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Development of a New Habitat Mimicking Tool for Assessing Larval Salamanders in Temperate Forest Streams

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Research Article

Development of a New Habitat Mimicking Tool for Assessing Larval Salamanders in Temperate Forest Streams

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ABSTRACT

Small streams are a high priority for conservation and an important target for biomonitoring. Stream salamanders are a useful indicator for biotic integrity of headwater streams; however, assessing stream salamanders is a challenging endeavor and existing methods can cause habitat disturbance or require expensive equipment. Our goal was to develop an artificial habitat that mimics the natural habitat that salamanders prefer, leading to rapid colonization and that also (1) represents a standardized area and (2) is easy to handle for rapid assessment. After developing a new artificial habitat (the Maloney Salamander Hotel), we tested the device in a variety of streams and compared our method with other techniques. After a series of field tests in a variety of streams through multiple seasons, we found that stream salamanders will colonize the apparatus and that this method yielded results similar or superior to other methods. For efficient and relatively simple assessment, our results indicate that three devices, left in streams for 3 d, should provide a reliable assessment of salamander presence. The device is relatively inexpensive, simple to build, easily handled for efficient deployment and collection, and does not harm the salamanders. We hope the development of this device provides a useful innovation for biological consultants, land managers, and researchers interested in assessing salamander presence in streams.

Index terms: headwater stream; Maloney Salamander Hotel; Plethodontidae; rapid assessment

INTRODUCTION

Small headwater streams are strongly influenced by surrounding landscape conditions and their biology is intimately connected to the landscape through material and energy subsidies. These headwater streams are critical to the ecology of larger waterways and broader watershed function (Vannote et al. 1980). In the United States, 50–80% of streams are primary headwater streams (drainage area <1 square mile), making them a high priority for conservation (Leopold et al. 1964; Meyer and Wallace 2001). These streams provide a wide variety of ecosystem services such as nutrient flux mitigation, flood control, and filtration for downstream human use (Palmer and Richardson 2009; Palmer et al. 2014). Small forested streams also provide resources for lotic food webs, which support the fishing industry and various forms of human recreation (Meyer et al. 2005). A variety of assessment tools are available that allow for estimation of the biotic integrity of headwater streams. Many rapid assessment 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 accurately represent the biological functioning of the system (Yoder and Rankin 1998). Physicochemical parameters such as dissolved oxygen, temperature, and pH are useful measures that 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 rapid bioassessment include methods that focus on sampling fish and macroinvertebrate populations; however, primary headwater streams are commonly fishless (Ohio EPA 2012), and macroinvertebrate identification relies on challenging taxonomic distinctions that can be time consuming and may require access to microscopes and other specialized equipment.

Salamanders that dwell in streams are a useful indicator for biotic integrity of headwater streams due to their longevity, relatively stable populations, small home ranges, and abundance (Petranka 1998; Jung 2002). Salamanders are often the most abundant vertebrate animal in a headwater stream and are the primary predators in fishless streams (Burton and Likens 1975). These salamanders rely on aquatic biota as a main food source and shifts in the quality or abundance of allochthonous inputs can have detrimental effects on salamander populations (Johnson and Wallace 2005). Adverse 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 (Orser and Shure 1972; Willson and Dorcas 2003; Schorr et al. 2013). Stream hydrology is a critical aspect of reproductive success for populations of salamanders with an obligate aquatic life phase and the larval phase of the life cycle can be 1–2 y or more. Aside from actual water quality issues, desiccation of the stream as a result of physical disturbance or destruction of the stream bed or associated hydrological sources such as springs and seeps can completely eliminate stream salamander populations. Alterations in stream temperature and degradation of stream habitat quality may also limit success of larval salamanders.

