

Examining habitat preferences of salamander species in Sandy Bottom Wetland Preserve

Holland Cook

Environmental Science
The University of North Carolina Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Mentor: Landon Ward

Abstract

Wetlands are declining at a pace that could result in irreversible habitat loss for countless species endemic to these ecosystems. Of these species, salamanders are especially at risk, with over half of the known species threatened with extinction. To mitigate this global loss of both species and their habitats, it is important to understand what ecosystem preferences allow this group to thrive. Prior research has examined restored wetland ecosystems, but there is a data gap regarding factors that can increase the resilience of amphibian populations in these habitats. This study examines the preferences of salamander species by conducting population surveys within vernal pools and a permanent pond during salamander breeding seasons in Western North Carolina. Tadpole and fish presence data were collected within the same ecosystem to identify any ecological relationships and effects. Both sites were divided into sections dependent primarily on vegetative habitat structure, with the temporary pools comprising five differing regions and the permanent pond containing three. This study conducted dip-netting

surveys once a week and collected data on the species, size, sex, and region of the specimens caught. The findings of this study suggest that diverse vegetation types and abundance throughout aquatic wetlands are imperative for accommodating the varied needs of different amphibian species. The majority of species sampled in this study preferred high vegetation and deeper water for both inhabiting and breeding. This information will help expand our understanding of the most effective repair methods for future wetland restoration projects, in hopes of protecting salamander biodiversity.

Introduction

Amphibian species are declining at the highest rate of any other vertebrate on the planet. Salamanders are especially vulnerable; over half of all species are considered endangered and it is estimated that over 200 species are already extinct (Re:wild et al. 2023). This decline is attributed to a plethora of factors, including climate change, disease spread, the introduction of invasive species, and, most detrimental, habitat loss (Collins 2010). Approximately 77% of amphibian species are directly affected by habitat loss due to agriculture (Luedtke 2023).

The sensitivity of salamander species to environmental disturbances demonstrates their ability to act as indicator species, meaning their abundance and fitness directly reflect the health and functioning of their environment (Gade and Peterman 2018). The rapid decline of this group is related to amphibians' reliance on freshwater wetlands, which are among the most threatened habitats globally. It is estimated that approximately 71% of global wetland habitats have been lost, with increased loss in the 20th and early 21st centuries (Davidson 2014).

To conserve both amphibian biodiversity and their wetland habitats, restoring damaged wetland areas is often necessary. A study conducted by Russel, Guynn, and Hanlin (2002) focused on wetlands in the South Carolina Coastal Plain and the essential role these areas play in herptile species diversity. The findings of this project showed that the diversity of herptile species did not correlate with the size of the wetland itself, but rather with the number and types of trees present in the surrounding area (Russel et al. 2002). Since the Southern Appalachians have the highest diversity of salamander species in the world, it is imperative to protect this biodiversity hotspot, which is home to many sensitive species.

Many wetlands in Southern Appalachia are seasonally flooded vernal pools. These systems tend to dry out every summer and are therefore typically free of fish that otherwise prey on or compete with larval amphibians (Zedler 2003). In some instances, such as during major flooding events, fish and other species that are typically absent from the pools can colonize them. Because vernal pools are characterized by their flooding and drying cycles, alterations to these cycles can easily change the ecosystem's ecological

functions. Species such as the green frog typically breed in permanent bodies of water because their prolonged tadpole stage would prevent them from metamorphosing before the pool dries (Woodford & Meyer 2003).

Within the vernal pool habitat used in this study, a significant portion of the salamanders present are members of the genus *Ambystoma*, commonly referred to as the mole salamanders. In Sandy Bottom wetland, there are three species within this genus that are commonly found utilizing vernal pools: *Ambystoma maculatum* (spotted salamander), *Ambystoma talpoideum* (mole salamander), and *Ambystoma opacum* (marbled salamander). All three species rely on these temporary pools for breeding and the aquatic phase of their life cycle (Semlitsch & Skelly 2007). Because competition and predation threaten juvenile salamanders, each species breeds at different times of the year. The earliest breeder is *A. opacum*, which breeds in early fall to gain a competitive advantage from size and age due to their early hatching (Boone et al. 2002). Both the *A. maculatum* and *A. talpoideum* breed during similar time periods of late winter to early spring, with the *A. talpoideum* beginning migration earlier (Semlitsch 1985) than *A. maculatum* (Stenhouse, et al. 1983). In addition to the mole salamanders, *Notophthalmus viridescens* (eastern newt) also uses these seasonally flooded wetlands.

