AQUATIC HABITAT SELECTION BY LARVAL AND PAEDOMORPHIC MOLE SALAMANDERS *(AMBYSTOMA TALPOIDEUM)* IN THE SOUTHERN APPALACHIANS

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology

By

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AQUATIC HABITAT SELECTION BY LARVAL AND PAEDOMORPHIC MOLE SALAMANDERS *(AMBYSTOMA TALPOIDEUM)* IN THE SOUTHERN APPALACHIANS

James Louis Cadolino
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The mole salamander *(Ambystoma talpoideum)* is listed as a species of Special Concern in the state of North Carolina. Populations within the state are disjunct and isolated. The main distribution of this species is in the Coastal Plain of the southeastern United States where much of our knowledge about its natural history comes from. In the mountains of North Carolina, mole salamanders exist at high elevations in a few isolated populations, suggesting that they could be a separate species. These populations are generally understudied and uncharacteristic for this species. At higher elevations, cooler air, water, and soil temperatures present conditions that are not common throughout its range. Mole salamanders are facultatively paedomorphic, meaning that individuals can forgo metamorphism and retain aquatic larval features as sexually mature adults. This means adults use their aquatic habitats more extensively than other species of ambystomatid salamander, such as the marbled salamander (*Ambystoma opacum*). There is little research on habitat use and the duration in which paedomorphic mole salamanders use their aquatic environments. Understanding habitat use within these environments is important for the conservation and management of this species and its habitat. This study focused on a population of mole salamanders in the Southern Appalachians at the highest known elevation of their range. I examined microhabitat selection and the effect of canopy openness on larval and paedomorphic mole salamanders within a complex of high elevation mountain bogs. Dip-net surveys were conducted using plots to examine the probability of detecting mole salamanders in relation to certain microhabitat characteristics found within these wetlands. Results determined that emergent vegetation was the strongest microhabitat characteristic in relation to the detection of mole salamanders during dip-net surveys, suggesting that this is an important microhabitat feature for mole salamanders in high elevation wetlands. There was also a positive correlation between canopy openness and the detection of mole salamanders during dip-net surveys. Canopy openness is essential for the growth of emergent vegetation in wetlands. Snout-vent-length measurements collected during field surveys showed that paedomorphic adults were smaller on average than terrestrial adults, suggesting that paedomorphic individuals were not reaching their maximum body size in this life stage. This data supports previous observations made on *A. talpoideum* by White et al. (2016) supporting Whiteman's (1994) "best of a bad lot" hypothesis, which states that smaller individuals forgo metamorphosis to reproduce earlier and maximize fitness under conditions that limit growth, while larger individuals metamorphose to escape an unfavorable aquatic habitat. However, it is not known how long these paedomorphic individuals remain in and use their aquatic habitats. This study provides a better understanding on the natural history and habitat use of mole salamanders within their aquatic environments and at the highest elevation of their range. Providing insight into microhabitat use will also aid in surveying and conservation efforts to bridge distributional gaps between the Coastal Plain and high elevation populations in the Southern Appalachians.

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INTRODUCTION

Mole salamanders (*Ambystoma talpoideum*) occur mainly in the Coastal Plain of the southeastern United States with several disjunct populations in the Southern Appalachian Mountains of North Carolina and Tennessee (Petranka, 1998) (Figs. 1, 12). In the Coastal Plain, populations are found in floodplain forests and cypress wetlands while populations further north are found in upland hardwood forests or mixed pine-hardwood forests (Petranka, 1998). As terrestrial adults, mole salamanders spend most of their lives underground in burrow systems with populations being found around suitable breeding ponds or wetlands (Williams and MacGowan, 2004; Shoop, 1964). Adults migrate to these wetlands in late autumn or winter when breeding takes place (Semlitsch, 1985a) and migrations are triggered by heavy rainfall (Semlitsch, 1985a). These wetlands provide crucial breeding habitat for mole salamanders and many other amphibians.

The mole salamander is often the most common species of ambystomatid salamander found in suitable wetland habitats throughout the Coastal Plain, while the species and its distribution become less common in wetlands further north and into the mountains (Williams and MacGowan, 2004; Petranka, 1998). Much of our knowledge on mole salamander natural history comes from the Coastal Plain, while disjunct Mountain populations, such as in North Carolina, are understudied. Because mole salamanders in North Carolina are disjunct from their core range and limited to several isolated populations within the Piedmont and Mountains (Beane et al., 2010) they are listed as a species of Special Concern in the state (NCWRC, 2021). These isolated mole salamander populations in North Carolina could potentially be a separate species than *A. talpoideum* populations found in the Coastal Plain. North Carolina presents differing habitat types and forest compositions compared to what is normally found for this species in the Coastal Plain and understanding how mole salamanders use these habitats is important for the conservation and management of this species in the state. Mountain populations are especially unique because they are found at elevations above 700 m where this species is thought to not occur. *A. talpoideum* populations in the Coastal plain typically occur between sea level and 700 m (2,296 ft.) (Lannoo, 2005). This study focused on a population of mole salamanders in the Standing Indian Basin of the Nantahala National Forest, which is the highest elevational extent of their range at 1073 m (3,520 ft.). The population here is found in a wetland complex of mountain bogs, which contrast to the cypress wetlands and Carolina Bays of the Coastal Plain in which this species is more commonly found (Beane et al., 2010; Williams and MacGowan, 2004) (Fig. 2).

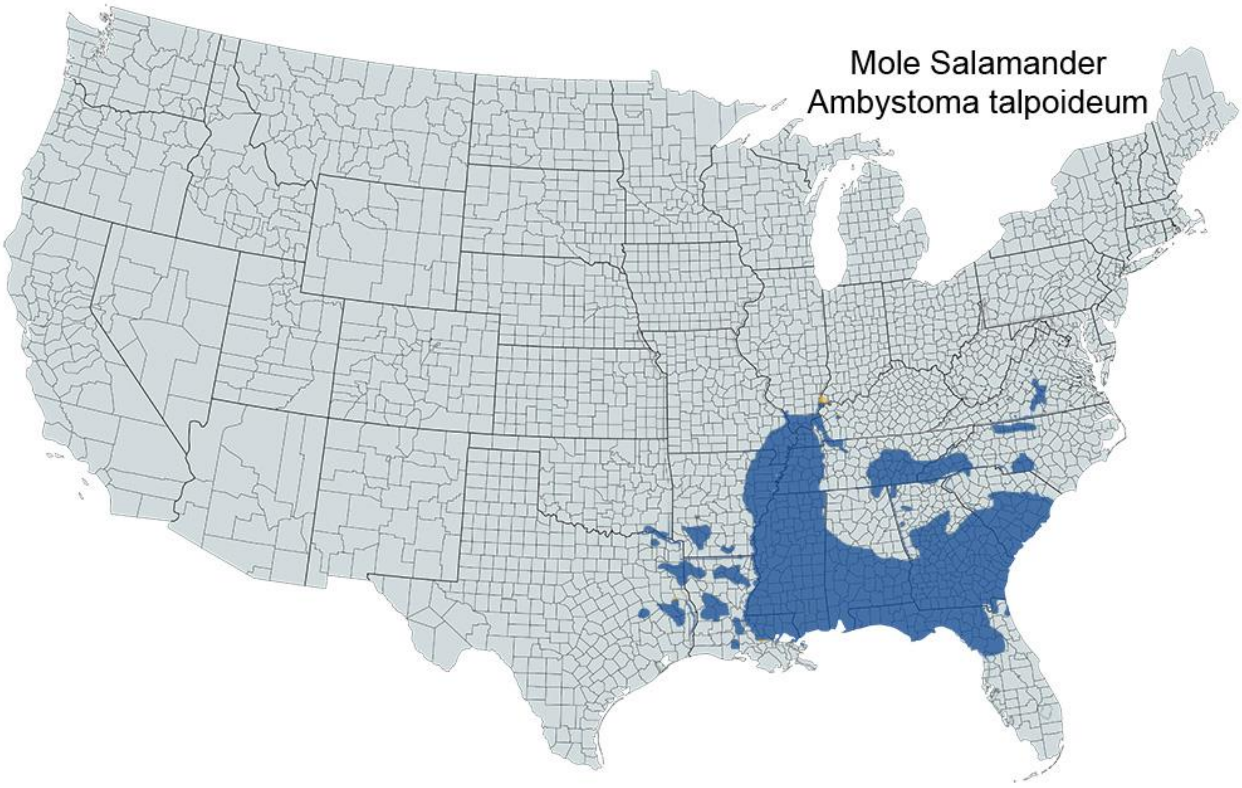


Figure 1. Distribution of the mole salamander (*Ambystoma talpoideum)* in the southeastern United States, showing disjunct populations in North Carolina, Tennessee, Virginia and to the west. (www.virginiaherpetologicalsociety.com).

Figure 2. Habitat and forest type comparison between *A. talpoideum* wetland habitat in the Coastal Plain, in Aiken County, SC (top photo) and in the Appalachian Mountains, at a wetland study site in Macon County, NC (bottom photo).

Mole salamanders are facultatively paedomorphic, meaning that individuals can forgo metamorphism and retain aquatic larval features as sexually mature adults (Gould, 1977; Whiteman, 1994). In the Coastal Plain, paedomorphic mole salamanders typically exist in fishless, permanent or semi-permanent ponds or wetlands (Semlitsch, 1985b) although the duration in which individuals remain in this life stage is not fully understood. Two commonly proposed explanations for why paedomorphism occurs in mole salamander populations are the "paedomorph advantage" (Gould, 1977) and the "best of a bad lot" theory (Whiteman, 1994). "Paedomorph advantage" is where favorable aquatic conditions result in large individuals, which can maximize their fitness by remaining in this favorable environment rather than metamorphosing and migrating to the terrestrial environment (Gould, 1977). In this scenario, smaller individuals metamorphose to avoid competition with the larger individuals that remain in aquatic habitats. This scenario suggest that the aquatic environment is favorable for growth and that environmental factors, such as water temperature, growing season, and the presence of predators, are not limiting factors. "Best of a bad lot" is where smaller individuals forgo metamorphosis to reproduce earlier and maximize fitness under conditions that do limit growth (Whiteman, 1994). These individuals become sexually mature as paedomorphs while larger individuals metamorphose to escape an unfavorable aquatic habitat. Both Gould's (1977) and Whiteman's (1994) hypotheses suggest that environmental differences among populations of the same species can result in different mechanisms for the selection and maintenance of facultative paedomorphosis. The "best of a bad lot" scenario assumes that the aquatic environment is unfavorable due to growth limiting environmental factors such as cooler water temperatures and a short growing season. Growth limiting environmental conditions such as these are common at higher elevations. Both the "paedomorph advantage" and “best of a bad lot” theories assume that many individuals remain paedomorphic throughout some duration during their lifetimes, resulting in two distinct life history pathways (Moore et al., 2020) but studies have not examined the duration in which individuals spend in the paedomorphic life stage.

Doyle and Whiteman (2008) demonstrated support for the "best of a bad lot” mechanism in a population of mole salamanders located in western Kentucky, which is at the northwestern edge of the range of this species. They predicted that shorter growing seasons and lower productivity at higher altitudes and elevations would create poor aquatic environments resulting in paedomorphosis through the “best of a bad lot” mechanism (Whiteman, 1994). Results showed that individuals who metamorphosed during the fall and following spring after hatching were larger on average as larvae than those that became paedomorphic and remained in the aquatic habitat (Doyle and Whiteman, 2008). This suggests that the largest larvae were metamorphosing to escape this unfavorable aquatic environment, which in this case was created by the colder climate and lower productivity found in Kentucky.

White et al. (2016) further demonstrated support for the “best of a bad lot” theory by conducting a mesocosm experiment with *A. talpoideum* larvae collected from a wetland site in NC to examine whether water temperature affected body size prior to metamorphosis or the expression of paedomorphism. The experiment found that water temperature did not affect body size or morphotype expression. Salamanders did not metamorphose before the first breeding season after hatching and 57% remained within their aquatic habitat to mature as paedomorphs (White et al., 2016). Fifteen percent metamorphosed the following spring with six of these individuals metamorphosing after becoming sexually mature as paedomorphs

(White et al., 2016). Individuals that did not become paedomorphic before metamorphosing overwintered as larva. White et al. (2016) also found that individuals who attained a larger snout-vent-length before the first spring metamorphosis event had a higher probability of metamorphosing. This further suggests that growth limiting conditions within aquatic environments, such as at higher elevations, influence paedomorphism in mole salamanders. To observe and document the average body size of both terrestrial and aquatic individuals at the highest elevation of their range in a natural setting, I collected body size measurements on paedomorphic and terrestrial mole salamanders during my study. Other notable observations from White et al. (2016) were paedomorphic *A. talpoideum* breeding with each other after most terrestrial adults had left the wetland. In the Coastal Plain, paedomorphic adults have been observed breeding with other paedomorphs prior to the arrival of terrestrial adults to the wetland (Scott, 1993; Krenz and Sever, 1995). Breeding early can increase offspring growth and survival (Ryan and Plague, 2004), while the advantages of paedomorphic *A. talpoideum* breeding after terrestrial adults have left the wetland are not clear. White et al. (2016) also observed female *A. talpoideum* depositing eggs singly in the NC mountains. Atlantic Coastal Plain populations are known to also lay eggs singly, while Gulf Coastal Plain populations lay eggs in clusters (Semlitsch and Walls, 1990) (Fig. 1).

Since the aquatic environment is key to understanding the factors influencing paedomorphism (paedomorph advantage vs. best of a bad lot), my study focused on environmental factors related to mole salamander habitats in the Mountains. There is little research on the aquatic habitat use of the mole salamander, so understanding its microhabitat selection within aquatic environments is important for identifying key habitat characteristics that may be associated with the occurrence of this species and its survival in this geographic region (Ayala et al., 2019). Pond-breeding amphibians are of special concern from a conservation standpoint due to data deficiencies on habitat use within aquatic environments in which these amphibians depend on for reproduction and larval development (Crawford and Semlitsch, 2008). Cushman (2006) emphasized the importance of determining a species' specific habitat use, which can be used to develop models and conservation plans aimed at understanding the effects of habitat loss and degradation on amphibian populations.

