



## Linking process to pattern: Causes of stream-breeding amphibian decline in urbanized watersheds

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### ARTICLE INFO

#### Article history:

Received 16 December 2009

Received in revised form 17 March 2010

Accepted 6 May 2010

Available online 26 May 2010

#### Keywords:

*Eurycea*

Flooding

Impervious surface

Stream

Urban

### ABSTRACT

Sufficient data have been collected to document negative effects of urbanization on many abiotic parameters, aquatic insects, and fish. Recently, stream-breeding salamanders have been observed to decrease in density in urban areas. We employed a two-step process to determine the mechanism(s) most likely responsible for salamander decline in disturbed streams. First, we conducted a 2-year survey of larval two-lined salamanders (*Eurycea cirrigera*) and abiotic variables among 12 streams in western Georgia, USA surrounded by land disturbed by urbanization and pasture. From these surveys we constructed hypotheses that might explain differences in observed larval salamander density among streams. A model in which increased watershed impervious surface causes an increase in spate (i.e., high water flow) frequency and magnitude, which then leads to decreased larval density had the most support. This analysis led to a second step in which we conducted an experiment using artificial streams lined with substrates commonly found in urban and forested streams (sand/sand with detritus and gravel-pebble/pebble-cobble, respectively). We placed salamander larvae in the artificial stream channels and subjected them to increasing water velocity. Larvae on sand-based substrates common to urban streams were flushed from the artificial stream at significantly lower velocities than larvae on rock-based substrates. The experimental data were consistent with the hypothesis generated from field sampling that high water velocities in urban streams result in decreased larval retention in streams. Our result emphasizes the need for upland habitat restoration in urban areas, which will reduce run-off and improve stream habitat quality.

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### 1. Introduction

Studies of stream systems have repeatedly documented shifts in the diversity and composition of a biota as a result of land development for agriculture or urbanization (Paul and Meyer, 2001; Allan, 2004). A recent review indicated a consistent loss of sensitive fish and invertebrate species in streams draining urbanized watersheds (Walsh et al., 2005). Many amphibians breed in streams, and several studies have documented amphibian assemblage response to agriculture or urban development (Orser and Shure, 1972; Willson and Dorcas, 2003; Price et al., 2006; Miller et al., 2007). These studies often have noted negative correlations between species abundance and percent of land cover existing as impervious surface or agriculture (Willson and Dorcas, 2003). Such correlations between land cover and a biotic response are noteworthy, but they do not provide a mechanistic explanation for species loss or decline with

development. Burcher et al. (2007) developed a framework for identifying the mechanism(s) that effect(s) stream biota following land use change. They proposed a cascading effects model in which multiple abiotic variables are evaluated as potential intermediates between land use change and biotic response(s). Competing a priori models can be constructed, each representing different combinations of cause and effect among intermediates; the best model can be identified based on collected data.

Creating multiple models representing connections between land use change and a biotic response is important because development within a watershed can lead to a number of shifts in the abiotic environment relative to undisturbed streams. Examples include long-term changes in measures of water quality, such as decreased organic matter, increased conductivity, or elevated suspended solid concentrations during stormflows (Walsh et al., 2005). Such altered abiotic environments can exert stress on stream communities; often leading to shifts in fish and invertebrate assemblages (Walsh et al., 2005), or decreased abundance of salamanders (Willson and Dorcas, 2003). A major disturbance in urbanized stream systems is hydrologic alteration (Schueler, 1995; Paul and Meyer, 2001; Schoonover et al., 2006). Increased

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impervious surface (i.e., roads and roofs) causes increased overland flow during rain events. As a result, urban streams often show stormflows that are of greater magnitude and frequency than those in areas with less impervious surface (Poff et al., 2006; Schoonover et al., 2006). Given the variety of abiotic shifts accompanying urban development, an attempt to identify specific mechanisms negatively impacting stream biota should be a priority.

We used a two-phase approach to evaluate the mechanism(s) that influence(s) the response of stream-breeding salamanders to urban and agricultural development. In the first phase, we adopted Burcher et al. (2007) cascading effects model to evaluate field data collected on the response of southern two-lined salamanders (*Eurycea cirrigera*; Plethodontidae) to landscape-scale disturbances. We estimated survivorship during the aquatic stage of the life-cycle for two cohorts of salamanders in 12 streams, which varied in the amount of disturbed land within their watersheds. We chose *E. cirrigera* because a previous study in the southern Piedmont of the United States (Barrett and Guyer, 2008) indicated it is the only salamander, of five species native to forested streams in this region, to persist in urban waters. By studying factors of land development that influence southern two-lined salamander survivorship during the larval phase, we hoped to generate hypotheses regarding factors leading to the extirpation of more sensitive species. We used salamander survey data in conjunction with measures of the abiotic environment to evaluate the relative importance of abiotic disturbances in explaining salamander persistence in streams. Our purpose was to move beyond a simple report of a negative correlation between species density and development, to a focus on potential mechanistic explanations for any observed differences in density among streams.

We then used artificial streams in a second phase of study that experimentally evaluated the effects of increased water velocity on salamanders (this was the strongest explanatory mechanism from the first phase of study). We conducted the experiments in a manner that allowed us to assess the prediction that the ability of larvae to remain in experimental streams would be reduced at high water velocities, especially when streambed substrates were constructed to resemble those found in urban streams. Results from this two-phase approach were used to create a more complete understanding of the mechanisms responsible for shaping salamander communities in urban environments.

