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Herpetological Review, 2008, 39(4), 438–442.

Density Estimation of Larval *Eurycea wilderae*: A Comparison of Mark-Recapture and Depletion Sampling

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Salamanders often are considered to be the most abundant vertebrate organisms in headwater-riparian ecosystems, contributing a significant amount of biomass to ecological and ecosystem processes (Burton and Likens 1975; Davic and Welsh 2004; Peterman et al. 2008). Research has shown that salamanders can exert significant top-down limitations on ecosystem processes, acting as predators of detrivorous organisms (Davic 1983; Wyman 1998), but larval salamanders are susceptible to bottom-up limitations of leaf litter in largely allochthonous-based headwater ecosystems (Johnson and Wallace 2005). Though salamanders are thought to be integral components to fishless headwater streams, acting as predators and prey, actual quantification of their importance has been limited by accurate estimation of population sizes and densities (Bailey et al. 2004; Dodd and Dorazio 2004).

Eurycea wilderae (Blue Ridge Two-lined Salamander) is the most abundant larval species encountered in many Appalachian headwater streams (WEP, unpubl. data). Like a number of other species, two-lined salamanders exhibit a variable activity pattern (Hairston 1949; Orser and Shure 1972; Petranka 1984). During the day, larval two-lined salamanders seek refuge under rock cover or in interstitial spaces in the gravel streambed and at night move about the stream feeding (Petranka 1984). These variable activity patterns can result in drastically different measures of abundance and habitat use (Crawford 2007). Previous estimates of larval salamander abundance and density have employed a variety of techniques that include passive sampling using leaf litter bags, drift nets, or funnel traps (e.g., Bruce 1986; Waldron et al. 2003; Willson and Dorcas 2003), daytime visual encounter surveys that include lifting of cover objects (e.g., Barr and Babbitt 2001; Lowe and Bolger 2002; Smith and Grossman 2003; Welsh and Ollivier 1998), active dipnetting (Willson and Dorcas 2003), or a combination of methods (Nowakowski and Maerz, *in press*). Data collected through these various methods have been analyzed using mark-recapture techniques (Johnson and Wallace 2005; Lowe 2003), removal equations (Jung et al. 2005; Spight 1967), or using replicated counts (Dodd and Dorazio 2004).

Though there are a variety of sampling and estimation options available, researchers are often restricted by time and resources in conducting surveys. In this study, we assess two population estimation techniques: passive trapping with mark-recapture and active depletion sampling. We compare both the time and resources involved with each of these techniques and population estimates

calculated from each method.

Methods.—Surveys for larval two-lined salamanders were conducted at four headwater streams in the southern Appalachian Mountains near Highlands, North Carolina, USA during June and July of 2007. All streams were located within 5 km of Highlands, and were separated by at least 300 m. The four study sites did not differ substantially with regards to habitat characteristics, which were measured at each trap location and then averaged across all sites; values are reported as means (± SD). All four of the gravel substrate streams were fishless headwater streams (i.e., first or second order) that flowed through mixed hardwood forests. *Rhododendron* spp. dominated the understory along the stream banks. Width and depth were measured with a meter tape and ruler, and were 3.21 m (± 0.72) and 105 mm (± 9.43), respectively. Canopy cover averaged 91.9% (± 1.78) and was measured from the center of the stream using a spherical crown densiometer. The area of streambed covered by surface sedimentation averaged 28% (± 3.18) and was measured using a 0.25 m^2 sampling grid divided into 25 sections. The average elevation of study streams was 1044 m (± 151).

Passive trapping of larval salamanders was done using leaf litter traps (Nowakowski and Maerz, *in press*), which differ from leaf bags in that they are generally easier to search (WEP, pers. obs.) and they cover a greater area of stream while utilizing less material than leaf bags. Traps were constructed from 53 × 26 × 6 cm plastic plant pallets. Each trap was covered with 1.9 cm^2 garden mesh, which was held in place using 10 cm plastic cable ties on three sides. Traps were then filled with leaf litter and the fourth side was secured with a metal binder clip. Three traps were deployed at each stream, at least 5 m apart. Each trap was placed on the streambed and weighted with one or two rocks to keep it in place and tied to a secure branch or root on the stream bank to prevent loss from drift. Traps were set for ten days prior to checking to allow for colonization by invertebrates and salamanders. Leaf traps were checked once every three days for a total of four sample periods (nine days between first and last sample). Though it is possible to make population estimates after just two sampling events (i.e. Lincoln-Peterson; Lincoln 1930), three or more samples are generally recommended (Mazerolle et al. 2007) and we chose to use four sample periods for this study with the goal of obtaining more precise estimates and narrower confidence intervals.

