

Response of stream-breeding salamander larvae to sediment deposition in southern Appalachian (U.S.A.) headwater streams

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SUMMARY

1. Increased fine sediment deposition is a prevalent threat to stream biodiversity and has been shown to impact stream-breeding salamanders negatively. However, their complex life histories make it difficult to determine which stage is affected.
2. We conducted field experiments from 26 August to 11 September 2010 and 11 October to 11 November 2010 in two southern Appalachian headwater streams (U.S.A.) to examine the response of larval salamanders to increased fine sediment deposition. Fine sediment was increased in artificial stream channels by 0, 33 and 67%. The number of larvae observed at the end of the experiments was used to determine whether larval microhabitat selection was influenced by fine sediment deposition. A concurrent survey of aquatic larvae in three nearby streams complemented this experiment. Stream substratum composition at survey sites was quantified to examine the effects of fine sediment on larval salamander abundance.
3. Increases in fine sediment deposition failed to explain the number of larval salamanders detected in stream channels. Similarly, a negligible effect of fine sediment was observed on abundance estimates.
4. These results suggest that fine sediment deposition has a minimal impact on aquatic salamander larvae. Therefore, the effects of increased fine sediment loads on stream-breeding salamanders may not be the result of deleterious effects on the aquatic larvae but instead may be the result of effects on other stages. Management efforts that consider these other stages are therefore needed to protect stream-breeding salamander communities.

Keywords: *Desmognathus quadramaculatus*, *Eurycea wilderae*, headwater stream, salamander, sediment

Introduction

Freshwater biodiversity is highly threatened, largely as a result of human appropriation of water resources and land-use practices that degrade freshwater habitat (Allan & Flecker, 1993; Allan, 2004; Vorosmarty *et al.*, 2010). A pervasive form of freshwater habitat degradation is caused by the increased inputs of fine sediment to streams (reviewed by Waters, 1995). While increases in fine sediment can alter streams in a variety of ways, a particularly important effect is when deposited fine sediment alters the composition of the substratum (i.e. sedimentation). Sedimentation can limit the availability of interstitial spaces that serve for foraging, cover and nesting habitats for benthic organisms (reviewed by

Waters, 1995). Fine sediment deposition can therefore reduce substratum heterogeneity and alter the species composition and abundance of primary producers (Matthaei *et al.*, 2006), macroinvertebrates (Angradi, 1999; Zweig & Rabeni, 2001; Matthaei *et al.*, 2006; Larsen & Ormerod, 2010), salamanders (Lowe & Bolger, 2002; Peterman & Semlitsch, 2009) and fish (Berkman & Rabeni, 1987; Sutherland, Meyer & Gardiner, 2002).

Headwater streams contribute significantly to overall freshwater biodiversity (Meyer *et al.*, 2007) and comprise by far the biggest proportion of channel length in drainage networks (Leopold, Wolman & Miller, 1964). Nevertheless, headwater streams often receive limited protection (Lowe & Likens, 2005) and may be particularly vulnerable to terrestrial environmental changes because of their

intimate connection to the riparian zone (Wallace *et al.*, 1997; Lowe & Likens, 2005). Given that fine sediment deposition is a chief consequence of such environmental changes, it represents a key threat to headwater stream biodiversity.

Where they occur, stream-breeding salamanders are abundant predators in headwater streams, and their biomass can exceed that of other vertebrates (Peterman, Crawford & Semlitsch, 2008). Surveys have generally, although not always, demonstrated a negative effect of sedimentation on salamander abundance (Lowe & Bolger, 2002; Smith & Grossman, 2003; Sepulveda & Lowe, 2009). However, it is unclear whether sedimentation exerts its main negative effects on the eggs, larvae or adults. Egg laying can occur in a variety of habitats, but, depending on the species, oviposition may be in the stream, such as beneath cobbles or in the hyporheic zone, or in riparian areas where eggs can be buried in the stream bank (Petranka, 1998). Sedimentation may therefore smother eggs laid in the stream or limit the availability of sites for egg deposition, as has been observed for some fish species (reviewed by Waters, 1995). In addition, bank erosion resulting from human activity potentially affects species that lay eggs in riparian areas. Aquatic larvae occupy interstitial spaces in gravel and cobble for cover and foraging (Petranka, 1998) and may therefore be directly affected by sedimentation. Sediments may also reduce the availability of macroinvertebrate prey for larvae. Adults are semi-aquatic and may be affected in a manner similar to that of larvae while using stream habitats, but, like the egg stage of some species, they are also susceptible to terrestrial habitat degradation (e.g. forestry, urbanisation, etc.).

