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Influence of Observers and Stream Flow on Northern Two-Lined Salamander (*Eurycea bislineata bislineata*) Relative Abundance Estimates in Acadia and Shenandoah National Parks, USA

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Abstract.—We investigated effects of observers and stream flow on Northern Two-Lined Salamander (*Eurycea bislineata bislineata*) counts in streams in Acadia (ANP) and Shenandoah National Parks (SNP). We counted salamanders in 22 ANP streams during high flow (May to June 2002) and during low flow (July 2002). We also counted salamanders in SNP in nine streams during high flow (summer 2003) and 11 streams during low flow (summers 2001–02, 2004). In 2002, we used a modified cover-controlled active search method with a first and second observer. In succession, observers turned over 100 rocks along five 1-m belt transects across the streambed. The difference between observers in total salamander counts was not significant. We counted fewer *E. b. bislineata* during high flow conditions, confirming that detection of this species is reduced during high flow periods and that assessment of stream salamander relative abundance is likely more reliable during low or base flow conditions.

Stream dwelling salamanders are difficult to survey because of their cryptic nature, small size, seasonal and weather-dependent movements, fossorial habits, and lack of vocalizations. Observer differences may influence salamander counts; researchers have attempted to minimize this bias during salamander population monitoring so that surveys are repeatable and results are transferable (Jung et al., 2000; Chalmers and Droege, 2002; Lowe and Bolger, 2002). Familiarity with the species and limiting the surveyor to one or two tasks or species per survey reduces observer bias (Marcus et al., 1995; Wang et al., 1996; Poole et al., 1997; Henke, 1998), although complete elimination of observer bias is difficult under some conditions, such as variable weather and light (e.g., cloud cover, canopy density). Attributing temporal or spatial differences in survey results to real differences in population estimates is possible only if sampling error and observer bias are known and quantified (Bailey et al., 2004a,b). Observer bias in stream salamander surveys has not been quantified.

Stream flow affects physical conditions and biological communities inhabiting lotic environments and can be a source of disturbance to benthic communities (Resh et al., 1988). Headwater and low order streams respond quickly to rain events, and high flows may be continuous during periods of abundant precipitation. High stream flow can affect salamander larvae by increasing passive drift, potentially increasing predation risk (Baumgartner et al., 1999; Sih et al., 1992). Thus, one might expect the abundance or detectability of stream salamanders to differ between periods of low- and high-stream flow.

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We investigated the effects of observer and streamflow differences on relative abundance of Northern Two-Lined Salamanders (*Eurycea bislineata bislineata*) in Acadia National Park (ANP), Maine, and Shenandoah National Park (SNP), Virginia. A member of the lungless salamander family Plethodontidae, *E. b. bislineata* is a widespread and abundant species in lower-order streams in the northeastern United States and is the only abundant stream salamander in ANP (Bank et al., 2006).

Our objectives were (1) to evaluate the influence of equally experienced observers on *E. b. bislineata* relative abundance surveys, and (2) to investigate the effects of stream flow condition (i.e., high flow vs. low flow) on *E. b. bislineata* relative abundance within a year (ANP) and among years (SNP).

Materials and Methods

We surveyed 22 streams for *E. b. bislineata* during May and June 2002 on Mount Desert Island and the Schoodic Peninsula in ANP, Maine. First order streams comprised ~80% of those surveyed; the remainder was second-order streams. Surveys were repeated in July 2002 at ANP. Surveyed stream segments ranged 50–250 m above mean sea level (AMSL). In SNP, Virginia, we surveyed nine first- and second-order streams in June and July 2001, 2003, and 2004 and 11 first- and second-order streams in July 2002. Elevation of surveyed streams ranged 290–700 m AMSL on west- and east-facing slopes in the Blue Ridge Mountains.

Belt transects.—We counted salamanders along belt transects in May and June and July 2002 at ANP and in July 2002 in SNP, with a cover-controlled active search method (Crump and Scott, 1994) similar to that described by Lowe and Bolger (2002). The first observer (OBS1) selected a random starting point at least 25 m upstream from the confluence with either a pond or higher order stream. At this starting point, OBS1 turned over 20 nonembedded rocks (3–36 cm diameter) at a constant rate along a 1-m transect perpendicular to and including the stream banks, edge, and channel in pools, riffles, and runs, and recorded all sightings of larval and adult *E. b. bislineata*. Salamanders were not captured, and cover objects were returned to their original position as each salamander was detected. Upon completing the transect survey, OBS1 moved upstream 20–30 m to locate the next stream survey area. Five minutes after OBS1 completed the survey, the second observer (OBS2) repeated the survey at that site. Rocks sampled by OBS2 were chosen independently from OBS1. We surveyed five transects per stream, with a total of 100 rocks searched by each observer in each stream. Observers alternated sequence between each survey stream.

