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Patterns in Caudate Abundance and Biodiversity at Sandy Bottom Preserve

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Abstract

The magnitude of Tropical Storm Helene in western North Carolina raises questions about the impacts of flooding on amphibians located near rivers and streams. This project aimed to quantify the changes in the diversity and abundance of salamanders historically present at Sandy Bottom Preserve following Tropical Storm Helene. The site has been monitored near-continuously since 2016 using a series of coverboards. Changes in diversity and abundance of three species, *Ambystoma opacum, Notophthalmus viridescens,* and *Pseudotriton ruber,* were evaluated by comparing capture rate, standardized by sampling effort, across years. The effects of precipitation and temperature on salamander abundance also were evaluated to investigate relationships between breeding cycle and weather. It was expected that significantly fewer salamanders would be found in the fall of 2025 than in previous fall seasons due to the impact of Tropical Storm Helene. No significant relationship was found between weather variables and salamander abundance, but mean abundance in the two years adjacent to extreme flooding were found to be significantly different with contrasting relationships between the three species.

Introduction

Declines in salamander abundance have been shown across multiple families throughout the United States (Caruso and Lips 2012, Wheeler et al. 2003, Milanovich et al. 2010). To slow these declines and conserve populations, it is important to understand their habits, including their seasonal habitat use and breeding cycles. Factors like temperature and rainfall affect seasonal habitat use in salamanders (Brooks et al. 2019, Grace 2003, Sexton et al. 1990). Benard and Greenwald (2023) found that temperature and rainfall varied across sites, and within sites across years, heavily impacting species interactions such that interactions were greater some years than others. This makes broad generalizations on the seasonal habitat use across sites and over time difficult to make. This suggests that, in order to understand temporal patterns of abundance at any specific site, we must examine that site's unique abiotic characteristics and species composition.

Abiotic conditons are especially important because they serve as the cues for migration to and from wetland sites. For example, photoperiod cues migration in winter-breeding Ambystomid salamanders, reducing the risk of laying eggs during early-thaws that are followed by dangerous freezes (Sexton et al. 1990). Photoperiod is a more reliable indicator of season than temperature; however, within seasons, rainfall and temperature are the environmental factors that influence when amphibians begin migration and breeding (Brooks et al. 2019). In an *Ambystoma* species, soil temperature has been shown to be a cue for migration, with air temperature correlated secondarily (Sexton et al. 1990). Further, *Ambystoma opacum* is known to lay eggs in dry floodplains before rain inundates the nest, allowing the eggs to hatch in an aquatic environment.

While these relationships have been widely documented, previous studies have largely examined the relationship between abiotic factors and salamander abundance under relatively normal weather conditions for their regions over the sampling period. Extreme weather events provide novel circumstances to further investigate the habits of salamanders. Flooding brought about by a tropical storm may impact the life cycles of sensitive species of herpetofauna in a variety of ways. North Carolina's state salamander, *Ambystoma opacum*, has been shown to have their nesting habits interrupted by flooding caused by hurricanes (Hall 2022). Under normal conditions, a vernal pool's ability to seasonally dry out actively inhibits almost all fish species from completing their life cycle within the habitat. Due to the lack of fish predators, amphibians have been able to thrive in vernal pools. However, major flooding that causes a connection between a vernal pool and another body of water could lead to the introduction of fish for a season. This could lead to adult and larval salamanders being predated upon, which has been established to have long term negative effects on salamander populations (Pope 2008, Smith et al 2001).

Sandy Bottom Preserve (Buncombe County, NC) is a Montane Alluvial Forest located in the French Broad River Floodplain (Boyd and Preusser 2016) and is designated as a North Carolina Unique Wetland. The property contains an ephemeral pool, a sensitive and rare habitat that allows amphibians to thrive. Sandy Bottom Preserve supports populations of at least forty species of reptiles and amphibians and serves as a critical habitat for several ecologically significant salamander species (Graeter and Hale 2020). Sandy Bottom Preserve has also been sampled for herpetofauna using a variety of traps for just under ten years.

In the Fall of 2024, Tropical Storm Helene swept through Asheville, North Carolina, causing the French Broad to reach historic flood levels. The flooding did extensive damage to infrastructure, human life, and wildlife habitats in the region (Associated Press 2024). Research is ongoing on the impacts to each. The unique ecological nature of Sandy Bottom Preserve and its high density of sensitive species require special attention to assess the impact of such unprecedented flooding.

Long term sampling at Sandy Bottom Preserve provides a unique opportunity to analyze the salamander population dynamics to determine the effects of extreme flooding, gain information on relationships between abiotic factors and salamanders, and better support the animals at the site. Wooden coverboard arrays were established at Sandy Bottom Preserve in 2016 to sample herpetofauna populations (McLeod 2017). Wooden coverboards have been shown to be an effective sampling tool for herpetofauna, with the most frequent sampling interval being weekly (Marsh and Goicochea 2003). In the first year of sampling herpetofaunal diversity at Sandy Bottom Preserve, McLeod (2017) found that caudates were in greatest abundance under coverboards in comparison to other herpetofaunal taxa. Caudate diversity at Sandy Bottom Preserve includes two North Carolina Species of Special Concern, *Ambystoma talpoideum* and *Hemidactylum scutatum,* well-recognized species such as *Ambystoma maculatum* and *Ambystoma opacum,* and evidence of 15 more (Graeter and Hale 2020).