This project focuses on the breeding and habitat preferences of salamander species in wetland areas in Western North Carolina. The primary goal of this study is to provide greater insight into the most effective conservation methods for wetland areas, with a focus on endemic and threatened herpetofauna. Similar studies have been conducted in this wetland, but have focused on overall population monitoring or predation of egg masses (Fullmer 2018; McLeod 2017). The hypothesis for this study was that the *Ambystoma* salamanders would have a strong preference for highly vegetated regions within the vernal pools compared to other amphibian and fish species present. The permanent pond, in comparison, would have a higher population of fish, tadpoles, and salamanders outside the *Ambystoma* genus that prefer higher-vegetation habitats to avoid predatory fish.

Methods

Study Site

This research was conducted at the Sandy Bottom Wetland Preserve, a natural wetland ecosystem in Arden, North Carolina. This ecosystem contains vernal pools, a permanent pond, and a small stream, making it a viable site to conduct research on a variety of amphibian species. Sandy Bottom Wetland Preserve is located south of Asheville, North

Carolina, and is a part of the French Broad River floodplain. The approximately 25-acre ecosystem is primarily composed of montane alluvial forests (Boyd & Preusser 2016).

The two habitats used for this study were the vernal pools and the permanent pond (Figure 1). Each habitat was divided into regions based on tree cover and aquatic vegetation, with the pools having five regions and the pond having three. The amount of plant growth and tree coverage was determined by the extent of plant coverage, either within the water or in the surrounding trees. Sections where water covered more than 75% of the plant or tree growth were classified as “High” coverage. Areas with less than 25% coverage were labeled “Low,” while those with 25% to 50% coverage were categorized as “Moderate.” Within the permanent pond, the regions were determined primarily based upon water accessibility and differing habitat types (Table 1). The vernal pool regions were divided based on tree coverage, trees growing in the water, tall grass in the water, the presence of root plates or logs, and the number of independent tree islands (Table 2).



Figure 1. Map of Sandy Bottom Wetland Preserve. The northern body of water is the vernal pools, and to the south is the permanent pond. (U.S. Fish and Wildlife Service, 2026)

Table 1. Amounts of different vegetation types within each permanent pond region.

Region	Plant Growth	Rocks	Tree Coverage
1	High	Yes	Moderate
2	High	No	Moderate
3	Low	Yes	Low

Table 2. Amounts or presence of different vegetation types within each vernal pool region.

Region	Plant/Tree Growth	Tall Grass	Root Plate/Log	Tree Islands	Tree Coverage
1	Moderate	No	Yes	No	Moderate
2	Moderate	No	Yes	Yes	High
3	High	Yes	No	Yes	Moderate
4	High	Yes	Yes	Yes	Moderate
5	Low	No	No	No	Low

Data Collection

At the beginning of October 2025, dip-netting began in both the vernal pools and the permanent pond within the wetland area. Due to a lack of filled pools, the salamander breeding season typically doesn't begin until early fall. A rain gauge was placed in an area with low tree cover and checked at each sampling period to obtain the most accurate rainfall data. A dipnet was used for approximately 15 minutes in each sampling section in both habitat areas. If the water levels in a section were below the required level to dipnet or were frozen, the inability to sample was noted. All amphibian species that were caught were measured by length (mm), life cycle identified, and sexed when possible. Fish species were also recorded and measured to determine whether there is a correlation with the presence of predators. Surveys were done once a week throughout the six-month period. During phases when breeding was likely to occur, such as warm, rainy evenings, or when egg masses were present, surveying occurred twice a week when able. The temperature and weather were recorded each time sampling occurred to further understand weather correlations. Sampling occurred between October 3rd, 2025, and March 31st, 2026, to observe the peak breeding times for the different species residing in the wetland.

Data Analysis

Following data collection, all analyses were conducted in RStudio using nonparametric tests. All species were analyzed using the Sheirer-Ray-Hare test, followed by a Dunn test, in RStudio. An analysis was conducted to compare the significance of species presence

between different regions of the two sites. The data was not compared between the pools and the pond, but only between the different regions within the same site. Species were compared based on the significance of the region preferred within the site.

