University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2020

Eastern newt (*Notophthalmus viridescens)* Monitoring at Sandy Bottom Preserve

Abigail Lynn Snow
Environmental Studies
The University of North Carolina Asheville
One University Heights Asheville, North Carolina 28804 USA

Faculty Advisor: Landon Ward

Abstract

Vernal pools are the known breeding grounds for a number of amphibian species. These temporary pools of water are a destination for species' seasonal migration for mating purposes. This research established data tracking the migration patterns of Eastern newts (*Notophthalmus viridescens*) within one such vernal pool habitat. The area of focus was Sandy Bottom Preserve located near Asheville North Carolina within the French Broad River floodplain. Currently the Eastern newt is not listed as a species of concern. These newts however are predators and secondary consumers that maintain balance within the vernal pool and surrounding deciduous forest ecosystem. Data collection on amphibian migration at this site spans intermittently back to 2004 with no studies focused solely on the Eastern newt. Methods of data collection in this research included the use of dip nets, coverboards, and rain gauges, all common and effective tools in previous studies on this site and in other similar ecological monitoring studies. The Eastern newts migration patterns observed suggest female newts may enter this vernal pool later in the breeding season than the males. This study established an Eastern newt specific migration data record and speculated about the influence of environmental factors such as seasonality on their migration. The data collection period spanned from November 2019 until March 2020. Monitoring occurred weekly during this period and was heavily influenced by weather conditions. The results of this study did not indicate any significant trends outside of the skewed sex ratio of newts observed and the mean difference in length of the two sexes. This research contributed to an updated inventory of the general amphibian population and may act as an indicator of changes in the migration patterns of the Eastern newt population in Sandy Bottom.

1. Introduction

The Eastern newt (*Notophthalmus viridescens)* occupies a range spanning from Nova scotia west to the great lakes and south into Northwest South Carolina, Georgia, and Alabama. Their diet consists primarily of worms, insects, small crustaceans, amphibian eggs and larvae[1]. The Southern Appalachian Mountains of western North Carolina they inhabit are a vastly heterogenetic topographic area that contains many microclimates. This topographic heterogeneity creates a variety of climatic areas co-occupied by Caudata that are both predators and prey to the Eastern newt. This newt is often the most abundant vertebrate in the North Carolina highland forested ecosystems[5]. Eastern newts are known to have aquatic larval development and mating stages[3]. These alternating life stages situate Eastern newts as a constituent of ephemeral wetland pools, maintaining prey populations along with influencing decay rates and nutrient cycling between terrestrial and aquatic substrates. They, along with other amphibians, are often bioindicators of environmental health[18]. The Caudata taxon in which *N. viridescens* belongs to has experienced a noticeable population decline in recent decades due to a variety of environmental factors[18].This taxon exhibits a decline trend that is more rapid than all other taxon on earth[2]. Over one third of the 6,300 known amphibian species are threatened with extinction[13]. The leading hypothesized causes of this decline in the southeastern United States includes climate altering urbanization (habitat loss, fragmentation, road mortality), and climate change[13]. Eastern newts are not listed as a species of concern currently[12]. Some of their prey species (ex: mole salamander larvae) are however dwindling rapidly in response to the aforementioned environmental factors.

There has been extensive research into the contemporary decline of amphibian populations globally[2]. Drier summer conditions due to climate change are considered one of the most probable reasons for this decline[2]. Drier patterns are of special concern to amphibians' high permeable integument and vernal pool habitat which are dependent on regular moist conditions to exist[4]. The sex ratio and mating success of *N. viridescens* is in part controlled by the availability of refuge edge habitat for females away from behaviorally aggressive males[5]. In consistent dry conditions, the availability of refuge edge habitat in vernal pools and population interactions of *N. viridescens* have been shown to alter significantly[11]. Some research points to female newts alternating or avoiding breeding years if the aquatic environment is assessed as unfit spatially for larval growth[19]. The immediate shore of these vernal pools has also been shown to be of importance in *N.viridescens* breeding habits, with most preferring open grassy areas edged by moderate tree cover[6]. The effect of deforestation on habitat suitability is thought to heavily influence Eastern newts current and future population numbers[3].

