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Reptile and Amphibian Monitoring at Sandy Bottom Preserve

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Abstract

The Southern Appalachian Mountains are among the most biodiverse temperate regions, particularly with regard to salamander diversity. However, many species in this region are endangered, threatened, or of special concern. Habitat loss, disease, and potentially climate change are major contributors to population declines of herpetofaunal species. Sandy Bottom Preserve is a wetland complex in the French Broad River floodplain near Asheville, North Carolina. A survey of reptile and amphibian populations at this site has not been taken since 2004, therefore the objective of this research is to establish a current herpetofauna inventory. This sensitive habitat is a known location for North Carolina Species of Special Concern including mole salamanders (*Ambystoma talpoideum*) and four-toed salamanders (*Hemidactylum scutatum*), and it serves as a breeding site for many other amphibians. There are also historical records of bog turtles (*Glyptemys muhlenbergii*), a federally threatened species. North Carolina Wildlife Resource Commission personnel have conveyed concern regarding the potential widening of Highway 191, which separates Sandy Bottom from the French Broad River. The development of this road could impact local herpetofauna populations. Therefore, establishment of an updated, systematically obtained record of diversity is critical before construction is proposed. Methods of data collection include the use of drift fences with pitfall traps and coverboards, both common and effective tools used in herpetofaunal monitoring. The monitoring schedule is largely dependent on weather conditions, with pitfall traps opened and checked every 24 hours during rain events and coverboards checked a minimum of six times per month. The study period spanned from September 2016 to March 2017. This research provides an updated inventory of herpetofauna in this ecosystem for the monitoring of rare or threatened species and those at the edge of their known ranges.

Keywords: Salamander, Southern Appalachia, Conservation

1. Introduction

The Southern Appalachian Mountains of eastern North America are among the world's most biodiverse temperate regions. This is largely due to the tremendous topographic heterogeneity, which creates microclimates and habitats for both boreal and subtropical species often at the boundaries of their ranges. The region is home to 58 species of reptiles and 76 amphibians, 55 of which are salamanders, and 21 of those salamanders are endemic species[1,2]. In Southern Appalachia, salamanders are often the most abundant vertebrates in forested ecosystems [3,4].

Reptiles and amphibians (collectively known as herpetofauna) are vital components to their ecosystems as predators, prey, controllers of decay rates and nutrient cycling including the transport of nutrients between aquatic and terrestrial habitats, and as bioindicators [3–7]. Both taxa have experienced major population decline globally over the last couple decades[8].

Amphibian populations are declining more rapidly than any other major vertebrate lineage [5,9]. Of the over 6,300 species identified globally [5], more than one-third are threatened with extinction and the number of critically endangered species worldwide is nearly double what it was in the 1980s [9]. In the southeastern United States, the leading causes of amphibian decline are the effects of urbanization (habitat loss, fragmentation, road mortality), chytridiomycosis (chytrid fungus), and potentially climate change [9].

Conversion of habitat for agriculture as well as urbanization place pressure on amphibian populations because many species rely on wetlands and ephemeral ponds to breed and will often return breed in their natal pond. Amphibians' low vagility and high habitat specificity limit dispersion, making small/isolated populations particularly vulnerable. Roadways, especially through wetlands, cause high mortality rates in reptiles and amphibians [10]. Roads create barriers, disrupting ecosystem structure and function, population demography and dynamics, and even sex ratios by obstructing migration [10,11]. Reptiles and amphibians are also often attracted to warm road surfaces for thermoregulation [10]. Models indicate that an annual mortality of as low as five percent would contribute to population decline. Aresco (2005)[11] found 100% mortality of 343 turtle crossings over a 40-day observation. The probability analysis resulted in 98% mortality rate with a 30% decrease in survival probability after roadway expansion.

Chytridiomycosis is a disease caused by the chytrid fungus (*Batrachochytrium dendrobatidis).* The fungus infects the skin of amphibians, impairing osmoregulation and resulting in electrolyte depletion. Some populations experience 100% mortality, while others seem to be more resistant. The first extinctions caused by the disease were observed in the 1990s in Australia, extinguishing the two species of gastric brooding frogs (*Rheobatrachus*) and in Costa Rica, exterminating the golden toad (*Bufo periglenes*) and Harlequin Frog (*Atelopus varius*). Chytrid fungus now affects populations worldwide and is considered a major driver in amphibian population declines [5,12]. A study from 1999-2006 sampled over 1,200 amphibians of 10 species from 30 sites in six southeastern states. They observed 17.8% prevalence of chytrid fungus with greater frequency of infection in anurans (39.2%) than caudates (5.5%) [13]. A second strain of the disease (*Batrachochytrium salamandrivorans sp. nov.*) has been identified on fire salamanders (*Salamandra salamandra*) with unclear implications for amphibian populations [14].