The limited existing evidence for stage-specific effects of sedimentation on salamanders indicates that adult salamanders are more affected by sediment deposition than aquatic larvae (Lowe, Nislow & Bolger, 2004). Peterman & Semlitsch (2009) showed that riparian buffer width had a greater effect than sedimentation on the larvae of the Blue-Ridge two-lined salamander (*Eurycea wilderae* Dunn), whose adults are highly terrestrial. Furthermore, they showed that larvae of the Black-bellied salamander (*Desmognathus quadramaculatus* Holbrook), whose adults are highly aquatic, appeared to be unaffected by either sedimentation or riparian buffer width. These results suggest that the effects of sedimentation on the aquatic stage (egg, larvae or adult) were slight and that declines in salamander abundance were the result of terrestrial habitat degradation affecting adults rather than fine sediment deposition.

Understanding potential stage-specific impacts of sedimentation on salamander populations has important management implications. Adult salamanders regularly use forest habitat >25 m from streams (Crawford & Semlitsch, 2007), and dispersal between streams often occurs over land rather than within the stream corridor (Grant *et al.*, 2010). Best management practices designed to protect stream ecosystems from increased inputs of fine sediment may therefore fail to protect salamander populations adequately if the terrestrial stage is most affected by sedimentation. For example, forestry practices aimed at reducing run-off in North Carolina recommend a riparian buffer of 9–15 m for permanent streams (Brogan *et al.*, 2006). While this practice may mitigate the effects of stream sediment inputs on most stream biota, it is unclear as to whether it adequately protects salamanders.

Our objective was to improve the understanding of the stage-specific effects of sedimentation on stream-breeding salamanders. We were specifically interested in examining the effects of increased sediment deposition on aquatic larvae. We therefore conducted two field experiments to examine the effects of increased sedimentation on larval salamander abundance. We hypothesised that larval salamander abundance would decrease in response to increased sedimentation in experimental stream channels. Concurrent larval population surveys were conducted in three southern Appalachian headwater streams to determine whether responses elicited in the sediment enrichment experiment were consistent with observations in nearby streams.

Methods

Study sites

This study was conducted in fishless Appalachian headwater streams (first or second order) in the Nantahala National Forest of south-western North Carolina (U.S.A.). The study reaches were at similar altitudes (760–920 m) and drain a dense canopy of mixed deciduous hardwood forest with an understorey dominated by rhododendron (*Rhododendron* spp.). Field experiments were conducted in two permanent headwater streams, Wolf Rock Branch and Snake Den Branch, located within the Coweeta Hydrologic Laboratory, Macon Co., North Carolina. Wolf Rock Branch is a first-order stream draining into Shope Fork, while Snake Den Branch is a second-order stream draining into Ball Creek. Surveys were conducted in three first-order unnamed streams located near Highlands, North Carolina, c. 30 km east of the experimental streams. Canopy cover over the study streams was estimated

using a densiometer and averaged 84%. The mean wetted width of the three study reaches was 1.53 m and the mean depth 3 cm. Discharge in the three survey streams varied over the course of the study but averaged 3.9 L s^{-1} ($\pm 2.5 \text{ SD}$). Although not measured in experimental streams, the latter were similar to the survey streams in their physical habitat characteristics.

Experimental methods

An initial experiment was conducted in Wolf Rock Branch from 26 August to 11 September 2010. To improve the overall generality of the results, the experiment was then repeated in Snake Den Branch from 11 October to 11 November 2010 after low numbers of larvae were found in the first experiment.