Urbanization is also known to contribute to the diversion of water flow from the hydrophilic soils of vernal pools[5]. The redistribution of this water results in declining periods of vernal pool temporal existence and limits habitat size and breeding periods for *N. viridescens*[5]. This variability in vernal pool existence further stresses *N. viridescens* populations and may be an indication to larger count of males per population[7]. High traffic roads are known to disrupt the migration patterns of *N. viridescens*. This migration disruption further skews the sex ratio of available mates in the ephemeral pools and increases female stress[5]. It is supposed that predation rates increase with dry conditions, especially for the juvenile red eft during migration out of forested areas[11]. Despite *N. viridescens* non-concern listing, this amphibian's range is still limited by a low vagility and high habitat specificity requirements[17]. Urbanization and its climate altering effects further isolate populations and disrupt ecosystem dynamics.

Vernal pools can be defined as temporary fishless bodies of water that fill and dry seasonally with winter precipitation rehydrating the soil[4]. *N. viridescens* is one among many aquatic amphibians whose phenology follow the fill-drain cycles of vernal pools to ensure breeding success. Variances to this cycle have been shown to negatively impact these populations breeding success[4]. The observation of amphibian migration patterns is crucial for vernal pool conservation efforts and a better understanding of regular fill-drain cycles. Sandy Bottom Preserve is a wetland area in the French Broad River floodplain containing one such vernal pool. This land area is owned by UNC Asheville and located south of the city of Asheville in Western North Carolina. This delicate habitat is a known host of *N. viridescens* along with many other sensitive amphibian species[10]. This location is unique in that it contains both a forested and vernal pool habitat required by many amphibian species for successful breeding and juvenile life stages. This vernal pool is a known Eastern newt breeding site, with documentation in the 2017 Mcleod study suggesting *N. viridescens* as the most abundant amphibian population present. There have been no Eastern newt specific studies in Sandy Bottom, so few specific details are known about their migration patterns, mating habits, or reasons behind the absence of terrestrial juvenile red efts (Landon Ward, personal communication with Abigail Snow, 11/7/2019). The establishment of this study allowed for a focused *N. viridescens* Sandy Bottom population analysis. The foundation of detailed record on any and all herpetofauna is critical in understanding the fundamental implications of habitat change and pertinence of conservation throughout wetland ecosystems.

N. viridescens maintain a role in population regulation of other species and reflect the health of their entire habitat. This research served as a closer observation of *N. viridescens* migration patterns and their ecological role in the Sandy Bottom vernal pool habitat. Data collection methods included the utilization of dip nets, coverboards, and monitoring of three distributed rain gauges. Rainfall gauges are a routine tool utilized to quantify site specific precipitation totals[4]. These three methods are common and were cited as effective in similar ecological studies[12]. Dip nets are a standard and effective tool utilized to understand aquatic breeding patterns and location of *N. viridescens* within a vernal pool[12]. The effectiveness of dip netting however is highly influenced by the skill of the data collector. Coverboards are a typical passive data collection method[8].

This research considered previous studies to investigate Eastern newt migration patterns. The question of fluctuating dry and wet conditions' effect on Eastern newt populations is pressing facing climatic changes in the near future[12]. This study aimed to gain a clearer understanding of the habits of *N. viridescens* throughout their breeding season within the physical boundaries of an ephemeral pool. This study resulted in an updated Sandy Bottom Preserve Eastern newt population and weather conditions catalog and provided insight on the migration habits of this specific population.

2. Methodology

The monitoring period of this study extended from November 2019 to March 2020. Data collection materials included rainfall gauges, coverboards, and dip nets. Dip net sampling occurred for forty-five minutes on a weekly basis in the vernal pool at varying depths. Coverboards were pre-existing and observed weekly. Rain gauges were distributed in three separate locations throughout the wetland complex near pre-existing drift fences. GPS data was not collected but utilized from a previous study to accurately distribute rain gauge locations. Two gauges were placed on the Northern end of Parkway Crescent, the road dividing the property. One gauge was located at the Southern boundary of the vernal pool parallel to an Eastern-facing slope, with the second located north and opposite of the pond, closer to highway 191. The third gauge was placed South of Parkway Crescent and in line with the placement of gauge one at the base of the Eastern facing slope. The differing locations of these rain gauges was intended to observe if precipitation totals within the wetland complex varied significantly. These gauges were monitored weekly and data was recorded utilizing in field paper copies along with Microsoft Excel. Previous research noted drier conditions at the position of rain gauge three and it was expected that precipitation totals here would be less. Weekly ambient temperatures were also recorded at the time of data collection.

