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A Retrospective Study on the Effects of Urbanization on the Plethodontid Salamander Species of Streams Surrounding Emory University

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B.S., Maryville College, 2021

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Abstract

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By Cooper Read

The percentage of the population living in urban areas has grown immensely across the world, and is only expected to grow over the coming decades. Urbanization has many impacts on the environment, especially freshwater systems, including habitat fragmentation, runoff, sedimentation, heat pollution, and overall biodiversity loss. Amphibians, especially salamanders, are among the most affected by this pollution, and are an integral component of many stream ecosystems. Atlanta offers interesting opportunities to study this impact. Fifty years ago, Orser and Shure performed a study on the populations of *Desmognathus spp.* salamanders in streams in and around the campus of Emory University. Opportunities to resample and compare populations to evaluate environmental changes are rare, especially in a region that has undergone drastic changes in recent decades like Emory University. Therefore, seven sites spread across five streams in and around Emory University were selected for sampling of salamander populations. Sampling took place from August to October, with each stream being subjected to a monthly sampling of environmental conditions and four monthly samples of the stream's salamanders. All observed salamanders were recorded, with the two most common salamander species in the area, the Spotted Dusky Salamander (*Desmognathus conanti*) and the Two-Lined Salamander (*Eurycea cirrigera*) being subjected to a mark-recapture population estimate. The data were then analyzed utilizing several statistical methods to compare the populations among the streams, the environmental conditions among the streams, and if there was any correlation between the two. While an abundance of data were collected that provided insight into the streams, statistical

significance was difficult to determine. However, the data that were collected aligned well with the findings of Orser, suggesting that the salamander populations remained stable in spite of urbanization. While these data do not offer concrete conclusions on their own, they offer a valuable starting point for more regular monitoring projects in the region.

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CHAPTER I

INTRODUCTION

As of 2018, 55% of the global population resides in urban areas, with that number expected to grow to 68% over the coming decade. Most of this existing urban population is in North America, with over 82% of its population living in heavily urbanized regions (United Nations, 2018). Recent decades have been a period of rapid growth for the American South, a region that has historically lagged behind the rest of the nation in terms of development. Between the years of 1970 and 2010, the population density of the region has doubled (Boustan, et al. 2013). To meet such a massive increase in urban population, large scale infrastructure had to be constructed. Urban growth and its resulting impact can cause detrimental effects on the local environment, especially waterways and other freshwater ecosystems (Peters, 2009).

Urban streams demonstrate a marked decrease in macroinvertebrate populations when compared to forested streams in the same geographical area, affecting processes such as litter decomposition and nutrient recycling. These essential factors for stream health are impacted by the diminished effect of decomposers in the ecosystem (Gao, et al. 2022). Overall biodiversity and species richness in freshwater habitats have a noticeable negative correlation with increased urbanization (Ma, et al. 2022). Despite the major impacts urban growth has on the health of these areas, streams and other freshwater systems often go overlooked by both the public and local shareholders in favor of more readily noticeable terrestrial environmental concerns (Higgins, et al. 2018). Urbanization has also demonstrated a strong correlation with habitat loss and habitat fragmentation with nearly all natural ecosystems involved in the urbanized areas. As urbanization increased, the surrounding natural ecosystems suffered from fragmented populations and an increase in edge effects among similar detrimental conditions (Liu, et al. 2016). While the negative impact of urbanization on freshwater ecosystems can originate from a variety of factors, one of the most readily apparent is the introduction of impervious surfaces via human development, such as concrete and asphalt. Impervious surfaces found in urban areas block water from flowing into aquifers as they would in soil or other natural ground cover, resulting in increased runoff (AMEC Earth and Environmental, et al. 2001). Surfaces such as these absorb a large amount of light, resulting in what is known as the urban heat island effect, producing thermal pollution in the streams that runoff flows into. The resulting increase in temperature could be lethal to sensitive species and poses increased danger when combined with climate change (Somers, et al. 2013). Runoff from impervious surfaces can also pick up industrial waste, atmospheric disposition from automobiles and other airborne pollution, post-consumer pollution and litter, and other harmful sources. The system of roads and other impervious surfaces funnels and directs this waste-ridden runoff into streams and other natural areas (Müller, et al. 2020). An increase in runoff also disrupts and destabilizes the stream bank, causing a compounding issue of erosion and sweeping silt and soil into the stream. Erosion and related issues such as increased silt in waterways are compounded in areas of high humidity and heat (Shikangalah, et al. 2016). An excess of silt and other pollutants results in a variety of negative impacts on ecosystems and their species (Niemelä, et al. 2011) (Price, et al. 2011).

While all organisms are affected by the stresses associated with an urban environment, amphibians are impacted at a noticeably higher rate (Hoffmann, et al. 2010). In a literature review of 32 urban amphibian populations studied across North America, 63% showed a negative impact correlated with urbanization, while less than 1% showed a positive impact. An increase in urbanization was found to affect life history stages, the breeding cycle, movement, and habitat selection across several amphibian species. (Scheffers, et al. 2012) While urbanization may help change an environment in ways that assist generalist species, the habitat destruction, fragmentation, and pollution have a much larger impact on more sensitive species (Schmidt and Garroway, 2020). Some studies have suggested that urbanization may assist amphibian populations due to thermal pollution raising temperatures to better match the desired temperature range of amphibian species. However, this would also be detrimental if the effect moved the temperature out of the natural preferred temperature range, therefore forcing the species out of their home range. Similarly, while some have suggested that urban landscaping of green spaces for human use can effectively counteract the negative impact of urbanization on the species of these spaces, this does not consider habitat fragmentation, noise pollution, light pollution, and several other detrimental effects (Yang, et al. 2022).

The order *Caudata*, consisting of salamanders and related species, have shown themselves to be both an essential component to many affected streams, and among those most noticeably affected by the detrimental impacts of urbanization. Salamanders often serve as high level predators for lower order stream ecosystems, especially lotic streams that include a large number of oxygenated points of fast-moving water and are essential for nutrient recycling. They in turn serve as prey for birds and small mammals. In forested areas, the biomass of such salamanders often outnumbers that of other vertebrates. Salamander populations were found to have a strong negative correlation with urbanization in both species abundance and diversity (Barrett, et al. 2014). This abundance can at least be partially attributed to salamander's status as ectotherms, which in turn makes them more sensitive to thermal changes such as those associated with urbanization and climate change (Buckley, et al. 2008).

Due to their integral role in the ecosystem and how impacted they are by changes to stream conditions, amphibians such as salamanders are among the first signs of damage to aquatic ecosystems, and any change to their population requires closer examination (Wake, 1991). Amphibians' permeable skin is sensitive to changes in several environmental factors, such as quality of water, ultraviolet light, and dissolved oxygen concentration (Stebbins, et al. 1997). These organisms exhibit a life cycle that consists of both terrestrial and aquatic stages that allow them to be affected by a wide range of environmental conditions, such as changes to weather patterns and pollution from both freshwater and terrestrial sources. (Barrett, et al. 2014). This major metamorphosis can be interrupted or damaged in easily noticeable ways by endocrine disrupting pollutants (Stebbins, et al. 1997).

The city of Atlanta, Georgia offers interesting research opportunities when examining the impact of urbanization on amphibian populations, and therefore on freshwater stream ecosystems. Regular monitoring studies have been undertaken to monitor the city's water quality since 2003, finding lowered pH levels and elevated levels of potentially harmful bacteria, heavy metals, and other such contaminants (Peters, 2009). However, research that specifically focuses on amphibian populations in such a heavily urbanized area is lacking. The earliest research on the topic of salamander populations in the Atlanta metro area (specifically the area surrounding the campus of Emory University) was performed by Orser and Shure (1972). This research focused on *Desmognathus spp.* salamanders, among the most populous taxa of salamanders in the American Southeast (Barrett, et al. 2014). This abundance allowed the populations of this species to serve as a noticeable marker of stream quality. In addition, plethodontid salamanders such as *Desmognathus spp.* have a wide range of tolerances across several environmental gradients, making them more likely to be found in degraded streams in need of study (Grover, 2000). Orser and Shure's research found a strong negative correlation between urbanization and salamander populations (Orser and Shure, 1972). In the fifty years since this study originally took place, the landscape of Emory University has undergone many large changes, with even more planned in the future (Jordan, Jones & Goulding, 2008). Therefore, resampling the salamanders in this area and evaluating the possible effects of changes that have taken place in these ecosystems over the past fifty years is an intriguing point for research. This kind of retrospective study is an opportunity that is rarely seen, and the ability to observe how the environmental conditions of these streams and the salamander populations within them may have changed over time is valuable.

This thesis used a species Orser identified as the Northern Dusky Salamander (*Desmognathus fucus fucus*) as the main focus of the research. However, this species has since been recategorized as the Spotted Dusky Salamander (*Desmognathus conanti*). *D. coanti* should be considered the same species that Orser referred to as *D. fucus fucus*. Recent research has suggested a reclassification of *Desmognathus spp.* (Pyron, et al. 2022). However, as *D. coanti* has yet to be recategorized this species will be identified according to current standards. Streams in areas where urban development has taken place in the last fifty years are expected to show higher levels of degradation compared to Orser's findings. In addition, the Two-Lined Salamander (*Eurycea cirrigera*), a species of similar abundance in the area of interest, was also sampled. This study is designed to examine the environmental conditions of these at-risk streams, observe the impact these conditions have had on the variation in their salamander populations, compare the conditions of the salamander populations and streams with Orser's findings whenever possible, and set a new baseline for future research in this area. Therefore, my research will consider two separate questions. Was there any correlation between the abiotic environmental factors affected by urbanization and plethodontid salamander populations in the streams in question, and if so, why? Can any differences be observed between the population dynamics of these streams today and when they were sampled by Orser fifty years ago?

CHAPTER II

METHODOLOGY

Site Description

Five streams were the focus of this study, each of which were located within woodland patches of Northeastern Atlanta's heavily urbanized matrix, which itself is part of Georgia's wider Southern Outer Piedmont region (Edwards, et al. 2012). Three of these streams were located in the Lullwater Preserve wildlife management area on the campus of Emory University. The other two were located between the Emory Clinic and a residential apartment area across from the main Center for Disease Control campus. These streams range from first or second order spring-fed streams to runoff from stormwater detention ponds. While four streams were chosen for their role in Orser's original work, the remaining stream was chosen due to its position in the environment, which could offer additional data to supplement the previously sampled areas (Figure 1)(Figure 2).