Experimental stream channels were constructed of 0.15-m-diameter polyvinyl chloride tubing divided into 1-m sections and longitudinally bisected so that tops were uncovered. Thirty channels were placed in streams at increments $>1 \text{ m}$ apart along the stream channel and were buried so that the bottom of each channel was flush with the surface of the streambed. A substratum mixture similar in composition to those used in other experiments with larval salamanders (e.g. Resetarits, 1991; Lowe *et al.*, 2004) was evenly distributed to a depth of *c.* 2 cm. It consisted of a 1 : 1 mixture of unbleached playground sand ($<2 \text{ mm}$) and gravel (2–60 mm) particles. Three leaf litterbags constructed of 1-cm plastic mesh and containing 10 g of air-dried mixed leaf material were added to each channel. Mixed litter was predominantly oak (*Quercus* spp.) and maple (*Acer* spp.) leaves, with other deciduous species (e.g. *Betula* spp., *Liriodendron* spp. and *Tilia* spp.) present to a lesser extent, and was collected haphazardly from stream banks. Three cobbles (60–256 mm) were collected from the stream and placed on top of leaf litterbags to secure them in place and provide cover for larval salamanders. Sediment treatments were randomly assigned to channels and consisted of no fine sediment added, fine sediment added to completely cover one cobble and leaf litterbag (*c.* 33% increase in sedimentation) and fine sediment added to completely cover two cobbles and leaf litterbags (*c.* 67% increase in sedimentation). Each treatment was replicated 10 times in both streams; however, two replicates each of the moderate and high fine sediment treatments were lost as a result of high flows following heavy rain.

Experimental channels were checked every 3 days throughout experiments, and fine sediment was added as needed to maintain treatments. At the conclusion of experiments, material in experimental channels was col-

lected while moving upstream. Contents of experimental channels were emptied into a large bucket, and stream water was added to completely cover this material. Bucket contents were then sieved through nested 1-mm and 500- μm sieves to remove salamanders. Larvae were identified to species, counted and kept individually in plastic bags containing stream water before being returned to the stream once all experimental channels had been examined.

To examine how well our fine sediment treatments replicated natural fine sediment deposition, we compared the proportion of coarse sand ($>500 \mu\text{m}$), medium sand ($>250 \mu\text{m}$), fine sand ($>125 \mu\text{m}$) and very fine sand ($<125 \mu\text{m}$) in the playground sand used with samples of natural fine sediment that had deposited on tiles ($5 \times 5 \text{ cm}$) collected from a separate experiment in Wolf Rock Branch. Dried playground sand was separated into 20 replicates of 100 g and sieved through nested sieves to separate it into the different size categories. Natural fine sediment was first placed in a muffle furnace ($550 \text{ }^\circ\text{C}$) for 3 h to remove organic material and then sieved. All samples were dried at $60 \text{ }^\circ\text{C}$ for 48 h and weighed to the nearest 0.01 g.

Survey methods

Larval salamanders were surveyed on three main dates (1 September, 18 October and 20 November 2010), with four consecutive secondary sampling days for each main period. This was done to meet the demographic closure assumptions of the model we used to estimate larval abundance. We chose a passive sampling technique to minimise disturbance to the streambed associated with more active sampling techniques (e.g. dip netting). Eleven larval 'traps' (Peterman & Truslow, 2008; Nowakowski & Maerz, 2009) were placed every 5 m along a 50-m reach in each stream. Traps were made using commercial plant nursery palettes ($53 \times 26 \times 6 \text{ cm}$), which are plastic trays with large holes on the sides and bottoms and an open top. The palettes were completely covered in coarse plastic mesh (1 cm^2) attached with plastic cable ties and filled with leaf litter collected from the stream bank (Peterman & Truslow, 2008; Nowakowski & Maerz, 2009). This allowed the traps to contain leaf litter throughout the sampling period while also allowing salamander colonisation, which they will readily do within a week of initial placement (Peterman & Truslow, 2008). Traps were held in place with one large cobble collected from the streambed.

Traps were checked beginning at the downstream end of a sampling reach by quickly removing it from the

stream and placing it in a large bucket. A dip net was then used to sample the area located immediately underneath the leaf trap by rapidly sweeping the dip net along the streambed (Peterman & Truslow, 2008). Stream water was then added to the bucket, and all the material in the trap was removed and kept in the bucket. This material was then carefully searched and returned to the trap, while the remaining material in the bucket was sieved through 1-mm and 500- μ m nested sieves to remove salamanders, which were identified, counted and returned to the upstream end of the trap following processing.