It is hypothesized that habitat disturbance caused by flooding from the French Broad River negatively impacts species that rely on ephemeral wetlands to breed. Such flooding introduces fish and may wash away active nests, increasing mortality among embryonic and larval salamanders. If my hypothesis is correct, a significant decline in capture rate of all species will be observed between 2024 and 2025 due to the extreme flooding event of Tropical Storm Helene in September 2024. Further, as the relationship between salamander abundance and the weather variables of temperature and rainfall are assessed, a relationship is expected to be found.

Methods

Study species

Species evaluated were *Ambystoma opacum*, *Pseudotriton ruber*, and *Notophthalmus viridescens*. These species were chosen due to their high abundance in the dataset, lending them to analysis of how their abundances vary with abiotic factors and across years.

Ambystoma opacum, the Marbled Salamander, is widely distributed across the eastern United States. They are stocky salamanders that are generally black to dark grey, with silver banding across the back. They begin migration to breeding sites in September and lay large clutches of loose embryos in nests under coverings (AmphibiaWeb 2026). These nests are located next to vernal pools in floodplain wetlands that are moist but not flooded. Females guard these nests until precipitation floods them (Croshaw and Scott 2005), allowing for larval individuals to hatch into an aquatic environment (Petranka 1982).

Notophthalmus viridescens, the Eastern Newt, is a species unique in its life cycle to the other two species studied. This is mainly due to the life cycle containing a separate and lengthy terrestrial juvenile phase, the red eft, along with the aquatic larval and adult stages (Petranka 1998). In their adult stage, they are green to a yellowish-brown color and have several rows of bright orange spots across their back (AmphibiaWeb 2026). Their breeding season is late winter to early spring and involves the female salamanders laying individual eggs on submerged plants (Behler and King 1996, AmphibiaWeb 2026).

Pseudotriton ruber, commonly known as the Red Salamander, is a plethodontid salamander native to the eastern United States. They are bright orange-red with black spots and spend most of their time under cover or in burrows (AmphibiaWeb 2026). They generally lay their eggs individually in water on the edge of streams on the underside of rocks and logs, with breeding time varying geographically (Petranka 1998).

Sampling methods

Forty-two coverboard arrays were established at the Preserve in 2016 (McLeod 2017) by UNC Asheville and the NC Wildlife Resources Commission (Figure 1). Each coverboard array contains 2 to 4 hardwood coverboards.

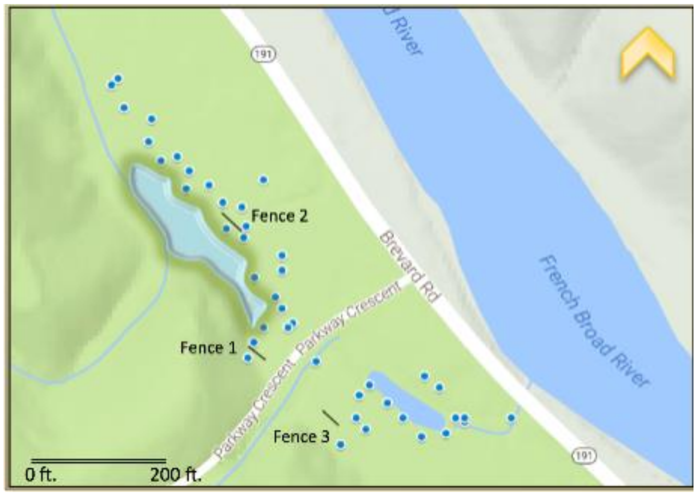

Figure 1. Map showing Sandy Bottom. Blue dots indicate location of coverboards. Black lines indicate drift fences that are no longer in use (map from McLeod 2017).

To sample herpetofauna, each coverboard in the array was slowly lifted and checked for the presence of animals. The species and number of individuals were noted along with what coverboard array the animals were found under. If a coverboard array did not house any animals, it was noted as empty. Any coverboard that was not found was noted as such and was not included in the analysis. Weekly or biweekly, each coverboard was sampled. Coverboards were sampled no more frequently than on a weekly basis, as to not excessively disturb the boards and to minimize impact on the number of individuals that may hide under the coverboards. A gap in sampling occurred from October 2024 to February 2025 due to Tropical Storm Helene's disturbance of coverboard arrays. Data prior to 2021 were excluded for two reasons. First, sampling was sporadic between 2016 and 2019, generally coinciding with course-based research activities. Therefore, the data do not provide a full account of abundance even within seasons. Second, a large gap in sampling occurred during the COVID-19 pandemic, between 2020 and 2021.

Daily maximum temperature, minimum temperature, and precipitation were sourced from NOAA Climate Data Online (National Centers for Environmental Information 2026) and merged with our coverboard sampling data to allow direction comparison of salamander abundances with the abiotic variables. Daily mean temperature, monthly mean temperature, and monthly total rainfall were calculated for use in graphing.