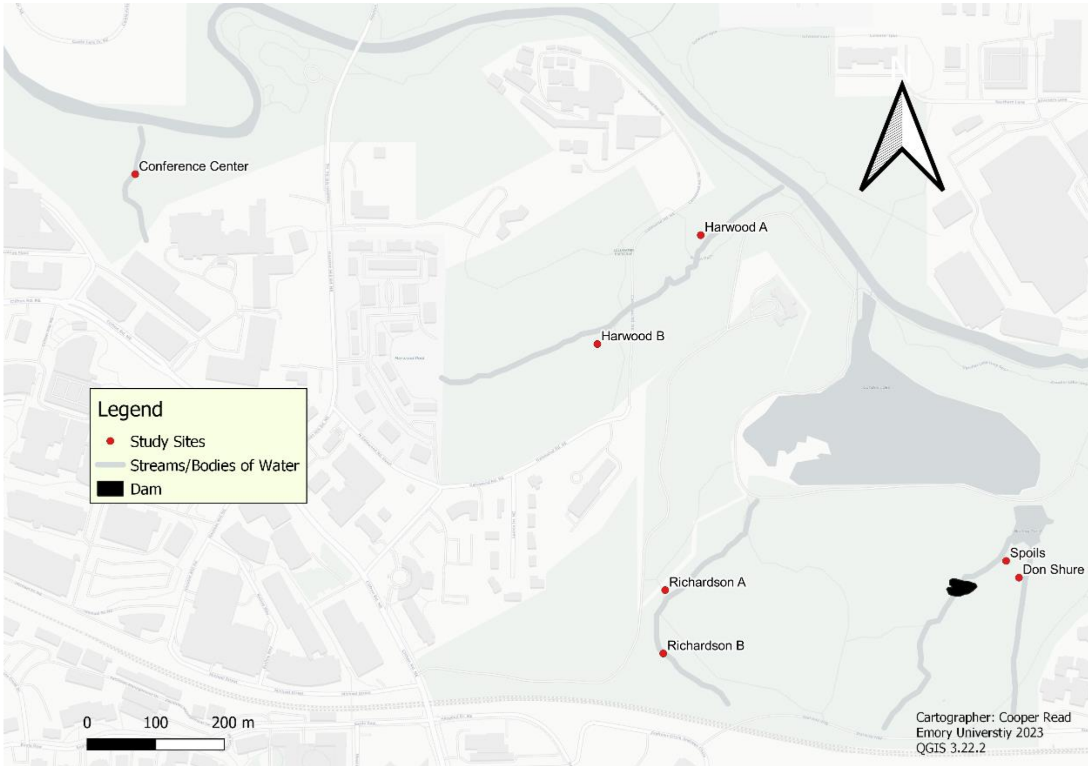


Figure 1: A Map of the Study Area Created with QGIS with Research Sites Labeled (QGIS.org, %Y. QGIS Geographic Information System. QGIS Association. http://www.qgis.org)

Don Shure Creek

Spoils Creek

Richardson Creek site A

Richardson Creek site B

Harwood Creek site A

Harwood Creek site B

Conference Center Creek

Figure 2: Images of the Stream Sites Facing Upstream. Captured September 14th-15th 2022.

Arrows Denote North

Site Selection

For each stream, an individual with expertise in sampling the area was consulted for the selection of sampling sites. Site selection criteria, including abundant cover such as rocks, logs, and leaf litter both on the stream banks and within the stream itself served as ideal areas for sampling. Finally, a flow rate fast enough to provide riffle points of oxygenated water but still slow enough to provide pools and clear water were also needed. Stream areas that met the criteria were selected to serve as study sites. A single site was chosen per stream for Don Shure Creek, Spoils Creek, and Conference Center Creek. However, due to the comparatively large size of Richardson Creek and Harwood Creek, two sites were selected for these streams, resulting in a total of seven sites. At each site, a square research area of ten meters per side was measured, marked with flags (Home Depot, *2455 Paces Ferry Rd. Atlanta, GA 30339)* and a GPS pin (W.W. Grainger Corporation, 100 Grainger Parkway, Lake Forest, IL 60045) was used to ensure that the same location was used for each sampling session.

The stream sampling sequence was randomized prior to the field season. Prior to any entry into the study area, the hands of all individuals involved, as well as any equipment that may encounter the salamanders, was disinfected using a diluted bleach solution to prevent the spread of harmful fungus and other pathogens (Huang, et al. 2013).

Salamander Sampling

All sampling and tagging procedures were approved by the Institutional Animal Care and Use Committee (PROTO202200038). The full text of this document may be accessed upon request.

Sampling took place over the course of three months utilized by Orser in his work: August, September, and October 2022. The sampling period consisted of the latter two weeks of the month, August 17-31, September 16-29, and October 16-29 respectively. For each of these periods two sites were sampled each field day with each site being sampled four times over the course of the two-week period. The only exception to this was in the month of August, in which torrential rains on the 30th of the month prevented sampling until the following day.

Prior to sampling, the stream, time, temperature (Etekcity, 1202 N Miller St Ste A, Anaheim, California, 92806), ambient humidity (General Tools, 75 Seaview Dr, Secaucus, New Jersey, 07094), and a qualitative analysis of the local weather were recorded. Sampling began between the hours of 3:00 and 4:00 PM and continued for a period of one hour. A digital stopwatch was used to ensure that only actively sampling in the stream counted towards the time measurement. Any time spent measuring and packaging salamanders for transport, taking down notes, daily environmental data, misadventures related to the researcher’s type 1 diabetes, and other unrelated activities were not included in this time measurement. During the sampling period, the researcher paced along the steam and surrounding area inspecting mud-banks, stones, and other common hiding spots for any salamanders. A small net and plastic bag were used to capture the salamanders. All captured salamanders were temporarily placed into an individual plastic bag (LK Packaging, 7515 Hartman Industrial Way #200, Austell, GA 30168) with the species, snout-vent length measurements taken via calipers (Walmart, 702 S.W. 8th St. Bentonville, AK 72716), and capture location recorded. Location of capture was denoted by the cover object the individual was found under, such as a rock, or lack thereof, in the case of a free swimming individual. The location of capture was further separated into stream, bank, and terrestrial categories, where stream was used when the capture location was fully within the stream water, bank was used in an area where the stream met the land, and terrestrial used for all other areas. Salamanders were kept in the bag for a period not exceeding five minutes, and separate bags were used for each individual to eliminate any chance of cross contamination. All salamanders that were not found to be Spotted Dusky Salamander (*Desmognathus conanti*) or the Two-Lined Salamander (*Eurycea cirrigera*) were then released, as these two species were subject to a mark-recapture population estimate study. It was suggested to only collect mark-recapture population data on the two most populous species, as if all captured salamanders were marked regardless of species, it was unlikely that the less common species would be sampled in sufficient numbers to provide any usable data. When an individual of either of these species was identified, the location in which it was captured was marked with a flag and picture to ensure it was returned to its original location. The individuals were then placed in a plastic meal prep container along with leaf litter for cover and a moist paper towel (Seventh Generation, 60 Lake St Ste 3N, Burlington, Vermont, 05401) to ensure the individual stayed hydrated (Dymit, 2019). To ensure the safety of organisms in both transport and the tagging process itself, only adult specimens completely lacking gills with a snout-vent length exceeding 30mm were retained for marking. If a specimen of either target species was captured that did not meet these criteria, it was returned to the location of its capture immediately after the snout vent length measurements were recorded.

Tagging and Release

Following the approved protocol, adult individuals identified as *D. conanti* or *E. cirrigera* were transported to the lab and given an initial wellness check to ensure the individuals were in good health and were not previously captured individuals recaptured erroneously. The well-being of the salamander was then recorded. All handling of salamanders was performed wearing a separate pair of rubber gloves for each individual. A separate bath of an anesthetic 0.2% solution of Tricaine (MS-222) was prepared for each animal (Crakir and Strauch, 2005). The salamanders were placed into the bath for a period not exceeding 20 minutes. During this period, the Visible Implant Elastomer Tag from Northwest Marine Technology, Inc. (4003 Airport Road Anacortes, WA, 98221) was prepared utilizing the manufacturer's instructions (Sanchez, et al. 2020). Separate syringes were utilized for each animal. Individuals were removed from their anesthetic and injected with a tag in the dorsal area running along the spine to ensure the tag was easily visible. The animal was then placed in a distilled water bath to remove any remaining anesthetic. The tag color, tag location, and any injuries sustained during tagging, if any, were recorded. This process was repeated separately for each individual until all were successfully tagged. The individuals were then returned to their initial capture containers for a period of one hour, after which a wellness check occurred to ensure that the individuals had completely recovered from the anesthetic treatment and were moving freely. The tags were inspected with a handheld ultraviolet flashlight to ensure that the tag fluoresced properly and was visible. The status of both the tag and the salamander were then recorded. Individuals were kept in the same containers that they were initially captured and transported in to prevent possible cross contamination, ensure the salamanders remained moist, and ensure individuals were returned to the correct point of capture. Individuals were returned to the areas of capture approximately twelve hours after the final wellness check of tagging.

Environmental Sampling

Prior to the two-week sampling period, two days were set aside for the collection of environmental data. Most environmental data were collected following the standardized Georgia Adopt-A-Stream Basic Visual form found at the state website (Adopt-A-Stream, 2020). The data from these surveys consisted of an analysis of water flow, water clarity, erosion and stability of the stream bank, local wildlife, ground cover, and approximate shade. For each of these criteria, the stream status was either described qualitatively, such as clear or cloudy water, or on a scale of 1-10, with less evidence of erosion and disturbance scoring higher. Additional environmental data were collected including ambient temperature (Etekcity, 1202 N Miller St Ste A, Anaheim, California, 92806), ambient humidity and soil moisture (General Tools, 75 Seaview Dr, Secaucus, New Jersey, 07094), pH (Xylem Corporation, 301 Water Street SE, Washington, DC, 20003), turbidity (Extech Instruments, *9 Townsend West Nashua, NH 03063*), flow rate (Xylem Corporation, 301 Water Street SE, Washington, DC, 20003), total dissolved solids (Honeforest, honeforest.net), water temperature, water pressure, and dissolved oxygen (Xylem Corporation, 301 Water Street SE, Washington, DC, 20003). Measurements on lead content of the soils at each site was obtained from a separate research project encompassing the same study area and added to the other environmental data to determine if this common pollutant could have any influence on the salamander populations. Stream depth and width were taken at each meter to give a general overview of stream size. These data were then compiled to compare stream measurements with their salamander population.

Invertebrate Sampling

During the two-day environmental sampling period, potential invertebrate prey items were also collected. A one square meter area was sampled for a period of 15 minutes. All captured invertebrates were then placed in a jar of 70% isopropyl alcohol for transport and identification. Streams were sampled for benthic macroinvertebrates using a Surber net sampler (Science First, 86475 Gene Lasserre Blvd., Yulee FL 32097) following the technique outlined in Storey, et al. (1991). These macroinvertebrates were then placed in a jar of 70% isopropyl alcohol for transport and identification along with their terrestrial counterparts. Two jars, one terrestrial and one aquatic, were collected at each stream each month.

Data Analysis

Sampled data were compiled into a series of tables and figures demonstrating the population makeup of captured salamanders, snout-vent length measurements, capture location, population estimates, stream measurements, invertebrate collection, and environmental conditions at each stream. These data were then statistically analyzed utilizing methods including t-tests, ANOVA tests followed by a post-hoc Bonferroni Correction to determine if there were discrepancies between the populations of the two target species, and if relationships existed between the environmental factors and population dynamics of the salamanders in the streams in question.

Population estimates were obtained from the use of the Schumacher and Eschmeyer Method, the equation of which is shown in Figure 3.

$$\hat{N} = \frac{\sum_{t=1}^{s}\left(C_t M_t^2\right)}{\sum_{t=1}^{s}\left(R_t M_t\right)}$$

Figure 3: The Equation Utilized for the Schumacher and Eschmeyer Method of Population Estimates

In this model, t=the sampling session for that stream (12 in total) s=total number of samples, C_t=total number of salamanders caught fitting the capture parameters, R_t=the number of tagged salamanders recaptured, and M_t=the number of tagged salamanders still at large. As the total number of recaptured salamanders in any of the streams did not exceed 50, confidence intervals were obtained from the table utilized for Poisson distribution as described in Krebs (1999). Correlation between these population estimates and environmental factors were analyzed utilizing a regression analysis followed by a Pearson Correlation Coefficient, which was used to obtain a t-value. This value was then compared to a t-chart to determine significant correlation.