## 2. Methods

### 2.1. Stream selection

We conducted salamander surveys in three counties in western Georgia, USA (Muscogee, Harris, and Meriwether). The study area was part of the West Georgia Project, an interdisciplinary effort by the Auburn University Center for Forest Sustainability to understand connections between ecological, sociological, and economic systems (Lockaby et al., 2005). We selected three 2nd- or 3rd-order streams (Strahler, 1952) in each of the following categories, which were established based a principal components analysis (PCA) performed on % land cover data (Table 1): forested, pasture, urban, and developing. The developing sites were in Harris County, Georgia, which is one of the fastest growing counties in the United States (Lockaby et al., 2005). Details on the PCA and support for the categorization of watersheds can be found in Barrett and Guyer (2008).

### 2.2. Salamander sampling

Southern two-lined salamanders typically inhabit riparian areas of 1st–3rd order streams as adults, and migrate to a stream in late

**Table 1**

Land cover and physical characteristics of study watersheds. IS = % impervious surface cover, pasture = % pasture cover, forest = % total forest cover, and LU/LC = dominant land cover in watershed (defined in methods, stream selection).

Site	Watershed size (km <sup>2</sup> )	IS	Pasture	Forest	LU/LC
SB1	20.1	2	20	73	Developing
SB2	6.3	3	20	73	Developing
SB4	26.6	3	28	64	Developing
BLN	3.6	1	19	76	Forest
MO	9.0	2	13	81	Forest
MU3	10.4	2	15	78	Forest
FS2	14.5	3	36	59	Pasture
FS3	3.0	3	34	62	Pasture
HC2	14.1	2	44	52	Pasture
BU1	25.5	40	23	34	Urban
BU2	24.7	25	25	46	Urban
RB	3.7	30	27	39	Urban

fall/early winter to mate (Smith, 2008). Eggs are deposited in late winter or early spring, and typically hatch in late spring (Smith, 2008). In the portion of the range where we studied this species, larvae remain in streams ~18 mo (KB, personal observation).

We sampled southern two-lined salamander density in 12 streams on four occasions: early summer 2006, spring and early summer 2007, and spring 2008. We used the spring sampling period to estimate density of salamanders immediately prior to metamorphosis (i.e., density of salamanders from the previous year's cohort). The summer sampling session was used to estimate hatchling density, as nearly all spring clutches had hatched by this time. Our spring pre-metamorphic sample was unlikely to have underestimated density due to individuals undergoing metamorphosis prior to the sample. Larval times vary with latitude, but in GA no population is known to undergo metamorphosis in less than 1 year (Smith, 2008). To sample salamanders, we established 10 permanent 15 m × 1 m transects, with one edge of the 1 m width established by the stream bank. Transect locations were set in a stratified random manner over a 500-m stream reach (two transects per 100-m stream reach). Salamanders were sampled by randomly selecting four transects in summer 2006 and spring 2007 and five transects in summer 2007 and spring 2008. Salamander density estimates were made using removal sampling (Jung et al., 2005). We used a small 15-cm wide aquarium net to scoop salamanders seen on the surface of the stream bed as well as to capture salamanders exposed as we searched under rocky cover or in root masses along the bank. We sampled each transect five consecutive times or until three consecutive passes yielded no animals. Animals caught on each pass were placed in a small plastic bag until all passes were complete. Any larvae observed on a pass, but not captured, were counted as captured for that pass as long as we were certain we did not later capture the escaped larva. It is unlikely, though not impossible, that animals escaped from the transect area during sampling or between passes. Salamanders are rarely found in the center of these streams, where cover objects are often less abundant (KB, personal observation), and time between passes was minimal (<1 min). Escapes from the sampling area would be of greatest concern for the pre-metamorphic larvae; however, there is no reason to assume such escapes would differ among streams for larvae of a particular size class. As a result, we believe our comparisons of density within a size cohort across streams and land use categories remain robust.

### 2.3. Land cover analysis and land use determination

We determined watershed boundaries and size from USGS 30-m resolution digital elevation models using ArcView 3.2a software (Environmental Research Systems Institute, Inc., Redwoods, Cali-

fornia). We obtained true-color 1-m resolution aerial photographs of the study watersheds that were taken during leaf-off in March 2003. All impervious surfaces (IS) in each watershed were manually digitized and remaining land cover was classified using a hybrid unsupervised/supervised technique, resulting in a land cover classification similar to the Anderson Scheme (Myeong et al., 2001). For each watershed we determined % categories of land use/land cover (LU/LC). We used % IS, % pasture, and % forest land cover for analyses (Table 1). Detailed image processing methods for the study watersheds are presented elsewhere (Lockaby et al., 2005).

#### 2.4. Stream hydrological variables

We quantified stream discharge ( $Q$ ) bimonthly from June 2003 to June 2004. This was the most recent detailed flow data available from the study streams. Flow was quantified using a Marsh-McBirney flow meter. Mini-Troll<sup>®</sup> pressure-transducer (In-Situ Inc., Ft. Collins, Colorado) data loggers recorded stage (water level above a reference point) every 15 min (0.01-m depth resolution) and stage- $Q$  rating curves were subsequently calculated from stage and discharge data to estimate continuous  $Q$  (Schoonover et al., 2006). We characterized the following elements of  $Q$  from each watershed hydrograph: (1) *magnitude* ( $Q$  for a given interval); and (2) *frequency* (number of occurrences of a given  $Q$ ). Ultimately, we calculated 10 hydrologic variables considered important in determining aquatic biota and separating sites based on LU/LC from past studies in these and other watersheds (Richter and Powell, 1996; Poff et al., 1997; Schoonover et al., 2006; Helms et al., 2009) (Table 2). All hydrological values were averaged over the period of record.

