Habitat Associations and Predictive Presence Modeling for Plethodontid *Eurycea hillisi* in West Georgia

by

Betsy Battistella Gratton

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Approved By

Sharon Hermann, Chair, Assistant Professor of Biological Sciences, Auburn University

Craig Guyer, Professor of Biological Sciences, Auburn University

Brain Helms, Assistant Professor of Biological and Environmental Sciences, Troy State University

Abstract

The purpose of this study was to identify habitat associations of *Eurycea hillisi* and use those characteristics to develop a predictive model. In Chapter 1, I gave an overview of the taxonomic history of the species and what general habitat usage has been observed. During preliminary data collection in stream floodplains, I consistently observed salamanders along one side of a given stream at several sites, rather than both sides. These "detected" and "undetected" stream sides served as a basis of comparison of species presence throughout the thesis.

In Chapter 2, I sampled sites during winter and summer seasons using a transect-based protocol within detected/undetected stream sides and used linear mixed models to determine the difference in mean values between the two sides. I found that habitat characteristics associated with the detected side tended to have association with moisture related vegetation. Soil moisture content exhibited a shift in associations between the seasons, likely due to flood potential and soil composition. I took the significant variables and ran a Non-metric Multidimensional Scaling (NMDS) analysis to demonstrate the similarities and dissimilarities of the habitat variables and the strength of the significance of each variable as they related to detected/undetected.

In Chapter 3, I used the significant variables to develop a predictive model using generalized linear mixed models for both winter and summer seasons. I discovered that the top winter model contained top of bank height, leaf litter depth, percent woody debris, percent sphagnum cover, and soil moisture content. The top two summer models were not significantly different from one another and both contained bank angle, percent woody debris cover, percent fern cover, soil moisture content, and soil pH. Percent sphagnum cover was also present in the second top model.

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Chapter 1. Introduction

Many amphibians have semi-aquatic life histories and undergo a significant niche shift during development which make them especially sensitive to habitat degradation (Ray et al. 2002). Not surprisingly, amphibians have been impacted by a global decline attributed to habitat fragmentation and degradation (Alford and Richards 1999). Thus, understanding species-specific habitat relationships can inform amphibian conservation and management practices.

Salamanders often play important ecological roles in their environments. Davic and Welsh (2004) discuss several ways that salamander species regulate food webs or contribute to ecosystem stability. Some of these ecological roles include controlling species diversity at lower trophic levels connecting energy pathways through migrations, facilitating soil dynamics though burrowing systems, supplying high-quality energy stores and nutrients to tertiary consumers, enhancing forest resilience through ecological succession, and providing a quantifiable metric of ecosystem health (Davic and Welsh 2004).

In the current study I focus on a recently named cryptic lungless salamander, *Eurycea hillisi* (Wray et al. 2017). Using known presence data for this species, my goal is to identify significant habitat associations and develop a model to predict salamander presence.

A Brief History of *Eurycea hillisi*

When my research began, my study species was listed as a member of the Dwarf salamander complex (*Eurycea quadradigitata*) that ranged throughout the Southeastern United States (Petranka 1998). In 2003, Harrison and Guttman (2003) described a new species within the Dwarf salamander complex named Chamberlain’s Dwarf salamander (*Eurycea chamberlaini*), which was previously thought to be a color phenotype (yellow belly) of the Dwarf salamander (silver belly). Later Lamb and Beamer (2012) identified three additional lineages within the dwarf salamander complex and designated them as the Panhandle, Central, and Western lineages.

Chamberlain's Dwarf Salamander was recently petitioned for listing under the U.S. Endangered Species Act, and as a result, there was an effort in Georgia to assess the conservation status of the species. Between 2005 and 2013, just 13 sites were known in west Georgia to support a yellow-bellied dwarf salamander assumed to be Chamberlain's Dwarf Salamander (Hermann et al. 2016). In 2014, S. Graham conducted surveys at 218 localities in 37 Georgia counties with an estimated total of 356 person hours of search effort. Salamanders were found at 43 sites in 25 counties covering what previously had been a distribution gap of this yellowbellied (Graham et al. 2017). A total of 115 salamander specimens were collected for genetic analysis and Graham et al. (2017) determined that the species belonged to the "Central lineage" described as differing phylogenetically, but not morphologically from Chamberlain's Dwarf Salamander (Lamb and Beamer 2012; Graham et al. 2016). This lineage was recently named *Eurycea hillisi*, Hillis's Dwarf salamander (Wray et al. 2017). Recently Graham et al (2017) suggested that *E. chamberlaini* may not exist in Georgia but rather that the distribution of that species may be restricted to North and South Carolina.

In summary, *Eurycea hillisi* is a small, semi-aquatic plethodontid now known from the Piedmont physiographic region as well as from Upper and Central Coastal Plain. Although little is understood about the habitat and distribution of this cryptic species (Means 2008), in the past few years, distribution records have accumulated extending its range, nearly contiguously, throughout the Upper Coastal Plain of Georgia.

Habitat Overview for *Eurycea hillisi*

The Piedmont region is characterized by silty sand soils with the mean grain size being approximately 0.075mm (Mayne et al. 2000), and the Coastal Plain is characterized by sandy loam soils that are coarser and have better drainage (Duffera et al. 2007). Within these physiographic provinces, the associated habitats for *E. hillisi* are typically floodplain areas, firstorder streams, and ponds. Many areas are characterized by a bay swamp that includes Sweetbay (*Magnolia virginiana*), Doghobble (*Leucothoe axillaris*), greenbrier (*Smilax spp.),* and thick sphagnum moss (Graham and Jensen 2011). The microhabitat usage of the species tends to be areas with leaf litter/debris build up, logs, and sphagnum moss. During a search of 19 sites, approximately 47% of individuals were found in sphagnum moss (Graham and Jensen 2011). During the collection of preliminary data in stream floodplains, I consistently observed the salamanders along one side of a given stream at several sites, rather than both sides. These "detected" and "undetected" stream sides served as a basis of comparison of species presence.

Current Study Objectives

The objective of the current study is to present a quantitative summary of the habitat usage and create a habitat model for predictive applications in management of the species. In Chapter 2, I established a basis for the habitat models by conducting a paired analysis between detected and undetected stream sides for winter and summer season. I compare various habitat characteristics between the two sides and explore differences in mean values for each variable. I also perform a non-metric multidimensional scaling analysis to demonstrate relationships of the variables to one another and to identify how variables associated with detected and undetected sides are similar or dissimilar to other variables. In Chapter 3, I used habitat variables that were found to have significant relationships in Chapter 2 with presence to develop a habitat model for both winter and summer seasons that may serve to predict presence of the species.

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Chapter 2. Habitat Associations of *Eurycea hillisi* in West Georgia Introduction

A vital part of understanding and conserving any species is to identify its distribution within a landscape. Different micro- and mesohabitat attributes play important parts in determining where a species resides. While larger-scale habitat characteristics, such as vegetative composition and climate variability, explain the coarse-grained distribution of a species, within each mesohabitat are microhabitats that provide specific requirements for a species' persistence (Yeiser and Richter 2015).

