GENERALIST HABITAT SELECTION BY COTTONMOUTHS (*AGKISTRODON PISCIVORUS*) IN A HYDROLOGICALLY-DYNAMIC WETLAND

By

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John H. Kauphusman 06/06/2020

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ABSTRACT

Wetlands are typically transitional habitats that represent a dynamic hydrologic gradient between terrestrial and aquatic systems. As hydrologic fluctuations within a wetland can cause the environment to become periodically unsuitable and resource-limited, species with traits that can adapt to these environmental changes will be favored. Populations inhabiting highly dynamic environments with high frequencies of disturbance may have extensive niche overlap and less habitat specialization among residents. This study examined the habitat selection of North Cottonmouths (*Agkistrodon piscivorus*) inhabiting an isolated riverine slough along the Cumberland River (Middle Tennessee). I hypothesize that cottonmouths use generalist habitat selection as an adaptation to hydrologically-dynamic ecosystems they are inhabiting and have extensive habitat niche overlap among the population as a result of temporal instability of habitat resources.

Field collection was by visual-encounter surveys, and environmental variables (abiotic and biotic) were recorded for each snake location (N = 149), and paired with a random location to assess available macro- and microhabitats within the study area.

Multivariate analysis found that cottonmouths have a broad affinity for a variety of microhabitats within the riverine slough. In addition, I found no intra-specific differences in habitat use between adult males, adult females, and juveniles. However, there was an ontogenetic shift with younger individuals selecting more terrestrial habitats with a higher availability of cover objects.

Random Forest modeling determined that cottonmouths tended to select sites associated near or within water, even though fully terrestrial habitats were available, emphasizing cottonmouths affinity for wetland ecosystems within the gradient between terrestrial and aquatic systems

My findings suggest that a fluctuating hydrologic regime may promote cottonmouths to exhibit a strategy of generalist habitat use, potentially to increase opportunities to obtain resources in a dynamic wetland environment.

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CHAPTER I

INTRODUCTION

Wetlands are productive and biologically diverse systems that provide resources for a variety of wildlife species during part or all of their life stages (Mitsch & Gosselink, 2000). Wetlands are often viewed as transitional ecosystems connecting terrestrial and aquatic systems, where fluctuations in hydrology can influence wetland boundaries to encroach or recede from the upland environment (Brinson, 1993). These wet-dry hydrologic fluctuations can cause these transitional ecosystems community structures to become periodically unsuitable and resource-limited, as areas can become either flooded or dry completely (Brinson, 1993). Fluctuations typically range from either seasonal to stochastic, and can cause some species to use wetland areas only when favorable conditions are available (i.e. specialists), or adopt more opportunistic use as the hydrological environment changes (i.e. generalists) (Devictor, Julliard & Jiguet, 2008; Poff & Zimmerman, 2010). As geomorphology shapes catchment area and water level retention in wetland systems, different wetland associations have varying hydroperiods which range from stable seasonal-depressional wetlands (Winter & Rosenberry, 1998; Mitsch & Gosselink, 2000) to highly variable riverine-floodplains (Junk, Bayley & Sparks, 1989). These differing wetland associations may conditionally favor certain strategies of habitat use, specifically, heterogeneous environments with frequent disturbance regimes are commonly occupied and rapidly colonized by generalist species (Macarthur & Wilson, 1967; Adler & Wilson, 1987; Fournier *et al.*, 2015; Büchi & Vuilleumier, 2016).

As environmental factors can shape life history strategies at the geographic and macrohabitat scale for species, resource availability and competition may play a role in determining habitat use at the microhabitat scale for species within their selected macrohabitats (Mayor *et al.*, 2009). Competitive exclusion forces organisms to alter their strategy of resource exploitation and adopt ecological strategies within the continuum of highly specialized to broad use of diets and habitats (Greenbaum, 2004; Steen *et al.*, 2014). As interspecific competition shapes evolutionary strategies to partition resource use among species (Levin, 1970), so too does intraspecific competition (Amarasekare, 2003). Intraspecific competition is thought to be more prevalent within species, and to avoid the limiting effects, inferior competitors tend to select resources not exploited by the superior (Bolnick, 2004).

A semi-aquatic species that is commonly found in a variety of wetland associations throughout the southeastern United States is the Northern Cottonmouth (*Agkistrodon piscivorus*). Cottonmouths are dietary generalists, consuming anurans, fish, invertebrates, lizards, snakes, turtles, carrion, and smaller conspecifics (Gloyd & Conant, 1990; McKnight *et al.*, 2014). Cottonmouths are considered "habitat generalists" (Gloyd & Conant, 1990; Enst & Ernst, 2011), as they are found in many different aquatic-associated habitats, including high elevation streams (Hill & Beaupre, 2008), depressional wetlands (Eskew, Willson & Winne, 2009; Delisle *et al.*, 2019), and river floodplains (Ford, Brischoux & Lancaster, 2004; Roth, 2005).

Cottonmouths are often in high abundance within appropriate environments (Ford, 2002), and in stable seasonal-depressional wetland systems they have been found to express subtle ontogenetic differences in habitat selection (Eskew *et al.*, 2009) and seasonal habitat selection differences between sexes (Delisle *et al.*, 2019). Ontogenetic shifts in habitat selection and diet are thought to be a common competitive exclusion strategy in reptiles to partition resources (Vitt, 2000; Shine, Shine & Shine, 2003), and differences in habitat use between sexes in pit-vipers is relatively common (Reinert, 1984; Pearson, Shine & How, 2002). However, populations inhabiting highly dynamic environments with high incidence of hydrologic disturbance may experience niche overlap and reduce habitat specialization among residents due to the instability of resources (Marvier, Kareiva & Neubert, 2004; Mason *et al.*, 2011; Durso, Willson & Winne, 2013; Smith *et al.*, 2018; Stireman & Singer, 2018). Because cottonmouth populations occupy a variety of wetland associations, populations inhabiting heterogenous wetlands with frequent hydrologic disturbance regimes should have habitat selection be driven predominantly by local environmental fluctuations rather than by population interactions (Mason *et al.*, 2011; Stireman & Singer, 2018).

In this study, I sought to understand the site-specific habitat niche space and selection strategy of an isolated population of cottonmouths found within a hydrologically-dynamic riverine wetland slough during their active season (April – October). I hypothesize that cottonmouths residing in riverine wetlands use generalist habitat selection as an adaptation to hydrologically-dynamic ecosystems and have extensive niche overlap among the population due to the temporal instability of available resources. I predict that cottonmouths studied in a hydrologically-dynamic wetland would express a generalist selection strategy in habitat use, and share many aspects of habitat use among demographics within the population. To examine cottonmouth habitat niche space and habitat selection strategy within the slough, I (i) investigated the realized niche space at the macro- and microhabitat scale from comparisons of the available habitat in the context of the transitional wetland gradient from aquatic to terrestrial zones, (ii) determined whether there was a presence of intraspecific differences in habitat use among conspecifics, and (iii) determined which environmental factors promoted (were most predictive of) the occurrence of cottonmouths.

CHAPTER II

STUDY AREA

Data were collected along the Cumberland River Bicentennial Trail (CRBT), Cheatham County, Tennessee, USA (36˚17’ N, 87˚05’W). The site is a mixture of lowland forest, slough wetlands, upland limestone bluffs, and rocky outcrops. Cottonmouths use the upland forest and limestone bluffs as overwintering sites and occupy the lowland forest slough during the active season (April - October; Smith, 2009). The site is bordered by limestone bluffs and backwater streams along the northern portions, the Cumberland River along the west, and crop fields and urban development along the southern portions (Figure 1). These landscape features appear to largely isolate the population from dispersing to other potential habitats along the Cumberland River, as unpublish telemetry data (Smith, 2009; Fulbright, 2014) has shown that snakes did not leave the approximately 76 hectare study area.

