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Seasonal Breeding Phenomena of Salamander Species in the Sandy Bottom Vernal Pools of Western North Carolina

Summer Vesper

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Abstract

Sandy Bottom Preserve is a 35-acre forest in Western North Carolina along the French Broad River that provides a glance into the ecosystem of vernal pools and the biodiversity that inhabits them. Among the many species that call it home are the Eastern newt (*Notophthalmus viridescens)*, Marbled salamander (*Ambystoma opacum)*, and the Spotted salamander (*Ambystoma maculatum)*. They breed in the vernal pools, providing a home and shelter for egg nests and juvenile salamanders, away from the threat of most predators. Vernal pools continuously drain and refill, making them unstable for fish, and crayfish which eat the salamanders and their larva. Previous research looked at these salamanders across the Southeastern United States, providing a background for the seasonal breeding variation among species and potential disturbances. This study aims to look at the correlation between rain levels, measured by gauge, and the arrival of salamander species, sampled in dip nets, breeding in the vernal pools. We hypothesized that the breeding seasons might vary due to the recent rainfall disruptions and timing in which the pools began to fill with many periods of unpredictable drought and flooding in between. After analysing the data we can see how it compared to previous research and how this might correlate with changes in the vernal pools' hydrologic patterns. The results concluded that the species-rain correlation, specifically among Eastern Newts, was potentially significant based on 0.08 p-value, but could not be concluded as significant until more research was conducted. More research would also be needed to further identify other salamander species patterns with rainfall, as this study did not capture enough individuals to be quantifiable.

Introduction

Western North Carolina is a hotspot for biodiversity of salamander species, particularly within its wetland and other aquatic habitats. Among the most critical are vernal pools, wetlands that dry up and replenish with water off and on throughout the seasons, allowing them to support an extraordinary quantity of these salamander species.[10] Vernal pools typically contain water in the winter and into the spring, becoming dry by summer.[3] The soil there is muddy and porous, with bedrock underneath that allows water to accumulate in the pools and acts as an impermeable surface.[6] The seasonal water fluctuations allow salamanders, as well as some aquatic macroinvertebrate species, and other amphibians to use the pools for breeding away from fish, crayfish, and other predators, maintaining a 'nursery refuge'. Aquatic predators cannot survive in these ponds or easily migrate to and from the pools because of the water fluctuations, so they are usually absent unless environmental changes or natural disasters occur, sometimes appearing if the water has stayed due to flooding.[1]

The Sandy Bottom vernal pools are habitats for many salamander species, including the Marbled salamander (*Ambystoma opacum*), Eastern newt (*Notophthalmus viridescens*), and Spotted salamander (*Ambystoma maculatum*). Marbled salamanders are known for their unique pattern of white or gray spots and black sleek body.[9] They mate in late August to late October in the dry and shallow pools. Males deliver sperm to the females, which they use to fertilize and lay egg nests in the leaf litter or mud. The female will stay with the nest until the young hatch in the waters of the pools in late fall, where the juveniles stay until they are old enough to survive in the forested areas.[9] The Eastern Newt has greenish-yellow skin with red dots on its dorsal side. They usually breed in late winter and early spring, sometimes continuing into early summer.[8] The females lay eggs in this duration in aquatic plants and leaf litter in small clutches, allowing for a higher survival rate. They do not provide any care for the eggs once laid, and continue on to the next clutch. When the juveniles are born in late spring to early summer, they eat small invertebrates and stay in water until they become Efts and migrate to terrestrial habitats.[8] The spotted salamander is brownish-black with orange-yellow spots or yellow lines on its dorsal side. They begin to mate in late February to mid-March, often after rainfall, and courtship happens in groups. The eggs are fertilized and laid at the same time on leaves and other debris in the water. They hatch in spring, sometimes in early summer. The juveniles have gills and stay in the water till they can breathe and live on land.[7]

In research done previously, Eastern Newts were shown to have a "false breeding" season in which courtship happens in the fall and females store sperm but do not fertilize the eggs until in the spring.[5] Marbled salamanders were found to have lower nesting rates due to hurricanes and flooding in the Southeastern United States, and spotted salamanders also dealt with these natural disasters while nesting.[2] This research has led to a pathway for more studies on salamander species and their breeding habits, with insight into outside obstacles. The research also serves to analyze Sandy Bottom Preserve species and how they might have changed over time with the effects of climate change and rainfall events.

