

Stream Salamander Species Richness and Abundance in Relation to Environmental Factors in Shenandoah National Park, Virginia

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ABSTRACT.—Stream salamanders are sensitive to acid mine drainage and may be sensitive to acidification and low acid neutralizing capacity (ANC) of a watershed. Streams in Shenandoah National Park, Virginia, are subject to episodic acidification from precipitation events. We surveyed 25 m by 2 m transects located on the stream bank adjacent to the water channel in Shenandoah National Park for salamanders using a stratified random sampling design based on elevation, aspect and bedrock geology. We investigated the relationships of four species (*Eurycea bislineata*, *Desmognathus fuscus*, *D. monticola* and *Gyrinophilus porphyriticus*) to habitat and water quality variables. We did not find overwhelming evidence that stream salamanders are affected by the acid-base status of streams in Shenandoah National Park. *Desmognathus fuscus* and *D. monticola* abundance was greater both in streams that had a higher potential to neutralize acidification, and in higher elevation (>700 m) streams. Neither abundance of *E. bislineata* nor species richness were related to any of the habitat variables. Our sampling method preferentially detected the adult age class of the study species and did not allow us to estimate population sizes. We suggest that continued monitoring of stream salamander populations in SNP will determine the effects of stream acidification on these taxa.

INTRODUCTION

Stream salamanders in the eastern United States, such as *Eurycea*, *Desmognathus* and *Gyrinophilus* species, are potential indicators of stream health (Rocco and Brooks, 2000; Ohio EPA, 2001; Barr and Babbitt, 2002). They can constitute significant biomass in eastern deciduous forests and are important in community ecology (*e.g.*, predator-prey interactions) and ecosystem energy flow and nutrient cycling (Spight, 1967; Burton and Likens, 1975; Petranka and Murray, 2001). In headwater streams, salamanders often replace fish as the dominant predator (Ohio EPA, 2001). These species lay their eggs in cryptic sites in streams or seeps. After metamorphosis, juveniles and adults forage in leaf litter and rocky substrates adjacent to and within streams. Compared to many anurans, salamanders are relatively long-lived, take longer to reach maturity and lay fewer eggs (Petranka, 1998). Some species, such as the northern spring salamander (*Gyrinophilus porphyriticus*), remain as larvae as long as 4 y (Petranka, 1998).

Stream salamanders are sensitive to acidification, drought, contaminants and habitat destruction or alteration such as urbanization, logging and road construction (Orser and Shure, 1972; Welsh and Ollivier, 1998; Corn and Bury, 1989; Rocco and Brooks, 2000). In Shenandoah National Park (SNP), located in the Blue Ridge Physiographic Province of Virginia, primary threats include habitat alteration (*e.g.*, past land use, including logging, agriculture, road and trail construction, tree defoliation and mortality caused by gypsy and balsam woolly adelgid moths) and acidification of soils and water (Mitchell, 1998). Gypsy moth

canopy defoliation from 1990–1992 affected the water chemistry of several SNP watersheds, causing increased levels of dissolved nitrogen (as NO_3^-) and a subsequent decrease in stream acid-neutralizing capacity (ANC), the ability of a watershed to neutralize acid inputs (Webb *et al.*, 1995; Eshleman *et al.*, 2000). When gypsy moth defoliation ceased, nitrogen export declined (Eshleman *et al.*, 2000), yet the ability of the watershed to neutralize acidic inputs was likely compromised (Webb *et al.*, 1995). The composition of bedrock strongly affects the susceptibility of streams to acid deposition, and stream acidity can be predicted by ANC (Bricker and Rice, 1989). In SNP, water in stream catchments underlain primarily by siliciclastic bedrock exhibit low ANC and pH values (as low as 5.0; Bulger *et al.*, 1999).