With the global decline in salamanders, it is important to conserve the species diversity that remains (Alford and Richards 1999). Salamanders serve important ecological roles, including controlling species diversity in lower trophic levels, connecting energy pathways through migrations, facilitating soil dynamics though burrowing systems, supplying energy and nutrients to consumers, enhancing forest resilience through ecological succession, and providing an indication of ecosystem health (Davic and Welsh 2004). Despite their important roles, only recently has there been a surge of research focused on the importance of microhabitat factors to salamanders (e.g. Farallo and Miles 2016; Basile et al. 2017; Manenti et al. 2017; Smith et al. 2017). Clipp and Anderson (2014) noted that salamander population abundance and diversity in riparian forests are influenced by both landscape-level features as well as microhabitat factors. Stream quality, leaf litter, woody debris, riparian buffer width, and soil characteristics were all identified as major environmental factors influencing salamander populations. Additionally, moisture and temperature requirements for salamanders play an important role in habitat selection and have been found to be variables that are consistently related to salamander abundance across species compared to other microhabitat variables (Rittenhouse et al. 2008; Peterman and Semlitsch 2013; Hyde and Simmons 2001).

Streamside salamanders are susceptible to dehydration and have a significant relationship with moisture and humidity (Crawford and Semlitsch 2008). With small body sizes, streamside salamanders are also more constrained in their movements as well as their distribution (Lee-Yaw et al. 2014). Crawford and Semlitsch (2008) investigated microhabitat use of streamside salamanders and found that soil moisture, distance from stream, leaf litter depth, and soil temperature had the strongest support for predicting stream salamander abundance. Amphibians are generally sensitive to differences in environmental factors (Chambers et al. 2006; Hatch and Blaustein 2000) so understanding the dynamics of streamside salamander microhabitat is likely to be important for improving understanding of life history, promoting conservation, and informing habitat management.

Eurycea hillisi is a recently-named streamside salamander of the *E. quadridigitata* complex (Wray et al. 2017). This complex includes some of the smallest salamander species known, including *E. hillisi* with a mean snout-vent length of 23.8 millimeters (Wray et al. 2017). Despite their small size, all members of this complex migrate during winter months to temporary wetlands where they breed (Wray et al. 2017) and *E. hillisi* is known to use flooded streamsides and seepages during this time period (Means 2008; Graham et al. 2016).

Eurycea hillisi was initially known from the Piedmont physiographic region and upper portions of the Central Coastal Plain. Means (2008) suggested that habitat and distribution of *E. hillisi* (as *E. chamberlaini*) was poorly known in Georgia due to its cryptic nature but noted that the species was found in ravines along the lower Chattahoochee River. Graham et al. (2016) provided distribution records that extended the species range contiguously across the Fall Line Hills of Alabama to Jefferson County, Georgia. Habitat descriptions have been limited to general statements, from which it was difficult to determine species relationships, perhaps because species delimitations within the complex had yet to be determined (Wray et al. 2017). Graham and Jenson (2011) initially suggested that *E. hillisi* was associated with seepage habitats, but later observations suggested occupancy was more likely related to shallow floodplain pools with mucky substrate rather than groundwater-fed seeps (Graham et al. 2016). In Georgia, Graham et al. (2016) found that sites most occupied by *E. hillsi* were located along the margins of floodplains or floodplain pools containing saturated leaf litter and no subsurface seepage flow. Wray et al. (2017) described microhabitat of the *E. hillisi* holotype as "… under small sphagnum mat on slope above floodplain amidst thin layer of hardwood leaf litter on sandy soil …". However, to date, no in-depth analysis demonstrating significance of the relationships between salamander presence and habitat variables suggested by Graham et al. (2016) has been conducted.

In this study, I examine habitat use by *E. hillisi* at sites in Georgia. In particular, I explore whether micohabitat factors, including leaf litter, sphagnum, and soil moisture, show a significant relationship with salamander presence. I also examine the relationship between overstory trees and stream morphology in generating habitat occupied by these streamside salamanders. Finally, I consider the role of season as it relates to features associated with site occupancy.

Methods

Site Description

Five study sites located in west central Georgia were chosen for sampling (Figure 1, Appendix B). Each was known to be occupied by *E. hillisi* from previous surveys (Graham et al. 2016). Each site encompassed both sides of a first-order stream bordered by bay swamp vegetation [dominated by sweetbay (*Magnolia virginiana*), doghobble (*Leucothoe axillaris*), and bamboo vine (*Smilax laurifolia*)]. The sites were selected because they were relatively undisturbed by human activities (dumping of rubbish, clearing of land, cattle ranching, or damage from recreational vehicles) and were accessible. Human interactions with forests and streams can be detrimental and alter habitat factors within riparian forests (Clipp and Anderson 2014). Thus, the sites selected for this study represent mesohabitat conditions in which *E. hillisi* are known to use.

Each site consisted of a shallow stream and riparian forest on either side of the stream. I visited each site in 2015 (June - July and November - December) to confirm salamander occupancy; both sides of the steams were visited repeatedly and evaluated for salamander occupancy and salamanders were detected only on the side of the stream indicated in Graham et al. (2016). This allowed me to designate one side of the stream as “detected” (known to be occupied by *E. hillisi*) and the opposite side as “undetected” (not known to be occupied by *E. hillisi*). These designated sides served as the basis of microhabitat comparisons associated with surveys that occurred during winter (December 2015 - February 2016) and summer (May 2016 – August 2016) seasons.

Transect Surveys

Habitat surveys consisted of a series of transects along a 30-m section of stream, with both sides of the stream being sampled identically. Within each sampling area, ten 20-m transects were established, one every 3 meters, with each beginning streamside and extending along a perpendicular line into the floodplain (Figure 2). The sampling area was buffered on both the upstream and downstream side by a minimum of 5-m of habitat that was not obviously disturbed by recent human activities. In cases where the stream consisted of braids, transects were oriented from the outer-most braid. At times, transects were truncated because of obstacles or inaccessibility. When this occurred an additional transect was added 3 meters from the 10th transect, extending the original 30 m survey area. Transects were sampled during winter (2015) and again during summer (2016).

Graham and Jensen (2011) observed that *E. hillisi* were often found in leaf litter, woody debris, and sphagnum. Based on this information, I included percent cover and depth of leaf litter, percent cover of woody debris, and percent cover and depth of sphagnum as key variables and predicted that they would be more prevalent on the side of the stream where *E. hillisi* was detected as compared to the undetected side. Additional vegetation-related variables included percent grass/forb cover, percent bare ground cover, percent fern cover, and a measurement of the basal area of trees. I expected detected sides to exhibit lower bank angle and lower top of bank (TOB) height as compared to the undetected sides. The increased bank heights and angles expected on the undetected side could indicate stream degradation limiting flood potential (Rosgen 1997), which would be important to maintain soil moisture and facilitate the growth of sphagnum to be used as microhabitat. Because of the importance of moisture to salamanders, I included bank angle and TOB as indicators of flood susceptibility. I also collected one soil sample along the midpoint of each transect to determine soil moisture, measured soil pH from the sample collection area, and water pH from the stream, which could affect habitat use.

Bank Measurements: Stream TOB and bank angle were recorded at the beginning of each transect. TOB was measured as vertical distance from the water's edge to the top of the bank (Helms et al. 2013). Bank angle was determined by placing a meter ruler at the TOB edge along the incline of the bank to the stream and using a Johnson™ pitch/slope locator on top of the flat surface of the ruler to find the approximate angle of the stream bank (Platts et al. 1987).