Removal sampling.—During June and July 2001–04 in SNP, we conducted removal sampling (2–3 passes conducted sequentially) at 1–2 transects per stream. Transects were 15 × 2 m, spanning 1 m along the stream bank and 1 m in the stream channel (Jung et al., 2005). Observers turned over the top layer of rocks within each transect and captured salamanders with dip nets. Salamanders were retained in zip-lock bags and released after the final pass. In 2001 and 2004, we compared detection probabilities between observers for total *E. b. bislineata* found along transects.

Stream flow.—We obtained ANP stream flow data (cubic meters per second, cms) from the Cadillac and Hadlock Brook USGS gauging stations and SNP stream flow data from the University of Virginia's Shenandoah Watershed Study (F.A. Deviney, pers. comm.). We initially partitioned stream flow into high and low rates based on field observations, with low rates characterized by reduced width and depth of the stream channel. We later statistically compared flow rates from gauged stations. Flow in Maine's low order streams varies seasonally, with greatest flows during early spring because of snow melt and rainfall; low flow occurs during mid- to late July when rainfall is minimal. Seasonal variation in stream flow in SNP is similar to ANP.

Statistical analyses.—We partitioned detections of larvae, adults, and total *E. b. bislineata* by park, stream, and age class and compared between observers with Mann-Whitney *U*-tests. We compared larval, adult, and total salamander relative abundances per 100 rocks between high and low flow from belt transect data at ANP with Mann-Whitney *U*-tests and across years and streams from 15 × 2 m transect data at SNP with two-way ANOVAs. Detection probabilities of different observers were compared using Mann-Whitney *U* and Kruskall-Wallis test. Monthly mean stream flow rates during high and low flow surveys in ANP were compared statistically with *t*-tests. Monthly (June through August) mean flow rates in SNP streams were compared among years (2001–04) and streams ($N = 5$) using a two-way ANOVA with a post hoc Duncan test to compare among years (Zar, 1999). Means are given ± 1 SE. We accepted statistical significance at $P < 0.05$.

Results

Stream flow differed at ANP between high flow (May and June 2002) and low flow (July 2002) surveys in the gauged streams (Cadillac Brook: $t_{60} = 3.71$, $P = 0.0004$; Hadlock Brook: $t_{60} = 4.28$, $P < 0.0001$; Fig. 1A). Mean low flow for gauged streams was 0.0004 m^3/sec. compared to a mean high flow 0.018 m^3/sec. Summer monthly average flow rates in five SNP streams in 2003 exceeded flow rates in 2001, 2002, and 2004 ($F_{3,40} = 8.42$, $P < 0.001$; Fig. 1B).

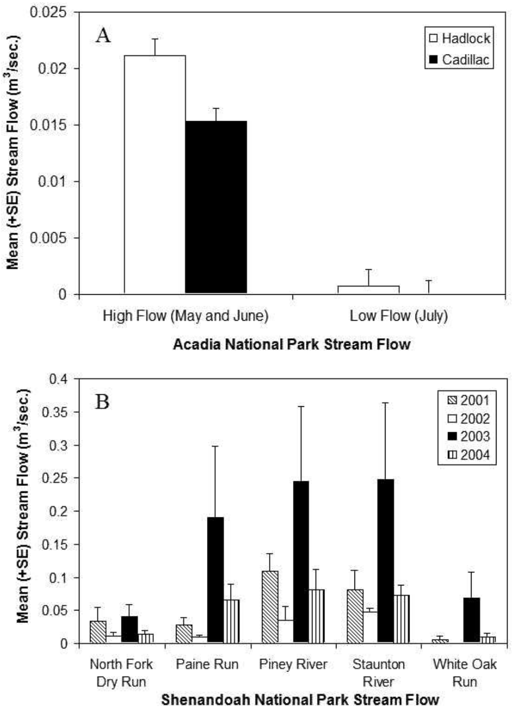

FIG. 1. Monthly mean stream flow (cubic meters/second, cms) during (a) high flow (May to June 2002) and low flow (July 2002) stream salamander surveys in Cadillac and Hadlock brooks, Acadia National Park, Maine, and (b) summer (June to August) during high flow (2003) and low flow (2001, 2002, and 2004) stream salamander surveys at five streams in Shenandoah National Park, Virginia.