Understanding the specific habitat requirements of a species throughout its entire life cycle is important for identifying critical habitat in its entirety for a species (Lefebvre et al., 2018; Van Drunen et al., 2020). Parmelee (1993) used cover objects to examine terrestrial microhabitat segregation around breeding ponds in four sympatric species of ambystomatid salamanders to determine if microhabitat segregation occurred between age groups or between these four species, which were the small-mouthed salamander *(A. texanum),* spotted salamander *(A. maculatum),* marbled salamander *(A. opacum),* and the mole salamander *(A. talpoideum).* Results showed that salamanders occurred more often under objects that were larger and more moist than other cover objects and that adults were found more often under larger cover objects compared to juveniles (Parmelee, 1993). Leaves were the most common cover object for all juveniles, except for *A. opacum.* Parmelee (1993) also found that substrate moisture also had a significant impact on what species was found under what cover object with certain species being found more often in moister conditions, such as *A. talpoideum,* showing that even small microhabitat differences in the terrestrial environment can impact how this species uses this habitat.

Forest compositions in the mountains of North Carolina present conditions for mole salamanders that are found nowhere else within their range. Williams and MacGowan (2004) recently described a disjunct northern population of mole salamanders in southwestern Indiana, which is one of the northernmost populations on record but still in the Coastal Plain geographic region. They noted that the forest composition and habitat in which this population was found in was similar to southern populations and consisted of cypress wetlands and extensive floodplain forests. The high elevation mountain bogs in our study present a completely different habitat type for the mole salamanders that is uncharacteristic for this species.

The rugged topography of Appalachian Mountains has created a series of mountain bog ecosystems that extend as far south as North Carolina (Schultheis et al., 2010). These bogs are defined as isolated wetlands surrounded by terrestrial communities (Spira, 2011) and are characterized by *Sphagnum* moss and saturated soils that are nutrient-poor and acidic (Schultheis et al., 2010). Spira (2011) described mountain bogs as three distinct types: southern Appalachian bog, swamp forest-bog complex, and cataract bog. The bogs or fens in this study most closely resemble swamp forest-bog complex under this description, which is associated with small streams that have level or gently sloping areas whose soils are water-saturated and acidic (Spira, 2011). Vegetation is characterized by a broken canopy with an open to dense shrub layer and small boggy openings, varying in amount of closed forest, shrub thickets, and boggy openings (Spira, 2011). The shallow roots of tree species associated with this bog type, such as red maple *(Acer rubrum)* and other canopy trees, result in treefalls that create openings or gaps within the canopy that provide habitat for species that require high light levels (Spira, 2011) which may be important for the presence of mole salamanders within these bogs.

It is generally thought that increasing canopy coverage decreases “pond breeding” amphibian abundance in aquatic environments due to the inability of sunlight to reach the water’s surface (Halverson et al., 2003). Open canopy conditions in wetlands allow sunlight to reach the water and create thermal gradients within the aquatic environment which may be necessary for the growth and development of ambystomatid salamanders, and other amphibians, at higher elevations (Heath, 1975). At higher elevations, these salamanders experience cooler air and water temperatures which can limit their growth and slow development in the larval stage (Berven et al., 1979; Patterson, 1978).

The wetlands focused on in this study are surrounded by northern hardwood forest with mountain tops reaching 1676 m (5,499 ft.) and culminating in spruce-fir forest. Deciduous tree canopy coverage is quite dense around bogs and wetlands in the mountains compared to the Coastal Plain, where coniferous pine forests with relatively open canopies surround wetlands (Kricher, 1998; Petranka, 1998) (Fig. 2). Besides natural treefall, succession at these bogs is controlled primarily by the American beaver (*Castor canadensis*). Historically, large megafauna such as elk (*Cervus canadensis*) and wood bison (*Bison bison athabascae*) would have also contributed to controlling succession primarily around the wetland edges through grazing (Yarnell, 1998; Spira, 2011). The forested edges along these wetlands are thick with trees and lined with rosebay rhododendron *(Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*). Dense woody coverage along wetland edges is known to increase water uptake and drying (Lilieholm, 1995). Large grazers likely would have played an important role in controlling succession in bogs by foraging on woody plants which would have kept bogs from becoming forested landscapes (Yarnell, 1998; Spira, 2011). Now, the American beaver is primarily responsible for controlling woody encroachment at our study sites and greatly influences the hydrology of these wetlands (Yarrow and Yarrow, 2005; Spira, 2011) by creating areas of permanent water, which the mole salamanders utilize, that are less susceptible to drying.

The western tiger salamander (*Ambystoma mavortium*), a facultatively paedomorphic species that relies on open canopy conditions at high elevations, inhabits the Rocky Mountains at elevations above 2700 m (8,858 ft.) where the active season for amphibians is very short (Heath, 1975; Whiteman, 1994). Salamanders in these populations breed in high elevation glacier ponds that are fed mostly by snow runoff and ground seepage. Individuals in these populations exist as a mixture of terrestrial and neotenic adults with terrestrial individuals overwintering in sub-alpine habitats and neotenic individuals remaining in their aquatic environments year-round. Heath (1975) observed that western tiger salamander larvae were reliably found in the warmest areas of high elevation ponds. Field and lab experiments designed to examine behavioral thermoregulation in these salamanders revealed that larvae, as well as paedomorphic and terrestrial adults, were selecting the warmest areas available in these ponds. (Heath, 1975). Larvae seemed to have greater thermoregulatory precision suggesting that this behavior may be essential for growth and development at higher elevations where cooler water temperatures can slow larval growth, making it difficult to grow big enough to metamorphose (Berven et al., 1979). Berven et al. (1979) demonstrated this in a montane population of the green frog *(Rana clamitans)* and showed that lower temperatures in montane ponds resulted in prolonged larval periods and increased size at metamorphosis. A temporary state of paedomorphism may be important for the fitness of individuals at higher elevations, which may apply to mole salamanders in the Southern Appalachians.

In addition to affecting thermoregulation and development, canopy openness can influence the distribution and performance of many pond breeding amphibians by providing more food resources. Werner and Glennemeier (1999) observed higher tadpole growth and survivorship in open canopy ponds for wood frogs *(Rana sylvatica),* leopard frogs *(Rana pipiens)*, and American toads *(Bufo americanus)*. Algal growth was abundant in open canopy ponds where tadpoles performed best. Open canopy conditions allow for the growth of algae, which is a large component of the diets of tadpoles and other aquatic organisms on which many amphibian larvae feed (Kupferberg et al., 1994). Conditions found in ponds with open canopies created a significant difference in the survival and performance of these amphibians and the availability of open canopy conditions within these ponds may be a determining factor for the presence and breeding success of these amphibians.

Open canopy conditions also allow for the growth of emergent vegetation (i.e., rushes and sedges) in wetlands, which is essential for many pond breeding amphibians and serves as refuge against predators (Altig and McDiarmid, 2017). Madison and Farrand (1998) found that adult tiger salamanders (*A. tigrinum*) in exposed breeding ponds preferred deep, vegetated areas for cover. Similarly, Gorman et al. (2009) found that the best habitat characteristics in breeding wetlands for the reticulated flatwoods salamander *(Ambystoma bishopi)* was herbaceous vegetation and open canopies. *Ambystoma bishopi* occurs in mesic, longleaf pine flatwoods and savannas in the northern coastal plain of the Gulf of Mexico (Palis, 1996) and requires wetlands with open canopies and dense herbaceous vegetation for breeding where they deposit their eggs (Gorman et al., 2009). Researchers suggest that managing breeding habitat for open canopies and dense herbaceous vegetation will contribute to the breeding success of this species, which is listed as endangered due to the loss of suitable wetland habitat in its range (Gorman et al., 2009).

Ayala et al. (2019) tested the relationship between vegetation presence and the distribution of captive-raised axolotls (*Ambystoma mexicanum)*. Results found that axolotls preferred vegetated microhabitats, especially during daytime hours when ambystomatid salamanders are less active (Ayala et al., 2019). They observed that axolotls were able to locate specific patches of vegetation which suggesting that axolotls can perceive differences in microhabitats, selecting certain areas more often than others. There is only one remaining area in which the Mexican axolotl is found in the wild and prior to the findings by Ayala et al. (2019), the habitat preferences of the axolotl were unstudied, expressing the importance of understanding habitat selection of threatened and endangered amphibian species.

In light of this background knowledge, the objectives of this study are to 1) observe the aquatic habitat use of larval and paedomorphic mole salamanders in high elevation wetlands, 2) identify important microhabitat characteristics and habitat features for mole salamanders within these high elevation wetlands that may be associated with their occurrence, and 3) provide a better understanding on the natural history of this species as a whole and at the highest elevation of its range.

I predict that larval and paedomorphic mole salamanders will select more open canopy areas in high elevation wetlands due to the availability of warmer water temperatures and higher amounts of emergent vegetation, which aid in growth, development, and predator avoidance. I also predict in support of Whiteman's (1994) "best of a bad lot" hypothesis and previous studies of mole salamanders in the Mountains and further north (White et al. 2016; Doyle and Whiteman 2008), that the average body size of paedomorphic individuals in this study population will be smaller on average than metamorphosed terrestrial adults due to an unfavorable aquatic habitat at high elevation compared to the Coastal Plain. To examine this, I collected body size measurements on paedomorphic and terrestrial individuals during field surveys.

METHODS

Study Site: This study focused on a high elevation population of mole salamanders in Macon County, North Carolina, found within a high elevation wetland complex of mountain bogs in the Standing Indian Basin of the Nantahala National Forest (Fig. 3). Three upper wetland study sites were selected which ranged from 1069 m (3,507 ft.) to 1283 m (4,209 ft.) in elevation (Table 1) (Fig. 3). The population of *Ambystoma talpoideum* here is currently the highest known elevation for this species. These wetlands run along the Nantahala River from Rainbow Springs in Macon County and up to the river's headwaters where Mooney Creek and Big Laurel Branch converge (Fig. 3). Wetland sites encompass a total 3.53 ha in combined area (Table 1).

Table 1. Site data from study sites in Macon County, North Carolina.

Study Sites	Elevation	Area (ha)
Site 1	1069 m	0.34
Site 2	1073 m	0.61
Site 3	1283 m	2.58

Wetlands here are made up of a series of mountain bogs and fens that occur along the Nantahala River (Fig. 3). These bogs are saturated primarily by rain and groundwater. Certain areas of these bogs more closely resemble semi-permanent riparian wetland due the proximity of the Nantahala River and due to manipulation by the American beaver (*Castor canadensis*) which has created areas of permeant water, or "beaver ponds" found throughout these wetlands that mole salamanders utilize. These "beaver ponds" remain mostly filled with water and fluctuate less throughout the year while the areas relying on flooding, rainfall, and groundwater seepage are more susceptible to drying. This creates a very complex and highly structured wetland habitat for many rare plant and animal species, including the mole salamander.

The upland forested habitat around these wetlands is northern hardwood forest and is composed primarily of red maple *(Acer rubrum)*, yellow birch *(Betula alleghaniensis)*, northern red oak *(Quercus rubra)*, American beech *(Fagus grandifolia)*, yellow poplar *(Liriodendron tulipifera)*, yellow buckeye *(Aesculus flava)*, red spruce *(Picea rubens)*, eastern white pine *(Pinus strobus)*, eastern hemlock *(Tsuga canadensis)*, and rosebay rhododendron *(Rhododendron maximum)*. The bogs themselves are a mixture of *Sphagnum*, sedges, and rushes with tussocks and deep-water pockets dispersed throughout. Some of these areas of deeper water were created by the American beaver, while some are formed by springs and rivulets that run throughout these wetlands.

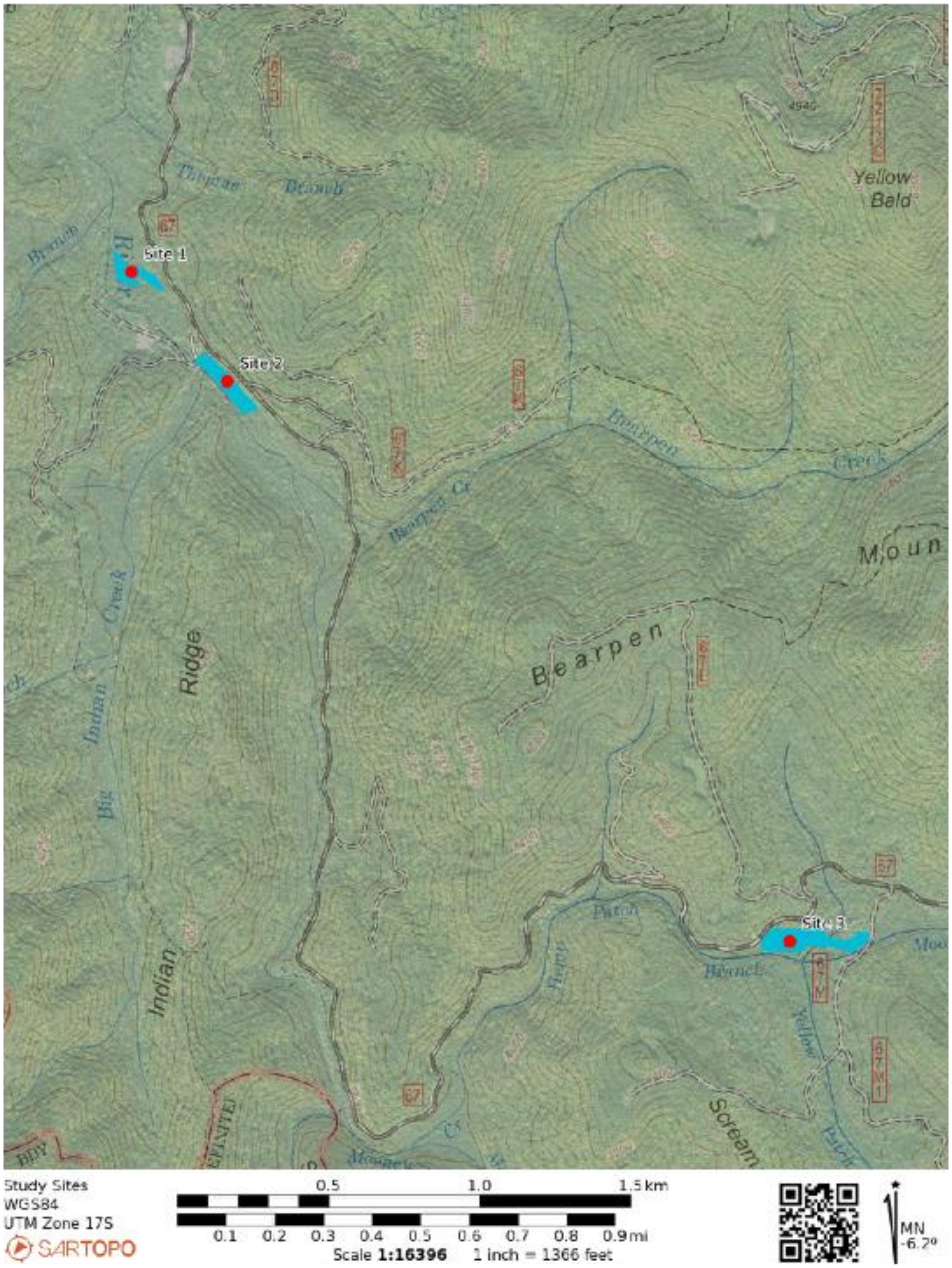


Figure 3. Topography map of study area and wetland sites in Macon County, NC. Wetland sites (shaded in blue and marked with red pins) run along the Nantahala River up to the river's headwaters where Mooney Creek and Big Laurel Branch converge.