CHAPTER III

RESULTS

A total of 1013 individuals were sampled across the seven sites in five streams. Of the salamanders collected, 461 were identified as Spotted Dusky Salamanders (*Desmognathus conanti*), 523 were identified as Two-Lined Salamanders (*Eurycea cirrigera*), and 29 were identified as other species. The non-target species identified were made up of the Northern Slimy Salamander (*Plethodon glutinosus*) found in Don Shure and Spoils Creeks, the Three Lined Salamander (*Eurycea guttolineata*) found in Spoils and Conference Center Creeks, the Seal Salamander (*Desmignathus monticola*) found solely in Conference Center Creek, and a single Red-Backed Salamander (*Plethodon cinereus*) found in Richardson Creek site B. All non-target salamanders captured were adults fully lacking gills. The overall capture totals for each site, in order of abundance, are shown in Table 1. All non-target species are collected under the heading "other".

Table 1: A Summary of Salamander Sampling from Each Site. Collected August 17th-October 29th, 2022

Stream	Spotted Dusky	Two-Lined	Other	Total
Don Shure	85	102	4	191
Conference Center	114	33	11	158
Richardson A	27	119	0	146
Spoils	103	21	13	137
Richardson B	47	87	1	135
Harwood B	58	76	0	134
Harwood A	27	85	0	112
Overall	461	523	29	1013

The data pertaining to the two target species, *D. coanti* and *E. cirrigera* were then compared in more detail (Table 2).

Table 2: Collection Dynamics of Spotted Dusky and Two-Lined Salamanders from Each Site. Collected August 17th-October 29th, 2022. Means with Standard Deviation in Brackets.

Stream/Month	Spotted Dusky Adult	Two-Lined Adult	Total Adult	Spotted Dusky Juvenile	Two-Lined Juvenile	Total Juvenile	Overall Totals
Don Shure Total	73	58	131	12	44	56	187
Don Shure Monthly Average	24.33 [2.08]	19.33 [13.87]	43.67 [12.10]	4.00 [6.93]	14.67 [11.59]	18.67 [18.18]	62.33 [9.07]
Spoils Total	102	19	121	1	2	3	124
Spoils Monthly Average	34.00 [6.56]	6.33 [5.03]	40.33 [2.08]	0.33 [0.58]	0.67 [1.15]	1.00 [1.73]	41.33 [3.06]
Richardson A Total	24	58	82	3	61	64	146
Richardson A Monthly Average	8.00 [0.00]	19.33 [15.57]	27.33 [15.57]	1.00 [1.73]	20.33 [17.93]	21.33 [19.66]	48.67 [7.77]
Richardson B Total	44	32	76	3	55	58	134
Richardson B Monthly Average	14.67 [3.06]	10.67 [7.09]	25.33 [8.96]	1.00 [1.00]	18.33 [9.71]	19.33 [10.41]	44.67 [3.21]
Harwood A Total	25	29	54	2	56	58	112
Harwood A Monthly Average	8.33 [4.51]	9.67 [2.08]	18.00 [5.29]	0.67 [1.15]	18.67 [8.39]	19.33 [9.07]	37.33 [6.03]
Harwood B Total	58	20	78	0	56	56	134
Harwood B Monthly Average	19.33 [4.62]	6.67 [0.58]	26.00 [4.36]	0.00 [0.00]	18.67 [11.55]	18.67 [11.55]	44.67 [7.23]
Conference Center Total	114	14	128	0	19	19	147
Conference Center Monthly Average	38.00 [2.65]	4.67 [4.04]	42.67 [6.11]	0.00 [0.00]	6.33 [6.81]	6.33 [6.81]	49.00 [1.00]
Overall Totals	440	230	670	21	293	314	984
Overall Monthly Averages	20.95 [11.84]	10.95 [6.07]	31.90 [10.14]	1.00 [1.39]	13.95 [7.52]	14.95 [7.91]	46.86 [7.94]

Although Two-Lined Salamanders were observed to be more common overall, adult Spotted Dusky Salamanders were captured much more often, at a rate of nearly two adult Spotted Dusky Salamanders for each adult Two-Lined Salamander. Sites in order of adult Spotted Dusky Salamander abundance were Conference Center Creek, Spoils Creek, Don Shure Creek, Harwood Creek site B, Richardson Creek site B, Harwood Creek site A, and Richardson

Creek site A. A one-way analysis of variance (ANOVA) test suggested significance (F=30.20, F-$crit$=2.22. df=5). A following post-hoc analysis utilizing the Bonferroni Correction showed significant differences in the abundance of adult Spotted Dusky Salamanders between Don Shure Creek and Richardson Creek site A (p=3.76E-6), Don Shure Creek and Harwood Creek site A (p=5.59E-6), Don Shure Creek and Conference Center Creek (p=4.79E-4), Spoils Creek and Richardson Creek site A (p=1.06E-9), Spoils Creek and Richardson Creek site B (p=4.29E-7), Spoils Creek and Harwood Creek site A (p=1.56E-9), Spoils Creek and Harwood Creek site B (p=7.08E-4), Richardson Creek site A and Conference Center Creek (p=6.17E-10), Richardson Creek site B and Conference Center Creek (p=1.10E-7), Harwood Creek site A and Conference Center Creek (p=8.55E-10), and Harwood Creek site B and Conference Center Creek (p=1.02E-4). Sites in order of adult Two-Lined Salamander abundance were Don Shure Creek and Richardson Creek site A (equal capture totals for these sites), Richardson Creek site B, Harwood Creek site A, Harwood Creek site B, Spoils Creek, and Conference Center Creek. While a one-way analysis of variance (ANOVA) test suggested significance between the sites in adult Two-Lined Salamander abundance(F=4.15, F-$crit$=2.22), a following post-hoc analysis utilizing the Bonferroni Correction did not find any significant difference between the streams that could not be explained by data variance. A two tailed paired t Test found statistically significant differences between the observed adult Spotted Dusky and Two-Lined salamanders in Spoils Creek (p=1.57E-5), Harwood Creek site B (p=6.60E-4), and Conference Center Creek (p=7.31E-6).

The juvenile populations observed also demonstrated some difference in their distribution amongst the sites. Sites in order of juvenile Spotted Dusky Salamander abundance were Don Shure Creek, Richardson Creek site A and B (equal capture totals for these sites), Harwood Creek site A, and Spoils Creek. No juvenile Spotted Dusky Salamanders were observed in

Harwood Creek site B or Conference Center Creek. A one-way analysis of variance (ANOVA) test did not suggest significance in the juvenile Spotted Dusky Salamanders observed in the sites. Sites in order of juvenile Two-Lined Salamander abundance were Richardson Creek site A, Richardson Creek Site B and Harwood Creek site A (equal capture totals for these sites), Don Shure Creek and Harwood Creek site B, Conference Center Creek, and Spoils Creek. A one-way analysis of variance (ANOVA) test suggested significance (*F*=3.85, *F-crit*=2.22). A following post-hoc analysis utilizing the Bonferroni Correction showed significant differences in the observed abundance of juvenile Two-Lined Salamanders between Don Shure Creek and Richardson Creek site A (p=4.34E-6), Richardson Creek site A and Harwood Creek site A (p=1.32E-4), Richardson Creek site A and Harwood Creek site B (p=3.32E-6), and Richardson Creek site A and Conference Center Creek (p=4.34E-4).

The collection dynamics between adult and juvenile Spotted Dusky Salamanders and Two Lined Salamanders among the sites over the three month sampling period are visualized in Figure 4 and Figure 5.

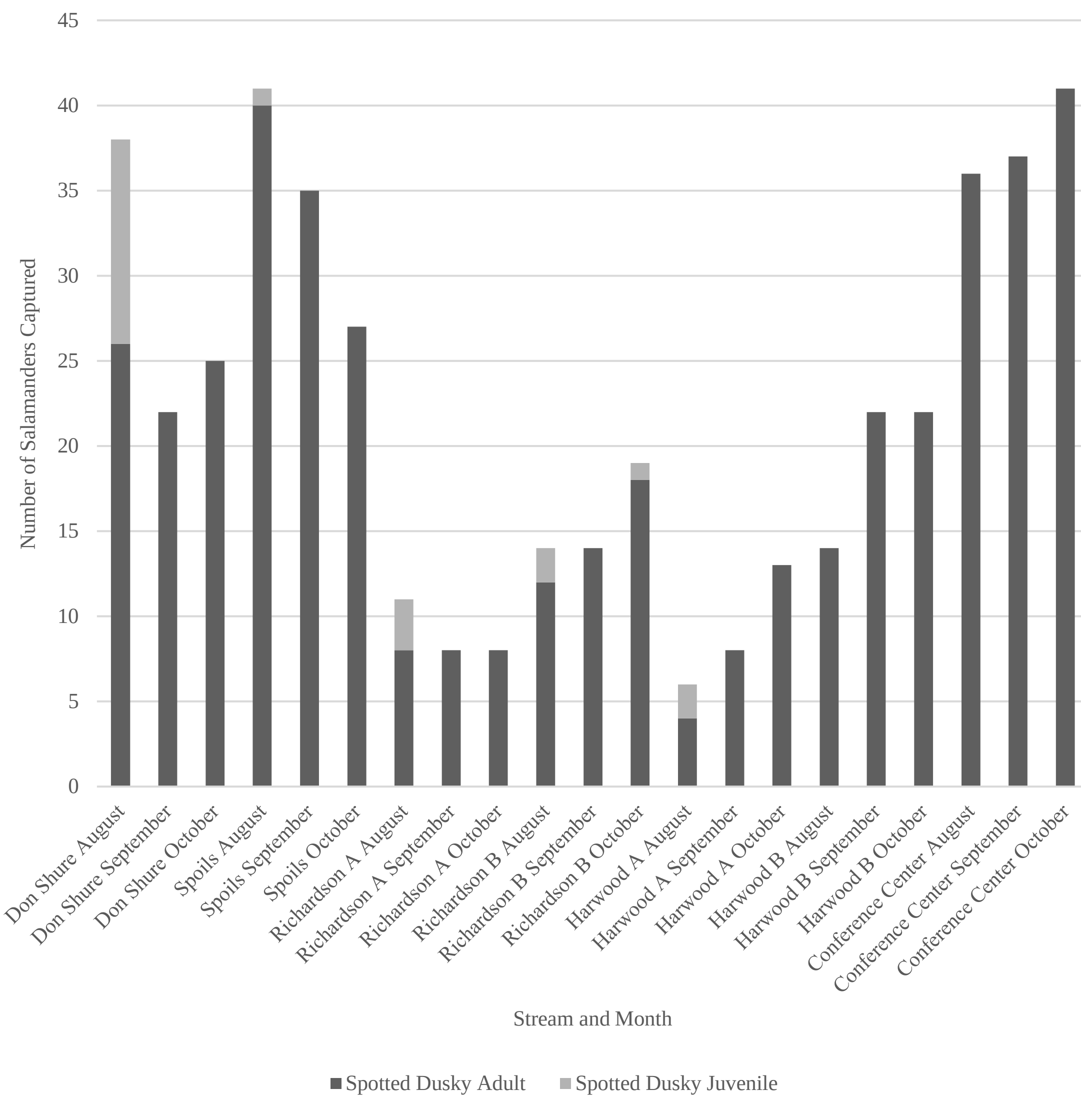


Figure 4: A Visualization of Collection Dynamics of Spotted Dusky Salamanders from Each Site. Collected August 17th-October 29th, 2022.