Mean observer difference of *E. b. bislineata* larvae and total individuals did not differ between observers, whereas OBS1's counts of adults exceeded those of OBS2 (Table 1). During low flow at ANP, means of *E. b. bislineata* adults, larvae and total individuals did not differ between observers. Similarly, there were no observer differences in counts during low-flow belt-transect surveys in SNP; OBS1 detected 34 and OBS2 detected 35 *E. b. bislineata* (Table 1). Detection probabilities (p) from removal sampling did not differ between two observers in 2001 ($U_1 = 2.0$, $P = 0.56$; OBS1: $P = 0.66 \pm 0.060$, $N = 2$; OBS2: $P = 0.74 \pm 0.087$, $N = 3$) or among three observers in 2004 ($X^2_2 = 1.2$, $P = 0.56$; OBS1: 0.67 ± 0.057, $N = 4$; OBS2: 0.64 ± 0.079, $N = 6$; OBS3: 0.51 ± 0.160, $N = 2$).

Relative abundance of *E. b. bislineata* detected per 100 rocks in ANP was greater in surveys conducted during low flow than during high flow ($U_1 = 308.0$, $P < 0.0001$; Fig. 2A). During low flow, OBS1 detected 79

TABLE 1. Mean (± SE) number of detection/100 rocks and mean (± SE) observer difference of *Eurycea bislineata bislineata* detected/100 rocks in high and low stream flow at Acadia and Shenandoah National Parks in 2002. Test results are presented for comparison of observer difference. U = Mann-Whitney test statistic. N = number of streams surveyed, [a] = mean (± SE) number of *E. b. bislineata* detection/100 rocks, [b] = mean (± SE) observer difference of *E. b. bislineata*/100 rocks, is the difference between the primary observer detections (first observer) and the detections of secondary observer (second observer) for each stream transect.

Stream condition and observer	N	Adults [a]	Observer difference adults [b]	U	P	Larvae [a]	Observer difference larvae [b]	U	P	Total [a]	Observer difference total [b]	U	P
Acadia National Park													
High water flow	22												
Observer 1		0.26 ± 0.12	0.059 ± 0.057	24.0	0.02	0.56 ± 0.21	0.0 ± 0.22	33.0	0.27	0.82 ± 0.26	0.059 ± 0.23	24.5	0.07
Observer 2		0.21 ± 0.08	0.49 ± 0.22			0.61 ± 0.22	0.20 ± 0.20			0.82 ± 0.26	0.60 ± 0.36		
Low water flow	22												
Observer 1		0.70 ± 0.22	0.41 ± 0.23	63.0	0.34	2.85 ± 0.64	0.47 ± 0.37	50.5	0.97	3.6 ± 0.77	0.58 ± 0.28	57.0	0.66
Observer 2		0.15 ± 0.07	0.83 ± 0.48			3.4 ± 0.71	0.10 ± 0.82			3.55 ± 0.72	0.16 ± 0.76		
Shenandoah National Park													
Low water flow	11												
Observer 1		0.18 ± 0.12	0.0 ± 0.0	12.0	1.00	2.9 ± 1.46	0.66 ± 0.33	16.0	0.40	3.09 ± 1.46	0.66 ± 0.33	17.0	0.28
Observer 2		0.2 7± 0.19	0.13 ± 0.35			2.9 ± 1.34	0.75 ± 0.99			3.18 ± 1.52	0.88 ± 0.95		