Dip-net Surveys: Dip-net surveys were used to examine the effect of canopy openness within wetlands on *Ambystoma talpoideum* and to examine the microhabitat preferences of larval and paedomorphic mole salamanders within their aquatic environments. Stratified random sampling was used to select 2x2 m survey plots within three wetland sites at the upper elevations of this wetland complex (Fig. 3). 48 plots total were placed throughout these wetlands and were marked with flagging and GPS coordinates. Plots were placed within a range of open and closed canopy areas and included different microhabitat characteristics found throughout these wetlands. Microhabitat characteristics included water depth, water temperature, pH, vegetation cover (as percent coverage within plots), and substrate composition of each plot. Additional data for each wetland site was collected including elevation, area of wetland, and presence of predatorial species such as minnows and other amphibian species also found in plots. Percent vegetation cover within plots was measured using the Daubenmire Cover Class Method (Daubenmire, 1959). Canopy cover was measured using a camera with a fisheye lens and photos were analyzed using Gap Light Analyzer software (Frazer et al., 1999) to determine the percent of canopy coverage for each plot.

Sites were surveyed in two separate survey periods. The first survey period took place June-July 2023 (Summer) and the second September-October 2023 (Fall). Additional dip-net surveys took place February-April 2024 (Spring) opportunistically since site access was limited over the winter months. During each survey period, wetlands and plots were surveyed three separate times each using dip-nets to determine the presence or absence of mole salamanders at each plot and wetland. Each plot was surveyed by one person using a dip-net for a total of ten minutes, covering the entirety of the plot. Using digital calipers and a digital scale, I measured the snout-vent-length (SVL), and mass of each salamander found while dip-netting during those ten minutes and recorded whether it was a larva or paedomorphic adult. Paedomorphic adults were sexed as male or female by examining cloacal development. Salamanders were returned to their capture location before moving on to survey the next plot.

Drift Fence Surveys: A drift fence was constructed (Gibbons and Semlitsch, 1981) at Site 2 (Fig. 4) to collect measurements on terrestrial individuals migrating down to the wetland from their upland habitat for breeding. These measurements were used to document the average body size of terrestrial adults in this population as well any paedomorphic adults found emigrating from the wetland during this time. This drift fence was used opportunistically on rainy nights during breeding season from August-December of 2023. The drift fence was constructed using silt fencing and wooden stakes. It was placed on a forested slope 15 m above the wetland and was 40 m across in length. The bottom of the fence was attached to the ground with landscape staples to minimize disturbance on the forest floor. I deployed funnel traps along the drift fence to capture any salamanders walking along the fence. Funnel traps contained a damp sponge to ensure moisture inside the traps and were checked actively while in use. Any salamanders captured were measured, weighed, and placed on the other side of the fence in the direction that they were heading.

Figure 4. Photo of upland habitat and drift fence constructed at Site 2 above the wetland edge (left) and a funnel trap placed alongside the fence to capture any salamanders walking along it (right).

Statistical Analysis: I performed logistic regression and analysis of deviance in RStudio v1.3.1093 (RStudio Team, 2020) to examine whether microhabitat characteristics such as canopy openness, water temperature, and emergent vegetation were associated with the probability of detecting mole salamanders within high elevation wetlands during dip-net surveys. There were no *A. talpoideum* detections at Site 3, so only Site 1 and Site 2 were used for this analysis. A repeated measure ANOVA was used to test for environmental differences between Site 3 and Sites 1 and 2 to determine why *A. talpoideum* wasn't detected here. A two-sample t-test was used on SVL measurements and mass measurements collected on individuals to compare differences in average body size between paedomorphic and terrestrial adults and to further test the "best of a bad lot" hypothesis.

RESULTS

Dip-net Surveys

Canopy Openness: The probability of detecting *A. talpoideum* in relation to canopy openness increased slightly during summer surveys at Site 1 ($\Delta x^2 = 1.01$, $P = 0.31$) (Table 2; Fig. 5) and increased significantly at Site 2 ($\Delta x^2 = 15.7$, $P < 0.001$) (Table 2; Fig. 5). The majority of *A. talpoideum* detections during summer surveys at sites 1 and 2 were between 50-75% canopy openness (Fig. 6). Detections were highest at Site 2 during all surveys and survey seasons.

Table 2. Odds of detecting *A. talpoideum* in relation to canopy openness at study sites 1 and 2 during dip-net surveys in summer, fall, and spring of 2023-2024, determined by logistic regression. Deviance chi-square compared a logistic model without canopy openness to a logistic model including canopy openness. The larger the deviance chi-square, the larger the association between this microhabitat feature and the odds of detection.

Predictor	Season	Site	Regression Coefficient (Log Odds)	Odds	Deviance X^2	*P*
canopy openness	summer	1	0.019	1.019	1.01	0.31
canopy openness	fall	1	0.015	1.015	0.7	0.39
canopy openness	spring	1	0.003	1.003	0.002	0.95
canopy openness	summer	2	0.16	1.180	15.7	<0.001
canopy openness	fall	2	-0.05	0.943	2.6	0.1
canopy openness	spring	2	0.083	1.086	1.1	0.29

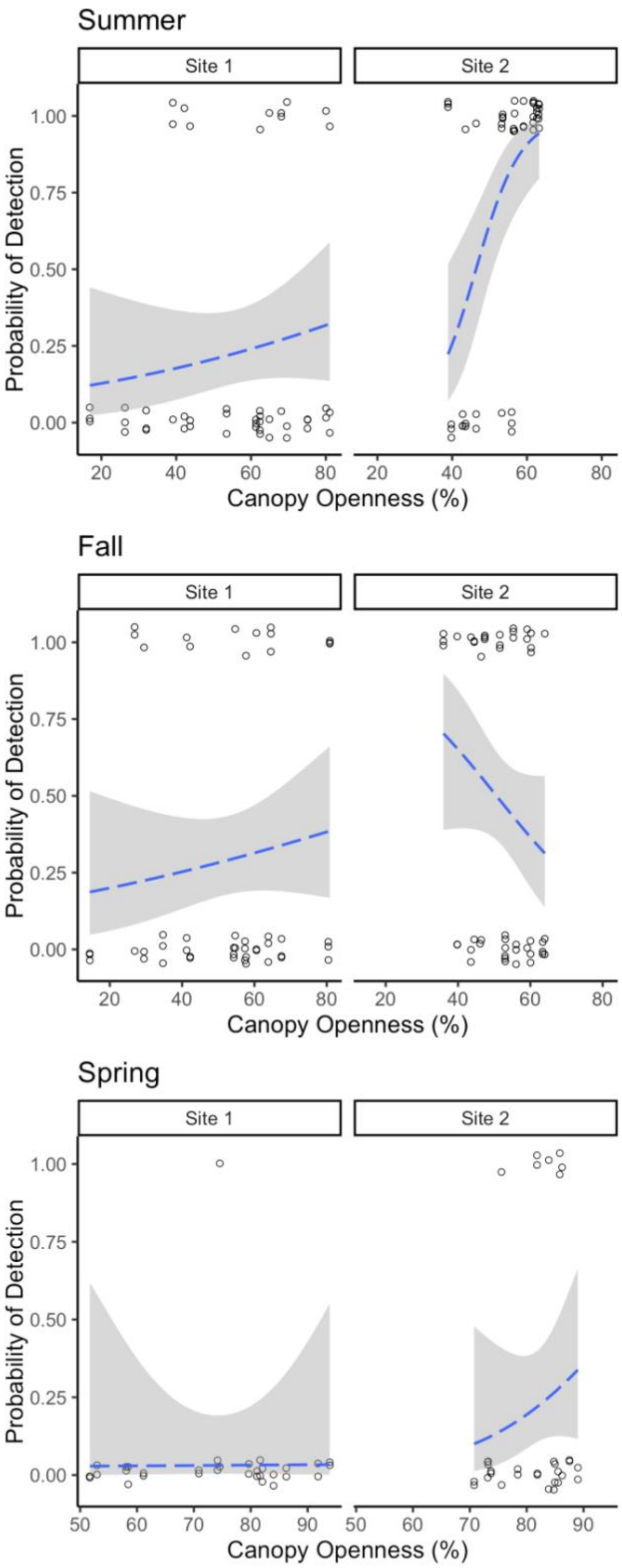


Figure 5. The probability of detecting *A. talpoideum* during summer, fall, and spring dip-net surveys in relation to canopy openness within wetlands at study sites 1 and 2.

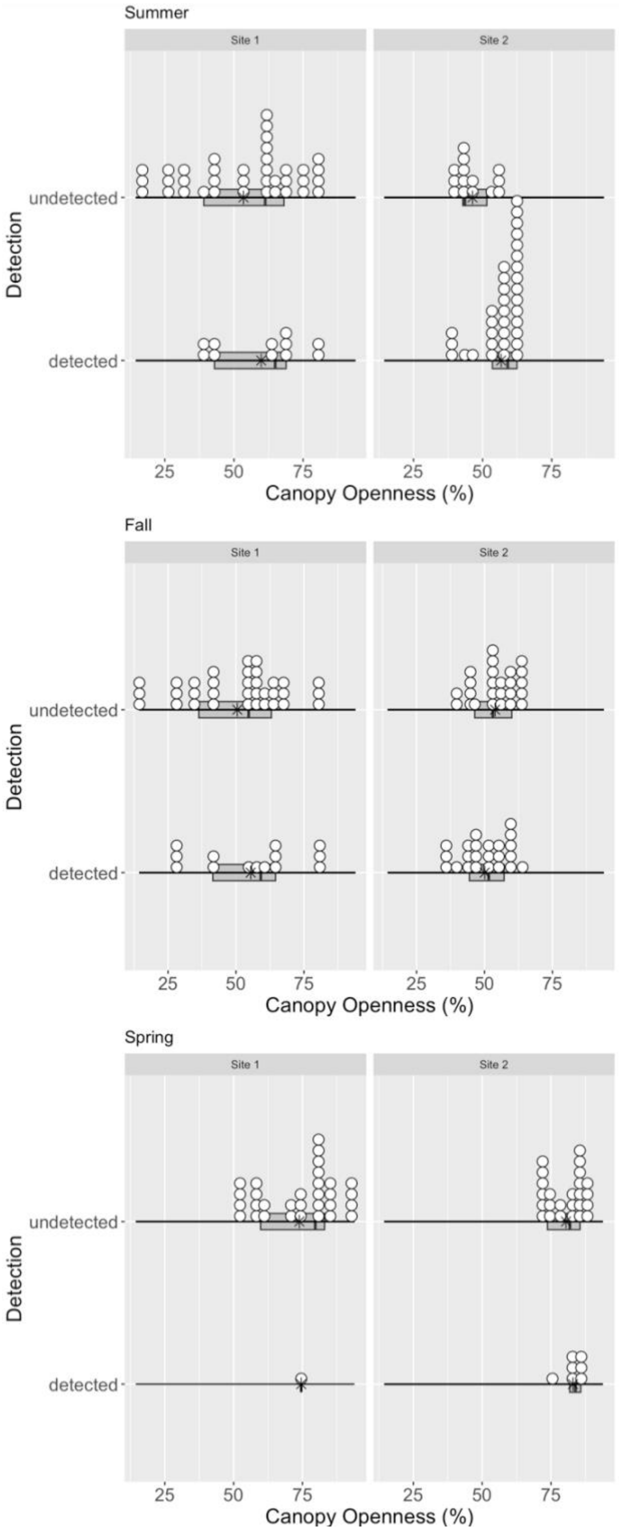


Figure 6. Detections and non-detections during summer, fall, and spring dip-net surveys plotted in relation to canopy openness within wetlands at study sites 1 and 2. Observed values (o), mean values (*), and quartiles (sideways boxplots).

During the fall, there was a slight association between detection and canopy openness at Site 1 ($\Delta x^2 = 0.7$, $P = 0.39$) (Table 2; Fig. 5), whereas there was a negative association at Site 2 (Table 2; Fig. 5). I suspect this negative association at Site 2 was due to detections being widespread in almost every plot during this survey period. Salamanders seemed to be utilizing the edges of the wetlands more during this time, possibly due to the onset of breeding season and the arrival of terrestrial adults from the upland habitat. Most detections during the fall at Site 1 were between 55-70% canopy openness whereas detections at Site 2 were between 45-60% (Fig. 6).

