STREAM-BREEDING AMPHIBIAN RESPONSES TO LAND USE DISTURBANCES

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STREAM-BREEDING AMPHIBIAN RESPONSES TO LAND USE DISTURBANCES

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STREAM-BREEDING AMPHIBIAN RESPONSES TO LAND USE DISTURBANCES

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Russell Kyle Barrett, son of Russell K. Barrett and Reita J. Naron, was born on January 4, 1977, in Shelbyville, TN, where his sister, Amy, was born also. He graduated Tullahoma High School in 1995. He attended Middle Tennessee State University in 1995, and graduate *summa cum laude* in May, 1999, with a B.S. in Biology. After working as a biology technician for the Nature Conservatory and Arnold Air Force Base, he entered graduate school at Missouri State University, in Springfield, MO in August, 2000. He graduated with a M.S. in Biology, and then entered Auburn University Graduate School in August, 2002 to pursue a PhD in Biology. While in Auburn, Kyle married Shannon Taylor on May 18, 2003. On April 9, 2005 Shannon and Kyle welcomed the birth of their son, Jonas Wells.

DISSERTATION ABSTRACT

STREAM-BREEDING AMPHIBIAN AND REPTILE RESPONSES TO LAND USE DISTURBANCES

Kyle Barrett

Doctor of Philosophy, May 9, 2009
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(B.S., Middle Tennessee State University, 1999)

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Conversion of land from an undeveloped state into agricultural or urban areas is widespread. Urban areas in particular are growing both in size and number globally. Such land use changes can potentially have negative consequences for organisms such as amphibians, many of which require aquatic and terrestrial environments to complete their life cycle. I conducted herpetofaunal richness surveys of several watersheds in western Georgia, USA, subject to varying degrees of urbanization or pasture land uses, which revealed amphibians are particularly sensitive to urban areas, but not pasture lands. Reptiles showed the opposite response (i.e., more reptile species were found in urban areas). The results of this survey prompted a more detailed study of the stream-breeding salamander, *Eurycea cirrigera* (two-lined salamander). I found that this species has a reproductive output in urban streams equal to conspecifics in reference environments, but that survivorship of larvae to metamorphosis was much lower in urban streams. Path analysis of potential environmental factors contributing to this putative decline in survivorship revealed frequent and intense flooding in urban environments is the most probable cause for observed declines. To validate this finding, I created a series of experimental streams (flumes), in which water flow and substrate were manipulated. I found salamanders were more likely to be eroded from experimental flumes at lower water velocities when flumes contained sandy substrates without rocky cover, a streambed condition common in urban streams. Taken together, field and experimental data strongly suggest an altered hydrology in urban areas is one of the leading factors causing stream-breeding salamander decline in urban habitats. In addition, I followed my studies on hydrological effects by evaluating growth of the two-lined salamander across an urban – forest gradient. I found that larvae in urban streams grew faster than larvae in forested streams. The benefit of faster larval growth could explain the persistence of the two-lined salamander in urbanized watersheds. Finally, I described shifts in consumed prey by two-lined salamanders that accompany urbanization. While these shifts were not dramatic, the descriptions I offer provide a foundation for describing food web dynamics in urban habitats. In total, urbanization dramatically alters herpetofaunal assemblages in and around streams. Those species that are not extirpated apparently suffer survivorship costs and shifts in growth and diet. Information from assemblage-wide and species-specific perspectives, provided here, is needed to increase our ability to ameliorate effects of urbanization on stream-dwelling amphibian species.

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CHAPTER 1. INTRODUCTION

Over half of the world's population resides in urban areas (Grimm et al. 2008), and the percentage of global urban inhabitants is expected to rise steadily over the next 20 years (Grimm et al. 2008). Urbanization of landscapes fundamentally alters a wide range of environmental factors including land use, nutrient cycling, climate patterns, and hydrological patterns (Paul and Meyer 2001, Walsh et al. 2005, Grimm et al. 2008). Shifts in such factors can then have effects on biotic communities, typically resulting in biotia in urban areas that do not resemble nearby communities in undisturbed habitats (Walsh et al. 2005, McKinney 2006).

Urban ecology emerged as a field of study from a desire to understand the relationship between human settlements and the resulting ecosystem in the developed area. Urban ecology can be defined as the study of organisms in areas densely populated by humans (Sukopp 2008); however, urban ecology is not just conducting ecological studies in urban settings. It also has developed into an interdisciplinary effort to examine the ecology of urban areas. Specifically, urban ecologists may also work under other titles such as sociologist, landscape planner, economist, health care worker, anthropologist, or ecologist, but they all share a common goal of understanding the relationships between human-dominated systems, the other organisms that inhabit such systems, and the energy and nutrients that move through and cycle within the system. It is now commonly recognized that the relationship among the parts of an urban ecosystem are complex and involve a number of indirect effects and feedback loops (Lockaby et al. 2005, Walsh et al. 2005, Grimm et al. 2008).

As a result of this complexity, the Center for Forest Sustainability at Auburn University developed the West Georgia Project. The impetus for the project is described in Lockaby et al. (2005). In brief, the West Georgia Project was developed from recognition that certain societal drivers (financial, demographic, historic) can lead to changes in land use across a given landscape, and that these land use shifts are accompanied by ecologically relevant changes (hydrology, nutrient cycling, biota). These interrelationships are brought full-circle in the above model, as the ecology of an area is thought to influence the societal drivers responsible for land use change (Lockaby et al. 2005, McDaniel and Alley 2005). Lockaby et al. (2005) approached understanding the interdisciplinary problem of urbanization's effects and feedback loops by constructing a research hierarchy where individual projects generate data from a variety of perspectives and subsequently the data from these perspectives are placed into more integrated, multidisciplinary papers.

My dissertation contributes to the above model by reporting on the response of amphibians (and in Chapter 2, reptiles) to urbanization (and relating these responses to data generated from studies on hydrology, temperature, prey availability, and amphibian predator density. The chapters of this dissertation use stream systems as the focal habitat to track the influence of urbanization on reptiles and amphibians. I chose to focus on these habitats because of the significant impact urban development has on streams (Paul and Meyer 2001, Allan 2004), and because of the significant number of imperiled reptiles and amphibians because of urban development (Hamer and McDonnell 2008, McKinney 2008).

Chapter 2 reports on a two-year survey of reptiles and amphibians inhabiting stream and riparian habitats in urban, developing, pasture, and forested watersheds. I determined that pasture and developing land uses had no appreciable effect on species richness of reptiles and amphibians relative to forested habitats; however, urban land uses resulted in a large shift in the herpetofaunal assemblage. Amphibian diversity was lower in urban habitats relative to the reference watersheds, while reptile diversity in urban areas was higher. In Chapter 2 I hypothesize this shift resulted from the widening and deepening of stream channels which results from the frequent and intense flooding following urbanization (Galster et al. 2008). These more open channels result in a warmer, more riverine type stream system favorable to basking reptiles, while potentially excluding many amphibians.

The results of species richness survey indicated that one species, the southern two-lined salamander (*Eurycea cirrigera*), persisted (at low densities) even in highly urbanized habitats. As a result, I focused the remainder of the dissertation on the mechanisms leading to amphibian decline in urban streams, and I used the two-lined salamander as the focal species for these studies. Two-lined salamanders are lungless salamanders in the family Plethodontidae. The species has a range that extends north into northern Indiana and Ohio and is bounded on the western side of its distribution by the Mississippi River. The species' range extends south to the Gulf of Mexico (though it is not found on peninsular Florida), and east to the Atlantic coast (Jacobs 1987). Much of the life history of the species, described below, is similar to other stream-breeding salamanders. As a result, data generated from study of this species may contribute to knowledge of other salamanders.

Two-lined salamanders have a biphasic lifecycle. As adults they have small, slender bodies ranging in total length from 65 – 120 mm (Smith 2008). They tend to be yellowish-brown in color with two dark lines that extend from the posterior of each eye, dorsolaterally onto the tail. Adults primarily inhabit riparian zones of small to mid-size streams and forage upon invertebrates. In the portion of the range where I studied this species, adults typically migrate from riparian habitat to the stream channel during the late fall or early winter and undergo courtship and oviposition from February to April. Eggs are deposited in the stream and attached to the underside of objects such as rocks, logs, and root masses. Females attend the eggs until larvae hatch. Aquatic larvae typically emerge from eggs from March to May and remain in the stream for ~ 18 months where they feed on benthic invertebrates. Chapters 3 – 6 of this dissertation describe the ecology of the larval two-lined salamander in the context of an urban-forested gradient. These chapters were written in collaboration with researchers working on ecologically related questions within the same watersheds I studied, and syntheses of our data represent a multidisciplinary contribution to the West Georgia Project.

Chapter 3 describes an observed decline in the density of two-lined salamanders from the period directly following larval emergence from eggs to the pre-metamophic period one year later. I explored several potential abiotic factors accompanying urbanization that might have led to the observed decline. I used path analysis to evaluate several hypothesized relationships between abiotic factors and larval density, and the magnitude and frequency of spates (floods) in urban areas emerged as the key driver for declining salamander density.

Chapter 4 describes an experimental test of the field-generated hypothesis from Chapter 3. For this chapter I constructed artificial streams (flumes) in which I manipulated streambed substrate and water velocity. This design allowed me to mimic urban stream-type substrates, (sandy with few large rocks), as well as reference stream-type substrates (mixed pebble and cobble). I examined the water velocities salamander larvae can withstand on different substrate types before being washed downstream. Results from these experiments supported the hypothesis that altered hydrology in urban habitats, which likely drives the change in substrate composition, is the mechanism for salamander declines in urban streams.

Chapter 5 focuses on the growth of two-lined salamander larvae along the urban-forested gradient. I examined growth in this context because many of the factors known to change with urbanization (temperature, prey-base, predator density) are the same factors hypothesized to influence amphibian growth. I found that salamanders in urban habitats quickly become larger than their reference stream counterparts. I explored the possible explanations for this size difference and determined that temperature, and possibly intraspecific competition, are the most likely candidate explanations for my observations.

Finally, in Chapter 6 I conducted a test to determine whether a shift in prey availability with urbanization influenced the diets of two-lined salamanders. I found slight shifts in dietary composition with land use change, and evidence that larval two-lined salamanders exhibited greater discrimination against available prey than has been previously described.

This dissertation makes significant progress toward understanding the mechanisms that impact stream-breeding salamanders subjected to urbanization, but much remains to be done to fully appreciate how stream environments change within urbanized watersheds. However, the subdiscipline of community ecologyhas yet to receive much attention in urban systems. To date, much of the research in urban streams focuses on abiotic processes or the diversity and/or abundances of an assemblage (but see Helms 2008). Tracking shifts in multi-trophic interactions and energy flows from terrestrial uplands to stream systems may reveal as yet unknown changes in community dynamics that occur during urbanization. Understanding such shifts could offer important insights during restoration/recovery efforts for aquatic and terrestrial systems.

Publicly funded stream restoration efforts have been commonplace in the US since in the 1930s (Riley 1998), and have been motivated by a recognition that healthy streams offer more than just habitat to wildlife such as amphibians. Sufficient evidence has accumulated to indicate that streams left (or restored to be) unchannelized and surrounded by some native vegetation can enhance urban tourism, improve residential home values (thereby increasing the tax base), and still offer services such as industrial water supply and recreational opportunities (Riley 1998). Often, streams surrounded by a dense urban matrix have progressed too far from their original state for restoration to be an option. In such cases, reconciliation ecology, the science of establishing new habitats to conserve species among the places where people live, work, and play (Rosenzweig 2003), may offer an alternative solution. These habitats may not resemble a reconstruction of native environments, but instead act as respites for a select group of species that can tolerate existing disturbances (mitigated or not) that exist in urban habitats. For reconciliation ecology to be a reality, we must first have a sound understanding of the various responses species exhibit when confronted by urban development. Knowing which species are at risk, and what mechanisms drive such responses, will help us understand the best way to enhance biological diversity in our increasingly urbanized society. This dissertation contributes to such knowledge by clearly documenting differences in the herpetofaunal assemblage with urbanization, and by identifying spate magnitude and frequency as a key driver responsible for the decline in the abundance of a stream-breeding salamander.

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CHAPTER 2. DIFFERENTIAL RESPONSES OF AMPHIBIANS AND REPTILES IN RIPARIAN AND STREAM HABITATS TO LAND USE DISTURBANCES IN WESTERN GEORGIA, USA

ABSTRACT

Urban and agricultural land uses have caused documented declines in diversity of many organisms. However, responses of stream- and riparian-dwelling amphibians and reptiles to anthropogenic land development are collectively understudied and, when studied, are often grouped together as though these two taxa respond in a similar fashion. We surveyed watersheds in four land use categories (reference, pasture, developing, and urban) for amphibian and reptile species richness over a two-year period in the southeastern United States. Total herpetofauna species richness was equivalent among all watershed types, but amphibians and reptiles responded differently to urbanization when analyzed separately. Urban watersheds had significantly fewer amphibian species than all other watershed types, but these losses were counterbalanced by significant increases in reptile species richness in these same watersheds. We also found that local, riparian-scale, habitat differences were strongly correlated with species composition differences between pasture and developing watersheds. While the difference in species composition between pasture and developing sites may have multiple, species-specific explanations, the dramatic differences between amphibian and reptile species richness in urban watersheds suggest broad trends that may be important to conservation planning. Specifically, our observations suggest that urbanization in the studied watersheds alters small streams from closed-canopy, shallow-water features of the forested landscape likely present before settlement by Europeans and favored by many salamanders and frogs to features associated with open vegetation and deeper, warmer waters favored by riverine turtles and snakes. We conclude that amphibians and reptiles, despite some physiological similarities, are not equivalent for monitoring purposes. Additionally, if future development in the Piedmont mirrors current urbanization, then significant faunal shifts seem assured.