Increasing severity of environmental conditions due to climate change poses a potential threat to amphibians. The extensive microclimates in the southern Appalachian region wherein many species occupy high elevation habitat, possibly at their thermal maximum, make populations particularly susceptible to climatic shifts [15]. There are two large families of salamanders in the Southern Appalachians: Plethodontidae, which are forest dwelling and comprise the largest family of salamanders[16], and Ambystomatidae, which breed aquatically and are among the top four most rapidly declining amphibian families globally [9]. Significant declines in suitable habitat for plethodontid salamanders in the Southern Appalachians were projected for as early as 2020[15]. Climatic changes such as shifts in precipitation patterns could have substantial impacts on ambystomatid populations as these animals breed in vernal/ephemeral pools. Ephemeral pools are temporary, fishless bodies of water that fill in the spring and dry out in the fall. Aquatic breeding amphibians' phenology is aligned with the fill regime of these pools and changes precipitation patters could impact reproductive success. The monitoring of these populations is important for further research and focusing conservation efforts.

Sandy Bottom Preserve is a UNC- Asheville owned wetland complex in the French Broad River floodplain just south of Asheville in Western North Carolina (35.494°N -82.591°W). The sensitive habitat is a known location for mole salamanders (*Ambystoma talpoideum),* and four-toed salamanders (*Hemidactylum scutatum*), both of which are North Carolina Species of Special Concern, and there are historical records for the presence of bog turtles (*Glyptemys muhlenbergii),* a state and federally threatened species[17]. Sandy Bottom is a unique location because it has both forested habitat accommodating woodland species, and the vernal pools required by aquatic breeding species.

North Carolina Wildlife Resource Commission personnel have conveyed concern regarding the potential widening of Highway 191, a two-lane road that experiences heavy traffic and separates Sandy Bottom from the French Broad River[18]. The development of this road could impact local herpetofauna populations; therefore, establishment of an updated, systematically obtained record of diversity is critical before construction is proposed. This research represents the first year of a long-term reptile and amphibian monitoring survey at the site.

Survey methods included drift fences with pitfall traps and coverboards, both effective tools commonly used in ecological monitoring. Drift fences are often used to quantify species diversity[19,20], abundance[21], and breeding patterns[22–26], as well as to determine the potential impact of hazards such as roads [11,27]. The use of coverboards is an efficient way to monitor herpetofauna, as the animals will use boards as refugia [28], providing means to quantify abundance and diversity through passive capture [29].

The objective of this study was to establish a quantitative data log of herpetofauna at Sandy Bottom Preserve. This will enable the monitoring of species of concern (*Ambystoma talpoideum* and *Hemidactylum scutatum*) and those species at the edge of their known ranges such as king snakes (*Lampropeltis spp*) or representing disjunct populations (*Ambystoma opacum*). In doing so, we hope to gain a better understanding of the migratory patterns of amphibians such as marbled salamanders (*A. opacum),* spotted salamanders *(A. maculatum)*, mole salamanders (*A. talpoideum*), eastern newts *(Notophthalmus viridescens),* and wood frogs (*Lithobates sylvaticus)*, which breed in the ephemeral pond. The data will also provide insight into the impact that widening Highway 191 would have on herpetofauna populations at Sandy Bottom. The anticipated results are a current catalog of the species present at this site as well as a clearer understanding of the spatial distribution, movement, and breeding patterns of resident amphibian species in relation to seasonality, temperature, and precipitation.