Vegetation: Vegetative measurements included diameter at breast height (DBH) of trees; percent cover of forbs and grasses, bare ground, woody debris, leaf litter, sphagnum, and ferns; leaf litter depth; and sphagnum depth. All trees within 1.5 m of the transect were measured for DBH, which was then used to estimate basal area of trees in the survey site. Due to the short time period (3 months) between winter and summer surveys, and because slight changes in DBH between the two seasonal surveys do not significantly affect basal area (Smith 1983), DBH was only measured during the winter season.

Percent cover measurements were made for vegetation below 1 m in height and were taken every 4 meters along the transect. A 1 m^2 frame made from PVC pipe was used to assess percent cover (Daubenmire method; Bonham et al. 2004). Cover percentages were estimated to the nearest five percent by visually assessing each of four equal 0.5 x 0.5m sections within the meter square. Leaf litter depth and sphagnum depth were measured to the nearest millimeter using a meter ruler at the deepest portion of leaf litter and/or sphagnum within the sample frame.

When transects overlapped due to the curvature of the stream, DBH measurements were only taken from trees not previously sampled. There was no overlap of areas sampled for percent cover of vegetation areas. In the event of a truncated transect, the number of samples that were not able to be collected on the truncated transect were collected along the additional transect to ensure uniform sample size between stream sides.

Soil and Water: Soil and water pH were assessed using a Hanna Direct Soil pH instrument. Water pH was measured from the stream at the start of the transect. Soil pH was measured at the 10 m mark along the transect. At that same point, a soil sample was collected, kept on ice to prevent water loss, returned to the lab, and processed the same day to determine moisture content. Soil moisture was measured using the gravimetric method (Faulkner et al. 1989). Wet weight was recorded, and each sample was oven dried at 105□C until a constant weight was reached (about 12-24 hours), and samples were reweighed. The difference in the before-drying and after-drying weights was considered the weight of the water. Moisture content (M_c) was calculated as a percentage of dry soil weight by dividing the weight of the water (W_w) by the dry weight of the soil (W_d) and multiplying by 100 % (Faulkner et al. 1989).

Statistical Analysis

I wanted to determine the differences in mean values for each habitat variable between the detected and the undetected stream sides. To best evaluate these data, each habitat variable was averaged across each transect. In the case of truncated transects resulting in more than 10 transect values on a single side, the additional values were averaged with the 10th transect value.
This allowed the data to be captured without compromising the structure of the analysis. Salamander detection was recorded binomially and was used in combination with habitat variables in a paired-design analysis to determine differences in mean values between detected and undetected steam sides. I tested the data for normality then ran mixed-effect linear models for each habitat variable using salamander presence as a fixed effect and site as a random effect. This structure allowed for a comparison of habitat variables as a factor of salamander presence and used the random effect of site to control for variation due to site locality. Sites were surveyed during both summer and winter seasons, but seasons were analyzed separately. I used the "lmerTest" package in program R (Kuznetsova et al. 2015; R Core Team 2017) to perform these comparisons

Non-metric Multidimensional Scaling: To best illustrate the relationships demonstrated in the mixed-effect linear model, I performed a non-metric multidimensional scaling (NMDS) analysis, an ordination technique that generates a plot for displaying the relative position of habitat components based on dissimilarities. This analysis was chosen over a PCA because it makes few assumptions about the data, and because it can handle a wide variety of data. I used the raw habitat data for each significant association and determined the relative positions of the habitat variables and then used a convex hull to determine differences between paired detected and undetected sides. NMDS was run for both seasons using only the variables found to be significant in the mixed-effect linear model analyses. I used the “vegan” package in program R (Oksanen et al. 2017; R Core Team 2017) to run these analyses.

Results

Paired Analysis:

Winter Surveys

Over all sites surveyed during the winter (Table 1, Appendix A), I found that detected sides had 3.3mm more leaf litter depth ($p= 0.008$; $\bar{x}$= 14.97mm), 8.5% more sphagnum cover ($p=0.0002$; $\bar{x}$= 5.37%), 6.4mm more sphagnum depth ($p=0.0001$; $\bar{x}$= 4.37mm), and 9.8% more moisture content ($p<0.0001$; $\bar{x}$= 18.36%) than undetected sides. Detected sides also exhibited 12.3° less bank angle ($p=0.001$; $\bar{x}$= 32.74°), 111.8mm less TOB height ($p=0.0001$; $\bar{x}$= 299.73mm), and 17.5% less bare ground cover ($p<0.0001$; $\bar{x}$= 15.75%). Percent grass/forb cover ($\bar{x}$= 11.17%), percent woody debris cover ($\bar{x}$= 15.54%), percent leaf litter cover ($\bar{x}$= 51.19%), basal area ($\bar{x}$= 0.26m^2/ha), water pH ($\bar{x}$= 6.3), and soil pH ($\bar{x}$= 6.0) were not found to have significant differences when comparing sides.

Summer Surveys

Over all sites surveyed during the summer (Table 1, Appendix A), I found detected sides had 4.8% more sphagnum cover ($p=0.002$; $\bar{x}= 3.57\%$), 7.0mm more sphagnum depth ($p= 0.007$; $\bar{x}= 7.4$mm), 1.7% more fern cover ($p<0.0001$; $\bar{x}= 1.77\%$), and 0.3 more soil pH ($p=0.002$; $\bar{x}= 6.4$). Detected sides also exhibited 24.3° less bank angle ($p<0.0001$; $\bar{x}= 37.78°$), 131.3mm less TOB height ($p<0.001$; $\bar{x}= 253.35$mm), 13.3% less bare ground cover ($p=0.0004$; $\bar{x}= 18.53\%$), 2.2% less woody debris cover ($p=0.0009$; $\bar{x}= 8.57\%$), and 11.6% less soil moisture content ($p<0.0001$; $\bar{x}= 22.76\%$). Percent forb/grass cover ($\bar{x}= 21.31\%$), percent leaf litter cover ($\bar{x}= 57.71\%$), leaf litter depth ($\bar{x}= 25.35$mm), and water pH ($\bar{x}= 6.5$) were not found to have significant differences when comparing sides.

Non-metric Multidimensional Scaling:

Winter Surveys

For the NMDS analysis, a two-dimension solution was reached in 31 runs with a stress of 0.12. The detected and undetected sides for the winter surveys were shown to be notably different when considering most cover variables. The distinction between the two sides is especially apparent when considering percent bare ground cover, bank angle, TOB height, percent sphagnum cover, and sphagnum depth.

Summer Surveys

For the summer NMDS analysis, the two-dimension solution was reached in 20 runs with a stress of 0.15 (Figure 4). There were strong similarities between bank angle and TOB height measurements related to the undetected sides as well as sphagnum depth and percent sphagnum cover measurements related to the detected sides. There was also dissimilarity between all vegetative cover values and percent bare ground cover. With the overlay of polygons representing the detected and undetected sides, I found a notable difference between sides where salamanders were detected and sides where salamanders were undetected during the summer season.

