University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2023

Sex Distribution of Eastern Newts at Sandy Bottom

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The focus of this study was on eastern newt sex distribution at Sandy Bottom wetlands in western North Carolina. We examined whether or not female newts could be found throughout their breeding season, or if there would be an observed period of time where only males would be found. The study took place at Sandy Bottom Wetlands in Buncombe County where the newts were caught and observed through means of dip-netting. During a previous study that also took place at Sandy Bottom and utilized dip-netting as the main method of data collection, it was observed that there was a period of time where only male eastern newts were being caught and female newts were not recorded as being in the vernal pools until a reappearance later on in the breeding season. This study aimed to see if there was a pattern in the sex distribution seen in the newts and identify whether female eastern newts were showing mate avoidance behavior. As part of the data collection, it was recorded where the newts were found in relation to the shore (noted as either near or far) as well as the length, sex, and whether females were gravid, and males had breeding tubercles. A Scheirer-Ray-Hare test was used on the data, looking at variables such as month and distance on the distribution variability of female and male newts. Our analysis showed that month and distance had no effect on where male newts were found in vernal pools, however female newts had a statistical significance when looking at month:distance variable. This could show that female newts were showing mate avoidance behavior and moving around the vernal pools to avoid groups of males.

Introduction

The eastern newt, *Notophthalmus viridescens*, has a range that spans across the east coast of the United States, over to the Great Lakes, and down into east Texas. Within North Carolina, the eastern newt is abundant, with adults living in lakes, ponds, or smaller bodies of water such as streams, rivers, and wetlands. The eastern newt relies on both the terrestrial and aquatic habitats found in the surrounding areas for its life cycle. The life cycle of the eastern newt typically consists of three stages. The eggs are laid in bodies of freshwater in late winter and hatch into a larval stage. In the summer or fall, the larvae typically enter the terrestrial red eft stage. During this stage, the eft can be a bright red orange in color to warn visually oriented predators that they are toxic. The terrestrial eft stage can last multiple years. Upon reaching maturity, newts migrate back to ponds and other standing water. This process may take anywhere from one year to eight years[1]. During their life, adult newts move between terrestrial and aquatic habitats but congregate in freshwater pools in the late winter to early spring to mate.

During the breeding season, adult newts migrate to standing bodies of water such as vernal pools. In a previous study done by an alumna of the University of North Carolina at Asheville, it was observed that there was a lack of females caught in January. As the breeding season for newts continues, male eastern newts may begin to have encounters where they can choose to pass by a courting pair or attempt an approach and interrupt. During times where the males choose to interrupt, this may begin to form a large gathering of males around a single female. To help find females or rival males, eastern newts use chemical signaling, which is especially useful to eastern newts that live in murky water. It is used for locating potential mates and rivals. It also allows for males to find the operational sex ratio (OSR) in their habitat. The OSR is the ratio of sexually active males to fertilizable females that are ready to mate[2]. At the beginning of the newts breeding season, a male may be less likely to approach another couple, as it is still possible to find another more available mate. However, as the breeding season continues, males are more likely to begin to interrupt mating couples as the competition for a mate increases[3]. As the competition increases, “dense patches of males are typically found near potential mates, and thus, it has been proposed that multiple males may be a reliable indirect cue to the vicinity of a female "[3]. Another explanation for finding fewer females is mate avoidance. Female eastern newts may avoid aggressive males during this time because their eggs are not fully mature. If female newts are moving around vernal pools more than males, who may form patches, it is easier for them to choose when they want to engage with the males.

Data from the previous study, which was also conducted at Sandy Bottom wetland in Buncombe County North Carolina, revealed that adult eastern newts seasonally use both forest and vernal pool habitats[4]. The study from 2019-2020 collected data on migration patterns in the eastern newt. Since the vernal pools are often dry in the summer and fall, it results in newts occupying the forest around the vernal pools. In late fall and early winter, the pools have standing water, and the adult newts migrate to the vernal pools. From December to March, the adult newts will not be found on land. During this study period, it was observed that from the end of January to mid-February, only male eastern newts were being caught in the vernal pools.