INTRODUCTION

Land uses such as agriculture and urbanization have been implicated as primary reasons for the loss of global biodiversity (Dobson et al. 1997, and Czech and Krausman 1997). Agriculture and urbanization are accompanied by a suite of factors that can directly or indirectly impact native species. For example, forested land cleared for grazing alters terrestrial habitats through deforestation, and results in increased sediment loads, nutrient inputs, and temperatures in receiving waters (Schoonover et al. 2006). In urban areas the obvious issue of habitat loss is compounded by issues of chemical (Borja et al. 2006), noise (Patricelli and Blickley 2006), and light pollution (Moore et al. 2000); replacement of native with invasive species (Loewenstein and Loewenstein 2005); changes in resource availability (Clergeau et al. 1998); and altered stream hydrologic regimes characterized by frequent and intense flooding (Schoonover et al. 2006).

The problem of land use disturbance, especially via urbanization, is relevant in the Piedmont ecoregion of the United States. The Piedmont experienced one of the highest-percentage increases of developed land area in the United States during the 1990s (Lockaby et al. 2005). The rapid loss of undeveloped land suggests that steps must be taken to (1) identify the species most imperiled by agricultural and urban land use, and (2) determine which types of land use (e.g., pasture or urban land) most strongly correlate with the presence of remaining species. Such information can lead to effective conservation planning by increasing focus on assemblages of species that are most severely impacted by land use change. For example, Stratford and Robinson (2005) reported that almost no Neotropical migrants are associated with plots containing > 15 % urban development. As a result, they argued that small woodlots embedded in an urban matrix are of little conservation value to many bird species, and instead, it is the remaining undeveloped regions that should be a priority for conservation planning.

Along with birds, amphibians and reptiles have been proposed as candidates for biodiversity monitoring (Dobson et al. 1997, Welsh and Ollivier 1998, Gibbons et al. 2000). Amphibians are often considered indicator species as a result of their semi-permeable integument (Vitt et al. 1990), which makes them sensitive to environmental pollution. Additionally, eggs of amphibians, which are covered only by a gelatinous membrane, may be sensitive to certain anthropogenically altered environmental conditions (e.g., increased UV-B radiation) and pollutants (Blaustein et al. 2003). In an assessment of the impacts of land use on amphibian diversity in Iowa and Wisconsin, Knuston et al. (1999) found that anuran diversity was negatively associated with the amount of urban land in their study watersheds; however, no strong conclusions regarding the impact of agricultural lands on anurans were reached. While some studies have assessed the utility of using reptile and amphibian diversity collectively as a surrogate measure of the overall biodiversity value of a region (Dobson et al. 1997), few studies have focused solely on reptile species richness or diversity in response to habitat degradation (Gibbons et al. 2000). Studies that have focused on reptile diversity suggest that this group may be just as sensitive to habitat loss as amphibians (Gibbons et al. 2000); however, which reptile species drop out of an assemblage in disturbed areas may not be intuitive. For example, Attum et al. (2006) reported on a desert lizard assemblage in which species specialized for existence in harsh environments persist in areas of human-induced desertification, while generalists, which are seemingly unable to endure the new, harsher environment, are extirpated.

The general sensitivity of amphibians and the understudied responses of reptiles to land development indicate the need for additional detailed studies of these organisms in a variety of habitat types. To date, many of the surveys that assess amphibian responses to urbanization have taken place in or around permanent or semi-permanent ponds (Knutson et al. 1999, Rubbo and Kiesecker 2005, Parris 2006). Surveys that do focus on stream systems typically assess only salamander abundance or diversity but do not include frogs (Orser and Shure 1972, Price et al. 2006). In short, there is a need for data on responses of stream-dwelling amphibians, and reptiles in general, to land use disturbance.

To address this need we assessed species richness of an entire herpetofauna in riparian zones and waters of 2nd and 3rd order streams in the Piedmont ecoregion across four land use/land cover categories. We coupled our herpetofauna surveys with data on land use/land cover at the watershed and local riparian scale to determine if specific species or groups were impacted by land use disturbances associated with grazing and urbanization at these scales.

METHODS

Study area and habitat assessment

We conducted herpetofaunal surveys within three counties in western Georgia, USA (Muscogee, Harris, and Meriwether). The areas we selected were also part of an interdisciplinary effort (known as the West Georgia Project) by the Auburn University Center for Forest Sustainability to understand feedback loops between ecological, sociological, and economic systems (Lockaby et al. 2005). In future studies, the data we report on here will be coupled with other ecological data sets (e.g., Helms et al. 2005) to contribute to a broader understanding of how anthropogenic disturbances impact natural systems. Further details on the study area and The West Georgia Project can be found in Lockaby et al. (2005) and Schoonover et al. (2006).

We selected for study three watersheds of 2nd- or 3rd-order streams in each of the following categories: reference, pasture, urban, and developing. Selection of these 12 sites (Fig. 2-1) was based on the predominant land cover present within each watershed and recorded by Lockaby et al. (2005) and Pan (unpublished data). Land cover was determined from aerial photography (1 m resolution) taken during March 2003. Details on image processing were described by Lockaby et al. (2005).

Reference watersheds were defined as those that had a minimum of 75% (mean = 79%, range: 76% – 81%) of the total watershed forested (evergreen + deciduous forests), pasture watersheds were those with at least 30% (mean = 38%, range: 34% - 44%) of the watershed under agricultural use (primarily cattle pasture), and urban watersheds were identified as having at least 25% (mean = 32%, range: 25% – 40%) impervious surface in the watershed. The developing watershed category was selected to represent watersheds with relatively low current development (mean impervious surface = 3%, range: 2% - 3%); however, these sites were situated in Harris County, Georgia, which is one of the fastest growing counties in the United States (Lockaby et al. 2005). Consequently, the developing watersheds represented sites subject to significant recent and ongoing development. These qualitative categorical delineations were supported by principle components analysis (described below under "*Data Analysis*").

We also collected data on riparian land use/land cover within a 15 x 100 m block centered on each of the focal stream drainages. These data were collected during the summer of 2006 along ten 15 m transects that were randomly placed along a 500 m reach of each stream. We collected the data by visually estimating the percentage (to the nearest 5%) of land use/land cover at each transect. We categorized riparian land use/cover as one or more of the following: mature forest (mixed-species stands with prominent canopy cover), immature forest (mixed-species stands with prominent midstory and/or shrubs), field (ungrazed grassland), pasture (grazed grassland), and disturbed (roads, housing, or industrial development).

Although random placement of treatments is always the desired goal, this goal cannot be reached in studies such as ours. The project was designed to focus on a single, rapidly growing city (Columbus, GA), which dictated that urban sites generally would be towards the city center, developing sites generally would be found along the periphery of the city, and pasture and reference sites would be further removed from these two treatments. Additionally, the city is situated at the southern extreme of the Piedmont in Georgia so that streams south of the city change some characteristics to those expected of the Coastal Plains (Wharton, 1978). This results in the decidedly non-random distribution of treatments in our study (Fig. 2-1). Nevertheless, we argue that this distribution of sites captures all species of amphibians and reptiles expected of the stream orders that we studied and that these species are part of a widespread herpetofauna found in closed-canopy, free-flowing, highly-oxygenated Piedmont streams and their borders (Jensen et al., 2008). As support for this argument we note that no species has a limit to its geographic range that traverses the study region and no narrowly-distributed endemic species is present. Thus, the expected species composition is identical for all sites (Jensen et al. 2008, Conant and Collins 1998) because no two sites are greater than 47 km apart, and on average sites are only 21.6 km apart. Although the two southern-most urban streams appear to be within the Coastal Plain, mean streambed slope is actually lower for reference sites (0.09^0) than it is for urban sites (0.19^0), which suggests that the urban sites are Piedmont-like in character. Additionally, mean maximum and minimum temperatures vary by only 2^0C and 3^0C among sites (Georgia State Climatology Office), and even these slight differences are most likely attributable to the heat island effect (Oke 1995), rather than any latitudinal differences in climate. Collectively, the above observations lead us to conclude that our study samples a Piedmont herpetofauna and that non-random placement of treatments does not affect our ability to detect biologically meaningful shifts in the reptile and amphibian assemblage associated with disturbance to the landscape, which was the main focus of the study.

Species richness surveys

We surveyed herpetofaunal species richness from June 2004 – September 2006. Surveys were centered on the stream drainage of our focal watersheds, tributaries into the drainage, and the riparian areas, which we arbitrarily, but consistently defined as habitat within 50 m of a stream bank. We sampled each of the twelve watersheds sixteen times over the twenty-seven month sampling period. Sampling sites on each watershed began at an access point on the stream drainage (road crossing) and continued upstream for approximately 500 m at all sampling sites, to ensure the area monitored at all streams was comparable. We employed two different survey methods for this study, as single survey methods are often biased to particular groups (Crump and Scott 1994, Scott and Woodward 1994), and yield lower values for species richness than those based on multiple sampling methodologies (Longino et al. 2002). On eleven of the visits to each site (June 2004 – September 2006), we surveyed for herpetofaunal species richness using a time-constrained search technique, which is predominately used to detect terrestrially-based species (Crump and Scott 1994, Burbrink et al. 1998), but has been used with success to detect turtles and other primarily aquatic amphibians (Mitchell 2006, 2007). During time-constrained search surveys, one to three observers walked in non-overlapping paths, searching under available cover objects both in the stream and adjacent 50 m of terrestrial habitat. Time-constrained searches were conducted for 30 person-minutes. On the remaining five visits to each of the sites, we collected data on species richness during stream surveys that were being conducted as part of a separate study. On these occasions we used dip nets to sample either two 25 m transects (all sites during the summer and fall of 2005) or ten 15 m transects (all sites during the winter, spring, and summer of 2006). During these transect surveys, sites were visited for approximately 1.5 hrs per visit. We recorded all species captured by dip netting, as well as any incidental captures while at the field site. This method was chosen to equilibrate our ability to detect members of the aquatic fauna relative to the terrestrial fauna (Scott and Woodward 1994). The total search time for each site was approximately 13 hrs, and the total search time for each land use category was 39 hrs. Amphibians and reptiles were recorded to be present if observed on site during any stage of development (egg, larva, adult), or, in the case of anurans, when vocalizations were heard during surveys.

Data analysis

To characterize vegetation and impervious cover of all watersheds we conducted a principal components analysis on the land cover data. This method allowed us to describe variables that differentiate each habitat type. Land cover data were log-ratio transformed to avoid the problem of a constant-sum constraint associated with ordination analyses conducted on percentage data (Kucera and Malmgren 1998).

Our primary goal was to assess the impact of current land use/land cover on herpetofaunal species richness; consequently, we pooled data from each of the three sites within a category, and simply report comparisons among land use/land cover categories. We had a total of forty-eight sampling occasions for each of the four categories. We estimated species richness among categories using EstimateS ver 7.5 (Colwell 2005). We chose to make all comparisons among land use/land cover categories using the Chao 2 estimator, because this algorithm resulted in species accumulation curves that reached near-maximum values with very few samples (i.e., the estimator was the least sensitive to undersampling; Colwell and Coddington 1994). The Chao 2 algorithm functions by inflating the observed species richness by a factor derived from the number of species observed only once or twice within a total sample. The estimator is calculated as $S_{Chao2} = Sobs + Q_1^2/2Q_2$, where Sobs is the observed species richness and Q_1 and Q_2 are the number of species detected only once or twice per sample area, respectively. Thus, this method accounts for the fact that species differ in detectability and uses the relative frequencies of species that are rarely detected to estimate the number of taxa that are present but not detected (Colwell and Coddington 1994, Chao 1987). We compared overall richness values among land use/land cover categories for all species, amphibians only, and reptiles only. We considered an estimate of species richness among categories or sites to be significantly different if 95% confidence intervals did not overlap. For each pooled category we also calculated coverage (number of species observed / number of species estimated x 100), percentage of exclusive species (species only observed in a given site or category), and completeness of a sample (species observed as a percentage of the landscape total; Gardner et al. 2007).

To assess whether or not the observed species composition shifted with land use/land cover, we performed a non-metric multidimensional scaling (NMDS) ordination to assess survey-site clustering in species space. We used PC-ORD for NMDS analysis (Mather 1976, McCune and Grace 2002). The resulting axis scores from this analysis were then correlated with a matrix of land use/land cover data at the watershed and riparian level to test for potential relationships between these variables and species composition.