#### 2.5. Stream physicochemical sampling

We quantified several stream-specific physicochemical parameters considered important in determining biotic assemblages

**Table 2**

Hydrological, physicochemical, and habitat variables used in analyses and their range of values recorded from June 2003 to June 2004 in the 12 study watersheds.

Variable	Description	Range
<i>Hydrological magnitude</i>		
MedQ	Median discharge ( $L s^{-1}$ )	0.01–0.90
MaxQ	Maximum discharge ( $L s^{-1}$ )	1.04–21.98
MinQ	Minimum discharge ( $L s^{-1}$ )	0–0.36
<i>Hydrological frequency</i>		
3×Med	# of times discharge exceeded 3× median flow	5–74
5×Med	# of times discharge exceeded 5× median flow	1–65
7×Med	# of times discharge exceeded 7× median flow	1–64
9×Med	# of times discharge exceeded 9× median flow	0–58
>75th	# of times discharge exceeded 75th percentile	25–115
>95th	# of times discharge exceeded 95th percentile	12–66
>99th	# of times discharge exceeded 99th percentile	2–35
<i>Physicochemistry</i>		
Temp	Median water temperature ( $^{\circ}C$ )	13.3–15.8
DO	Mean dissolved $O_2$ ( $mg L^{-1}$ )	8.6–14.5
TDS	Mean total dissolved solids concentration ( $mg L^{-1}$ )	17.1–61.1
TSS	Mean total suspended solids concentration ( $mg L^{-1}$ )	2.1–8.1
<i>Habitat</i>		
BOM	Benthic organic matter (g)	0.3–1.2
Substrate	Median substrate size (cm)	0.7–1.8
Habitat	Habitat assessment index score	54–126
Cover	Mean % substrate area of cobbles, pebbles, root mass, or detritus	11–33

(Willson and Dorcas, 2003; Table 2). We measured stream temperature continuously with HOBO<sup>®</sup> Temp data loggers placed near pressure transducers. Dissolved oxygen (DO) and stream water pH were measured seasonally (4 times per year) with a YSI 55 and pH 100 respectively (YSI Incorporated, Yellow Springs, OH). Total dissolved solids (TDS) and total suspended solids (TSS) were determined from grab samples collected monthly from each watershed (see Schoonover and Lockaby, 2006). All chemical values were averaged over the hydrological period of record (June 2003–June 2004).

To determine available habitat quality, we used a multimetric habitat index from the Georgia Environmental Protection Division (GA Department of Natural Resources) designed for use in biomonitoring (GA DNR, 2005). This habitat index included estimates of available cover, substrate, pool morphology, channel alteration, channel sinuosity, sediment deposition, flow status, bank condition and riparian condition (GA DNR, 2005). This index involved taking the average of three observers' summed scores (1–10 or 1–20, depending upon parameter) of the different habitat parameters to obtain an overall habitat quality value for the representative reach, with high average score indicating high habitat quality. We used the same three observers at all sites.

We further assessed stream habitat by quantifying benthic organic matter abundance (BOM) and substrate cover in each stream reach. We quantified BOM by determining the ash-free dry mass of nine replicate  $2.5 \times 10$  cm benthic cores taken in transitional areas between the runs and pools to standardize efforts. During summer 2007 we sampled substrate cover by estimating the % of substrate in each reach covered by cobble (65–256 mm), pebble (33–64 mm), exposed live root masses, or leaf packs along each of the transects sampled for salamanders. Values were averaged among the 10 transects for each stream.

#### 2.6. Data analysis and model building

Salamander densities were estimated using the variable probability removal estimator (Pollock and Otto, 1983) option of the Removal task in Program Capture (software available online at <http://www.mbr-pwrc.usgs.gov/software.html>). This algorithm uses transect-specific detection probabilities to estimate density. For example, if salamander captures do not decrease appreciably from the first to second sampling pass, then one could assume poor detection probability, and thus a higher density estimate than the number of individuals actually observed. We chose to sample streams intensely over multiple seasons, so we did not evaluate enough streams to warrant statistical comparison of mean salamander density among land cover categories. Instead, we plotted mean salamander density (mean of data from streams within a land cover category). Data between years were combined for these plots as all pair-wise comparisons within land cover categories among years were not significantly different ( $p > 0.05$  for all  $t$ -tests). Plots were created for hatchlings and pre-metamorphic individuals and allowed us to qualitatively explore density trends prior to the quantitative assessment described below.

Path analysis is a general form of multiple regression that allows for more than one dependent variable in the model, and dependent variables are allowed to influence one another. Such an analysis begins by diagramming or hypothesizing, a priori, causal relationships among variables (Mitchell, 2001). We used path analysis to determine the abiotic factors that could have generated the estimated density trends. The environmental data we used in our models predate data on salamander densities. While it would be ideal to have collected all data from the same years, this was not possible due to logistical constraints. We make an explicit assumption that the environmental data describe general features of streams that were consistent across the years up through the

**Table 3**

Categories of predictor variables used to select variables in constructing path models.