Rainfall plays an important role in species abundance in the vernal pools. Vernal pools ebb and flow with rainfall as they require it alongside groundwater to fill. Climate change is leading to more periods of drought, flooding and freezing temperatures, changing the flow regimes of the pools. Species within the pools need them to breed and survive, potentially having to adapt their breeding seasons around these new variables. Species might even enter the pools in correspondence with rainfall, instead of seasonality over time.

This study aims to analyze salamander species abundance in the vernal pools, along with other amphibious and non-permanent species, in correlation with rainfall. It looked at how water level changes and rainfall events have potentially separated from the normal vernal pool hydrology. These hydrological changes could cause salamander species migrating to and from the vernal pools to follow alongside different rainfall events instead of seasonality.[7] In the study, dip netting methods were used to capture and record salamander species data in the vernal pools, measuring temperature, while taking weather, time, and date into account. Dip netting allowed for the effectiveness of surveying the area in multiple sections and ensuring a more accurate set of data. The rainfall was also captured in a gauge to monitor the vernal pool depth as the waters fluctuated and species arrived during the sampling period. We also took note of drought and ice freezing within the vernal pools.

Methods

The research project took place over the course of the fall and spring 2025-2026 seasons, surveying the entire vernal pool ecosystem. During this time, we collected data in Sandy Bottom, NC, a vernal pool preserve that is located along the French Broad River. The environment is forested with the vernal pools having partial plant coverage and partial sunlight fluctuating based on the time of year. The pools also had periods of fallen leaf litter debris accumulation and ice coverage in the water. We collected data including the length of the species, the species name, sex, temperature, and rainfall. These were recorded on a spreadsheet to later use for calculations and analysis.

In order to ensure accurate data, we separated the vernal pools into sections, marked by orange flags. Dip nets were used to collect species, and the same amount of time was spent dip netting in each section. We spent roughly 20 minutes in each section to further ensure we got an equal data spread. Collection started in section I and moved through to sections II, III, IV, and V, making sure we dip-netted in multiple areas of each section to get data representative of the entire habitat. The sections were not separated in the data analysis; they were only separated to ensure the entire area was surveyed equally during collection.

When a salamander was found, we would first identify its sex, seeing if it was male or female, and if it was in a breeding phase. The males were identified by their testes or nuptial pads, and the females by whether they were egg-bearing or had a rounder body shape. The length of each individual was also measured in millimeters and recorded. Fish, such as Western Mosquitofish and Bluegill Sunfish, were also measured, but the sex was not taken, alongside frog and toad tadpoles found. Sex was used in determining breeding phases, which was not needed in measuring species besides salamanders. After measuring the different individuals, they were released where we originally found them, and we did not dip net the same spot again to ensure little to no recapture.

Environmental data was also collected, including temperature, rainfall, weather, and the time. The temperature was obtained using a thermometer, and the rain was measured using a gauge that was checked every time we took data, roughly twice a week between October and March.

The data was analyzed by being entered into the R programming language using the Spearman Rank test, which showed the significance and any correlation. Afterwards, the data for rainfall was compared to salamander species, specifically Eastern Newts, presence in the pools, and any relations were further identified. Non-permanent species, and green frog tadpole presence were also analyzed to analyze any patterns that were uncommon. Eastern Newt data was most prevalent, so we looked at how their presence and absence correlated with other species and rainfall in further depth.