Substratum composition at each trap site was characterised using a 50 $\times$ 50 cm clear acrylic sampler divided into 5 $\times$ 5 cm grids. The sampler was placed flush with the streambed, and the percentage of fine sediment (<2 mm), gravel (2–60 mm), cobble (60–256 mm) and boulder (>256 mm) was visually estimated to the nearest 5% (Peterman & Semlitsch, 2009). We recognise that particle size classes included in what we call fine sediment encompass particles ranging from silt to coarse sand as classified by the Wentworth scale (Bunte & Abt, 2001). While these particle sizes may differ in their effects on stream biota, we were specifically interested in the potential effects of sediment deposition, and previous studies have demonstrated a negative effect of fine sediments classified as particles <2 mm on stream biota (e.g. Zweig & Rabeni, 2001; Matthaei *et al.*, 2006; Larsen, Vaughan & Ormerod, 2009; Sepulveda & Lowe, 2009). However, we acknowledge that including coarser sediment particles in the fine sediment category may underestimate the impact of fine sediment if these larger particles differ in their impact on larval salamanders. The percentage of riffle, run and pool habitat was visually estimated to the nearest 5% for every 5-m reach along each transect. We are therefore assuming that the number of individuals within a trap is related to the surrounding habitat characteristics and representative of larval preferences for those characteristics.

Statistical analysis

Experiments were analysed using generalised linear models (GLM) with a Poisson error distribution. Separate analyses were conducted for *D. quadramaculatus* and *E. wilderae*, which were the two most abundant species encountered. Data were pooled for the analysis of *E. wilderae* larvae after first testing for a significant interaction between stream and fine sediment treatment (*P*-values for interaction terms were >0.05). We used data from only Snake Den Branch in the analysis of *D. quadramaculatus*, because of the small number of these larvae ($n = 3$)

captured in Wolf Rock Branch. Chi-square tests and Akaike's information criterion adjusted for small sample size (AIC_c) were used to examine the importance of including sedimentation as a factor in GLMs (Burnham & Anderson, 2002). A Hotelling's T^2 test was used to examine differences in arcsine-square-root-transformed proportions of sand categories for playground and natural sand. A significant difference was detected ($F_{4, 35} = 240.20$, $P < 0.001$), so we performed multiple Welch's *t*-tests, which do not assume equal variance, with a Bonferroni adjusted *P*-value (0.0125) to examine differences in size categories between natural and artificial sand. Statistical analyses were performed in R version 2.12.2 (R Development Core Team, 2011). Model comparisons were made using the AICcmodavg package (Mazerolle, 2011).

Hierarchical N-mixture models for estimating the number of individuals based on spatially replicated count data were used to estimate the number of larval salamanders at a site (Royle, 2004; Kery & Schaub, 2012). To avoid issues of multicollinearity among substratum variables, we first conducted a principal component analysis (PCA) on arcsine-square-root-transformed substratum data. We retained the first two principal components based on the broken-stick criterion (McGarigal, Cushman & Stafford, 2000), which together explained 84% of the variation, to use as site-level covariates in hierarchical models. The first principal component (PC 1) explained 60% of the variation and was positively associated with fine sediment ($r = 0.98$, $P < 0.001$) and boulder ($r = 0.09$, $P = 0.65$), but negatively associated with gravel ($r = -0.72$, $P < 0.001$) and cobble ($r = -0.67$, $P < 0.001$). This axis appeared to be driven largely by differences in the percentage of fine sediment versus gravel and cobble, so sites with a high PC 1 value were areas with a high percentage of fine sediment and a low percentage of gravel and cobble. The second principal component (PC 2) explained 24% of total variation and was positively associated with fine sediment ($r = 0.10$, $P = 0.59$) and gravel ($r = 0.69$, $P < 0.001$), but negatively associated with cobble ($r = -0.61$, $P < 0.001$) and boulder ($r = -0.48$, $P = 0.005$). This axis was driven largely by differences in the percentage of gravel versus the percentage of cobble and boulder, so sites with high PC 2 scores had a large amount of gravel but low amounts of cobble and boulders. A third site-level covariate describing the macrohabitat of a site (riffle, run or pool) was also included because there is evidence that this may affect abundance (Smith & Grossman, 2003). Other important features of these models were random effects for abundance and detection to account for potential overdispersion (Kery & Schaub, 2012). The goodness of fit was assessed using a Bayesian

P-value and visual examination of the discrepancy in simulated data versus discrepancy in observed data (Kery & Schaub, 2012). Analyses were performed using uninformative priors in WINBUGS version 1.4.3 (Lunn, Thomas & Best, 2000) with the R2WinBUGs package (Sturtz, Ligges & Gelman, 2005). We ran 950 000 iterations, with a burn-in period of 50 000, a thinning rate of 300 and three Markov chains. Convergence of the Markov chains was checked with Rhat values and a visual examination of time-series plots (Kery & Schaub, 2012).

