4-2018

If You Build It, They Will Come: A Habitat Mimicry-based Assessment Tool for Estimating Larval Salamander Density in Temperate Forests Streams

Margaret E. Maloney

If You Build It, They Will Come:

A Habitat Mimicry-based Assessment Tool for Estimating Larval Salamander Density in Temperate Forests Streams

Honors Thesis

Margaret E. Maloney
Department: Biology

Advisor: Ryan McEwan, Ph.D.

April 2018

If You Build It, They Will Come:
A Habitat Mimicry-based Assessment Tool for Estimating Larval Salamander Density in Temperate Forests Streams

Honors Thesis

Margaret E. Maloney

Department: Biology

Advisor: Ryan McEwan, Ph.D.

April 2018

Abstract

The biology of small, forested streams is critical to the ecology of larger waterways and broader watershed function. Because of their size, these "headwater streams" are strongly influenced by surrounding landscape conditions and their biology is intimately connected to the landscape through material and energy subsidies. In the United States, 50 to 80 percent of streams are primary headwater streams, making them a high priority for conservation. Often, stream salamanders are a useful indicator for biotic integrity of headwater streams due to their longevity, relatively stable populations, small home ranges, and abundance. Assessing stream salamanders is a challenging endeavor and existing methods often underestimate the abundance of salamanders present. In this project, we sought to develop and assess a new method for estimating salamander density in headwater streams. Our goal was to develop a device that mimics the habitat that salamanders prefer, leading to rapid colonization that also (a) represents a known area for estimating density and (b) is easy to handle for rapid assessment. The project sought to meet the following three aims: Aim_1 invent a prototype device for quantification of salamander density in Ohio streams, Aim_2 validate this prototype through field trials and against other methods, and Aim_3 validate this prototype in various headwater streams and seasons. Field trials revealed that this method was superior for estimating salamanders than other methods including the standard Visual Encounter Survey (VES) and allows for estimation of population density. After placing the artificial habitat in a variety of streams through multiple seasons, we found that stream salamanders will colonize the apparatus and we can estimate a fair density of salamanders present in streams.

Acknowledgements

I would like to thank the University of Dayton Biology Department, specifically Dr. Ryan McEwan and Julia Chapman. I would also like to thank the National Science Foundation, Five Rivers Metroparks, the Ohio Invasive Plant Council, and Chris Skalski from the Ohio EPA. Lastly, I would like to thank Eric Borth, and Tristan Schramer for their work, inspiration and guidance throughout this project.

UNIVERSITY *of* DAYTON

Table of Contents

Abstract	Title Page
Introduction	1
Methods	3
Results	6
Discussion	9
Literature Cited	13
Appendix	16

Introduction

The biology of small, forested streams is critical to the ecology of larger waterways and broader watershed function (Vannote et al., 1980). Because of their size, these “headwater streams” are strongly influenced by surrounding landscape conditions and their biology is intimately connected to the landscape through material and energy subsidies. In the United States, 50 to 80 percent of streams are primary headwater streams, making them a high priority for conservation (Leopold et al. 1964; Meyer and Wallace 2001). A variety of assessment tools are available that allow for estimations of the biotic integrity of a headwater stream including rapid assessment tools. Many of these methods rely on the geomorphological characteristics of the stream including factors such as sinuosity, flow and bankfull depth (Rosgen, 1985; Barbour et al., 1999). While effective for a rapid assessment, these geomorphological characteristics do not always capture the biological functioning of the system (Yoder and Rankin, 1998). Assessment of physicochemical parameter such as DO, temperature, and pH are a useful way to augment a geomorphological-based assessment, but represent only a moment in time and may not reflect factors that impair the longer-term biology of the stream (Barbour et al., 1999). Common modes of biotic assessment include sampling fish and macroinvertebrate populations; however, primary headwater streams are commonly fishless, and macroinvertebrate identification relies on challenging taxonomic distinctions and can be time consuming and often require access to microscopes.