Results

Throughout the sampling period, we found a total of 4 salamander species, as well as one paedomorphic sub-species (Figure 1). During the fall, we found 2 species, with a total of 45 individuals, from October 3rd to December 19th. In the late winter to early spring, we found 5 species with a total of 27 individuals between February 10th and March 15th. These numbers were significantly higher than those between December 31st and January 29th, in which we only found 1 species and 7 salamanders (Figure 1). We also found 2 frog species, the Wood Frog and Green Frog, that were only found as tadpoles; adults live terrestrially. The Green Frog tadpoles were found in the fall, and we captured 52 individuals, while we captured 7 Wood Frog tadpoles in the spring only (Figure 5). The vernal pools also had non-permanent species in the fall, which included 4 Western Mosquitofish and 14 Bluegill Sunfish (Figure 4).

When the data was analyzed, we began by conducting a Spearman Rank Test in R using the data collected for Eastern Newts. The other species had too little data to conduct this test on; they would not have had a quantifiable p-value. The test indicated a correlation between rainfall and Eastern Newts presence being 0.08 on the p-value scale of 0.0 to 1.00, showing a possibility for significance in the data set, but no significance yet. The p-value indicates that a correlation cannot be determined, so the null hypothesis failed to be rejected.

The data also presented salamander species abundance and the number of individuals within these species that were found during the sampling period (Figure 1). It indicated a higher abundance of species in late fall and into late winter, with an absence of most species in the earlier part of winter. Early winter had periods in which the vernal pools were frozen, and ice covered the top of the water, potentially playing a role in these periods of lower salamander abundance.

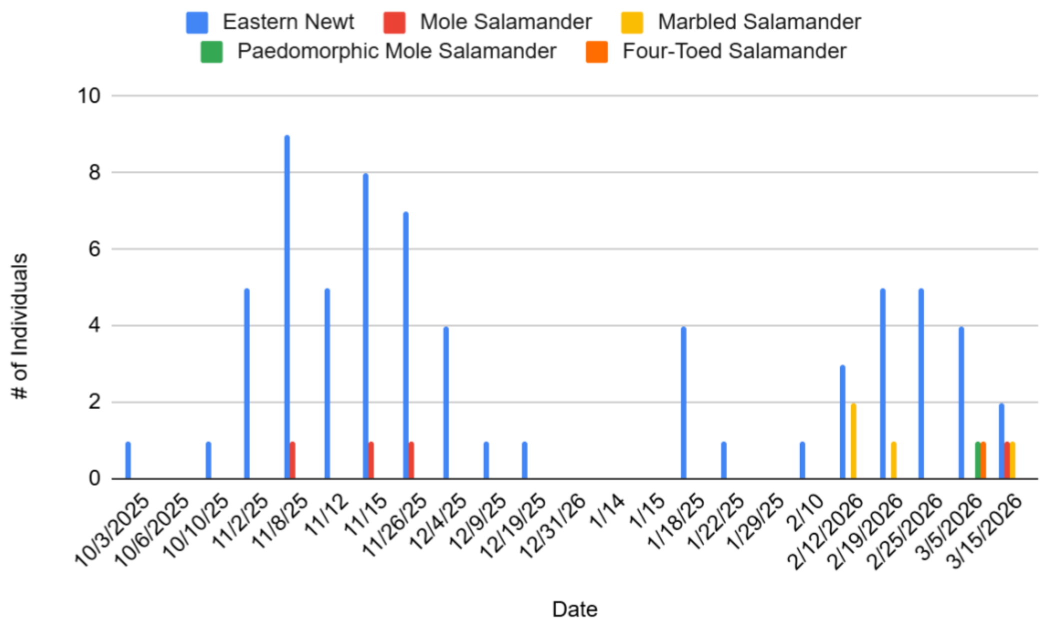

Figure 1. Abundance of salamander species in the vernal pools

Eastern Newt abundance followed a similar trend as other salamander species found in the vernal pools (Figure 2). There was a higher presence indicated in the fall and late winter into early spring, coinciding with their normal breeding season.