Stream salamanders are negatively affected by low soil and water pH (Roudebush, 1988; Wyman, 1988; Kucken *et al.*, 1994), with eggs and larvae thought to be the most sensitive life stages. Responses may include reductions in fertilization and hatching success (Freda, 1986), survival of larvae (Pough, 1976) and larval growth rates (Freda and Dunson, 1985). In New Hampshire, Barr and Babbitt (2002) found lower abundances of northern two-lined salamanders (*Eurycea bislineata*) in streams with lower pH, while Mushinsky and Brodie (1975) found that northern dusky salamanders (*Desmognathus fuscus*) preferred substrates at pH 7.7 over pH 5.5. In a limited study in SNP, Mitchell (1999) found no difference in stream salamander species richness and abundance in three streams differing in ANC. In contrast, stream ANC affects the demographics, distribution and abundance of fish (Bulger *et al.*, 1999; Dolloff and Newman, 1999) and invertebrate fauna (Feldman and Conner, 1992) in SNP.

In this study we examine stream salamander richness and abundance in relation to environmental parameters. Specifically, we hypothesized that streams with lower pH or ANC values would have fewer species and a lower abundance of individuals than streams with higher pH or ANC.

METHODS

From 3 June to 12 July 1999, we surveyed 49 stream sites throughout SNP (Fig. 1). Stream sites were selected using a stratified random design (Cochran, 1977) based on elevation, underlying bedrock and aspect. In the few cases where stream sites were located within the same watershed, sampling was conducted at least 500 m apart along the stream channel. Twenty-three of the stream sites were at low elevation (≤ 700 m) and 26 were at high elevation (> 700 m); site elevations ranged from 378–1081 m. Approximately one-third of the sites were associated with either basaltic ($n = 16$), granitic ($n = 17$) or siliciclastic ($n = 16$) bedrock, and distributed among north ($n = 12$), south ($n = 14$), east ($n = 12$) and west ($n = 11$) aspects. Survey sites were located at headwater seeps ($n = 5$), first- ($n = 33$), second- and third- (combined $n = 11$) order streams, and were constrained to within one-half mile of a road or trail. The Park has approximately 515 km of first-order and 241 km of second-order streams (J. Atkinson, pers. comm.), so our sampling design was representative of the stream order distribution in the Park.

We used area-constrained, visual encounter streamside transects. Two people turned over rocks and other objects within two $25 \text{ m} \times 1 \text{ m}$ transects (one on each side of a stream channel). This survey method sampled primarily the terrestrial habitat immediately adjacent to the wetted stream channel, and preferentially captured adult salamanders, though we did capture larvae in the hyporheic zone. Salamanders were captured by hand or using dipnets and were removed for the duration of the survey to avoid duplicate sampling of individuals. When a salamander was captured, we recorded the species, age class (those with gills were recorded as larvae, and those without, including both recently metamorphosed juveniles and sexually mature adults, were classified as adults), snout-vent length (SVL, mm) and total length (TL, mm), and the size of the cover object [length, width, depth (cm)] under which the salamander was found. About 45% of the time salamanders escaped capture, but in the