Our study explored the possible explanation for pattern of sex distribution observed in the previous study. We explored sex distribution by distance from shore to find if any sex-based patterns that may exist. Our hypothesis was that female newts were showing mate avoidance behaviors and would avoid male newts until they were ready to breed. From the end of January and through March, we went out to survey the salamanders at Sandy Bottom. The main method of observation was dip-netting, which was also used in the previous study.

Methods

The study took place at the Sandy Bottom wetlands, located in Buncombe County North Carolina. This site is classified as a Montane Floodplain Slough Forest. The vernal pool wetlands are considered a unique wetland habitat and are protected. It is located close to a road and has generally flat topography. The dominant vegetation is made up of woody plants and trees that border the wetlands. The wetlands themselves contain tall grasses and sedges.

From January of 2023 to March 2023, surveying took place at the wetlands approximately twice a week for around an hour each time. The study took place during the same time frame as the 2019-2020 study to better draw a comparison between the data. Covering 25 meters, five transects of five meters were measured and sampled randomly each time. Flags on the shore marked the beginning and end of each transect. Within each transect, a general standard for distance was used. Areas within 2 meters of shore were near, and areas that occurred more than 2 meters from shore were far. The transects did not fully go across the wetland due to obstacles such as sedges in the middle of the wetlands and rhododendron growth on the opposite shore. Using waders, long handle dip nets, 10 cm rulers, and small acrylic tubs to catch newts. Transect areas including both near-shore and far-shore were sampled weekly. For each newt that was caught, the sex, length (mm), gravidness (females only), width (mm, females only),

whether breeding tubercles were present (in males), the transect meter at which the newt was caught, and the distance from shore (measured as NEAR or FAR), were recorded. The distance recorded was correlated with water depth. Areas within the transect were sampled randomly every week. Each sampling period included both near and far sampling.

Data were statistically analyzed using a Scheirer-Ray-Hare (SRH) test in RStudio. A Scheirer-Ray-Hare test is similar to a non-parametric test used for two-way factorial design[5] and also similar to a two-way analysis of variance. We looked at the connection between male/female distribution using month and distance as variables, as well as any connection between the month and distance variables themselves. This was used to compare male and female presence over time at both NEAR and FAR locations. Only adult newts were included in the statistical analysis.

Results

A total of 36 newts were found over the course of the current study. Of those, 58% of the newts were males. In table 1, a total of 81 newts were found and male newts made up 80% of the total number observed. In both tables (Table 1 and 2), all females found were gravid and all sexually mature males were found to have breeding tubercles. In table 1 there were three juvenile males found while none were found in the previous study.

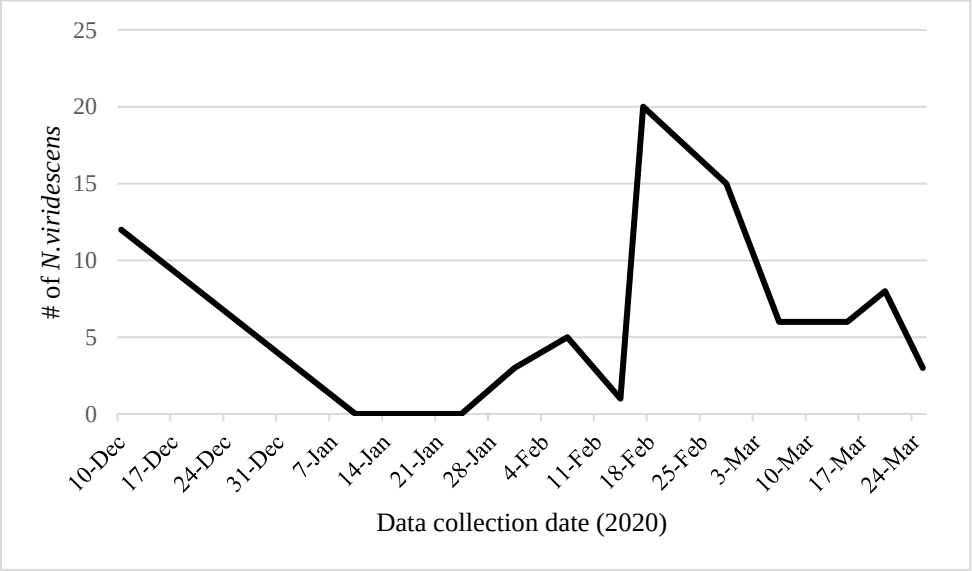

Figure 1. Previous Sandy Bottom study, number of newts found over each day of collection[4].