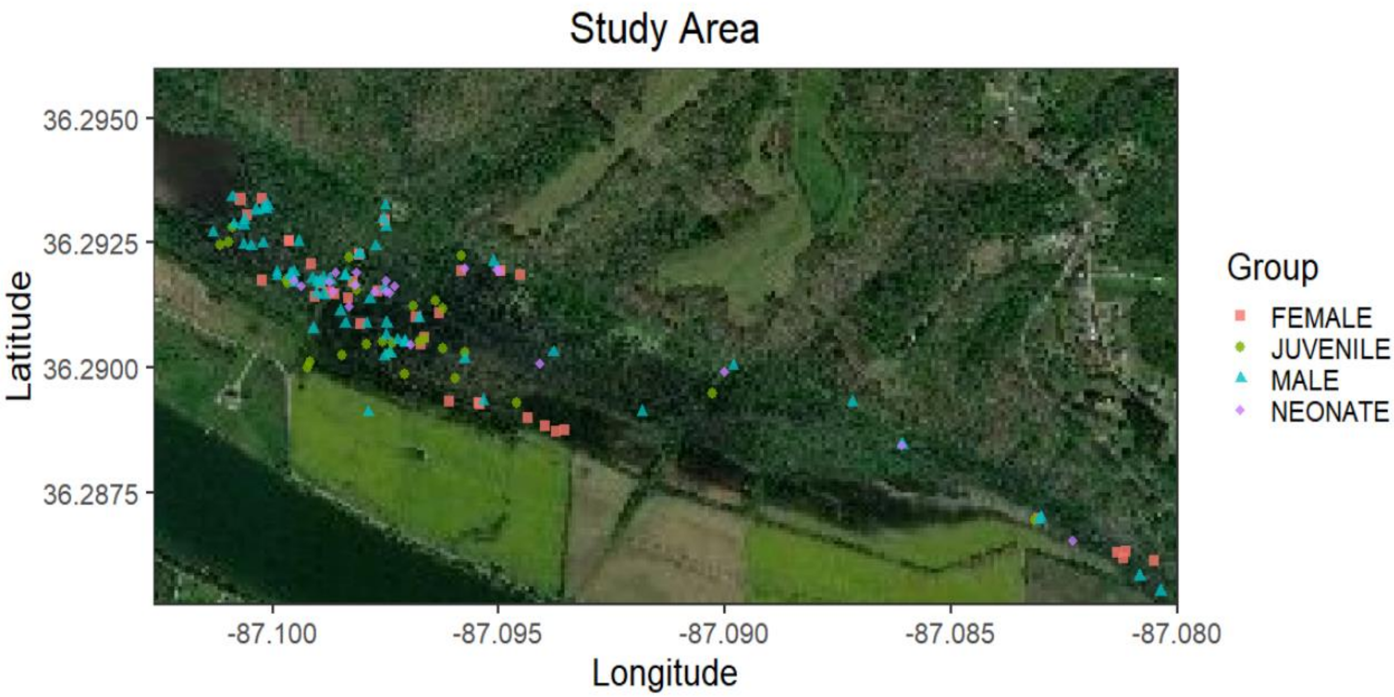

FIGURE 1: Map of the study area showing the locations of captured individual snakes within and along the slough south of the Cumberland River Bicentennial Trail, Cheatham County, TN. Group represents the conspecific category for each individual cottonmouth within the population; adult female (red square), juvenile (green circle), adult male (blue triangle), and neonate (purple diamond).

The study area is predominately buttonbush (*Cephalanthus occidentalis*) slough in the central portion (12.6 ha), having a relatively open canopy, deep water (50-250 cm), and mats of floating vegetation (*Hydrocotyle ranunculoides & Ludwigia palustris*) scattered throughout. The seasonally-flooded lowland forest (20.9 ha) is dominated by red maples (*Acer rubrum*) giving dense canopy cover, and functions as a transition area between the slough and upland forest containing limestone bluffs and hibernacula. Toward the southern portion between the slough and crop fields is edge habitat (15.4 ha) dominated by dense grass vegetation that occasionally floods (<10 cm) throughout the year. The upland forest habitat (16.3 ha) is dominated by oak trees (*Quercus* spp.) with dense over story canopy cover and high percentage of bare soil on the forest floor. Sycamore Creek flows into the slough from the upland forest and provides varying inflows of water to the center of the slough, along with periodic flooding from the Cumberland River, and groundwater seepage. During seasonal precipitation events, the multiple points of inflow cause periodic flooding within the slough. Both the Cumberland River and Sycamore Creek follow a general trend of high flow in the early spring and low flows in the fall, although, both have stochastic flooding events annually (Figure 2). Because the Cumberland River and Sycamore Creek are hydrologically connected to the slough, it can be assumed that the slough follows a similar hydroperiod of flooding and drying events.

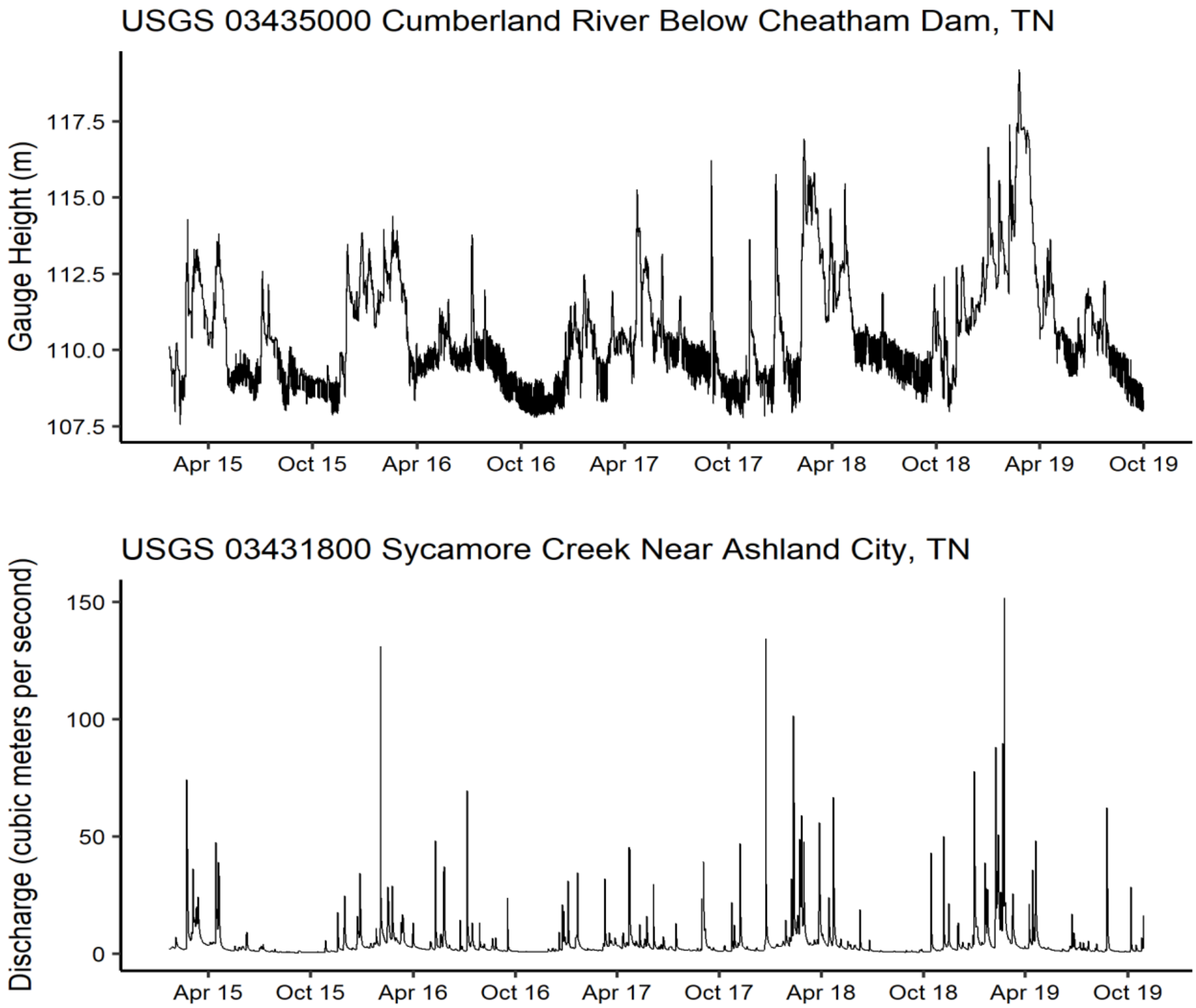

FIGURE 2: (above) Hydrographs of a five year hydrologic cycle (from April 2015 – October 2019) of the Cumberland River below Cheatham Dam, TN recorded at 36°19'22.26" N & 87°13'41.73" W, and Sycamore Creek Near Ashland City, TN (below) recorded at 36°19'11.89" N, & 87°03'04.29"W. Both waterways flow into Cumberland River Bicentennial Trail riverine wetland slough and were recorded within 8 km from the study site. Information was provided by the USGS National Water Information System (https://waterdata.usgs.gov/nwis).