Two nylon dip nets with four to eight-foot handles were utilized throughout the study and time spent using the dip net was quantified. The inclusion of these nets allowed for aquatic data collection and observations of Eastern newt mating and migration patterns. This method involves the sampler extending their arms as far as possible and submerging the net entirely in the water. A raking motion in the direction of the sampler is completed until the net reaches the samplers feet. The net is then is taken out from underneath the water for observations to occur. Dip net samples were individually examined by hand for *N. viridescens*. If present Eastern newts were transferred to an individual container for later analysis. The remaining leaf litter and aquatic life were returned to the vernal pool by re-submerging and gently shaking the net. A typical dip net period lasted 45 minutes and sampled various depths within the pool.

Coverboards were observed before the 45-minute dip net sampling period began. This study utilized the coverboard set up described in Mcleod's 2017 herpetofauna diversity inquiry at Sandy Bottom. Of the original 43 arrays of coverboards in Mcleod's research, 25 were utilized in this assessment of newt migration patterns. This discrepancy occurred due to multiple arrays having decayed, been moved with weather events, or otherwise made unidentifiable. The 25 that were utilized consisted of four boards varying in size. Coverboard observation consisted of manual lifting of plywood coverboards, and then visually assessing if newts were present. If present, newts were collected, and all measurements mentioned in the previous paragraph were noted. The boards were returned to their original position with newts placed near the board to allow them to go underneath at a self-directed pace.

After the 45-minute period, sample specimens were weighed, observed, and measured on land. A pocket scale with two petri dishes were utilized to approximate the weight (g) of each newt. The utilization of this scale was not uniform throughout the study, resulting in multiple individuals having no associated weight recorded. Observed and noted were *N. viridescens* readiness for breeding (breeding tubercles and tales on males), sex, or signs that breeding had occurred (females swollen with eggs). Length (mm) was determined by placing each newt onto a metal ruler and observing the stretch from tail tip to jaw. Individuals were then returned to the water.

Data was translated from paper in field copies to an online spreadsheet and analyzed at the end of the data collection period. The relationship between newt position in the wetland complex and seasonality was examined in Microsoft Excel utilizing a T-test. Additional analysis focused on the sex ratio of newts in relationship to seasonality. The breeding readiness of each newt was also recorded. Individual regressions were completed to analyze the link between total newt mass and sex, along with newt length and sex. An inquiry into the hypothesized difference in the number of individuals captured per sampling session was completed utilizing a 95% CI G-test.

3. Results

Data in this study collectively represented an individual *N. viridescens* population exhibiting a skewed sex ratio. The majority were male (65/81) with a minority of females (16/81) found throughout the study period. All newts collected were encountered in the vernal pool, with most being located at the base of aquatic vegetation or feeding within amphibian egg masses. All newts sampled displayed signs of readiness for breeding (Table 1). Supplementary data from previous studies was utilized to assess seasonal migration patterns to and from the vernal pool. The specimens were in their adult phase and no juvenile red efts were observed during this study **(**Figure 2). There was no significant difference in the number of individuals captured per session as assessed utilizing a 95% CI G-test (95% CI=6.85$\pm$3.97) (Figure 1). Additionally, there was a visible trend of newt location and seasonality with newts being found terrestrially from June until December (Figure 2).

There was no significant difference of mean newt weight between the sexes (P=.58, F=.31, df=1) (Figure 3). There was however a significant difference in mean length between males and females (P=.01, F=6.79, df=1) (Figure4). There was no significant difference in mean female and male abundance throughout the late winter and early spring months according to a T-test assuming unequal variances (P two-tail=.18, df=3) (Figure 5). Noticeable is the almost complete absence of *N. viridescens* within the month of January, followed by a spike in observations during February 2020 (Figure 5).