Figure 1. shows the total number of newts collected in a 2020 study starting in December and continuing through March. From December until late January, the number of newts collected shows a downward trend, with no newts collected between January 7th and January 21st. In mid-February there is a spike in the number of newts collected, followed by a decrease in March.

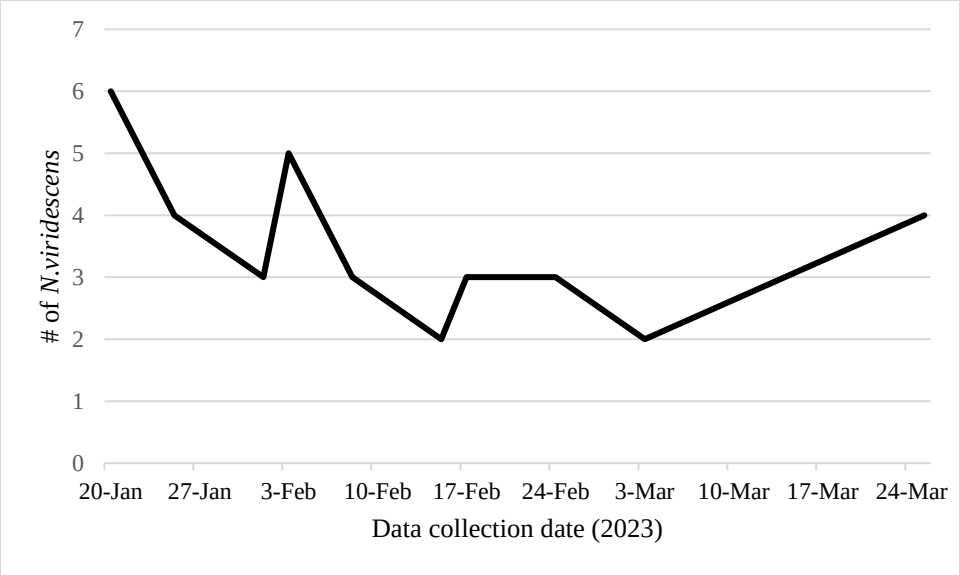

Figure 2. Number of eastern newts found each day of data collection at Sandy Bottom.

Figure 2. shows the 2023 trends in newts sampled. The highest newt capture rates occurred in mid-to-late January and early February. Newts were found on all days when data collection occurred.