To check for larvae, one researcher would quickly remove the leaf trap from the water and place it over a 60 × 40 × 22 cm Rubbermaid® container. Immediately after the trap was lifted from the streambed, a second researcher would use a 15 × 20 cm baitnet with <1 mm mesh to sweep directly under the trap (an area approximately 0.75 × 0.50 m). One researcher would then shake and agitate the trap over the large container while another poured approximately 35 liters of water over the trap. The resulting water and sediments were then poured from the large container through the 15 × 20 cm baitnet. The consolidated contents were then transferred into a white tray (40 × 30 × 8 cm) and sorted. Larvae were identified, counted, and given a cohort mark by clipping the tail fin at a 45° angle. All salamanders were then released at the upstream edge of the trap after it was reset. Following the fourth and final trapping period, all salamanders were released at the trap location after traps were removed from the stream.

Depletion sampling was conducted in plots centered on the area where leaf traps were previously set (i.e., three plots per site). Flags were placed in the stream bed to delineate the search area (0.75 m × 0.75 m) for depletion sampling. Depletion sampling began three days after the last trapping session. Each site was searched twice a night by two researchers (at least 45 minutes between searches) for three consecutive nights (six total samples). Sites were sampled in a different order each night to accommodate potential temporal variation in surface activity. Surface active larvae were captured using dipnets and turkey basters. No rocks or other cover objects were lifted or disturbed during depletion sampling. Following each sample, larvae were released at least 15 m downstream from the most downstream sample location. This distance effectively removed larvae from the depletion plots as most larval salamander movement occurs in the downstream direction, and movements upstream are generally less than 3 m (Johnson and Wallace 2005).

Mark-recapture data collected from trapping were analyzed using a Schumacher-Eschmeyer estimator (Seber 1982). Data from the three traps were pooled together for a single abundance estimate for each site. Abundance estimates were converted into density•m^{-2} estimates, assuming that the total area covered by each trap was 0.375 m^2 (0.75 × 0.50 m per trap). This area is greater than the trap size to account for the area searched with the dipnet. Removal data were analyzed in Program CAPTURE using Pollock and Otto's closed population estimator (Pollock and Otto, 1983) and abundance estimates were converted to density•m^{-2} estimates by dividing by the depletion plot area (0.75 × 0.75 m) .

Results.—We caught a total of 211 larvae with leaf litter traps and 237 were captured using active depletion sampling. Most of the captured larvae (93% and 92% for trapping and depletion, respectively) were smaller than 15 mm snout–vent length and are likely representative of the new cohort of two-lined salamanders, which generally have a larval period lasting about a year. The vast majority of larval captures in traps were two-lined salamanders, but traps captured a low number of larval Red Salamanders (*Pseudotriton ruber*) and larval Black-bellied Salamanders (*Desmognathus quadramaculatus*) at some sites. Density estimates could not be made using depletion sampling at Site 2 because of a lack of captures; nor could estimates be made using mark-recapture at Site 4 because of a severe storm and high water that disturbed the traps. For all other sites, density estimates calculated using mark-recapture and depletion sampling did not differ significantly (Table 1, Fig. 1). Estimates made through mark-recapture ranged from 75 larvae•m^{-2} (95% CI = 69.34–79.58) to 137 larvae•m^{-2} (95% CI = 113.87–160.80) and estimates from depletion sampling ranged from 50 larvae•m^{-2} (95% CI = 40.30–73.48) to 99 larvae•m^{-2} (95% CI = 83.83–128.43; Table 1, Fig. 1).

During the course of data collection, the time needed to complete a sample was recorded, where a sample is equal to the time needed to check three traps at a site or search three plots during depletion sampling. Both methods required about 0.50 h per site per sample period (two researchers per site per sample period; ~1 total man h; Table 2). More sample periods (six samples) were required to get estimates using depletion methods than for the mark-recapture methods (four samples; Table 2), as population estimation via depletion is dependent on reducing the population size (i.e. capturing fewer animals in each subsequent sample period). Following four sample periods, capture numbers at each site

were only reduced 55% on average, but were reduced 83% following six sample periods. As a result of our use of six sample periods, the total effort for depletion sampling was substantially greater and the cost in time per salamander was lower using depletion sampling (Table 2).

Discussion.—We successfully obtained density estimates for larval two-lined salamanders using two different closed capture techniques. In order for the results obtained from these techniques to be valid, four assumptions needed to be met: 1) the population was closed from immigration or emigration; 2) all animals had the same chance of being caught in a sample (i.e., must be a non-biased sample); 3) marking animals did not effect their catchability or survivability; and 4) animals did not lose marks between sampling periods (White et al. 1982). We are confident that these assumptions were satisfactorily met, as both studies (once started) were completed in nine days or less. The marks could not be lost over such a short time period and movement into or out of sample populations was likely minimal. Survivability and catchability are harder to estimate, but these same techniques have been used in longer term studies (WEP, unpubl. data) without apparent adverse effects on survivability.