Due to their biology, plethodontid (lungless) salamanders are excellent bioindicators of overall stream biology. Larval salamanders of the Plethodontidae family breathe through their skin and have a larval stage that can last 1–5 y (Moore 2010), making them sensitive to changes in stream conditions and excellent indicators of biological integrity. These salamanders exhibit high sensitivity to pollutants and extreme selectiveness of streams for their breeding site (Welsh and Ollivier 1998; Jung 2002; Moore 2010) and are reliable indicators of water quality. For example, Willson and Dorcas (2003) found a strong inverse relationship between the percentage of disturbed habitat within a watershed and the number of salamanders present in streams. Long-term data have shown that plethodontids are strong indicators of intact forest in New England (Siddig et al. 2019) and Schorr et al. (2013) found extreme sensitivity of salamanders to disturbance from acid mine drainage associated with coal mining. Due to vulnerability to all of these disturbance processes, salamanders in the family Plethodontidae are a highly sensitive indicator of longer-term stream integrity.

Assessing stream salamanders is a challenging endeavor and common methodologies for sampling salamanders often require specialized skills or access to expensive equipment (Peterman and Truslow 2008; Nowakowski and Maerz 2009). Salamander larvae are small, cryptic, and mobile making them challenging to survey in the field (Burton et al. 1975). Perhaps the most common method for assessment is the Visual Encounter Survey (VES), which involves disturbing ("flipping") all potential salamander habitats in a stream including stones, leaves, and woody debris to find salamanders within a prescribed length of stream reach over an allotted time interval (Barr and Babbitt 2001; Ohio EPA 2012). When these salamanders are found, they are collected and identified to life stage and species after which all salamanders are released. These surveys are time consuming, labor intensive, require clear water, and may be disruptive to the stream. Dip net sampling is another technique used by researchers to sample salamanders in streams; however, dip net sampling can also be disruptive to the stream and requires experience and training to be effective (Skelly and Richardson 2010). More recently, studies have utilized leaf packs to assess populations of larval salamanders (Peterman and Turslow 2008; Nowakowski and Maerz 2009). These habitat-mimicking methods are an effective tool; however, many salamanders colonize beneath the leaf pack apparatus resulting in underestimations (Pauley and Little 1998). Our field observations indicate that salamanders prefer stony materials over leaf material and Chalmers and Droege (2002) hypothesized that more salamanders would colonize leaf packs if there were a large flat rock placed on top of the leaf pack. Previous work has found that while the leaf pack method has some potential for estimating species richness, larval salamanders did not seem to colonize the bags equally to other substrates and that the use of leaf packs most likely results in underestimates of salamander numbers (Chalmers and Droege 2002; Moore 2010). New techniques for salamander observation may be useful, especially to natural areas managers and others involved with practical land stewardship. In this project, we sought to develop and assess a new method for estimating salamander abundance in headwater streams. Our overall objective was to develop an artificial substrate that mimics the habitat that salamanders prefer, leading to rapid colonization that also (1) could be standardized for research applications and (2) is easy to deploy and retrieve for rapid assessment. We conducted a variety of field tests to validate our method, assess colonization period, compare the prototype device to other methods, and assess the colonization across seasons.

METHODS

Description of Maloney Salamander Hotel

Our overall objective was to create a sampling apparatus that mimicked habitats that salamanders prefer while being easy to handle and providing a known surface area. After experimenting with various models, we settled on an apparatus that is constructed using a standard dishwashing rack (Figure 1, top left). This type of rack is easy to handle, provides a frame for colonization substrate, and is both relatively inexpensive and readily available to researchers. The artificial habitat was standardized in the following ways: (1) two stacked tempered hardboards were placed in the artificial habitat to provide a known surface area (in our model, 0.06 m^2) and (2) a measured amount of gravel or other substrate found in the stream bed was spread on each board to mimic natural salamander habitat (in our model, 300 mL of gravel; Figure 1, top left). These artificial habitats allow salamanders to freely swim in and out of the front of the device and provide cover that makes for an attractive habitat for the organisms (Figure 1, top right). The device can be easily retrieved, and the rack makes for stable handling even when wet (Figure 1, bottom left). To ensure that salamanders were not lost or injured during sampling, (1) the mesh at the bottom of the device was slightly buried into the stream bed to prevent salamander colonization beneath the structure, (2) as the device was removed from the stream it was tilted so the salamanders would drift to the back of the apparatus, and (3) the entire device was placed in a large clear plastic bin to facilitate sorting (Figure 1, bottom right) while preventing loss of salamanders. The shelves inside the device were carefully sorted in the bin, and water was drained through a sieve to ensure that no larval salamanders were missed in the process (Figure 1). Specifications for the sampling apparatus are outlined in Appendix A.