RESULTS

The first two principal component axes explained 95% of the variation in watershed land use/land cover data (Fig. 2-2a). PC1 was primarily explained by a contrast between sites with a large percent of forested area and those with a large percentage of impervious surfaces in the watershed; decreasing values along PC2 represent an increase in agricultural land use in the watershed (Table 2-1). The three replicate sites within each habitat type all clustered, on average, more closely with each other than with sites of other categories; however, limited separation of developing from reference sites was observed along the two axes (Fig. 2-2a). This limited separation reflected the low levels of impervious surface that were present in the developing areas, indicating that the developing category represented a very early stage of urbanization. Urban sites were separated from the other three habitat types along PC1 and pasture sites were separated from the other three habitat types along PC2 (Fig. 2-2a). The degree of separation between pasture and urban sites was approximately equal to that of each of these habitat types from the combined reference and developing sites.

We captured a total of 37 reptile and amphibian species during the course of the study (Table 2-2). We made no statistical comparisons among individual sites, since data on sample coverage suggested such an analysis might not be informative; however, the coverage of pooled samples was high for all land use/land cover categories (≥ 73 %; Table 2-3). We did not detect a significant difference among the Chao 2 estimated species richness values for the four land use/land cover categories (Fig. 2-3a, Table 2-3) when all species were analyzed together. However, the pooled urban sites displayed greater exclusivity followed by pooled pasture sites, which were elevated relative to reference and developing sites (Table 2-3). When amphibians were analyzed alone, reference, developing, and pasture sites had significantly more species than did urban sites (Fig. 2-3b, Table 2-3). The difference between urban sites and other land use/land cover categories resulted from an absence of all salamanders except *Eurycea cirrigera* and of all hylid frogs from the urban sites (Table 2-2). The results for reptile species richness were surprising, as urban sites were estimated to have significantly more species than developing and pasture sites (Fig. 2-3c, Table 2-3). Urban sites were estimated to have approximately 10 more reptile species than reference sites, but the 95 % confidence intervals for these estimates overlapped. The elevated reptile species richness in urban areas resulted from accumulation of four species of snakes and four species of turtles that were not observed in any of the other habitat types (Table 2-2).

NMDS analysis indicated that a two-dimensional solution best fit the data (Fig. 2-2b). The observed stress reduction in the ordination of the data (final stress = 11.0) was significantly less than that expected by chance (Monte Carlo simulation, $p = 0.02$). The results from the Monte Carlo simulation, coupled with the relatively low observed stress value provide us with reasonable confidence in interpreting the outcome of the ordination (McCune and Grace 2002). The two-dimensional solution explained 87% of the variation in herpetofaunal species composition among sites (number of iterations = 44). Axis 1 correlated negatively with the percentage of impervious surface in the watershed and the percentage of disturbance in the riparian area ($r = -0.70$, $p = 0.01$ and $r = -0.58$, $p = 0.05$, respectively; n = 12) and positively with the percentage of forest cover in the watershed and the percentage of mature forest in the riparian area ($r = 0.67$, $p = 0.02$ and $r = 0.58$, $p = 0.05$, respectively; n = 12). Axis 2 correlated positively with the percentage of immature forest cover in the riparian area ($r = 0.51$, $p = 0.09$; n = 12) and negatively with the percentage of pasture in the riparian area ($r = -0.52$, $p = 0.08$; n = 12).

DISCUSSION

Our data, coupled with previous studies from these same watersheds (Helms et al. 2005, Schoonover et al. 2006, Burton and Samuelson 2008) suggest a strong effect of land use/land cover on herpetofauna diversity within the Piedmont study area. We observed the greatest assemblage shift in urban habitats relative to the reference watersheds but only if effects on amphibians and reptiles are considered separately. The concordance between the land cover analysis and NMDS ordination was clear for species found at two urban sites, which separate from the faunas of the other habitats by accumulation of rare reptiles associated with larger, more-open stream systems and loss of common amphibians associated with smaller, closed-canopy streams. Concordance between the two ordinations was not reflected in the third urban replicate, in which three ranid species were detected, thus causing the site to cluster with the majority of reference and developing sites. It should be noted that several of the species responsible for the urban sites' separation from other land use types in the NMDS analysis were only observed once or twice (Table 2-2). Nevertheless, all of these species were similar ecologically (i.e., large bodied snakes and riveriene turtles), which we feel strengthens our interpretation of the ordination.

The streams reported on here represent habitats similar to the centers of origin for many species in the Plethodontidae (Means 2000). Our data suggest that urbanization causes a near total faunal collapse of this radiation. Similarly, urbanization of our stream habitats, and other habitats reported in the literature (Babbitt et al. 2003, Resetarits 2005), yields a loss of hylid frogs that require fish-free temporary wetlands, or, in a few cases, slow-flowing streams for breeding (RKB personal obs.,). The habitat differences between urban and reference land use/land cover categories most likely impact reptiles and amphibians through shifts in stream and forest structure and quality. Within our study site, Schoonover et al. (2006) demonstrate significant changes in hydrology of urban streams relative to streams in all other land use/land cover categories. Specifically, urban streams experience severe flood events frequently. In addition, Helms et al. (2005) found significantly more lesions and tumors on fish inhabiting urban streams relative to reference streams, suggesting degraded water quality. These observations provide plausible hypotheses for the near absence of plethodontids in our urban study areas and the appearance of reptiles associated with riverine habitats. Such disturbances may extirpate salamander populations and then inhibit population establishment by increasing the mortality of eggs and larvae, as has been shown in other habitats (Welsh and Ollivier 1998, Knuston et al. 1999, Willson and Dorcas 2003, Riley et al. 2005, Price et al. 2006). However, the apparent losses of woodland salamanders, which have direct development, and hylids, several of which breed in wetlands other than streams, suggest that detrimental effects of urbanization on amphibians are not mediated exclusively through alteration of water quantity or quality in streams. The disappearance of these amphibians from urban areas likely results because the urban riparian zones in our study have a low density of woody vegetation and have more open canopies relative to the reference riparian zones (Burton and Samulson 2008). The open nature of urban riparian habitat may increase desiccation rates, and concomitantly decrease survivorship, in some amphibian species (Rothermel and Semlitsch 2002, Rothermel and Luhring 2005).

Remarkably, the loss of species within the plethodontid and hylid families is offset nearly exactly by greater reptile species richness in the urban watersheds relative to all other land use/land cover categories. All of the reptile species that were uniquely associated with urban sites are characteristic of larger, open streams (*Graptemys barbouri*, *Pseudemys concinna*, *Sternotherus minor*, *Nerodia erythrogaster*, *Regina septemvitatta*) or open riparian zones (*Elaphe obsoleta*, *Heterodon playrhinos*). The effects of urbanization impacting stream and forest structure and quality should be reduced in reptiles, which, because of their larger size, can re-colonize areas faster and, because of their thick skin and amniotic eggs, are affected less directly by altered water quality. In addition, the open riparian habitat in our urban study sites coupled with the widening of stream beds that results from urban hydrology (Schoonover et al. 2006), contributes to the explanation for the appearance of snakes and turtles in the urban areas since opportunities for basking would be increased under such conditions. In summary, urbanization within this area alters small streams from closed-canopy, shallow-water features of the pre-developed landscape favored by many plethodontids and hylids to features associated with open vegetation and deeper, warmer waters favored by riverine turtles and snakes.

We did not find strong evidence to suggest that creation of pasture sites within our study area significantly alters the herpetofaunal assemblage relative to reference sites. The differences we did observe are associated with replacements of species among sites and not the significant assemblage differences observed between reference and urban habitats. For example, two pasture sites contained a herpetofauna composed of an accumulation of rare (within our samples) species associated with forest openings or farm ponds (*Hyla gratiosa*, *Kinosternon subrubrum*, *Sceloporus undulatus*). Thus, the increased canopy openness of streams in pasture settings appears to alter the local herpetofauna associated with this land use, except for one site which retained a fauna that is similar to the majority of developing and reference sites. The subtle compositional shifts in assemblages we observed between the pasture and reference sites may offer some insights into the way amphibians and reptiles respond to habitat disturbance. For example, assemblages in areas disturbed by cattle production do not resemble those assemblages in areas disturbed by urban development. This suggests that habitat fragmentation, which is present in both disturbance types, is not solely responsible for the composition shifts seen in urban habitats. Additionally, historical land cover could explain the persistence of a diverse herpetofauna within the pasture sites. Historical records (Jones 1974, Radford and Martin 1975) indicate open-canopy habitat, rather than dense forest, was present in parts of our study area. Consequently, pasture lands, for some fauna, may mimic ecosystems present prior to European settlement.

The developing sites in this study clustered closely with the majority of reference sites in land use and species ordinations. One developing site was further removed from references sites in the NMDS than the others. The site accumulated species associated with floodplain wetlands (*Ambystoma opacum*, *Pseudacris feriarum*, *Agkistrodon piscivorus*) that were not observed at any of the other sites. Nevertheless, the general clustering of species assemblages in developing and reference sites is noteworthy since the developing sites are undergoing a high current rate of housing development in these watersheds. If reptiles and amphibians are able to maintain sufficient population densities in watersheds with some development, it will be important for future studies to determine if particular patterns of housing and commercial development allow for the persistence of these groups over time periods longer than we document here. However, it is highly likely that the similarity between reference and developing sites results because insufficient time has passed since the initiation of heavy housing development for population crashes to occur (Lofvenhaft et al. 2004). To detect such a result, long-term studies monitoring population density across watershed types will be necessary.

Maintaining herpetofaunal diversity hinges on maintaining suitable habitat (Price et al. 2006, Crawford and Semlitsch 2007) and maintaining connections among habitat patches (Parris 2006). An emerging synthesis suggests protection of riparian buffers is generally insufficient for maintenance of stream-breeding amphibian populations, and instead whole-watershed protection is needed (Willson and Dorcas 2003, Miller et al. 2007). In contrast, Crawford and Semlitsch (2007) have recently suggested that it may be sufficient to greatly expand stream buffers in order to protect the core habitat of certain stream- and riparian-dwelling amphibians. In either case, preserving large patches of habitat in high-diversity watersheds will be most productive for conserving reptile and amphibian assemblages. Because such conservation goals are not always possible to implement, it is noteworthy that we have shown herpetofaunal diversity can be high in some disturbed habitats within our study system. Recognizing this fact is the first step toward determining if the observed individuals are part of viable populations. For example, if reptile populations in these urban areas are viable, then in some disturbed habitats it may be necessary to develop conservation goals that maintain the assemblages tolerating a given disturbance by protecting riparian corridors in these landscapes. This strategy, in conjunction with urban planning guidelines that provide for some entirely or nearly undeveloped watersheds will most likely maximize regional diversity.

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Table 2 - 1. Results from a principle components analysis of land cover variables quantified at 12 watersheds within western Georgia, USA.“Other” refers to land cover categories comprising less than 5% of the land cover in any given watershed. Raw land cover data were log-ratio transformed prior to analysis.

	Component	
	PC1	PC2
Eigenvalue	3.27	1.46
Variance explained (%)	66	29
Cumulative variance (%)	66	95
Variables	Factor loadings	
Impervious surface	-0.53	0.23
Evergreen forest	0.54	-0.06
Deciduous forest	0.51	-0.20
Agriculture	-0.05	-0.81
Other	0.41	0.51

Table 2 - 2. Species recorded during surveys of 12 watersheds in western Georgia, USA.

Species	Code	Taxon	Ref*	Past*	Dev*	Urban*
Acris gryllus	ACGR	Anura	7	3	4	0
Bufo fowleri	BUFO	Anura	5	4	8	18
Hyla cinerea	HYCI	Anura	1	0	0	0
Hyla gratiosa	HYGR	Anura	0	2	0	0
Hyla chrysoscelis	HYCH	Anura	2	0	3	0
Pseudacris crucifer	PSCR	Anura	0	1	0	0
Pseudacris feriarum	PSFE	Anura	1	0	7	0
Rana catesbeiana	RACA	Anura	2	3	5	2
Rana clamitans	RACL	Anura	2	4	7	2
Rana sphenocephala	RASP	Anura	5	2	5	2
Ambystoma opacum	AMOP	Caudata	0	0	2	0
Desmognathus conanti	DECO	Caudata	11	13	1	0
Eurycea cirrigera	EUCI	Caudata	27	20	21	17
Eurycea guttolineata	EUGU	Caudata	6	1	3	0
Plethodon glutinosus	PLGL	Caudata	1	2	0	0
Plethodon websteri	PLWE	Caudata	2	0	1	0
Pseudotriton ruber	PSRU	Caudata	0	1	0	0
Agkistrodon piscivorus	AGPI	Squamata	0	0	7	0
Anolis carolinensis	ANCA	Squamata	3	3	7	4
Diadophis punctatus	DIPU	Squamata	1	0	1	1
Elaphe obsoleta	ELOB	Squamata	0	0	0	1

Eumeces fasciatus	EUFA	Squamata	4	1	2	4
Eumeces inexpectatus	EUIN	Squamata	1	0	0	0
Heterodon platirhinos	HEPL	Squamata	0	0	0	1
Nerodia erythrogaster	NEER	Squamata	0	0	0	1
Nerodia sipedon	NESI	Squamata	2	2	5	8
Regina septemvittata	RESE	Squamata	0	0	0	1
Sceloporus undulatus	SCUN	Squamata	0	2	0	0
Scincella lateralis	SCLA	Squamata	1	1	1	0
Storeria dekayi	STDE	Squamata	2	0	0	1
Chelydra serpentina	CHSE	Testudines	0	0	0	2
Graptemys barbouri[†].	GRBA	Testudines	0	0	0	1
Kinosternon subrubrum	KISU	Testudines	0	2	0	0
Pseudemys concinna	PSCO	Testudines	0	0	0	2
Sternotherus minor	STMI	Testudines	0	0	0	2
Terrapene carolina	TECA	Testudines	3	1	6	0
Trachemys scripta	TRSC	Testudines	0	0	2	0
Summed incidence			89	67	98	70

* The number of visits during which a species was recorded as present in reference, pasture, developing, and urban watershedsheds. Each watershed category was visited 48 times.