CHAPTER III

METHODS

Field Collection

I conducted visual encounter surveys during the active seasons (April – October) and snakes were captured using tongs and retained in plastic restraining tubes (Midwest Tongs, Greenwood, MO, USA). To maximize the opportunity to identify individuals, visual encounter surveys were conducted with a minimum of two surveyors each survey day for a minimum of 4 hours. Surveys occurred between 06:30 – 22:00 hours, and were conducted once or twice a week for each week during the populations' active season. All aspects of habitat use for the cottonmouth population, such as seasonal, year, and time of day were combined for analyses, as the goal was to identify the populations' overall selection strategy for macro- and microhabitats within the riverine slough. To certify that the study area was sampled equally, a 200 m^2 grid was created using the Fishnet Tool in ArcMap 10.6.1 (ESRI, Redlands, CA, USA) for the 78 ha site, and a minimum of one 200 m^2 area was surveyed for each field day systematically moving to a new grid cell for each event.

For individual identification, each snake was implanted subcutaneously with a Passive Integrated Transponder (PIT) tag (Biomark, Boise, ID, USA) on the ventral side, posterior to midbody. Tags and implant syringes were soaked in Benzalkonium Chloride (Benz-all; VetPro, Northfield, MN, USA) for 2 – 3 minutes prior to tag implantation, and an antiseptic liquid bandage (Vetclose, Portland, ME, USA) was applied to the insertion area to facilitate healing. Snout-vent length (SVL) and tail lengths (TL) were measured, and snakes were sexed by either everting hemipenes or by probing (Blanchard & Finister, 1933). Individuals were considered adults if their SVL was greater than 500 mm, individuals with an SVL between 300 – 500 mm were considered juveniles (Burkett, 1966; Eskew *et al.*, 2009), and neonates were considered to be individuals less than 300 mm SVL (Koons *et al.*, 2009). Gravid females if captured were removed from analysis to reduce the effect of habitat selection being affected by gravidity. Body mass was measured with a Pesola spring scale. All handling and tagging activities were approved by the Institution Animal Care and Use Committee at Austin Peay State University (IACUC Protocol Approval Numbers 12.005 and 19.015).

Habitat selection was quantified at the location in which the individuals were first sighted. There I recorded a suite of biotic and abiotic environmental variables to identify what was the extent of the populations' affinity to sites within the transitional wetland gradient from the terrestrial to aquatic system (macrohabitats). In addition, these environmental variables included different aspects of cover objects and vegetation to identify microhabitat preferences within each macrohabitat (Table 1). A 1 m^2 quadrat was used to assess the habitats selected by cottonmouths compared to those available in the environment. A randomly chosen comparison site was paired with each snake location using the Random Points Tool in ArcMap 10.6.1 within the study area. Secondarily, fixed sites at each cell corner of the survey grid were quantified to assure all available macro- and microhabitats in the study area were quantified for comparison (Figure 3).

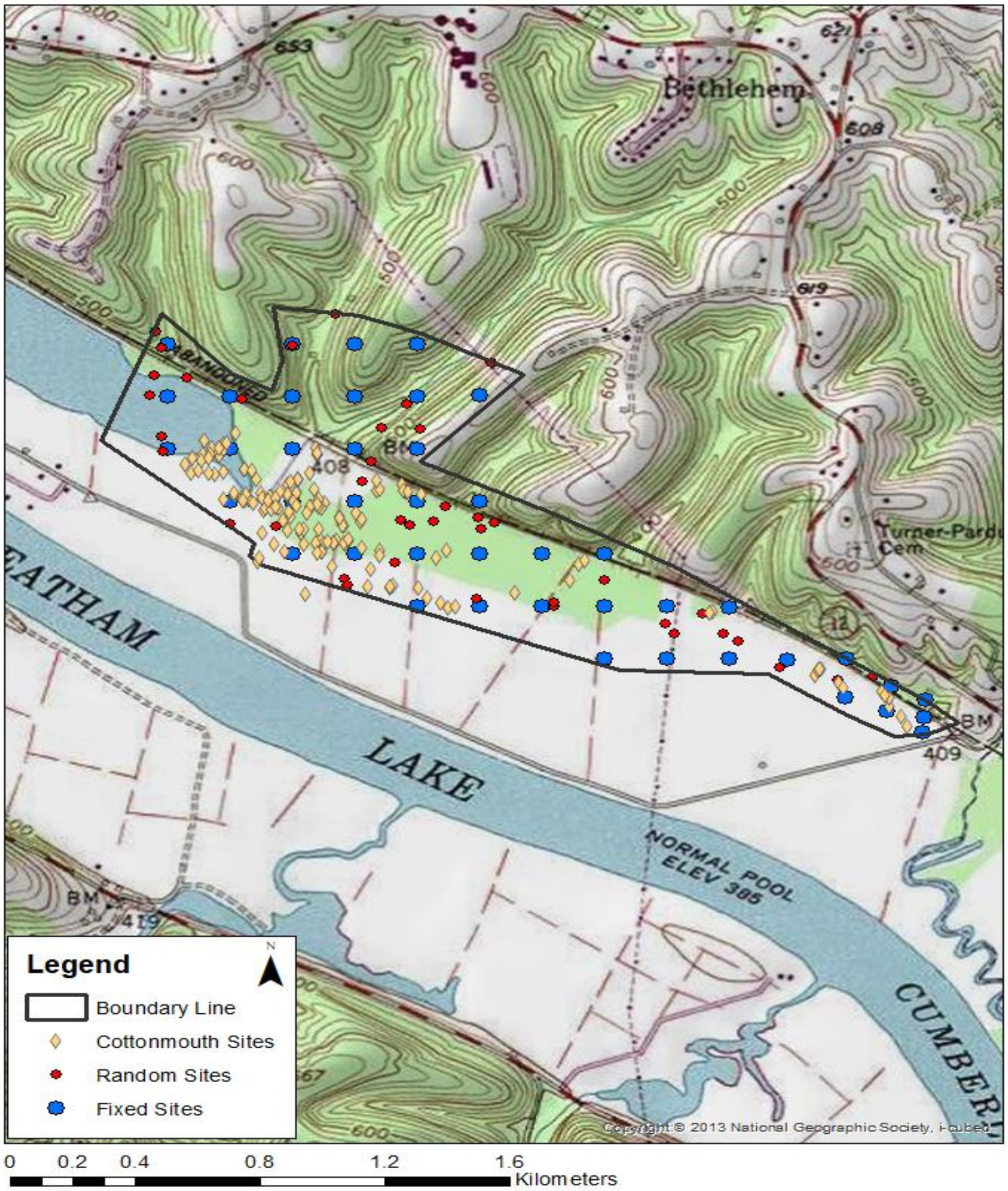

FIGURE 3: Map of the cottonmouth and available habitat (random and fixed) sites quantified during field collection for 2012, 2013, and 2019. The boundary line indicates that total area (78 ha) of the riverine slough and its adjacent upland and deep aquatic environments.