Discussion

With the increasing decline in salamander diversity (Alford and Richards1999), understanding habitat usage is an important component to conservation. I found many of the microhabitat variables predicted to be associated with *E. hillsi* presence were significantly different between detected and undetected sites. Detected sides were found to have smaller bank angle and lower TOB than undetected sides. Lower TOB and angles could be an indicator of increased flood potential as higher TOB could indicate incised banks, decreased flooding frequency, and hydrologically disconnected floodplains (Rosgen 1997). Hydrologic connection of the floodplain would facilitate maintenance of consistent soil moisture and the creation of seasonal pools and boggy, mucky patches used by *E. hillisi* as noted by Graham et al. (2016). Higher banks would be less susceptible to flooding during periods of increased water levels in the nearby streams, minimizing available *E. hillisi* habitat.

Soil moisture was found to be a significant variable for both seasons, however, the undetected side showed greater soil moisture during the summer while the detected side showed greater soil moisture during the winter. It was expected that the detected side would be consistently wetter than the undetected side throughout the year, but the results did not support this prediction. This could also be a result of the shallow floodplain pools with mucky substrate being dispersed throughout the sampling area, but not specifically targeted during sampling. The pools may have retained soil moisture more effectively than the surrounding areas but, using the transect protocol, soil samples were typically collected in areas surrounding the pools rather than in the pools themselves. Additionally, there could be small differences in composition of the soil samples (such as a larger component of organic material) that could affect moisture retention and movement, which could have impacted the results. Including soil composition in future analysis and measurements over multiple years would be helpful in determining the significance of this pattern.

At a microhabitat scale, Graham and Jensen (2011) made observations on salamander use of woody debris as cover. Percent woody debris cover was predicted to have a significant association with salamander presence, however, this relationship was not supported by the statistical analysis, as the relative amount of woody debris cover was not significantly different between stream sides. The significance of the relationship may be related to size of woody debris and specific moisture requirements of the species. Large branches or fallen trees have generally been observed to be used as cover by salamanders, but woody debris was not categorized by size, which may have affected the results. Many smaller twigs were included in percent woody debris cover, but small twigs may not provide sufficient cover or retain adequate moisture levels to benefit the salamander like larger woody debris. In future studies, accounting for size of woody debris may be beneficial to determining the significance of woody debris as an important microhabitat.

I also found significant differences between salamander-detected and salamanderundetected sides for three vegetation metrics; sphagnum depth, percent sphagnum cover, and percent fern cover were all more common on the side where *E. hillisi* was detected. Sphagnum results in the current study enhances the general observation of Graham and Jensen (2011) when they noted 9 of 19 sites supporting salamanders also contained sphagnum moss. A positive association between sphagnum depth and salamander presence may be explained by the importance of moisture to salamander species, as they are prone to dehydration (Crawford and Semlitsch 2008) and sphagnum generally grows in moist soils. Often found in sphagnum mats like *E. sphagnicola* (Wray et al. 2017), *E. hillisi* has been detected on stream sides with both high cover of sphagnum and fern species indicating increased soil moisture. This potentially important relationship with sphagnum has been documented for salamanders (e.g. Chalmers and Loftin 2006) and frogs (e.g. Baldwin et al. 2006). With global decline of amphibian species, one of the first steps in conservation is to determine species' habitat use and biological needs. The current study provides a basis in determining which microhabitats may be critical for conservation of *E. hillisi* during both summer and winter seasons and how this species may be affected by changes in habitat variables.

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Table 1. Differences in means of habitat variables in relation to salamander detection, p-value, and confidence intervals. $\Delta\boldsymbol{x}_{(\boldsymbol{D}-\boldsymbol{U})}$ is the difference in mean values for the detected and undetected stream sides. Positive means indicate higher values of the habitat variable on the detected sides (Appendix A). Negative means indicate higher values of the habitat variable on the undetected sides. Significant variables are indicated with an asterisk. Ferns were not found during the winter surveys and were not included in the winter analysis.

Variance of Habitat Variables

	Winter				Summer			
Habitat	$\Delta\boldsymbol{x}_{(\boldsymbol{D}-\boldsymbol{U})}$	p	Lower C.I.	Upper C.I.	$\Delta\boldsymbol{x}_{(\boldsymbol{D}-\boldsymbol{U})}$	p	Lower C.I.	Upper C.I.
Bank Angle (°)	-12.259	0.0012*	-17.318	-31.242	-24.280	0.0000*	-5.062	-19.455

Bank Height (mm)	-111.820	0.0001*	-75.32	-187.2	-131.260	0.0000*	-57.813	-165.821
Grass/Forb Cover (%)	0.166	0.899	2.6 -	-2.565	0.018	0.989	2.722	-2.39
Bare Ground Cover (%)	-17.488	0.0000*	6.196	-20.464	-13.330	0.0004*	-9.712	-25.265
Woody Debris Cover (%)	-2.407	0.117	-0.926	-3.374	-2.150	0.0009*	0.578	-5.393
Leaf Litter Cover (%)	-0.905	0.8180	8.723	-9.113	-0.195	0.966	6.777	-8.587
Leaf Litter Depth (mm)	3.291	0.0079*	9.685	-1.557	4.064	0.160	5.669	0.913
Sphagnum Cover (%)	8.525	0.0002*	7.775	1.805	4.790	0.0022*	12.796	4.255
Sphagnum Depth (mm)	6.372	0.0001*	11.924	1.976	6.950	0.0074*	9.317	3.428
Fern Cover (%)	-	-	2.631	0.841	1.736	0.0003*	-	-
Basal Area (m^2/ha)	0.051	0.117	0.113	-0.012	0.051	0.117	0.113	-0.012
Soil Moisture Content (%)	9.835	0.0000*	-6.483	-16.621	-11.553	0.0000*	14.329	5.341
Water pH	-0.251	0.2411	0.634	0.049	0.342	0.024	0.166	-0.668
Soil pH	-0.123	0.5598	0.492	0.113	0.302	0.0024*	0.29	-0.536

Appendix A. Mean measurements of habitat variables for detected and undetected side for winter and summer season.

Mean Measurements Per Side

	Winter		Summer	
Habitat Variable	$\bar{x}_d$	$\bar{x}_u$	$\bar{x}_d$	$\bar{x}_u$
Bank Angle (°)	26.46	39.76	25.64	49.92
Bank Height (mm)	173.02	284.59	187.72	319.02
Grass/Forb Cover (%)	11.24	10.88	12.32	12.3
Bare Ground Cover	6.94	24.59	11.86	25.19
(%)	14.25	16.47	7.49	9.64
Woody Debris Cover	50.65	51.59	57.62	57.81
(%)	16.62	13.23	30.39	26.32
Leaf Litter Cover (%)	9.68	1.08	5.97	1.18
Leaf Litter Depth	7.59	1.14	10.87	3.92
(mm)	-	-	2.64	0.91
Sphagnum Cover (%)	0.29	0.24	-	-
Sphagnum Depth	23.32	13.65	16.98	28.53
(mm)	6.1	6.5	6.7	6.3
Fern Cover (%)		6.1	6.5	6.2
Basal Area (m^2/ha)				
Soil Moisture Content (%)				