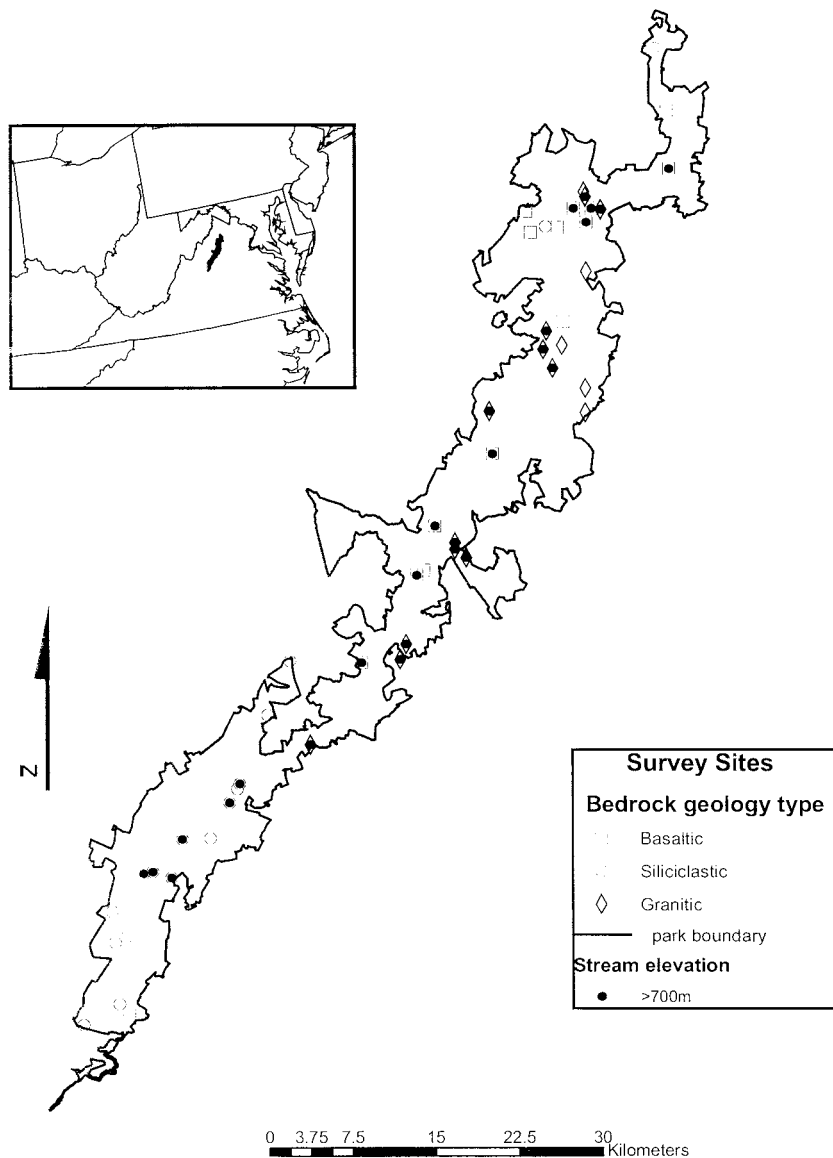


FIG. 1.—Locations of stream salamander survey sites in Shenandoah National Park, Virginia, USA, by bedrock geology type (basaltic, siliciclastic, granitic). Filled symbols indicate sites at high elevation (n = 26 sites >700 m) and open symbols indicate sites at low elevations (n = 23 sites ≤700 m)

majority of these cases genus or species could be determined. We combined counts of northern dusky (*Desmognathus fuscus*) and seal salamanders (*D. monticola*) into the variable DESMOG for analyses. Salamanders were returned to the stream transect after the survey was completed. We recorded the number of crayfish encountered and the number of overturned objects (rocks and logs).

At each site we recorded water temperature, specific conductance and pH. We measured water temperature using a digital Hanna K-Type Thermocouple. Water samples collected in 250 ml Nalgene HDPE sample bottles from the field were kept on ice and brought back to the laboratory for same-day measurements of specific conductance (YSI Conductivity Instrument 3100-115V) and pH (Cole Parmer 59003-20 pH 500 series with a Broadley-James Corp. probe E-1439-EC2-A03BC). ANC was not measured directly but was estimated using relationships between H^+ and ANC from data collected in June and July 1999 at streams throughout SNP on the three bedrock types (Shenandoah Watershed Study, J. R. Webb, pers. comm.). Based on the pH measurement and the underlying bedrock type, we determined ANC at each site as follows:

$$\text{Siliciclastic ANC} = -13.7 \ln(\text{pH}) + 20.9 \text{ (median} = 10.4)$$

$$\text{Granitic ANC} = 5.8 (\text{pH}) + 86.9 \text{ (median} = 91.7)$$

$$\text{Basaltic ANC} = -201.6 (\text{pH}) + 330.9 \text{ (median} = 291.0)$$

We divided ANC into three classes (Lynch and Dise, 1985; Schindler, 1988) which roughly corresponded with bedrock types: "extremely sensitive" to acidification (range: -7.21 to 43.9 $\mu\text{eq/liter}$), "sensitive" to acidification (88 to 200 $\mu\text{eq/liter}$) and "not sensitive" to acidification (201 to 322 $\mu\text{eq/liter}$).