Analysis of Habitat Selection: Ecological Niche

The concept of the ecological niche was used as a framework to analyze the range of environmental variables cottonmouths selected within the habitats available within the study area. A correlation matrix was used to identify collinear environmental variables, and variables with a coefficient above |0.70| were removed from analysis (Dormann *et al.*, 2013). I found land cover and water cover to be highly correlated, as well as three temperature variables: ambient, surface, and substrate temperature. Therefore, I chose to remove land cover and substrate temperature, while averaging both surface and ambient temperature to a single mean temperature variable, leaving 16 environment variables for analysis (Table 1). I used Principal Component Analysis (PCA) on correlations to reduce dimensionality of the environmental variables and to elucidate trends of cottonmouth realized habitat niche width. Each dimension (Principal Component axis) was ranked based on explained variance (Peres-Neto, Jackson & Somers, 2003) and I retained the first three principal components for further analysis, using the scree method on the eigenvalues (Jackson, 1993). The scree method retains eigenvalues that explain more variance than would be expected by chance. Therefore, the largest eigenvalues above the average eigenvalue score, along with the first subsequent component below the largest eigenvalues, are retained (Jackson, 1993). PCA was performed using R (R Core Team, version 3.6.1, 2019. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Australia) and plotted using the *ggplot2* package (Wickham, 2016).

As PCA is predominantly a visual ordination technique, I used a Permutational Multivariate Analysis of Variance (PERMANOVA; 9999 permutations) and Permutational Analysis of Multivariate Dispersion (PERMDISP; 9999 permutations) on Euclidean distances to determine whether cottonmouth use of habitat was significantly different than habitat available in multivariate space. PERMANOVA tests for discrimination between groups within multivariate space, and a significant p-value (using $\alpha = 0.05$) indicates that groups differ as a function of their distances between centroid locations, their relative dispersion of variance, or both (Anderson, 2001). The advantages of PERMANOVA are that it does not assume normality and homogeneity of variance compared to a MANOVA (Delgado-Baquerizo *et al.*, 2015). PERMDISP tests for homogeneity of multivariate dispersion among groups, and is used to determine whether differences in dispersion is the primary cause of significance in the PERMANOVA test (Anderson, Ellingsen & McArdle, 2006; Assis *et al.*, 2013). A significant p-value from PERMDISP indicates that dispersion is heterogeneous among groups in multivariate space (Anderson *et al.*, 2006). PERMANOVA/PERMDISP tests were computed in R using the *vegan* package (Oksanen *et al.*, 2019).

Analysis of Habitat Selection: Intra-specific Differences

To test for intra-specific differences in habitat selection, an ANOVA (Analysis of Variance) was used to compare conspecific group PCA scores (adult males, adult females, juveniles, and neonates) and was followed by Tukey's HSD post-hoc tests. Linear regression was used to evaluate the presence of an ontogenetic shift in habitat use by comparing snake body size (SVL) with individual component scores on the first three PC-axes (Eskew et al. 2009).

Analysis of Habitat Selection: Modeling Habitat Preference

Random Forest (RF) classification (Breiman, 2001) was used to identify the important environmental variables that best predict cottonmouth habitat selection. RF is a nonparametric classification method that builds an ensemble model based on a large set of decision trees. For each tree, a bootstrap sample of approximately 64% (i.e., bag sample) was used as a training set, and the remaining data withheld as a validation set (i.e., out-of-bag [OOB]) (Breiman, 2001; Severson *et al.*, 2017). RF uses all trees constructed within a model to predict and estimate the overall OOB error rate, which is treated as a metric of model accuracy (Breiman, 2001; Cutler *et al.*, 2007; Severson *et al.*, 2017).

I constructed an optimal model for classifying cottonmouth habitat selection from the environmental variables by comparing sites of cottonmouth occurrence to available habitat sites as pseudo-absences. I applied 30-iterations of RF models, using the *randomForest* package (Breiman *et al.*, 2018), where each iteration used 6,000 decision trees on a 10-fold cross-validation training set (similar to Severson et al. 2017). Next, a cross-validation test set was applied to calculate mean model accuracy and identify a minimal error rate model. To improve model performance, I ran the first RF model applying the Model Improvement Ratio (MIR, Murphy et al., 2010) technique to increase model parsimony by identifying the minimum number of variables to include. The model with the minimum number of variables and the minimum OOB error rate was selected for model iterations.

RF Model performance was evaluated by the minimum, maximum, and mean OOB accuracy from the 30-iterations. The independent validation set from cross validation was used to assess model accuracy, sensitivity (the proportion of cottonmouth habitat correctly classified), specificity (the proportion of general habitat presence correctly), the area under the curve of a Receiver Operator Characteristic (AUC; a measure of how evenly the model predicts sensitivity and specificity), and the Kappa statistic (a measure of how much better the model predicted cottonmouth and general habitat than would be expected by random chance) (Cutler *et al.*, 2007).

To assess the importance of each environmental variable in predicting cottonmouth occurrences I used the minimal error rate model to produce relative variable importance values based on the Mean Decrease Accuracy (MDA). This is a variable importance metric within a RF model that is calculated as follows; for each tree, the initial prediction error on the OOB portion of the data is recorded, then the OOB prediction error is permuted for each predictor variable. The difference between the initial prediction error and permuted prediction error is then averaged over all trees. (Han, Guo & Yu, 2016). The MDA for variables classifying cottonmouth habitat was used to calculate relative importance in predictability of cottonmouth occurrence for each environmental variable. Partial dependence plots were constructed from the six most important environmental variables (~50% cumulative relative importance) to illustrate the relationship of the environmental variables to predict cottonmouth occurrence.

CHAPTER IV

RESULTS

Two observers surveyed on 96 days during three-years (2013, 2014, & 2019) of field study and recorded sixteen environmental variables for 149 individual cottonmouths and 174 available habitat comparison sites (134 random and 40 systematic sites). Captured individuals included 60 adult males, 39 adult females, 28 juveniles, and 22 neonates. Adult males averaged 744 ±132 mm in SVL, adult females were 620 ± 60 mm, juveniles were 408 ± 55 mm, and neonates averages 251 ± 24 mm. During spring (April – May) surveys, I found 48 individuals during 20 outings to the study area, 84 individuals during 64 outings in the summer (June – August), and 17 individuals during 12 outings in the fall (September – October).

Habitat Selection: Ecological Niche

The first three Principal Components describing cottonmouth habitat choice collectively explained 43.7% of the variation within the environmental variable structure (Table 1). Environmental variables associated with PC-1 describe a terrestrial to aquatic gradient (Figure 4), where distance to water and canopy closure were positively associated, and percent water and water depth were negatively associated. PC-2 described availability of cover objects, where log diameter and fallen log cover were positively associated. PC-3 described herbaceous cover, where vegetation cover, cover height, and woody stem height were negatively associated. Collectively, the three components explain an environmental gradient between flooded and non-flooded habitats along with available refuge cover where cottonmouths had broad preferences for sites within the wetland system between the extremes of aquatic and terrestrial sites, and slight preferences for microhabitats with more cover objects.

TABLE 1: Factor loadings from Principal Component analysis of environmental variables measured within a 1-m^2 quadrat of cottonmouth sites and random selected sites for the available habitat comparison. These 16 environmental variables describe the macrohabitats of the study area of the transitional zone between the terrestrial and deep-aquatic system, and the microhabitats within each macrohabitat.