LU/LC	Hydrology	In-stream habitat	Physicochemical
Agriculture	Median discharge	Habitat index	pH
Forest	Max discharge	Width:depth	Total dissolved solids
	Spate frequency <sup>a</sup>	Bank height	Total suspended solids
		In-stream cover	BOM

<sup>a</sup> Spate frequency measured as the number of events that were three or more times greater than the median flow as recorded from June 2003 to June 2004.

time in which all data could be accumulated. This assumption is supported by the hydrological data. We had access to hydrology measures for an additional year beyond the environmental data used in our models. Measures of spate frequency (Table 3) and median discharge were highly correlated between the 2 years ( $R = 0.86$ ,  $p < 0.0005$ , and  $R = 0.96$ ,  $p < 0.0005$ , respectively). Maximum discharge was not correlated between years ( $R = 0.27$ ,  $p = 0.40$ ); however, this variable was not present in the final models constructed for path analysis. We measured several environmental variables that could potentially affect salamander density. To reduce the set of predictor variables used in the path analysis, we examined the correlation matrix for categories of predictors (sensu Burcher et al., 2007). Categories of predictors included land use/land cover, in-stream habitat measures, general water quality measures and hydrology measures (Table 3). When two or more variables were highly correlated, we chose to use the variable that was either normally distributed (or could be transformed to normality), or that was most commonly recognized as potentially affecting a biota (Burcher et al., 2007). Data normality was a higher priority in choosing variables than was potential affect on salamanders. After eliminating highly correlated measures within a category, we were left with five or fewer predictors per category (Table 3). We also reduced the data set by combining variables representing water quality using principle components analysis. We performed principal components analysis on BOM, TDS, and conductivity. All variables loaded heavily on PC 1, which described a contrast between sites with high conductivity, high TDS, and low BOM and sites with low conductivity, low TDS, and high BOM. We considered PC 1 to be a general measure of water quality; lower values of PC1 represented higher water quality.

Based on the trends observed from our density estimates, we constructed seven plausible models for how land cover could affect pre-metamorphic salamander density (Table 4) via effects of the reduced abiotic variable set. We did not use all variables within the reduced data set; rather, we chose variables that would contribute to a priori models describing ways salamanders might re-

spond to land use change. Our original intent was to use% impervious surface as the land cover variable to represent urban development; however, % impervious surface was not normally distributed and no transformation of the data resulted in a normal distribution. Because the land cover variables% forest and% impervious surface were highly negatively correlated ( $R = -0.85$ ), we used% forest cover as a proxy for urban development in all of our models (i.e., less forest cover corresponds to more impervious surface). All models assumed the land cover cascade framework of Burcher et al. (2007), which proposes that changes in land cover manifest themselves through changes in hydrology, chemistry, and/or in-stream habitat, which, in turn, impact a biota (Table 4). In brief, we evaluated models hypothesizing single factor and multi-factor effects. Single-factor models included the effects of spates, water quality, suspended solids (as a measure of potential gill fouling in larvae), or in-stream cover availability on salamander density. Multi-factor models incorporated two or more of the above elements into a single model. Each of these hypotheses was tested using path analysis (Amos 4.0), and models with sufficient support ( $p > 0.05$  and explaining at least 40% of the variation in salamander density) were compared against one another using Akaike's Information Criterion (AIC).

## 2.7. Artificial stream experiments

We collected larvae of southern two-lined salamanders from 2nd and 3rd order streams in Lee and Macon Counties, AL during February 2008 (first trial) and April 2008 (second trial). Larvae were maintained in the lab for 2–4 weeks prior to the experiment. All larvae were measured (snout-vent length and total length) and weighed prior to trials.

We conducted experiments at Auburn University's North Auburn Upper Fisheries Research Station. Artificial streams (flumes) were constructed from a 1-m section of 15.25-cm (diameter) PVC pipe cut in half along its length. We lined the resulting channel with natural substrates and delivered water through it from a 3.80-cm (diameter) PVC pipe that had a cutoff valve to control out-flow volume and velocity. The water was gravity fed from an existing pond. The artificial stream had a 2° slope, and water drained from the channel through a 10.16-cm (diameter) reducer that was placed at the end of the length of PVC pipe. A net was placed at the end of the reducer to capture salamanders as they were washed from the experimental area.

We used flumes to test the effect of stream substrate type on the ability of a larva to resist being washed downstream. We established the following four substrates: sand, sand with detritus (leaves and coarse woody debris), gravel–pebble mix (particles

**Table 4**

Seven hypothetical models describing how a decrease in forest cover (and concomitant increase in impervious surfaces) can have cascading effects on salamander density. We failed to reject the null hypothesis that the model fit the data for models with  $p$ -values  $> 0.05$ . All models that were not rejected were compared using Akaike's Information Criterion (AIC).