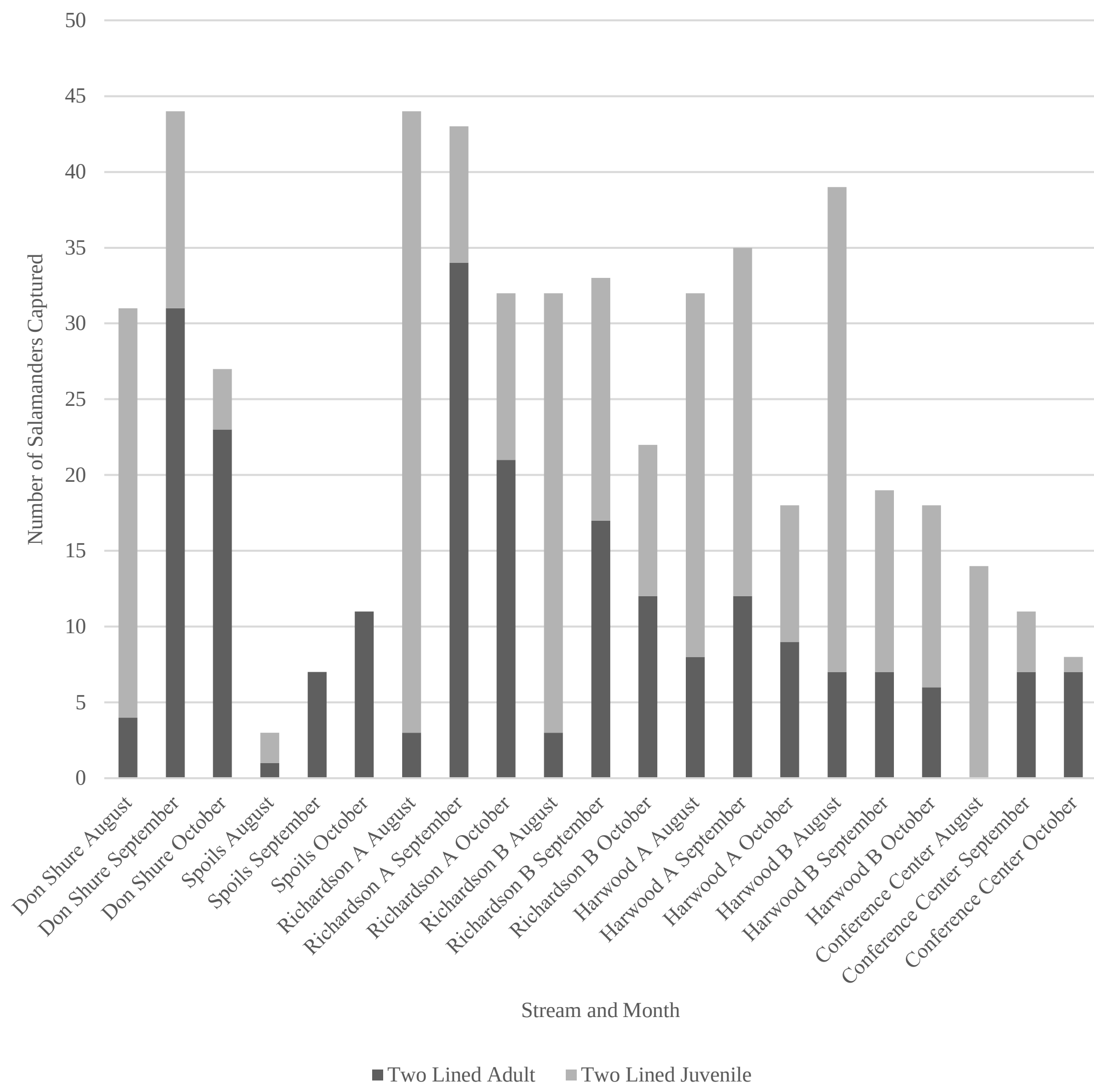
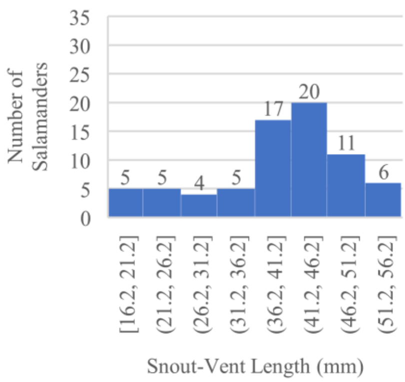


Figure 5: A Visualization of Collection Dynamics of Two-Lined from Each Site. Collected August 17th-October 29th, 2022.

These figures assist in showing the discrepancy between juvenile and adult populations of both target species. As can be seen in Figure 2, juvenile Spotted Dusky Salamanders made up a very small percentage of the total number of Spotted Dusky Salamanders collected. Juvenile Spotted Dusky Salamanders were found almost solely in the month of August, with the only exception to this trend being a single individual observed in October in Richardson Creek site B in October. In contrast, Juvenile Two-Lined Salamanders made up a much larger percentage of the Two-Lined Salamander collection dynamics. In the month of August, Juvenile Two-Lined Salamanders made up the majority of the species observed at all sites, with a complete lack of adults being observed this month at Conference Center Creek. The number of adults observed increased in the following months.

In Figures 6-9, the histogram grids compare the snout-vent length of salamanders captured at the study sites, with Don Shure Creek designated by a 1, Spoils Creek designated by a 2, Richardson Creek site A designated by a 3, Richardson Creek site B designated by a 4, Harwood Creek site A designated by a 5, Harwood Creek site B designated by a 6, and Conference Center Creek designated by a 7. The final histograms in the lower right corner of each grid compare the snout-vent length of salamanders from all study sites. The x-axis shows the snout-vent length of salamanders in millimeters and the y-axis is the total number of salamanders captured.



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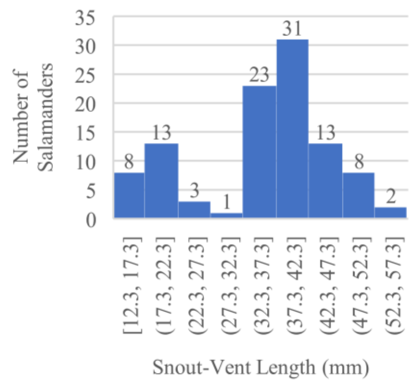

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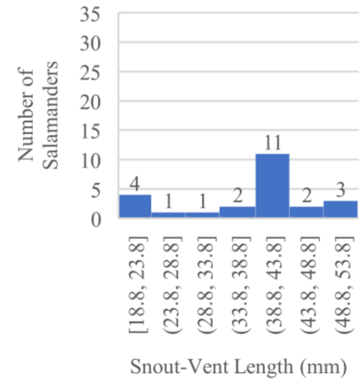

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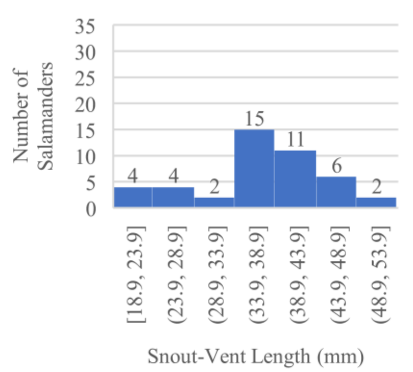

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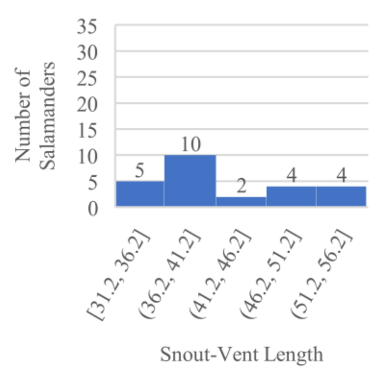

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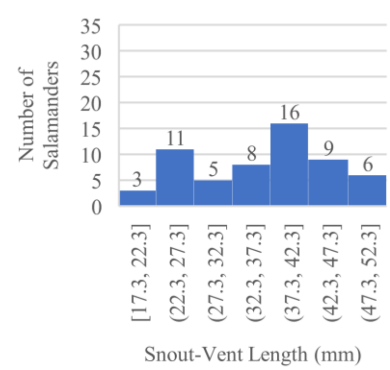

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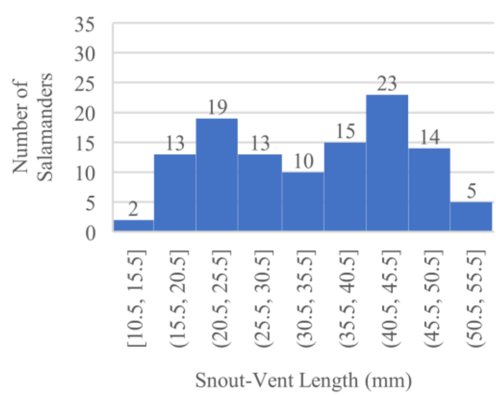

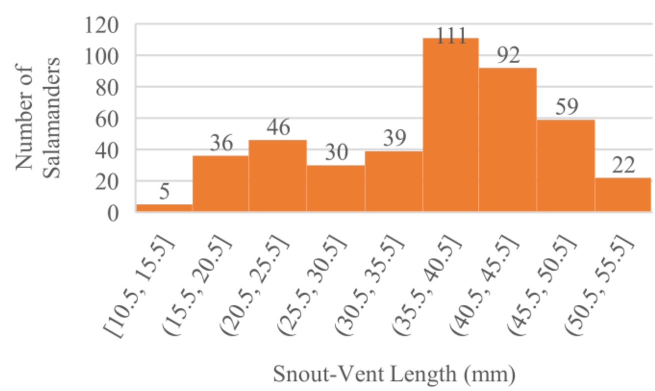

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Figure 6: A Histogram Grid of the Snout-Vent Length of Adult Spotted Dusky Salamanders Collected August 17th-October 29th, 2022