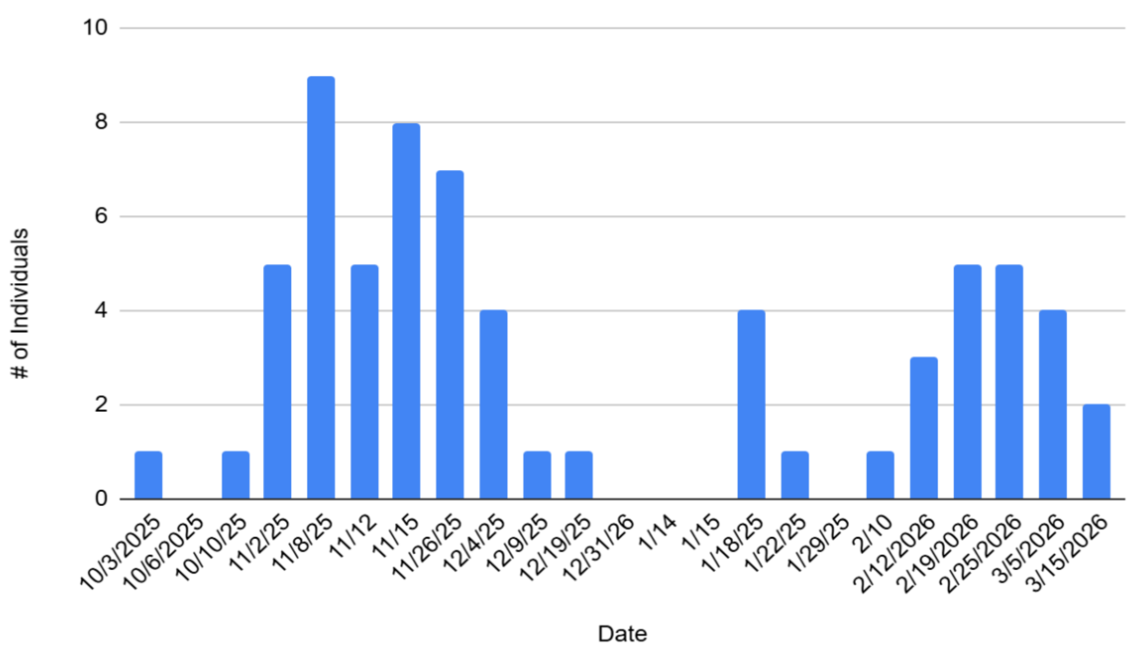

Figure 2. Abundance and absence of Eastern Newts

Rainfall in the vernal pools fluctuated, with some dates having no rain at 0mm and also rising, with the highest being 120mm (Figure 3). There was not a clear pattern as rainfall was random, although it seemed to slightly line up with species presence, in early fall, although not being close enough to prove correlation.