Model	Path	Hypothesis	$\chi^2$	$p$ -Value	AIC	$\Delta$ AIC
Spate frequency	A	Urbanization increases spate frequency, which flushes salamanders from the stream	0.18	0.67	16.18	0
Water quality (direct)	B	Point-source pollution accompanying urbanization alters the water quality, which negatively influences salamanders	1.82	0.18	17.82	1.64
Water quality (indirect)	C	Runoff from increased overland flow and from non-point-source pollution alters water quality and negatively influences salamanders	2.97	0.23	26.97	10.79
Multi-factor 1	D	Models A and C, plus altered in-stream habitat in urban streams (larger channels, decreased bank stability, etc.) negatively influence salamanders	4.54	0.34	36.54	20.36
Multi-factor 2	E	Models A and C, plus decreased in-stream cover (refugia, oviposition sites) negatively influence salamanders	8.98	0.06	40.98	24.8
Gill fouling	F	Increase in total suspended solids from point-source pollution and increased overland flow negatively influences salamanders	13.16	<0.05		
In-stream cover	G	Urbanization increases spate frequency, which flushes in-stream cover from the channel, which negatively influences salamanders	18.51	<0.05		



ranging from 2 to 60 mm diameter), and pebble–cobble mix (particles ranging from 30 to 150 mm diameter). Sand and sand with detritus treatments mimicked substrates commonly observed in the urban study streams, while gravel–pebble and pebble–cobble mixes mimicked substrates commonly observed in the forested streams we evaluated.

We placed one salamander larva in a flume and regulated water flow to approximate base flow values of 2nd and 3rd order streams where *E. cirrigera* are known to be present (0.02–0.04 m/s; JES, unpublished data). Water depth at the start of trials ranged from 1 to 3 cm. A porous Plexiglass divider was inserted in the upper quadrant of each flume to retain larvae during a 2-min acclimation period. In the sand-only treatment, we placed a leaf in the acclimation quadrant, as well as each of the remaining three quadrants, to serve as cover, thereby preventing larvae from simply leaving the flume because of exposure. After the acclimation period, the Plexiglas divider was removed to provide a salamander access to the full length of the flume. Base flow was retained for 45 s, at which point flow was increased incrementally every 45 s until either the salamander was washed from the flume into a net or the salamander remained in the flume after 45 s of exposure to a mean water velocity of 0.83 m/s (range 0.43–2.00 m/s). These maximum water velocities were equal to or greater than maximum observed velocities in 2nd and 3rd order forested streams (BSH and JES, unpublished data). At the end of each trial we recorded water velocity at the front, middle, and end points of the flume with a Marsh-McBirney Flo-Mate 2000 flow meter, and used the mean of these recordings as the velocity at trial termination.

Experiments were performed using a Latin-Squares design so we could detect any effect of test sequence on larvae, as each larva was subjected to each of the four substrate types. Larvae were assigned to one of six blocks, with each block containing four animals. Each of the animals within a block experienced a different sequence of substrates during the tests. Tests were conducted so that larvae had at least 1 day separating trials. The experiment was conducted twice to increase replication, each time using 24 unique larvae (i.e., 48 total larvae were used). Results from the two experiments were evaluated separately using a three-way ANOVA with velocity at trial termination as the response variable, and block (as described above), Day (1, 2, 3, or 4), substrate, and subject as independent variables. Subject was entered as a random effects factor, which allowed us to conduct the test using a repeated-measures framework. Tests were conducted in Minitab (version 13.0, Minitab, Inc., State College, PA).

During the trials, some individuals either actively swam or were washed from the sand-based substrates within the first 2 min of the trial. To verify that the results we obtained from the experiment described above were the result of water velocity, rather than habitat avoidance, we conducted a short-term habitat selection experiment. Here, flumes identical to those described above were created with two separate substrate arrangements. Each arrangement consisted of three equally divided sections, and each section contained either sand with a small amount of detritus or a gravel–pebble mix. One flume was lined with sand–detritus in the upstream section, gravel–pebble in the middle section, and sand–detritus in the last, downstream section; the second flume was set up with the opposite arrangement. Water flowed through the flume at base flow and a salamander larva was introduced into the middle chamber. The larva was allowed to move freely through the flume and its location was recorded after 5 min. We reasoned that salamanders engaging in short-term habitat avoidance of sandy-based substrates would move from the middle section more frequently when it contained sand and detritus than they would when it was a gravel–pebble mix. We conducted 20 trials with each of the two substrate arrangements. We tested the hypothesis that the probability of moving from one substrate type to the other

would be higher for the trial where animals were initially placed on sand–detritus using a goodness-of-fit test.