Variable (units)	**Description**	**PC-1**	**PC-2**	**PC-3**
Canopy Closure (%)	Percentage of canopy above quadrat	0.334	0.144	0.166
Rock Cover (%)	Coverage within quadrat	0.169	-0.048	0.410
Leaf Cover (%)	Fallen leaf coverage within quadrat	0.328	-0.257	-0.004
Vegetative Cover (%)	Terrestrial plant coverage within quadrat	-0.051	-0.131	-0.556
Fallen Log Cover (%)	Coverage within quadrat	0.065	0.478	0.157
Woody Stem Height (cm)	Height of largest living woody stem nearest to quadrat	0.120	-0.001	-0.301
Cover Height (cm)	Height of cover object closest to quadrat	-0.028	0.131	-0.429
Distance to Cover (cm)	Distance to nearest cover object	-0.087	-0.437	0.039
Log Diameter (cm)	Diameter of largest log within quadrat	0.054	0.517	0.093
Distance to Water (cm)	Distance to nearest water source	0.395	-0.281	-0.025
Water Depth (cm)	Water depth measured within quadrat	-0.406	0.017	0.042
Percent Water (%)	Coverage within quadrat	-0.459	-0.037	0.126
Percent Woody Debris (%)	Woody material less than 4 cm in diameter within quadrat	0.157	0.204	-0.247
Percent Floating Vegetation (%)	Floating and emergent aquatic vegetation within quadrat	-0.351	0.023	-0.077
Percent Bare Soil (%)	Coverage within quadrat	0.189	0.185	-0.282
Mean Temperature (°C)	Mean ambient and surface temperature within quadrat	-0.074	0.183	-0.145
PCA Statistics				
Eigenvalue		1.90	1.38	1.22
Explained Variance		22.60	11.81	9.27

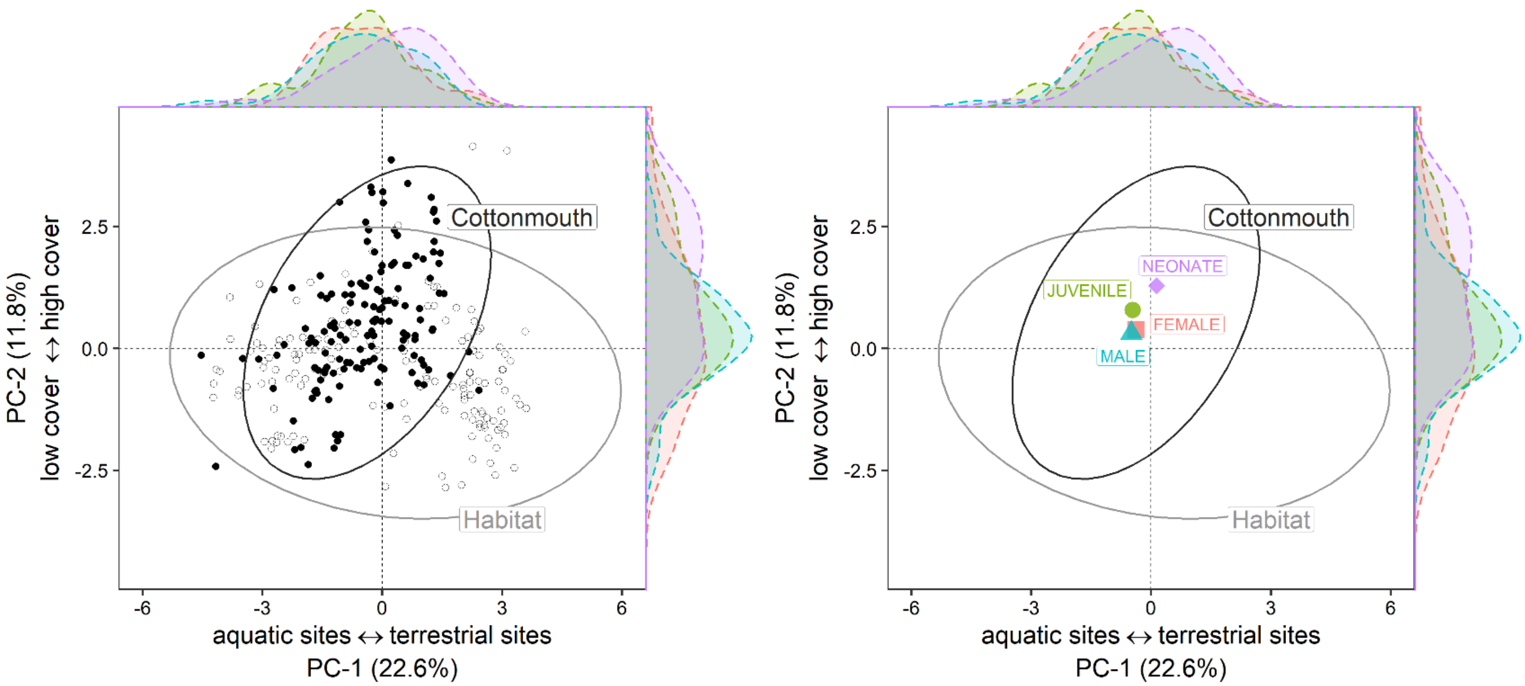

FIGURE 4: Principal Component Analysis (PCA) of 16 environmental variables measured for *Agkistrodon piscivorus* (cottonmouth) and available habitat comparison sites. Ellipses represent 95% confidence intervals for cottonmouth (black) and available habitat (open circle). (left) PCA comparing the observation of cottonmouth and available habitat sites. (right) PCA with mean component scores of the intra-specific cottonmouth conspecifics; adult males (blue), adult females (red), juveniles (green), and neonates (purple). Point density curves for conspecific groups are represented for PC-1 (above), and PC-2 (right). PCA expresses cottonmouths to share broad similarities with the available habitat comparison sites, while neonates expressed some degree of separation toward the other cottonmouth conspecifics.

The PERMANOVA/PERMDISP tests found a difference between cottonmouth habitat selection and available habitat within multivariate space (PERMANOVA; P= 0.001; Pseudo-$F_{1,314}$ = 20.4). The results from the PERMDISP test showed that differences between cottonmouth sites and available habitat sites were attributed to the heterogeneous distribution of variance of the environmental variables between the two groups, where cottonmouth habitat was nested within the available habitat measured (PERMDISP; P<0.001; $F_{1,314}$ = 25.02). In multivariate space, the mean distance to centroid locations was 4.02 for available habitat, while cottonmouth habitat was 3.26 (Figure 4). The PERMANOVA/PERMDISP tests supported results of the PCA, in that the heterogeneous dispersion between cottonmouth habitat selection and available habitat showed that cottonmouths habitat resources deferred slightly than the occurrence of available habitat in the environment (Figure 4). Specifically, the restricted distribution of sites used by cottonmouths, in relation to available habitat, was due to avoidance of upland and deep aquatic areas during the active season in favor of occupying transitional-associated sites (likely foraging and ambush sites) within the more hydrologically-dynamic wetland.

Habitat Selection: Intra-specific Differences

There were no differences among conspecific groups (adult males, adult females, juveniles, and neonates), in mean PCA component scores of habitats used, within PC-1 ($F_{3,145}$ = 1.44, P = 0.23) or PC-3 ($F_{3,145}$ = 1.08, P = 0.36; Table 2), however, there were differences in cover object microhabitat preferences among conspecifics within PC-2 ($F_{3,145}$ = 3.6, P = 0.02; Table 2). Component scores of adult females and adult males were lower than neonates (Tukey's HSD female-neonates; P = 0.048; Tukey's HSD male-neonates: P=0.015). Specifically, neonates mean component scores were 0.98 higher than adult males and 0.91 higher than adult females, indicating neonates were more positively associated with selecting fallen log cover. Adult males, females, and juveniles were found to use a wide variety of the available microhabitats, as density curves of PC-1 and PC-2 had wide distributions (Figure 5). Females and juveniles expressed more of a preference for the wetland edge areas than males (Table 2).

TABLE 2: Mean component scores of principal component analysis on intra-specific habitat selection within cottonmouths with ANOVAs used to compare conspecific groups. ANOVA showed that on PC-1 and PC-3 there was no difference in habitat selection. However, on PC-2 there was a significant difference in habitat selection among the population.