Analysis

The number of individuals of each species were totaled for each day sampled and divided by the number of coverboards sampled, getting a rate of capture that adjusts for sampling effort. The daily rate of capture for each species was filtered for the months in which each of the three species historically appeared most frequently at the site (June-

December), as to remove any periods of time where they are never actually found. These filtered data were subject to a Spearman's rank correlation test against daily mean temperature and daily total rainfall for the entire August 2021 to December 2025 period (R Core Team 2026). In addition, interannual variation in abundance was evaluated by calculating mean daily capture rate from August to December and comparing this value across years from 2021 to 2025 using ANOVA. Tukey HSD post-hoc tests were performed to identify pairwise differences among years.

Results

Statistical Testing

No significant relationship was found between precipitation and rate of capture for all three species (Table 1). Similarly, no significant relationship was found between mean temperature and rate of capture for all three species. While ignoring outliers in capture rate and precipitation, Figures 2, 3, and 4 each indicate a non-linear relationship with peak capture rates associated with around 5cm of precipitation.

The ANOVA shows *N. viridescens*, *P. ruber,* and *A. opacum* each varied significantly across years (Table 2). Tukey HSD post hoc tests revealed that *A. opacum* (Table 3) and *N. viridescens* (Table 4) significantly declined in abundance between 2024 and 2025, whereas *P. ruber (*Table 5) significantly increased in abundance within the same year pair. Abundance of *Ambystoma opacum* in 2021 was significantly different from 2024. *Notopthalmus viridescens*' 2024 was significantly different from its 2021 and 2022. 2025 was significantly different from 2021 for *Pseudotriton ruber*.

Table 1. Results from Spearman's Ranked Correlation Tests.

Weather Variable	Species	Cor. Coef.	*p*
Temperature	*P. ruber*	-0.0104	0.076
	A. opacum	0.001	0.965
	N. viridescens	0.006	0.844
Precipitation	*P. ruber*	-0.029	0.372
	A. opacum	-0.046	0.164
	N. viridescens	0.015	0.642

Table 2. Results from ANOVA. Effect of year on rate of capture.

	F	df	*p*
A. opacum	3.577	4, 39	0.014000
N. viridescens	5.825	4, 39	0.000895
P. ruber	3.406	4, 39	0.017600

Table 3. Results from post-hoc Tukey HSD testing on ANOVA for *Ambystoma opacum*. Significant pairwise comparisons are **bold.**

Year pair	Difference	p adj
2021-2022	0.024	0.462
2021-2023	0.018	0.712
2021-2024	**0.068**	**0.008**
2021-2025	0.004	0.999
2022-2023	-0.006	0.997
2022-2024	0.043	0.252
2022-2025	-0.02	0.746
2023-2024	0.049	0.149
2023-2025	-0.014	0.910
2024-2025	**-0.064**	**0.031**

Table 4. Results from post-hoc Tukey HSD testing on ANOVA for *Notophthalmus viridescens*. Significant pairwise comparisons are **bold.**

Year pair	Difference	p adj
2021-2022	0.021	0.743
2021-2023	0.033	0.339
2021-2024	**0.100**	**0.001**
2021-2025	-0.005	0.998
2022-2023	0.012	0.976
2022-2024	0.079	0.023
2022-2025	-0.027	0.688
2023-2024	0.067	0.074
2023-2025	-0.039	0.337
2024-2025	**-0.106**	**0.001**

Table 5. Results from post-hoc Tukey HSD testing on ANOVA for *Pseudotriton ruber*. Significant pairwise comparisons are **bold.**

Year pair	Difference	p adj
2021-2022	0.013	0.961
2021-2023	0.017	0.916
2021-2024	-0.016	0.968
2021-2025	**0.066**	**0.015**
2022-2023	0.003	1.000
2022-2024	-0.030	0.826
2022-2025	0.053	0.163
2023-2024	-0.033	0.764
2023-2025	0.050	0.214
2024-2025	**0.083**	**0.041**

Descriptive Statistics

The month of highest mean temperature was July 2025 at 29°C, and the month of lowest mean temperature was January 2025 at -6°C (Figure 5). The month with the highest cumulative precipitation was September 2024 with 50cm of rainfall. January and February 2025 were recorded with 0cm of precipitation. The highest daily rate of capture for *A. opacum* was 0.16 on 09/19/2024 (Figure 6). The highest daily rate of capture for *N. viridescens* was 0.25 on 06/17/2024 (Figure 7). The highest daily rate of capture for *P. ruber was* 0.28 on 10/21/2025 (Figure 8).