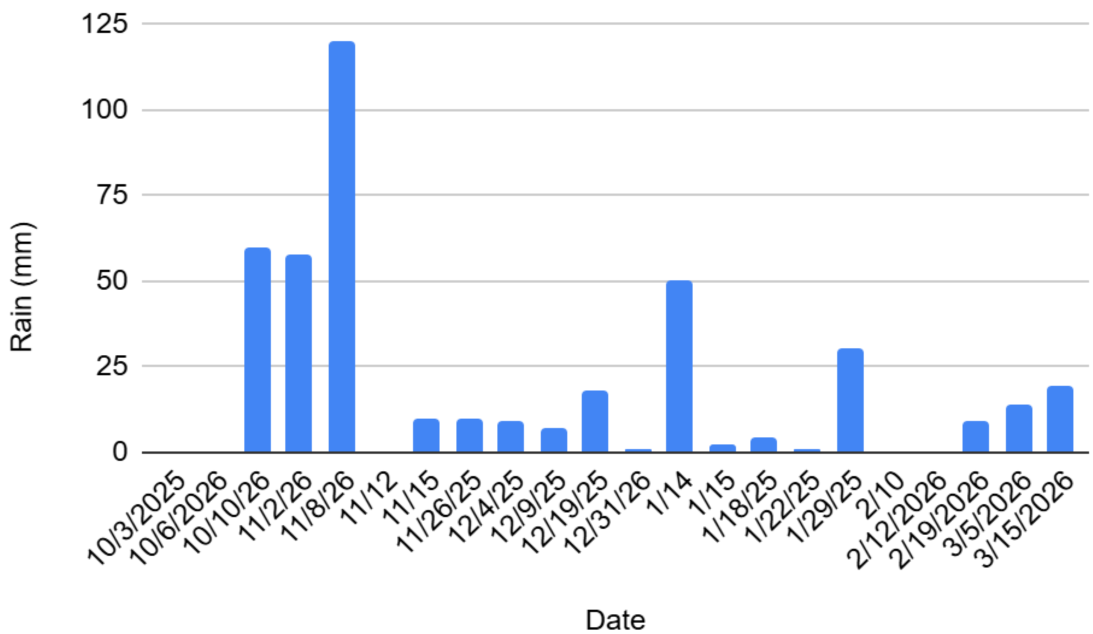

Figure 3. Rainfall captured in the vernal pools, measured in millimeters

Fish species also colonized and bred in the vernal pools during the higher water levels in the fall before a period of drought (Figure 4). The species found in the vernal pools were Western Mosquitofish and Bluegill Sunfish, both being analyzed due to them usually not found at Sandy Bottom. They are non-permanent species, meaning they are not found in the vernal pools under normal conditions. They migrate to these pools when water levels are high, and they migrate out, if possible, or are killed off in the pools when the water is too shallow. The data obtained shows that they were likely in the pools for a longer time than the sampling period, and due to less rainfall during a period of drought in November, they were killed or left to seek safer areas with more oxygen and water. Gaps in data occurred among Bluegill abundance were potential due to during the start of the drought period, the pools could have had small areas where fish could live for small amounts of time, until a short intermittent rainfall event. Afterwards, they could have returned until the pools dried up completely.

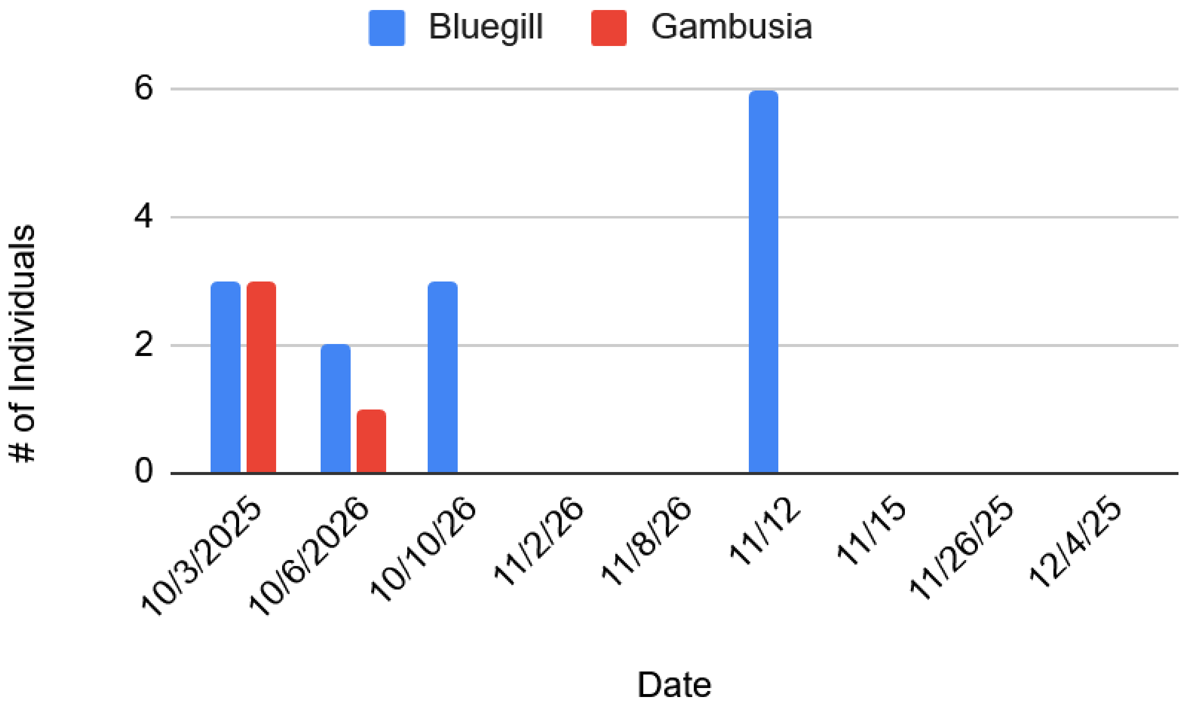

Figure 4. Abundance of non-permanent fish species in the vernal pools

Green Frog Tadpoles were found in the vernal pools during the fall and became absent the rest of the sampling period (Figure 5). This absence coincided with a period of drought during November (Figure 3).