Field Methods

We conducted four field tests to assess the viability of the apparatus, which we colloquially called the Maloney Salamander Hotel (MSH), for assessment of salamanders in primary headwater streams (Figure 1; Appendix A). Our first field tests occurred during February and March of 2016 (Maloney et al. 2018b). In each trial, three MSH were installed in a first-order perennial stream (locally known as Patty Falls; drainage area 0.04 square miles) located at Englewood Metropark in southwestern Ohio (39°53′09.9″N, 84°16′44.7″W, WGS84). The sampling site is ideal for stream-dwelling salamanders as it is located near a consistent groundwater seep with clean, cool water and had a gravel substrate with large pieces of cobble. The MSH were staggered between runs and riffles. For the first experiment three MSH were installed on 8 February 2016, MSH were checked on

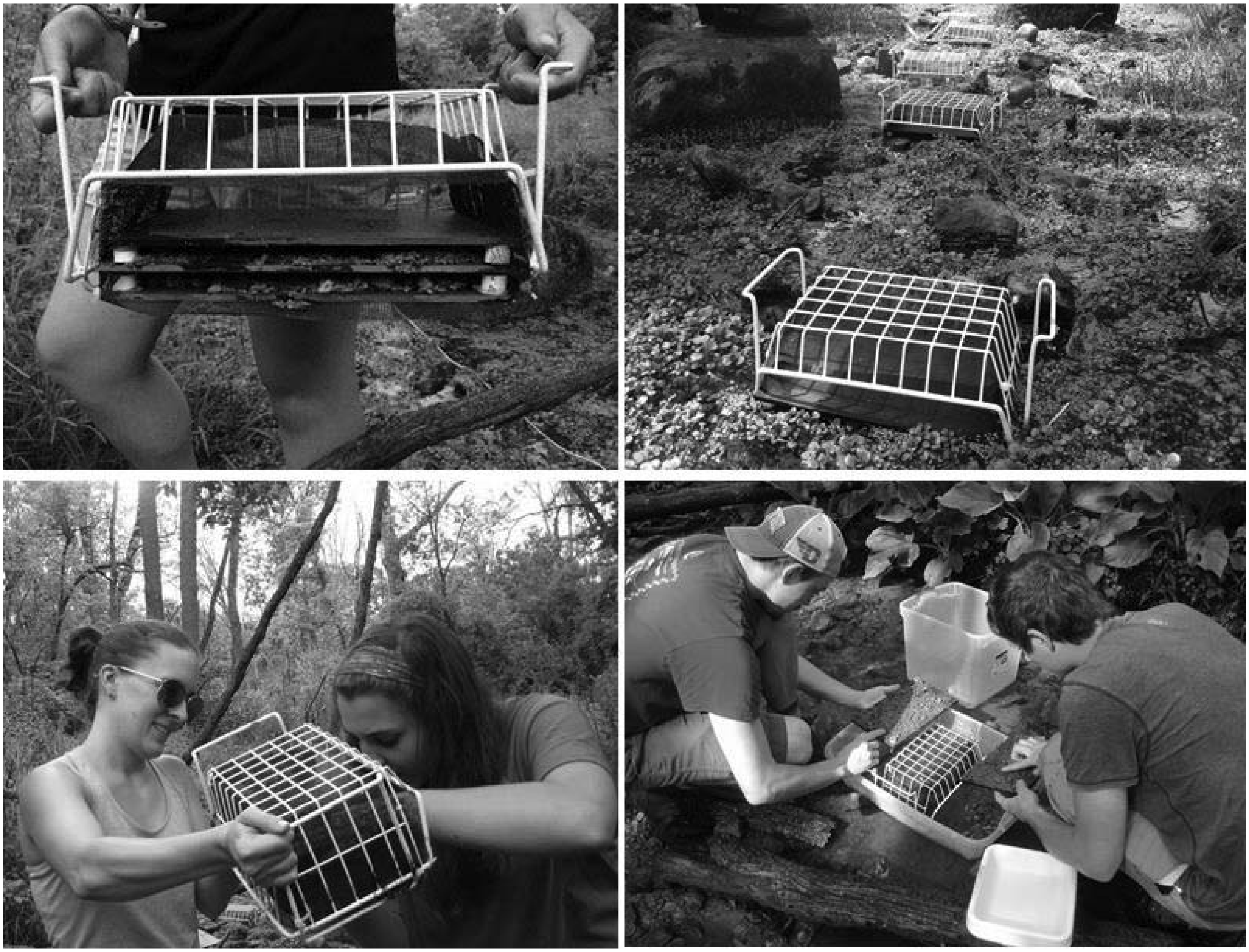

Figure 1.—Application of the Maloney Salamander Hotel (MSH) in a forested headwater stream near Dayton, Ohio (USA). The MSH is constructed from a dishwashing rack lined with fine window mesh (left panel). Three tempered hardboards are lined with 300 mL of stream gravel and inserted into the hotel to provide habitat (right panel). The MSH is placed facing upstream. Construction details are provided in Appendix A.

seven dates ending 23 March 2016 (Table 1), and all salamanders within each MSH were identified and released.

Data from field trials were summarized and detection probabilities calculated to assess the number of samples needed to confidently detect a target species. A sample was defined as a single visit to an MSH to count the number of salamanders within it. The detection probability (DP) is the probability that *Eurycea cirrigera* will be found in a single MSH check, assuming the species is present at the site. We focused on *E. cirrigera* because it was the most numerous species in our study stream. We averaged the DPs for all three MSH to calculate the number of samples needed for 95% confidence in detecting the species (Table 1).