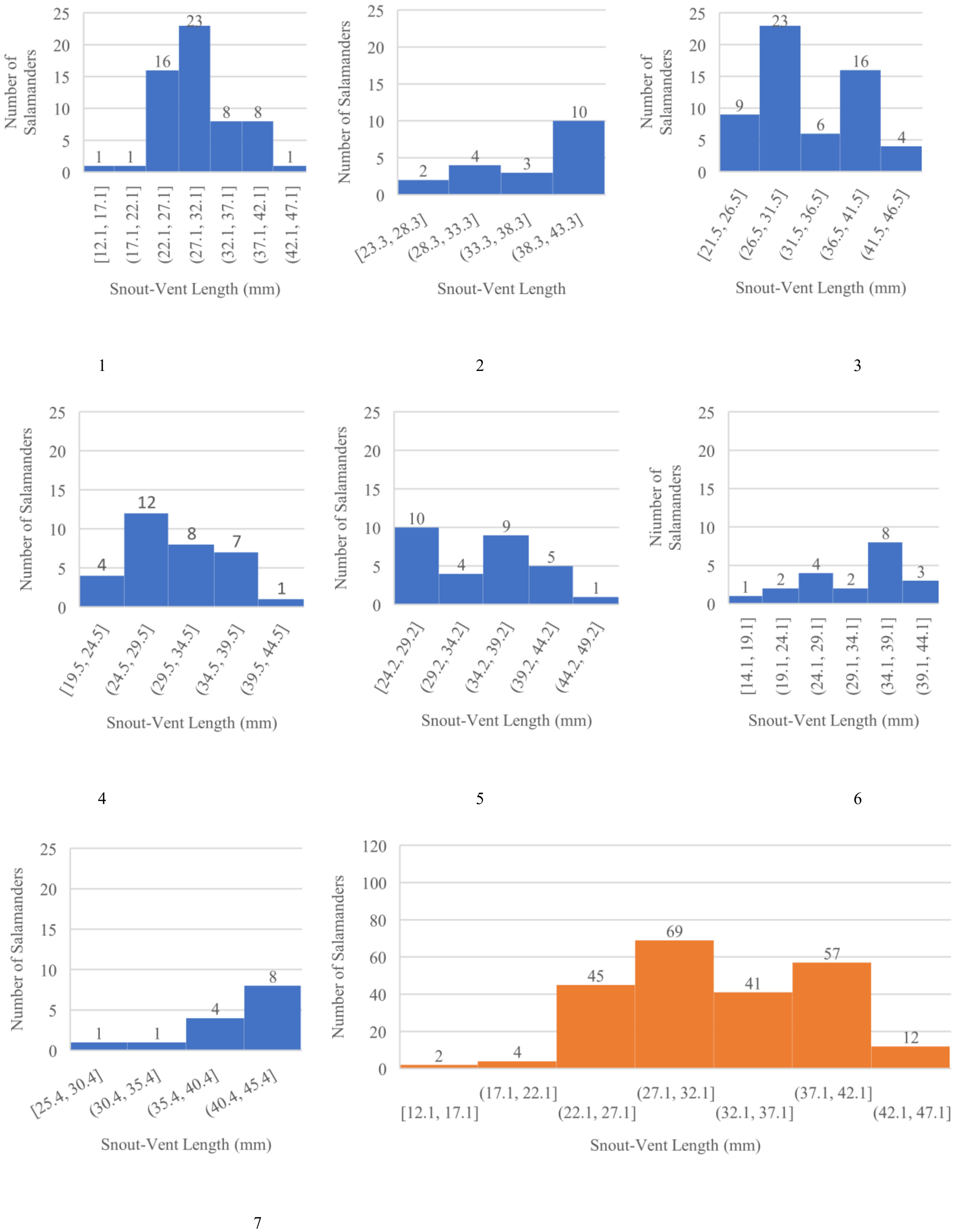


Figure 7: A Histogram Grid of the Snout-Vent Length of Adult Two-Lined Salamanders

Collected August 17th-October 29th, 2022

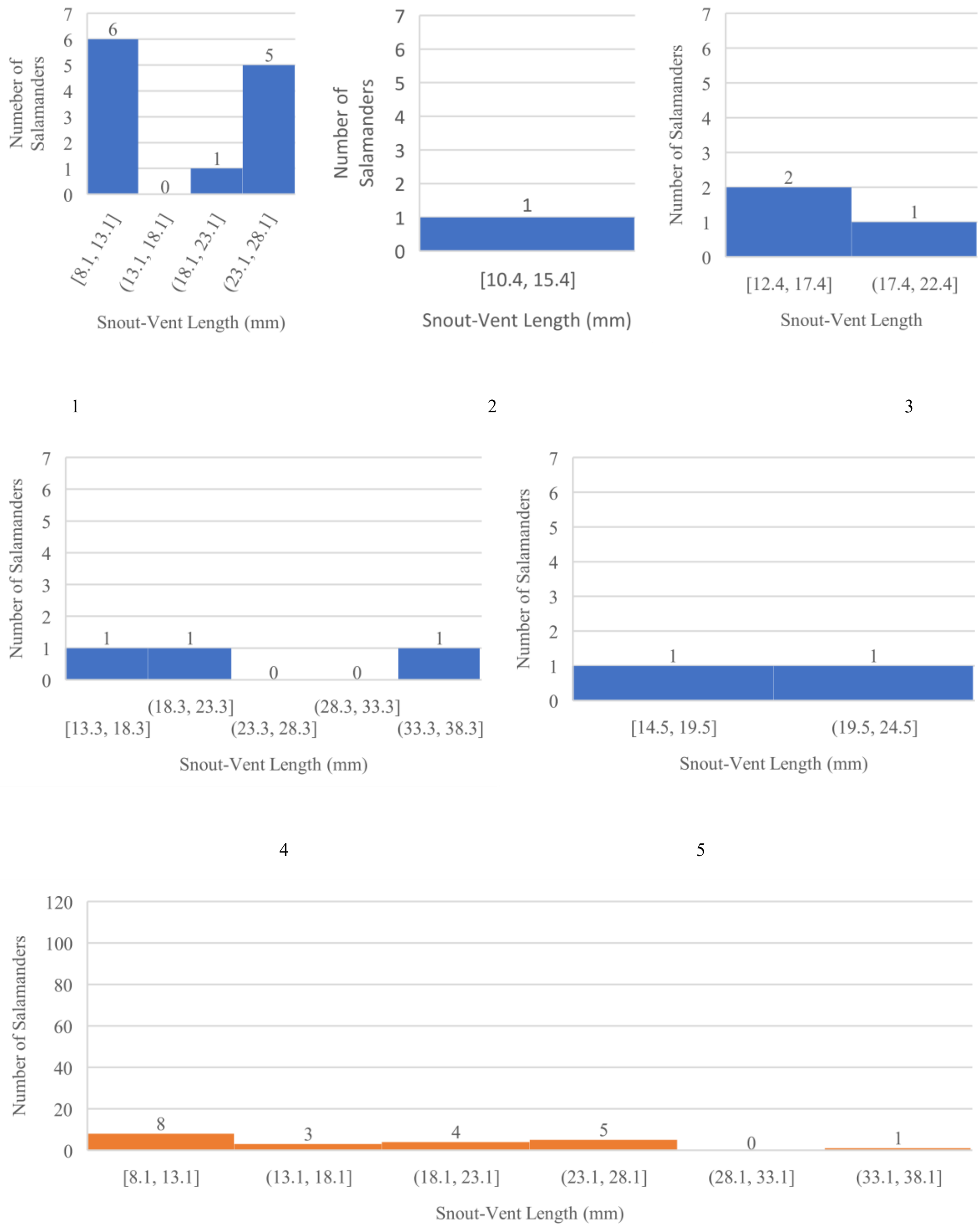
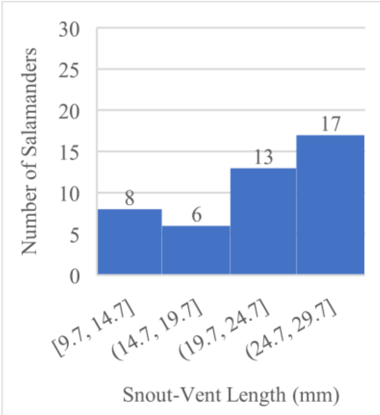
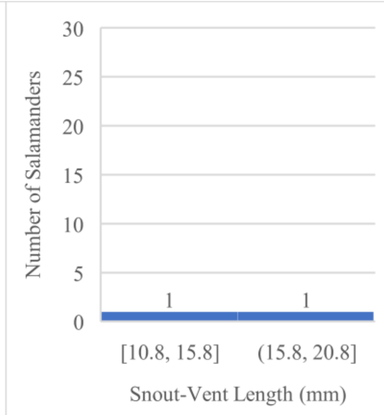
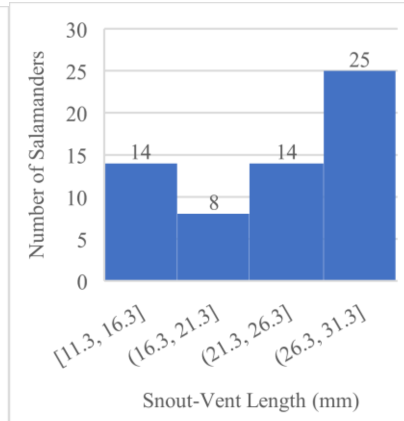


Figure 8: A Histogram Grid of the Snout-Vent Length of Juvenile Spotted Dusky Salamanders Collected August 17th-October 29th, 2022







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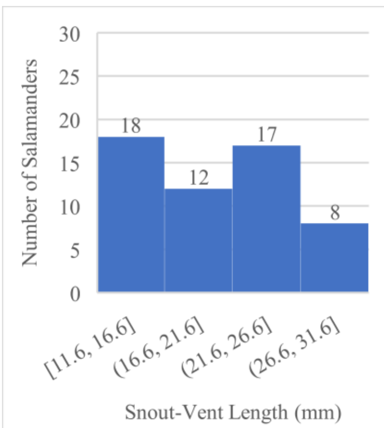

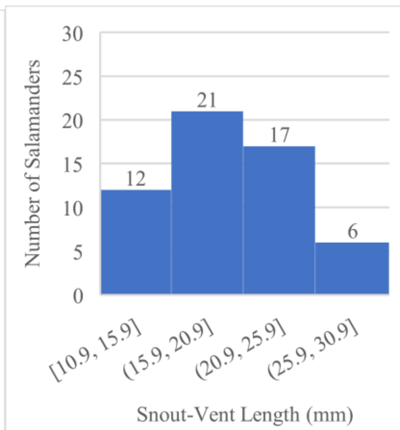

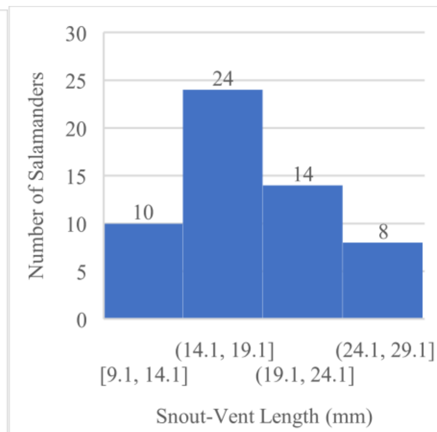

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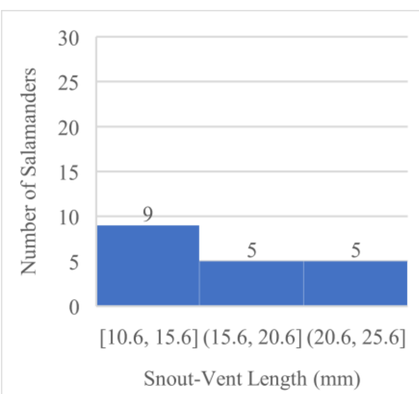

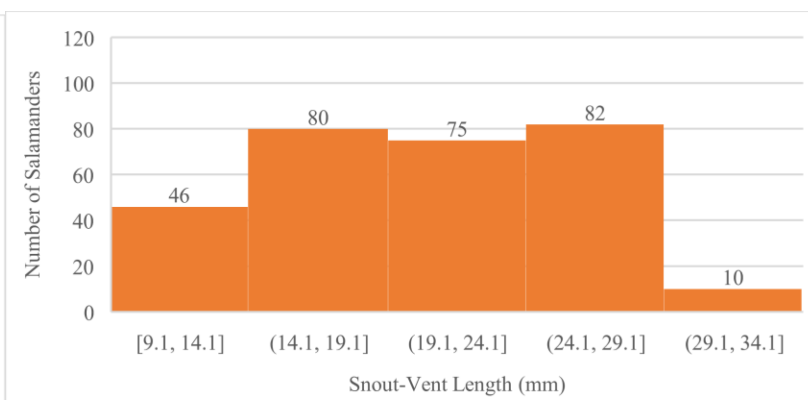

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Figure 9: A Histogram Grid of the Snout-Vent Length of Juvenile Two-Lined Salamanders Collected August 17th-October 29th, 2022

These histogram grids reveal some differences between the snout-vent length size distribution between the streams. In Don Shure Creek, adult Spotted Dusky and Two-Lined Salamanders demonstrated similar size distributions, but Spotted Dusky Salamanders trended towards larger sized individuals. In Spoils Creek, adult Spotted Dusky Salamanders had a larger size distribution when compared to Two-Lined Salamanders, as well as trending larger. This may have been impacted by the comparatively small number of adult Two-Lined Salamanders captured in this stream. In Richardson Creek site A, while adult Spotted Dusky Salamanders had a larger size distribution and larger size, it was more evenly spaced along the size gradient when compared to adult Two-Lined Salamanders in the same site. Size range and distribution for adult Spotted Dusky and Two-Lined Salamanders was similar in Richardson Creek site B and Harwood Creek site B. Harwood Creek site A found adult Spotted Dusky Salamanders trending slightly larger and adult Two-Lined Salamanders trending slightly smaller. Conference Center Creek found adult Spotted Dusky Salamanders with a larger size distribution than adult Two-Lined Salamanders. This may have been impacted by the comparatively small number of adult Two-Lined Salamanders captured in this stream. Overall, while adult Spotted Dusky and Two-Lined Salamanders demonstrated a similar range of sizes, Spotted Dusky Salamanders demonstrated a more even distribution. Juvenile Two-Lined Salamanders featured similar size distribution in Don Shure Creek and Richardson Creek site A. Both sites located in Harwood Creek featured similar size distribution as well. The small number of juvenile Spotted Dusky Salamanders observed makes comparison difficult and may have impacted distribution.