Water pH	5.9
Soil pH	

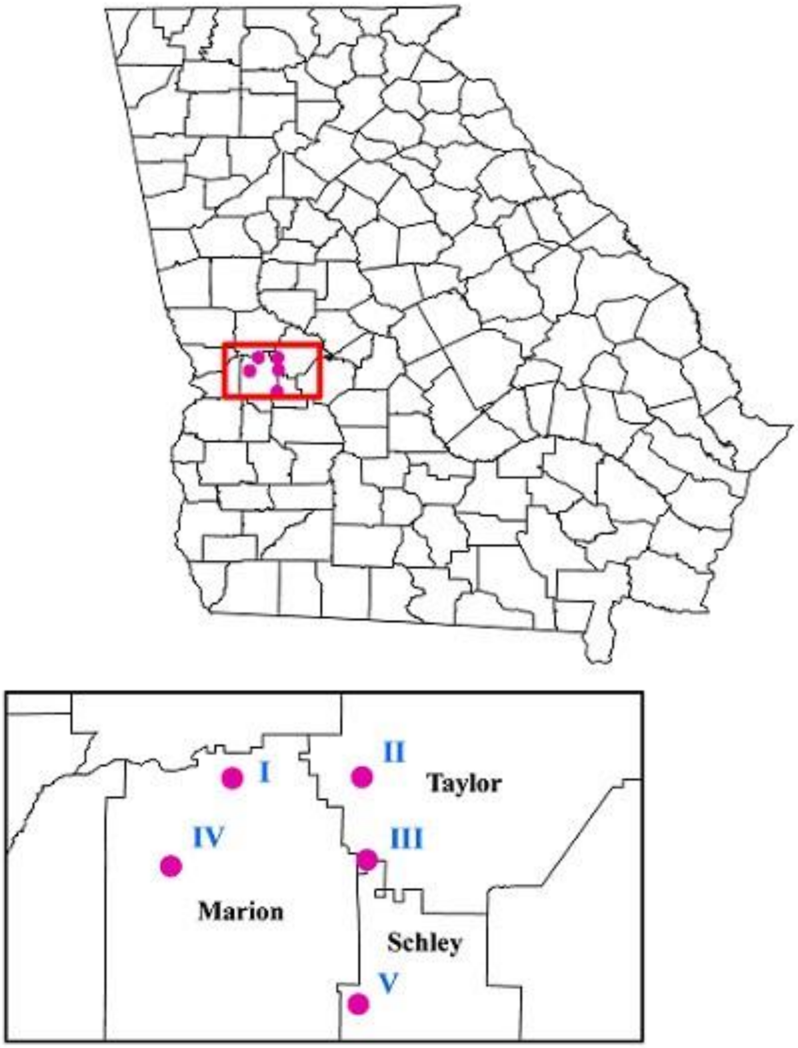

Figure 1. Survey sites located in Western Georgia (Appendix B).

Appendix B. Surveyed site localities with coordinates, *E. hillisi* presence and abundance, notes, and sympatric species observed.

Surveyed Site Localities

County	Latitude	Longitude	Presence	Site Notes	Additional Comments	Other Sp.
Marion	32.52647	-84.5704	yes	Not tons of sphagnum, but plenty of leaf/stick pacts to look under	7 hillisi were found. All females were spent. 1 was found under a small log, with the rest being found in leaf pacts.	E. cirrigera, D. punctatus
Talbot	32.58828	-84.5044	yes	Habitat seems degraded. Mostly pine needles and logs.	2 hillisi were found. 1 adult, and 1 metamorph.	
Marion	32.54192	-84.4507	yes	Site is a pond with no sphagnum. Lots of pine needles and logs. Record of some hillisi having orange bellies.	2 hillisi were found, both of which were metamorphs.	Larval newt, P. crucifer metamorph
Taylor	32.52631	-84.4103	no	Site looked ideal, but no		

				hillisi were found.		
Taylor	32.5167	-84.3871	yes	Very deep sphagnum that grows in thick carpets	18 hillisi found. Some had orange and almost red bellies. All found in deep sphagnum.	2 unidentified amphiumas. E. cirrigera, Acris sp.
Taylor	32.52495	-84.3098	no	Very degraded habitat. Little leaf litter, and very little sphagnum.		P. ruber (deceased)
Taylor	32.61863	-84.3233	yes	250-300m backwater creek they can be found. Habitat degraded otherwise	1 hillisi found	A. piscivorous, E. cirrigera, Acris sp.
Taylor	32.61765	-84.2828	yes		1 hillisi found	A. piscivorous, E. cirrigera, D.apalachicolae

Taylor	32.63812	-84.2354	yes	Great site on L side of road. Lots of small/dense sphagnum patches with wire grass growing in them.	2 metamorph hillisi found	
Taylor	32.61939	-84.1896	yes	Habitat looked degraded from road, but looked pretty good inside forest line.	1 metamorph found under short sphagnum	A. piscivorous
Taylor	32.59997	-84.1887	no	Beaver pond Lots of leaf/stick pacts but very little sphagnum		E. cirrigera, D. punctatus, L. sphenocephala
Taylor	32.59154	-84.1578	yes	Beaver pond. Dry, no sphagnum on pond side. Other side of the road looks degraded, but has tons of sphagnum 20m within forest line.	2 hillisi found in sphagnum	

Marion	32.51548	-84.5244	yes	Lots of sphagnum, lookds good but not many found.	1 hillisi found in sphagnum	E. cirrigera
Marion	32.4142	-84.4745	yes	Back water slope areas away from the creek have the best sphagnum spots. Small scattered beds throughout the area.	1 metamorph hillisi	E. cirrigera
Marion	32.37771	-84.4455	no	Sparse sphagnum on slope by creek, overall habitat looks degraded		C. serpentina, E. cerrigera
Marion	32.4405	-84.6481	no	Tons of sphagnum, but no hillisi found.	Site has been surveyed in winter. Would be a good negative control	E. cirrigera

Schley	32.4405	-84.6481	no	Habitat was degraded, stream was between beaver pond and farm pond. Sphagnum was short, deep rooted variety.		Nerodia sp.
Schley	32.30929	-84.2932	no	Looks like river floods and washes too often. Sphagnum found was very short and sparse. The most common type was the bunch, short, deep rooted variety.		
Schley	32.29606	-84.0678	no	No sphagnum found, stream coming from beaver pond. Some leaf pacts, but overall crappy habitat		Nerodia sp./A. piscivorous

Schley	32.27584	-84.391	yes	On the right side of the stream there are beds of sphagnum.	2 hillisi found. 1 confirmed as male.	E. cirrigera
Schley	32.15884	-84.2918	no	No sphagnum found. Area seemed very dry, sparse leaf pacts.		
Talbot	32.83872	-84.5387	no	Creek has very sleep sloping sides (clifflike). No sphagnum found, and minimal leaf pacts present		
Talbot	32.8193	-84.6458	no	Cliff slopes into river, no sphagnum or nice seepage areas		
Taylor	32.47663	-84.2313	no	Sphagnum found was very saturated and sparse, beaver pond area		
Talbot	32.71011	-84.3782	no	Area looked good from the		P. crucifer

				outside, was no real sphagnum and some leaf/stick pacts.		
Talbot	32.71965	-84.3405	no	Banks were very high, leaf/stick pacts abundant but were quite dry. No sphagnum found		
Stewart	32.1992	-84.6829	no	Very briary, but no bamboo briar or sweetbay inside. By beaver pond, no sphagnum, some leaf/stick pacts that were relatively dry		
Crawford	32.68597	-83.9675	no	Some sparse sphagnum found, but degraded habitat. Beaver pond stream.		
Crawford	32.68163	-83.945	yes	Good habitat, sparse sphagnum near pond, but	1 hillisi	E. cirrigera

				probable honey hole further down. Beaver pond.	
Crawford	32.68525	-83.8883	no	Lots of sphagnum	D. aeneus
Crawford	32.64332	-83.8973	yes	Lots of stick and leaf pacts, but not much sphagnum.	