Stream salamanders are a useful indicator for biotic integrity of headwater streams due to their longevity, relatively stable populations, small home ranges, and abundance (Jung, 2002). Salamanders are the most abundant vertebrate animal in a headwater ecosystem and are the primary predator in fishless streams (Burton et al., 1975). These salamanders rely on aquatic biota as a main food source, and shifts in allocthonous inputs can have detrimental effects on salamander populations (Johnson and Wallace, 2005). *Plethodontid* (lungless) salamanders exhibit high sensitivity to pollutants and extreme selectiveness of streams for their breeding site (Jung, 2002; Moore, 2010; Welsh and Ollivier, 1998). Stream salamanders breathe through their skin, and rely on excellent water quality in order to thrive. If there are changes in temperature, DO, or chemistry in the stream, salamanders are often the first to respond (Orser & Shure, 1972; Willson & Dorcas, 2003). For instance, detrimental effects on salamander populations have been documented in response to perturbations such as those associated with acid mine drainage or nutrient input from runoff which can cause serious population declines and render the stream uninhabitable to these organisms streams (Orser & Shure, 1972; Schorr et al., 2013).

Assessing stream salamanders is a challenging endeavor and existing methods often underestimate the abundance of salamanders present (Nowakowski & Maerz, 2009; Peterman & Turslow, 2008). Salamander larvae are small, cryptic and mobile making them challenging organisms to survey in the field and their population density is often grossly underestimated (Burton et al., 1975). Perhaps the most common method for assessment is the visual encounter survey (VES) which involves flipping every stone, leaf, and piece of woody debris to find salamanders within a prescribed length of stream reach over an allotted time interval (Ohio EPA, 2012; Barr and Babbitt, 2001). When these salamanders are found, they are collected and identified to life stages and species after which all salamanders are released. These surveys are time consuming, labor intensive, and relatively destructive to the stream. More recently, studies have utilized leaf packs to estimate population density of larval salamanders (Nowakowski & Maerz, 2009; Peterman & Turslow, 2008). These habitat-mimicking methods are an effective tool; however, salamanders can colonize beneath the leaf pack apparatus resulting in underestimated density (C. Skalski, Ohio EPA, *unpublished data*). Moreover, although leaf litter studies are good for estimating species richness, these studies do not accurately estimate abundance and are not the best sampling method for population density (Chalmers and Droege, 2002). Previous work has found that while this method has some potential estimating species richness, larval salamanders did not seem to colonize the bags in comparison to other substrates and a good population estimate was not able to be calculated (Chalmers and Droege, 2002; Moore, 2010). Lastly, researchers often use dip net sampling in order to sample salamanders in streams. However, dip net sampling can be disruptive, and requires experience in order to maintain a consistent technique to estimate population density (Skelly & Richardson, 2010). Moreover, dip net sampling does not readily allow for estimates of population density (Werner et al., 2007).

In this project, we sought to develop and assess a new method for estimating salamander density in headwater streams. Our goal was to develop a device that mimics the habitat that salamanders prefer, leading to rapid colonization that also (a) represents a known area for estimating density and (b) is easy to handle for rapid assessment. The project sought to meet the following three aims: Aim_1 invent a prototype device for quantification of salamander density in Ohio streams, Aim_2 validate this prototype through field trials and against other methods, and Aim_3 validate this prototype in various headwater streams and seasons.

Methods

The device (which we call the Maloney Salamander Hotel (MSH)) is positioned in the headwater stream and provides artificial habitat for larval stream salamanders (Figure 1, left panel). This device was then standardized in the following ways: (a) two stacked tempered hardboard are placed in the device to provide a known surface area and (b) 300 mL of gravel from the stream bed into which the device will be placed are spread on each board to mimic natural salamander habitat (Figure 1, right panel).

Figure 1: Maloney Salamander Hotel is constructed from a dishwashing rack lined with fine window mesh (left panel). 3 tempered hardboards are lined with 300 mL gravel and insert into the hotel to provide habitat (right panel) and placed facing upstream.