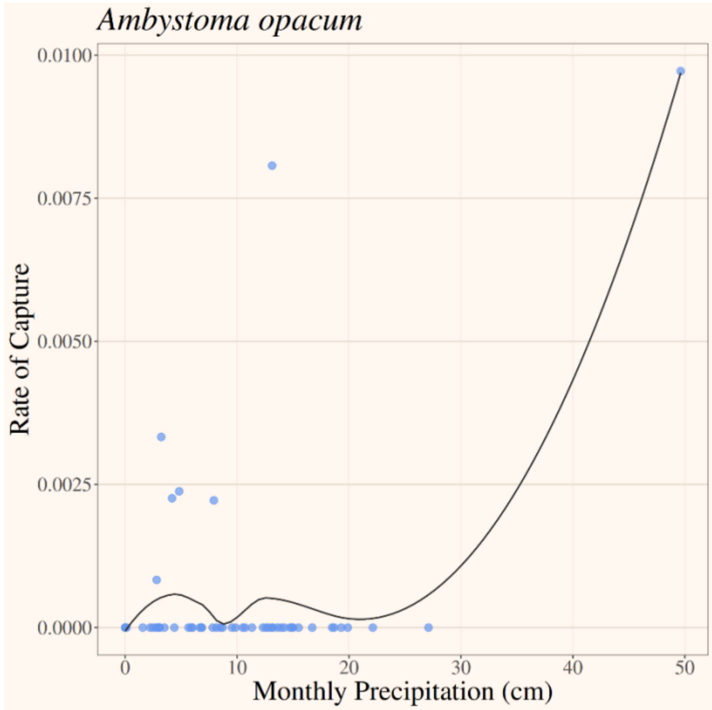

Figure 2. Monthly mean rate of capture for *Ambystoma opacum* plotted against total monthly precipitation (cm) from August 2021 to December 2025. Each point represents one month.

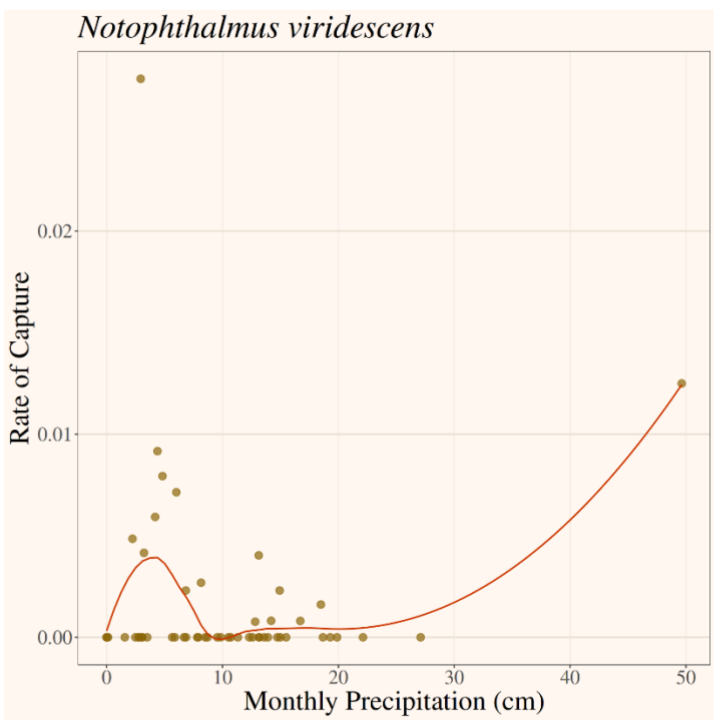

Figure 3. Monthly mean rate of capture for *Notophthalmus viridescens* plotted against total monthly precipitation (cm) from August 2021 to December 2025. Each point represents one month.

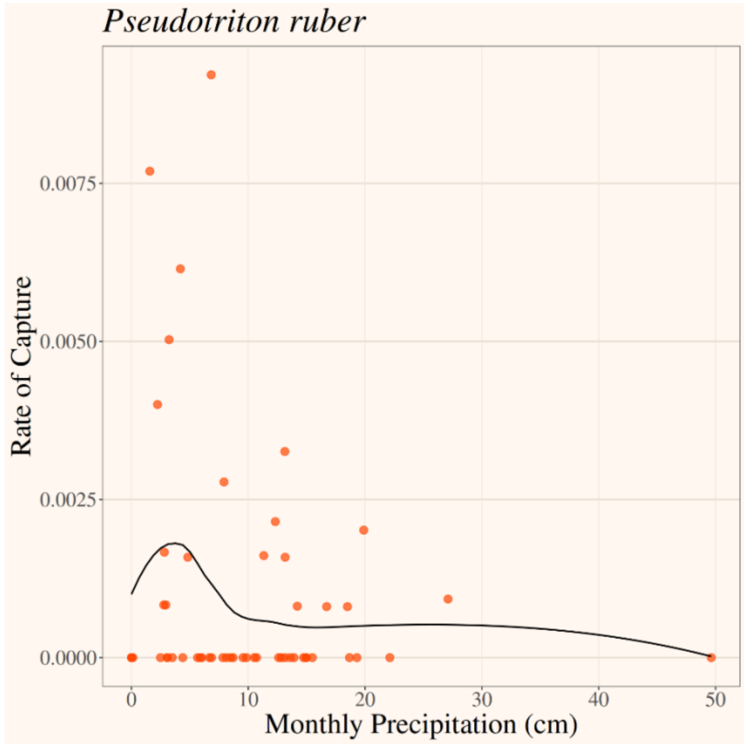

Figure 4. Monthly mean rate of capture for *Pseudotriton ruber* plotted against total monthly precipitation (cm) from August 2021 to December 2025. Each point represents one month.