Table 1. Number of *N. viridescens* observed with breeding structures present

Sex	# Individuals	Breeding Tubercles	Breeding Tail	Swollen with Eggs
M	65	65	65	N/A
F	16	N/A	N/A	16

Table 1 is representative of the total number of Eastern newts encountered throughout the study within the vernal pool and breeding structures presence. M represents the males, whereas F represents the females.

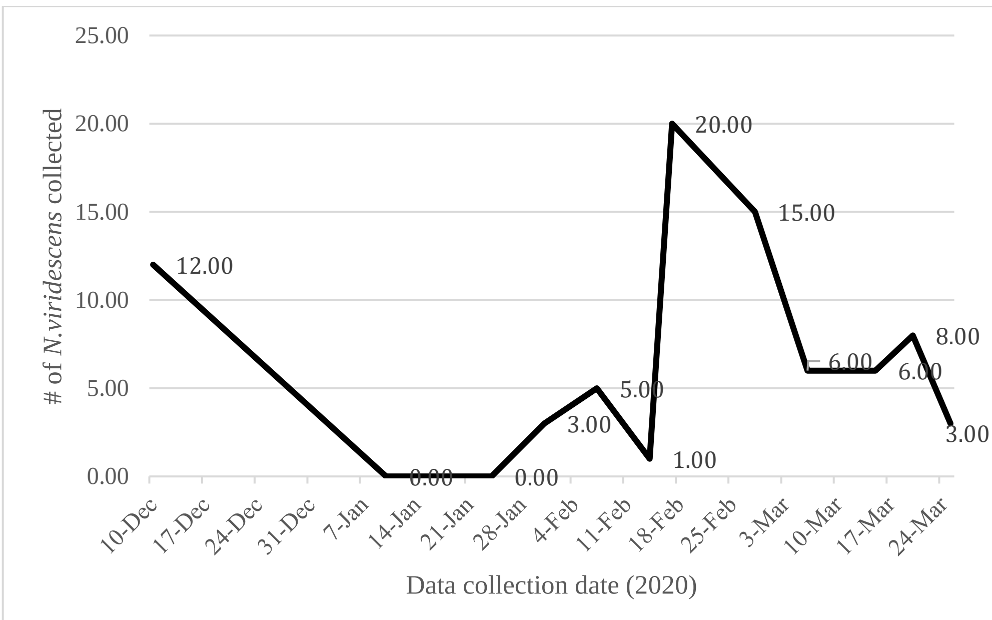

Figure 1. *N. viridescens* captures per sampling session

Figure 1 displays the number of captures of Eastern newts throughout the vernal pool per sampling session (95% CI=6.85$\pm$3.97). Sampling session dates are graphed on the x-axis with individual data points representing the number of newts collected. The y-axis is the number of individual newts captured per sample day.

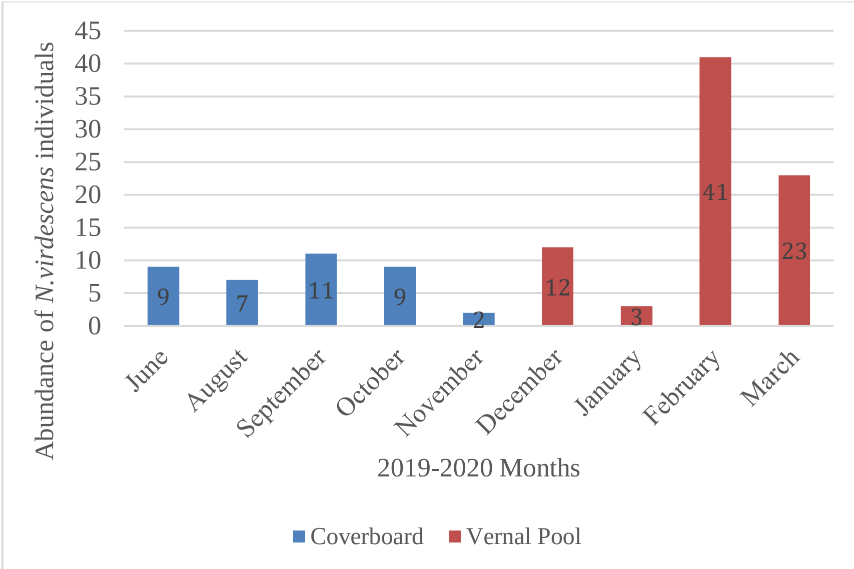

Figure 2. Total *N. viridescens* abundance and distribution in relation to time of year

Figure 2 features the temporal and spatial distribution of Eastern newts throughout the summer, fall, winter, and spring seasons. The x-axis is labeled with the months of sampling, with blue bars indicating newts found terrestrially and red bars indicating newts found within the vernal pool. The y-axis is the number of individuals found per month. Data displayed here is from all dip net collections and previous studies coverboard observations.