The average estimated number of larvae at a site for all three primary periods was used in GLMs with a Poisson error distribution to examine the relationship between estimated larval abundance and arcsine-square-root-transformed fine sediment. Nonlinear relationships between salamanders and substratum cover have been observed in previous studies (Davic & Orr, 1987), so we tested for this possibility in our data using generalised additive models (GAM) with a Poisson error distribution (Ficetola & Denoel, 2009). We considered an increase in the estimated degrees of freedom of ≥ 2 to represent a significant nonlinear relationship (Ficetola & Denoel, 2009). GAMs were run using the mgcv package (Wood, 2011).

Results

Habitat information

Sites varied considerably in their substratum composition (Table 1). The amount of fine sediment at a site ranged from 0 to 100%, gravel from 0 to 94%, cobble from 0 to 80% and boulder from 0 to 84%. There was also variation in substratum composition among streams (Table 1). Overall, sites were located more in riffle ($n = 13$) and run ($n = 15$) habitats than in pools ($n = 5$). However, this bias towards riffles and runs reflects the visually estimated percentage of these habitats (Table 1).

Experiment

Playground sand used for simulating fine sediment deposition was composed of small amounts of coarse

sand (playground mean = 0.06, stream mean = 0.44, $t_{17.78} = -21.47$, $P < 0.001$) and very fine sediment (playground mean = 0.22, stream mean = 0.29, $t_{24.08} = -5.03$, $P < 0.001$) compared with *in situ* stream sediment samples. Playground sand had large amounts of medium sand (playground mean = 0.82, stream mean = 0.76, $t_{36.4} = 2.69$, $P = 0.01$) and fine sand (playground mean = 0.70, stream mean = 0.52, $t_{32.93} = 8.06$, $P < 0.001$). The inclusion of fine sediment failed to improve models for either *E. wilderae* ($\chi^2 = 0.56$, $P = 0.62$) or *D. quadramaculatus* ($\chi^2 = 2.43$, $P = 0.39$). The inclusion of fine sediment also failed to reduce AIC_c values for either species (Table 2). A comparable trend was observed in stream-averaged predictions for both species in response to sediment treatment (Fig. 1). The predicted number of *E. wilderae* larvae was similar for low (0.35 larvae per channel) and high (0.33 larvae per channel) sediment treatments but was higher for the moderate (0.56 larvae per channel) treatment. Similarly, the predicted number of *D. quadramaculatus* larvae was reduced for low (0.60 larvae per channel) and high (0.38 larvae per channel) sediment treatments but was higher for the moderate (1 larvae per channel) treatment.

Survey

The hierarchical N-mixture models produced an adequate fit to the data for both *E. wilderae* (Bayesian *P*-value = 0.48) and *D. quadramaculatus* (Bayesian *P*-value = 0.50). The mean estimated number of larvae was three for *E. wilderae* and two for *D. quadramaculatus*, which corresponds to a density of 22 and 15 larvae m⁻², respectively (determined by dividing the estimated count by the trap area). The mean number of estimated larvae declined noticeably for *E. wilderae* throughout the sampling period (September = 4, October = 2, November = 2), but not for *D. quadramaculatus* (September = 2, October = 2, November = 2). Mean estimates of the probability of detection were similar for both species (*E. wilderae* = 0.35 and *D. quadramaculatus* = 0.34) but varied strongly temporally, ranging from a mean of 0.24 to 0.42 for *E. wilderae* and 0.26

Table 1 Mean percentage (± 1 SE) of different substratum and habitat types. Substratum composition was estimated at individual sites, while habitat type was characterised for a 5-m reach