There was no noticeable association between canopy openness and the probability of detection during spring surveys at Site 1 or Site 2 ($\Delta x^2 = 1.1$, $P = 0.29$) (Table 2; Fig. 5). Site access was limited over the winter and springs months and fewer detections were made. The single detection made at Site 1 during spring surveys was at 75% canopy openness while the range of canopy openness for detections at Site 2 was 70-90% (Fig. 6).

Water Temperature: Water temperature had no effect on the probability of detection during summer surveys at Site 1 and Site 2 (Table 3; Fig. 7). During fall surveys, there was no association at Site 1 whereas Site 2 had a slight increase in detection ($\Delta x^2 = 2$, $P = 0.14$) (Table 3; Fig. 7). There was a decrease in detection during spring surveys at Site 1, however, only one detection was made at this site during spring. No association was found at Site 2 during spring (Table 3; Fig. 7). Results did not show a strong selection for warmer areas of water during surveys (Fig. 8).

Table 3. Odds of detecting *A. talpoideum* in relation to water temperature at study sites 1 and 2 during dip-net surveys in summer, fall, and spring of 2023-2024, determined by logistic regression. Deviance chi-square compared a logistic model without water temperature to a logistic model including water temperature. The larger the deviance chi-square, the larger the association between this microhabitat feature and the odds of detection.

Predictor	Season	Site	Regression Coefficient (Log Odds)	Odds	Deviance X^2	*P*
water temperature	summer	1	0.07	1.072	0.2	0.53
water temperature	fall	1	-0.10	0.904	0.3	0.54
water temperature	spring	1	-0.35	0.704	1.8	0.17
water temperature	summer	2	0.03	1.030	0.06	0.79
water temperature	fall	2	0.23	1.258	2	0.14
water temperature	spring	2	0.05	1.051	0.2	0.63

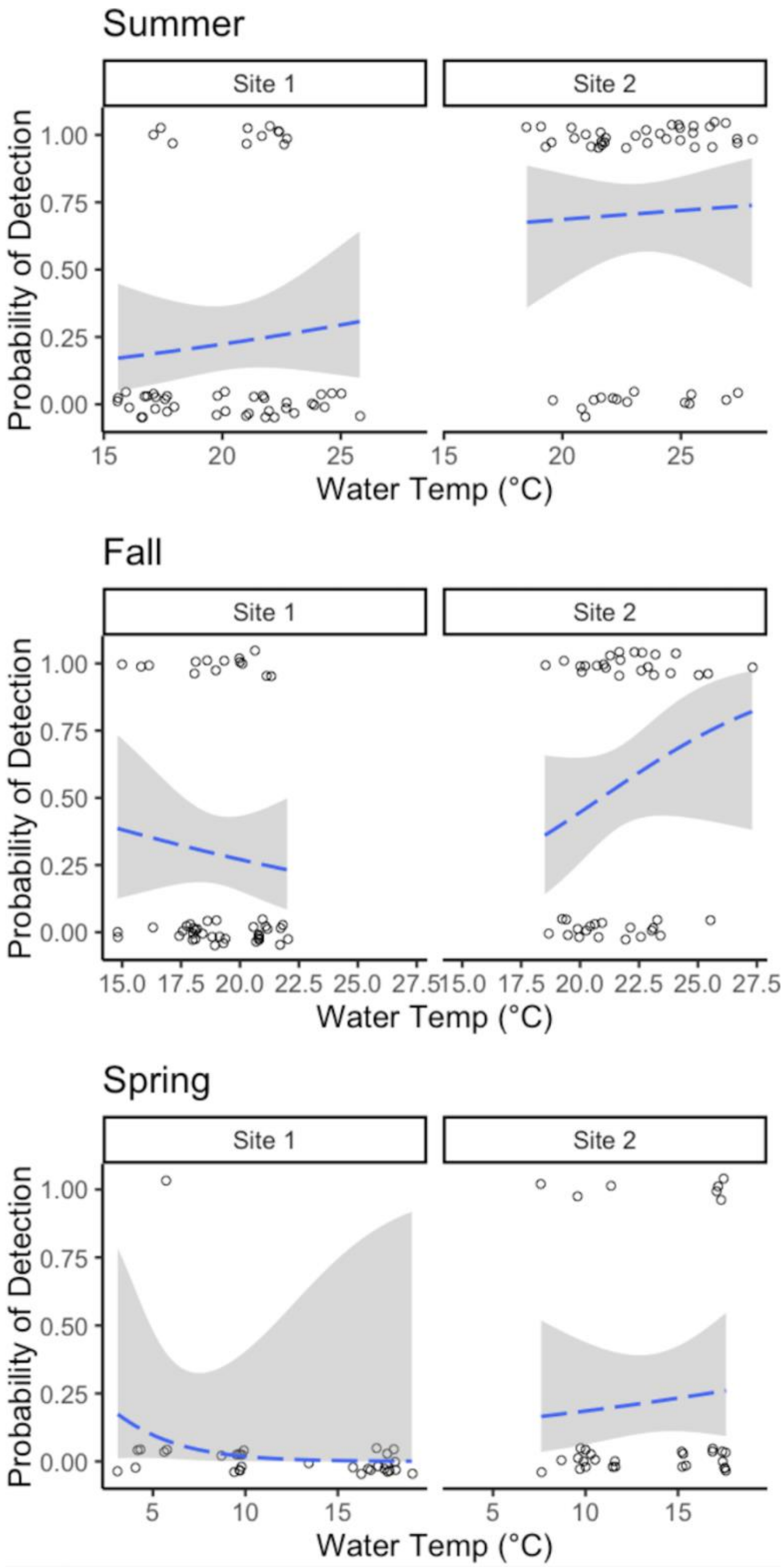


Figure 7. The probability of detecting *A. talpoideum* during summer, fall, and spring dip-net surveys in relation to water temperature within wetlands at study sites 1 and 2.

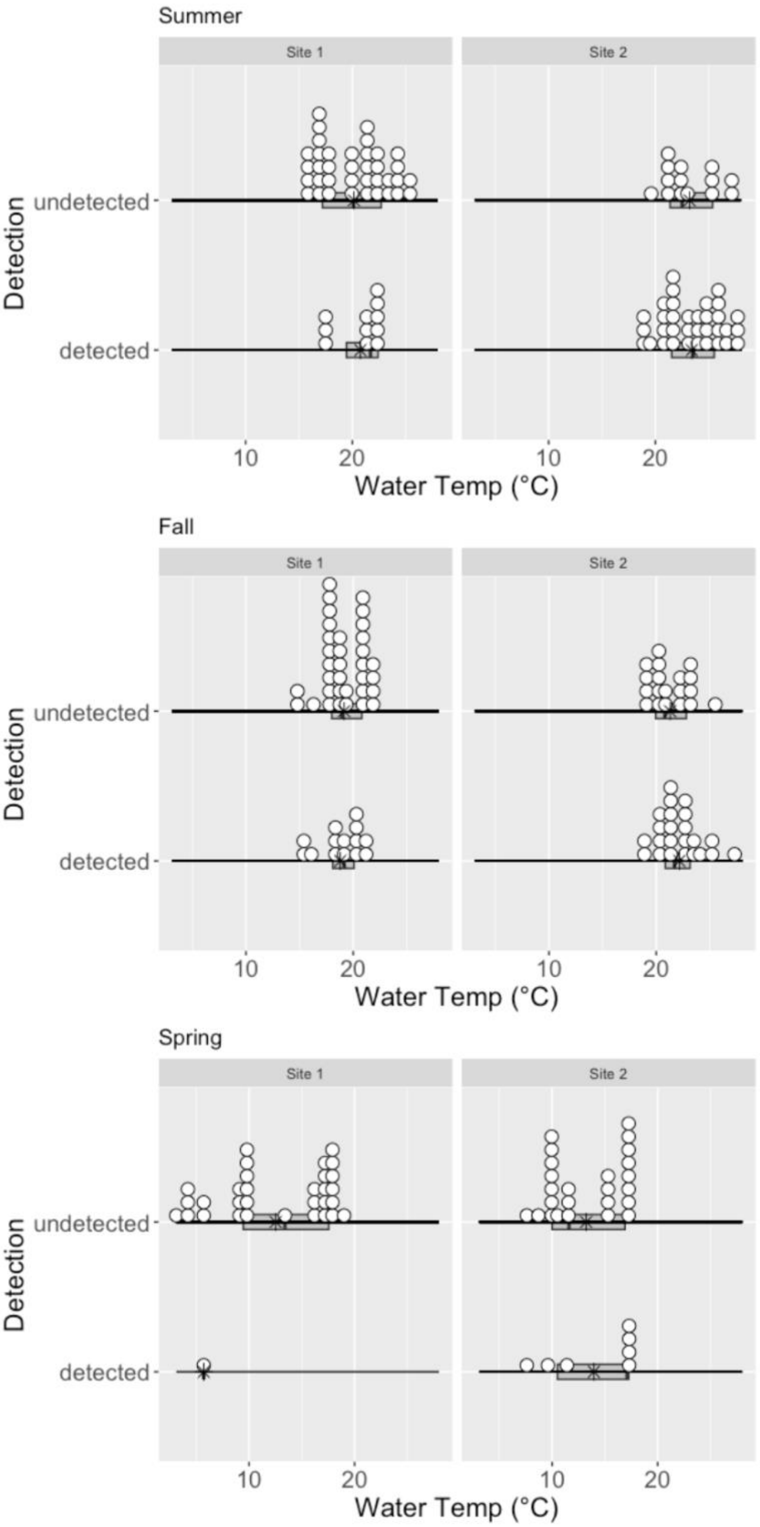


Figure 8. Detections and non-detections during summer, fall, and spring dip-net surveys plotted in relation to water temperature within wetlands at study sites 1 and 2. Observed values (o), mean values (*), and quartiles (sideways boxplots).

Emergent Vegetation: Emergent vegetation had the strongest effect on the probability of detecting *A. talpoideum* during dip-net surveys, with detection increasing significantly in association to higher percentages of emergent vegetation at both sites during surveys, apart from Site 2 during fall surveys (Table 4). A strong increase in detection was seen at sites 1 and 2 during summer surveys ($\Delta x^2 = 6.1$, $P = 0.01$) ($\Delta x^2 = 19.5$, $P < 0.001$) (Table 4; Fig. 9) with detections being more frequent in areas with higher percentages of emergent vegetation (Fig. 10).

Table 4. Odds of detecting *A. talpoideum* in relation to emergent vegetation at study sites 1 and 2 during dip-net surveys in summer, fall, and spring of 2023-2024, determined by logistic regression. Deviance chi-square compared a logistic model without emergent vegetation to a logistic model including emergent vegetation. The larger the deviance chi-square, the larger the association between this microhabitat feature and the odds of detection.

Predictor	Season	Site	Regression Coefficient (Log Odds)	Odds	Deviance X^2	*P*
emergent vegetation	summer	1	0.02	1.020	6.1	0.01
emergent vegetation	fall	1	0.02	1.020	7.1	0.007
emergent vegetation	spring	1	0.84	2.316	3.4	0.06
emergent vegetation	summer	2	0.05	1.051	19.5	<0.001
emergent vegetation	fall	2	-0.003	0.997	0.2	0.64
emergent vegetation	spring	2	0.07	1.072	12.8	<0.001

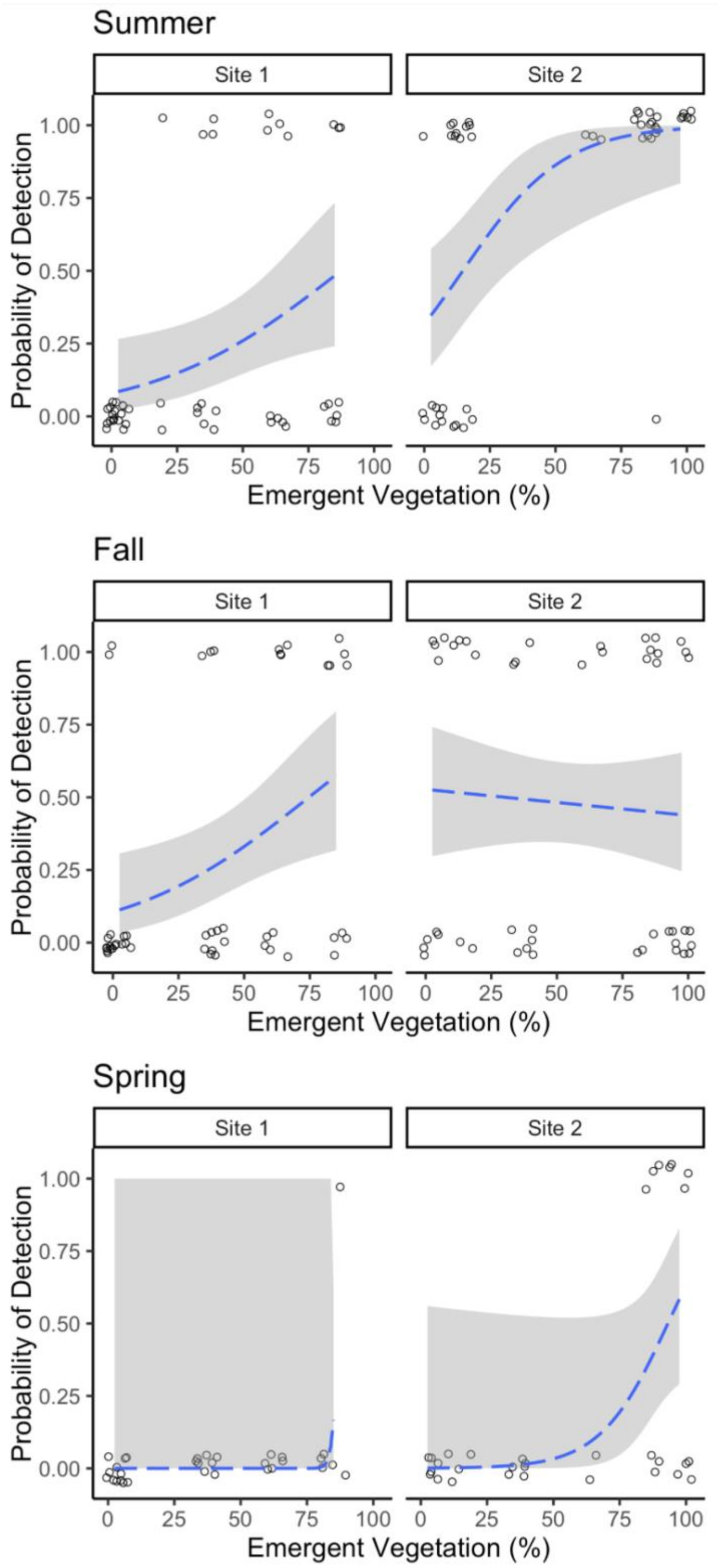


Figure 9. The probability of detecting *A. talpoideum* during summer, fall, and spring dip-net surveys in relation to emergent vegetation within wetlands at study sites 1 and 2.