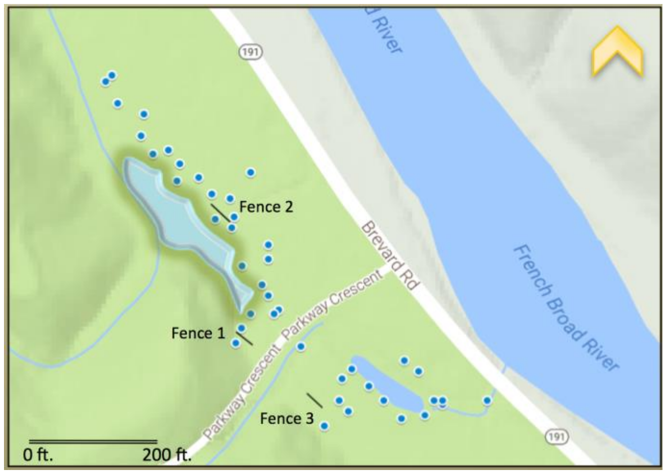
2. Methodology

The monitoring period spanned from September 2016-March 2017. Three drift fences with pitfall traps and 43 cover board arrays were established at the site in early August. Ambient conditions including air temperature and precipitation were recorded. The fences measured 8 meters long, extending above ground roughly 18 inches with the bottom edges buried roughly 6 inches deep. All fences were oriented along a north-south axis. Drift fences1and 2 were located on the north side of Parkway Crescent road, which divides the property. The first was installed at the south margin of the ephemeral pond, parallel with the base of an east-facing slope, and the second to the north and on the opposite side of the pond, nearer to Highway 191 and the French Broad. The juxtaposition of these two fences was intended to yield data regarding movement patters of migrating animals. Captures at fence 1 should reflect the composition of animals moving from the upland forest into the wetland to breed, and fence 2 that of those moving in from the direction of the highway. Fence 3 was installed on the south side of the wetland complex, and inline with fence 1 at the base of the slope. Conditions on this side of the property were visibly drier and a different species composition was expected (See map).

Each fence was equipped with 12 pit fall traps, one at either end labeled north and south, and five on each side labeled east and west 1-5. Each trap contained a moist sponge to prevent desiccation of trapped animals. Traps were covered with standing wooden tents to shelter the animals, and each cover was equipped with a twine escape rope for small mammals (designed by Lori Williams, NCWRC). Pitfall traps were checked every 24 hours during times of use by researchers and were closed during times of non-use. Captures were identified to species, photographed beside a ruler to later measure snout-vent length with ImageJ, location recorded (fence number and trap), and released on the opposite side of the fence from which they were collected.

A total of 43 arrays of untreated wooden coverboards were distributed randomly on either side of the trail running through the property (See map). Arrays were numbered in ascending order with 1-24 on the North side of the property and 25-40 on the south side. Each array included four boards, two larger (labeled A and B), and two smaller (labeled C and D). Coverboards were checked a minimum of six times per month, in varying weather conditions and times of day. Sampling protocol involved lifting boards to check for presence of animals. Encountered animals were identified, photographed, and location (array number and board letter) recorded. The board was returned to its original position and the animal released beside the board to go back under on its own.

Temporal distribution was graphed to illustrate species activity patterns throughout the study period. The spatial distribution of ambystomatid salamanders was analyzed using GPS points for trap locations and a Clark-Evans test was run to assess significance of clustering. The relationship between animal abundance (fence and board data combined) and air temperature, and animal abundance and precipitation were analyzed in RStudio using regression analyses. Individual regressions were run for the ambystomatid species (*Ambystoma opacum* separately and *A. maculatum*, and *A. talpoideum* combined due to overlapping breeding times) analyzing the relationships of abundance to temperature and precipitation. T-tests were run comparing species richness and abundance between the two trap types.



Map of Sandy Bottom Preserve

The blue dots represent coverboard arrays and the black lines represent the three drift fences. The approximate area of the ephemeral pond is shown in light blue with green margins.

3. Results

The majority of the data collected are representative of the Caudata taxon (salamanders). One American toad (*Anaxyrus americanus*), one green frog (*Lithobates clamitans*), two spring peepers (*Pseudacris crucifer*), one garter snake (*Thamnophis sirtalis*), and two worm snakes (*Carphophis amoenus amoenus*) were the only non-caudates found. Ten species of Caudata were captured. The total number of individuals representing each species encountered is recorded in Table 1. Adult eastern spotted newts (*Notophthalmus viridescens*) were the most abundant species with the greatest number of individuals encountered at both drift fences and coverboards. Marbled salamanders (*Ambystoma opacum*) were the second most abundant. The three ambystomatids were the only species more prevalent at fences than boards. There were no spotted salamanders (*A. maculatum*) found under coverboards and no dusky salamanders (*Desmognathus carolinensis*) captured at drift fences (Table 1). The *Plethodon metcalfi* may have been misidentified and are more likely *P. cylindraceus* without spots.