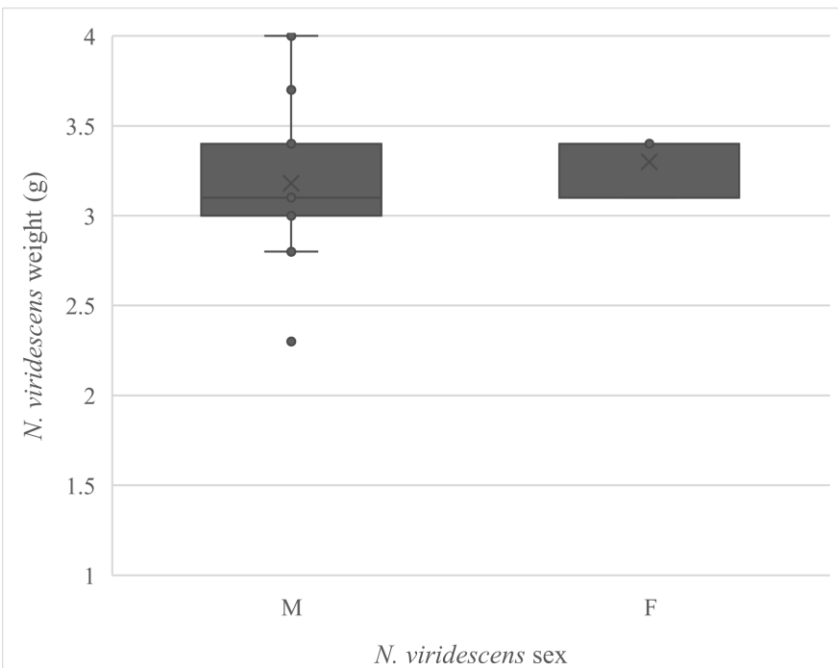

Figure 3. Female and male *N. viridescens* mass

Figure 3 presents the difference in mass between female and male Eastern newts in this study. Newt mass (g) is graphed on the y-axis. Data displayed on the x-axis accounts for the number of individuals of each sex weighed throughout the study period and is not representative of the entire data set.