Cottonmouth conspecifics	PC-1	PC-2	PC-3
Female	-0.384	0.380	0.047
Juvenile	-0.457	0.784	-0.208
Male	-0.499	0.306	-0.383
Neonate	0.137	1.291	-0.153
ANOVA			
$F_{3,145}$	1.443	3.573	1.083
P-value	0.233	0.016*	0.358

Regression of PCA scores relative to SVL indicates that body size was related to site selection along PC-1 and PC-2 axes (Figure 5A; $F_{1,147} = 5.28$; $P < 0.023$; $r^2 = 0.35$; Figure 5B; $F_{1,147} = 7.06$; $P = 0.008$; $r^2 = 0.046$). This suggests an ontogenetic shift with larger individuals found more frequently in flooded sites containing less cover objects compared to smaller individuals tending to choose more terrestrial sites in the wetland with prominent available cover objects, specifically fallen logs. There was no apparent relationship between body size and PC-3 component scores (Figure 5C; $F_{1,147} = 0.47$; $P = 0.49$; $r^2 = 0.0035$), suggesting all individuals shared microhabitat preferences for vegetative cover.

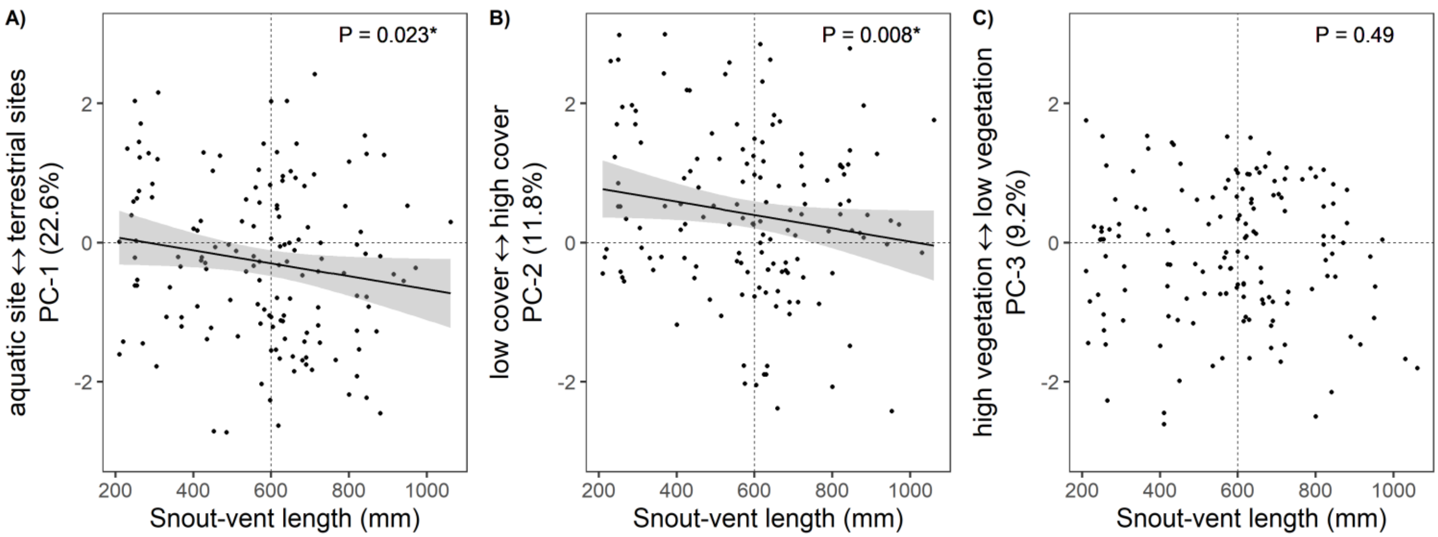

FIGURE 5: Linear regression of Principal Component scores showing ontogenetic shift in habitat selection as a function of body size (snout-vent length). Linear regression shows a significant negative slope for PC-1 and PC-2 expressing an ontogenetic shift in the population, however there was no relationship of an ontogenetic shift on PC-3.

Habitat Selection: Modeling Habitat Preference

Based on the Model Improvement Ratio, no environmental variables were excluded from RF analysis as OOB error rates all increased with removal of each environmental variable when compared to the global RF model. Cumulatively, RF models (30-iterations) had a mean accuracy of 89.0% from 10-fold cross-validation and an 84.4% mean accuracy from the OOB (Table 3). The maximum RF model had a cross validation accuracy of 89.3%, an 85.1% OOB accuracy, and 94.4% AUC, suggesting that I was able to derive a good model for predicting cottonmouth occurrence (Table 3). Error rates for each RF model stabilized at approximately 3,000 trees, indicating that the 6,000 trees used in calculations were sufficient to determine what areas were cottonmouth sites compared to available habitat sites. Throughout all 30-iterartions of RF models, available habitat was misclassified at approximately the same rate as cottonmouth habitat (Mean RF model class error rate was 15.5% for available habitat; 15.7% for cottonmouth occurrences), suggesting that about 15.6% of sampled sites were indistinguishable as either cottonmouth sites or unused available habitat sites.

TABLE 3: Performance metrics for the 30-iteration random forest model on cottonmouth habitat preference within the study area. Overall, modeling metrics show that the random forest model was very accurate at predicting cottonmouth selected sites from available habitat sites.

Random Forest Models	Accuracy	Kappa	Sensitivity	Specificity	OOB	AUC
Maximum	0.893	0.786	0.867	0.923	0.851	0.944
Mean	0.890	0.781	0.862	0.923	0.844	0.934
Minimum	0.857	0.716	0.800	0.923	0.837	0.928
N(trees) = 6000						
OOB = Out of Bag Accuracy						
AUC = Area Under the Curve						

The maximum-accuracy RF model predicted the most important environmental variables in determining the occurrence of cottonmouths in the backwater slough study area were distance to water, bare soil, rock cover, and water depth. In addition, the environmental variables with secondary relative importance were percent water and distance to a cover object (Figure 6). These six most influential environmental variables accounted for roughly half of the RF modeling predictions, and further supports the multivariate analysis, indicating cottonmouths' strong association within and near the wetland system. Although available cover objects were an important component of cottonmouth microhabitat in the multivariate analysis, the RF modeling indicated that there was no specific preference for a particular cover object type; terrestrial vegetation cover, floating vegetation cover, and log cover as all had low relative importance. Partial dependence plots (Figure 7) indicate that the probability of cottonmouth occurrence was negatively related to distance from a water source, as areas within 100 cm of water had the highest probability of occurrence (> 25%; Figure 7B). In addition, modeling found that areas that had bare soil cover had a 30% probability of cottonmouth occurrence, decreasing as amount of bare soil cover increased (>40%; Figure 7A). Rock cover was found to have a negative relationship with predicting cottonmouth occurrence, as rock cover increased from zero, the probability that the area would be inhabited by a cottonmouth became increasingly less likely (Figure 7C). Modeling found that the highest probability of occurrence for water depth was between 1 to 25 cm, and occurrence decreased as depth increased past 15 cm (Figure 7D). Areas with water cover between 10 to 90%, were found to predict a roughly 15% probability of cottonmouth occurrence (Figure 7E). As distance to cover increased, modeling found that there was an increasing negative association with predicting cottonmouth occurrence (Figure 7F).