1 metamorph P. gluttinosus, G. carolinensis, P. chamberalini cruicifer

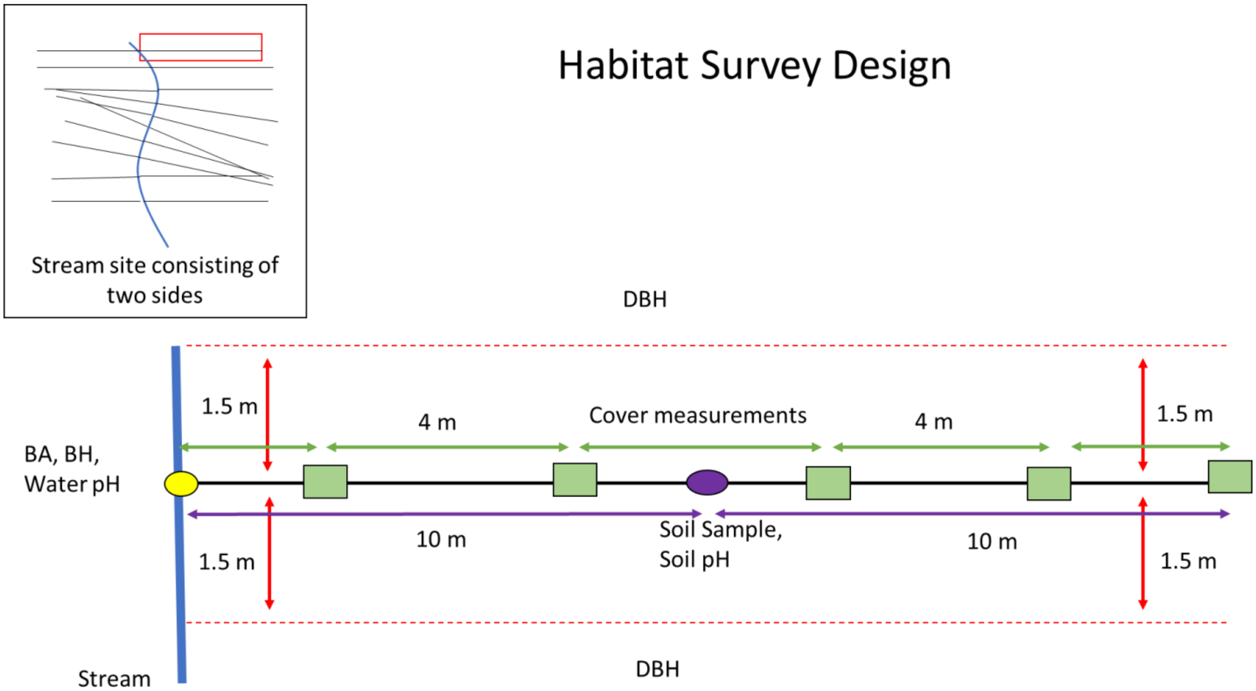

Figure 2. Habitat survey design. Thick blue line is the stream, black line is a 20m transect, green boxes are 1 m^2 Daubenmire squares, area within dotted outline is diameter at breast height (DBH) measurement area. DBH was not resampled for overlapping transects. Green arrows indicate the distances at which the Daubenmire squares were placed, and the red arrows indicate the distance at which DBH was sampled. The yellow circle is the start of the transect where bank angle, top of bank (TOB), and water pH measurements were taken. The purple circle is where soil pH and soil samples were taken for moisture content analysis, and the purple arrows indicate the distance along the transect at which samples were taken.

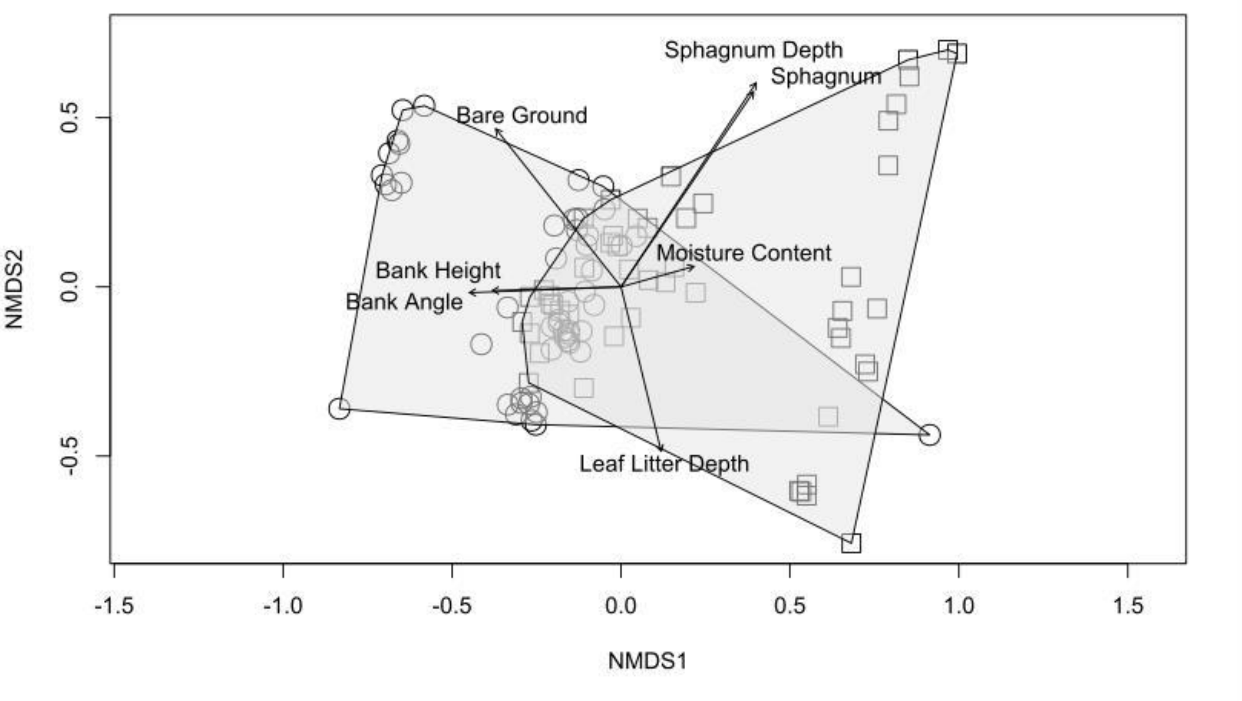

Figure 3. Non-metric multidimensional scaling analysis of the winter season. The individual convex hulls demonstrate the similarity and dissimilarity of "detected" and "undetected" sides of the sampled sites based on the variable measurements. Labels denote the centroid for each habitat variable, and open circles are relative position of each measurement. BG= percent bare ground cover, BA= bank angle, TOB= top of bank height, MC= soil moisture content, LLD= leaf litter depth, SD= sphagnum depth.