These devices allow salamanders to freely swim in and out while providing cover that makes for attractive habitat for the organisms. The device can be easily retrieved; the salamanders are counted in the MSH and then promptly released. In addition, in order to ensure that salamanders were not lost or injured during sampling: (a) the mesh at the bottom of the MSH was slightly buried into the stream bed to insure that salamanders do not colonize beneath the structure; (b) as the MSH was removed from the stream, it was tilted so the salamanders would drift to the back of the apparatus and (c) the MSH was placed in a clear plastic bin afterward for easy sorting. The shelves inside the MSH were carefully sorted in the bin, and a sieve was used to drain the water out to ensure that no larval salamanders were missed in the process. Specifications for the MSH are outlined in Appendix A.

We conducted three experiments to assess the viability of the MSH for rapid assessment of populations in headwater streams. The objective of the first experiment was to assess salamander colonization time for the MSH. Three trials were performed in the summer of 2016 to assess the length of time needed for salamanders to colonize the MSH. The colonization trial was performed in a first order perennial stream (known as Patty Falls) located at Englewood Metropark (39°53'09.9"N 84°16'44.7"W**).** We had previously visually observed a dense and species rich community of salamanders in this stream. On a 9 meter transect, 9 prototypes were placed at random distances. Next, 3 prototypes were then randomly sampled after 3, 6, and 9 days. During each sampling, the salamanders were counted and identified to species and life stage. This trial was repeated three times.

In the second experiment, we compared the results of our new MSH to results obtained with established sampling methods to verify the its utility for assessing (a) species richness and (b) population density. This experiment was conducted in two primary headwater streams. The first stream was Patty Falls detailed in experiment one. The second stream (known simply as Englewood) was also located at Englewood Metropark (39°52'57.3"N 84°17'01.5"W**)** and was a first order, perennial stream with similar geomorphological features to Patty Falls, in which we had previously visually observed a lower abundance but similar richness of salamanders. Thus, the experiment was designed to assess performance of the MSH in comparison to other methods with both high and low densities of salamanders. First, a visual encounter survey was conducted using guidelines from the Ohio EPA Primary Headwater Habitat Assessment (Ohio EPA, 2002). A 9 meter reach was sampled with all salamanders collected, identified to species and life stage, and released after the entire reach had been sampled. Second, a quadrat-style sampling was done using a Hess sampler. Along a 9 meter transect in both streams 3 random locations were selected, the Hess sampler was installed in those locations and all salamanders were identified to species and life stage and released. Lastly, the MSH was installed in each of the streams for 3 days. After 3 days, the MSH were collected and salamanders were identified to species and life stage. Each method was tested on each stream during the summer, with three days between each method performed.

Lastly, the MSH was validated by placing them out in headwater streams throughout Montgomery County, Ohio. Three primary headwater streams were identified throughout the county. These headwater streams had a variety of conditions one might come across when sampling. The first stream was located at Englewood Metropark (39°52'44.2"N 84°16'18.4"W) and was a second order stream. The substrate of the stream was mostly cobble and gravel. The second stream was also located at Englewood Metropark (39°52'55.7"N 84°16'56.9"W) and was a first order stream. The substrate of this stream was composed of leaf litter, wood debris and silt primarily. The last stream was located off of the Buckeye Trail Bike Path (39°54'52.0"N 84°10'06.4"W) and was a first order stream. The substrate of this stream was primarily muck with minimal cobble and woody debris. Three MSH were placed randomly on a 9 meter transect within each stream for 3 days. A trial was repeated throughout each season to verify the best time to sample and to ensure salamanders would colonize the hotel.

Results

The number of salamanders found within the MSH during the colonization trial was relatively stable through time indicating rapid colonization and a stable estimate of numbers. In the first trial, the number of salamanders detected was 2 ± 0.5 at all three sampling points (Figure 2, top panel). During the second trial, the number detected declined slightly on day 9, and there was an increase of average number of salamanders on day 9 during the third trial, though there were no significant differences among the dates (P = 0.623; Figure 2). Throughout the duration of the trials, 39 larval salamanders were caught, 37 were *Eurycea cirrigera* and the other two were *Desmongnathus fuscus*. There was variation in temperature throughout the sampling event, which is why some of the fluctuations in the number of salamanders that were caught over the nine days (Figure 3).