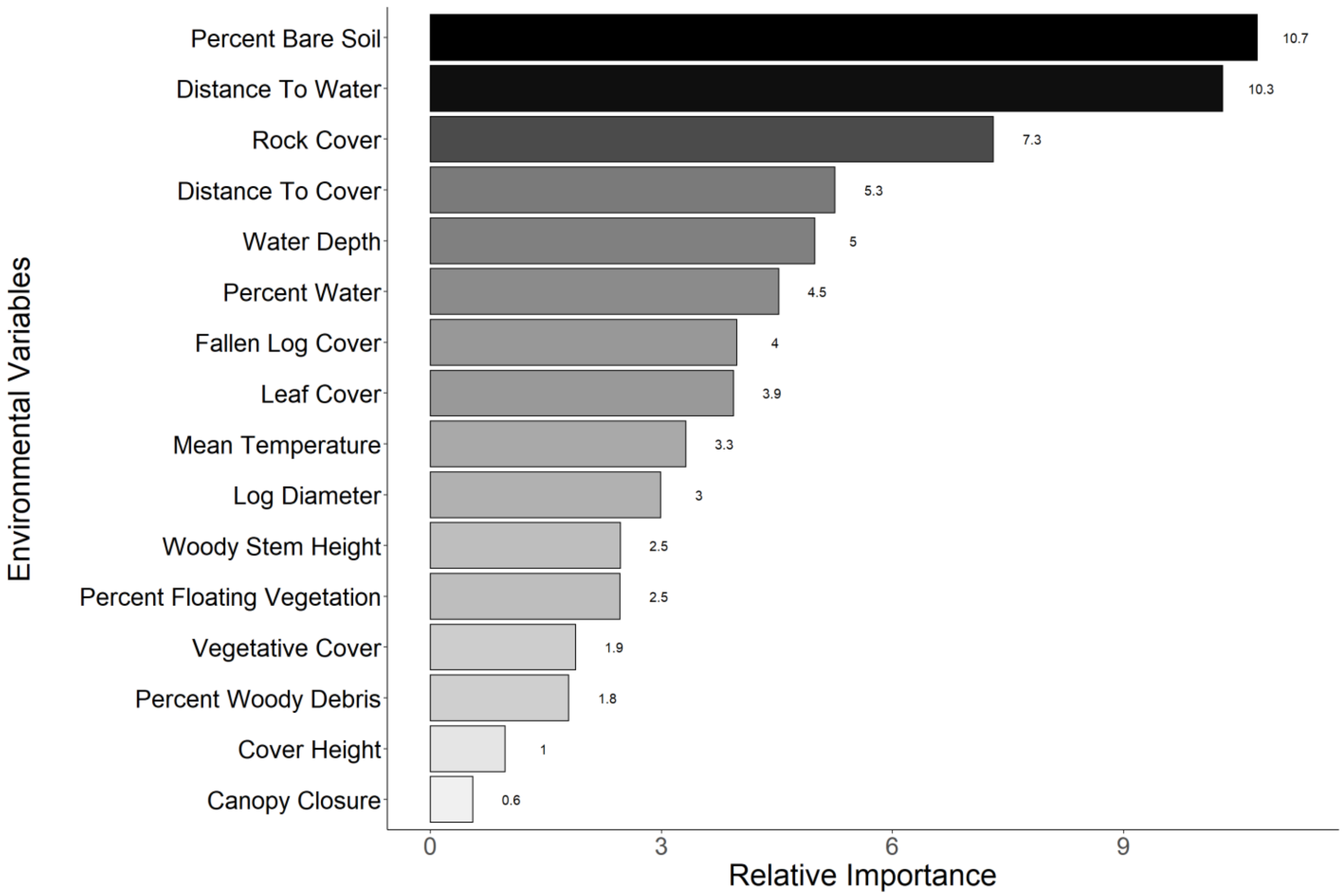

FIGURE 6: The relative importance on the environmental variables on cottonmouth (*Agkistrodon piscivorus*) habitat selection based on the Mean Decrease Accuracy in the minimum error rate Random Forest Model. The six most predictive environmental variables described the locations of sites within the transitional gradient between terrestrial and deep-aquatic macrohabitats, while the other ten variables described microhabitat features and had low predictability describing a use for a variety of microhabitats.

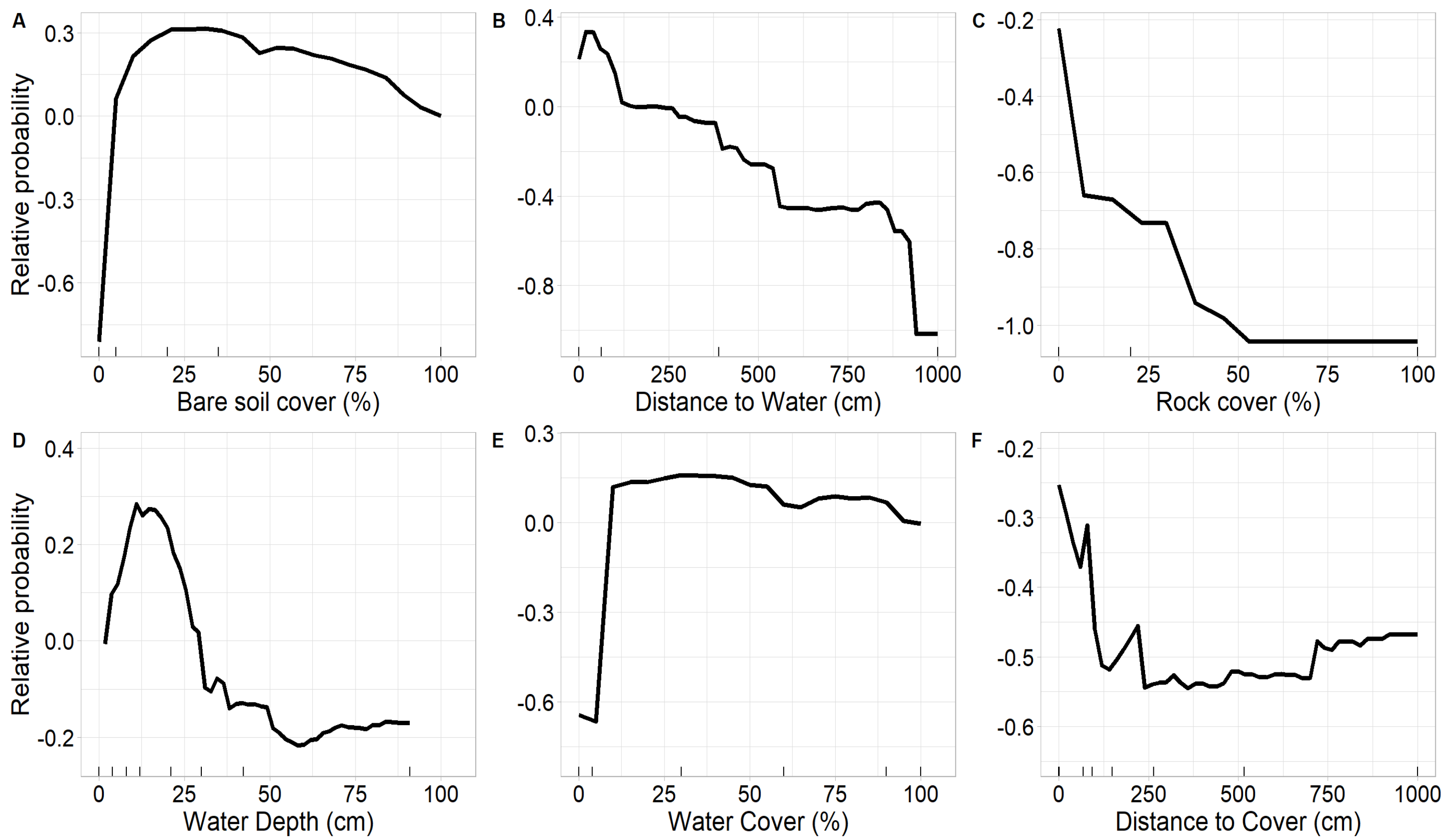

FIGURE 7: Partial dependence plots with relative probability of cottonmouth occurrence for the six most important contributing variables (based on mean decrease accuracy). Bare soil cover (A), distance to water (B), rock cover (C), water depth (D), water cover (E), and distance to cover (F) were measured at sites of cottonmouth locations during visual encounter surveys. Upward ticks on x-axis indicating mean grouping of cottonmouth occurrence for each environmental variable used to calculate relative probability for specific values.

CHAPTER V

DISCUSSION

I found that an isolated population of cottonmouths expressed a generalist selection strategy in microhabitat use within the hydrologically-dynamic riverine slough system. Sites selected by snakes were similar to available habitats measured in the study area, describing habitat use for a wide variety of microhabitats. Although selected sites were more restricted in multivariate space compared to the available habitats, this can be attributed to cottonmouths avoiding upland and deep aquatic macrohabitats during the active season and instead remaining within the wetland-slough system.