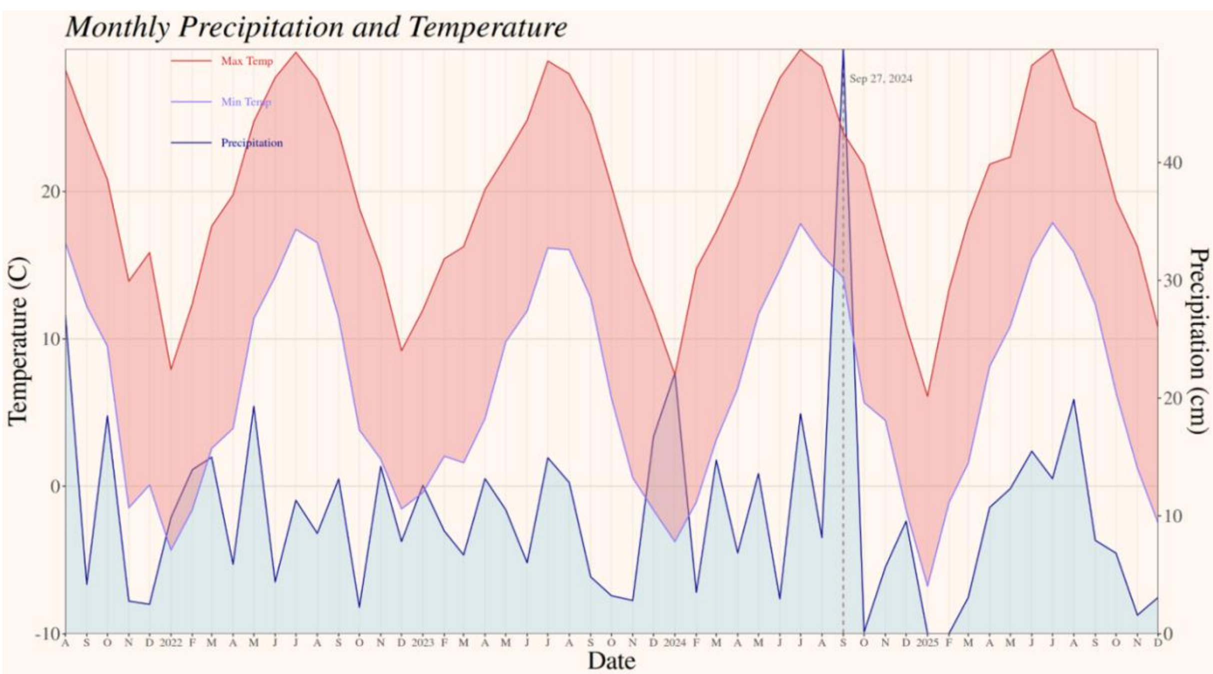

Figure 5. Monthly maximum and minimum temperature make up the upper and lower bounds of red ribbon. Cumulative rainfall plotted in blue. The dotted line indicates September 27, 2024, the date of flooding.