Noteworthy observations regarding species' temporal distribution were: the greatest abundance of *Ambystoma opacum* was in late September-October, with another peak in November. *Notophthalmus viridescens* were found in highest concentrations in October and practically not at all after January. *A. talpoideum* were observed November-March with abundance peaking in January (Fig. 1). *Ambystoma spp.* occurred throughout the site but were significantly clustered at the fences, particularly fence 1 (R=0.105, P=0.001) (Fig. 2).

Abundance of animals showed significant positive correlation with temperature (P=0.005, T=2.96, df=38) (Fig. 3) and marginally significant positive correlation with precipitation (P=0.056, T=1.97, df=38) (Fig. 4). There was no significant relationship between *Ambystoma opacum* (temp:P=0.332, precip:P=0.928) or *A. maculatum* and *A. talpoideum* (temp:P=0.186, precip:P=0.246) abundance and temperature or precipitation.

There was significantly greater species richness associated with coverboards than drift fences (P=0.003, T=2.99, df=99.04) (Fig. 5). Abundance of animals did not differ significantly between capture methods (P=0.883, t=0.148, df=33.62)(Fig. 6).

Table 1: Number of Caudata species encountered

Species	Drift Fences	Coverboards	Total
Ambystoma opacum	38	9	47
A. maculatum	10	0	10
A. talpoideum	13	6	19
Desmognathus carolinensis	0	10	10
Eurycea cirrigera	4	14	18
Pseudotriton ruber	0	11	11
Plethodon metcalfi	7	1	8
P. cylindraceus	0	3	3
Hemidactylium scutatum	2	3	5
Notophthalmus viridescens	25	77	102

Table 1 shows the number of individuals of each Caudata species encountered at drift fences, under coverboards, and in total.

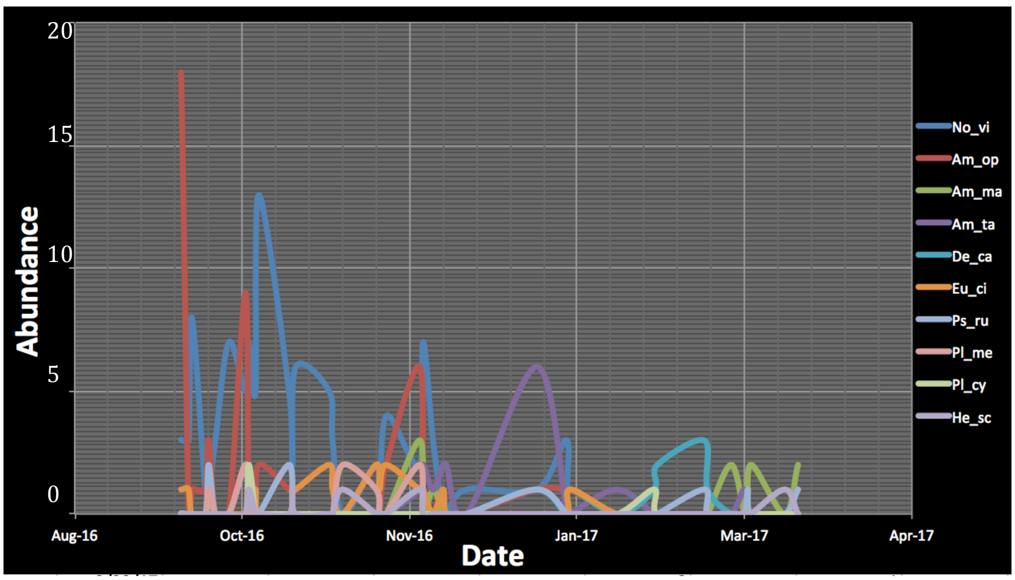


Figure 1: Temporal distribution of Caudata abundance

Figure 1 illustrates the temporal distribution the 10 Caudata species abundance over the study period. Data was combined for drift fences and coverboards.