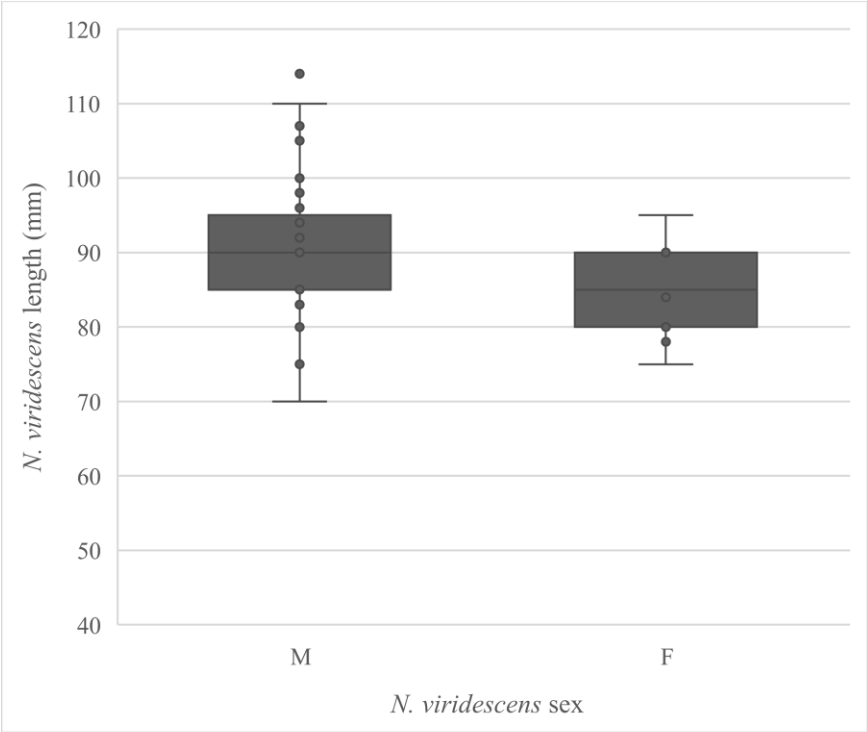

Figure 4. Female and male *N. viridescens* length

Figure 4 represents the difference in length of female and male Eastern newts throughout this study period. Newt length (mm) is graphed on the y-axis. Data displayed on the x-axis is the number of individuals both male (65) and female (16) measured throughout the study period and is representative of the entire vernal pool dataset.

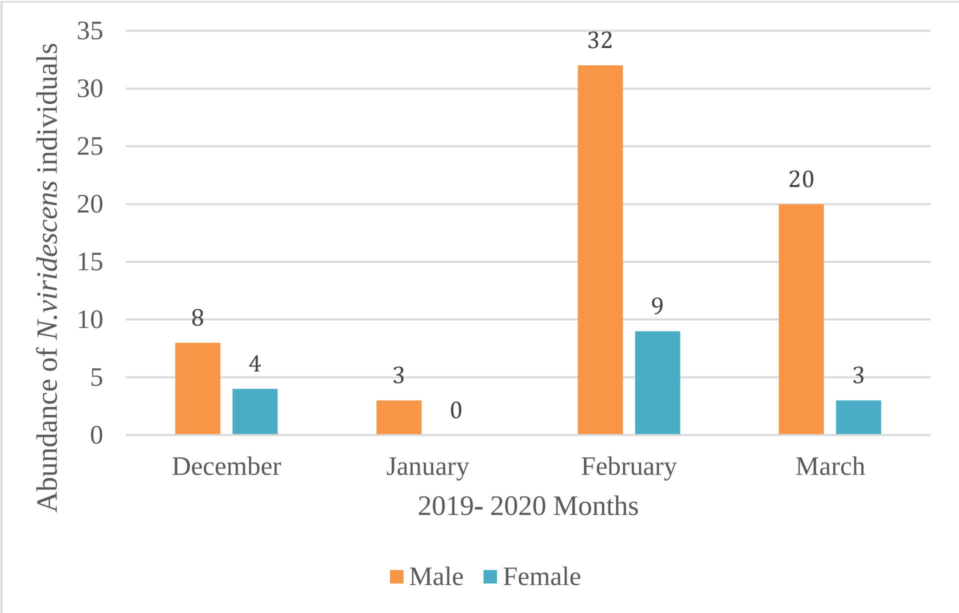

Figure 5. Temporal distribution of *N. viridescens* within the vernal pool

Figure 5 features the temporal distribution of total Eastern newt abundance throughout the study period with weekly data collection being grouped per month. Data displayed here is from all dip net collections.

4. Discussion

This data demonstrates a short representative study of the Eastern newt population located within Sandy Bottom Preserve. It indicates a large abundance of male individuals with no significant difference from the number of females present in comparison with representative population dynamics from literature on the species. This study displays no correlation between total newt abundance and seasonality. These results are likely inaccurate representations of the relationship between the seasonality and migration due to the four-month duration of this study and single sampling method selected. The data however can be accepted as a limited representation of the population dynamics of this location's *N. viridescens* inhabitants. The inclusion of more on land coverboard observations might result in a higher number of individuals observed, along with the extension of the observation period from late fall into the early summer. Often *N. viridescens* were observed at shallow depths that the large dip net was unable to effectively collect from, a previously thought skew in the datum to favor newts observed in deeper waters. This hypothesis was however not supported by the 95% CI G-test that the dataset was subjected to.

The data analysis in this study did include a cross comparison of weight and length between males and females and found only a significant difference in the length of *N. viridescens* sexes. The entire data set is exceedingly small, and further analysis would require a larger sample size to produce meaningful results. The moderately significant difference of mean length between each sex was expected, as other studies have recorded a relatively consistent Eastern newt body size with males averaging slightly longer[1]. The mean difference in length between male and female individuals may suggest slight female stress due to an overabundance of males present[7]. One study suggests female newts will reduce weight and alter foraging habits to avoid the high number of male individuals during breeding season[7]. This correlates with our finding of females having a lower average body weight and length than males and could explain their general absence during the time of data collection.