Salamander metrics at each stream site included: (1) TOTIND (total count of all salamander individuals including escapes), (2) SPECIES (number of salamander species), (3) EBIS (count of *Eurycea bislineata*), (4) DESMOG (summed count of *Desmognathus fuscus* and *D. monticola*) and (5) lengths (TL and SVL) of EBIS or DESMOG. Presence or absence of *Gyrinophilus porphyriticus* was analyzed in relation to environmental variables using a logistic regression model. Salamanders that escaped capture were included in TOTIND and in counts of specific species or species' groups when the genus and/or species could be determined.

STATISTICAL METHODS

Salamander metrics were analyzed using Spearman rank correlations, analyses of variance (ANOVA) and nonparametric Kruskal-Wallis tests. Normality of each variable was assessed using Kolmogorov-Smirnov tests and data were transformed when necessary to satisfy assumptions of normality. TOTIND, EBIS, DESMOG and lengths (TL and SVL) of DESMOG and EBIS were square-root transformed. SPECIES did not normalize with transformation and was, therefore, analyzed in relation to environmental variables using non-parametric Spearman rank correlations and Kruskal-Wallis tests for independent samples.

The stratified random design permitted us to use ANOVAs or Kruskal-Wallis tests for independent samples to investigate relationships between salamander metrics and the stratified habitat variables (elevation, aspect), and ANC classes. Separate analyses were run on each habitat stratification variable for all eight salamander metrics. For ANOVA tests, we used Tukey's multiple comparison test to determine group similarities. We analyzed untransformed data using Spearman rank correlations to evaluate relationships between salamander metrics and habitat [number of crayfish (CRAY), number of rocks (ROCKS)] and water quality variables [temperature (TEMP), pH].

RESULTS

The number of site occurrences out of 49 streams were: *Desmognathus monticola* (40), *D. fuscus* (41), *Eurycea bislineata* (43) and *Gyrinophilus porphyriticus* (33). A single *Eurycea longicauda* was found. We found 568 desmognathine salamanders, 174 *Eurycea* and

TABLE 1.—Captured salamanders at 49 stream sites in Shenandoah National Park showing species, age class, count (n), snout-vent (SVL) and total lengths and rock dimensions. Data presented as mean \pm standard error

Species	Age class	n	SVL (mm)	Total length (mm)	Rock area (cm ²)
<i>E. bislineata</i>	Larva	24	21 \pm 1.2	37 \pm 1.7	357 \pm 54.3
	Adult	81	30 \pm 0.8	58 \pm 1.6	345 \pm 40.1
<i>D. fuscus</i>	Larva	37	23 \pm 0.8	41 \pm 1.6	550 \pm 132.5
	Adult	145	43 \pm 1.0	76 \pm 1.9	596 \pm 51.3
<i>D. monticola</i>	Larva	8	22 \pm 1.4	41 \pm 3.1	526 \pm 218.1
	Adult	119	42 \pm 1.4	79 \pm 2.8	442 \pm 41.7
<i>G. porphyriticus</i>	Larva	28	45 \pm 2.2	79 \pm 2.8	802 \pm 151.4
	Adult	17	62 \pm 4.2	103 \pm 6.4	575 \pm 141.4

95 *Gyrinophilus* larvae and transformed individuals. *Desmognathus* spp. comprised 68% of the total number of salamander individuals encountered. We captured and measured 459 salamanders, including 362 adults and 97 larvae (Table 1). An additional 378 salamanders (261 adult, 117 larvae) were found but not captured.