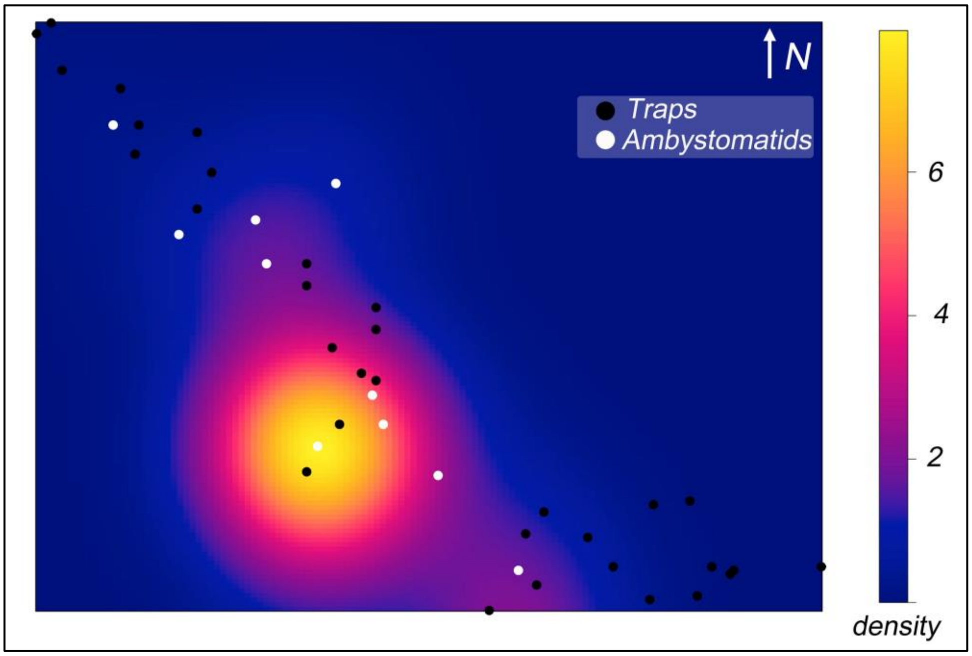


Figure 2: Spatial distribution and density of *Ambystoma spp.*

Figure 2 illustrates the distribution of *Ambystoma spp.* at Sandy Bottom. Black dots represent trap locations (compare with map) and white dots represent traps at which *Ambystoma spp.* were captured. The figure also shows the density of where animals occurred. Results based on 1,000 simulations of random distribution.

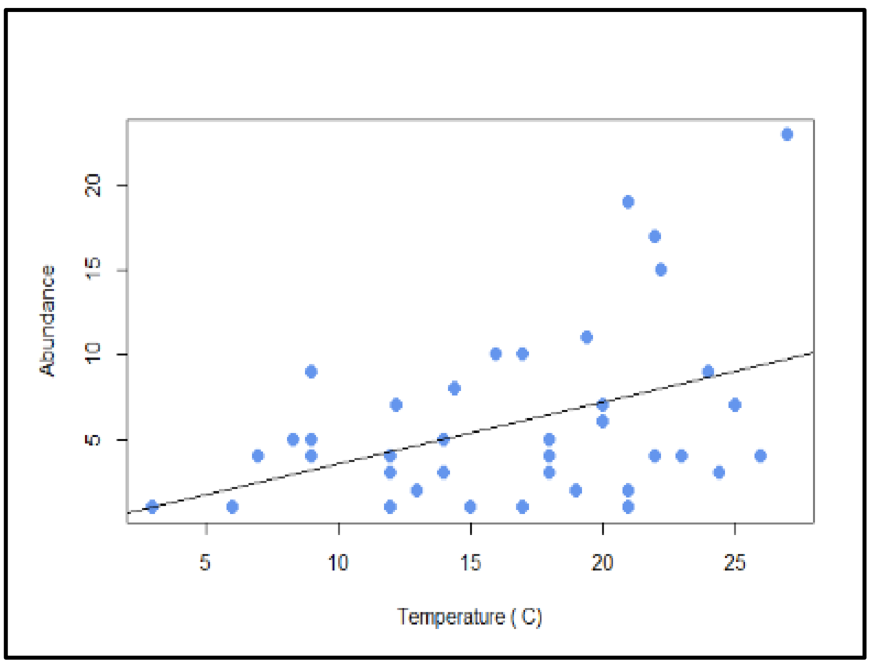


Figure 3: Relationship between animal abundance and air temperature

Figure 3 illustrates a significant positive correlation between animal abundance and air temperature (y=-0.14x+0.368). Dots represent days on which animals were found and the number of animals found that day is plotted on the y-axis. The air temperature for that day at the time of recording is plotted on the x-axis.