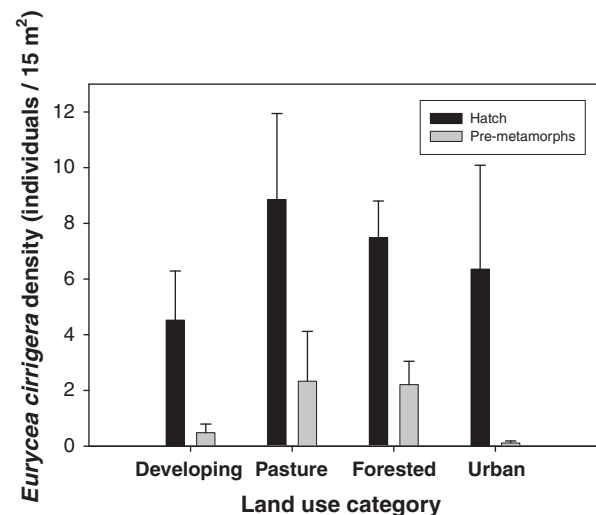
### 3. Results

#### 3.1. Salamander density and model comparison

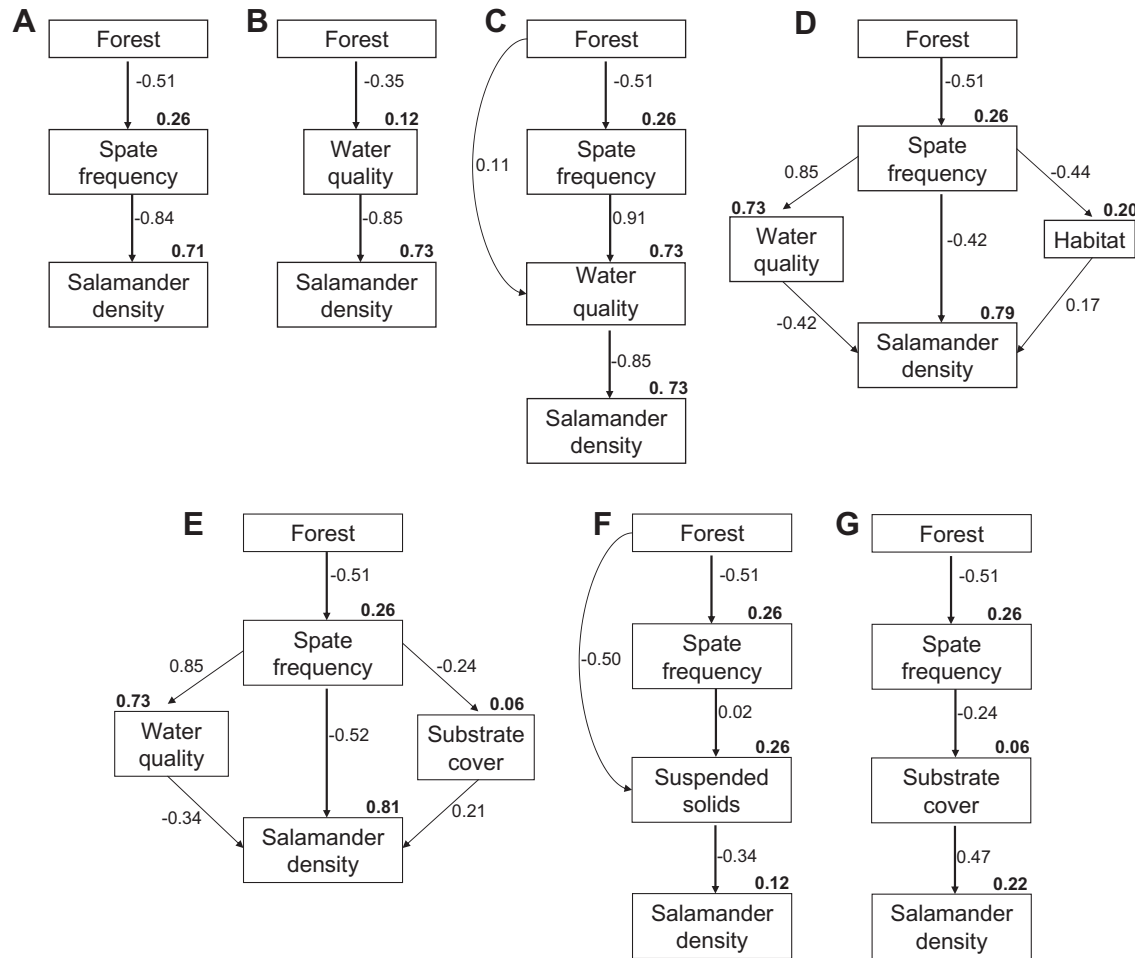
There was no appreciable difference in density of hatchlings among the four land cover categories (Fig. 1), suggesting that reproductive effort was not influenced by land cover category during the 2 years we monitored hatchling density. A large reduction in density occurred in all land cover categories over the nine months between sampling of hatchlings and pre-metamorphic individuals (Fig. 1); however, decline in density was far more pronounced in urban and developing streams (89% and 98% decrease, respectively) relative to pasture and forested streams (74% and 70% decrease, respectively). Pre-metamorphic salamander density in pasture streams was not appreciably different from density in forested streams, so all path analyses focused on how urban and developing landscapes affect salamanders.

Path analysis of a priori models indicated strong support (Table 4) for a path linking increased urbanization (as indicated by decreasing forest) to an increase in spate frequency, which, in turn, decreased the density (i.e., survivorship) of salamanders prior to the pre-metamorphic stage (Fig. 2a). This spate frequency model was the top ranked model by AIC; however, the water quality (direct) model in which decreasing forest cover leads to decreased water quality, which then causes decreased salamander density also should be considered likely (Burnham and Anderson, 2002; Table 4, Fig. 2b). The Water Quality (direct) model had support, but the path coefficient between decreasing forest and water quality was extremely low (Fig. 2b), indicating little bivariate correlation between these two variables. Nevertheless, this model cannot be rejected based on the data.

None of the remaining five models had sufficient support to be considered as plausible explanations for the observed decrease in pre-metamorphic density. The multi-factor models that incorporated several abiotic variables acting in concert (Fig. 2d and e) had very little support. The least supported models were the gill



**Fig. 1.** Mean ( $\pm$ SE) hatchling and pre-metamorphic mean density estimates of the southern two-lined salamander (*Eurycea cirrigera*) from four land cover categories (three replicate streams per category). Density estimates for hatchling and pre-metamorphic size classes represent the mean from two cohorts. Means were not compared statistically, as we only had three streams in each watershed category. Statistical assessment of a disturbance effect on salamander larvae was performed using path analysis (see text).