It is likely that the absence of soil temperature data, infrequency of data collection (once per week), and length of observation period significantly influenced these results. Future research could expand the period of data collection and incorporate outside NOAA climate data to more accurately address the influence of these factors. This season was particularly warm, and experienced typical rainfall totals[11]. Precipitation is considered to play a larger role in the migration patterns of Eastern newts than temperature and could explain why no noticeable difference in migration were observed[15]. If the newts were not experiencing drought in this season, it is unlikely that an observable change in their migration behavior would occur[17]. The entire data set is bias towards male individuals and explains little about the habits or physiological traits of females. This skew can be explained by the population dynamics of newts favoring a larger male sex ratio, and a female that tends to avoid conglomerations of males[5]. Interestingly, data points taken at night instead of mid-day resulted in a higher female to male ratio. Other studies suggest a temporal separation of foraging as female's strategy to avoid high stress high male environments[7]. Future studies could sample at varying times of day to assess this conjuncture.

The newts remained within the vernal pool throughout the study, something typical of their six-month long breeding period[19]. They begin migration in late fall and extend breeding into early summer, a process that is highly aquatic and consistent with our results of solely aquatic encounters from December to March. The absence of terrestrial individual observation could be explained by this phenomenon as well[14]. Additional sampling methods such as additional coverboards, drift fences with pitfall traps, and longer or variable dip net sampling periods could allow for a better understanding of this newt population. The spatial distribution of the Eastern newt could be accounted for by GPS mapping of specimen encounter locations. Sex ratio throughout the mating season could be better observed through an extended monitoring period. As suggested earlier, the absence of the red eft stage at this site is still unexplained and could be addressed by these additional sampling methods. The implications of this data in wetland conservation could be expanded by such future inquiries.

The results of this research provide an understanding of the Eastern newt population at Sandy Bottom Preserve, and a snapshot of their interaction with ambient conditions. There is useful insight to the effectiveness of dip net survey methods and frequency of survey periods. Imperative to the legitimacy of this study is work expanding the methods utilized and time spent observing Eastern newts. The correlation suggested in the introduction of this study between eastern newt behavioral patterns and suitable habitat availability cannot be supported by the data of this research alone. This work serves to establish a larger catalog on Eastern newt behavior patterns and suggest inquiry into their relationship to changing habitat availability.

It is critical to remember the reliance of many amphibian species on the presence and flooding of ephemeral pools such as Sandy Bottom Preserve. This water table is sensitive to rainfall patterns and times of drought have been shown to negatively impact amphibian breeding success[7]. The possible reduction of this area over time could create high stress environments for the Eastern newt and alter their reproductive behaviors[7]. Sandy Bottom is among few areas in

Western North Carolina that can support vernal pools and the myriad of amphibians which utilize them as reproductive grounds[10]. The conservation of this area has implications for maintain populations of the Eastern newt as well as species of high concern.

This data provides insight to a short period and a generally average newt population. *N. viridescens* in this area are not yet suffering population decline but exhibit marginal indicators of high stress environments. Human activity known to further the effects of habitat destruction could over time increase the stress experienced by these newts which in turn may have a cascade effect on the ecosystem in which they inhabit[7]. It is pressing that research continues to observe the patterns of these and other amphibians. This Southern Appalachian region is known globally as a biodiversity hotspot and requires consistent conservation and monitoring efforts to maintain this position for a plethora of species.

5. Acknowledgements

I thank Landon Ward tremendously for his commitment to reptiles and amphibians and the passion he has inspired in me for environmental sciences. A thanks is extended to all undergraduate students who aided in data collection. Thank you to Grace Mcleod for her work at Sandy Bottom in 2017. A deep appreciation goes to UNC-Asheville's Undergraduate Research Department along with Bill and Peg Steiner who funded this research. Special thanks to Bill and Peg, who continue to believe in the work of young scientists.

