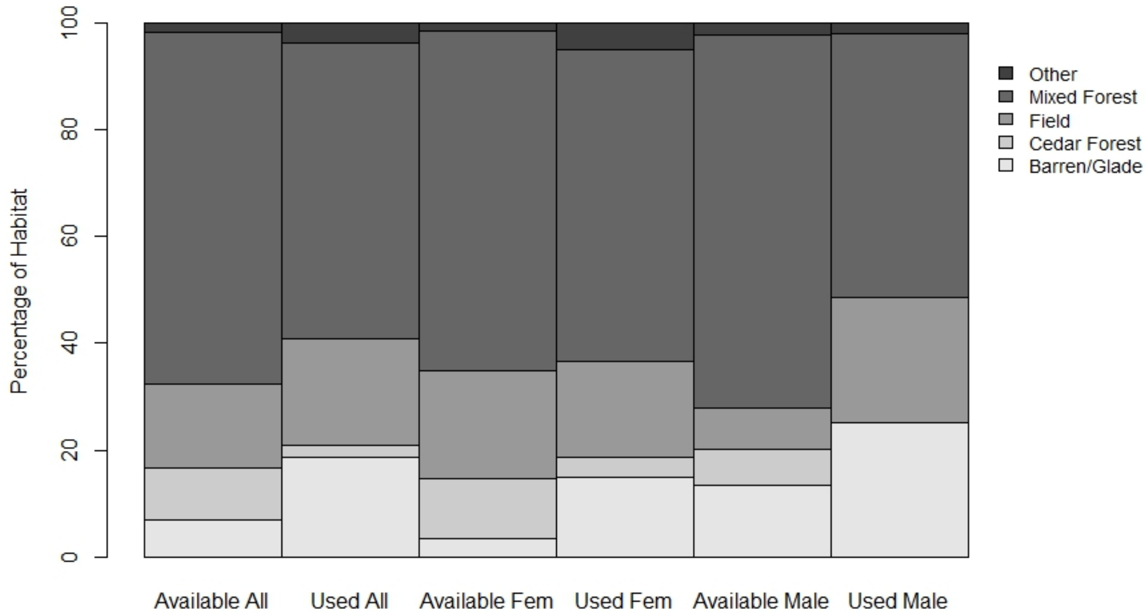

Figure 4: Percentage of habitat types used at each relocation by *Crotalus horridus* within the core activity ranges. The (Available) category refers to the average percentages of each habitat within core active season areas. (All) refers to the average percentages of each habitat type within core active season area based on female and male snakes combined. (Used) refers to the percentage of relocations in each habitat type. The sample size of gravid females was too small for compositional analysis. All snakes combined were significantly different from the available habitat.

APPENDIX

APPENDIX A: IACUC APPROVAL FORM

May 2, 2012

Investigator(s) Name: Vincent Cobb
Investigator(s) Email: vincent.cobb@mtsu.edu
Department of Biology

Protocol Title: "Timber Rattlesnake Ecology"
Protocol Number: 12-009

Dear Investigator,

The MTSU Institutional Animal Use and Care Committee has reviewed your research proposal identified above and has approved your research under the PHS definition of animal, pending the compliance office receives a copy of your TWRA permit before you begin.

Approval is granted for three (3) years. **Please note you will need to file a Progress Report annually regarding the status of your study in order to keep the study active.**

According to MTSU Policy, an investigator is defined as anyone who has contact with animals for research purposes. Anyone meeting this definition needs to be listed on the protocol and needs to provide a certificate of training to the Office of Compliance. If you add investigators to an approved project, please forward an updated list of investigators and their certificates of training to the Office of Compliance before they begin to work on the project.

Any change to the protocol must be submitted to the IACUC before implementing this change. Any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

You will need to submit an end-of-project report to the Office of Compliance upon completion of your research. Complete research means that you have finished collecting data and you are ready to submit your thesis and/or publish your findings. Should you not finish your research within the three (3) year period, you must submit a Progress Report and request a continuation prior to the expiration date. Please allow time for review and requested revisions. Your study expires **May 2, 2015**.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Emily Born

Emily Born
Compliance Officer
615-494-8918
emily.born@mtsu.edu