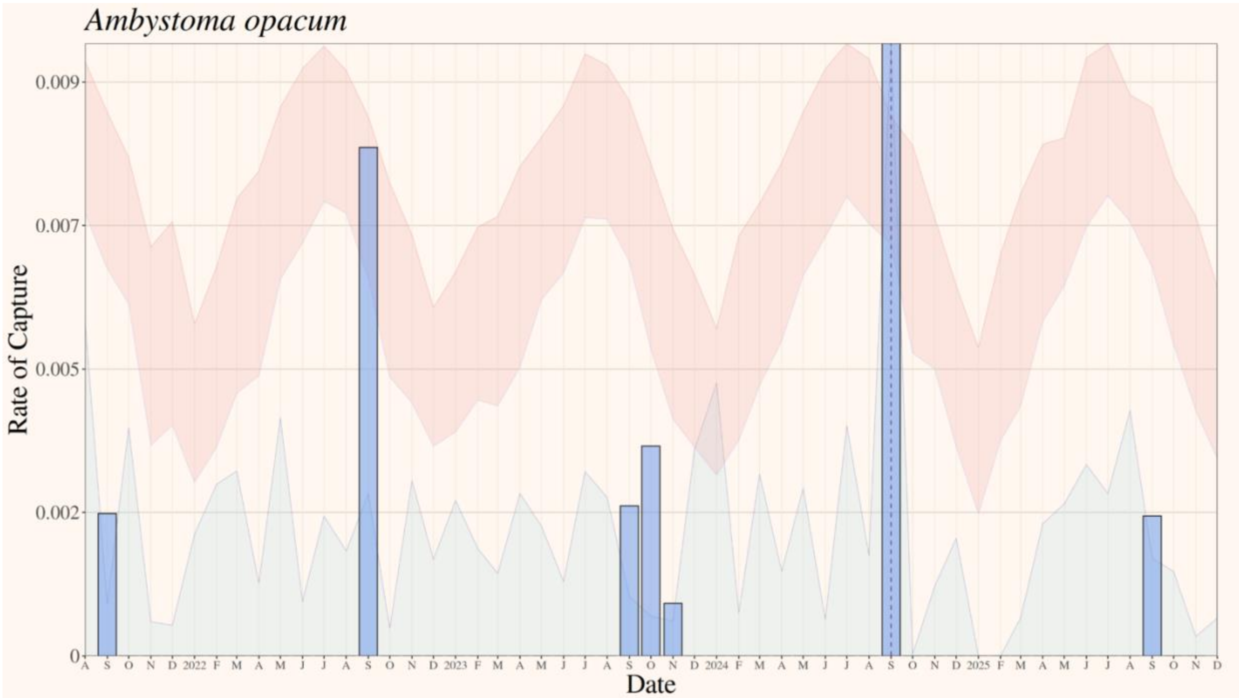

Figure 6. Monthly mean rates of capture for *Ambystoma opacum* with the temperature and precipitation data from Figure 5 in the background. The dotted line indicates September 27, 2024, the date of flooding.

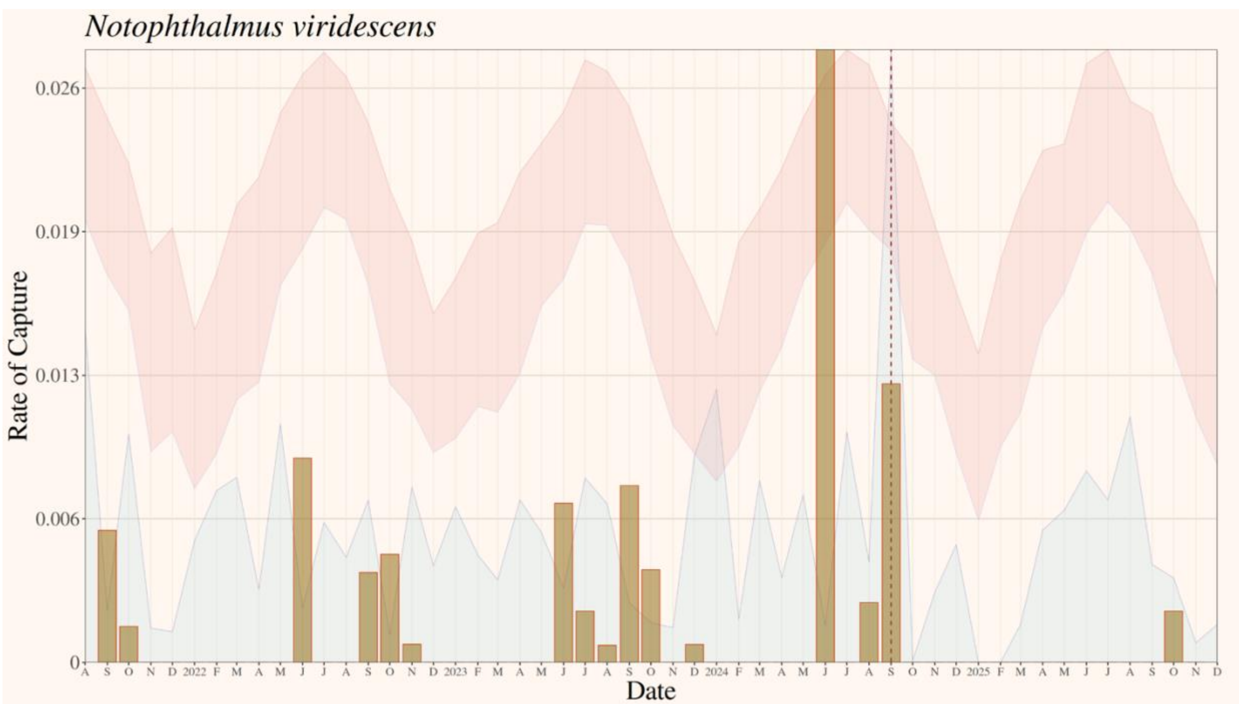

Figure 7. Monthly mean rates of capture for *Notophthalmus viridescens* with the temperature and precipitation data from Figure 5 in the background. The dotted line indicates September 27, 2024, the date of flooding.