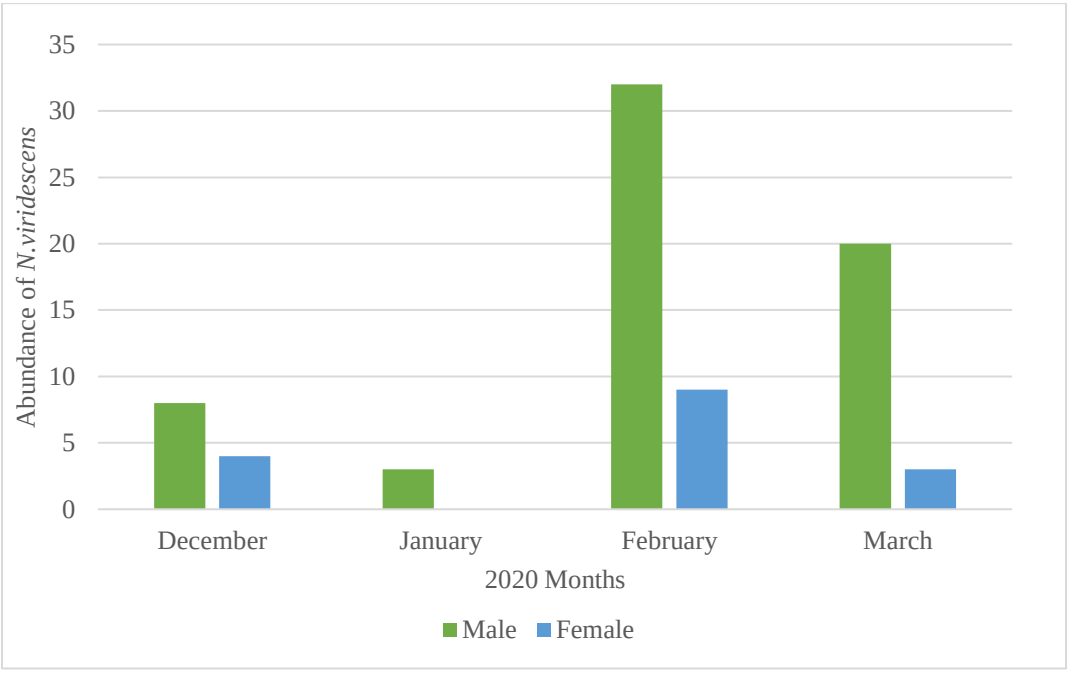

Figure 3. Comparison of the abundance of male, female newts collected at Sandy Bottom in a 2019-2020 study[4].

Figure 3 shows the abundance of the sexes of newts sampled in the 2019-2020 study. In December, relatively few male and female newts were collected in the vernal pools at Sandy Bottom. In January, no females were collected. There was an increase in males collected during the month of February, with a few females collected during the same sampling period. During that time, there were over three times as many males found than females. In March, there is a decrease in males as well as females, however there are still nearly six times as many males to females that were collected.

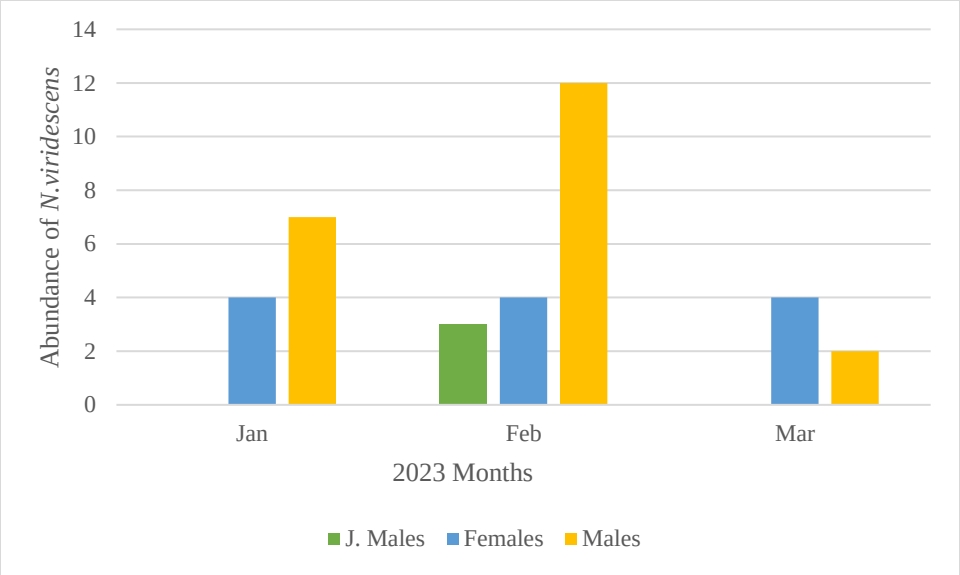

Figure 4. Comparison of the abundance of male, female, juvenile newts collected at Sandy Bottom in 2023.

Figure 4, Reveals that the same number of females were found each month during the 2023 study. In comparison, the number of males found increased by nearly twice the amount found in January and triple the amount found in March. Juvenile males were also found only in the month of February.

Figure 3 and figure 4 both show an increase in males in February. In contrast to the previous study, figure 4 shows that female newts were caught in January, while no females were observed in the previous study. In both studies, a similar number of female newts were observed in the month of March.

Table 1. Comparison of number of individuals of each sex found and status of sexual maturity, previous study[4].

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

Table 2. Comparison of number male/female/juvenile newts collected and status of sexual maturity.

Sex	# Individuals	Breeding Tubercles	Gravid
M	21	21	N/A
F	12	N/A	12
Juvenile	3	NO	N/A

Table 3 shows there was no effect between female newt distribution and the month or the distance (p-value > 0.05). However, there was an effect between the month and the distance (p-value = 0.03), suggesting that female newts were possibly moving around different parts of the vernal pool over the course of the breeding season. Distance refers to how near or far an eastern newt was caught from shore. A newt that was near to shore was under two meters from the shore and far was any distance over two meters from shore.