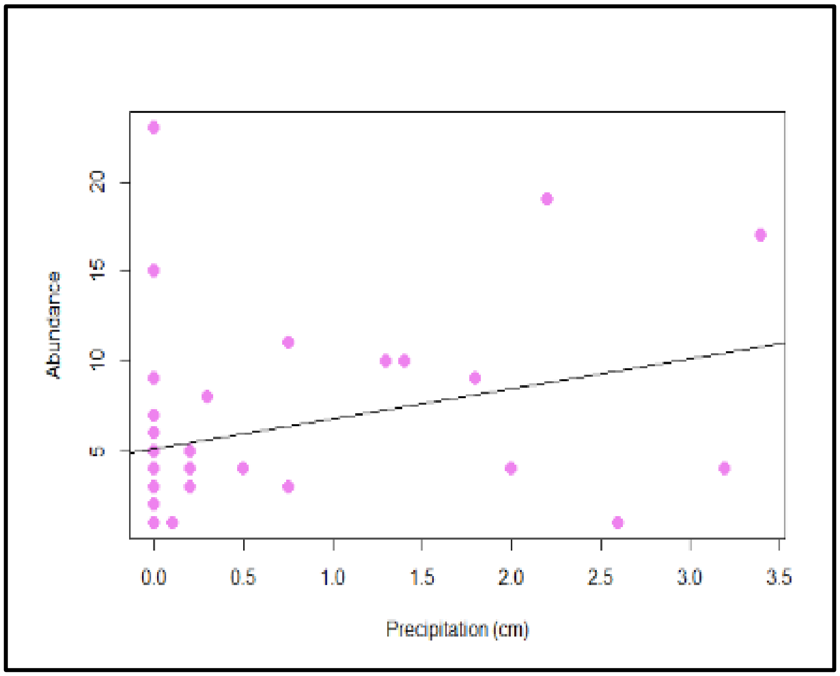


Figure 4: Relationship between animal abundance and precipitation

Figure 4 illustrates the marginally significant positive correlation between animal abundance and precipitation (y=5.13x+1.66). Dots represent days on which animals were found and the number of animals found that day is plotted on the y-axis. The amount of precipitation for that day is plotted on the x-axis.

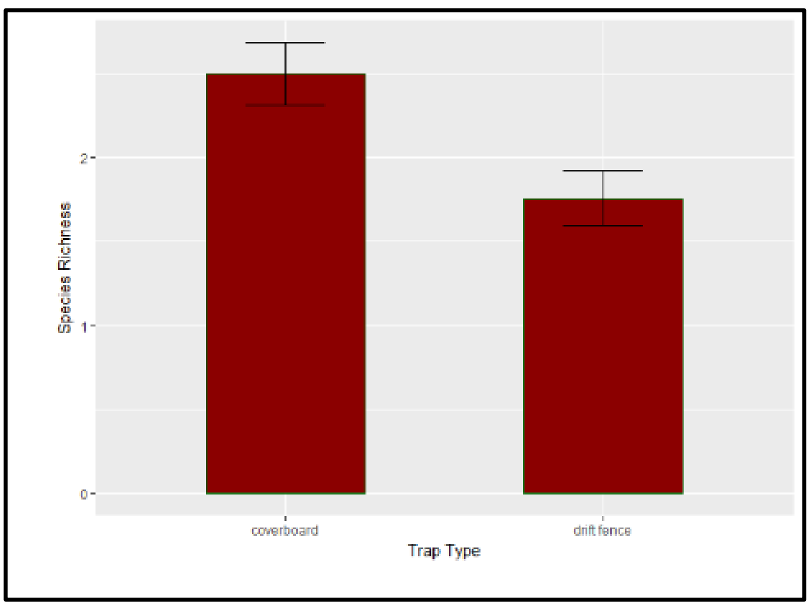


Figure 5: Comparison of species richness between two capture methods

Figure 5 illustrates the significant difference in species richness (the number of different species) associated with drift fences and coverboards. Data is displayed as mean number of species captured per day.