The objective of the second field test was to assess salamander colonization time for the MSH (Maloney et al. 2018d). Three trials were performed in the summer of 2016 to assess the length of time needed for salamanders to colonize the MSH. The colonization trials were also performed in Patty Falls (see above). Using a transect, nine MSH were placed 1 m apart, and three of the nine prototypes were randomly sampled after 3, 6, and 9 d. During each sampling event, the salamanders within the artificial habitat were counted and identified to species and life stage. This trial was repeated three times for a total of 27 sampling events.

In the third field test, we compared the results of the MSH to results obtained with established sampling methods (Maloney et al. 2018a). This experiment was conducted in two primary headwater streams. The first stream was the Patty Falls location described in experiment 1. The second stream (referred herein as Englewood; drainage area 0.04 square miles) was also located at Englewood Metropark (39°52′57.3″N, 84°17′01.5″W, WGS84) and was a first-order, perennial stream with similar geomorphological features to Patty Falls. In this stream, during field surveys, we observed a lower abundance, but similar taxa, of stream salamanders as compared to Patty Falls. Thus, the experiment was designed to assess performance of the MSH in streams with both high and low abundance of salamanders. The

Table 1.—Naïve detection probabilities (DP) for occupancy estimates. Average depth of stream 10.0 cm, average width 1.7 m, main substrate cobble and gravel, surrounded by mature forest, riparian width is wide (over 10 m). Stream source is from groundwater spring 2 m above reach. MSH were set in the same 9 m for each experiment. Formula for *N* samples at 95%: Minimum number of samples needed = LOG(significance level)/LOG(1−DP).

Hotel	Survey	Date	*E. cirrigera*
1	1	10 February 2016	1
1	2	13 February 2016	1
1	3	17 February 2016	1
1	4	14 March 2016	1
1	5	16 March 2016	1
1	6	18 March 2016	1
1	7	23 March 2018	1
		DP	1.000
2	1	10 February 2016	0
2	2	13 February 2016	0
2	3	17 February 2016	1
2	4	14 March 2016	1
2	5	16 March 2016	1
2	6	18 March 2016	1
2	7	23 March 2018	1
		DP	0.714
3	1	10 February 2016	0
3	2	13 February 2016	1
3	3	17 February 2016	1
3	4	14 March 2016	1
3	5	16 March 2016	1
3	6	18 March 2016	0
3	7	23 March 2018	1
		DP	0.714
	Average DP		0.810
	N samples at 95%		2.391