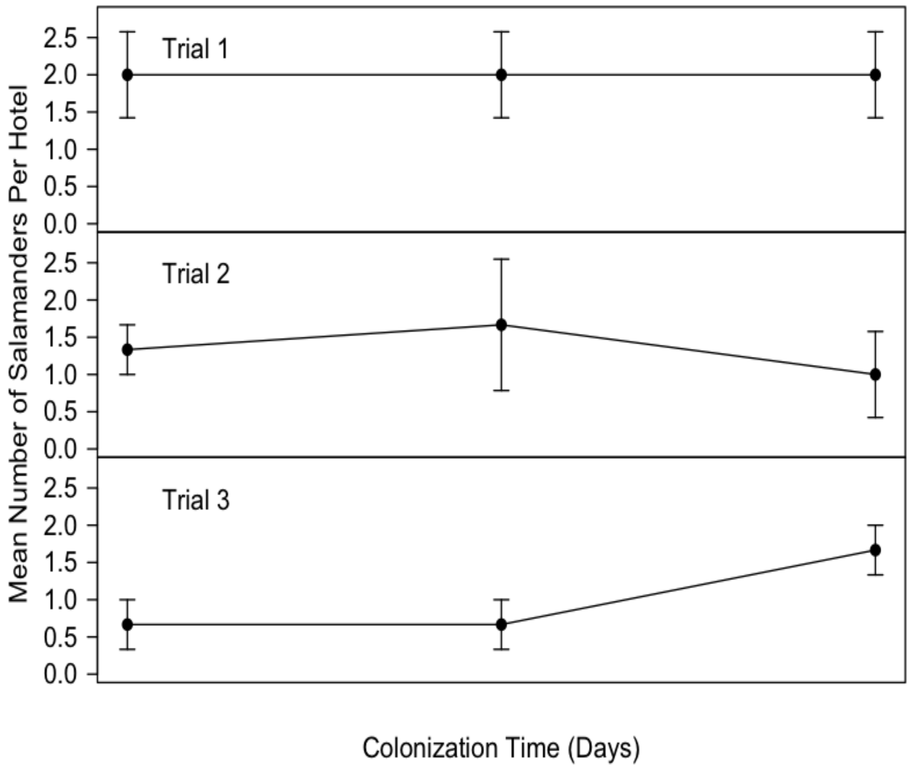

Figure 2: Mean number of salamanders found per Maloney Salamander Hotel over a 9 day trial period which was repeated three times.

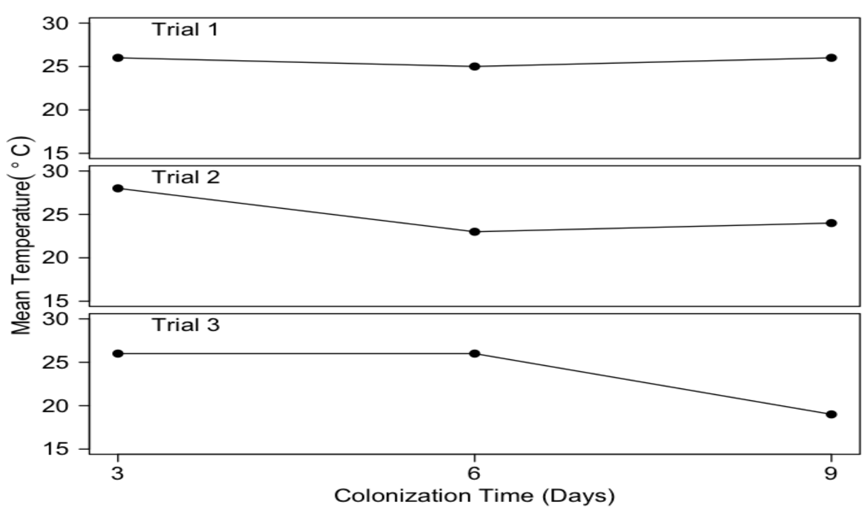

Figure 3: Mean temperature during the collection day of each Maloney Salamander Hotel.