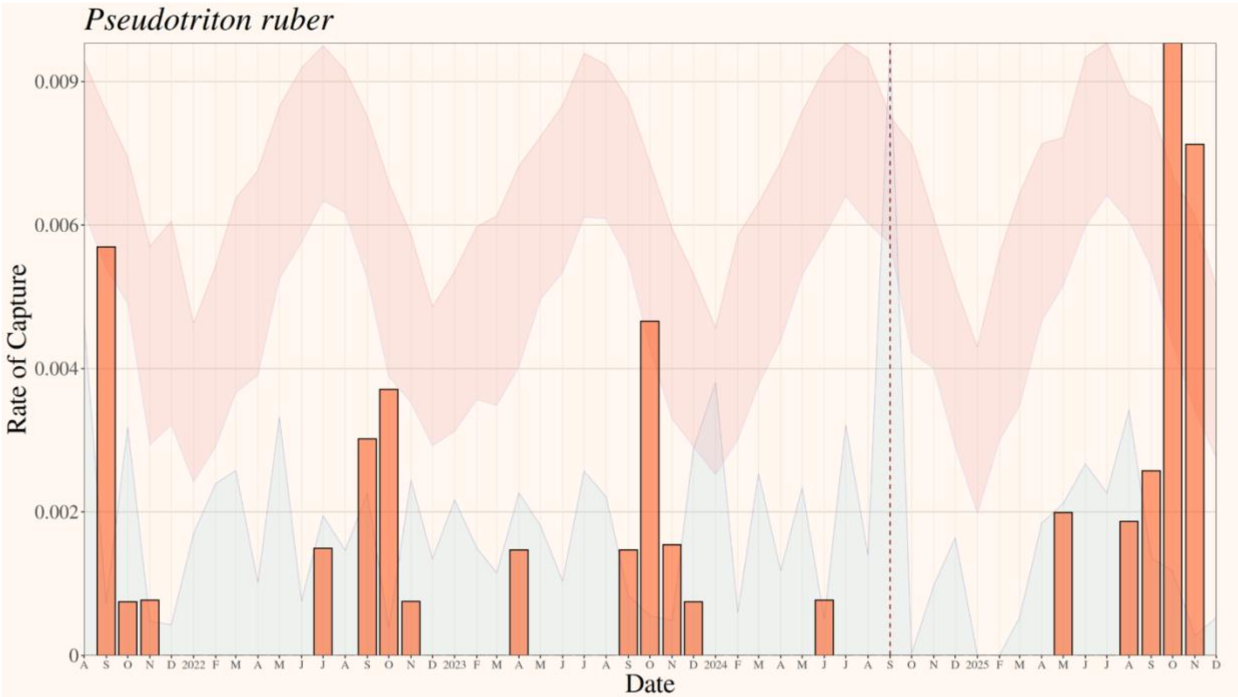

Figure 8. Monthly mean rates of capture for *Pseudotriton ruber* with the temperature and precipitation data from Figure 5 in the background. The dotted line indicates September 27, 2024, the date of flooding.

Discussion

Although it is widely documented that salamanders migrate toward ponds during evening rains (DeLisle and Grayson 2011, Brooks et al. 2019, Sexton et al. 1990, McLeod 2017), the data in this study were not able to distinguish such a pattern. The lack of relationship found by Spearman tests could also be due to the relationship between weather variables, specifically precipitation, and salamander abundance being non-linear. Brooks et al. (2019) suggests that there is a sort of "Goldilocks" zone of conditions that trigger movement in *Ambystoma bishopi,* with the peak they found to be around 43mm of precipitation. The patterns seen in figures two through four are consistent with this Goldilocks zone, showing that rainfall around 40-50mm was associated with a peak in salamander abundance for all three species.

Further, coverboard sampling occurred weekly or bi-weekly, and salamander abundance measured this way may have a loose association with specific weather events. Daily sampling of drift fences may be able to represent a more accurate count of the daily number of individuals migrating and therefore show a greater link between specific rainfall events and salamander abundance. McLeod (2017) shows that coverboards yielded a higher species richness than drift fences, but a lower overall number of individuals.

In addition to the patterns found connecting rainfall to abundance, these data reveal further temporal patterns in abundance. Both *A. opacum* and *N. viridescens* spiked during the year of, but prior to, the tropical storm. The largest numbers of *A. opacum* were found two weeks before the weather event and *N. viridescens* in the preceding June. While the endogenous mechanisms that allow salamanders to coordinate the timing of migratory behavior with suitable weather conditions are not fully understood (Grace 2003, Brooks 2019), it seems unlikely that there were any environmental cues of a storm available that far in advance.

Fall 2022 and Fall 2024 were standout high years for *A. opacum*. This may suggest that *A. opacum* breeds on a biannual cycle, with exceedingly high numbers migrating in alternate years. Consistent with this explanation, *A. opacum* has been documented to have some individuals skip a year of breeding (Gamble et al. 2009). In the population located at Sandy Bottom Preserve, this behavior could produce a biannual breeding cycle. Abundance of *A. opacum* in Fall 2025 was low, but consistent with the low years between biannual spikes in rate of capture. This could suggest that the population has not been severely damaged by the flooding event and is merely a natural dip in the biannual breeding cycle. The validity of this suggestion will be unknown until more Fall data are collected for *A. opacum* in even numbered years and we see the continuation (or lack thereof) of a pattern of high rates of capture. The low abundance of *A. opacum* in 2025 can also possibly be explained by the interaction of observed breeding cycles with the major flooding event in 2024. If 2024 was a year in which an abundant species on the site had an infrequent and massive migration event, large numbers of them would have been killed or unable to breed and previously laid eggs may have been washed away, having a devastating effect on the population. Male *Ambystoma* have been shown to migrate earlier than females (Sexton et al. 1990) and females have been shown to stay at the site to guard eggs beyond the initial breeding events (Croshaw and Scott 2005). As the flooding event happened late into the breeding season of *A. opacum*, not only could it have disproportionately affected female *A. opacum* but also washed away females guarding egg masses. The loss of adult individuals of either sex would affect the number of individuals returning to the pond in subsequent years, hampering breeding success (Gamble et al. 2009), and the washing away of eggs would have also impacted the number of embryonic *A. opacum* that were able to complete their life cycle.