Applying the Bonferroni adjustment for multiple correlation comparisons (α level = 0.002), DESMOG ($r_s = 0.45$, $P < 0.001$) and TOTIND ($r_s = 0.43$, $P < 0.002$) were correlated only with ROCKS. Neither EBIS nor SPECIES showed any relationship with independent habitat variables. A plot of ROCKS versus TOTIND and DESMOG suggests a linear relationship ($r^2 < 0.20$). Therefore, we included the number of rocks as a covariate in the ANOVAs examining the relationship with these dependent variables and ANC class, elevation and aspect.

We found few significant associations between salamander metrics and stratification variables. We found no relationship between SVL or TL of any species of stream salamander and ANC class. The variable DESMOG was significantly related to ANC class including the covariate ROCKS ($F_{2,48} = 3.21$, $P < 0.05$). Evaluation of the pairwise comparisons revealed no significant difference at $\alpha = 0.05$ between the ANC classes, though the mean counts of DESMOG in “sensitive” (13.6 ± 2.3) and “not sensitive” (10.9 ± 1.2) streams are more similar than in “extremely sensitive” (9.6 ± 1.9) streams ($P < 0.15$). Because ANC is related to pH (by equations presented in Methods section), we plotted the abundance of DESMOG vs. stream pH, and found a weak but significant positive relationship (Fig. 2; $r^2 = 0.17$, $P < 0.01$). DESMOG was also positively related to elevation ($F_{2,48} = 3.40$, $P < 0.05$). SPECIES, TOTIND and EBIS were not related to any of the stratification variables.

Using a logistic regression model, we found no relationship between the independent habitat variables and the presence or absence of *Gyrinophilus porphyriticus* in the study streams.

DISCUSSION

We did not find overwhelming evidence of stream salamander response to stream acid-base status in streams at Shenandoah National Park. *Desmognathus monticola* and *D. fuscus* were abundant at all sites and constituted a large percentage of all salamander captures, though *Eurycea bislineata* was more ubiquitous among the surveyed streams. Salamanders such as *Gyrinophilus porphyriticus* and *E. bislineata* with extended larval periods (e.g., 2 y or more), are exposed to acidic stream water for a longer period during development than desmognathine salamanders (9 mo larval period; Petranka, 1998). If chronic stream acidification was

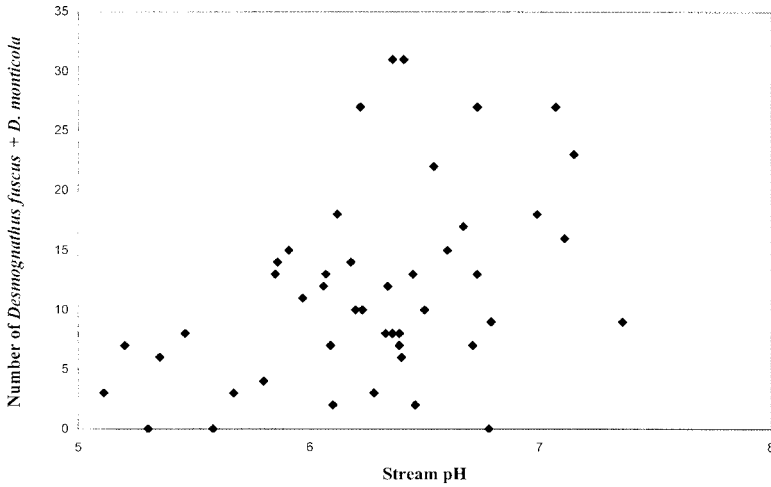


FIG. 2—Relationship between the combined counts of *Desmognathus fuscus* and *D. monticola* (DESMOG) and stream pH ($r^2 = 0.17$) at 49 stream sites in Shenandoah National Park, Virginia, USA. Three sites were missing pH data

affecting their larval growth or survival, we might expect smaller individuals and lower counts of these species.