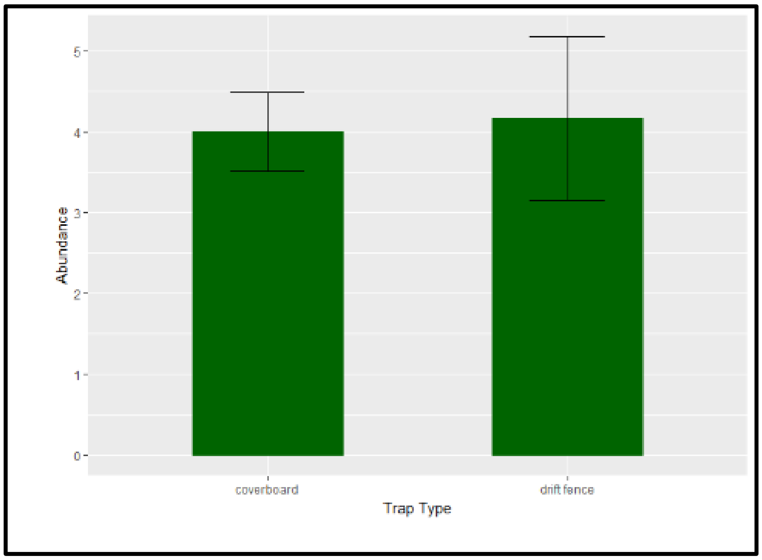


Figure 6: Comparison of animal abundance between two capture methods

Figure 6 illustrates the insignificant difference in abundance of individual animals at drift fences and coverboards. Data is displayed as mean number of animals captured per day.

4. Discussion

The data recorded during this period indicate a far greater abundance of Caudata species at Sandy Bottom than other amphibians or reptiles. These results likely are not an accurate representation of the herpetofauna composition as sampling methods may have been biased towards Caudata[30] and the sample set only includes half the year when reptiles are less active. The implementation of plastic or metal tin covers could possibly yield higher abundance results for reptiles[30], along with monitoring during summer months when conditions are warmer. Wood frogs (*Lithobates sylvaticus*) were observed breeding in the ephemeral pond in late January, but drift fences were not being run the week prior, which is likely why none were captured.

The species' temporal distribution is representative of their phenology. *Ambystoma opacum* breed and oviposit in dried down ephemeral ponds during September-November, which appears to be consistent with our results [16]. In this part of their range, *Notophthalmus viridescens* usually breed in the spring, but more generally from fall to early summer. The breeding period can last as long as six months. This explains the high abundance observed in October when the animals were migrating into the wetland, and tapering off in January because adults were in the pond breeding [16]. Research at the Savanna River Site in South Carolina supports our results in observing that *A. talpoideum* immigrate from September-March, with arrival peaking from November-January[16]. Shoop (1960)[31] found that most breeding occurs on cold nights with heavy rainfall and many females do not migrate to breed during years with low levels of precipitation. Though we did observe *A. talpoideum* migrating into the pond, recording of individual's sex was not consistent. Therefore, considering the extremely dry conditions experienced this year[32], it is possible that a disproportionate number of males migrated than females. Continued data collection should be careful to record sex for future ratio analyses.

The significant clustering of *Ambystoma spp.* is representative of the high abundance of these species at drift fences. Density was greatest at fence 1 on the southern margin of the ephemeral pond. Density at fence 2 may have been greater had the fence not been temporarily decommissioned from 11/6/2016 until 2/22/2017 due to reoccurring bear damage. This data illustrates that the ambystomatids were predominantly captured at fences because they migrate into the ephemeral pond to breed, and were mostly migrating in from the direction of fence 1.

It is important to note that the regression analyses do include the non-caudate animals encountered, but as they make up such a small portion of the sample set, the data is predominantly representative of Caudata. The significant positive correlation between abundance and air temperature was expected, as herpetofauna are ectothermic. A study by Sexton et al. (1990)[33] in Missouri observed that 98.3% of *A. maculatum* immigration occurred at or above 5.5°C. This is consistent with our results, as the lowest temperature at which we encountered *A. maculatum* was 9°C. One *A.*

talpoideum under a cover board was the only animal found when temperature was less than 5°C. Though our study did not take into account soil temperature, Sexton et al. (1990)[33] suggested a correlation between air and soil temperature in dictating salamander activity. They observed over 99% of animal movement when air temperature became warmer than soil temperature at 30cm below ground, thus reversing the thermal profile. The relationship between abundance and temperature would have been more accurately represented had temperature been recorded as a daily average rather than at varying times of day. This could provide more stable temperature data as well as account for overnight temperature, which likely also drive surface activity. Future research could use the National Oceanic and Atmospheric Administration (NOAA) regional average temperature data.