Table 3. Statistical results of female newts and the effect of month and distance tested using a Scheirer-Ray-Hare test.

Female Newt SRH Results				
	Df	Sum sq	H	p. value
Month	2	85.333	3.0277	0.220061
Distance	1	45	1.5966	0.20638
Month:Distance	2	190.333	6.7532	0.034164
Residuals	14	214.833		

Table 4 shows that there was no effect on male newt distribution over the course of the study. The pattern of males by distance per month was the same and had very low variability. Neither the month nor distance affected male newt distribution (p-value > 0.05).

Table 4. Statistical results of male newts and the effect of month and distance tested using a Scheirer-Ray-Hare test.

Male Newt SRH Results				
	Df	Sum sq	H	p. value
Month	2	112.71	3.5961	0.16562
Distance	1	12.8	0.4084	0.52278
Month:Distance	2	114.91	3.6663	0.15991
Residuals	14	355.08		

Discussion

Since the figures show a pattern of male newts being found in January and then spiking in February before decreasing a month later in March, with female newts seeming to peak in February, this could mean that male newts begin to move into breeding pools before female newts. This data is also backed up by looking at the patterns in the overall collection of newts, where there is a dip in the number of newts being collected before showing an increase in mid-February. These patterns could also be due to the different dates of sampling. Both studies generally sampled Sandy Bottom on different days, and while a schedule of sampling two days per week was attempted for the current study, it did not always turn out that way. The previous study also began a month before the current study started and so extra data was collected.

However, despite the increases in male newts, capture rates could also be driven by an increase in female newts, the pheromones of which could lead to males collecting in large numbers. Rohr et al., also found that after oviposition occurred, male newts would be more likely to engage larger mating groups because it would not be guaranteed that a male would be able to find a suitable female later. With an increase in population OSR, this would mean that there would be less chances for male newts to find an available female partner and could lead into areas with a large number of males. During times of collection, this behavior may have an impact, as the chance of catching a male newt would be higher than catching a female.

Our study was also done over a shorter period of time and did not include the month of December as in the previous study. However, where it was recorded that no females were found in January, our study did find females present at Sandy Bottom during that time. Our study also came across a mating pair in January. This could mean that female eastern newts have a prolonged egg laying season and may seek and avoid males during the breeding season. Temperature also may have an effect on the time of the breeding season. The 2023 season may have been different from the 2020 season. If eastern newts use temperature cues as part of their breeding season, large or noticeable fluctuations may impact sex distribution. If male newts move into the breeding pools before females and then act aggressively to each other, female newts may begin to show mate avoidance and move to other parts of the pool to protect their eggs, before coming back later in the season.

Mate avoidance movements may be reflected in the findings. Mate avoidance among amphibians is generally under-researched and in those studies mate avoidance is usually described as an individual avoiding mating with a close relative. However, the movement of female newts may show that there is some mate avoidance behavior happening that is unrelated to being related to other newts. In the case of the eastern newts at Sandy Bottom, the

movements of the females may show that they avoid the high male areas and once they are more receptive to mating, begin to move into the areas occupied by the males.

Conclusion

The results of this study may show that female eastern newt sex distribution at Sandy Bottom could be influenced by mate avoidance behaviors. Using data from a previous study in 2020, this study was able to look at when female and male newts enter breeding pools and their distribution over time. We found that male newt distribution remained unchanged over the course of the study which may have led to higher density areas that female newts avoid until they are ready to mate. We concluded that female newts would move throughout the vernal pools over the course of the breeding season, which may have been due to mate avoidance behaviors. Further studies can also be done to better understand the distribution of eastern newts and their behavior, such as looking at temperature as a factor for when the breeding season begins, as well as surveying different parts of the wetlands to see if females are going elsewhere in the vernal pools. Another possibility for future research is studying the sex ratio of the eastern newt population outside of the breeding season to see if it is still as skewed as it is during the breeding season.

Acknowledgements

The author wishes to express their appreciation to Professor Landon Ward for advising, supporting, and assisting in this research, to Dr. Jennifer Ward for helping to run the statistical analyses, and to all of their friends (Megan Huske, River Rico, Hailey Wunder, and Jaden Ballard) who took time out of their schedules to help catch newts.

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