[†] This species was the only state-listed (threatened) amphibian or reptile found at any of the sites. No species was federally listed.

Table 2 - 3. Summary species richness data for herpetofauna in four land use/land cover categories in western Georgia, U.S.A. (n = 3 sites per category).

Land Use	Taxon	Sobs[a]	Chao 2 est. richness[b]	95 % Lower CI[b]	95 % Upper CI[b]	% Coverage[c]	% Exclusive species[d]	% Completeness[e]
Reference	All	21	24	22	38	86	5	57
	Amphibians	13	14	13	20	96	6	76
	Reptiles	8	11	8	29	73	5	40
Developing	All	20	22	20	32	93	8	54
	Amphibians	12	13	12	20	96	6	71
	Reptiles	8	8	8	14	96	10	40
Urban	All	18	21	19	33	86	22	49
	Amphibians	5	5	5	5	100	0	29
	Reptiles	13	21	15	55	61	40	65
Pasture	All	19	21	19	32	90	14	51
	Amphibians	12	13	12	20	94	18	71
	Reptiles	7	8	7	15	91	10	35

[a] Number of species observed

[b] Number of species estimated to be present (and associated 95 % confidence intervals) based on the Chao 2 estimator

[c] Number of species observed as a percentage of the average estimated species richness (Chao 2)

[d] Number of species not found elsewhere as a percentage of the landscape total

[e] Number of species observed as a percentage of the landscape total

Figure 2 - 1. Locations of study sites (black circles) and waterways (gray lines and polygons) located within the Chattahoochee Watershed of western Georgia, USA. Sites were placed in one of four land use categories, which are listed in Table 3 and described in the text. R = reference, D = developing, U = urban, and P = pasture.

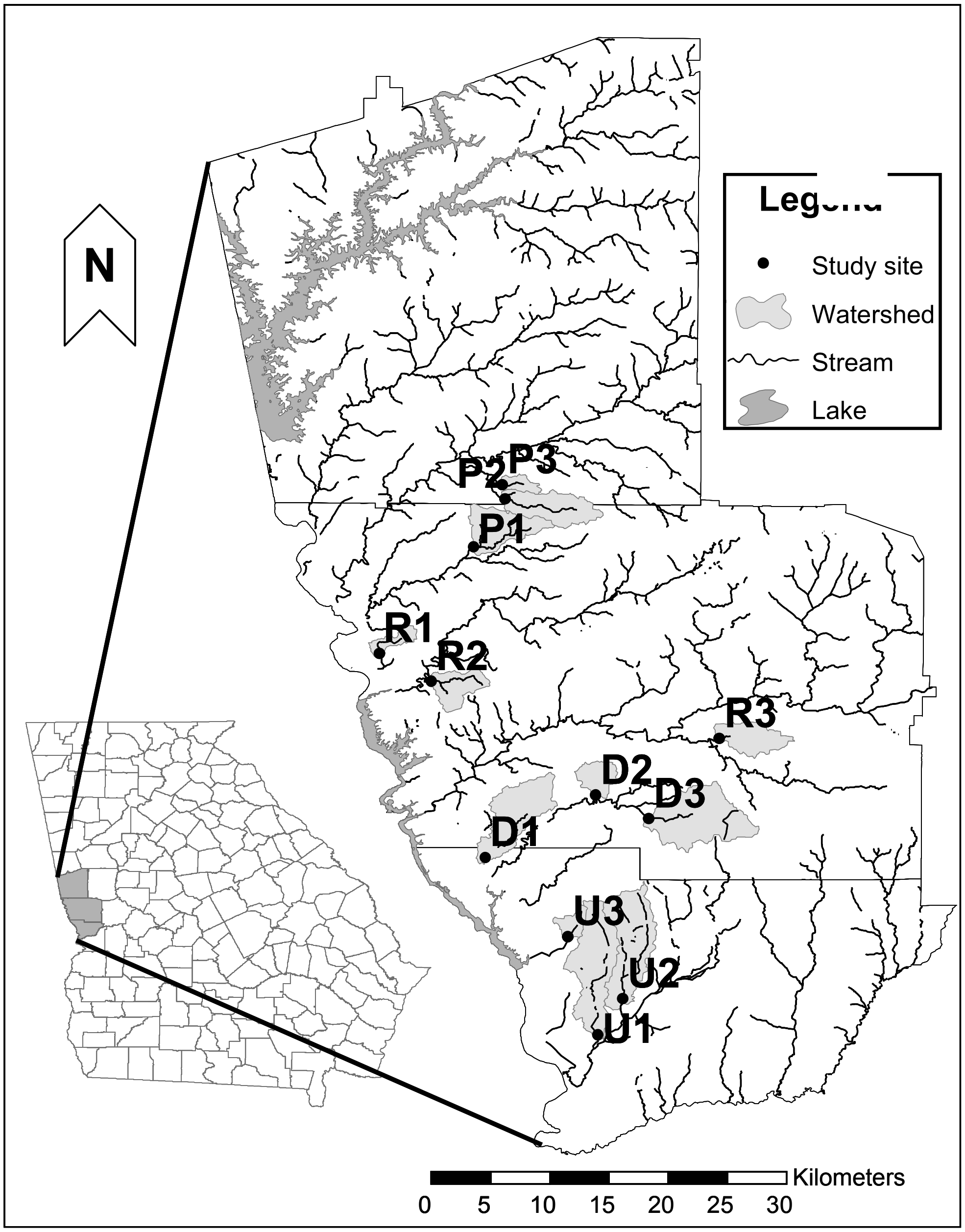


Figure 2 - 2. Multivariate ordination of environmental and biotic variables. (a) The relationship between land use/land cover variables and site categorization based on principal components analysis (variables are listed in Table 2-1). (b) NMDS species scores from a non-metric multidimensional scaling plot for species (plotted as four-letter codes; reptile species codes are in gray, amphibians in black) sampled among the four watershed categories. Watersheds are plotted as symbols in species space as defined by the first two axes in the analysis. Land use/land cover variables that correlated strongly with NMDS axes are labeled on the axis. (WS = watershed-scale variable, R = riparian-scale variable). See Table 2-2 for species code definitions.

Figure 2-2

(a)

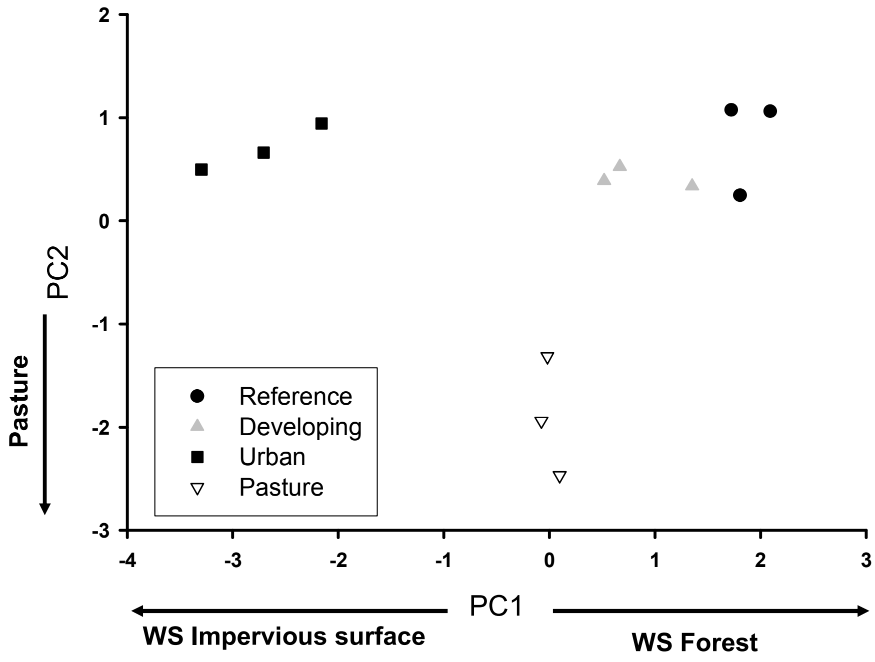

2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
PC2
Pasture
PC1
WS Impervious surface
WS Forest
● Reference
▲ Developing
■ Urban
▽ Pasture

Figure 2-2

(b)

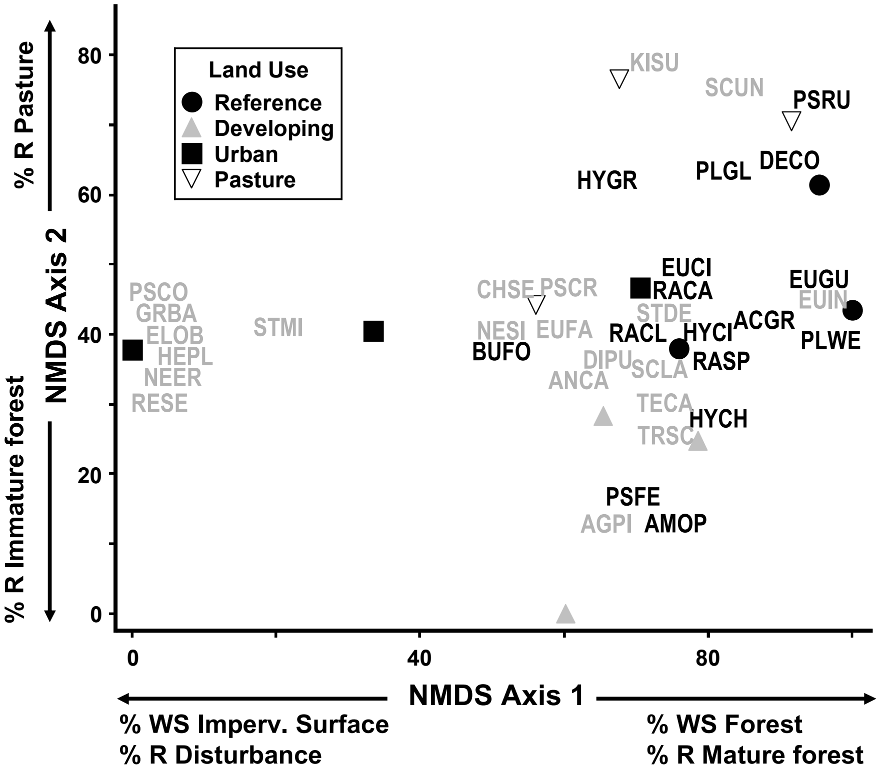

Land Use
● Reference
▲ Developing
■ Urban
▽ Pasture
% R Pasture
NMDS Axis 2
% R Immature forest
80
60
40
20
0
0
40
80
NMDS Axis 1
% WS Imperv. Surface
% R Disturbance
% WS Forest
% R Mature forest
KISU
SCUN
PSRU
PLGL
DECO
HYGR
EUCI
RACA
EUGU
EUIN
PSCO
GRBA
ELOB
HEPL
NEER
RESE
STMI
CHSE
PSCR
STDE
ACGR
NESI
EUFA
RACL
HYCI
PLWE
BUFO
DIPU
SCLA
RASP
ANCA
TECA
HYCH
TRSC
PSFE
AGPI
AMOP

Figure 2 - 3. Sample-based species accumulation curves for (a) all herpetofauna, (b) amphibians, and (c) reptiles sampled within four land use/land cover categories in western Georgia, USA.