Overall, more salamanders were found per square meter in the MSH than found in the other two methods. The MSH estimated a higher density of 22 salamanders per square meter than the Visual Encounter Survey. The MSH also estimated a higher density of 26 salamanders per square meter than the quadrat sampling (Figure 4). There were 3 larval *E. cirrigera* that were found at the high density site (Patty Falls) while only 1 larval *E. cirrigera* was found at the low density site. In addition, when conducting at VES survey, 188 salamanders were found at the high density site and 13 were found at the low density site. While conducting each test, the Hess sampler ensured that no salamanders escaped study area. However, 5 salamanders were lost (escaped capture) when conducting the VES survey.

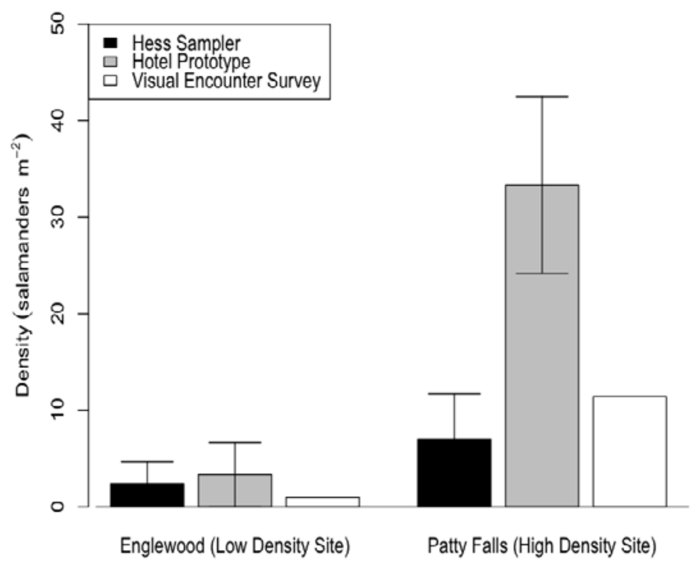

Figure 4: Density of salamanders found within a high and low density streams in Montgomery County, OH. Three methods were compared to understand which method estimated the most accurate density.

Lastly, the Salamander Hotel was placed in a variety of headwater streams to understand ideal time to use the device as well ensuring the device could withstand a variety of stream conditions. The best time to sample salamanders with this device is during the winter and spring months (Figure 5). While summer did attract some larval salamanders (n=2), there were not nearly as many larval salamanders present. In the fall months, there were not any salamanders found at any of the sites due to the ample leaf litter present. Moreover, the MSH was also able to withstand a variety of stream conditions. The MSH held up through some strong summer storms in July, and some flash flood events in the spring. The MSH was also able to attract salamanders into the hotel (during peak sampling time) despite the differing stream conditions. This proves that salamanders will colonize the hotel in most streams.

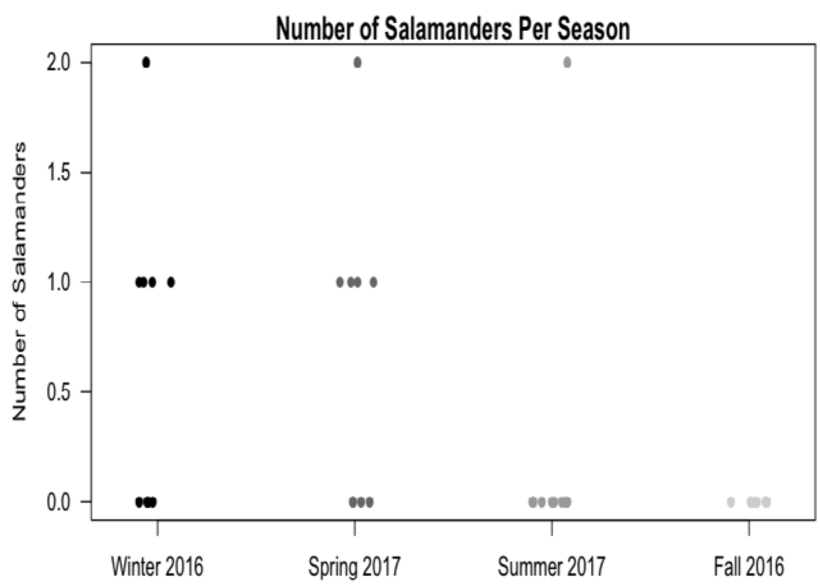

Figure 5: Mean number of salamanders found for each season in the Maloney Salamander Hotel. The best time to sample salamanders would be late winter and spring of each year.