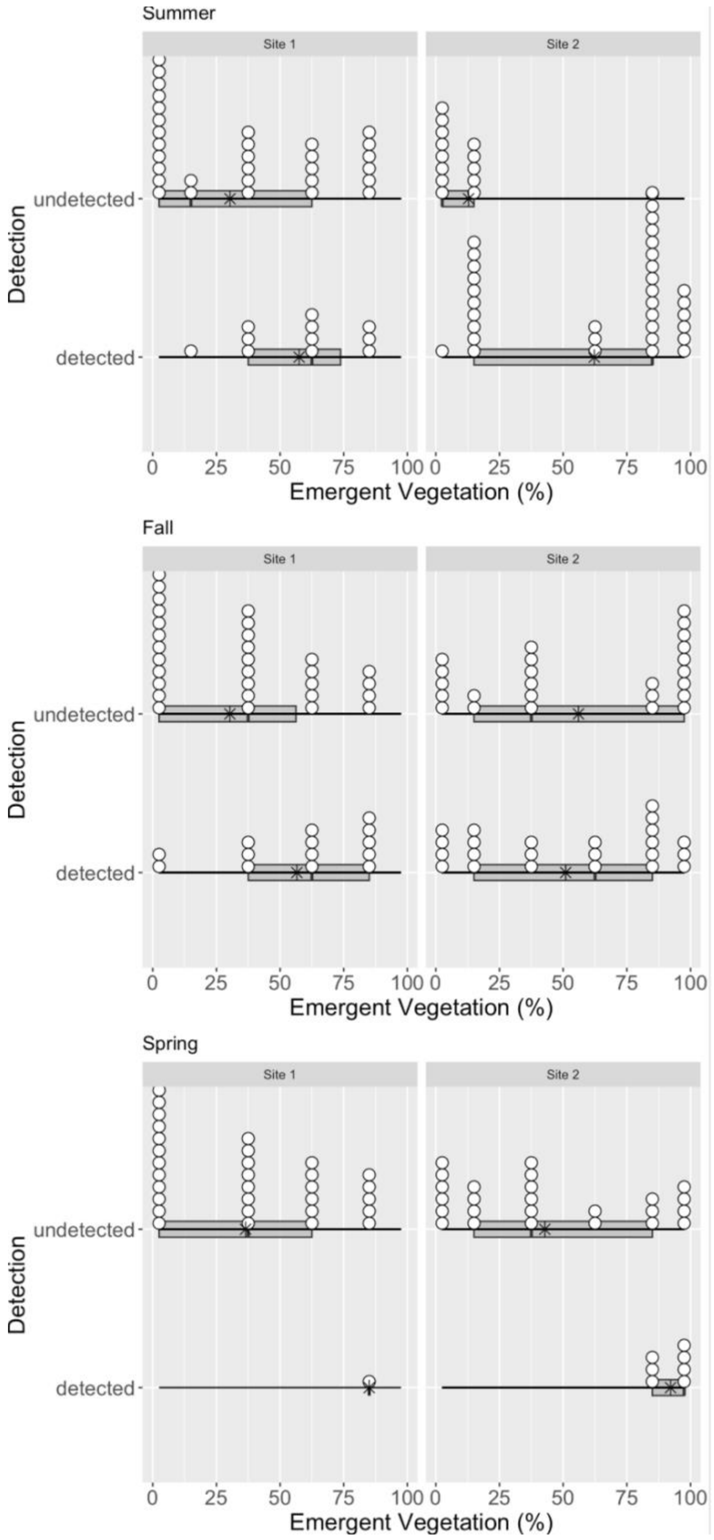


Figure 10. Detections and non-detections during summer, fall, and spring dip-net surveys plotted in relation to emergent vegetation within wetlands at study sites 1 and 2. Observed values (o), mean values (*), and quartiles (sideways boxplots).

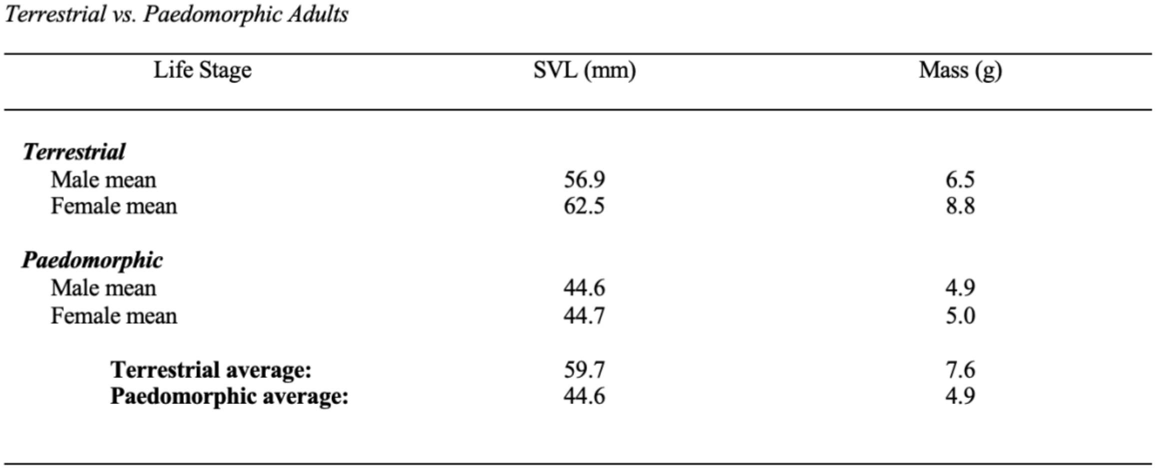
During fall surveys, there was an increase in detection at Site 1 in relation to higher percentages of emergent vegetation ($\Delta x^2 = 7.1$, $P = 0.007$) while there was no noticeable association at Site 2 (Table 4; Fig. 9).

There were fewer detections overall during spring surveys compared to summer and fall with only one detection being made at Site 1 ($\Delta x^2 = 3.4$, $P = 0.06$) while there was a noticeable association between emergent vegetation and detection at Site 2 ($\Delta x^2 = 12.8$, $P = 0.0003$) (Table 4) (Fig. 9, 10). Overall, emergent vegetation was the strongest microhabitat characteristic in association to the detection of mole salamanders in these high elevation wetlands.

Drift Fence Surveys and Body Size Measurements

Snout-vent-length measurements collected on terrestrial and paedomorphic adults (Fig. 12) during field surveys showed that paedomorphic males were smaller on average than terrestrial males (SVL mean = 44.6 mm vs 56.9 mm, $P < 0.001$) and paedomorphic females were smaller on average than terrestrial females (SVL mean = 44.7 mm vs 62.5 mm, $P < 0.001$) (Table 5; Figs. 11). Terrestrial adults averaged 59.7 mm in SVL while paedomorphic adults averaged 44.6 mm in SVL (Table. 5). The average mass of paedomorphic males was smaller than terrestrial males (mean = 4.9 g. vs 6.5 g., $P = 0.05$) and the average mass of paedomorphic females was less than terrestrial females (mean = 5 g. vs 8.8 g., $P < 0.001$) (Tables 5-7; Fig. 11). This suggests that paedomorphic individuals are not reaching their maximum body size in the paedomorphic life stage and further supporting Whiteman's (1994) "best of a bad lot" hypothesis suggesting that larger individuals metamorphose, and smaller individuals become paedomorphic.

Table 5. The average SVL and mass of males and females of both terrestrial and paedomorphic individuals compared. Measurements on terrestrial individuals were collected during drift fence surveys. Measurements on paedomorphic individuals were collected during dip-net surveys.



Terrestrial vs. Paedomorphic Adults

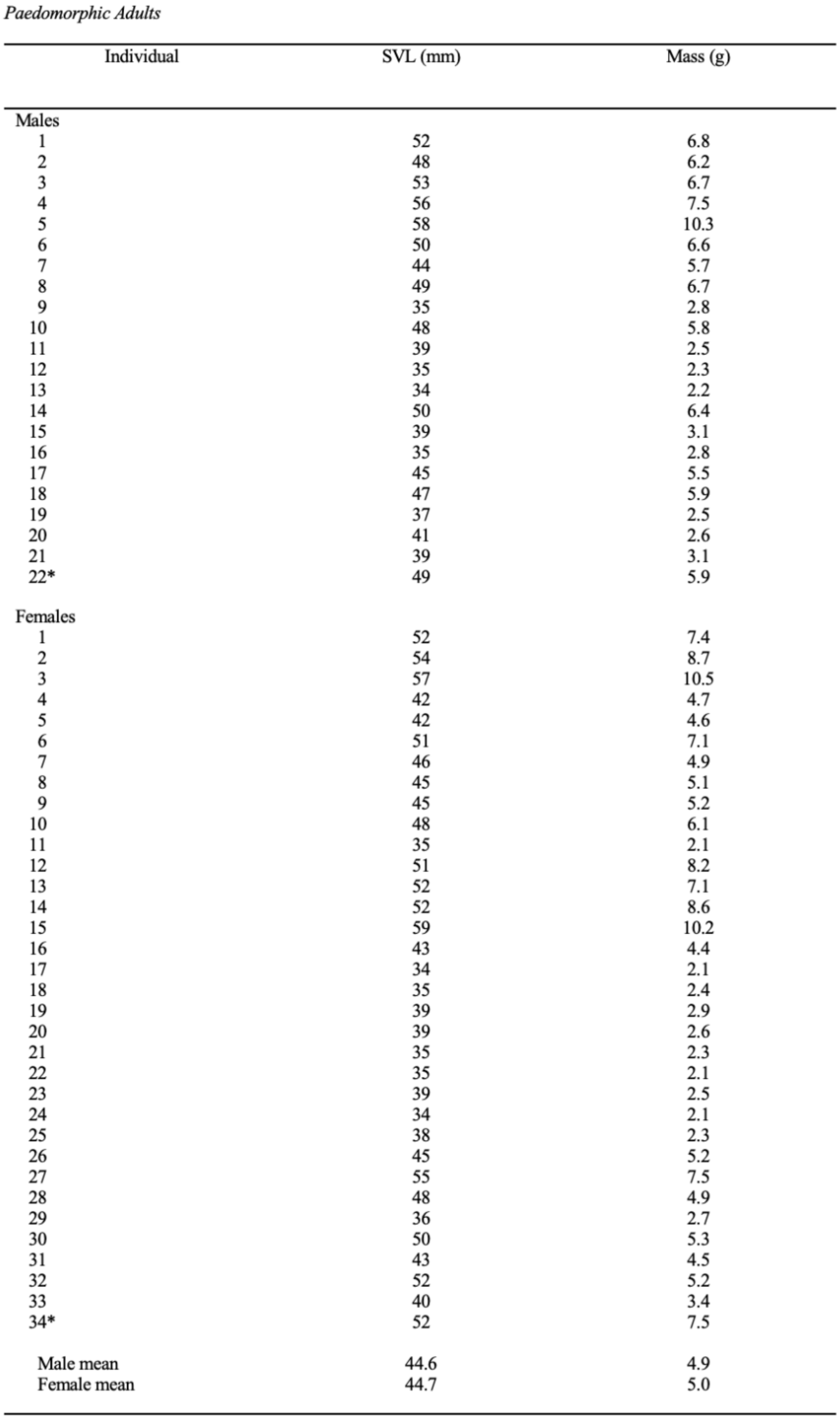
Life Stage	SVL (mm)	Mass (g)
Terrestrial		
Male mean	56.9	6.5
Female mean	62.5	8.8
Paedomorphic		
Male mean	44.6	4.9
Female mean	44.7	5.0
Terrestrial average:	59.7	7.6
Paedomorphic average:	44.6	4.9

Table 6**.** Total number of individuals found during drift fence surveys along with SVL and weight data. Metamorphs were included in paedomorphic adult means, since they were sexually mature paedomorphs that metamorphosed to leave the wetland.

Drift Fence Data/Terrestrial Adults

Individual	SVL (mm)	Mass (g)	Notes:
Males			
1	54	5.3	
2	53	5.7	
3	63	8.6	
4	64	8.2	
5	52	4.4	
6	54	5.3	
7	53	5.4	
8	61	8.8	
9	52	4.7	
10	61	9.0	
11	59	6.4	
Females			
1	61	7.4	
2	57	7.7	
3	66	9.2	*gravid*
4	65	9.3	*gravid*
5	63	9.8	*gravid*
6	63	9.2	*gravid*
7	65	9.8	*gravid*
8	58	7.9	*gravid*
9	65	8.7	*gravid*
Metamorphs			
1	52	7.5	*female*
2	49	5.9	*male*
Male mean	56.9	6.5	
Female mean	62.5	8.8	

Table 7. Paedomorphic adult SVL and weight measurements taken during dip-net surveys to compare to terrestrial adult measurements taken during drift fence surveys. * indicates metamorphs found leaving the wetland.