(a)

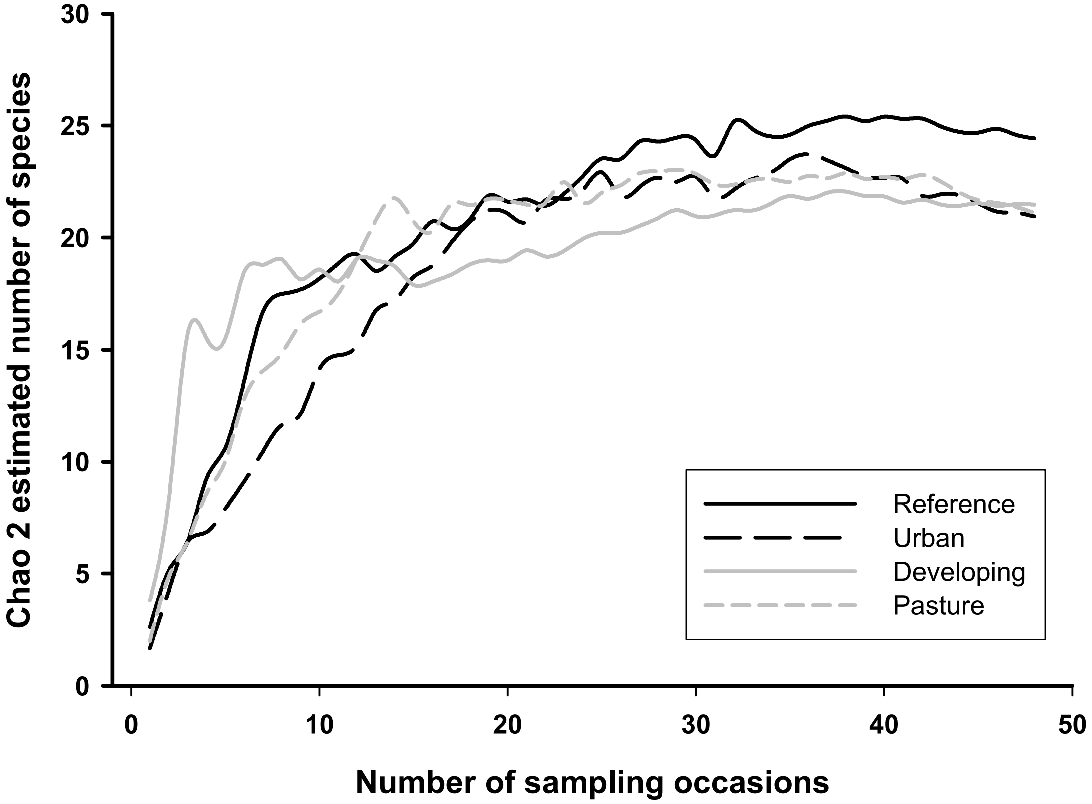
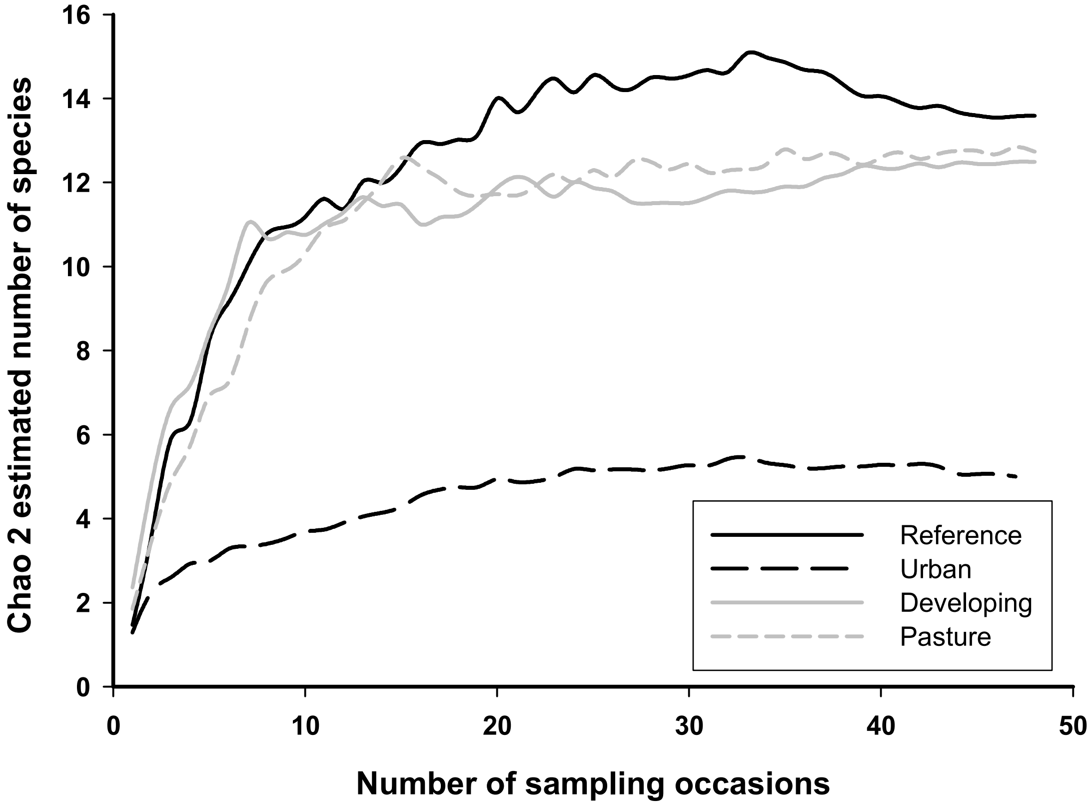


Figure 2-3

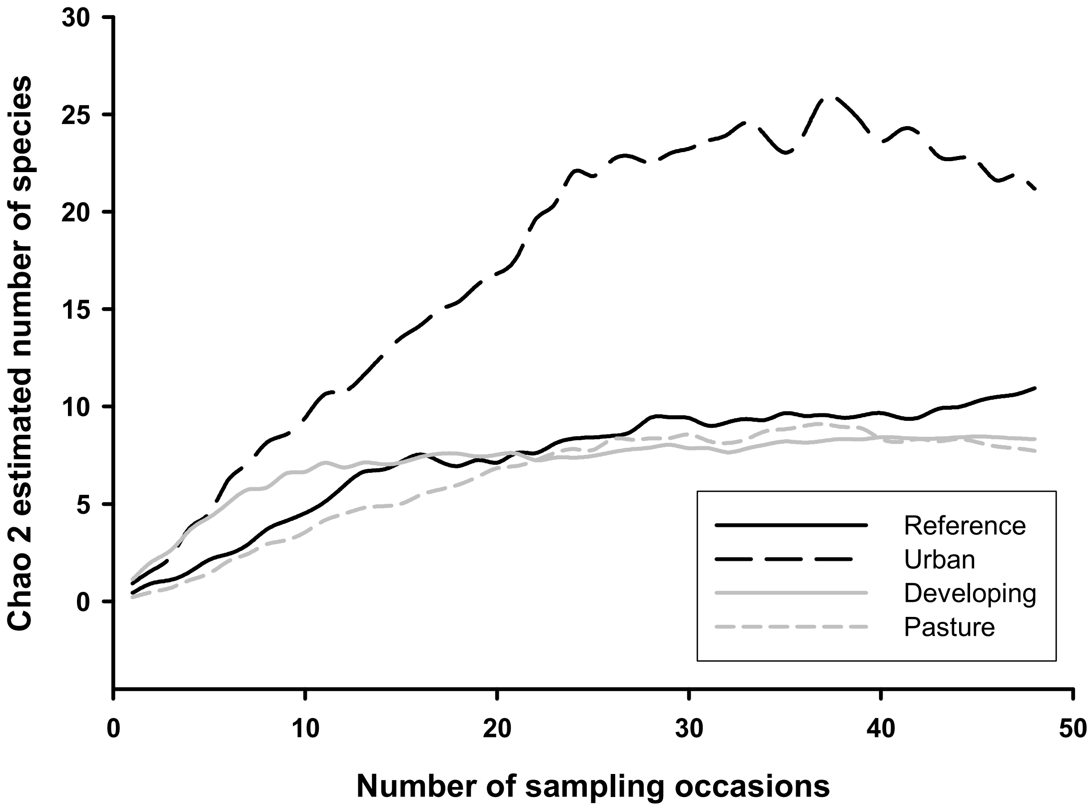
(b)



16
14
12
10
8
6
4
2
0
Chao 2 estimated number of species
0
10
20
30
40
50
Number of sampling occasions
Reference
Urban
Developing
Pasture

Figure 2-3

(c)


Chao 2 estimated number of species
30
25
20
15
10
5
0
0
10
20
30
40
50
Number of sampling occasions
Reference
Urban
Developing
Pasture

CHAPTER 3. INCREASED SPATE FREQUENCY DECREASES SURVIVORSHIP OF A STREAM-BREEDING SALAMANDER IN URBANIZED WATERSHEDS

ABSTRACT

Sufficient data have been collected to document repeated patterns in urbanized streams for many abiotic parameters, aquatic insects, and fish. More recently, stream-breeding salamanders have been observed to decrease in density in urban areas. We found that southern two-lined salamanders in western Georgia, USA exhibited no change in reproductive output between urban and reference habitats. However, repeated sampling throughout the larval period revealed a large decline in density of larvae in urban areas prior to metamorphosis, whereas a similar decline was not seen in reference habitats. We evaluated several hypotheses that might explain the observed decline in urban areas, and a model in which increased watershed impervious surface causes an increase in spate frequency and magnitude, which then leads to decreased larval density had the most support. This study is the first attempt to compare multiple hypotheses as to why salamander density and diversity decreases in urban habitats. By describing larval density at the beginning and end of the larval period, and by identifying a likely mechanism for the observed decline in density, species-specific and stream restoration efforts can be enhanced.

INTRODUCTION

Studies of stream systems have repeatedly documented shifts in the diversity and composition of 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 mapping the most probable cause-effect path linking land use change to biotic response. They investigated multiple categories of abiotic responses to land use change that might, in turn, affect biotic variables. We adopted Burcher et al.'s (2007) cascading effects model to determine mechanism(s) that influence the response of a widely distributed salamander to urban and agricultural development.

Development within a watershed can lead to a number of shifts in the abiotic environment relative to undisturbed streams. Examples include chronic 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 shifts in the abiotic environment 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 impervious surface (i.e., roads and roofs) cause increased overland flow during rain events. As a result, urban streams often show storm flows that are of greater magnitude and frequency than those in areas with less impervious surface (Poff et al. 2006, Schoonover et al. 2006). Understanding the type of disturbance that impacts an organism should allow for a more focused assessment of how biota will respond to certain types of management and/or restoration efforts.

We monitored survivorship during the aquatic stage of the life-cycle for two cohorts of southern two-lined salamanders (*Eurycea cirrigera*) in 12 streams categorized by land cover. We chose this species because a previous study in the southern Piedmont of the United States (Barrett and Guyer 2008) indicated it is the only salamander species persisting in urban waters relative to four stream-breeding salamanders found in nearby forested streams. By studying factors of development that influence southern two-lined Salamander survivorship during the larval phase, we hope to provide insight into 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 for 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.

METHODS

Stream selection

We conducted salamander surveys within three counties in western Georgia, USA (Muscogee, Harris, and Meriwether). The study area was also part of an interdisciplinary effort (known as the West Georgia Project) by the Auburn University Center for Forest Sustainability to understand feedback loops 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 on % land cover in the watershed and current housing development trends (Table 3-1): reference (at least 75 % forested cover), pasture (> 30% pasture), urban (> 20 % impervious surface), and developing. The developing sites are located in Harris County, Georgia, which is one of the fastest growing counties in the United States (Lockaby et al. 2005). Details on and support for the categorization of watersheds can be found in Barrett and Guyer (2008).

Salamander sampling

Southern two-lined salamanders (Plethodontidae) typically inhabit riparian areas of 1st through 3rd order streams as adults, and migrate to a stream in late fall/early winter to mate. Females deposit eggs in the stream by attaching an egg mass to the underside of rocks, logs, or exposed root masses (Guy et al. 2004). Eggs typically hatch in late spring.

In the portion of their range where we studied this species, larvae remain in streams ~ 18 mo (KB, personal observation).

We sampled southern two-lined salamander density in the 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. To sample salamanders, we established 10 permanent 15-m transects 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 during summer 2007 and spring 2008. Salamander density estimates were made using removal sampling. We used a small 15 cm wide aquarium net to scoop salamanders that were seen on the surface of the stream bed as well as to capture salamanders from under cover that we lifted or from 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.

Land cover analysis and land use determination

We determined watershed boundaries and size from US Geological Survey 30-m resolution digital elevation models using ArcView 3.2a software (Environmental Research Systems Institute, Inc., Redwoods, California). During leaf-off in March 2003, true-color 1-m resolution aerial photographs of the study watersheds were taken. 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 estimated % IS, % pasture, and % forest land cover for analyses (Table 3-1). Detailed image processing methods for the study watersheds are presented elsewhere (Lockaby et al. 2005).

Stream hydrological variables

We quantified stream discharge (Q) bimonthly from June 2003 to June 2004 using a Marsh McBirney flow meter. Mini-Troll ® pressure-transducer (In-Situ Inc., Ft. Collins, Colorado) data loggers recorded stage 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 1996, Poff et al. 1997, Schoonover et al. 2006, Helms et al. in press) (Table 3-2). All hydrological values were averaged over the period of record.

Stream physicochemical sampling

We quantified several stream-specific physicochemical parameters considered important in determining biotic assemblages (Table 3-2). We measured stream temperature continuously with HOBO® Temp data loggers placed near pressure transducers. Dissolved oxygen (DO) and streamwater pH were measured seasonally 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.

To determine available habitat quality and quantity, we used a multimetric habitat assessment from the Georgia Environmental Protection Division (GA DNR) 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 assessment involves taking the average of 3 individuals' 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 3 observers at all sites.

We further assessed stream habitat by quantifying benthic organic matter abundance (BOM) and substrate cover in each stream reach. We estimated BOM by sampling transitional areas between the runs and pools to standardize efforts. We quantified BOM by determining the ash-free dry mass (AFDM) of 9 replicate 2.5 x 10 cm benthic cores. We sampled substrate cover by estimating the % of substrate covered by cobbles (size range), pebbles (size range), live root masses, or leaf packs during the summer of 2007 along each of the transects sampled for salamanders. Values were averaged among the 10 transects for each stream.