Justifying all the assumptions of closed population models is difficult as few detailed, long-term studies of larval salamanders have been conducted. Johnson and Wallace (2005) found that only 35.7% of larvae moved more than one meter from the point of capture and the mean movement for all marked salamanders at their reference stream was 2.38 m (N = 122). These data were collected while conducting an 18-month mark-recapture study using monthly sampling. Taking the results of Johnson and Wallace (2005) into consideration, movement into or out of our seemingly confined sample plots was likely minimal.

Trapping with mark-recapture and depletion sampling provided estimates of population density that were not significantly different, though success per hour effort was about 33% less using depletion sampling. Confidence intervals and estimates for both techniques could likely be refined with the inclusion of more sample periods. Trapping and mark-recapture of larval salamanders proved to be relatively cost effective, time efficient, and required no previous experience in locating and effectively capturing salamanders. All of these are important and attractive attributes for implementing replicated studies using minimally trained personnel. One drawback to this method is the initial cost in getting materials to make and check traps. The plant pallets can be purchased from plant nurseries for ~ US $0.40 each, while garden mesh can be purchased from the hardware store in bulk (65 m^2) for ~$30.00. The Rubbermaid® container, white sorting tray, and dipnet cost an additional $18.00. Also, it is difficult to quantify the extra time involved with having to set traps and waiting to check them (10 days in this study).

Nighttime depletion sampling required only a dipnet, a turkey baster (~$10.00 total) and a headlamp, but there is a significant amount of researcher effort and experience required, which are also hard to objectively quantify. In

TABLE 1. Reported larval *Eurycea* densities from fishless 1st and 2nd order headwater streams. Passive Trapping (PT) with leaf litter traps, Area Constrained Dipnetting (ACD), Area Constrained (AC) searches, and Time Constrained□

Species	Larvae•m-2	95% CI	Capture Method	Est. Method	Site Characteristics	Location	Reference
E. cirrigera	1.11–3.81	2.63–4.99	PT & ACD	Count	70% substrate embededness[a]	GA	Nowakowski and Maerz, *in press*
E. cirrigera	10.75–72.81	6.77–108.23	PT & ACD	M-R	70% substrate embededness[a]	GA	Nowakowski and Maerz, *in press*
E. bislineata	0.5–0.76	0.29–1.16	AC (pool)	M-R	Extensive organic detritus, cobble / boulder substrate	NH	Burton and Likens (1975)
E. bislineata	0.7–1.30[b]	N/A	AC (quadrat) & TC (pool / riffle)	Count	Cobble dominate (64–256 mm)	NH, ME	Barr and Babbitt (2002)
E. wilderae	1.16[c]	0.74–1.75	AC	M-R	1.25 m wide, 80% mixed substrate, 20% bedrock[a]	NC	Johnson and Wallace (2005)
E. wilderae	75–137	69.34–160.80	PT & ACD	M-R	3.2 m wide, gravel substrate, 28% surface sedimentation[a]	NC	This study
E. wilderae	50–99	40.30–128.43	AC (quadrat)	Depletion	3.2 m wide, gravel substrate, 28% surface sedimentation[a]	NC	This study

[a] Mean values from all sites included in study
[b] Estimates from fishless streams only.
[c] Study included nutrient manipulations of streams; the reported value is from the unaltered reference site.

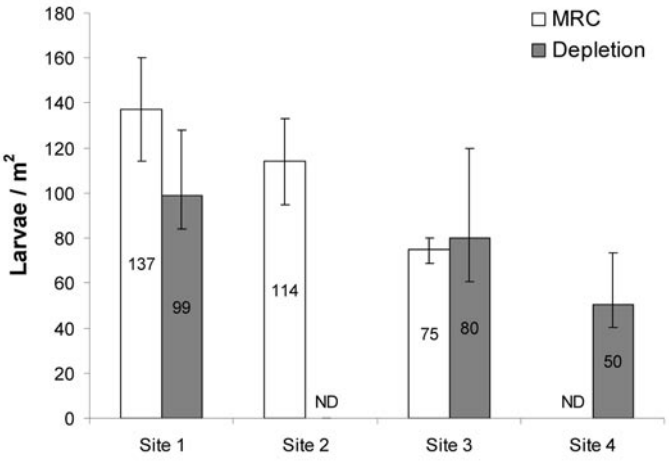

FIG. 1. Estimated larval density and 95% confidence intervals for the four study sites and the two estimation methods. No data were available to make estimates at Sites 2 and 4 using depletion and mark-recapture sampling, respectively.

order to get accurate estimates from depletion sampling, sample sizes need to show a steady, progressive decline over time (White et al. 1982), which can be variable depending on weather conditions, stream conditions, or time of sample period (Barr and Babbitt 2002; Hairston 1949; Orser and Shure 1972; WEP, pers. obs.). After four sample periods in this study, capture numbers were only 55% lower than the first sample, but were decreased 83% after the sixth sample.