first assessment method we tested was the VES conducted using guidelines from the Ohio EPA Primary Headwater Habitat Assessment manual (Ohio EPA 2012). Using a small fishing net, researchers moved upstream flipping all substrate within a 9 m reach. Any salamanders found were collected and identified to species and life stage, and then promptly released after the entire reach had been sampled. Second, a quadrat-style sampling was performed using a Hess sampler, which is a metal cylinder of a known area that can be pressed into the stream substrate creating a sampling area. The Hess sampler was implemented by pressing the cylinder securely to the stream bed and then a small fish net was used to capture the salamanders inside (Muenz et al. 2008). This process was replicated at five random locations along a 9 m transect in both streams and all salamanders within the Hess sampler were subsequently captured, identified to species and life stage, and released. Finally, five MSH were deployed in each of the streams for 3 d, then collected and the salamanders contained within were identified to species and life stage. Each method was tested on each stream during the summer, with at least 3 d between each method performed. Because the VES does not allow for replication, the number of salamanders/area are reported but no formal statistical comparison was made.

In the final field test, MSH effectiveness was tested within streams with varying characteristics across a full year (Maloney et al. 2018c). Three primary headwater streams were identified that represented varying conditions typical of our study region, southwestern Ohio, where stream beds commonly have high clay and gravel content along with some large limestone bedrock and detritus from surrounding forests. The first stream was located at Englewood Metropark (39°52′44.2″N, 84°16′18.4″W, WGS84) and was a second-order stream with a drainage area of 0.220 square miles. 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, WGS84) and was a first-order stream with a drainage area of 0.0439 square miles. The substrate of this stream was primarily composed of leaf litter, woody debris, and silt. The last stream was located off of the Buckeye Trail Bike Path (39°54′52.0″N, 84°10′06.4″W, WGS84) and was a first-order stream with a substrate that was primarily composed of a thick silty layer with minimal cobble and woody debris with a drainage area of 0.100 square mile. In each of these streams, three MSH were placed randomly on a 9 m transect within each stream for 3 d. These trials were repeated in winter, spring, summer, and fall to assess the utility of the MSH in varying conditions and seasons. The objective of this experiment was to assess the utility of the MSH for salamander detection across seasons in a variety of streams, not to formally compare the number of salamanders detected; therefore no statistical analyses were conducted.

RESULTS AND DISCUSSION

Initial Field Tests

The first set of trials indicate strong utility for the use of MSH in assessing occupancy in *E. cirrigera*. In each of seven trials in February and March of 2016, in a stream in which previous visual surveys had indicated an abundant salamander population, we recorded *E. cirrigera* colonization in at least one of the three MSHs (Figure 2). The colonization time ranged from <2 to <9 days (Figure 2). Detection probabilities (Mazerolle et al. 2007) ranged from 1.000 to 0.714 (average 0.810), with three samples required for 95% confidence in detection (Table 1).

Salamander Colonization Time

Time spent in the field to conduct sampling is an important consideration for biological assessments. We found that the number of *E. cirrigera* captured within MSHs during the colonization trial was relatively stable through time indicating rapid colonization and continued occupancy. We performed nine surveys of three MSH each from 29 July to 1 September 2016. MSH were set on 26 July and checked approximately every third day (Table 2). In each check, we counted and identified to species the number of larval salamanders in each MSH. In total, 39 larval salamanders were detected of which 37 were *E. cirrigera* and two were *Desmongnathus fuscus*. Within 3 days *E. cirrigera* colonized the MSHs, which were occupied through 1 September with detections in seven or eight of the nine samples. Overall, we recommend the retrieval of the MSH following a period of 3 days; however, if the bioassessment required a longer duration our data suggest the sampling would yield similar results. According to Keitzer and Goforth (2013), the leaf pack method also indicates a flexible time of retrieval, with a recommendation of 3 days. One caveat with longer colonization periods is the