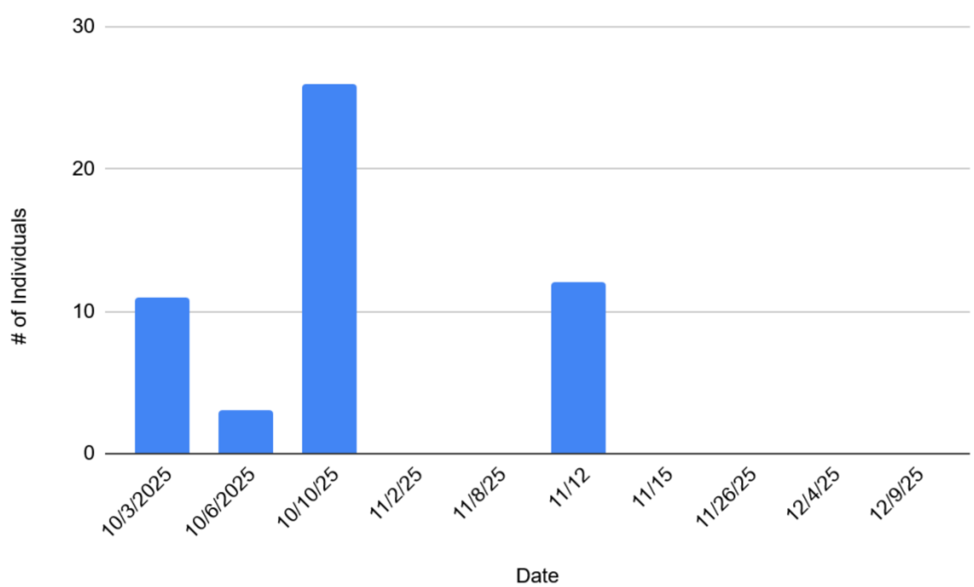

Figure 5. Green Frog Tadpole abundance over the sampling period

Further data collection and analysis would allow for further investigation into any correlation between rainfall and species presence, as well as serve as a gauge for vernal pool ecosystem biodiversity. The data collected in this study acted as a baseline for further discussion and research.

Discussion

Data taken at the Sandy Bottom Vernal Pools indicated a potential for correlation between rainfall and salamander abundance, although there is no relation that has been found at present. Flooding and drought data indicated the possibility of potential changes in salamander abundance as non-permanent fish species declined abruptly due to a period of drought, lowering salamander predators within the pools.[10] Green frog species also might potentially decline over future breeding seasons, as many tadpoles were killed within the drought period. Salamander species tended to follow their normal breeding patterns, especially the Eastern Newt, which was most abundant within the vernal pools.

When analyzing the data, we ran a Spearman Rank Test on Eastern Newts found. The p-value was indicated to be 0.08. This indicates that there is no significant correlation between rainfall and species abundance in the vernal pools. This could potentially change due to it being almost significant, with further research added to the existing data. The data set we collected only covered a part of the spring breeding season and could potentially have a higher correlation if the entire breeding season were researched, or multiple breeding seasons. Research conducted previously in Sandy Bottom had higher salamander abundance in the middle of spring, with 41 eastern newts found during April, but they did not look at rainfall alongside abundance.[6] This proposes that with higher abundances and rainfall being assessed alongside, a relationship might be found. Higher numbers of individuals allow for more data that can be quantifiable while being analyzed.