Paedomorphic Adults

Individual	SVL (mm)	Mass (g)
Males		
1	52	6.8
2	48	6.2
3	53	6.7
4	56	7.5
5	58	10.3
6	50	6.6
7	44	5.7
8	49	6.7
9	35	2.8
10	48	5.8
11	39	2.5
12	35	2.3
13	34	2.2
14	50	6.4
15	39	3.1
16	35	2.8
17	45	5.5
18	47	5.9
19	37	2.5
20	41	2.6
21	39	3.1
22*	49	5.9
Females		
1	52	7.4
2	54	8.7
3	57	10.5
4	42	4.7
5	42	4.6
6	51	7.1
7	46	4.9
8	45	5.1
9	45	5.2
10	48	6.1
11	35	2.1
12	51	8.2
13	52	7.1
14	52	8.6
15	59	10.2
16	43	4.4
17	34	2.1
18	35	2.4
19	39	2.9
20	39	2.6
21	35	2.3
22	35	2.1
23	39	2.5
24	34	2.1
25	38	2.3
26	45	5.2
27	55	7.5
28	48	4.9
29	36	2.7
30	50	5.3
31	43	4.5
32	52	5.2
33	40	3.4
34*	52	7.5
Male mean	44.6	4.9
Female mean	44.7	5.0

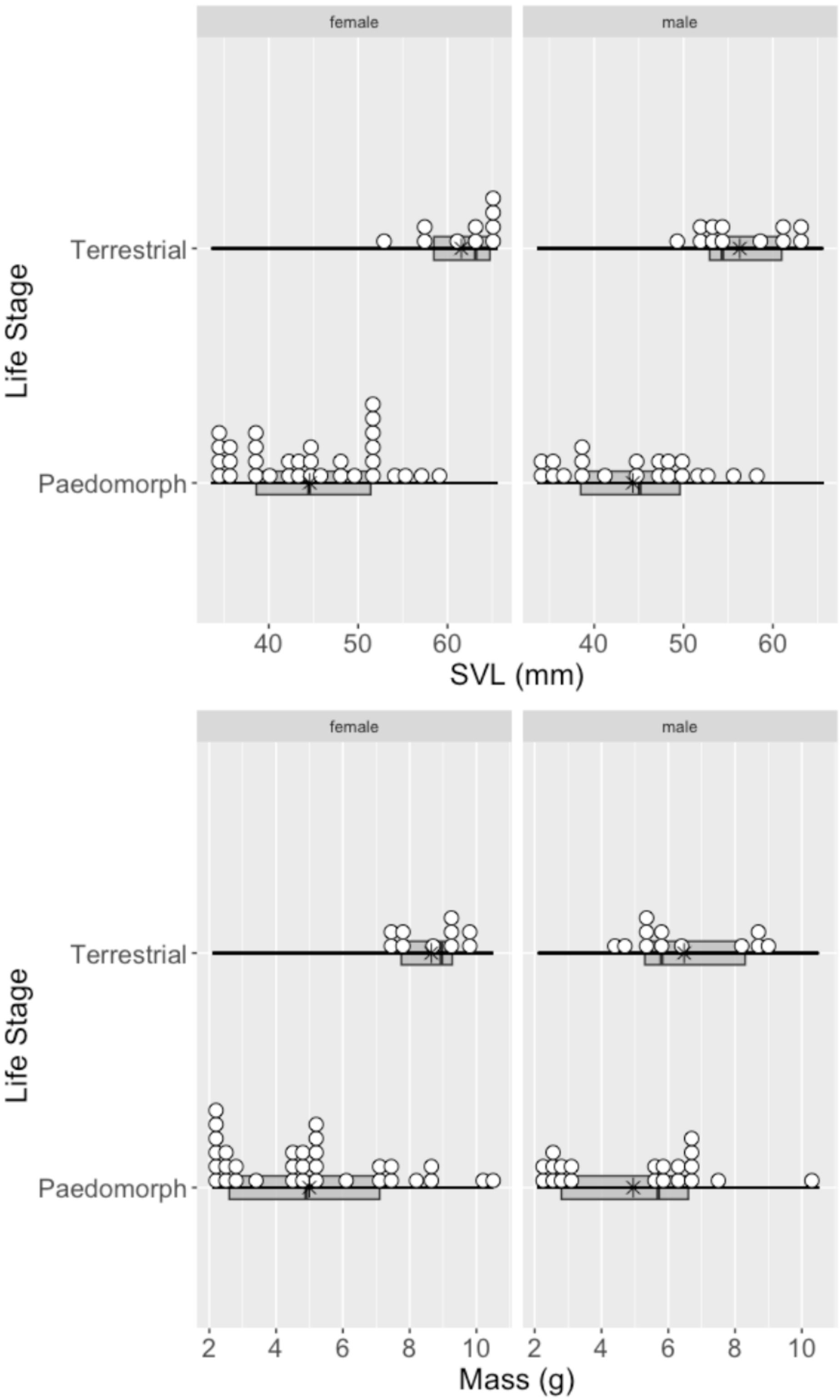


Figure 11. Comparison between the average SVL (top) and mass (bottom) of terrestrial and paedomorphic male and female *A. talpoideum* found during dip-net and drift fence surveys. Observed values (o), mean values (*), and quartiles (sideways boxplots)

Figure 12. A terrestrial female mole salamander (top photo), a terrestrial male (middle photo), and a paedomorphic female mole salamander (bottom photo) found during drift fence and dip-net surveys.

Two metamorphosed individuals were found leaving the wetland during drift fence surveys (Fig. 13). These individuals were determined as sexually mature adults by SVL and by examining cloacal development. The male had an SVL of 49 mm while the female had an SVL of 52 mm (Table. 6, 7). Both individuals had recently absorbed their gills, and gill nubs were present and visible on the female (Fig. 13). Both individuals would have been paedomorphic for some duration of time before leaving the wetland since they were sexually mature before metamorphosing.

Figure 13. Two metamorphosed individuals found leaving the wetland during drift fence surveys. Both male (top photo) and female (bottom photo) had recently absorbed their gills, with gill nubs visible on the female. Both appeared to have been previously paedomorphic.

There was also an unusually large paedomorphic individual found while dip-netting outside of surveying plots at Site 1 in a deep beaver pond area. This individual was female and had an SVL of 61 mm and a weight of 12.5 g (Fig. 14) which was larger in both SVL and weight when compared to the average of terrestrial adults and paedomorphic adults (Table 5). However, it was the only large individual like this found while dip-netting and was not included in body size averages since it was found outside of survey plots.

Figure 14**.** A large female paedomorphic mole salamander found while dip-netting outside of surveying plots at Site 1.

Elevational Extent and other Environmental Variables

There were no detections of *Ambystoma talpoideum* during dip-net surveys at Site 3, which was the highest elevation site in this wetland complex at 1283 m (4,209 ft.) (Table 1, Fig. 3). Non-standardized dip-netting was done outside of plots throughout other areas of Site 3 to confirm the absence of mole salamanders at this site. The highest elevation in which I detected *A. talpoideum* was at Site 2 at 1073 m (3,520 ft.) (Table 1, Fig. 3).

This suggest that there could be an elevational limit for mole salamanders in the Southern Appalachians, however, I suspect that this top wetland site (Site 3) was not occupied by mole salamanders due to factors such as predation and competition for resources with other salamander species, such as the spotted salamander (*A. maculatum*), whose egg masses and larvae were more abundant in this upper wetland compared to Site 1 and Site 2 ($F = 15.7$, $DF_{res} = 379$, $P < 0.0001$) (Fig. 15). Other salamander species found in plots with *A. talpoideum* during surveys included eastern newt (*Notophthalmus viridescens*), red salamander (*Pseudotriton ruber*), Blue Ridge two-lined salamander (*Eurycea wilderae*), and spring salamander (*Gyrinophilus porphyriticus*). Site 3 had a higher average number of tadpoles per plot during dip-net surveys when compared to Site 1 and Site 2 ($F = 49.0$, $DF_{res} = 379$, $P < 0.0001$) (Fig. 15) which are known to feed on salamander eggs. Green frog (*Rana clamitans*) tadpoles were the most common species found during surveys. Other tadpole species included American bullfrog (*Rana catesbeiana*), wood frog (*Rana sylvatica*), and spring peeper (*Pseudacris crucifer*). Site 3 was also the only site where crayfish where commonly found, however, the average number found per plot was less than one ($F = 6.5$, $DF_{res} = 379$, $P = 0.002$) (Fig. 15). There were no minnows found in plots during dip-net surveys at Site 2 or Site 3. However, creek chub (*Semotilus atromaculatus*), white sucker (*Catostomus* *commersoni*), and brook trout (*Salvelinus fontinalis*) were found outside of plots at these two sites. In contrast, Site 1 averaged 3.5 minnows per plot (F = 53.7, $\mathrm{DF}_{\mathrm{res}} = 379$, *P* < 0.0001) (Fig. 15) with creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersoni*) being found alongside *A. talpoideum* at this site. This could explain the fewer detections at Site 1 compared to Site 2, along with a higher average of eastern newts (*N. viridescens*) per plot compared to Sites 1 and 2 (F = 17.5, $\mathrm{DF}_{\mathrm{res}} = 379$, P < 0.0001) (Fig. 15).

I also speculate that environmental factors such as water temperature and soil temperature at this higher elevation could contribute to why *A. talpoideum* was absent from this upper site. Site 3 had a cooler water temperature on average during summer and fall surveys, but not during spring (Summer F = 17.5, $\mathrm{DF}_{\mathrm{res}} = 139$, P < 0.0001; Fall F = 14.0, $\mathrm{DF}_{\mathrm{res}} = 131$, P < 0.0001; Spring F = 2.7 $\mathrm{DF}_{\mathrm{res}} = 84$, P = 0.07) (Fig. 16). The pH of water did not differ significantly on average between sites (Summer F = 0.7, $\mathrm{DF}_{\mathrm{res}} = 139$, P = 0.5; Fall F = 0.7, $\mathrm{DF}_{\mathrm{res}} = 131$, P = 0.5; Spring F = 1.3 $\mathrm{DF}_{\mathrm{res}} = 84$, P = 0.3) (Fig. 16). Soil temperature measurements were not taken during this study, but I suspect that the over wintering conditions and soil temperatures at this upper wetland (Table 1, Fig. 3) may have been too extreme for *A. talpoideum* at this elevation. In the Coastal Plain, terrestrial adult *A. talpoideum* are known to be a shallow burrowers compared to other ambystomatid species (Shoop, 1959) and below freezing temperatures during winter at high elevation in the Southern Appalachians may be a limiting factor for *A. talpoideum* here. In the Coastal Plain, freezing temperatures are less frequent, and winters are mild compared to winters at high elevation in the mountains. Canopy openness was highest on average at Site 3 compared to sites 1 and 2 (Summer F = 16.7, $\mathrm{DF}_{\mathrm{res}} = 45$, P < 0.0001; Fall F = 23.4, $\mathrm{DF}_{\mathrm{res}} = 45$, P < 0.0001; Spring F = 12.9, $\mathrm{DF}_{\mathrm{res}} = 45$, P < 0.0001) (Fig. 17). I suspect that this higher degree of canopy openness was due mostly to the larger size of Site 3 (2.58 ha) compared to Site 1 and Site 2 (Table. 1). There was no significant difference in emergent vegetation coverage between Site 3 and Sites 1 and 2 (Summer F = 0.8, DF_{res} = 45, P = 0.4; Fall F = 0.8, DF_{res} = 45, P = 0.4; Spring F = 0.8, DF_{res}= 45, P = 0.4) (Fig. 17).

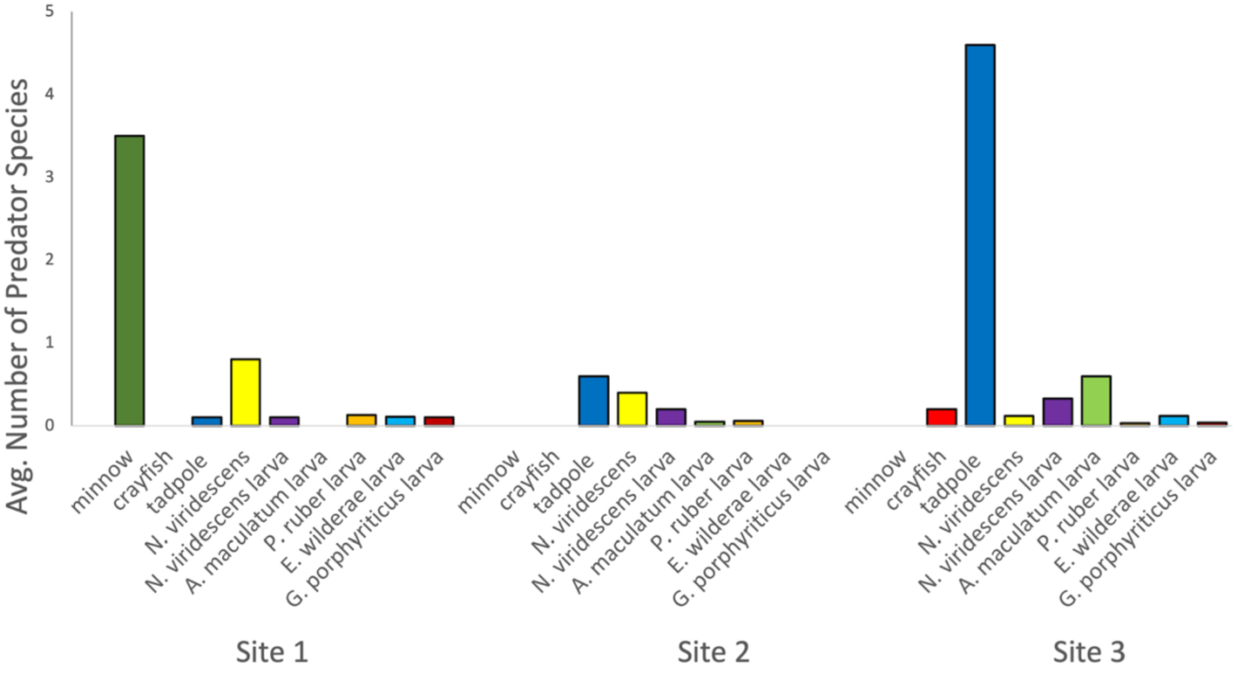


Fig 15. Average number of predatorial species found per plot during dip-net surveys compared between study sites. Minnows, crayfish, and tadpoles were not separated by species. Minnow species included creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersoni*). Tadpole species included green frog (*Rana clamitans*), American bullfrog *(Rana catesbeiana*), wood frog (*Rana sylvatica*), and spring peeper (*Pseudacris crucifer*). Salamander species included eastern newt (*Notophthalmus viridescens*), spotted salamander (*Ambystoma maculatum*), red salamander (*Pseudotriton ruber*), Blue Ridge two-lined salamander (*Eurycea wilderae*), and spring salamander (*Gyrinophilus porphyriticus*).