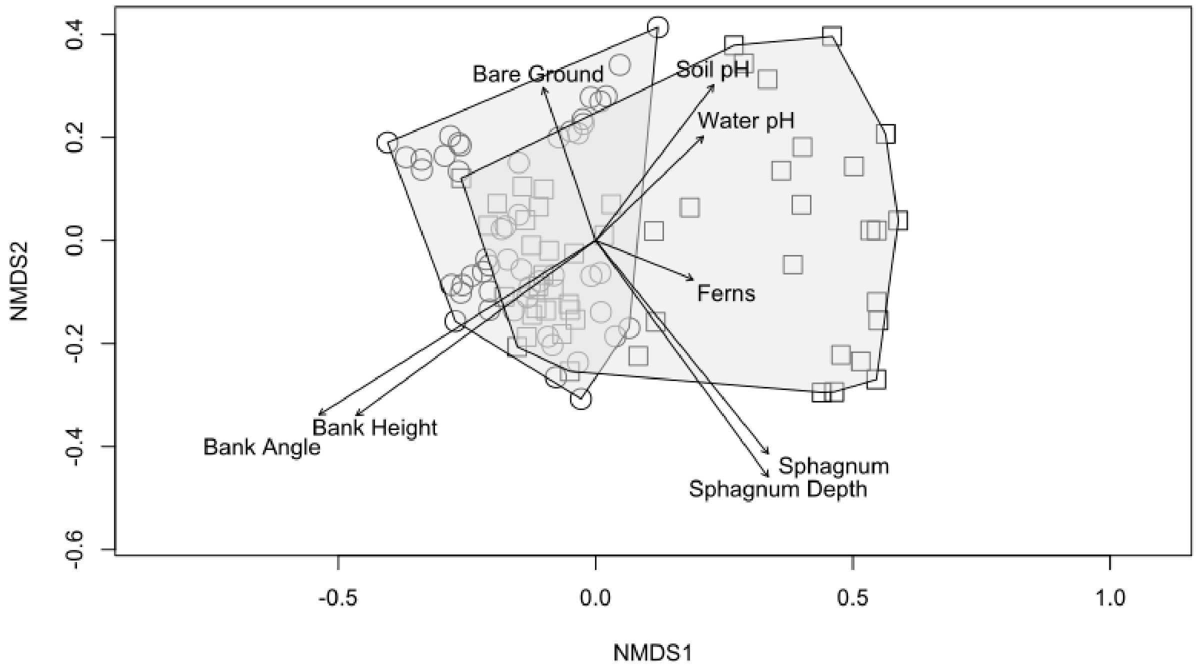

Figure 4. Non-metric multidimensional scaling analysis of the summer season. The individual convex hulls demonstrate the similarity and dissimilarity of “detected” and “undetected” sides of the sampled sites based on the variable measurements. Labels denote the centroid for each habitat variable, and open circles are relative position of each measurement. BG= percent bare ground cover, BA= bank angle, TOB= top of bank height, WD= percent woody debris cover, MC= soil moisture content, SD= sphagnum depth.

Chapter 3.

Models for Based on Habitat Associations for Predicting Salamander Presence in West Georgia

Introduction

As technology advances, scientists often consider predictive modeling to better understand species distributions (Guisan and Zimmermann 2000). In recent years predictive models have been used to understand many complex salamander species-habitat relationships. For example, Gustafson et al. (2001) used predictive models to assess impacts of forest management on terrestrial salamander populations and Milanovich et al. (2010) was able to predict loss of salamander diversity due to global climate change. Although there has been some modeling of salamander populations, to date use of predictive models to understand occurrence and distribution of semi-aquatic salamanders has been limited.

As noted in Chapter 2, *Eurycea hillisi* is known from the Piedmont physiographic region and upper portions of the Central Coastal Plain and previously classified as member of the Dwarf Salamander complex (Wray et al. 2017). Recent records extended the species range contiguously across the Fall Line Hills of Alabama to Jefferson County, Georgia (Graham et al. 2016). *Eurycea hillsi* has been described as occupying the margins of floodplains or floodplain pools containing saturated leaf litter and no subsurface flow and generally residing under small sphagnum mats (Graham et al. 2016; Wray et al. 2017). Using this information, I performed an analysis of habitat associations with *E. hillisi* presence (Chapter 2). The goal of the study is to use identified habitat relationships to generate a predictive model that could be used for understanding species distribution and inform conservation measures.

Methods

Using five sites in west Georgia (Chapter 2, Figure 1), I performed habitat surveys during the winter of 2015 and summer of 2016. Sites consisted of a stream with two stream sides. Each stream side was evaluated for salamander presence and labeled “detected” for sides where salamanders were found and “undetected” where salamanders were not located. Presence was recorded binomially for analysis. Using a transect-based approach I collected various habitat measurements such as bank angle; top of bank (TOB) height; percent cover of grasses/forbs, bare ground, woody debris, leaf litter, sphagnum, and ferns; depth of leaf litter and sphagnum; basal area; percent soil moisture content; water and soil pH (for more detail on survey methods and measurements, see Chapter 2). I then used these data in mixed-effect linear models to determine the differences in mean values for habitat variables on the detected versus the undetected stream sides (Chapter 2, Table 1).

Using the data presented in Chapter 2, I used habitat variables with significant associations with salamander presence as the basis for a model that predicts salamander occupancy of a site. The habitat measurements were collected on different scales and for the current project were standardized to a mean of zero and unit variance. To construct the predictive models, I used 'lme4' package in program R (Bates et al. 2014; R Core Team 2017) to run generalized linear mixed models (GLMM). GLMM allows the model to incorporate a random effect for variation in the data from site locality and can handle non-normal data associated with lower sample sizes and does not carry the assumption of normal distribution of residuals and linearity like the linear models (Bolker et al. 2008). Using the differences in mean values for the habitat variables calculated in Chapter 2, I identified variables that displayed significant associations with salamander presence. Winter and summer seasons were analyzed separately due to the environmental changes that occur between the two seasons. I ran models that contained all significant variables and did a subset analysis to find the best fit model for predictive applications. Sphagnum depth and percent sphagnum cover were found to be highly correlated ($r= 0.94$, $p<0.00001$); therefore, sphagnum depth was excluded in model development. Additionally, water pH and soil pH measurements were also found to be highly correlated ($r=0.84$, $p<0.00001$) and so water pH was excluded in model development. Percent sphagnum cover and soil pH were chosen over sphagnum depth and water pH to best reflect microhabitat usage as availability of sphagnum and soil characteristics would be most likely to influence presence over depth of microhabitat and stream pH values.

After running the models, red, ranked them, and then calculated AIC values (Akaike's information criterion) as a basis for comparison as well as AICc (Akaike's information criterion corrected) values, for use in analysis of small sample sizes (Burnham and Anderson 2004). I then calculated ΔAICc (the difference between the current model and the best fit model) and model weights (ω_i) to determine the probability that the model was the best fit compared to the other models (Akaike 1974). The conditional R^2 and marginal R^2 value were then calculated to determine variance that is explained by both the fixed and random factors in the model and variance that is explained by only the fixed factors in the model, respectively (Nakagawa and Schielzeth 2013). These values were calculated using the 'piecewiseSEM' package in program R (Lefcheck 2016; R Core Team 2017).