The marginally significant positive correlation between abundance and precipitation was expected, as amphibians skin needs to stay moist to prevent desiccation and allow for gas exchange [4]. Precipitation is often thought to play a greater role in initiating migration than temperature[33–35]. The more significant relationship found between abundance and temperature than abundance and precipitation is likely due to the extremely dry conditions this year. According to NOAA, during the fall of 2016, 36% of the southeastern U.S. experienced extreme and exceptional drought[32]. Though our data show a positive correlation between abundance and precipitation, a seemingly unusual amount of animals were found on days with zero centimeters of rainfall. Animals were likely forced to migrate by their phenology despite non-ideal conditions. The insignificance of temperature and precipitation on *Ambystoma* spp. abundance at drift fences is likely due to the small sample size.

The greater species richness associated with coverboards was likely because drift fences catch migrating animals while coverboards serve as refugia [28]. Drift fences showed bias towards the three ambystomatid species and newts, as these animals immigrate into the ephemeral pond to breed. This also explains the evenness of abundance between the two trap types, as fences caught a greater number of *Ambystoma* spp., but coverboards resulted in more individuals of different species. Coverboards proved a more effective method for monitoring species diversity while drift fences are valuable tools for targeting ambystomatids and gaining details on amphibian migration.

Suggestions for future work include the addition of aquatic sampling to gain a better understanding of population size and phenology of ephemeral pond breeding amphibians such as ambystomatid salamanders, and the implementation of tin covers for reptiles. A more thorough analysis of species' spatial distribution throughout the site could be done using the trap location data. It could be determined whether animals prefer smaller or larger coverboards, and directionality of movement could be analyzed by drift fence and pitfall trap location. Additionally, the snout-vent length recorded for each animal could be used to assess age demography. It has been suggested that the *Hemidactylum scutatum* at Sandy Bottom may be a genetically distinct population; therefore, genetic testing could be valuable. A couple of frogs were observed dead or dying at the site, and testing amphibians for chytrid fungus is another area for future work.

The data obtained through this study provides a better understanding of herpetofauna species composition, temporal and spatial distribution, and movement patterns in response to ambient conditions at Sandy Bottom. It also provides insight into the effectiveness of and type of data yielded by different capture methods. Furthermore, a detailed impact assessment for roadway expansion, possibly necessitating conservation measures, along with the monitoring of populations' response to changes in climate is imperative.

The establishment of this catalog will enable monitoring of populations' responses to continuing climatic change. This is particularly important for aquatic breeding species due to their dependence on the presence and flood regime of the ephemeral pond. Fluctuation of the water table in this habitat is highly sensitive to precipitation patterns. Periods of prolonged drought such as those experienced this year could have a negative impact on the reproductive success of aquatic breeding amphibians, including ambystomatid salamanders. There are few other locations in the area that can support ambystomatids, particularly so for *A. opacum* [16].

The monitoring of populations at this site is critical for consideration in the case of potential roadway expansion as well. An analysis of road mortality hotspots found that reptile and amphibian road-kill was greatest at sites associated with wetlands located within 100 meters of a road [10]. At Sandy Bottom, the coverboard array in closest proximity to Highway 191 is only 15m away and animals were found under this board during the study period. Additionally, continued monitoring at the site, though the data is not included in this paper, found relatively large quantities of *A. talpoideum* at drift fence 2 (approximately 90m from the road) as of April 2017. This data shows that *A. talpoideum* are emigrating from the ephemeral pool in the direction of the road. Immigration of this species may have been observed from the same direction had fence 2 not been temporarily decommissioned. As *A. talpoideum* are a NC Species of Special Concern, further observations may necessitate conservation efforts.

Human activity severely impacts herpetofauna populations both directly and indirectly. Mitigation of direct impact such as roadway expansion is possible through policy. In order to better understand the extent to which human activity indirectly impacts herpetofauna populations in terms of shifting climate and promoting the spread of disease, a long-term, systematically recorded data set is required. Therefore, the continued monitoring of herpetofauna at Sandy

Bottom is essential for focusing management and conservation efforts, as well as furthering ecological knowledge. Reptiles and amphibians are key ecosystem components and with populations under heavy decline, conservation of these animals and the sensitive habitat that supports them is critical. The biota of the Southern Appalachians merit monitoring and conservation to insure the persistence of the region as one of the world's temperate biodiversity hotspots.

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