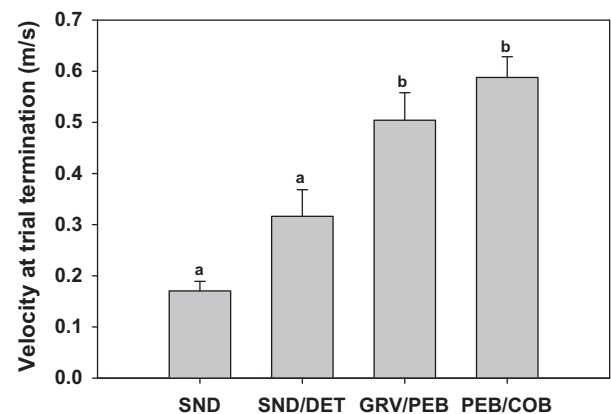
**Fig. 2.** Path diagrams representing a priori models describing how land cover change impacts salamanders in urban streams. Models were based on knowledge of the species and published studies on salamanders in urban streams. Spate frequency (A) was the top model among those compared by AIC from Table 4. Boldface numbers represent multiple correlation coefficients (analogous to  $r^2$ ) describing the amount of variance explained by the preceding predictor variables. Numbers adjacent to arrows are standardized path coefficients and represent direct bivariate effects. Curved arrows represent evaluated paths where an intermediate variable is omitted from the path.

clogging model, which tested the idea that overland flow increases total suspended solids, which then clog gills (or reduce prey-detectability), and the in-stream cover model, which posited reduced in-stream cover (i.e., refugia) correlates with decreased pre-metamorphic density (Fig. 2f and g, respectively).

### 3.2. Artificial stream experiments

Salamander larvae were flushed from the sand and sand with detritus treatments at significantly lower velocities than larvae in the gravel–pebble and pebble–cobble mixes ( $p < 0.0005$ ). This result was nearly identical in both iterations of the experiment (Fig. 3; Table 5).

The short-term habitat selection experiment did not support the hypothesis that larvae selected against sand or sand–detritus based substrates during the first few minutes of a trial (i.e., at or slightly above base flow). Specifically, the probability of a larva leaving a sand–detritus substrate was no different than the probability that a larva would move from a gravel–pebble substrate ( $df = 1$ ,  $p < 0.05$ ). In fact, only seven of the 40 test larvae moved to a different substrate than the one upon which they were initially placed during the short-term habitat selection trials, indicating larvae tended to remain in one place in the experimental flumes, irrespective of substrate.



**Fig. 3.** Mean ( $\pm$ SE) water velocity at trial termination for the first experimental trial in which salamander larvae were placed on one of four different substrates within a constructed flume and water velocity was slowly increased until the salamander was flushed from the channel. Salamanders were flushed at significantly lower velocities from sand (SND) and sand–detritus (SND/DET) substrates relative to larvae on gravel–pebble (GRV/PEB) and pebble–cobble (PEB/COB) substrates. Letters above bars represent statistical significance based on pair-wise comparisons using Tukey's 95% confidence intervals. The trial was run a second time using a new group of larvae. Statistical results were identical to what is presented here.

**Table 5**

Treatment effects from each of two experiments testing the effect of substrate on a larval salamander's ability to withstand high velocity stream flows. All salamanders in the experiment were exposed to each of four different substrate types over the course of 4 days. The experiment was conducted using a Latin-squares design so we could assess whether testing over subsequent days affected the outcome of the trials.

Factor	df	SS	F	p
<i>Trial one</i>				
Day	3	0.11	0.89	0.45
Substrate	3	2.54	21.23	<0.0005
Subject (block)	18	1.24	1.73	0.06
Block	5	0.22	0.63	0.68
Error	66	2.63		
<i>Trial two</i>				
Day	3	0.27	1.67	0.18
Substrate	3	4.27	26.84	<0.0005
Subject (block)	18	1.61	1.69	0.06
Block	5	0.58	1.30	0.31
Error	66	3.50		

#### 4. Discussion

We demonstrate a strong link between increased spate frequency (and the correlated variable spate magnitude) and a decline in southern two-lined salamander density during the larval stage. A model describing alterations in water quality as a result of land use change also maintained support. The effects of altered water quality on fish and invertebrates have been well documented (Paul and Meyer, 2001; Allan, 2004). Our data suggest such effects extend to amphibians as well. Relative to water quality, the negative effects of urban flood events on amphibian larvae has received far less attention. Our data strongly suggest salamander larvae are likely flushed from the low-order streams we studied, and such flushing is exacerbated by the loss of in-stream habitat. Repeated monitoring of a 500-m reach in each stream indicates the study areas were not recolonized in subsequent seasons after larvae were washed from the focal reaches. It is possible that salamanders simply colonize downstream reaches; however, this scenario should essentially result in loss of larvae within the study reach coupled with a replacement of those larvae by animals displaced from upstream of the study reach. We did not observe density estimates that would support such a hypothesis. As a result, we conclude that the reduction of density we observed in a common species, *E. cirrigera*, represents a decrease in survivorship. The same process acting to reduce density of *E. cirrigera* may be responsible for the lower species richness of salamanders observed in urban habitats (Barrett and Guyer, 2008).