Salamander species in the vernal pools followed the standard seasonal breeding times during sampling, often being more abundant in the late fall and early spring. They were less abundant in the winter when the pools were often icy, and as the eggs were still unhatched. Salamander species that stayed with their eggs were hidden among the copious amounts of leaf debris, while other species left to land and laid eggs in the early spring when the vernal pools were not iced over. Rainfall sometimes seemed to lead to a higher abundance, but often the relation was not identifiable among most dates. For example, rainfall on November 8th hit 120mm, the highest number collected, while we found 2 species and 10 individual salamanders, the highest abundance collected. But, afterwards, the rainfall declines, while salamander abundance stays consistent for a while longer. The potential relations might be coincidences in the data rather than correlations.

The Eastern Newt, *Notophthalmus viridescens,* was the most common of the salamander species found within the vernal pools. The data indicated that they entered the pools when the water levels were higher, often corresponding with rain events. This could potentially be due to there being more habitat to breed and find mates, alongside the fact that there was more shelter to hide. If the water is too low, predators from on land can easily spot them from above and eat them or their larvae. Predators in the water also require higher visibility levels to find their prey, and rain leads to turbidity, causing difficulty for them to find prey. Rainfall also brings in nutrients that could help nurture juveniles, including increased macroinvertebrate spawning and leaf litter abundance. The rainfall, although sometimes seeming to bring increased Eastern Newt abundance, might not be the reason. Instead, they are most likely continuing to follow their seasonal patterns, while dealing with the effects of uncommon periods of drought and flooding.

Seasonality also played an important role in Eastern Newt presence, as they were most likely found when it was fall, their breeding season, and juveniles were found in the spring when they hatched. This matches with research done in the vernal pools before, indicating their breeding cycle to be similar.[6] Snow found eastern newt abundance followed a downward trend from late fall, and throughout winter. They stated that the newts increased in abundance in early spring as they found 23 individuals in March.[8] During the study, 6 eastern newts were found in March, but sampling only occurred during the first half of the month. This was an increase from having found none for much of the winter.

The data indicated the non-permanent fish species Western Mosquitofish (*Gambusia affinis*) and Bluegill Sunfish (*Lepomis macrochirus*) were found in the vernal pools during the fall breeding season. It also shows the rainfall data from the times the non-permanent fish species were found in the pools and into when they were absent. From the data, it is indicated that potential low water levels could have led to them leaving and not returning when the water rose again. A paedomorphic mole salamander was found in early spring potential indicating that with the loss of non-permanent fish predators allowed for enough safety to stay aquatic instead of losing its gills. The lengths of the Gambusia and Bluegill indicated they were juvenile, meaning the species had to have been there for a longer amount of time and had been breeding in the area. Bluegill take time to colonize new environments, and this could indicate water having been in the pools since the last flood event. It could also indicate that the vernal pools potentially faced a change in hydrology, perhaps having no periods of drought and not drying up for a while.[10]

The Green Frog tadpoles were another example of the presence of non-permanent species in the vernal pools during early fall, which became absent as rainfall decreased and a drought period occurred. Green Frog Tadpoles were found in the vernal pools between October 3rd and November 12th, with a short absence between November 2nd and November 8th. After these dates, there were no more green frog tadpoles found in the vernal pools. This indicated that they were using the pools to breed during their usual breeding season, and due to the drought, they might not have survived in the pools as they dried out and became extremely shallow.[5] The tadpoles were found commonly until the rainfall was below 15mm for multiple sampling dates, where they became fully absent. They need higher water levels for the first part of their lives, as they are aquatic, and without water, they were unable to continue to develop.

This study provided a foundation for preserving and understanding salamander species among the vernal pools. Further research would be needed in order to indicate a direct correlation between rainfall and species abundance, although the p-value of 0.08 was almost significant. Water levels often change in the vernal pools, and by looking at species abundance, we can begin to see patterns and where outlying effects, such as periods of drought and flooding, might change the normal hydrology and ecology of these wetlands.

Vernal Pools are biodiversity hotspots for species of salamanders, and as climate change causes periods of drought and flooding, their abundance could potentially fluctuate, which is why rainfall must be analyzed, in order to look at any future changes.

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