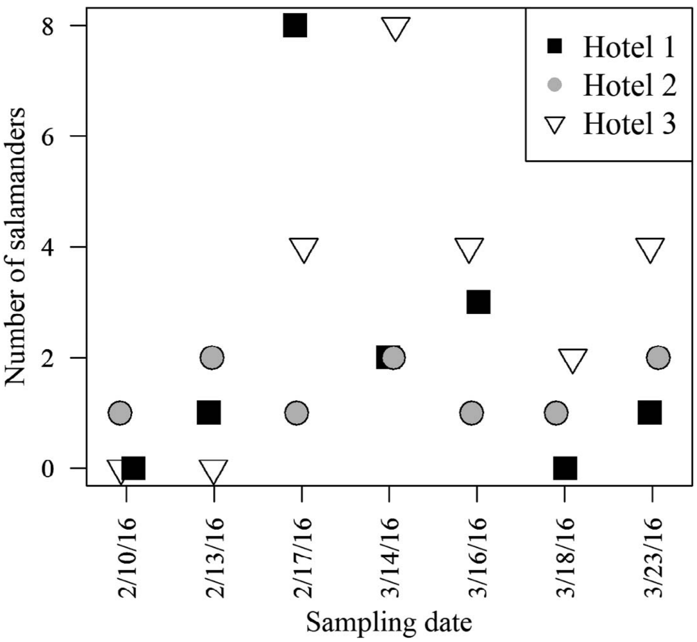

Figure 2.—The number of larval *E. cirrigera* occupying each of three concurrently deployed Maloney Salamander Hotels during seven field trials in February and March of 2016 at Patty Falls (southwestern Ohio, USA). MSH were first set on 8 February 2016.

potentially increased risk of the MSH being lost due to vandalism or disturbance by wildlife.

Comparison to Other Methods

Stream salamanders are often the most common vertebrate in headwater streams (Burton and Likens 1975; Peterman and Truslow 2008), but are cryptic and mobile hence difficult to survey. More salamanders were observed in MSH compared to the VES or use of a Hess sampler in both test sites (Figure 3). The larval salamanders detected in our assessment were mostly *E. cirrigera*, with four *D. fuscus* found during the VES sample and one during the Hess sampling. While conducting each test, the Hess sampler ensured that no salamanders escaped the study area; however, five salamanders were noted then lost (evaded capture) while conducting the VES. Previous studies have shown the abundance of salamanders is often underestimated (Taub 1961; Dodd and Dorazio 2004). Using the mark and recapture method, it was estimated that 10.7–72.8/m^2 *E. cirrigera* larval salamanders were present in Appalachian streams (Nowakowski and Maerz 2009). Compared to VES and Hess sampling, MSH is less disruptive to streams and requires very little actual field time. The VES may be disruptive to the stream and requires the researcher to capture salamanders by hand potentially in murky water. It is also difficult to perform a VES when there are high densities of salamanders or complex substrates. Salamanders colonize the MSH, which simply rests on the stream bed creating minimal disturbance and mimicking natural substrates (Figure 1). The large opening in the MSH allows salamanders to enter and exit the device. Our objective was to assess larval salamanders and only larval salamanders were observed in our various field trials. The leaf pack method, which we did not test, is similar to the MSH in that salamanders colonize an artifical structure and are free to come and go; however, salamanders will often colonize under leaf packs resulting in missed detections (in

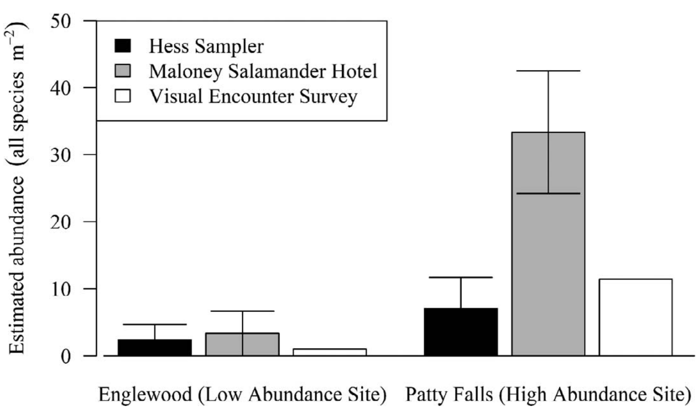

Figure 3.—Salamander counts within high and low salamander abundance headwater streams near Dayton, Ohio (USA), using the Maloney Salamander Hotel. Three methods were compared to assess sampling efficacy.