Results

Species Diversity

There was a total of seven amphibian species found within the water at Sandy Bottom (Table 3). The vernal pools had the highest species richness, with four members of the order Caudata and two of the order Anura. In contrast, the pond had only two members of the order Caudata and one of Anura. Both habitats contained two smaller fish species; however, the vernal pools did not contain any after the first complete drying. The pond included three additional larger species: bullhead catfish, chain pickerel, and swamp darters.

Table 3. Abundance of amphibian species within Sandy Bottom vernal pools and permanent pond.

Species	Abundance of Species in Pools	Abundance of Species in Pond
Eastern Newt	74	25
Marbled Salamander	7	0
Spotted Salamander	1	0
Mole Salamander	5	0
Red Salamander	0	23
Green Frog Tadpole	52	4
Wood Frog Tadpole	89	0

Abundance Within Regions

The abundance of some of the amphibian species studied was correlated with the wetland region (Figure 2). There was a significant correlation between the occurrence of eastern newts and Region four (p-value = 0.0001), and green frog tadpoles inhabiting the same region (p-value = 0.0247). There was no significant preference for other Caudata species, such as the paedomorphic mole salamander or the juvenile marbled salamander.

The two other species caught, the juvenile mole and juvenile spotted salamanders, were collected only once and therefore could not be analyzed statistically with confidence.

The permanent pond had much lower Caudata diversity than the vernal pools (Figure 3). Within the permanent pond, all species showed a strong preference for Regions one and two. Region three only contained eastern newts over the study period. The juvenile red salamanders preferred Region one but were also found in neighboring Region two, especially toward the end of the study.