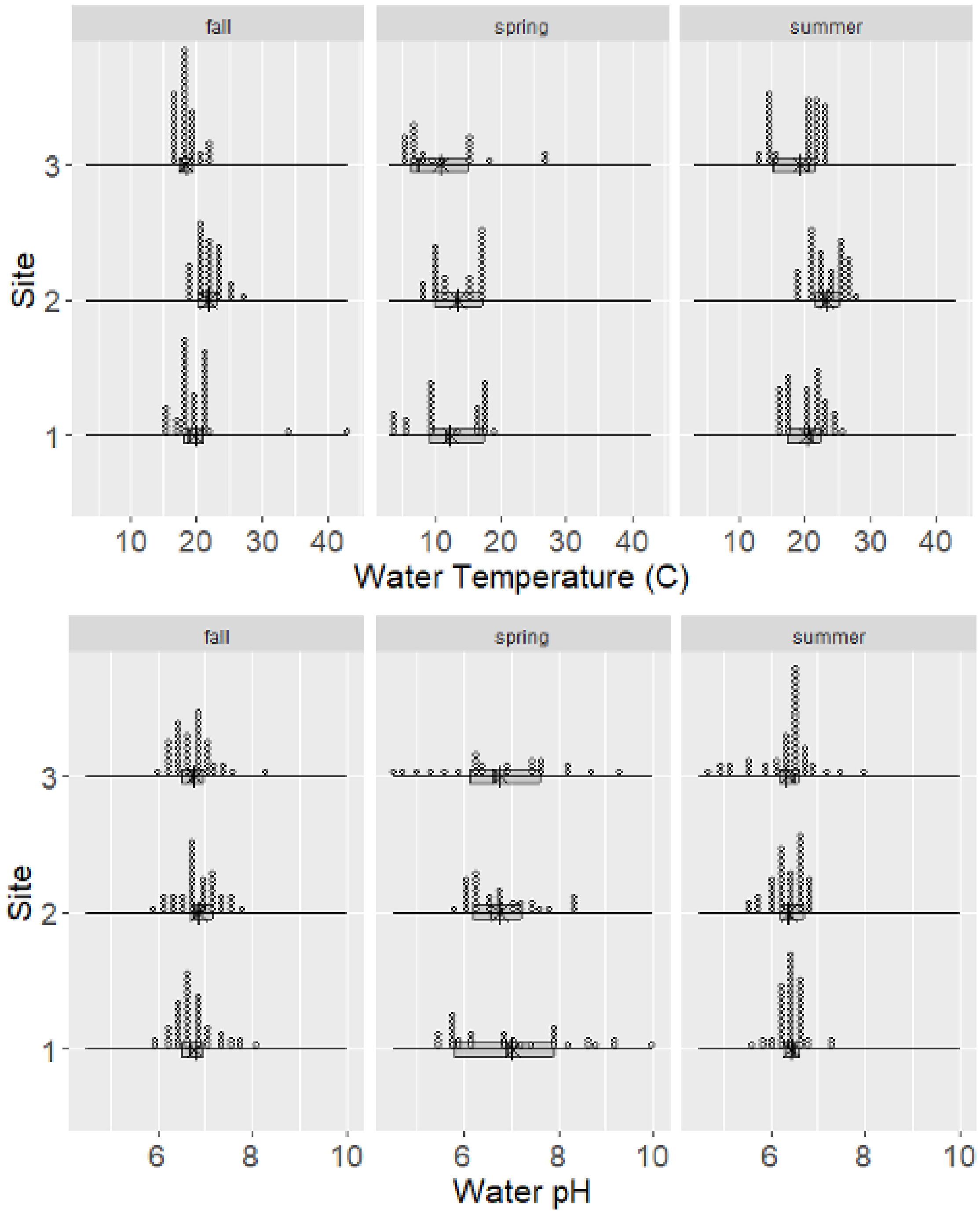


Fig 16. Observed values (o), mean values (*), and quartiles (sideways boxplots) of water temperature (top) and pH (bottom) within 16 plots at each wetland site.

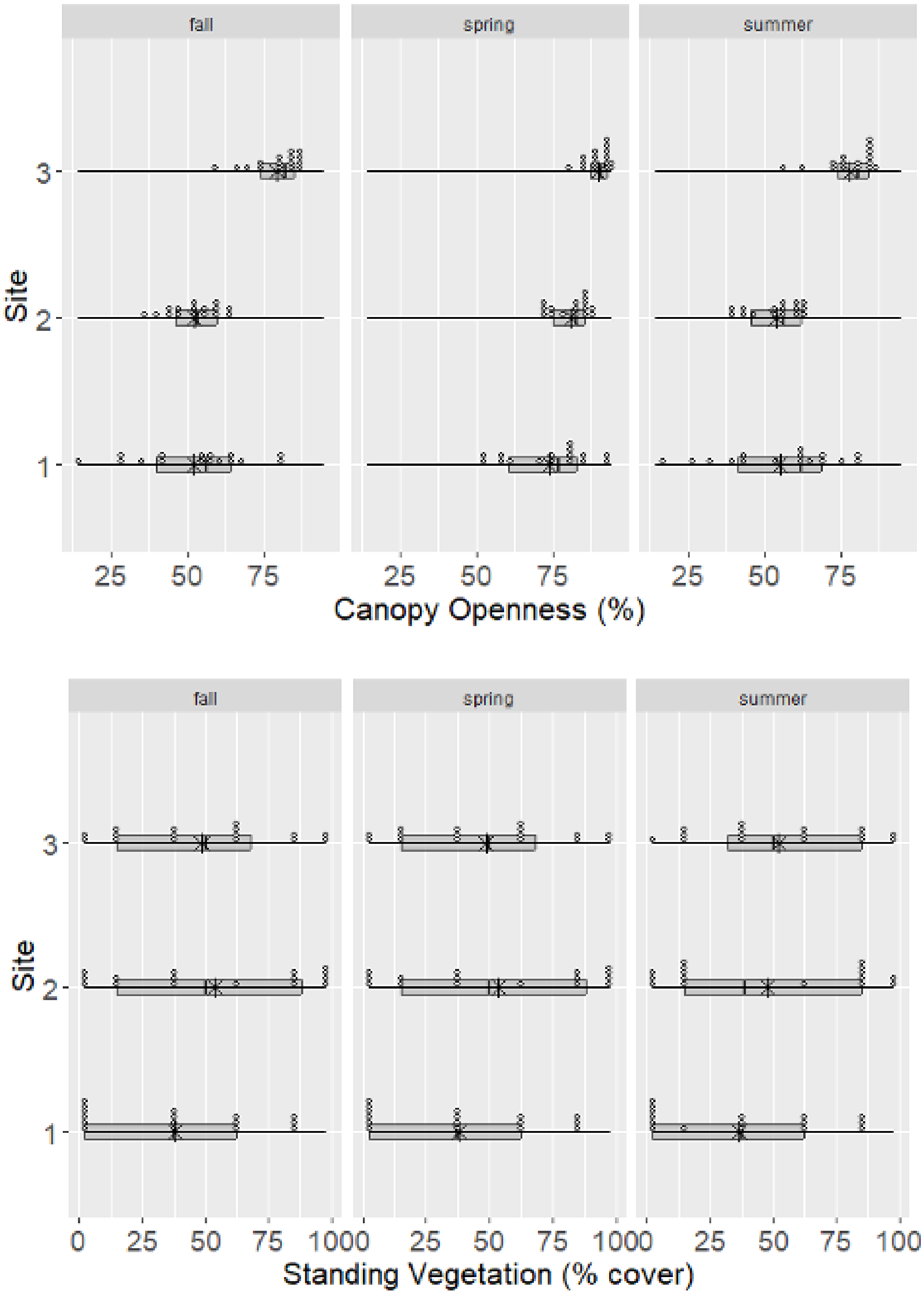


Figure 17. Observed values (o), mean values (*), and quartiles (sideways boxplots) of canopy openness (top) and standing (emergent) vegetation (bottom) within 16 plots at each wetland site.

DISCUSSION

Emergent vegetation was the strongest microhabitat characteristic for *A. talpoideum* in these high elevation wetlands in association to the probability of detection during dip-net surveys (Figs. 9, 10). Canopy openness allows for the growth of early successional plants and grasses, such as emergent vegetation (i.e., rushes and sedges), that are shade intolerant (Yarrow & Yarrow, 2005). Areas with dense emergent vegetation provide refuge against predators for *A. talpoideum* in these wetlands and minnows were commonly found within the same plots as *A. talpoideum* during dip-net surveys. It is uncommon for mole salamanders to coexist with fish (Petranka, 1998) and fish species such as bluegill (*Lepomis macrochirus*) are known to predate on *A. talpoideum* eggs and larvae in the Coastal Plain (Semlitsch, 1988). White et al. (2016) reported that redbreast sunfish (*Lepomis auritus*) and creek chub (*Semotilus atromaculatus*) were observed in high elevation NC wetlands with *A. talpoideum* and speculated that this coexistence was possible because of the abundant refuges in these wetlands, such as leaf packs and aquatic vegetation, that provide cover against predation.

The positive association between detection and emergent vegetation found during this study can be useful for surveying efforts aimed at discovering new populations of this species in the Southern Appalachians. Having these microhabitat features present within high elevation wetlands may increase the probability of detecting *A. talpoideum* at new locations and knowing their microhabitat preferences may be useful for conservation efforts focused on bridging the distributional gap between the Coastal Plain and disjunct populations to the north. Understanding microhabitat preference and use of for this species in its aquatic breeding habitat is essential for the conservation of this listed amphibian and many individuals were observed utilizing this microhabitat feature for refuge within these wetlands. Increasing and maintaining emergent vegetation for *A. talpoideum* within wetlands may also increase their population numbers, which is important for the conservation of this species.

Many wildlife management practices for wetland breeding amphibians with specific habitat requirements such as the gopher frog *(Lithobates capito)*, reticulated flatwoods salamander *(Ambystoma bishopi)*, and the striped newt *(Notophthalmus perstriatus)* focus heavily on promoting and enhancing the growth of emergent vegetation within wetlands (Means et al., 2008; Gorman et al., 2009) which is essential for the survival of these threatened species since they rely on it for cover, egg/larvae protection, and food resources. This is achieved through maintaining open canopy conditions within wetlands, which is controlled anthropogenically (i.e., cutting and burning) for many areas currently in the southeastern United States. Findings from my study may suggest that wildlife management agencies in the Southern Appalachians should consider managing and monitoring the upland habitat around high elevation wetlands and mountain bogs to promote canopy openness within these ecosystems at sites that have experienced heavy succession. The availability of open canopy areas is beneficial for many rare and endangered bog species, such as Gray's lily *(Lilium grayi)*, swamp pink *(Helonias bullata)*, and the bog turtle *(Glyptemys muhlenbergii)* (Byers et al., 2007; Spira, 2011).

Likewise, monitoring and maintaining American beaver populations within these wetlands is also important for conservation efforts since they play a major role in controlling canopy openness and woody encroachment in these wetlands. Throughout summer, fall, and spring dip-net surveys, areas of pooled or dammed water created by the American beaver were less susceptible to drying during dryer periods, which was essential for any larval or paedomorphic individuals living in these aquatic habitats. Many individuals were observed congregated in these deeper pools of water created by beaver and seemed to use these areas as refuge during dryer periods. A terrestrial male and paedomorphic female were also observed utilizing the dugout entrance of a beaver den where the water was deep and pooled while the surrounding habitat was dry. The deeper areas of water created by beaver throughout these wetlands may allow paedomorphic individuals to persist within these aquatic environments for longer periods of time.

Measurements collected on terrestrial and paedomorphic adults during this study lend support to the findings of Whiteman (1994) and White et al. (2016) and show that paedomorphic individuals are smaller on average than terrestrial adults (Figs. 11, 12). Paedomorphic individuals do not seem to be reaching maximum body size in their aquatic habitats, which indicate a growth limiting environment. In future studies, paedomorphic adults in this population could be marked to monitor their growth over time and to examine the longevity of the paedomorphic life stage in this species. It is not clear how long facultatively paedomorphic adults remain within their aquatic habitats before presumably metamorphosing. Individually marking paedomorphic individuals would help to determine the average body size at which paedomorphs metamorphose to become terrestrial. Marking with visual elastomer, which is commonly used to mark and monitor amphibians over time, could be used to track individuals (Grant, 2008). The two metamorphosed individuals that were found leaving the wetland during drift fence surveys appeared to have both been paedomorphic for some duration of time before metamorphosing since they were sexually mature, which was determined by cloacal development and SVL, and were both similar in size to the average of paedomorphic adults found in this population (Fig. 13). However, an exceptionally large paedomorphic female found during dip-net surveys at Site 1 was larger in SVL and mass compared to both terrestrial adults and average paedomorphic adults (Table. 5) (Fig. 14). This suggests that certain individuals could be remaining in and using these aquatic habitats for longer periods of time, allowing them to grow larger. Marking individuals in this population would be beneficial to understanding the average longevity of the paedomorphic life stage for this species.