The various capture location of salamanders was also recorded in Table 3 for adults and Table 4 for juveniles.

Table 3: Capture Location of Adult Salamanders in Each Site. Collected August 17^{th}-October 29^{th}, 2022

Stream	Standing on Stream Rock	Swimming in Stream	Under Bank Leaf Litter	Under Bank Rock	Under Stream Leaf Litter	Under Stream Log	Under Stream Rock	Under Terrestrial Leaf Litter	Under Terrestrial Log	Under Terrestrial Rock	In Bank Mud	Standing on Stream Bank	Under Bank Log	Total
Don Shure	2	2	1	79	3	0	37	1	5	5	0	0	0	135
Spoils	1	1	1	68	3	0	51	0	7	1	1	0	0	134
Richardson A	1	2	0	45	1	0	22	0	0	9	0	2	0	82
Richardson B	0	4	0	31	4	6	19	2	2	0	1	8	0	77
Harwood A	0	3	1	28	1	0	17	0	1	0	2	0	1	54
Harwood B	1	2	2	33	1	14	12	1	1	0	1	1	9	78
Conference Center	1	1	4	101	1	0	27	0	0	2	0	2	0	139
Total	6	15	9	385	14	20	185	4	16	17	5	13	10	699

Table 4: Capture Location of Juvenile Salamanders from Each Site. Collected August 17th-October 29th, 2022

Stream	Swimming in Stream	Under Bank Rock	Under Stream Rock	Under Stream Log	Total
Don Shure	10	2	44	0	56
Spoils	1	1	1	0	3
Richardson A	12	0	52	0	64
Richardson B	36	1	19	2	58
Harwood A	36	1	19	2	58
Harwood B	28	0	28	0	56
Conference Center	12	0	7	0	19
Total	135	5	170	4	314

Adult salamanders greatly favored utilizing rocks along the stream bank as cover, with rocks in the stream itself serving as secondary cover options in nearly every stream. In contrast, while juveniles also preferred using rocks as cover, they more often chose stream rocks farther from the shores, with a sizable section swimming in the steams without cover whatsoever. In Richardson Creek site B, Harwood Creek site A, and Conference Center Creek these free swimming juveniles outnumbered those seeking cover.

The Spotted Dusky Salamanders fitting the capture criteria (adult, snout-vent length over 30mm) were subject to a mark-recapture population estimate. Utilizing these data, population estimates were obtained for each stream utilizing the Schumacher-Eschmeyer technique as described in Krebs (1999). As Two-Lined Salamanders were only recaptured in Richardson Creek and none of the other sites, no population estimates were calculated for this species as there could be no comparison made between the streams (Table 5).

Table 5: Schumacher-Eschmeyer Population Estimates of Spotted Dusky Salamanders from Each Site. Collected August 17th-October 29th, 2022.

Stream	Population Estimate (Rounded to nearest whole number)	Upper 95% Estimate	Lower 95% Estimate
Don Shure	84	144.04	50.86
Spoils	81	129.35	56.68
Richardson A	28	69.55	9.90
Richardson B	65	140.94	33.64
Harwood A	19	37.44	9.96
Harwood B	57	90.55	27.34
Conference Center	104	187.98	64.09

Given these estimates, the sites in order of adult Spotted Dusky salamander population size are Conference Center Creek, Don Shure Creek, Spoils Creek, Richardson Creek site B, Harwood Creek site B, Richardson Creek site A, and Harwood Creek site A. These population estimates and their confidence intervals are visualized in Figure 10.

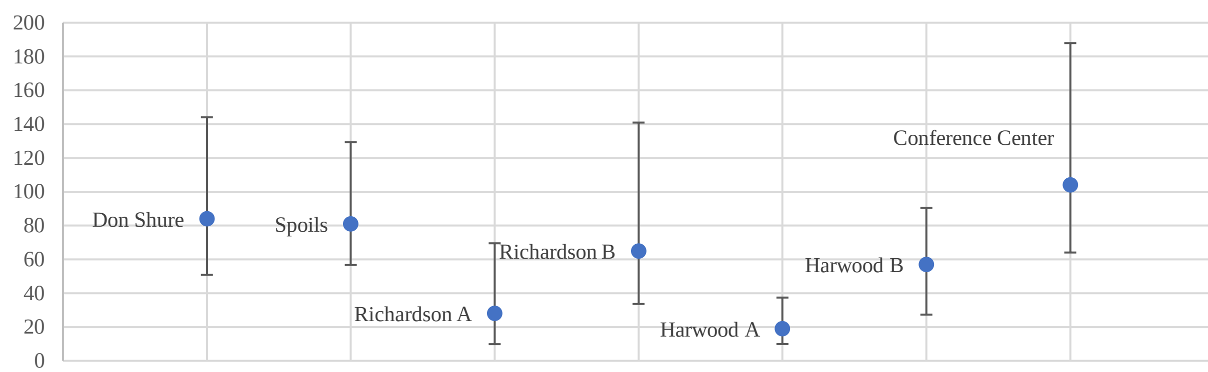

Figure 8: Salamander Population Estimates from Each Site. Collected August 17th-October 29th, 2022

Despite the large discrepancy between the population estimates between the sites, the large confidence intervals make a determination of significance between the populations difficult to determine. However, the two sites with the smallest estimated populations, Richardson Creek site A and Harwood Creek site A have the smallest amount of overlap when compared to the other five sites, making these the most noticeably different among the populations.

Environmental data were collected at each site during the two days prior to each sampling period. The measurements of stream depth and width at each meter of the site from meter one at the most downstream point to meter ten at the most upstream point. The averages from the three monthly samples are collected in Table 6, along with the averages and standard deviation for the depth and width of each stream in general.

Table 6: Stream Measurements from Each Site. Collected August 15th-October 14th, 2022. Means with Standard Deviation in Brackets

Meter	1	2	3	4	5	6	7	8	9	10	Average
Don Shure Depth (cm)	4.67	9.33	5.17	3.08	10.83	4.25	13.00	7.75	5.00	9.83	7.29 [3.33]
Don Shure Width (m)	2.24	2.20	1.65	1.42	1.94	1.03	1.39	1.06	0.71	0.74	1.44 [0.56]
Spoils Depth (cm)	8.83	1.67	4.17	2.92	1.75	1.50	2.83	1.92	3.67	9.92	3.92 [3.02]
Spoils Width (m)	0.48	0.53	0.44	0.24	1.06	1.37	1.50	1.03	2.28	1.01	1.00 [0.61]
Richardson A Depth (cm)	5.33	8.72	9.67	5.92	7.83	9.09	6.72	6.03	2.53	3.00	6.48 [2.43]
Richardson A Width (m)	1.03	1.03	0.92	0.92	2.28	1.26	1.50	0.87	0.35	0.31	1.05 [0.56]
Richardson B Depth (cm)	5.07	3.03	3.98	22.77	13.67	14.62	3.18	6.63	3.01	11.63	8.76 [6.67]
Richardson B Width (m)	1.53	2.02	1.97	2.03	1.05	0.67	0.67	0.56	0.62	0.67	1.18 [0.64]
Harwood A Depth (cm)	4.48	5.05	5.32	5.93	5.45	6.20	4.50	13.37	4.03	8.82	6.32 [2.82]
Harwood A Width (m)	0.69	0.56	0.58	0.76	0.66	1.02	0.95	1.14	1.06	1.05	0.85 [0.22]
Harwood B Depth (cm)	11.08	5.07	4.07	6.62	10.33	2.00	9.02	6.28	6.80	7.00	6.83 [2.78]
Harwood B Width (m)	0.88	0.68	0.53	1.45	1.49	0.17	0.28	0.60	1.77	1.16	0.90 [0.54]
Conference Center Depth (cm)	3.73	9.92	6.92	4.67	3.87	8.38	4.78	7.47	9.58	9.63	6.90 [2.47]
Conference Center Width (m)	1.08	0.97	1.32	1.55	2.09	1.72	0.78	1.48	0.97	0.88	1.28 [0.42]

Overall, Richardson Creek site B was on average the deepest site, with Harwood Creek site A being the shallowest. Richardson Creek site B was also the creek with the most varied depths, while Harwood Creek site A was the most consistent. The widest streams site on average were found in Conference Center Creek, with the narrowest streams being located in Harwood Creek site A. Richardson Creek site B had the most variability in width, with Harwood Creek site A having the smallest variability in width.

Samples of invertebrates were also collected in the terrestrial and aquatic areas of each stream during the environmental sampling period. These data are collected in Table 7.

Table 7: Invertebrate Collection from Each Site. Collected August 15th-October 14th, 2022

Stream	August Terrestrial	August Aquatic	September Terrestrial	September Aquatic	October Terrestrial	October Aquatic	Total Terrestrial	Total Aquatic	Total Invertebrates
Don Shure	3	1	7	1	8	1	18	3	21
Spoils	5	2	8	1	5	2	18	5	23
Richardson A	2	0	7	3	7	0	16	3	19
Richardson B	8	4	3	2	3	2	14	8	22
Harwood A	8	3	4	2	4	1	16	6	22
Harwood B	5	21	7	27	5	0	17	48	65
Conference Center	4	2	5	0	4	2	13	4	17

On average, terrestrial invertebrates were more common than aquatic invertebrates, being most commonly found in Don Shure Creek and Spoils Creek. Aquatic Invertebrates were most commonly found at Harwood Creek site B. In order of overall invertebrate abundance, the sites are Harwood Creek site B, Spoils Creek, Richardson Creek site B and Harwood Creek site A (with equal counts), Don Shure Creek, Richardson Creek site A, and Conference Center Creek.

The remaining average measurements calculated from the three monthly measurements at each site are compiled in Table 8, with standard deviation displayed in brackets. The Adopt-A-Stream Measurements is an overall measure of environmental conditions based upon a stream scorecard provided by the state of Georgia, as these measurements did not change during the sampling period, there is no standard deviation to record for this category.