Data analysis and model building

We chose to sample streams intensely over multiple seasons, so we did not evaluate enough streams to warrant statistical comparison of means among land cover categories. Instead, we plotted mean salamander density (mean of data from streams within a land cover category and between years) for hatchlings and pre-metamorphic individuals. 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). Essentially, the algorithm incorporates transect-specific detection probabilities into density estimates. For example, if salamander captures do not decrease appreciably from the first to second pass, then one could assume poor detection probability, and thus a higher density estimate than the number of individuals actually observed. We explored the abiotic factors that could have generated the estimated density trends using path analysis.

We measured several environmental variables that could potentially affect salamander density. To reduce the set of predictor variables for path analysis, we began by examining 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 3-3). When two or more variables were highly correlated, we chose to use the variable that was either most commonly recognized as potentially affecting biota (Burcher et al. 2007), or that was normally distributed (or could be transformed to normality). After eliminating highly correlated measures within a category, we were left with, at most, five predictors per category (Table 3-3). We also reduced the data set by combining variables representing water quality using principle components analysis. We performed principle component 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 and that lower values of this composite measure represented increased water quality.

Based on the descriptive statistics from our density estimates, we constructed seven plausible models for how land cover could affect pre-metamorphic salamander density (Table 3-4) via the reduced abiotic variable set. We did not use all variables within the reduced data set, but rather chose variables that would contribute to *a priori* models describing ways salamanders might respond to land use change. The land cover variables % forest and % impervious surface were negatively correlated. Because no transformation of the data resulted in a normal distribution for % impervious surface, 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 via changes in hydrology, chemistry, or in-stream habitat (or some combination thereof), which then impact biota (Table 3-4). 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 Criteria (AIC).

RESULTS

There was no appreciable difference in density of hatchlings among the four land cover categories (Fig. 3-1), which suggests reproductive effort was not influenced by land cover category during the two years we monitored hatchling density. A large reduction in density occurred in all land cover categories over the nine months between samplings of hatchlings and pre-metamorphic individuals (Fig. 3-1); however, the 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). Because salamander survivorship in pasture streams was not appreciably different from survivorship in reference streams, all path analyses focused on how urban landscapes affect salamanders.

Path analysis of *a priori* models indicated strong support (Table 3-4) for a path linking increased urbanization (concomitant with decreasing forest) to an increase in spate frequency, which in turn decreases the density (i.e., survivorship) of salamanders prior to the pre-metamorphic stage. 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 a decrease in salamander density should also be considered likely (Burnham and Anderson 2002; Table 3-4). While this model had support, the path coefficient between decreasing forest and water quality was extremely low, indicating little bivariate correlation between these two variables. As a result, even though the model cannot be rejected based on the data, we do not see it as a strong contender for explaining how increasing urbanization effects salamander density.

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. 3-2D and E) had very little support; however, the least supported models were the gill 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. 3-2F and G, respectively).

DISCUSSION

The term “urban stream syndrome” has been used to describe predictable abiotic and biotic consequences to streams that result from urbanization (Walsh et al. 2005). While both fish and aquatic invertebrate assemblages were found to shift as part of the syndrome, the response of stream-breeding amphibians was not addressed by Walsh et al. (2005). Only recently have sufficient data emerged to enable biologists to confirm a consistent negative impact on stream-breeding amphibians with disturbance in general, and urbanization in particular (Willson and Dorcas 2003, Price et al. 2006, Barrett and Guyer 2008). The results we present here add a new feature to the urban stream syndrome. Specifically, salamander larvae are flushed from streams and this lowers density of common species and likely lowers species richness (Barrett and Guyer 2008).

Two other studies have examined the response of southern two-lined salamanders to urbanization. Willson and Dorcas (2003) demonstrated a strong, negative correlation between the amount of disturbed habitat around a stream and the number of southern two-lined salamanders captured. The key finding of this study was that watershed-scale disturbance was more predictive of salamander number than % disturbance at any of the smaller scales they examined. Similarly, Miller et al. (2007) identified negative correlations between salamander abundance and percent impervious cover in the watershed. They also identified several abiotic correlates with abundance, but made no attempt to determine the relative strength of any one factor with regards to its influence on southern two-lined salamander density. Finally, Miller et al. (2007) use mean salamander abundance from three sampling occasions as a response variable. One of these sampling occasions was from April, so hatchlings were likely to be quite abundant. On subsequent sampling occasions, in our experience, density of animals decreases considerably. Analyzing the mean from multiple seasons may have inhibited their ability to identify some trends.

The next step toward understanding the amphibian-related symptom of the urban syndrome involves identifying the mechanisms leading to loss in amphibian species richness and/or abundance. We demonstrated a strong link between spate flow frequency and the decline in southern two-lined salamander density. We also evaluated models that posited other disturbances such as water quality changes would influence density, but none of these models compared favorably to the spate frequency hypothesis. The strong influence of flooding on southern two-lined salamander survivorship is seemingly at odds with many fish species that have been shown to recover quickly from even the most extreme flood events (Jurajda et al. 2006). However, how a species responds to hydrological changes depends on the evolutionary history of the taxa. Shifts in flow regime have been identified as a major problem for many stream- and river-dwelling species (Lytle and Poff 2004). Because salamander larvae primarily inhabit low-order streams (Petranka 1998), they presumably have not evolved mechanisms such as those present in many fish species that allow them to survive and/or re-colonize after extreme flow events encountered in urban stream systems.

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 two years of monitoring. This pattern suggests that adult salamanders have not abandoned these urban areas. Should spate frequency and intensity be reduced it is likely that populations of this salamander would increase recruitment. Other salamander species that have likely been extirpated from the urbanized streams we evaluated may not recover as quickly, as no adult populations 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; 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 is strongly related to decreases in salamander abundance. Decreasing overland flow, and thus in-stream flow, in urban areas is a daunting management task, it is perhaps more manageable than dealing with issues such as sedimentation, non-point source pollution, or conductivity of the water. Additionally, effects of in-stream restoration efforts will be minimal with respect to biota if upland processes such as overland flow are not addressed (Charbonneau and Resh 1992).

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Table 3 - 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^2)**	**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

Table 3 - 2. Hydrological, physiochemical, and habitat variables used in analyses and their range of values recorded from June 2003 to June 2004 in the 12 watersheds.

Variable	**Description**	**Range**
	Hydrological Magnitude	
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
	Hydrological Frequency	
3xMed	# of times discharge exceeded 3x median flow	5 – 74
5xMed	# of times discharge exceeded 5x median flow	1 – 65
7xMed	# of times discharge exceeded 7x median flow	1 – 64
9xMed	# of times discharge exceeded 9x 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
	Physicochemistry	
Temp	Median water temperature (^{o}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
	Habitat	
OM	Benthic organic matter (g)	0.3 – 1.2
Substrate	Median substrate size (cm)	0.7 – 1.8
Tg	Tractive force	2.7– 73.9
Instability	Tg / Substrate	0.002– 0.05
Habitat	Habitat assessment index score	54 – 126
Cover	Mean % substrate area of cobbles, pebbles, root mass, or detritus	11 – 33

Table 3 - 3. Categories of predictor variables used to select variables in constructing path models.

LU/LC	**Hydrology**	**In-stream habitat**	**Physicochemical**
agriculture	median discharge	Instability	pH
forest	max discharge	Habitat	total dissolved solids
	spate frequency[a]	width:depth	total suspended solids
		bank height	organic matter
		in-stream cover	

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

Table 3 - 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 models with p-values > 0.05. All models that were not rejected were compared using AIC.

Model	**Path**	**Hypothesis**	χ^2	***p*-value**	**AIC**	**Δ 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 and 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		

Figure 3 - 1. Southern two-lined salamander hatchling and premetamorphic mean density (±SE) estimates from four land cover categories (3 replicate streams per category). Density estimates for hatchling and pre-metamorphic size classes represent the mean from two cohorts.

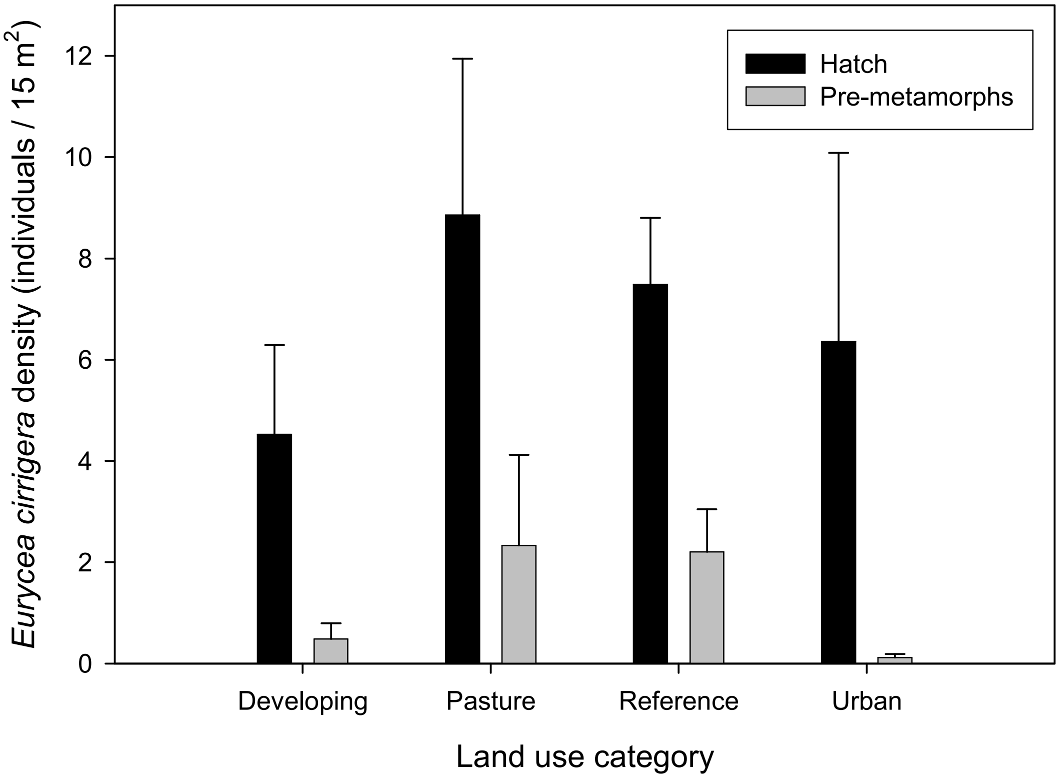


Figure 3 - 2. Path diagrams representing a priori models describing how land cover change impacts salamanders in urban streams. Models were based on knowledge of the species and published studies on salamanders in urban streams. Spate frequency (A) was the top model among those compared by AIC from Table 3-4. 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.