Discussion

Larval salamanders of the *Plethodontidae* breathe through their skin and have a larval stage that can last from 1 to 5 years (Moore, 2010) making them sensitive to changes in stream conditions and an excellent indicator of biological integrity. We developed a new method to assess stream salamander populations that has a number of advantages for use in both research and rapid assessment. For a method to provide valid estimates of salamander populations, immigration or emigration must be minimized and all animals must have the same likelihood of being sampled. Furthermore, the method should ensure that salamanders are not lost during the sampling and the sampling should not impact their survivability (White, 1982; Peterman & Turslow, 2008). Previous studies have shown that salamanders are unlikey to move more than 1 meter from where they are captured (Johnson & Wallace, 2005); therefore, it is unlikely that immigration or emmigration are problematic for salamander estimates using the MSH. Because the MSH is placed within the stream and organisms colonize on their own, this method provides equal opportunity for sampling any particular organism in the stream. Lastly, no salamanders were harmed during any sampling. It is also important to note that due to how well the MSH attacts salamanders into the hotel, rarely were salmanders found colonizing underneath the hotel or were lost during sampling.

Seasonaility is an important consideration for assessing streams using salamanders as an indicator species. When there is an abundant amount of leaf litter present (mostly in the fall months), it leaves ample areas for salamanders to seek shelter and there will not be many salamanders present in the hotels. It is also difficult because as the temperature drop, salamander seek shelter in areas underground (Taub, 1961). Therefore, it is best to sample salamanders in the late winter or spring. During the early winter, adult salamanders begin to lay egg masses in the stream (Smith 2008). In the late spring, larval salamanders hatch and there are a more abundant population of larval salamanders to sample (Smith, 2008). We have also found larval salamanders in muddy areas using the MSH, but the population density is far less than in higher quality streams.

Estimating the density of salamanders in a headwater stream is challenging and the MSH provides some advantages. Previous studies have shown that current sampling methods usually underestimate the actually density of salamanders present (Taub, 1961; Dodd and Dorazio, 2004). The MSH estimated a higher density of salamanders present which we believe is a truer representation of the amount of salamanders present. Because most salamanders dwell underground and are also the most common vertebrate in headwater streams, the higher the density estimate the truer the actual estimation is to populations. Visual Encounter Surveys are extremely disruptive and often miss salamanders that are in a certain area of the stream. In contrast, this MSH method has minimal disturbance on the stream and allows for salamanders in the known area not to be missed. Because the salamanders do not have a preference in colonization time, the researcher can place the hotel out for any time that is convinent and retrieve based on the researchers availability.

This is far more convienent than a VES, which can be time consuming if the stream has a higher density of salamanders. It is recommended the hotels are left out for at least 24 hours but they can be retrieve at convienence. To set the Salamander Hotel out takes about 10 minutes for each Salamander Hotel and approximately 20 minutes each for retrevial. In addition, these Salamander Hotels have weathered a multitude of conditions such as flash floods in July and heavy snows in January. It is recommended that if the streams has flash flooding, that the Salamander Hotel is anchored using zipties and inert stakes within the stream.

Lastly, while adult salamanders did not lay egg masses within the hotel, there is reason to believe that this could be possibility. During the sampling, there was an egg mass of another organism that was layed on the bottom of one of the hardboards.

Optimized sampling with the MSH would take into consideration local habitat conditions. For other researchers interested in using this sampling method, it is recommended that the researcher is strategic about the placement of the Salamander Hotel. Larval salamanders have a preference of areas they tend to dwell in, streams with cool, bubbly, rocky areas are prefered instead of deep pools or muddy areas (Moore, 2010; Ohio EPA, 2012). Therefore, the placement of the Salamander Hotel should be considered before placing the prototype into the stream.

Literature Cited

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers. *USEPA, Washington*.