6. References

1. Behler, J.L. 1979. "The Audubon Society field guide to North American reptiles and amphibians." *The Indiana University Catalog*. 276-277.
2. Blaustein, A.R., J.M. Kiesecker. 2002. "Complexity in conservation: lessons from the global decline of amphibian populations." *Ecology Letters.* 5: 597-608.
3. Chambers, D.L. 2008. "Logging Road Effects on Breeding-Site Selection in Notophthalmus Viridescens (Red-Spotted newt) and Three Ambystomatid Salamanders in South-Central Pennsylvania." *Northeastern Naturalist* 15.1: 123-30.
4. Feder, M.E., P.L. Londos. 1984. "Hydric Constraints upon Foraging in a Terrestrial Salamander, Desmognathus ochrophaeus (Amphibia: Plethodontidae)" *Oecologia* 64.3: 413-418.
5. Fred, V. D., Berthel, A., Harju, S. M., Lamb, R. L., Thompson, D., Ryan, J., . . . Dreyer, G. 2017. "Amphibians in forest pools: Does habitat clustering affect community diversity and dynamics?" *Ecosphere,* 8.2: 1-17.
6. Gates, J., & Thompson, E. 1982. "Small Pool Habitat Selection by Red-Spotted newts in Western Maryland." *Journal of Herpetology,* 16.1: 7-15.
7. Grayson, K.L., et al. 2012. "Behavioral and Physiological Female Responses to Male Sex Ratio Bias in a Pond-Breeding Amphibian." *Frontiers in Zoology* 9: 24-34.
8. Jenkins, C. L., K. McGarigal, and L.R. Gamble. 2003. "Comparative effectiveness of two trapping techniques for surveying the abundance and diversity of reptiles and amphibians along drift fence arrays." *Herpetological Review* 34: 39-42.
9. Marion, Z. H., & Hay, M. E. 2011. "Chemical defense of the eastern newt (notophthalmus viridescens): Variation in efficiency against different consumers and in different habitats." *PLoS One,* 6.1: 1-9.
10. McLeod, M.G. 2017. "Reptile and Amphibian Monitoring at Sandy Bottom Preserve." *UNC Asheville Journal of Undergraduate Research:* 239- 247.
11. NOAA National Center for Environmental Information. 2019-2020. National Weather Service. Accessed 2019-2020 from https://www.weather.gov
12. North Carolina Wildlife Resource Commission. 2014. "Protected wildlife species of North Carolina." Accessed 11.10.2019 from http://www.ncwildlife.org/Portals/0/Conserving/documents/protected_species.pdf
13. Petranka, J.W. 1998."Salamanders of the United States and Canada" *Smithsonian Institute Press*. 1:89,97,157,453.
14. Rohr, Jason R., and Dale M. Madison. 2003. "Dryness Increases Predation Risk in Efts: Support for an Amphibian Decline Hypothesis." *Oecologia* 135.4: 657-64.

15. Schrank, A. J., Resh, S. C., Previant, W. J., & Chimner, R. A. 2015. “Characterization and classification of vernal pool vegetation, soil, and amphibians of pictured rocks national lakeshore.” *The American Midland Naturalist*, 174.1: 161-179.

16. Semlitsch, R.D., D.E. Scott, J.H.K Pechmann, and J.W. Gibbons. 1993. “Phenotypic variation in the arrival time of breeding salamanders; individual repeatability and environmental influences.” *Journal of Animal Ecology* 62: 334-340.

17. Sherman, E., K. Van Munster. 2012. "Pond pH, Acid Tolerance, and Water Preference in newts of Vermont." *Northeastern Naturalist* 19.1: 111-22.

18. Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.L. RodrSigues, D.F. Fishman, and R.W. Waller. 2004. “Status and trends of amphibian declines and extinctions worldwide.” *Sciences, New Series* 306.520: 1783-1786

19. Takahashi, M. K., & McPhee, C. 2016. “Facultative opposition of eastern newt (notophthalmus viridescens) in response to water reduction of aquatic habitats.” *The American Midland Naturalist,* 175.1: 73-81.

20. Whitmore, S. S., S. Losee, S.L. Meyer, and T.A. Spradling. 2013. “Conservation genetics of the central newt (notophthalmus viridescens) in Iowa: The importance of a biogeographic framework.” *Conservation Genetics*, 14.4: 771-781.