Our survey method focused on the stream banks, and subsequently we did not capture many larval salamanders. The larval stage of stream salamanders is likely the most sensitive life stage to acidification (Kucken *et al.*, 1994), though chronic acidification of stream habitats in SNP would also be expected to alter the distributional patterns and abundance of the more terrestrial adult salamanders. We found suggestions that adult *Desmognathus* sp. are negatively affected by stream ANC class. Abundance of *Desmognathus* sp. was also greater at higher elevations.

While stream ANC status did not explain abundance of *Eurycea bislineata* or presence of *Gyrinophilus porphyriticus*, our results indicated that desmognathine salamander abundance may be affected by stream acidification. Roudebush (1988) reported sensitivity of *Desmognathus* species to pH levels below 5.0. None of the SNP streams that we sampled had a pH lower than 5.1, but a plot of the numbers of *Desmognathus* sp. versus pH suggest a similar pattern (Fig. 2).

Our results are consistent with Davic *et al.* (1987), who found that the density of rock cover was related to salamander density. Barr and Babbitt (2002) found that *Eurycea bislineata* presence was positively related to temperature and pH, and abundance was positively related to elevation, temperature, pH, canopy closure and stream gradient. We did not observe the same relationship with elevation, temperature (often correlated with elevation) or stream pH, though Barr and Babbitt (2002) captured primarily larval salamanders, while our study method was designed to sample primarily adults.

In this synoptic but geographically extensive study, we did not estimate salamander population sizes. Since 2000, we have been using removal sampling (two or three removal passes) in SNP to estimate stream salamander populations at a reduced number of streams (three streams on each of the three bedrock types) to determine whether long-term changes in populations are evident and whether changes in populations over time may be related to environmental changes, such as stream acidification. As salamanders are long-lived

organisms [*e.g.*, *Desmognathus monticola* can live up to 11 y (Castanet *et al.*, 1996)], it is possible that the number of adult salamanders has not yet responded to stream acidification, even if the population is declining due to decreased recruitment from larval age classes. Because we did not sample the larval age class efficiently, we cannot determine whether the proportion of larval and adult salamanders is different among streams with different ANC status. However, acid precipitation has presumably affected the acid-base status of streams in SNP since before 1980 (Webb *et al.*, 1995), and thus, the lack of a consistent pattern in the distribution and abundance of adult salamanders may indicate that stream salamanders in SNP are not strongly affected by historic or current watershed acidification.

The summer of 1999 was an extremely dry year, with rainfall 31.8 cm below normal and an extended period of lower than average precipitation (SERCC, 2003). Petranka and Murray (2001) found that surface activity of *Eurycea bislineata* was related to rainfall events, with greater captures recorded in high moisture conditions. Increasing captures may have clarified differences in amphibian distribution with respect to the habitat variables measured in this study. Typically, stormflow events with pulses of NO_3^- entering the stream would create temporary periods of decreased ANC in the stream water (*i.e.*, episodic acidification, *see* Vertucci and Corn, 1996; Buffam *et al.*, 2001). Stream salamanders may be affected by episodic acidification if these events were in synchrony with breeding phenology events, such as egg deposition or larval development. In a normal year, precipitation events occur throughout the early life stages of stream salamanders, and thus, the capacity to neutralize acidic inputs to a stream might be expected to affect recruitment.

Despite the dry summer and sampling focus on adult rather than larval salamanders, we found suggestions of stream salamander response to the acid-base status of streams in SNP. We suggest that monitoring of stream salamander populations in relation to stream acid-base status continue, especially in federally protected areas of the northeastern United States. Studies of acidic precipitation on stream habitats (*e.g.*, Galloway *et al.*, 1999) indicate effects on stream biological communities. More study is needed to directly test potential effects of watershed acidification on stream salamander populations.

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