Barr, G. E., & Babbitt, K. J. (2001). A comparison of 2 techniques to sample larval stream salamanders. *Wildlife Society Bulletin*, 1238-1242.

Burton, T. M., Likens, G. E., Brook, H., Forest, E., Hampshire, N., Burton, T. M., & Likens, G. E. (1975). Salamander Populations and Biomass in the Hubbard Brook Experimental Forest , New Hampshire. *Copeia*, *1975*(3), 541–546.

Chalmers, R. J., & Droege, S. (2002). Leaf Litter Bags as an Index to Populations of Northern Two-Lined salamanders (Eurycea bislineata). *Wildlife Society Bulletin*, *30*(1), 71–74. Retrieved from http://www.jstor.org/stable/10.2307/3784637

Dodd Jr, C. K., & Dorazio, R. M. (2004). Using counts to simultaneously estimate abundance and detection probabilities in a salamander community. *Herpetologica*, *60*(4), 468-478.

Johnson, B. R., & Wallace, J. B. (2005). Bottom-up limitation of a stream salamander in a detritus-based food web. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(2), 301–311. http://doi.org/10.1139/f04-197

Jung, R. E. (2002). *Streamside Salamanders Inventory and Monitoring*. Retrieved from http://www.pwrc.usgs.gov/nearmi/nearmi/projects/Stream Salamander Protocol.pdf

Leopold, L. B., M. G. Wolman, AND J. P. Miller. 1964. Fluvial processes in geomorphology. Dover Publications, New York.

Meyer, J. L., AND J. B. Wallace. 2001. Lost linkages and lotic ecology: rediscovering small streams. Pages 295–317 in M. C. Press, N. J. Huntly, and S. Levin (editors). Ecology: achievement and challenge. Blackwell Science, Oxford, UK.

Moore, E. L. (2010). *An Index of Biotic Integrity for Macroinvertebrates and Salamanders in*

Primary Headwater Habitat Streams in Ohio. *Electronic Thesis or Dissertation*. Ohio State Univeristy. Retrieved from https://etd.ohiolink.edu/

Nowakowski, A. J., & Maerz, J. C. (2009). Estimation of Larval Stream Salamander Densities in Three Proximate Streams in the Georgia Piedmont. *Journal of Herpetology*, *43*(3), 503–509. http://doi.org/10.1670/07-128R2.1

Ohio EPA. (2012). *Field Evaluation Manual for Ohio ' s Primary Headwater Habitat Streams* (Vol. 2012). Retrieved from http://www.epa.state.oh.us/portals/35/wqs/headwaters/PHWHManual_2012.pdf

Orser, P. N., & Shure, D. J. (1972). Effects of Urbanization on the Salamander Desmognathus Fuscus Fuscus. *Ecology*, *53*(6), 1148–1154. Retrieved from http://www.jstor.org/stable/1935428

Peterman, W., & Turslow, S. (2008). Density estimation of larval Eurycea wilderae: a comparison of mark- recapture and depletion sampling. *Herpetological Review*, *39*(4), 438–442. Retrieved from http://petermanresearch.weebly.com/uploads/2/5/9/2/25926970/peterman_truslow_2008--euryceadensity.pdf

Rosgen, D. L. (1985, April). A stream classification system. In *Riparian Ecosystems and Their Management. First North American Riparian Conference. Rocky Mountain Forest and Range Experiment Station, RM-120* (pp. 91-95).

Schorr, M. S., Dyson, M. C., Nelson, C. H., Van Horn, G. S., Collins, D. E., & Richards, S. M. (2013). Effects of stream acidification on lotic salamander assemblages in a coal-mined watershed in the Cumberland Plateau. *Journal of Freshwater Ecology*, *28*(3), 339–353. http://doi.org/10.1080/02705060.2013.778219

Skelly, D. K., & Richardson, J. L. (2010). Larval sampling. *Amphibian ecology and conservation: a handbook of techniques. Oxford Univ. Press, Oxford, UK*, 55-70.

Smith, S. N. (2008). Two-lined salamanders: Eurycea bislineata complex. *Amphibians and Reptiles of Georgia. The University of Georgia Press, Athens, GA*, 186-189.