Table 2.—Naïve detection probabilities (DP) for occupancy estimates. Average depth of stream 10.0 cm, average width 1.7 m, main substrate cobble and gravel, surrounded by mature forest, riparian width is wide (over 10 m). Stream source is from groundwater spring 2 m above reach. MSH were set in the same 9 m for each experiment. Formula for *N* samples at 95%: Minimum number of samples needed = LOG(significance level)/LOG(1—DP).

Hotel	Survey	Date	*E. cirrigera*
1	1	29 July 2016	1
1	2	1 August 2016	1
1	3	4 August 2016	1
1	4	12 August 2016	1
1	5	15 August 2016	1
1	6	18 August 2016	0
1	7	26 August 2016	1
1	8	29 August 2016	1
1	9	1 September 2016	1
		DP	0.889
2	1	29 July 2016	1
2	2	1 August 2016	1
2	3	4 August 2016	1
2	4	12 August 2016	1
2	5	15 August 2016	0
2	6	18 August 2016	0
2	7	26 August 2016	1
2	8	29 August 2016	1
2	9	1 September 2016	1
		DP	0.778
3	1	29 July 2016	1
3	2	1 August 2016	1
3	3	4 August 2016	1
3	4	12 August 2016	1
3	5	15 August 2016	1
3	6	18 August 2016	1
3	7	26 August 2016	0
3	8	29 August 2016	0
3	9	1 September 2016	1
		DP	0.778
	Average DP		0.815
	N samples at 95%		1.776

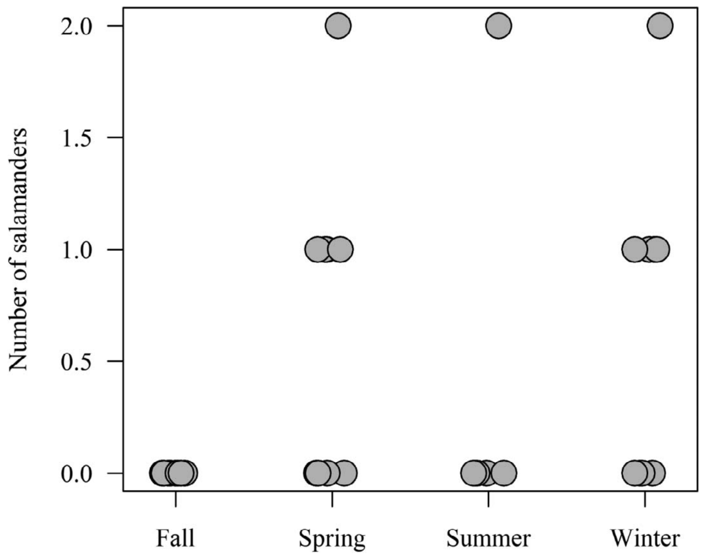

Figure 4.—Number of larval salamanders collected in forested headwater streams near Dayton, Ohio (USA), using the Maloney Salamander Hotel in different seasons.

some cases 33–77% of salamanders were not found within the leaf pack; Pauley and Little 1998; C. Skalski unpublished data) and both creation and handling of artificial leaf packs can be challenging (C. Skalski personal observation).

Seasonality

Seasonality is an important consideration for assessing streams when using salamanders as biological indicators. The MSH was tested in a variety of headwater streams across the growing season to understand the best times to deploy the samplers and to ensure the artificial habitat was effective in a variety of stream conditions. Our data indicate the best time to sample salamanders with the MSH is during the late winter to early summer (March–June; Figure 4). While larval salamanders were drawn to the MSH in late summer, there were far more larval salamanders observed in late winter and late spring. During the fall months, no Plethodontidae salamanders were collected in the MSH at any of the sites. This is likely due to the abundant leaf litter present and cooler temperatures in fall. Maximum seasonal salamander observations in our trials in early spring aligns with the life history timing of reproductive activity of these organisms (Smith 2008; Barrett et. al 2010).