Table 8: Environmental Measurements from Each Site. Collected August 15th-October 14th, 2022. Means with Standard Deviation in Brackets.

Stream	Ambient Temperature (Celcius)	Adopt-A-Stream Measurements	Ambient Humidity	Soil Moisture	pH	Turbidity (ntu)	Flow Rate (ft/sec)	TDS (ppm)	Water Temperature (Celsius)	Water Pressure (mmHg)	Dissolved Oxygen (DO %RTB)	Soil Lead Concentration (ppm)
Don Shure	23.0 [2.8]	77	73.3% [12.0%]	95.8% [3.5%]	7.3 [0.6]	0.0	0.2	53.7 [9.0]	20.7 [3.1]	758.1 [31.6]	73.3 [4.7]	34.1 [6.9]
Spoils	21.6 [3.1]	61.5	72.3% [12.3]	94.6% [3.3%]	7.5 [0.2]	6.2 [10.7]	0.2	65.0 [13.9]	20.7 [3.6]	739.8 [3.7]	64.2 [9.2]	24.5 [2.9]
Richardson A	23.3 [3.2]	57.5	75.2% [6.5%]	92.3% [5.7%]	7.7 [0.4]	9.4 [16.2]	0.8 [0.2]	106.0 [6.1]	21.3 [3.8]	740.5 [6.2]	86.4 [3.2]	26.3 [4.4]
Richardson B	22.2 [2.8]	67	77.3% [10.7%]	96.3% [4.9%]	7.8 [0.1]	10.4 [18.0]	0.3 [0.1]	112.7 [20.6]	20.6 [2.5]	739.2 [2.2]	80.0 [2.1]	24.1 [2.7]
Harwood A	23.2 [3.8]	71.5	63.6% [14.6%]	94.3% [5.0%]	7.7 [0.2]	23.2 [3.8]	0.3 [0.1]	46.3 [13.3]	21.4 [2.5]	737.6 [1.4]	84.4 [1.2]	18.7 [4.8]
Harwood B	21.5 [4.3]	70	75.2% [9.2%]	89.4% [7.2%]	7.4 [0.8]	1.4 [24.2]	0.3 [0.2]	48.0 [23.1]	19.8 [3.1]	737.0 [1.5]	78.7 [3.6]	22.5 [5.0]
Conference Center	19.5 [3.8]	75.5	69.0% [0.2]	92.5% [2.0%]	7.7 [0.1]	0.0	0.3 [0.1]	92.7 [21.0]	20.1 [2.6]	739.9 [1.2]	86.9 [1.3]	26.7 [3.9]

With the exception of the Adopt-A-Stream score, each of the environmental factors measured in Table 9 were analyzed utilizing a one-way analysis of variance (ANOVA) test followed by a post-hoc analysis utilizing the Bonferroni Correction. The initial ANOVA test suggested significant differences among the sites for four of the tested factors: flow rate (F=6.87, F-$crit$=2.22, df=5), total dissolved solids (F=8.91, F-$crit$=2.22, df=5), dissolved oxygen (F=10.12, F-$crit$=2.22, df=5), and soil lead concentration (F=12.65, F-$crit$=2.22, df=5). The following post-hoc analysis did not find any significant difference between the streams that could not be explained by data variance for flow rate or dissolved oxygen levels. Significant differences were found in the total dissolved solids levels between Don Shure Creek and Richardson Creek site A (p=1.12E-3) and Richardson Creek site A and Harwood Creek site A (p=2.12E-3). Significant differences were found in the soil lead levels between Don Shure Creek and Spoils Creek (p=2.06E-4), Don Shure Creek and Richardson Creek site B (p=1.22E-4), Don Shure Creek and Harwood Creek site A (p=2.23E-6), Don Shure Creek and Harwood Creek site B (p=1.07E-4), Spoils Creek and Harwood Creek site A (p=0.00173), Richardson Creek site A and Harwood Creek site A (p=0.000528), and Harwood Creek site A and Conference Center Creek (p=0.000449). Lead was the only unnatural pollutant measured during the environmental sampling period. Lead levels ranged from a low of 18.7 parts per million in Harwood Creek site A to a high of 34.1 parts per million in Don Shure Creek. Both of these measurements are under 50 parts per million, well within the CDC's designation for natural lead levels and far less than the 200 parts per million needed to be deemed hazardous (CDC.gov, 2022).

The Georgia Adopt-A-Stream Visual Scorecard measured a series of habitat parameters such as sediment embeddedness, bank stability, channel flow and sinuosity, vegetation cover, human impact, and the presence of the three main instream habitats: riffles, runs, and pools. The values for these factors were compiled into a single score of overall stream habitat health. Given the guidelines provided in the form, Spoils Creek and both Richardson Creek sites were considered in good condition (score of 46-68) while Don Shure Creek, the Henderson Creek Sites, and Conference Center Creek were considered in excellent condition (score of 69-90) (Georgia Adopt-A-Stream, 2020). As these values did not change throughout the course of the sampling period, an One Way ANOVA test could not be performed as it was with the rest of the environmental data.

These measurements were then compared to the previously obtained population estimates (Figure 11).

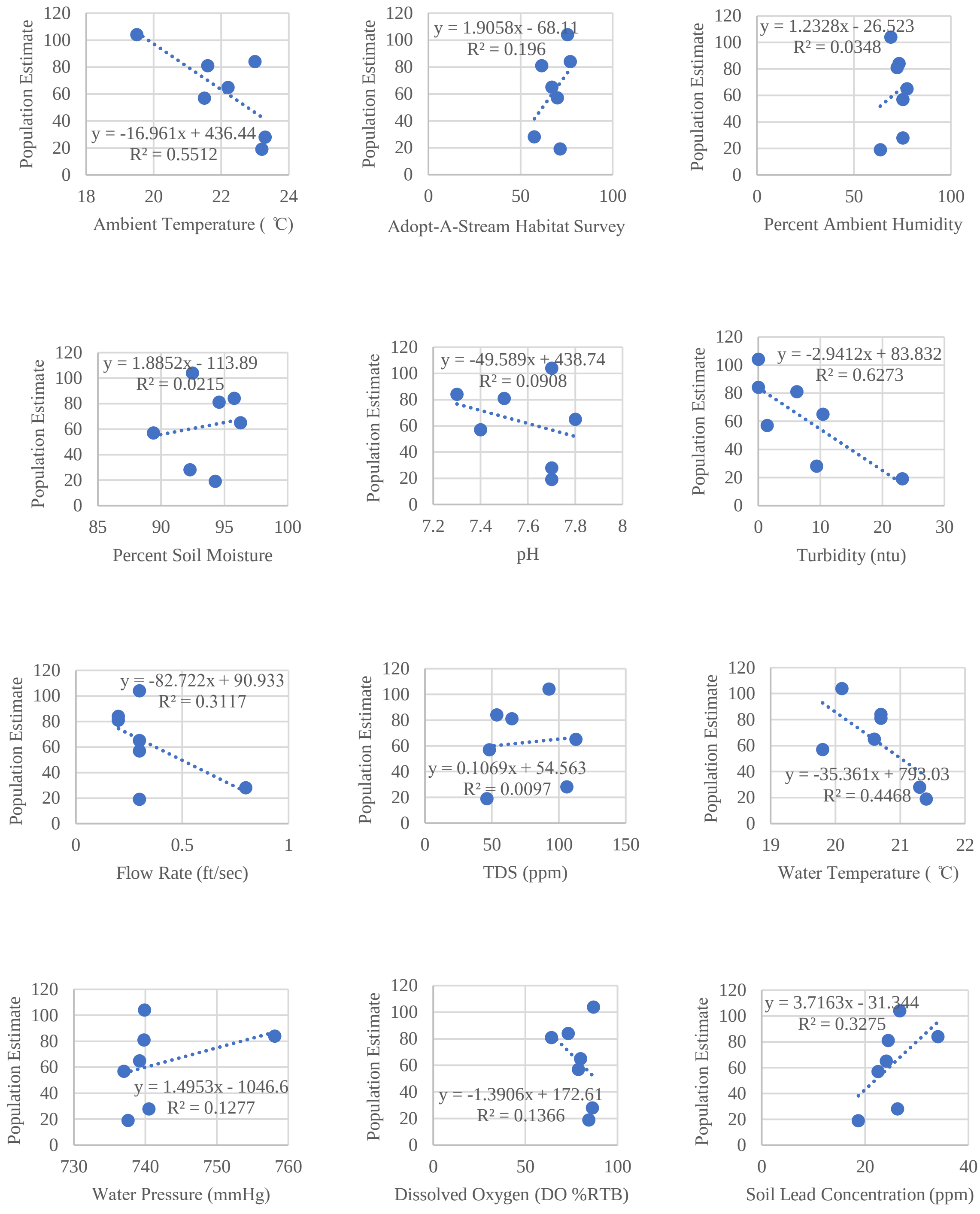

Figure 11: A Comparison of Several Environmental Conditions Along the Salamander Population Gradient

Of the twelve environmental parameters compared, only ambient temperature and turbidity demonstrated an R^2 value higher than 0.5 (0.55 and 0.63 respectively), suggesting a correlation between the two. In both cases, there was a negative correlation between population size and the environmental condition measured, with higher population being correlated with both lower ambient temperature and low levels of turbidity. A follow-up Pearson Correlation Coefficient test only found significant correlation with turbidity (t=-2.90), but not with ambient temperature (t=-2.47) utilizing a two tailed t-chart at 5 degrees of freedom.

CHAPTER IV

DISCUSSION

Significance was found between the overall number of adult Spotted Dusky and Two-Lined Salamanders captured in three of the seven sites sampled in this study: Spoils Creek (p=1.57E-5), Harwood Creek site B (p=6.60E-4), and Conference Center Creek (p=7.31E-6). While no significant difference was found between the adult Two-Lined Salamanders observed among the sites, there was significance among the adult Spotted Dusky Salamanders in the streams. Significance was found between Don Shure Creek and Richardson Creek site A (p=3.76E-6), Don Shure Creek and Harwood Creek site A (p=5.59E-6), Don Shure Creek and Conference Center Creek (p=4.79E-4), Spoils Creek and Richardson Creek site A (p=1.06E-9), Spoils Creek and Richardson Creek site B (p=4.29E-7), Spoils Creek and Harwood Creek site A (p=1.56E-9), Spoils Creek and Harwood Creek site B (p=7.08E-4), Richardson Creek site A and Conference Center Creek (p=6.17E-10), Richardson Creek site B and Conference Center Creek (p=1.10E-7), Harwood Creek site A and Conference Center Creek (p=8.55E-10), and Harwood Creek site B and Conference Center Creek (p=1.02E-4). While this suggests a wide variability between the adult Spotted Dusky Salamanders observed in the streams, it should be kept in mind that salamanders could be observed multiple times between the sampling sessions, and therefore could sway the results.

The non-target species captured during the sampling period: the Northern Slimy Salamander (*Plethodon glutinosus*), the Three Lined Salamander (*Eurycea guttolineata*), the Seal Salamander (*Desmignathus monticola*), and the Red-Backed Salamander (*Plethodon cinereus*), were all of the family Plethodontidae, or lungless salamanders. This is the same family as the two target species: the Spotted Dusky Salamander (*Desmognathus conanti*) and the Two-Lined Salamander (*Eurycea cirrigera*). Therefore, it is assumed that these salamander species prefer similar environmental conditions to the two target species chosen for their observed abundance in the streams of interest and can provide little additional data.