The strong influence of flooding on southern two-lined salamander survivorship is seemingly at odds with many fish species that recover quickly from even the most extreme flood events (Jurajda et al., 2006). However, how a species responds to hydrological changes may depend on the evolutionary history of the taxon. Shifts in flow regime have been identified as a major obstacle for many stream- and river-dwelling species (Lytle and Poff, 2004). Salamander larvae primarily inhabit low-order streams (Petranka, 1998), and have seemingly evolved upstream movement behaviors to counter downstream drift that can occur at low flows (Lowe, 2003; Cecala et al., 2009). They presumably have not evolved mechanisms, such as those present in many fish species, which allow them to survive and/or re-colonize after extreme flow events encountered in urban stream systems.

Other studies have documented species that are susceptible to spates; however, these studies have been performed largely in the context of increased flows that occur with increasing stream order (Baumgartner et al., 1999; Leipelt, 2005; but see Dudgeon, 1993). For example, Leipelt (2005) used artificial streams to evaluate the response of four species of Odonata to a high-flow stream

environment. The two species in Leipelt (2005) that were more prone to drift were species found in lower order streams less prone to spates, which he interpreted as evidence for hydrological factors shaping species distributions. Baumgartner et al. (1999) used field data to argue that larval fire salamanders (*Salmandra salamandra salamandra*) prefer lower current speeds within a given stream, and they found fewer salamanders in streams with higher mean stream discharge. Collectively, these studies support the idea that spate frequency and/or magnitude can influence abundance and distribution of stream organisms.

Urbanization can have a strong effect on stream physical conditions (Galster et al., 2008) and biota (Barrett and Guyer, 2008) within a single stream, and that effect is analogous to changes observed among streams as they increase in stream order. For example, Galster et al. (2008) demonstrated an increase in stream width and depth that accompanied urbanization. Barrett and Guyer (2008) noted a shift in the herpetofaunal assemblage with urbanization from one that was amphibian-dominated to one dominated by basking turtles and snakes more typical of riverine systems. An altered hydrology is most likely a key driver leading to these shifts within streams that suffer from watershed urbanization.

This study adds to the evidence that poor water quality resulting from urbanization can negatively affect amphibians. The study also provided two independent lines of evidence supporting the hypothesis that extreme flow events in urban areas wash larvae from streams. We fully recognize that our flume experiments represented a highly simplified stream system. Nevertheless, they likely provide a relative (but not absolute) assessment of how larvae respond to flooding, especially when it is coupled with a loss of rocky habitat. Observation of larvae in more complex, natural environments (i.e., sinuous streams with porous stream banks) was simply not possible. In such environments, it is possible that larvae are not completely washed from streams, but rather flushed partially downstream and become more susceptible to mortality from secondary factors (i.e., predation, unsuitable foraging areas) due to absence of familiar habitat. Further work will be required to test such hypotheses.

The use of field data, coupled with an experiment explicitly designed to test the effects of spate magnitude on larvae, allowed us to elucidate at least one mechanism responsible for lower salamander density in urban streams. Several studies have documented a decline of salamander diversity and/or density with urbanization (Willson and Dorcas, 2003; Price et al., 2006; Barrett and Guyer, 2008); however, no study has directly explored a mechanistic explanation for such declines. With amphibian populations under increasing pressure from urbanization (Hamer and McDonnell, 2008), it is imperative to understand the underlying processes responsible for generating the pattern of declining species richness and density in urban habitats.

By assessing the type of disturbance response exhibited by southern two-lined salamanders and the relative importance of specific disturbances we have provided insight into how this species might respond to restoration efforts. First, we found no difference among land cover categories for salamander reproductive output during the 2 years of monitoring. This pattern suggests that adult salamanders have not abandoned these urban areas. Nevertheless, survivorship appears to be low during the larval phase of the life-cycle, which results in few salamanders undergoing metamorphosis into the adults. Should spate frequency and intensity be reduced and some in-stream habitat restored, it is likely that populations of this salamander would increase recruitment of larvae into the breeding population. Future work will be required to understand how adults of this species are able to maintain high reproductive output despite the low numbers of metamorphic larvae we observed. Other salamander species that have likely been extirpated from the urbanized streams we evaluated may not re-

cover as quickly, as no adult populations of other species have been recorded in the urban habitats (Barrett and Guyer, 2008). In addition, loss of these species may be due to more than spate frequency or magnitude. The other salamander species in this area all occur at lower densities (KB, personal obs.), and therefore populations may be more sensitive to any number of habitat disturbances. However, we believe the set of candidate models we put forth here provides a sound starting point for evaluating the mechanisms behind other amphibian losses due to urban development. Second, we have shown that increased spate frequency and poor water quality are both strongly related to decreased salamander abundance. Our work suggests in-stream restoration efforts will have a minimal effect on the biota if upland processes such as increased overland flow due to impervious surfaces are not addressed (Charbonneau and Resh, 1992).

## Acknowledgments

We would like to acknowledge significant assistance in the field from S.T. Samoray, C.M. Romagosa, D. Ratajsky, V.M. Johnson, S.E. Samoray, S.K. Hoss, M.I. Williams, and J.D. Peterson. Jackie Crim provided hydrology data from 2004 to 2005. We are grateful to Auburn University's Fisheries Department for providing access to the facilities necessary to conduct these experiments. This research was approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2007-1207). The Center for Forest Sustainability at Auburn University provided funding.

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