In 2024, *N. viridescens* were found under coverboards in the highest numbers throughout the study period. A pattern similar to that of the possible biannual cycle of *A. opacum* could explain the extremely high number of *N. viridescens* in Fall 2024; however, the temporal scale of their migratory cycle is unknown and may be even larger. With sampling gaps during the COVID-19 pandemic, and inconsistent data collection prior to the pandemic, a conclusion is unable to be drawn as to what the duration of this cycle may be. As illustrated by the abundance data, *N. viridescens* has been abundant at the site during the Fall season. Not only did the flooding happen in the fall, but in the year that an incredible number of individuals were found under coverboards.

Decline in *N. viridescens* can be further illustrated by Collazo's (2025) study assessing behavior of *N. viridescens* at Sandy Bottom Preserve. Data collection for his study took place in the summer of 2024, and only seven individuals of *N. viridescens* were found for the study (Collazo 2025). The small number of newts found using minnow traps in a time when no newts were found under coverboards may be representative of the overall decline seen in 2025 for *N. viridescens*.

The data indicate that *P. ruber* are found in Sandy Bottom Preserve's terrestrial habitat in the fall. Bruce (1978) reported them in their terrestrial habitats near breeding sites from early spring to late summer, but in his review of *P. ruber* ecology, Petranka (1998) described their breeding season as varying geographically and taking place within streams and seeps that feed into ponds like the one at Sandy Bottom Preserve. In *A. opacum* and *N. viridescens*' abundant 2024, *P. ruber* may have been intentionally migrating in lower numbers in the month of September, as salamanders have been shown to sit out breeding years when conditions are not optimal (Gamble et al. 2009). Salamanders that would have been present after the month of September would have been excluded due to the gap in sampling in 2024. In contrast to the 2024 spikes of *A. opacum* and *N. viridescens* abundance, *P. ruber* had its lowest abundance of the study in the fall of 2024. In contrast, *P. ruber* had an extremely abundant 2025, whereas *A. opacum* and *N. viridescens* had a significantly lower rate of capture. The data suggest that before 2025, *P. ruber* generally had a steady annual abundance, with no year pairs being significantly different from each other except for 2025 against 2024 and 2021 (2025 being higher). *P. ruber* was largely not seen under coverboards in 2024 and may have mostly avoided the flooding event. Seeing as *P. ruber* generally uses streams and seeps as their primary habitat (Petranka 1998), the individuals found around the pond may be a small portion of the individuals that actually reside within the area around and including Sandy Bottom Preserve. If this is the case, a large number of *P. ruber* may have avoided the flooding event entirely by being uphill around the streams that feed the pond. This would allow *P. ruber* to possibly find greater habitat availability in and around the pond, more *P. ruber* individuals moving down from their typical habitat than usual, and to be found in higher abundance around the pond in 2025 as represented in the dataset. Again, literature on the competition between *P. ruber* and the other two species is limited, so this is speculative.

Patterns in abundance across species, namely a rise in abundance of *P. ruber* coincident with declines in *A. opacum* and *N. viridescens*, could be explained by interspecific competition between the three species in conjunction with the impact of the flooding on each species as they each share a habitat under normal weather conditions. Interactions between salamanders have been shown to be influenced by seasonal habitat use. Specifically, Anderson et al. (2015) found that *Ambystoma annulatum* larvae compete with *Ambystoma maculatum* larvae in shared breeding sites. In the case of Sandy Bottom Preserve, *A. opacum* can be used as a fall breeding analog to *A. annulatum*, and *N. viridescens* can be used as a winter breeding analog to *A. maculatum*. The unique nature of Sandy Bottom Preserve has *A. opacum* and *P. ruber* adults sharing the habitat at a similar time, possibly introducing competition between the two. However, due to this unique nature, literature on such interactions is lacking, possibly because they rarely share habitats as they do at Sandy Bottom Preserve.

Performing data analysis in the spring of 2026 comes with limitations on the ability to draw conclusions about the effects of Tropical Storm Helene. Timescales of salamander habitat use are so large, and the flooding event happened so recently that it makes it difficult to draw conclusions from the data. Data will be collected for this season and beyond, and years down the line more conclusions may be drawn. Future work with these data should include performing more statistical tests on the relationship between the weather data and abundance data, as the Spearman tests performed are limited to linear relationships. Plotting the data provides insights into interesting relationships that would have their validity strengthened by further statistical testing.

It would be beneficial for future student projects to assess a wider range of species, specifically those that breed in the spring or summer. Species that have been historically found under coverboards in the fall were chosen to be analyzed due to the sampling gap after Tropical Storm Helene. Hopefully, there are no more catastrophic events that cause large gaps in sampling. With more opportunities for sampling, data can be analyzed across a larger variety of species and with more distance from Helene, allowing for more room to draw conclusions based on analysis.

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