Most adult salamanders were captured using rocks along the stream bank as cover, nearly twice as many as the next most abundant category, which was under rocks within the stream itself (385 under bank rocks compared to 185 under stream rocks). None of the other eleven observed categories of adult salamander capture locations contained over twenty individuals. However, in Richardson Creek site B and Harwood Creek site B had small but sizable sections of their captured populations found under the prominent tree-falls present in the streams. The categories of under terrestrial/aquatic leaf litter and in stream mud made up a small proportion of the overall captured salamanders, but this is due to these categories only being added in the month of October to account for the environmental (an abundance of leaf litter in several streams) and behavioral (salamanders beginning the stages of brumation) changes that the population was experiencing.

Juvenile salamander capture locations were nearly entirely located within the stream itself, with individuals found under stream rocks making up 54.1% of the overall total and individuals found swimming in the stream itself making up 43.0% of the overall total. This is unsurprising, as the gilled juveniles are more dependent on water than their skin-breathing adult forms. ANOVA analysis did not find significance among the observed juvenile Spotted Dusky salamanders between the sites. Significance was found in the observed the abundance of juvenile Two-Lined Salamanders between Don Shure Creek and Richardson Creek site A (p=4.34E-6), Richardson Creek site A and Harwood Creek site A (p=1.32E-4), Richardson Creek site A and Harwood Creek site B (p=3.32E-6), and Richardson Creek site A and Conference Center Creek (p=4.34E-4). As with the adult Spotted Dusky Salamanders, it must be kept in mind that individuals could have been counted multiple times between the sampling sessions, possibly swaying the data.

Collected juvenile Two-Lined Salamanders greatly outnumbered collected juvenile Spotted Dusky Salamanders, making up 93% of the 314 juvenile salamanders collected overall. The low number of juvenile Spotted Dusky Salamanders collected (with none being observed in Harwood Creek site B and Conference Center Creek) made site-to-site comparison with juvenile Two-Lined Salamanders difficult. All observed nests (found in Don Shure Creek, Spoils Creek, and Harwood Creek site B) were laid by Spotted Dusky Salamanders, who were found along with their eggs. No Two-Lined Salamander nests were observed during the sampling period. This further confuses the lack of observed juvenile Spotted Dusky Salamanders. A larger proportion of Spotted Dusky Salamanders with a snout-vent length of less than 20mm were considered adults (due to the lack of visible gills) when compared to Two-Lined Salamanders (in which many of the observed juveniles were in this size range). This may suggest that subadult Spotted Dusky Salamanders lost their gills earlier than Two-Lined Salamanders, serving as a possible explanation for the observed lack of juvenile Spotted Dusky Salamanders. However, no data in the literature could be found to corroborate this claim.

The adult Spotted Dusky Salamander population estimates obtained by the Schumacher-Eschmeyer method showed that the sites in order of descending population were Conference Center Creek, Spoils Creek, Don Shure Creek, Harwood Creek site B, Richardson Creek site B, Harwood Creek site A, and Richardson Creek site A. However, the large confidence intervals of these estimates makes any conclusions on statistical significance between the stream populations difficult to ascertain. This is most likely due to the small number of captured salamanders that fit the tagging criteria: adults with a snout-vent length over 30mm. The maximum number of tagged Spotted Dusky Salamanders was 36 in Spoils Creek, with 23 of the tagged salamanders being recaptured across the 12 sampling sessions. The minimum number of tagged Spotted Dusky Salamanders was 7 in Richardson Creek site A, with 4 being recaptured across the 12 sampling sessions. While the lack of collected data is regrettable, the tagging criteria that limited the taggable individuals available were deemed necessary for the safety of the salamanders during transport, tagging, and overnight observation.

The results of the population estimate are slightly different from those found in the overall collection of adult Spotted Dusky Salamanders, in which the sites in order of descending population were Conference Center Creek, Don Shure Creek, Spoils Creek, Richardson Creek site B, Harwood Creek site B, Richardson Creek site A, and Harwood Creek site A. This can most likely be assumed to be the result of the mark-recapture section of the research only capturing adults over 30mm in snout-vent length, therefore possibly missing a section of the overall population. Marked Two-Lined Salamanders were only recaptured in the two sites located in Richardson Creek, therefore making it impossible to compare their populations across the seven sites. This lack of data, combined with their lower overall adult capture count despite having similar environmental preferences to Spotted Dusky Salamanders as fellow plethodontids raises interesting questions as to their differing habitat preferences.

Invertebrates were sampled to assess potential prey for the salamander populations. This included several species of spiders, snails, millipedes, aquatic and terrestrial annelids, and ants among others. The number of captured individuals were relatively consistent among the streams, ranging from a low count of 17 total invertebrates in Conference Center Creek to a second-to-largest count of 23 total invertebrates in Spoils Creek. One site served as an outlier, Harwood Creek site B, with a total of 65 total invertebrates. However, this was made up almost entirely of floating patches of dead Argentine ants (*Linepithema humile*) that drifted into the net. While crayfish were observed to be abundant in all sampled streams, they were not included in the invertebrate samples due to the presence of the endangered Chattahoochee Crayfish (*Cambarus howardi*) in the area. Observed crayfish, apart from Richardson Creek site B, were often similar in size with the salamanders of the stream, and therefore were more likely to serve as competition rather than a food source.

Only two of the environmental conditions sampled were found to have a statistically significant correlation with the population estimates of adult Spotted Dusky Salamanders: ambient temperature (R^2=0.55) and turbidity (R^2=0.63). A Pearson Correlation Coefficient test found significant ($p<=0.05$) correlation with turbidity (t=-2.90), but not with ambient temperature (t=-2.47). Despite ambient temperature showing significant correlation, water temperature did not (R^2=0.45). Since bank rocks were shown to be the preferable capture location of salamanders, the temperature outside of the water could have an impact on these populations even if the temperature of the water itself did not. The streams that had the smallest population estimates: Richardson Creek site A and Harwood Creek site A were both in areas in which increased temperatures could be expected. Richardson Creek site A was located in an area with comparatively little tree cover when compared to other streams and was next to a major paved pedestrian path. Harwood Creek site A was located next to a major road near the Emory Primate Research Center, an unforested meadow, and two major pedestrian footpaths. In contrast, the two streams with the highest population estimates, Conference Center Creek and Don Shure

Creek were both located in areas with heavy tree cover and a negligible level of paved surfaces or pedestrian traffic. The correlation with turbidity was expected, as research indicates that higher levels of turbidity are negatively correlated with population size for most amphibian species (Brodman, et al. 2003). While it is generally accepted that increased turbidity is associated with an increase in urbanization, the work of Miguel-Chinchilla, et al. (2019) demonstrated that this correlation is not consistent across all forms of urbanization. However, the same study does indicate that an increase in stream bank vegetation and other sediment-stabilization effects can consistently mitigate turbidity increases. The ANOVA statistical analysis found no significance between the ambient temperature and turbidity of the sites, which raises some doubt onto the significance of the correlation. Soil lead levels were not found to be present in large enough quantities to have an effect on the environment at any sampled sites.

Comparing the data obtained in this research with the work of Orser fifty years ago provides some interesting differences. Orser sampled four of the five streams utilized in this study: Don Shure Creek (referred to by Orser as “L1”), Spoils Creek (referred to by Orser as “L2”), Richardson Creek (referred to as “B” with 3 sites, “B1”, “B2”, and “B3”), and Conference Center Creek (referred to by Orser as “WW”). L1, L2, and WW were considered “less urbanized” in Orser’s work. Harwood Creek was not examined by Orser in his research, another stream was utilized instead, which was deemed to not be of interest for this study after consulting with regional experts. Orser’s research also included a large element of analyzing the age distribution of the stream, which was not possible to repeat in this study due to the difference in tagging methods. Orser found significant correlations between salamander population and air temperature (R^2=0.016) as well as salamander population and dissolved oxygen (R^2=0.019). There was not significant correlation between salamander population and water temperature

(R^2=0.062) as well as salamander population and relative humidity (R^2=0.071). Turbidity levels were also found to have significance (R^2=0.05). Orser found WW, L1, and L2 to be the most abundant sources, which is consistent with this research, in which Conference Center Creek, Don Shure Creek, and Spoils Creek were found to have the highest estimated populations. This is unsurprising for Conference Center Creek and Don Shure Creek, as these streams have been kept in a similar condition to how they were found in Orser's time. However, Spoils Creek underwent dramatic changes over the 50 years between Orser's work and this study. During the dredging of Chandler Lake, sediment was pumped to the headwaters of the stream. Over time, the dam holding back this sediment breached, introducing a large amount of rust in the sediment to the stream. This was expected to have a much larger impact on the stream's salamander populations. While Orser's research focused solely on Dusky Salamanders, he did make note of the non-target salamander species that he observed. Orser found two individuals identified as *Eurycea bislineata* (likely identified as *Eurycea cirrigera* according to modern standards) each at sites L1, L2, and B1. At L1 and B1, each site also contained a single salamander identified as a Red Salamander (*Pseudotriton ruber*). While no red salamanders were observed in this study, a vastly larger number of Two-Lined Salamanders were found when compared to Orser (1972). Discussion with individuals experienced in studying the area's salamander populations noted that Two-Lined Salamanders were commonly found in recent years. Further study into the life history differences between Two-Lined and Spotted Dusky salamanders will be needed to explain this discrepancy.

One of the main goals of this research was to provide a new baseline for continuing research and monitoring of these salamander populations. While the methodology used in this study serves as an acceptable baseline standard, several options for future expansion have made themselves apparent during the research process. The visual implant elastomer tagging method utilized is currently the safest option for tagging amphibians and related organisms (Sapsford, et al. 2015). However, it is limited in scope and cannot safely facilitate tagging of smaller and more sensitive individuals such as juveniles. Juvenile individuals were captured in abundant numbers during the tagging period, and a form of tagging that could not only track their population size, but also monitor their growth into adults. A juvenile tagging technique could also possibly give insight into urbanization-based pollution's impact on the metamorphosis process and other endocrine systems of these organisms (Thambirajah, et al. 2019). The environmental conditions measured in this study are connected to urbanization, especially the two conditions with correlation to salamander populations: ambient temperature and turbidity (Ackall, 2022) (Somers, 2013). However, there was no direct correlation made between urbanization and the status of the streams. A future study working in conjunction with a study monitoring the current impervious ground cover of the surrounding area, emissions from local motorways, and/or nearby wastewater sources could assist in determining if there is a noticeable impact on the populations of these streams. In this study, marking of individuals was halted in the month of October, as it was unclear how quickly the tagged individuals would intermix back into the population. While the effect that this has had on the project is minimal, it is recommended that future studies following this work as a baseline continue to tag individuals throughout the project, ceasing only after the penultimate tagging session, in this study the third October sampling session or the eleventh session overall. The work of Huang, et al. (2013) suggests the presence of the chytrid fungus, *Batrachochytrium dendrobatidis* in Northern Georgia. While testing for this fungus fell outside of the parameters of this specific research, it would be a worthwhile avenue for future work due to the possible impact such a fungus could have on these communities.

While direct comparison and correlation between the salamander populations and environmental conditions were less prominent than expected, the data were found to be consistent with historical data, implying that the streams sampled in both studies have remained healthy despite the increase in urbanization. The results of this research still hold value as a snapshot of the current status of the populations of these streams. The data could also serve as a possible starting point for a consistent sampling regime that could more closely monitor the streams themselves and their salamander populations for impacts from one of the Southeastern United States' most urbanized areas.

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