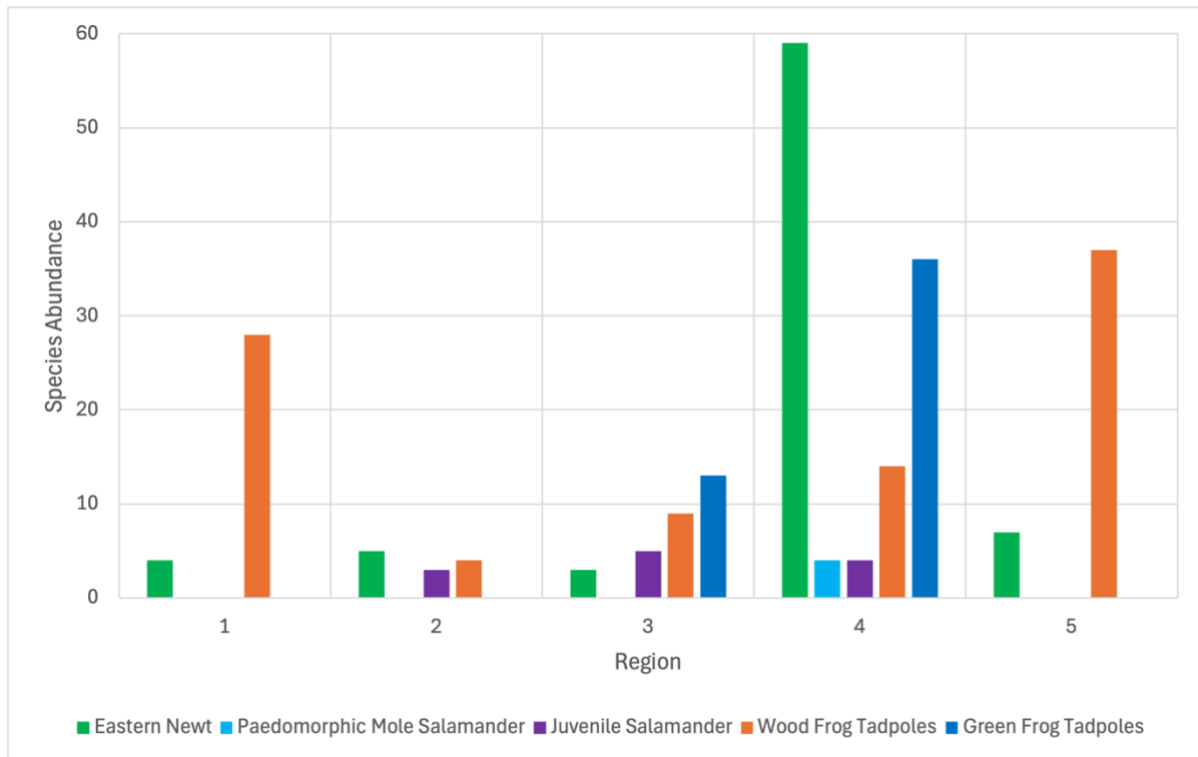


Figure 2. Abundance of each species caught within vernal pool

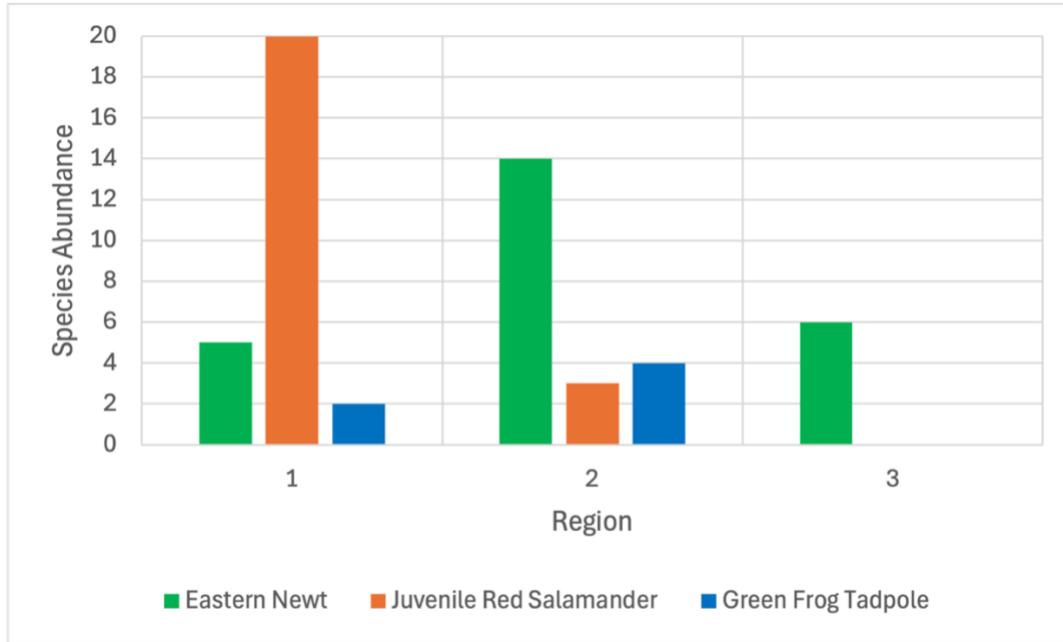


Figure 3. Abundance of each species caught within the permanent pond regions.

Abundance Over Time

The recorded numbers of each Caudata species in the vernal pools changed over the study period, as shown in Figure 4. Events of complete pool drying and freezing are shown by the red and blue lines, respectively. The eastern newt population had the highest abundance throughout the study. Paedomorphic mole salamanders were observed periodically throughout the study, with higher occurrence before the pools froze. Juvenile marbled salamanders and spotted salamanders appeared toward the end of the project, during late winter and early spring, respectively. The abundance of other species within the order Anura and fish from the class Actinopterygii is shown in Figure 5. As in Figure 4, the red line indicates complete drying, and the blue line indicates complete freezing of the pools. Prior to the pools drying, there is a high population of fish and green frog tadpoles. After drying, a small number of bluegills and tadpoles survived but were not present soon after. There were no other fish found within the pools, and no tadpoles were seen until the wood frog tadpole hatchlings appeared in late winter.