Paedomorphic mole salamanders use their aquatic habitats more extensively than other species of ambystomatid salamanders that metamorphose quickly, such as the marbled salamander (*Ambystoma opacum*) and the spotted salamander (*Ambystoma maculatum*) which are generally non-neotenic (or non-paedomorphic) meaning that these species of ambystomatid salamanders undergo metamorphosis before becoming sexually mature adults (Lyons and Arbuckle, 2024). Neoteny is defined as a condition in which an adult salamander has certain traits that are typically found in aquatic larvae and is often used interchangeably with paedomorphosis (Wilson et al., 2024). Everson et al. (2021) described the "neotenic states" of ambystomatid salamanders as non-neotenic (always undergoing metamorphosis), obligate neotenic (rarely or never metamorphosing), and facultatively neotenic (variation of metamorphosing and neotenic individuals within populations). Our focal species, the mole salamander (*A. talpoideum),* would be described under this classification as facultatively neotenic (or facultatively paedomorphic) with populations existing as a mixture of both paedomorphic and metamorphosed terrestrial individuals with paedomorphic individuals presumably metamorphosing at some point (Petranka, 1998). In comparison, the Mexican axolotl *(Ambystoma mexicanum)* would be described as obligate neotenic, remaining permanently in its aquatic habitat and rarely metamorphosing (Everson et al., 2021). The

Mexican axolotl is listed as endangered, yet little was known about its aquatic habitat use prior to observations made by Ayala et al. (2019).

The higher density of tadpoles, presence of *A. maculatum,* cooler on average water temperatures, and the harsh overwintering conditions at this higher elevation could all be factors in why mole salamanders were not detected at Site 3. The most notable difference at Site 3 was in the average water temperature, which was colder on average during summer and fall surveys (Fig. 16). The highest elevation in which mole salamanders were detected in this study was 1073 m (3,520 ft.) (Table 1; Fig. 3). However, I believe that it may be possible for *A. talpoideum* to exist at higher elevations if important microhabitat characteristics such as emergent vegetation exist within the aquatic environment and if other environmental factors, such as predation and competition with other amphibian species, do not diminish their numbers.

The upland habitat surrounding these wetlands present conditions that could limit the terrestrial dispersal of *A. talpoideum* around these aquatic breeding habitats. The complex topography of northern hardwood forests in the mountains greatly contrasts with the relatively flat and open forests of the Coastal Plain. In the Coastal Plain in South Carolina, the terrestrial home range of *A. talpoideum* is usually located 13-280 m away from breeding ponds or wetlands (Semlitsch, 1981). I suspect that the topography of the Southern Appalachians would limit terrestrial dispersal away from breeding ponds in the mountains and that populations are more condensed around their wetland habitats here. Madison and Farrand (1998) used radio-implanted transmitters in *A. tigrinum* to examine terrestrial and aquatic habitat use by individuals during breeding and emigration. They were able to track individuals for up to a year to gain insight on adaptive flexibility in habitat use and showed that it is possible to track individuals from the breeding pond onto land. Future studies could individually mark and monitor paedomorphic *A. talpoideum* over time to examine the longevity of the paedomorphic life stage and to gain further understanding on the habitat use of this species throughout all of its life stages.

Findings from this study provide a better overall understanding on the mole salamander as a species and provide insight into the microhabitat use of this species within its aquatic environment and at the highest elevation of its range. Mountain populations of mole salamanders are an important component of biodiversity in the Southern Appalachians, where terrestrial and stream-associated plethodontid salamanders are most abundant. Better understanding the habitat preferences of mole salamander populations and the niche they fill in the mountains will help in protecting this species of concern into the future.

References

Altig, R., & McDiarmid, R. W. (2017). *Handbook of larval amphibians of the United States and Canada*. Cornell University Press.

Ayala, C., Ramos, A. G., Merlo, Á., & Zambrano, L. (2019). Microhabitat selection of axolotls, Ambystoma mexicanum, in artificial and natural aquatic systems. *Hydrobiologia*, *828*(1), 11-20.

Beane, J. C., Braswell, A. L., Mitchell, J. C., Palmer, W. M., & Harrison III, J. R. (2010). *Amphibians and Reptiles of the Carolinas and Virginia*. Univ of North Carolina Press.

Berven, K. A., Gill, D. E., & Smith-Gill, S. J. (1979). Countergradient selection in the green frog, Rana clamitans. *Evolution*, 609-623.

Byers, E.A., J.P. Vanderhorst, and B.P. Streets. (2007). Classification and Conservation Assessment of High Elevation Wetland Communities in the Allegheny Mountains of West Virginia. West Virginia Division of Natural Resources, Elkins.

Crawford, J. A., & Semlitsch, R. D. (2008). Abiotic factors influencing abundance and microhabitat use of stream salamanders in southern Appalachian forests. *Forest Ecology and Management*, *255*(5-6), 1841-1847.

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological conservation*, *128*(2), 231-240.

Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. *Northwest Science* 33:43-64

Doyle, J. M., & Whiteman, H. H. (2008). Paedomorphosis in Ambystoma talpoideum: Effects of initial body size variation and density. *Oecologia*, *156*(1), 87–94. https://doi.org/10.1007/s00442-008-0977-2

Earl, J. E., Luhring, T. M., Williams, B. K., & Semlitsch, R. D. (2011). Biomass export of salamanders and anurans from ponds is affected differentially by changes in canopy cover. *Freshwater Biology*, *56*(12), 2473-2482.

Everson, K.M., Gray, L.N., Jones, A.G., Lawrence, N.M., Foley, M.E., Sovacool, K.L., Kratovil, J.D., Hotaling, S., Hime, P.M., Storfer, A., Parra-Olea, G., Percino-Daniel, R., Aguilar-Miguel, X., O'Neill, E.M., Zambrano, L., Shaffer, H.B., Weisrock, D.W., (2021). Geography is more important than life history in the recent diversification of the tiger salamander complex. Proc. Natl. Acad. Sci. USA 118(17), e2014719118. 10.1073/pnas.2014719118.

Frazer, G. W., Canham, C. D., & Lertzman, K. P. (1999). Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. *Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York*, *36*.

Gibbons, J. W and R. D. Semlitsch. 1981. Terrestrial drift fences with pitfall traps an effective technique for quantitative sampling of animal populations. Brimleyana 7:1-16.

Gorman, T. A., Haas, C. A., & Bishop, D. C. (2009). Factors related to occupancy of breeding wetlands by flatwoods salamander larvae. *Wetlands*, *29*, 323-329.

Gould, S. J. 1977. Ontogeny and phylogeny. Harvard University Press, Cambridge, Massachusetts.

Grant, E. H. C. (2008). Visual implant elastomer mark retention through metamorphosis in amphibian larvae. *The Journal of Wildlife Management*, *72*(5), 1247-1252.

Halverson M.A., Skelly D.K., Kiesecker J.M. & Freiden- burg L.K. (2003) Forest mediated light regime linked to amphibian distribution and performance. Oecologia, 134, 360–364.

Heath, A. G. (1975). Behavioral Thermoregulation in High Altitude Tiger Salamanders, Ambystoma tigrinum. *Herpetologica*, *31*(1), 84–93. http://www.jstor.org/stable/3891993

Krenz, J. D. and D. M. Sever. (1995). Mating and oviposition in paedomorphic *Ambystoma talpoideum* precedes the arrival of terrestrial males. Herpetologica 51(4):387-393.

Kricher, J. C. (1998). *A field guide to eastern forests, North America* (Vol. 37). Houghton Mifflin Harcourt.

Kupferberg, S. J., Marks, J. C., & Power, M. E. (1994). Effects of variation in natural algal and detrital diets on larval anuran (Hyla regale) life-history traits. *Copeia*, 446-457.

Lannoo, M. (2005). *Amphibian Declines: The Conservation Statues of United States Species*. Berkeley, CA: Berkeley University of California Press.

Lemieux Lefebvre, S., Landry-Cuerrier, M., & Humphries, M. M. (2018). Identifying the critical habitat of Canadian vertebrate species at risk. *Canadian Journal of Zoology*, *96*(4), 297-304.

Lilieholm, R. (1995). Sustainable Forestry: Philosophy, Science and Economics. *American Scientist*, *83*(6), 578-580.

Lyons, T. A., & Arbuckle, K. (2024). The neoteny goldilocks zone: The evolution of neoteny in Ambystoma. *Ecology and Evolution*, *14*(4), e11240.

Madison, D. M., & Farrand III, L. (1998). Habitat use during breeding and emigration in radio-implanted tiger salamanders, Ambystoma tigrinum. *Copeia*, 402-410.

Means, D. B., Means, R. C., & Means, R. P. (2008). Petition to list the striped newt (Notophthalmus perstriatus), as a federally threatened species under the Endangered Species Act of 1973. *Coastal Plains Institute, Tallahassee, FL.*

Moore, M. P., Pechmann, J. H. K., & Whiteman, H. H. (2020). Relative size underlies alternative morph development in a salamander. *Oecologia*, *193*(4), 879–888. https://doi.org/10.1007/s00442-020-04723-8

[NCWRC] North Carolina Wildlife Resources Commission. Protected wildlife species. (2021). https://www.ncwildlife.org/Portals/0/Conserving/documents/Protected-Wildlife-Species-of-NC.pdf

Palis, J. G. (1996). Flatwoods salamander(Ambystoma cingulatum Cope). *Natural Areas Journal*, *16*(1), 49-54.

Patterson, K. K. (1978). Life history aspects of paedogenic populations of the mole salamander, Ambystoma talpoideum. *Copeia*, 649-655.

Parmelee, J. R. (1993). *Microhabitat Segregation and Spatial Relationships Among Four Species of Mole Salamanders (Genus" Ambystoma")*. University of Kansas.

Petranka, J. W. (1998). *Salamanders of the United States and Canada*. Smithsonian Institution Press.

Powell, R., Conant, R., & Collins, J. T. (2016). Peterson field guide to reptiles and amphibians of eastern and central North America.

RStudio Team. (2020). *RStudio: Integrated development for R*. RStudio, PBC. http://www.rstudio.com/

Ryan, T. J. and G. R. Plague. (2004). Hatching asynchrony, survival, and the fitness of alternative adult morphs in *Ambystoma talpoideum*. Oecologia 140(1):46-51

Schultheis, E. H., Hopfensperger, K. N., & Brenner, J. C. (2010). Potential impacts of climate change on Sphagnum bogs of the southern Appalachian Mountains. *Natural Areas Journal*, *30*(4), 417-424.

Scott, D. E. (1993). Timing of reproduction of paedomorphic and metamorphic *Ambystoma talpoideum*. 20 The American Midland Naturalist 129(2):397-402.

Semlitsch, R. D. (1981). Terrestrial activity and summer home range of the mole salamander (Ambystoma talpoideum). *Canadian Journal of Zoology*, *59*(2), 315-322.

Semlitsch, R. D. (1985a). Analysis of Climatic Factors Influencing Migrations of the Salamander Ambystoma talpoideum. *Copeia*, *1985*(2), 477–489. https://doi.org/10.2307/1444862

Semlitsch, R. D. (1985b). Reproductive Strategy of a Facultatively Paedomorphic Salamander Ambystoma talpoideum. *Oecologia*, *65*(3), 305–313. http://www.jstor.org/stable/4217534

Semlitsch, R. D. (1988). Allotopic distribution of two salamanders: effects of fish predation and competitive interactions. Copeia 1988:290-298.

Semlitsch, R. D. and S. C. Walls. (1990). Geographic variation in the egg-laying strategy of the mole salamander, *Ambystoma talpoideum*. Herpetological Review 21:14-15.

Shoop, C. R. (1959). *The breeding habits of the mole salamander, Ambystoma talpoideum(Holbrook) in Southeastern Louisiana.*. (Master's thesis, Tulane University of Louisiana).

Shoop, C. R. (1964). Ambystoma talpoideum. *Catalogue of American Amphibians and Reptiles (CAAR)*.

Spira, T. P. (2011). *Wildflowers and plant communities of the southern Appalachian Mountains and Piedmont: A naturalist's guide to the Carolinas, Virginia, Tennessee, and Georgia*. Univ of North Carolina Press.

Van Drunen, S. G., Linton, J. E., Bogart, J. P., McCarter, J., Fotherby, H., Sandilands, A., & Norris, D. R. (2020). Estimating critical habitat based on year-round movements of the endangered Jefferson Salamander (Ambystoma jeffersonianum) and their unisexual dependents. *Canadian Journal of Zoology*, *98*(2), 117-126.

Werner, E. E., & Glennemeier, K. S. (1999). *Influence of Forest Canopy Cover on the Breeding Pond Distributions of Several Amphibian Species* (Vol. 5, Issue 1). https://about.jstor.org/terms

White, E. C., Pechmann, J. H. K., Hyman, J., & Davis, R. W. (2016). *The Influence of abiotic and biotic environmental factors at high elevation on the life cycle of a facultatively paedomorphic salamander.* Western Carolina University.

Whiteman, H. H. (1994). Evolution of facultative paedomorphosis in salamanders. *The Quarterly review of biology*, *69*(2), 205-221.

Williams, R. N., & MacGowan, B. J. (2004). Natural history data on the mole salamander (Ambystoma talpoideum) in Indiana. In *Proceedings of the Indiana Academy of Science*(Vol. 113, No. 2, pp. 147-150).

Wilson, L., Gibbons, W., & Mitchell, J. C. (2024). *Salamanders of the eastern United States*. The University of Georgia Press.

Yarnell, S. L. (1998). *The southern Appalachians: a history of the landscape* (Vol. 18). Diane Publishing.

Yarrow, G. K., & Yarrow, D. T. (2005). Managing Wildlife: on private lands in Alabama and the Southeast. Alabama Wildlife Federation.