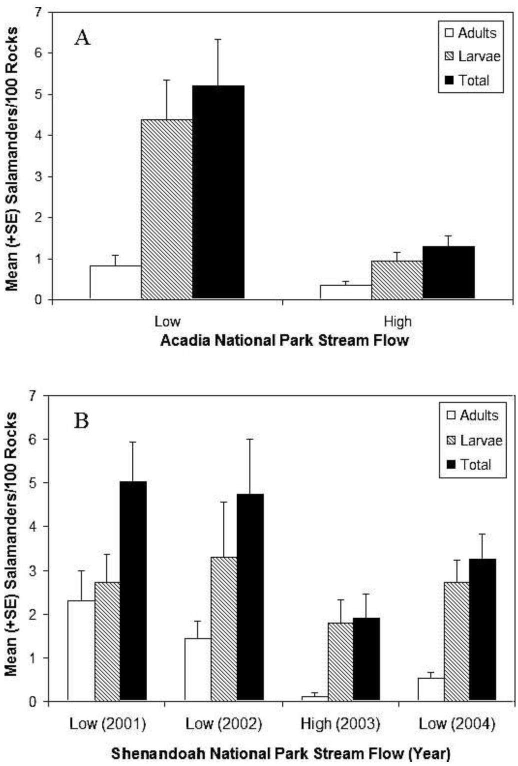

FIG. 2. Mean (± SE) number of adults, larvae, and total Northern Two-lined Salamanders detected per 100 rocks in (A) 22 streams during high (May to June 2002) and low (July 2002) flow in Acadia National Park, Maine, and (B) along 15 × 2 m transects conducted at eight streams during high (June 2003) and low (July to August 2001, June to July 2002, July 2004) flow in Shenandoah National Park, Virginia.

and OBS2 detected 77 salamanders, whereas each observer found 28 salamanders during high flow at the same streams. More larvae were detected during low flow compared to high flow surveys (U_1 = 162.0, $P < 0.0001$), whereas relative abundance of adults did not differ with flow (U_1 = 180.5, P = 0.114).

In SNP, observers detected more adult and total *E. b. bislineata* per 100 rocks during removal sampling transect surveys conducted in low flow than in high flow (Fig. 2B). Relative abundance of larvae did not differ among streams or years. We found more adults during low flow (2001, 2002, 2004) than during high flow surveys (2003; $F_{3,32}$ = 8.88, $P < 0.001$). Relative abundance of adults also differed among streams ($F_{7,32}$ = 2.96, P = 0.016). We found more salamanders (adults and larvae total) during low flow in 2001 than during high flow in 2003 ($F_{3,32}$ = 3.46, P = 0.028), with relative abundance differing among streams ($F_{7,32}$ = 3.56, P = 0.006). Detection probabilities for total individuals did not differ across years or between high (2003) and low (2001, 2002, 2004) flow (see Jung et al., 2005). Small sample size prohibited statistical comparisons of detection probabilities for adults and larvae.

Discussion

Quantifying factors affecting temporal and spatial variation in population estimates and detection probabilities is crucial in amphibian monitoring programs. If a monitoring program is to provide comparative data for population trend analysis and detecting effects of human activities on amphibian populations, then a standardized technique is critical (Heyer et al., 1994).

Stream flow affected our salamander relative abundance estimates within and among years, indicating that this factor should be taken into account when monitoring populations of salamanders that seek refuge in stream bottom cobble. High stream flow temporally disturbs stream benthic communities by increasing riffles and runs (Resh et al., 1988) and reducing the occurrence of pools and slow current, the preferred habitat for *E. b. bislineata*; larvae are rarely found in fast currents (Petranka, 1998). Benthic communities recover quickly after high flows, suggesting that refugia are important in lotic systems (Lancaster and Hildrew, 1993) and that larval salamanders may seek refuge during periods of high flow. In our study, *E. b. bislineata* relative abundance per 100 rocks differed between high and low flow at ANP, in part because of differences in larval and adult counts. During high flow, larvae may avoid drifting by taking refuge deep in stream bed cobble; this would explain their low relative abundance in high flow surveys. Our results indicate that stream flow affects salamander relative abundance within and among years; surveys completed in low flow provide higher counts and perhaps more reliable estimates of relative abundance of salamanders dwelling in stream cobble.

Relative abundance of *E. b. bislineata* larvae and adults during low flow in SNP and ANP streams was consistent between observers. Adult salamanders tended to flee when their cover was removed by the first observer; the second observer detected adult salamanders within 5 min in the same general stream location although we are not certain these were the same individuals. Fewer adults were detected by the second observer at ANP during high flow conditions, however, perhaps because the salamanders may have been displaced by the flow or sought refuge in the substrate out of the observer's view. Fleeing behavior of adult stream salamanders may be a defense against predators (Petranka, 1998). In contrast, larvae typically remained motionless when their cover was removed. Resetarits (1991) reported that *E. b. bislineata* larvae were less active in the presence of predators and that their small size and more cryptic coloration may provide protection from predation in lieu of fleeing.

The most useful sampling methods are those that are unbiased and show the least variation or measurement error (Jung et al., 2000). Methods that are not observer biased are necessary if the goal of the survey is to monitor population change over space and time. Although completely eliminating observer bias from relative abundance estimates may be difficult to achieve, our results indicate that minimizing differences attributable to observer experience and stream flow will lead to more reliable population monitoring.

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