The multivariate results identifying cottonmouths' generalist habitat affinity for microhabitats within the riverine slough gives support to the hypothesis that the generalist strategy is an adaptive response to the hydrologically-dynamic ecosystem. Because the study site is hydrologically driven by inundation from the Cumberland River and Sycamore Creek, causing periodic high and low inflows, the generalist strategy should be more beneficial than the specialist strategy in coping with periodic local resource disturbances. Ecosystem disturbances (flooding, droughts, fire, & anthropogenic) reduce available resources for unadapted species, thus decreasing species richness within an area, especially among habitat specialists (Marvier *et al.*, 2004; Devictor *et al.*, 2008; Beesley *et al.*, 2012). However, generalist species have been found to routinely tolerate environmental perturbations (Beesley *et al.*, 2012; Rossetti *et al.*, 2017), specifically within areas that have regular flood regimes (Magalhães *et al.*, 2002; Gerisch *et al.*, 2012; Turić *et al.*, 2015). Because the generalist strategy is not structured to specific habitats and diets within an environment, generalist species are more tolerant of disturbance and adopt opportunistic strategies to acquire resources for survival (Balcombe *et al.*, 2005). Other studies of semi-aquatic species exhibiting broad habitat preferences have attributed this behavior as a mechanism for maintaining residency in fluctuating hydrological environments (Roe, Kingsbury & Herbert, 2004; Luiselli, 2006; Durso *et al.*, 2013). As water tables oscillate between flooding and drought conditions (Junk *et al.*, 1989), species that express ecological plasticity in resource use will be able to fully exploit habitats, regardless of complexity (Marvier *et al.*, 2004; Devictor *et al.*, 2008; Sol, Lapiedra & González-Lagos, 2013).

I found that the cottonmouths did not have differences in habitat selection between sexes of adults, but did identify an ontogenetic shift in microhabitat selection. Because the results found adult sexes to share many aspects of habitat use in the riverine slough, this yielded some support for the hypothesis that hydrologically-dynamic environment promotes niche overlap within a population due to instability of resources. Dynamic environments have been shown to promote sympatry in a variety of taxa; such as alpine plants (Callaway *et al.*, 2002), marine mammals (Elliser & Herzing, 2016), and semi-aquatic snakes and turtles (Escoriza & Hassine, 2017). The findings by Delisle *et al*. (2019a) of cottonmouth sexes sharing broad habitat preferences in stable depressional wetlands are similar to my results for cottonmouths within a riverine slough. However, Delisle *et al*. (2019a) were able to identify seasonal shifts in preferences between sexes, with females selecting more concealed refuge sites around edge habitats during the spring. The authors suggest this was likely a concealment strategy related to emaciation brought on during the prior year's gestation and parturition. In contrast, males were found to predominently select aquatic habitiats in the middle of the wetland during the summer, which they attributed to optimal foraging for large aquatic prey (Delisle *et al.*, 2019). When sexually dimorphic species are shown to have sex-specific differences in habitat use (Ardia & Bildstein, 2001; Butler & Losos, 2002), the relationship is usually driven by reproductive behaviors (Reinert, 1984; Roth, 2005). The findings of indistinguishable habitat niche occupancy between adult conspecifics add support for the conclusion that dynamic wetlands with stochastic processes facilitates niche overlap because of the instability of resources (Durso et al. 2013).

I found evidence for an ontogenetic shift in habitat use. Ontogenetic shifts in habitat use or dietary preferences are usually attributed to competitive exclusion between older and younger individuals, especially when older/larger individuals are cannibalistic (Foster, Garcia & Town, 1988; Keren-Rotem, Bouskila & Geffen, 2006). The observed ontogenetic shift parallels findings that of Eskew et al. (2009), in that younger cottonmouths tend to prefer terrestrial habitats more than adults. This supports the notion of habitat selection being shaped by competition between larger and smaller individuals, as cottonmouths are opportunistically cannibalistic (Glaudas *et al.*, 2007). Other studies of ontogenetic shifts have attributed the phenomena to younger individuals being morphologically constrained to prey resources and microhabitats until they reach adult size (Vincent, Herrel & Irschick, 2004; Barriga & Battini, 2009). Snakes are morphologically constrained to prey items that can fit in their mouths; because of this younger cottonmouths have been found to select smaller prey such as amphibians and arthropods (Vincent *et al.*, 2004; Eskew *et al.*, 2009). In addition, smaller cottonmouth individuals are limited in swimming performance, and are more vulnerable to aquatic predators such as fish (*Micropterus* spp.), snapping turtles (*Chelydra serpentina*), and wading birds (e.g., *Ardea* spp.) compared to larger conspecifics (Winne & Hopkins, 2006; Isaac & Gregory, 2007; Enst & Ernst, 2011). Within the study area, the edges of the riverine slough and lowland mixed forest habitats provided the highest density of fallen logs, root masses, and tall vegetation cover. Amphibian prey remain in moist refugia during overland migration to aquatic sites, and use similar microhabitats, such as downed trees, as migratory pathways to the wetland edges from forested systems (Gibbs, 1998). Because younger cottonmouths have more morphologically constrained diet and poorer swimming ability, they select sites along the wetland edge. These sites along the wetland edge provide concealment from potential predators, and as an added benefit allow for natural pathways to attract and ambush their preferred prey.

Since one of the strongest habitat predictors was a proximate association to water, it is likely that an adaptation to fluctuating hydrology facilitates broader microhabitat preferences, as most habitats within the study area were subjected to flooding during some point within the active season. The results of cottonmouths having a strong association with aquatic sites mirrors Roth's (2005), findings that cottonmouths were generally located near the water/shore interface, having linear home ranges along a stream system. While other semi-aquatic reptiles often leave wetland systems when conditions become dry and switch to foraging in upland habitats (Mushinsky, Hebrard & Vodopich, 1982; Bodie & Semlitsch, 2000) or move to adjacent areas with suitable wetland systems (Roe, Kingsbury & Herbert, 2003; Willson *et al.*, 2006), the cottonmouths remained within the slough choosing sites that follow the flood regime. Since riverine habitats support a high diversity and abundance of prey items (Robertson & Weatherhead, 1992; Durso *et al.*, 2013), this may explain cottonmouths role as one of the top predators in wetland systems (Himes, 2003). The tendencies of cottonmouths to colonize aquatic habitats as they become resource rich and high tolerance to drought conditions (Willson *et al.*, 2006) may explain how the population can persist in a variety of wetland environments. This adaptation to tolerate droughts may enable the species to occupy foraging and ambush sites during dry conditions, and be readily positioned for returning prey species when conditions again become more hydrologically favorable (Semlitsch & Bodie, 2003; Vincent *et al.*, 2004). In addition, this strategy to remain near fluctuating water sources may promote better access for the species to use more suitable thermal microhabitats (Mueller & Gienger, 2019), and provide the ability to escape to aquatic refugia from potential predators (Weatherhead & Robertson, 1992).

Overall, the results indicate that cottonmouths opportunistically use microhabitat selection to counteract limited resource availability due to habitat disturbance from hydrologically-dynamic environments. As environmental disturbance temporally influences resource availability, successful predators have been found to adapt generalist strategies to survive unfavorable conditions by adjusting to different prey items and foraging habitat use (Steenhof & Kochert, 1988; McHuron *et al.*, 2016; Spencer, Newsome & Dickman, 2017). Moreover, generalist species have been found to anticipate favorable conditions in habitats that express seasonal environmental changes (Peres, 1994; Willson *et al.*, 2010). Since cottonmouths utilize a broad array of habitat features and microhabitats, they remain a common component of many wetland systems of the southeastern United States, and a model species to understand the advantage of the generalist strategy in stochastically disturbed environments.

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APPENDIX

Locations for recorded data and analysis script

Data can be found on the herpetology Dropbox shared drive for the herpetology lab under the advising of Dr. Gienger. The R script for the project can be found on my hosted github repository (https://github.com/jkauphus/Generalist-Cottonmouth). The vegetative community was documented on the iNaturalist group for the Cumberland River Bicentennial Trial (https://www.inaturalist.org/projects/flora-of-cumberland-river-bicentennial-trail-tn).