Estimated densities for larval salamanders are rare in the literature. Previous density estimates for larvae of the *Eurycea bislineata* complex have varied substantially with nearly all studies reporting densities less than 5 larvae•m^{-2} (Table 1). The lone exception to this is the study of Nowakowski and Maerz (*in press*), on which our leaf trapping methodologies were based. It is hard to determine whether this variation is attributable to geography, species, or the quality of habitat. Some likely factors contributing to the variable density estimates include the area of stream surveyed (i.e., selected quadrats vs. entire pools or reaches), the presence of fish or other predators, and whether or not surveys were conducted during the day or night (e.g., Barr and Babbit 2002; Burton and Likens 1975; Johnson and Wallace 2005). The costs in time and effort among the different studies listed in Table 1 are difficult to quantify, but search time would largely be affected by the complexity and amount of suitable, searchable habitat. In order to gain a greater understanding of how larval densities differ among species and regions, standardized sampling needs to be conducted. We have shown that *E. wilderae* densities in North Carolina are comparable to *E. cirrigera* densities in Georgia using similar techniques. Future research on *E. bisleanata* in northern regions utilizing leaf traps and area constrained dipnetting may provide clarity to the question of larval density in headwater streams as well as explain or alleviate the variability in density estimates currently in the literature.

Our estimates of 75 (95% CI = 69.34–79.58) to 137 (95% CI = 113.87–160.80) *E. wilderae*•m^{-2} using passive trapping are among the highest reported, and support claims that salamanders are likely important and influential organisms in headwater streams (Burton and Likens 1975; Davic and Welsh 2004; Peterman et al. 2008). Though high in comparison to previous studies, we feel our density estimates are describing the three-dimensional nature of headwater streams. As suggested by Ward (1989), there are four dimensions to lotic systems: the lateral, longitudinal, vertical, and time dimensions, and it is the vertical dimension within streambed interstices that larval salamanders often inhabit. It is widely understood that terrestrial salamanders are fossorial in nature, and Petranka and Murray (2001) demonstrated that only a small fraction of the population is surface active at any point in time, thus requiring several successive sample periods to adequately deplete population numbers. Similarly, only a fraction of the larval salamanders were surface active or inhabiting our traps during a given sample period, and only with repeated sampling and marking of animals could accurate estimates be made.

The importance of salamanders to headwater stream communities is becoming more evident (Peterman et al. 2008), further reinforcing their ecological importance (Davic and Welsh 2004). From the results of our study, we suggest that larval salamanders can be effectively sampled passively using either a leaf litter trapping technique with mark-recapture or by active depletion sampling, but there are costs and benefits to both. The quality and resolution of the subsequent results are largely dependent upon the number of sample periods used. Here we have demonstrated that four sample periods can be sufficient to get estimates using simple cohort marking and Schumacher-Eschmeyer estimation methods, while six sample periods were sufficient for removal sampling. The number of samples needed is likely to vary, depending on several factors and may be more or less dependent on one's study design and research objectives, but we feel that there is much less variability involved with in trapping, allowing for a more equitable comparison among study sites. With refined sampling techniques and estimation capabilities, future research should address ecological and ecosystem processes that include both larval and adult salamanders.

TABLE 2. Estimated effort involved with each sampling method. The mark-recapture study was concluded after four sample periods; depletion was concluded after six sample periods. Time per sample is the time needed for two researchers to search three traps or three depletion plots at a single site; time per site is the cumulative time needed to sample a site (time per sample x number of samples); total effort is the cumulative time needed to collect data for abundance estimates at four sites (time per site x number of sites); and salamanders per hour are the total number of salamanders that were captured divided by the hours of total effort.

Method	Time per sample (h)	Time per site (h)	Total Effort (h)	Salamanders/h
Mark-recapture	0.48	1.92	7.68	27.47
Depletion	0.54	3.24	12.96	18.29

Acknowledgments.—We thank J. Crawford, L. Eggert, C. Rabeni, R. Semlitsch, M. Mahoney, and three anonymous reviewers for constructive comments and criticism on this manuscript. We also thank J. Costa, L. Davis, and the staff of Highlands Biological Station for use of facilities. Materials and discussions were provided by J. Maerz and J. Milanovich. A special thanks to J. Crawford and J. Wisdom for assistance with field data collections. This research was partially funded by a Highlands Biological Station Fellowship (WEP) and a cooperative agreement with the United States Forest Service Southern Research Station (WEP). This research was conducted under a U.S. Forest Service permit (HIG6493) and animal care protocol 3951 approved by the University of Missouri Animal Care and Use Committee.

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