	Fine Sediment	Substratum composition			Habitat type		
		Gravel	Cobble	Boulder	Riffle	Pool	Run
Stream A	10.36 (± 3.17)	54.95 (± 8.79)	26.29 (± 8.46)	8.44 (± 7.60)	29.50 (± 8.86)	32.50 (± 6.34)	38.00 (± 7.08)
Stream B	32.03 (± 10.19)	41.73 (± 8.60)	19.38 (± 6.93)	6.85 (± 3.81)	46.50 (± 4.72)	11.50 (± 2.89)	42.00 (± 5.88)
Stream C	44.71 (± 11.55)	36.07 (± 8.07)	15.45 (± 6.32)	3.76 (± 2.55)	34.00 (± 6.18)	20.50 (± 4.50)	45.5 (± 7.54)
Overall	29.03 (± 5.66)	44.25 (± 4.95)	20.38 (± 4.15)	6.35 (± 2.88)	36.67 (± 4.02)	21.5 (± 3.11)	41.83 (± 3.89)

Table 2 Experimental results for the effects of sedimentation on the abundance of larval salamanders. The number of parameters (K), Akaike's information criterion adjusted for small sample size (AIC_c), change in AIC_c (ΔAIC_c), Akaike's weight (w_i), log likelihood ($LogL$), χ^2 value and P -value (P) are shown. The combined evidence of the AIC_c value and the χ^2 test indicates that fine sediment had a negligible effect on larval salamander abundance for either species

	K	AIC_c	ΔAIC_c	w_i	$LogL$	χ^2	P ($\geq \chi^2$)
<i>Eurycea wilderae</i>							
Null	2	131.02	0	0.86	-63.4		
Fine Sediment	4	134.58	3.56	0.14	-62.9	0.56	0.62
<i>Desmognathus quadramaculatus</i>							
Null	2	56.97	0	0.78	-27.4		
Fine Sediment	4	59.46	2.49	0.22	-26.19	2.43	0.30

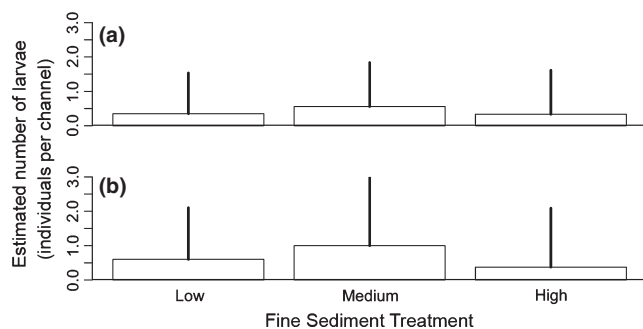


Fig. 1 Mean (+1 SE) estimated number of (a) *Eurycea wilderae* and (b) *Desmognathus quadramaculatus* larvae in experimental stream channels.

to 0.45 for *D. quadramaculatus*, with a seasonal decline in detection probability for both species. The amount of fine sediment appeared to have a slightly positive effect on the number of *E. wilderae* larvae observed at a site (Fig. 2). In contrast, the number of *D. quadramaculatus* at a site was negatively associated with the amount of fine sediment (Fig. 2). Results of GAMS suggested that this relationship was linear for both species, as the estimated degrees of freedom were 1 for both.

Discussion

Field experiment

Increases in fine sediment deposition had a limited impact on the number of larval salamanders observed in experimental channels. This was somewhat surprising, given that surveys have generally found a negative association between larval salamander abundance and fine sediment (Smith & Grossman, 2003; Peterman & Semlitsch, 2009). The mechanisms behind this relationship are unknown, but it has been suggested that increased fine sediment may negatively impact larvae by limiting access to

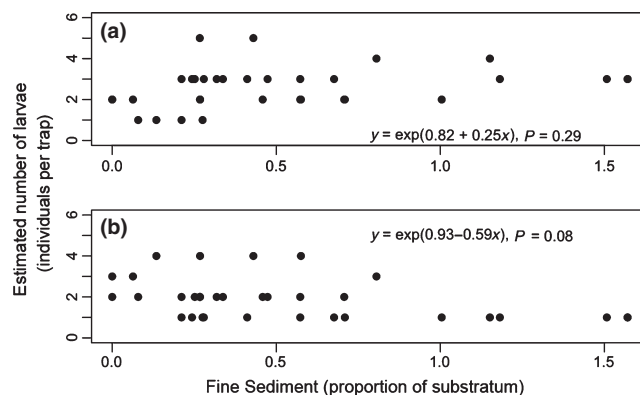


Fig. 2 Relationship between estimated number (individuals per trap) of (a) *Eurycea wilderae* and (b) *Desmognathus quadramaculatus* larvae and the proportion of fine sediment. The proportion of fine sediment was arcsine-square-root-transformed.