Results

Winter

I ran a total of 5 models using winter survey data, compared their AICc values (Table 1) and determined that the top model contained TOB, leaf litter depth, % woody debris cover, % sphagnum cover, and soil moisture content; AICc=84.84, ω_i=0.798, marginal R^2=0.930 and conditional R^2=0.953. Estimates from the top model indicated that for each unit increase in TOB, leaf litter depth, % woody debris cover, % sphagnum cover, and soil moisture content, salamanders are 0.1135 (p=0.0002), 10.203 (p=0.0011), 0.345 (p=0.0129), 1154.39 (p=0.0079), and 7.309 (p=0.0087) times as likely to be present, respectively (Table 2). The second top model contained the variables bank angle, TOB, % bare ground cover, leaf litter depth, % woody debris cover, % sphagnum cover, and soil moisture content; AICc= 88.81, ω_i= 0.109, marginal R^2=0.922 conditional R^2=0.951.

Summer

I ran a total of 5 models using summer survey data, compared their AICc values (Table 3) and determined that the top model contained the variables bank angle, % woody debris cover, % fern cover, soil moisture, and soil pH; AICc=97.54, ω_i= 0.537, marginal R^2=0.642, and conditional R^2= 0.842. Estimates from the top model indicated that for each unit increase in bank angle, % woody debris cover, % fern cover, soil moisture content, and soil pH, salamanders are 0.165 (p=0.0014), 0.508 (p=0.0475), 5.045 (p=0.0401), 0.223 (p=0.0006), and 4.109 (p=0.1279) times more likely to be present, respectively (Table 4). The second highest rated model contained the variables bank angle, % woody debris cover, % fern cover, % sphagnum cover, soil moisture content, and soil pH; AICc= 99.3, ω_i=0.222, marginal R^2=0.662, and conditional R^2=0.846.

Discussion

Predictive models can be useful for identifying key areas for conservation actions or assessing impacts on habitat. Variables included in each of the current models varied by season, which was to be expected due to seasonal changes. The top winter model contained most variables predicted to be associated with salamander presence. The top summer model varied from winter and contained most variables that indicated moisture but did not contain sphagnum cover. The exclusion of sphagnum cover in the top model could be due to sampling protocol and/or the patchiness of the sphagnum. An increase in sample size and sampling area would likely be beneficial to capture a more accurate estimate of sphagnum cover at a site.

The AICc difference between the summer top model and the secondary model is within 2 AICc values, indicating that the top model may not be significantly better than the secondary model. Comparing the conditional R^2, we found that the top model explained 84.24% of variance, whereas the second model explained 84.6% of the variance. This discrepancy could be a result of the percent cover sphagnum variable being significant but with a small sample size the impact of this variable may not have been significant enough to be included in the top model. The AICc values for the top two winter models varied greater than 2 AICc from one another. This indicates that the top model is significantly better than the other models and explains 93.04% of the variance with the second model explaining 92.25% of the variance. Due to both summer and winter models having high conditional R^2 values, they may both be effective in predicting salamander presence, when used during their respective seasons.

Based on the life history traits of close members in the dwarf salamander complex, *E. hillisi* is thought to migrate between sites for breeding purposes during the winter months (Wray et al. 2017). Salamanders, to date, have not been located at the surveyed sites during the winter season and potential winter habitats have not yet been identified. Because of the habitat uncertainty associated with the life history of this salamander, the winter predictive model is only effective in predicting presence during the summer season rather than the winter season. Although the winter model cannot be used to predict occupancy during the winter months, it may still be a useful tool for analyzing impacts of management activities as well as allowing the identification of potentially occupied habitats throughout the year.

Future Studies

Overall, there were significant patterns associated with the detected and undetected stream sides found in the paired analysis, and these patterns were matched by the predictive models. Because the models were generated using the patterns that were already exhibited in the paired analysis, further development of the model would benefit from data from a new, previously excluded site and testing the accuracy of the model with these parameters. Pilot surveys could serve as field tests before full implementation of use for conservation or management purposes. Furthermore, this model could potentially be molded into a spatial model for use in GIS modeling, and used for impact analysis. With the global decline of amphibian species (Alford and Richards 1999), the benefits of implementing such models could be considerable.

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Table

1. AIC results for models run with winter survey data. BA= bank angle, TOB= top of bank, BG= % bare ground cover, WD= % woody debris cover, LLD= leaf litter depth, S= % sphagnum cover, MC= soil moisture content, and SpH= soil pH.

Models	Parameters (K)	AICc	Delta AICc (Δi)	Akaike Weight (ωi)	Marg. R^2	Cond. R^2
TOB+LLD+WD+S+MC	5	84.84	0.000	0.797	0.930	0.952
BA+TOB+BG+LLD+WD+S+MC	7	88.82	3.979	0.109	0.922	0.951
TOB+LLD+S+MC	4	90.12	5.282	0.056	0.887	0.924
BA+TOB+BG+LLD+WD+S+MC+SpH	8	91.08	6.244	0.035	0.925	0.953
BA+TOB+BG+S+MC+SpH	6	98.20	13.364	0.001	0.862	0.898

Table

Table
2. Beta estimates, standard errors, and p-values for variables contained in the top model for winter surveys. Estimates >1 indicate a positive relationship with salamander presence, estimates <1 indicate a negative relationship with salamander presence.

Parameter	β Estimates	Standard Error	*p*
Top of Bank	0.1135	1.7885	0.0002*
Leaf Litter Depth	10.2032	2.0423	0.0011*
Woody Debris	0.3498	1.5258	0.0129*
Sphagnum	1154.3584	14.2435	0.0079*
Moisture Content	7.3090	1.9410	0.0087*

Table 3. AIC results for models run with summer survey data. BA= bank angle, TOB= top of bank, BG= % bare ground cover, WD= % woody debris cover, S= % sphagnum cover, FE= % fern cover, MC= soil moisture content, and SpH= soil pH.

Models	Parameters (K)	AICc	Delta AICc (Δi)	Akaike Weight (ωi)	Marg. R^2	Cond. R^2
BA+WD+FE+MC+SpH	5	97.53	0	0.537	0.642	0.842
BA+WD+FE+S+MC+SpH	6	99.30	1.764	0.222	0.661	0.846
BA+FE+MC+SpH	4	100.4	2.882	0.127	0.627	0.821
BA+FE+S+MC+SpH	5	101.5	4	0.072	0.658	0.818

Table

BA+TOB+BG+WD+S+FE+MC+SpH	8	102.6	5.144	0.041	0.682	0.804

Table
4. Beta estimates, standard errors, and p-values for variables contained in the top model for summer surveys. Estimates >1 indicate a positive relationship with salamander presence, estimates <1 indicate a negative relationship with salamander presence.

Parameter	β Estimate	Standard Error	*p*
Bank Angle	0.1625	1.7672	0.0014*
Woody Debris	0.5084	1.4069	0.0475*
Ferns	5.0451	2.2002	0.0401*
Moisture Content	0.2234	1.5489	0.0006*
Soil pH	4.1094	2.5305	0.1279

Table