Taub, F. B. (1961). The Distribution of the Red-Backed Salamander , Plethodon C . Cinereus , within the Soil. *Ecology*, *42*(4), 681–698.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137. http://doi.org/10.1139/f80-017

Welsh, Hartwell H., Ollivier, L. (1998). Stream Amphibians as Indicators of Ecosystem Stress : A Case Study from California ’ s Redwoods Author (s): Hartwell H . Welsh , Jr . and Lisa M . Ollivier Published by : Ecological Society of America Stable URL : http://www.jstor.org/stable/2640966. *Ecological Applications*, *8*(4), 1118–1132.

Werner, E. E., Skelly, D. K., Relyea, R. A., Yurewicz, K. L., Werner, E. E., Skelly, D. K., … Yurewicz, K. L. (2007). Amphibian Species Richness across Environmental Gradients, *116*(10), 1697–1712. Retrieved from http://www.jstor.org/stable/10.2307/3784637

White, G. C. (1982). *Capture-recapture and removal methods for sampling closed populations*. Los Alamos National Laboratory.

Willson, J. D., & Dorcas, M. E. (2003). Effects of Habitat Disturbance on Stream Salamanders: Implications for Buffer Zones and Watershed Management. *Conservation Biology*, *17*(3). http://doi.org/10.1111/een.2010.35.issue-4/issuetoc

Yoder, C. O., & Rankin, E. T. (1998). The role of biological indicators in a state water quality management process. *Environmental monitoring and assessment*, *51*(1-2), 61-88.

Appendix A

Artificial Habitat Method for Salamander Sampling:

Building the Artificial Habitat:

Materials:

1. Dishwashing rack (Ensure that it is made of inert materials)
2. Window mesh (Charcoal fiberglass screen mesh, 20 feet)
3. 1 Tempered Hardboard (3/16 inches, 8 feet by 8 feet)
4. PVC Pegs *Optional* (3/4 inch)
5. Invert 12 inch stakes (4 per hotel)
6. Zip-ties
7. 1ft by 2 ft by 0.5 inch Rubber-made container

Procedure (15 minutes per hotel):

1. Cut window mesh to cover the inside of the dishwashing rack completely.
2. Zip-tie window mesh to the inside of the dishwashing rack and ensure that no holes are present.
3. Cut tempered hardboard to fit as shelving units in the inside of the dishwashing rack. Each hotel should have between 2-3 shelving unis.
4. Optional: Glue PVC pegs on each corner of the tempered hardboard to help the shelving stay upright.

Sampling the Artificial Habitat:

Setting Up the Hotel (5-10 minutes per hotel):

Description: When sampling a headwater stream, look for areas with cobble and gravel present. These areas will usually have higher populations of salamanders present. While salamanders *will colonize hotels after 24 hours, it is recommended that the hotels remain in the water for 3-5 days before sampling.*

1. Place 300 mL of gravel from the stream bed onto 2-3 shelves. Place one shelf with no gravel on top of the shelves.
2. Stack the shelves within the hotel.
3. Place the hotel apparatus facing upstream so water flows through the hotel.
4. Bury the bottom of the hotel slightly into the bottom of the stream to avoid salamanders swimming underneath the hotel.
5. If needed, stake the hotel down into the stream bed using an inert stake and zip-ties.
6. Add rocks and leaf litter atop the hotel to help hotel blend into the natural environment.

Sampling the Hotel Apparatus (10-15 minutes per hotel)

1. Start at the most downstream hotel to ensure the habitat is not disturbed. Approach the hotel and tip it backwards so that the contents fall to the back.
2. Immediately place the hotel in clear plastic rubber-made bin to help no salamander escape.
3. Slowly take out each shelving unit and sort through the gravel carefully.
4. Examine the leftover contents in the hotel. Most likely the salamanders have fallen backwards and are sitting on the window mesh hidden beneath rocks that have fallen off the shelves.
5. Drain excess water through a small sieve to ensure no salamanders are missed.
6. Record the species and life stage of every salamander found.
7. Repeat the process with the next downstream hotel.