Summary and Application

We developed a novel method to assess stream salamander populations that has a number of advantages for use in both research and rapid assessment. Optimized sampling with the MSH should take into consideration local habitat conditions. Transect-based establishment protocols may be most appropriate for placement of the devices in research; however, for rapid assessment we recommend that the MSH be installed in riffle habitats and avoid muddy areas where no cobble is present (Moore 2010; Ohio EPA 2012). Optimally, samping should take place in the spring and our results indicate that establishing three devices in the stream and allowing for a 3 d colonization period should provide a viable way to detect larval salamander populations in temperate headwater streams. Detection probability analysis indicated that deployment of three MSHs was sufficient to detect *E. cirrigera* with 95% confidence. In terms of practical application, the MSH held up through strong summer storms in July and flash flood events in the spring. The MSH also attracted salamanders despite the differing stream conditions such as varying substrates, water temperature, and leaf litter. This provides evidence that salamanders will colonize the MSH in a variety of streams.

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Chris Skalski is a graduate of the University of Dayton who has spent the last 20 years developing assessment tools for biomonitoring of headwater streams in Ohio.

Ryan McEwan is a Professor in the Department of Biology at the University of Dayton. His research focuses on forest ecology in temperate, tropical, and arctic ecosystems.

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Appendix A: Artificial Habitat Method for Salamander Sampling: The Maloney Salamander Hotel

Construction Details of the Artificial Habitat

Materials

1. Dishwashing rack (ensure that it is made of inert materials; size used in this study was 30 cm × 20 cm × 9 cm, but any size can be used)
2. Window screen (charcoal fiberglass screen mesh, 20 feet)
3. 1 tempered hardboard (3/16 inches, 8 feet × 8 feet)
4. PVC pegs *optional* (3/4 inch)
5. 12 inch stakes (4 per hotel)
6. Zip-ties
7. Plastic container 1 foot × 2 feet × 5 inches

Construction 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 to be used as shelving units inside the dishwashing rack. Each hotel should have 3 shelving units that can be placed within the dishwashing rack. In this study, our shelving units were 25 cm × 15 cm.
4. Optional but recommended: Glue PVC pegs (3/4 inch) on each corner of the tempered hardboard to keep the shelving upright.

Deployment and Retrieval of the Artificial Habitat

Setting Up the Hotel (5–10 min per hotel)

When sampling a headwater stream, look for areas with cobble and gravel present. These areas will usually have higher abundance of salamanders. While salamanders will colonize hotels after 24 hr, it is recommended that the hotels remain in the water for 3–5 d before sampling. Be sure to record the date and time of deployment and retrieval.

1. Place 300 mL of gravel (or bottom substrate from stream) from the stream bed onto 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. Ensure that the shelving units are completely submerged. However, the top of the hotel does not need to be submerged.
4. Bury the bottom of the hotel slightly into the bottom of the stream to prevent salamanders from swimming underneath the hotel.
5. If needed, stake the hotel down into the stream bed using a stake and zip-ties. If the stream is bedrock, use a cinderblock or roots to secure the hotels into the stream.
6. Add rocks and leaf litter atop the hotel to help hotel blend into the natural environment.

Sampling the Hotel Apparatus (10–15 min per hotel)

1. Start at the most downstream hotel to ensure the habitat is not disturbed. Approach the hotel from behind and tip it backwards so that the contents fall to the back.
2. Immediately place the hotel in clear plastic bin to prevent salamander escape.
3. Slowly remove each shelving unit and carefully sort through the gravel.
4. Examine the leftover contents in the hotel. Frequently, the salamanders fall to the back of the unit and are found on the window mesh hidden beneath gravel that has fallen from the shelves.
5. Drain excess water through a small sieve (1 mm recommended) to ensure no salamanders are missed.
6. Record the species and life stage of all captured salamanders.
7. Repeat the process with the next most downstream hotel.