Figure 3-2

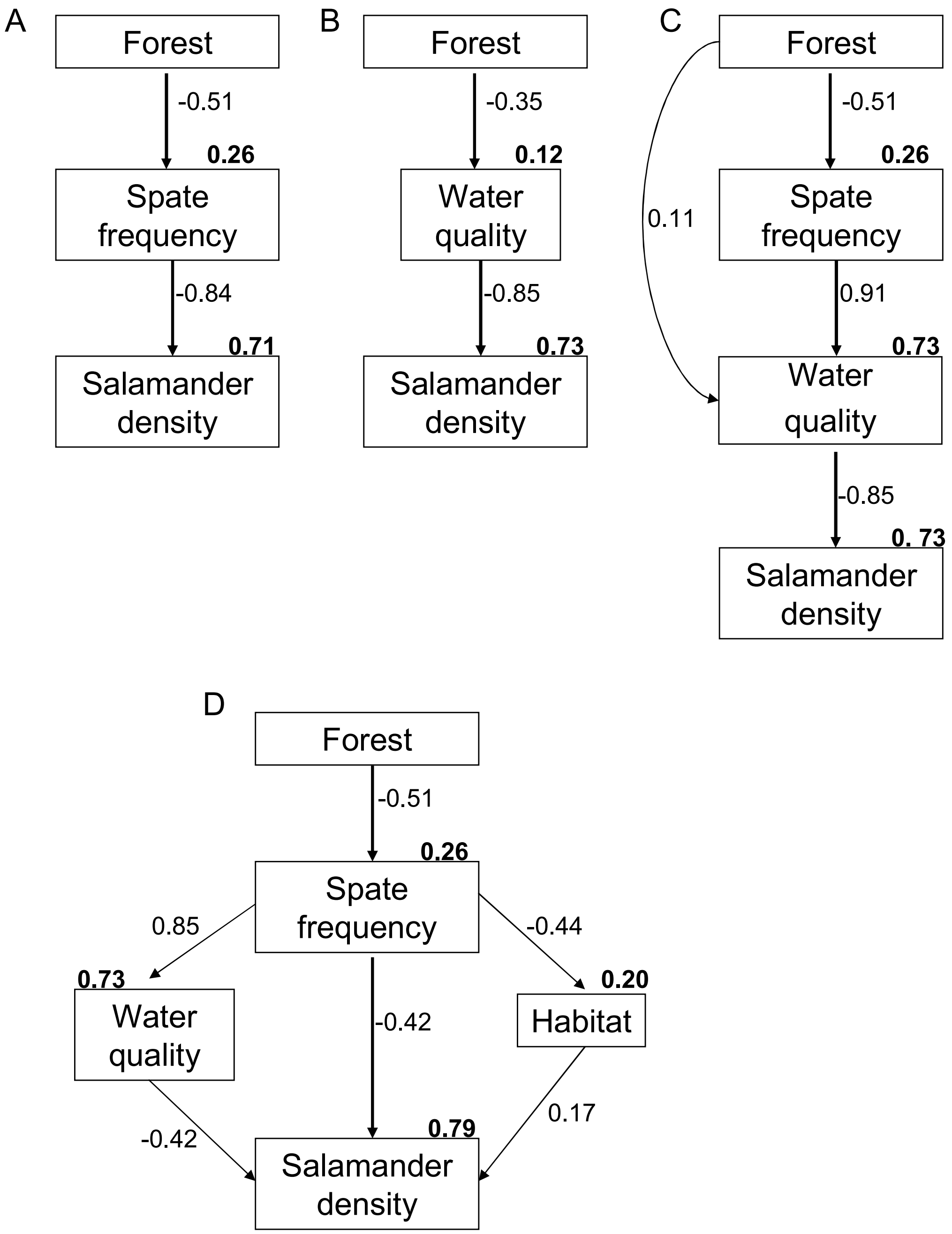
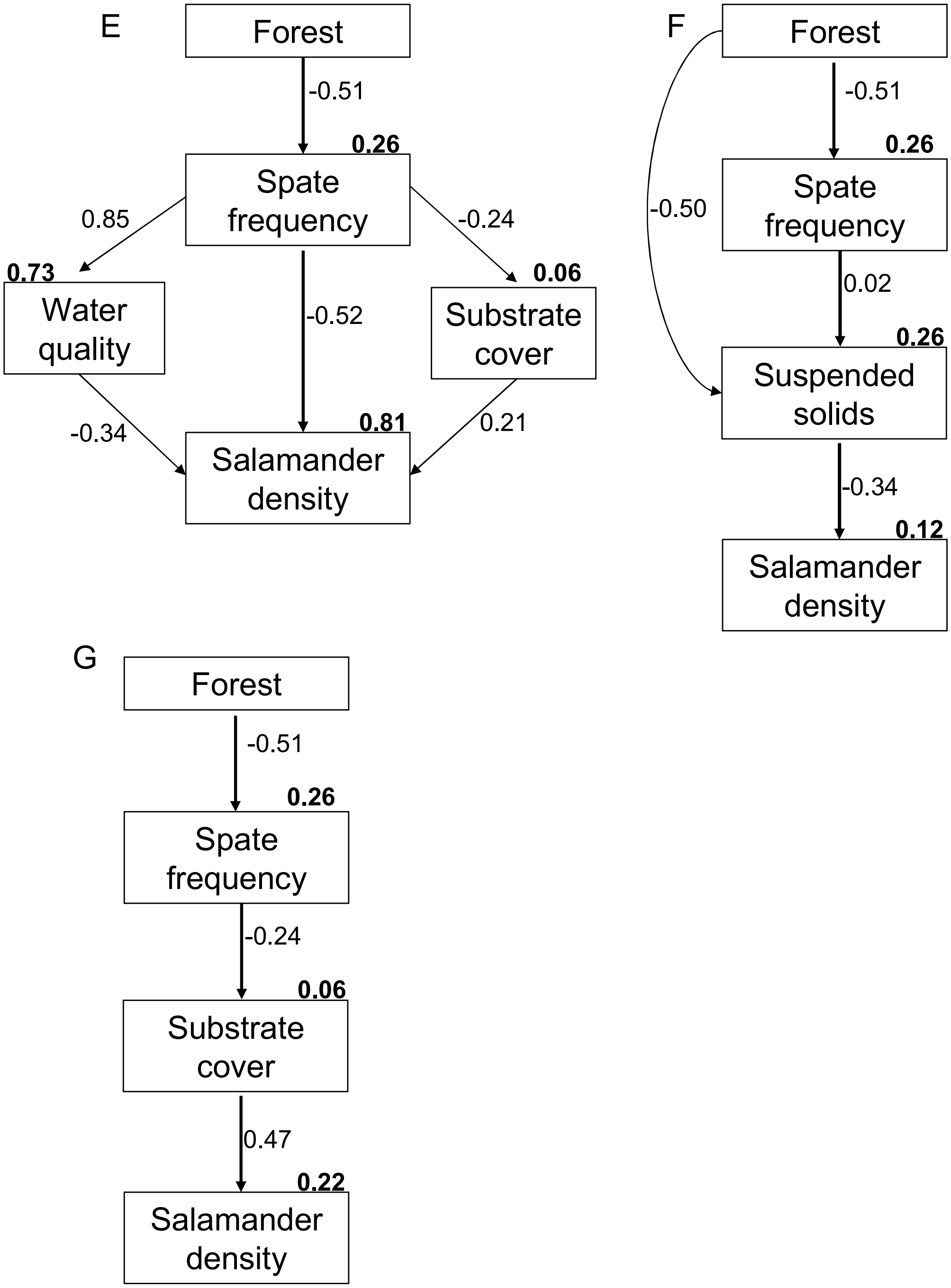

A
Forest
-0.51
0.26
Spate frequency
-0.84
0.71
Salamander density
B
Forest
-0.35
0.12
Water quality
-0.85
0.73
Salamander density
C
Forest
0.11
-0.51
0.26
Spate frequency
0.91
0.73
Water quality
-0.85
0. 73
Salamander density
D
Forest
-0.51
0.26
Spate frequency
0.85
-0.44
0.73
Water quality
-0.42
0.20
Habitat
-0.42
0.17
0.79
Salamander density

Figure 3-2


E
Forest
-0.51
0.26
Spate frequency
0.85
-0.24
0.73
Water quality
-0.52
0.06
Substrate cover
-0.34
0.81
0.21
Salamander density
F
Forest
-0.51
-0.50
0.26
Spate frequency
0.02
0.26
Suspended solids
-0.34
0.12
Salamander density
G
Forest
-0.51
0.26
Spate frequency
-0.24
0.06
Substrate cover
0.47
0.22
Salamander density

CHAPTER 4. EXPERIMENTAL EVIDENCE FOR INCREASED LARVAL SALAMANDER DISPLACEMENT DURING HIGH FLOW EVENTS ON SUBSTRATES COMMON TO URBAN STREAM BEDS

ABSTRACT

We conducted an experimental test of the hypothesis that spates in urbanized streams, which result from increased impervious surface in the watershed, lead to lower survival of salamander larvae because larvae are dislodged from available substrate. We established artificial stream channels lined with substrates commonly found in urban and forested streams (i.e., sandy- and rocky-based substrates, respectively). We placed larval two-lined salamanders (*Eurycea cirrigera*) in these artificial channels and subjected them to increasing water velocities to evaluate the magnitude of flow required to dislodge the animal on a given substrate type. Larvae on sandy-based substrates common to urban streams were flushed from the artificial stream at significantly lower velocities than animals on rocky-based substrates. These experimental results are consistent with field data that suggest hydrological changes can impact the distribution and abundance of species. Our results emphasize the need for upland habitat restoration in urban areas that will reduce run-off, in conjunction with in-stream restoration efforts designed to improve stream habitat quality.

INTRODUCTION

Urban areas are increasing across the globe, and in the US the size of urban areas is expected to increase in the future (Pickett et al. 2001). Urbanization changes many facets of the native landscape, and this situation is especially true for streams draining urban watersheds (Walsh et al. 2005). An altered hydrology is one of the more significant changes occurring in streams surrounded by urban lands (Arnold and Gibbons 1996, Paul and Meyer 2001). Such alteration results from an increase in the amount of impervious surface (i.e., roads, rooftops, etc.) in a watershed, which causes increased overland flow into streams, which in turn leads to increased spate frequency and magnitude in urban drainages (Arnold and Gibbons 1996, Schoonover et al. 2006).

Abiotic changes to the stream channel accompany shifts in hydrological regime. Perhaps most dramatically, overall stream channel morphology can shift with urbanization (Hammer 1972, Galster et al. 2008). In conjunction with (and/or as a result of) this large-scale change in stream morphology, in-stream sediment loads increase (Nelson and Booth 2002). An increase in sedimentation has been linked to a decrease in the amount of larger size substrata such as cobble and pebble, and corse woody debris (Davis et al. 2003).

The effect of altered hydrology on stream biota has not been addressed for many species. Some studies have examined the effect of natural flooding on species composition. For example, Meffe (1984) provided evidence that flash flooding in streams within the western United States allowed the persistence of a native topminnow, while this same species was extirpated by an introduced mosquitofish in streams not subject to flooding. Flooding also has been demonstrated to alter survivorship of brook trout, presumably by decreasing habitat for fish and for prey items (Elwood and Waters 1969). Fewer studies have identified the impact that spates caused from urban runoff have on stream biota. Such disturbance is typically of lower magnitude and of a greater frequency than the increased flows evaluated in the studies above. Chapter 3 identified spate frequency (and correlated measures of spate magnitude) as a significant predictor of salamander density declines in urban areas. In short, we examined several probable pathways by which increased urbanization might affect salamander survivorship in 2nd and 3rd order streams, and the model using spate frequency/magnitude as a primary link between increasing impervious surface and decreasing salamander density garnered the most support. Here, we explicitly test the hypothesis that an altered hydrology and stream bed substrate composition affects salamanders in urban streams. Specifically, we evaluated the ability of two-lined salamander (*Eurycea cirrigera*) larvae to remain in artificial streams when the larvae were subjected to simulated spates. We conducted the experiments in a manner that allowed us to assess the prediction that larval retention in experimental streams would be a function of both substrate type and water velocity.

METHODS

We collected larvae of southern two-lined Salamanders (*Eurycea cirrigera*) from 2nd and 3rd order streams in Lee County and Macon County, AL during February 2008 (first trial) and April 2008 (second trial). These 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 were constructed from one-meter of 15.25 cm PVC pipe cut in half length-wise. We lined the resulting channel with one of four substrates and the channel received water from a 3.80 cm PVC pipe (with cutoff valve to control outflow volume and velocity), which was gravity fed from an existing pond. The artificial stream had an approximate 2^0 slope, and water drained from the channel through a 10.16 cm 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 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 (rocks ranging in diameter from 2-60 mm), and a pebble-cobble mix (particles ranging in diameter from 30-150 mm). Sand and sand with detritus were intended to mimic substrates more commonly observed in urban streams, which tend to have less rocky cover than forested streams (t-test, $t = 2.51$, $p = 0.02$; Fig. 4-1). This characterization of urban and forested substrates was based on data collected by visually assessing the stream bed along ten 15-m transects (spread over a 500-m stream reach) in three urban and three forested streams in western Georgia, USA, that were surveyed as part of another project (Chapter 3) during spring 2006. We estimated the percent cover (to the nearest 5 %) of the following categories: silt, sand, detritus, gravel, pebble, and cobble.

We placed a salamander larva in the flume with water flow approximating base flow values (0.02-0.04 m/s) recorded in several 2nd and 3rd order streams where *E. cirrigera* are known to be present. Water depth at the start of trials ranged from 1 – 3 cm. A porous Plexiglass divider was inserted in the upper quadrant of the 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 salamanders 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 flume velocities were equal to or greater than maximum observed velocities of Helms and Schoonover in 2nd and 3rd order forested streams, 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. The experiment was conducted twice to increase replication, each time using 24 unique larvae (i.e., 48 total larvae were used).

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 4 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 one day separating trials. The results from the two experiments were evaluated separately using a three-way ANOVA with velocity at trial termination as the response variable. The following independent variables were entered in the model: Block (as described above), Day (1, 2, 3, or 4), Substrate, and Subject. Subject was entered as a random effects factor and 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 two minutes of the trial. To verify that the results we obtained from the experiment described above were the result of water velocity, rather than habitat selection, we conducted a sort-term habitat selection experiment. In this experiment, 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 selection against 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.

RESULTS

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. 4-2; Table 4-1).

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 larvae tested had moved to a different substrate than the one they were initially placed on during the short-term habitat selection trials, indicating larvae tended to remain in one place in the experimental flumes, regardless of substrate, at low flows.

DISCUSSION

Data from our experiments supported the hypothesis that an altered hydrology in urban streams results in lower salamander survivorship in those habitats. Specifically, from the above hypothesis we tested the prediction that salamanders can not maintain position in a stream channel when water velocities reach levels recorded during spates in urban habitats. Additionally, we demonstrated that the ability to withstand high flows is related to presence or absence of rocky substrates. When gravel, pebble, and cobble are present in a stream bed, salamanders are provided with refugia that allow them to maintain position during times of elevated water velocity.

We caution against inferring that salamander response to velocities generated within flumes can be extended to natural (or urbanized) streams. Typically, all but the most heavily impacted streams exhibit some rocky cover and sinuosity. Both of these features within a stream would mediate the impact of high flows on salamanders. Our flumes were straight channels with (purposefully) homogenized substrates. We do believe the relative outcome of our experiment can be interpreted to mean that (1) salamanders can be dislodged and washed from a refuge at high water velocities, and (2) the magnitude of water velocity required to wash a salamander downstream is significantly lower in the absences of rocky substrates.

The data from this experiment support data that demonstrated spate frequency (which was highly correlated with spate magnitude) is the best predictor of salamander density across an urban-rural stream gradient. In Chapter 3 I compared several plausible path models that hypothesized how increasing impervious surface could cause cascading effects through several abiotic variables to impact salamander larval density. The model with the most support was one in which increasing impervious surface caused an increase in spates which in turn decreased salamander density. The experiment we conducted explicitly tested salamander response to high flows. Together, these two data sets suggest salamanders do not have the ability to maintain position in the stream during high flows and that recolonization after elevated flows subside must be low.