interstitial spaces in gravel and cobble that provide refuge from predation as well as habitat for macroinvertebrate prey (Smith & Grossman, 2003; Peterman & Semlitsch, 2009). However, Lowe *et al.* (2004) found that increased sedimentation had no effect on larval growth rate or survival of spring salamanders (*Gyrinophilus porphyriticus* Blatchley), despite the presence of a predator. Although we studied different species, this suggests that increased sediment deposition may not necessarily increase predation risk in larval salamanders. Unfortunately, we were unable to assess the potential importance of the effects of sedimentation on macroinvertebrate prey, as we did not examine macroinvertebrate prey available in experimental stream channels. However, while the effects of sedimentation on macroinvertebrates are well documented, it is unclear how these changes will affect larval salamanders.

Sedimentation has been shown to reduce the abundance and diversity of Ephemeroptera, Trichoptera and Plecoptera that prefer heterogeneous mixtures of gravel and cobble (reviewed by Waters, 1995). However, benthic taxa tolerant of fine sediment, such as Chironomidae and Oligochaeta, typically benefit from increased sedimentation (reviewed by Waters, 1995). Fine sediment deposition may therefore alter community composition but not cause declines in the overall density of macroinvertebrates (reviewed by Waters, 1995). Larval salamanders are generalist predators capable of exploiting a variety of macroinvertebrate taxa (Petranka, 1998), so changes in macroinvertebrate community composition may not negatively impact larvae as long as macroinvertebrate densities are adequate to support them.

We should not conclude that sedimentation is not detrimental to larval salamanders based on this experimental evidence alone because of several potential issues. First,

sediment loads above those simulated in these experiments could have negative impacts. We observed a decline in the number of larvae at high sediment compared to moderate sediment for both species, suggesting that more sediment may have a negative impact. Additionally, our experiment simulated sediment deposition at a fine spatial scale (c. 0.15 m²), and while salamander larvae have been shown to respond to sedimentation at local scales (e.g. Smith & Grossman, 2003; Sepulveda & Lowe, 2009), it is possible that larger-scale sediment deposition negatively impacts larvae. Scale-dependent impacts of sediment deposition have been observed for macroinvertebrate taxa, with effects detected at finer scales sometimes disappearing at larger scales (Larsen *et al.*, 2009). Multiscale relationships have been observed for stream-breeding amphibians (Lowe & Bolger, 2002; Welsh & Lind, 2002; Sepulveda & Lowe, 2009; Ficetola *et al.*, 2011), but we are unaware of studies that have explicitly examined potential scale-dependent effects of sedimentation on larval salamanders. Finally, we note that the sediment used in experiments contained smaller fractions of coarse and very fine sands than naturally deposited sediment. While we believe that the experimental fine sediment adequately simulated the process of fine sediment deposition, it is possible that the smaller and larger particles observed in naturally deposited sediment might differ in their impacts on larval salamanders.

Field survey

There are few estimates of the density of larval salamanders in streams, and those that are available are variable. For example, density estimates for *E. wilderae* range from 1.16 to 137 larvae m⁻² in south-western North Carolina (Johnson & Wallace, 2005; Peterman & Truslow, 2008; Milanovich, 2010). It is difficult to know whether this variation is because of actual ecological differences in the abundance or because of differences in sampling methods. Our estimates for *E. wilderae* density (20 larvae m⁻²) are within the observed range seen in previous studies, suggesting that they are reasonable. Additionally, we found considerable temporal variation at a site in estimates as well as between sites (1 to 64 larvae m⁻²), which shows that the variation in estimates seen in other studies might at least partially reflect true differences in larval abundance rather than methodological differences in sampling. There are even fewer estimates of *D. quadramaculatus* larval density, but estimates vary from 2.27 to 32.23 larvae m⁻² (Davic, 1983; Milanovich, 2010), and ours (15 m⁻²) are within this range.