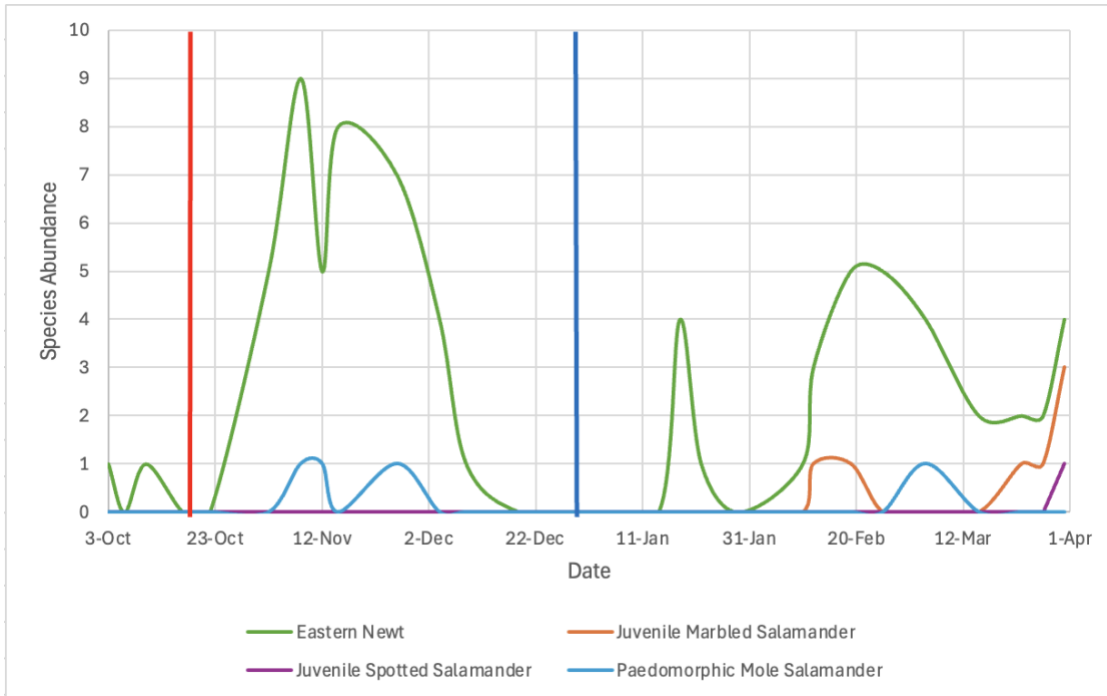


Figure 4. Abundance of Caudata species caught within the vernal pools and occurrence of complete drying (red) and complete freezing (blue) over study period.

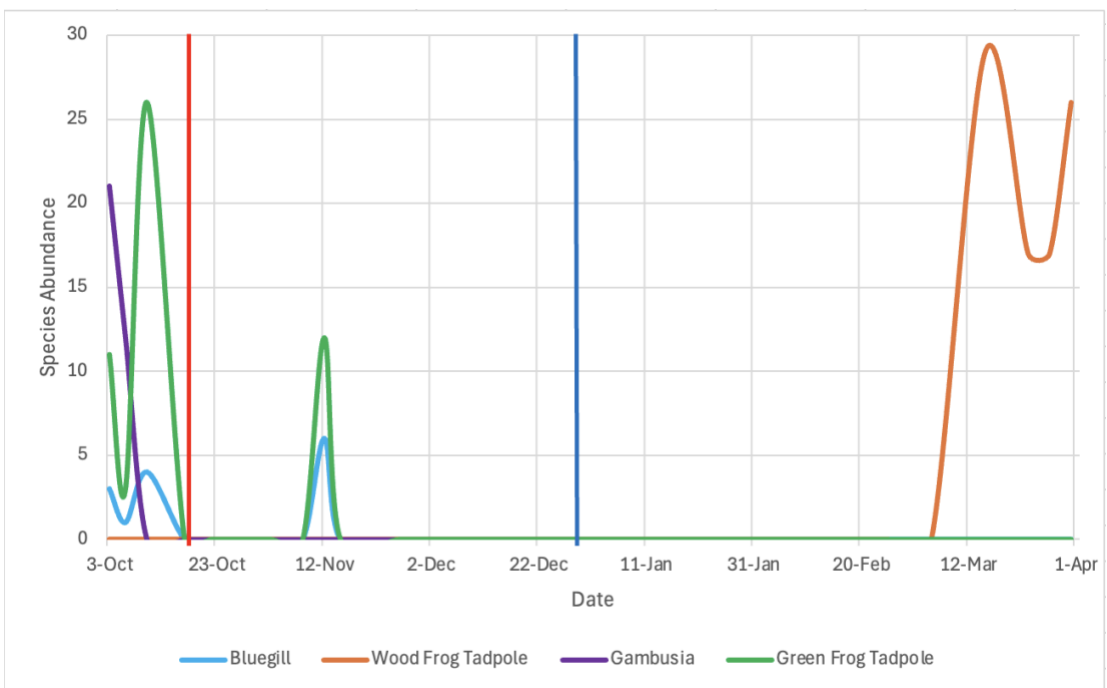


Figure 5. Population abundance of Anura and fish species within the vernal pools with indicators of complete drying (red) and complete freezing (blue) during study period.

Discussion

The higher concentration of species within the vernal pools is expected among *Ambystoma* species and winter-breeding *Anura* species. Since these populations rely on temporary pools for reproduction to avoid fish predation, it is expected that there would be higher abundance and diversity within the pools during breeding periods. In contrast to most *Ambystomatidae* salamanders, red salamanders in the family *Plethodontidae* instead breed within permanent bodies of water to support their long maturation period. Since they remain in the larval stage for over two years, temporary pools would lead to high mortality during dry periods (Bruce 1978).

Since this preference depends primarily on the absence of fish in these bodies of water, fish populations are uncommon in the pools. The presence of gambusia and bluegill was likely due to the pools not fully drying until the first drying in October. The fish were likely able to colonize and reproduce in this area during flooding from Tropical Storm Helene and survived due to the lack of routine drying during the warm seasons. This extended period of flooding also altered the typical breeding pattern of green frogs. Due to their prolonged larval stage, permanent bodies of water are necessary for juvenile survival (Woodford & Meyer 2003). Since the pools were unusually full during their typical breeding period, late spring through summer, this habitat was likely mistaken for a permanent body of water (Gaul & Mitchell 2007).

Spotted salamander populations had a strong preference to lay egg masses primarily within Regions three and four of the vernal pools. Since these regions had higher tree coverage and vegetation, it is likely that accessibility to branches for egg mass stabilization was greater (Kern et al. 2013). Branches present within the pools are used by salamander species to lay egg masses. Using a branch or stick to hold their eggs prevents them from falling to the depths of the water, which often results in hypoxia due to low oxygen and a lack of sunlight needed for the symbiotic green algae within the egg mass (Kerney et al. 2011). Previous studies in wetland ecosystems have shown that spotted salamanders typically deposit eggs in deeper, shaded areas, likely to protect against drought (Egan & Paton 2004).

In contrast to the spotted salamander, the wood frog egg masses were only found within Regions one and five. A previous study found that wood frogs prefer to deposit eggs in areas with higher sunlight to ensure sufficient warming of the eggs, given their early breeding (Calsbeek et al. 2022). This contradicts the demonstrated behavior in wood frog tadpoles, which often choose habitats to avoid direct sunlight, likely to avoid UV radiation (Connolly et al. 2011). The addition of wood frog egg masses led eastern newts to shift to these regions to feed on the egg masses. This shift contradicts the habits we had

previously seen from this species. Throughout the study period, there was also a higher abundance of males at both sites until early spring, when mating began.

The results of this study show that Region four within the vernal pools and Region one within the permanent pools had the highest diversity and abundance among the regions. This aligns with previous research on amphibians in vernal pools (Egan & Paton 2004; Shrank et al. 2015), suggesting that habitats with greater sedge and other vegetation are typically preferred for protection from drying. Across the two sites, these regions had the highest levels of aquatic vegetation and tree cover. Both regions also had the deepest water of the surrounding regions. This for the vernal pools could reduce the likelihood of drying and provide more habitat opportunities for the pond-dwelling population.

The findings of this study suggest that vegetation, hydric regime, and water depth are crucial for the success of the amphibian biodiversity within wetland ecosystems. Diverse vegetation types and abundance throughout wetland habitats are necessary to accommodate the needs of each species. The altered hydric regime observed following Helene will likely become more frequent as climate change increasingly impacts natural ecosystem functions and patterns. Most species are unable to adapt rapidly enough to survive within these shifting environments and will likely experience a major loss in the near future. To protect these biodiversity hotspots and the vulnerable species that reside in them, additional research is needed on the specific preferences of species within these habitats to inform future conservation efforts and mitigate the effects of climate change.

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