We found that increasing amounts of fine sediment were not associated with large declines in the estimated number of larvae for either species (Fig. 2). These results are similar to those of the study of Peterman & Semlitsch (2009), who found no effect of sedimentation on *D. quadramaculatus* larvae, although our results differ in that we did not observe a negative effect of sedimentation on *E. wilderae*. However, Peterman & Semlitsch (2009) found this effect to be relatively minor compared with the effect of riparian buffer width, which agrees with the negligible effect of sedimentation we observed. In contrast, Smith & Grossman (2003) found that larval southern two-lined salamanders (*Eurycea cirrigera* Green), a species morphologically similar and closely related to *E. wilderae*, avoided areas of high sedimentation. It is possible that these two species use different microhabitats despite their morphological similarity. It is also possible that *D. quadramaculatus*, which were not present in the streams sampled by Smith & Grossman (2003), exclude *E. wilderae* from preferred areas of low fine sediment through either competition or predation risk. However, we saw no relationship ($r = -0.05$, $P = 0.77$) between these species' predicted larval abundances, suggesting that this was not the case. Unfortunately, little is known about the microhabitat preferences of larval salamanders, let alone how interactions with other species affect those preferences, and it is therefore difficult to determine the cause of the apparent difference in microhabitat choice between these two closely related species.

Our results point towards a potential species-specific effect of sedimentation. We observed a negative trend in larval abundance estimates for *D. quadramaculatus* associated with increasing sediments (Fig. 2). In contrast, almost no effect of sediment was observed for *E. wilderae*. Species-specific effects of sedimentation have been observed for macroinvertebrates and fish, with some species benefiting from more sediment (Angradi, 1999; Sutherland *et al.*, 2002). We suspect that such species-specific responses are likely for other salamander species, and studies examining the responses of other stream-breeding species to sedimentation are needed to evaluate the generality of our results.

Management implications

A number of studies have documented declines in stream-breeding salamander populations associated with increasing sedimentation (Corn & Bury, 1989; Lowe & Bolger, 2002; Smith & Grossman, 2003). However, the impacts of sedimentation may have been confounded with the landscape disturbance causing increased sediment inputs

in these studies. The combined evidence of our experimental and survey results suggests the aquatic larval life stage is only moderately affected by sedimentation. However, we caution against concluding that sedimentation has no deleterious effects on aquatic stages because of several potentially important limitations of our surveys and experiment. First, it is possible that the negative effects of sedimentation occur at the egg stage, which is aquatic for many species, which would have a large impact on salamander populations. Negative effects of sedimentation on egg survival have been observed in many fish (reviewed by Waters, 1995). While this phenomenon is well documented in fish, particularly salmonids, we are unaware of any studies that have investigated this potential issue for stream-breeding salamanders and suggest it is in need of research. Additionally, this study examined fine-scale effects of sedimentation, and it is possible that large-scale effects will differ from those we observed (Larsen *et al.*, 2009). Finally, we did not consider other effects of increased sediment input, such as increased turbidity or suspended particles, which may negatively affect larval growth and survival. To our knowledge, the effects of suspended sediment on larval stream-breeding salamanders have not been studied, but evidence from fish suggests it is a potentially important factor. Although direct mortality is rare, sublethal effects on fish are well documented (Waters, 1995). The reduced foraging and respiratory ability as well as increased susceptibility to disease that has been seen in fish may also prove problematic for larval salamanders. We believe that larval salamanders, which have external gills, may be particularly vulnerable to reductions in respiratory ability.

With these caveats in mind, our results suggest that sedimentation at fine spatial and limited temporal scales has little impact on aquatic larvae. This suggests that the terrestrial life stages may be the most vulnerable to the processes causing sedimentation. A number of studies have documented declines in salamander populations associated with terrestrial habitat degradation (e.g. timber harvest), including many species that are completely terrestrial (Corn & Bury, 1989; Petranksa, Eldrige & Haley, 1993; Ash, 1997; Crawford & Semlitsch, 2008). Current best management practices in the southern Appalachians, which recommend a 9- to 15-m riparian buffer to mitigate the impacts of run-off and sedimentation into streams (Brogan *et al.*, 2006), will therefore fail to protect stream-breeding salamander communities adequately. A larger riparian buffer is needed, as recommended by Peterman & Semlitsch (2009), to protect the terrestrial adults that use areas >15 m from the stream channel (Crawford &

Semlitsch, 2007). The biphasic life history of stream-breeding salamanders complicates the management of these species, and our results suggest that careful consideration of the habitat requirements of the terrestrial adult stage is needed to more appropriately manage headwater stream salamander communities.

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