Other studies have documented species that are susceptible to spates; however, these studies are largely in the context of hydrological changes 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 (1999) used field data to argue that larval fire salamanders (*Salamandra 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.

Previous studies have demonstrated that urbanization can have an effect on streams (Galster et al. 2008) and stream biota (Barrett and Guyer 2008) that is analogous to increased 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 the key driver leading to these shifts. The loss of low-order stream habitats and the associated biota allows riverine species to move further up the stream network and increases stream network homogeneity. Preventing this cascading series of events will require restoration efforts that are focused on upland areas of the watershed (i.e., reducing impervious surfaces) as well as in-stream restoration efforts in urbanized habitats.

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Table 4 - 1. 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 four 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*
		Trial One		
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		
		Trial Two		
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		

Figure 4 - 1. Mean (± SE) availability of pebble/cobble substrate between forested and urban streams in western Georgia, USA (n = 3). Data are based on visual estimates from ten 15-m transects stratified over a 500 m stream reach. Reference streams had significantly more pebble and cobble on the stream bed than urban streams (p = 0.02).

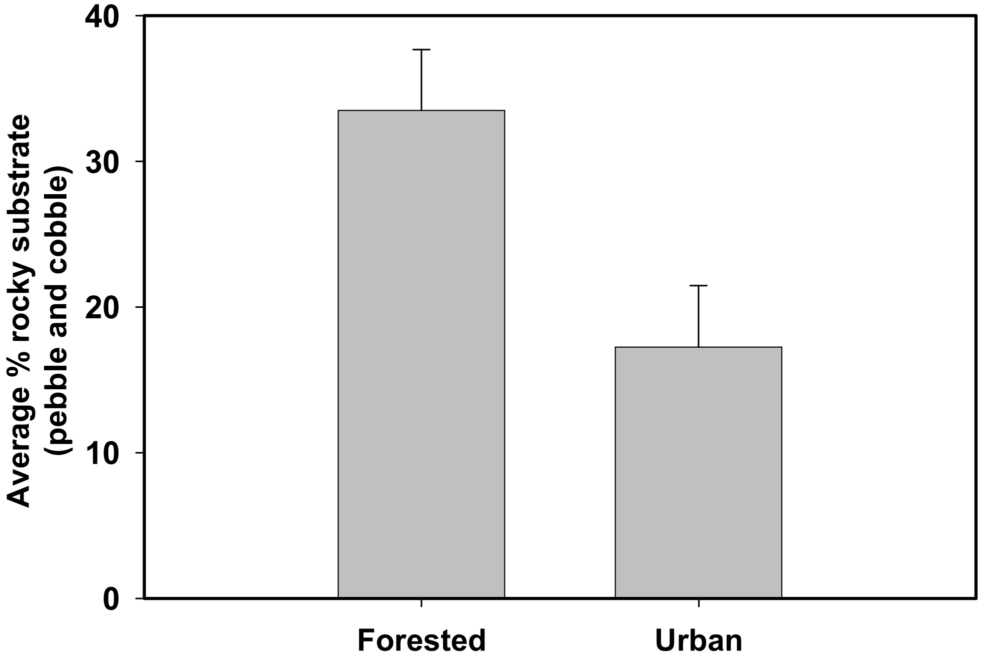


Figure 4 - 2. Mean (± SE) water velocity at trial termination for two experimental trials (a and b) in which salamander larvae were placed on one of four different substrates within a constructed flume and water velocity was slowly increased until the salamander was flushed from the channel. Salamanders 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 pairwise comparisons using Tukey's 95% confidence intervals.

(a)

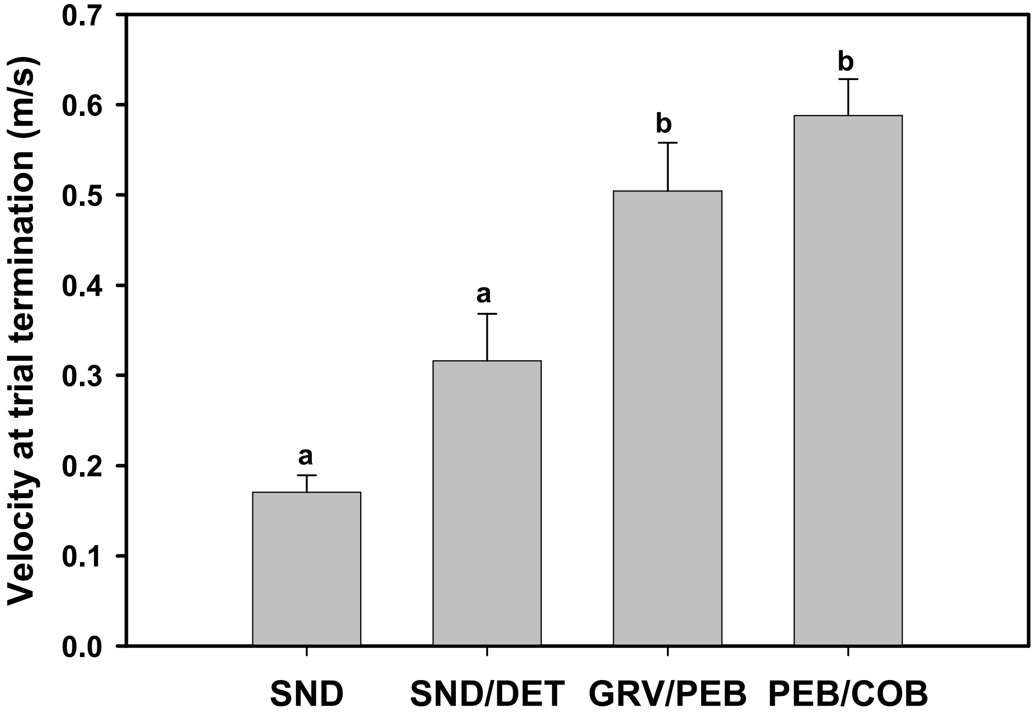
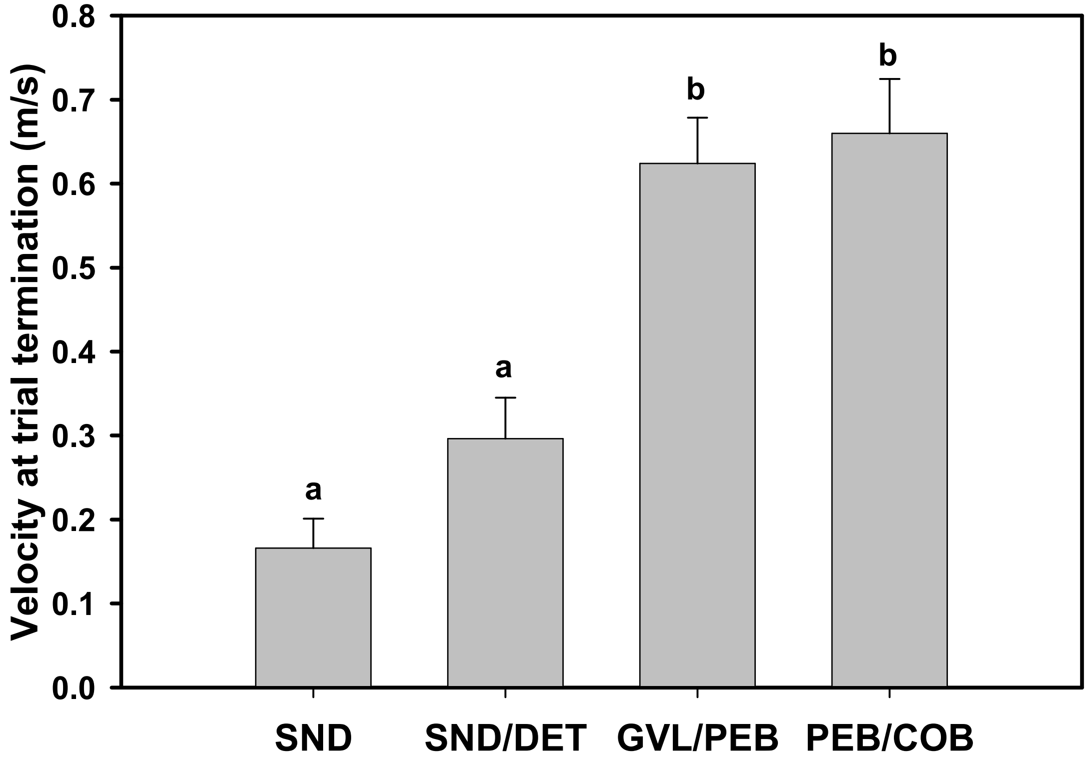


Figure 4 – 2

(b).


Velocity at trial termination (m/s)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
a
b
b
SND
SND/DET
GVL/PEB
PEB/COB

CHAPTER 5. MULTIPLE FACTORS ACCOMPANYING URBANIZATION AFFECT GROWTH OF TWO-LINED SALAMANDERS (*EURYCEA CIRRIGERA*)

ABSTRACT

Studies have begun to accumulate that document a decline in species richness and abundance of amphibian species within areas experiencing urban development. A logical next step toward understanding why urbanization negatively impacts amphibians is to track species-specific demographic responses to urban development. We monitored growth of two-lined salamander larvae over two complete cohorts (2006 and 2007). We found that salamanders in streams surrounded by urbanized and developing watersheds hatched at the same size as their reference-stream counterparts, but quickly achieved larger sizes. We evaluated the effect of four variables that correlate with the urban-forest gradient in an effort to elucidate mechanisms yielding increased growth. We found that elevated temperatures in the urban environment, coupled with decreased intraspecific competition due to lower survivorship in these same habitats, were two of the most likely explanations for increased growth. Such an increase in growth of surviving larvae may maintain population viability in urban areas where it has been shown survival is difficult because of increased in-channel flow during flood events. Because larvae that do survive in urban streams undergo metamorphosis at large sizes, they may recoup a component of fitness through growth. However, our results may not represent a general explanation for

survival of plethodontids in urban streams because other species are unlikely to withstand the elevated water flows and/or elevated water temperatures of urban environments.

INTRODUCTION

A global decline in amphibian species richness has brought considerable attention to the need for studies that will enhance our understanding of which species are imperiled and what factors put these species at risk (Simon et al. 2004, Brito 2008). Habitat loss ranks high on the list of contributors to the loss of amphibian species (Cushman 2006), and urbanization in particular has been cited as a key threat to this group of animals (Hamer and McDonnell 2008). To date, many studies have noted an overall decline in amphibian species richness or in abundances of focal species examined across areas of increasing urbanization (Willson and Dorcas 2003, Miller et al. 2007, Barrett and Guyer 2008). Documentation of such trends is vital; however, in a review on the impact of urban areas on amphibians, Hamer and McDonnell (2008) suggest the effect of urbanization should not be assessed solely by composite measures such as diversity. Likewise, Cushman (2006) suggests an urgent need for noting species-specific responses to fragmentation, which can offer key insights into conservation strategies applicable to a particular species. This recommendation of species-specific information was echoed by Hamer and McDonnell (2008) for species impacted by urbanization.

Among amphibians (particularly larvae stages), differential growth in body size of individuals in urban and forested habitats is a species-specific response that could prove important to evaluate for some species. Growth differences along the urban-forest gradient, as with other habitat gradients, may arise due to differential resource availability

(Bernardo and Agosta 2003) or habitat quality (Gillespie 2002, Johnson and Wallace 2005, Peterson et al. 2008) among habitats. For example, Johnson and Wallace (2005) found that *Eurycea wilderae* larvae had reduced growth rates, density, and biomass after litter was experimentally reduced in streams. Their manipulations provided a strong indication that habitat alterations can impact higher trophic levels in a bottom-up fashion. In addition to resource availability and habitat quality, several studies have noted the importance of temperature on amphibian larval growth (Newman 1998, Alvarez and Nicieza 2002, Gillooly et al. 2002). This factor has particular relevance in urban habitats, where a heat island effect (Oke 1995), coupled with heat pulses from runoff during rain events (Pluhowski 1970), and a decrease in riparian vegetation elevates stream temperatures in urban habitats above regional norms (Pluhowski 1970, Paul and Meyer 2001). Warmer stream temperature could lead to faster larval growth. Additionally, differential predation pressure can affect larval growth. For example, larvae grow slower in the presence of predators (Currens et al. 2007, Collier et al. 2008), a response that is thought to represent a tradeoff between foraging time and predator avoidance.

The two-lined salamander has been the focus of two previous studies (Barrett and Guyer 2008, Chapter 3), which suggested this species would be well-suited for assessing urban land use effects on growth of a stream-dwelling species. First, Barrett and Guyer (2008) indicated that salamanders and frogs were particularly sensitive to urban development. The two-lined salamander was one of the few amphibian species persisting in urban habitats. As a result, a second study (Chapter 3) was conducted demonstrating that two-lined salamanders hatch in urban streams in equal or greater numbers than conspecifics in reference (forested) streams, and that survivorship declines far more rapidly in urban environments than forested ones. In sum, even though two-lined salamanders persist more effectively in urban environments than many other amphibian species, they must overcome decreased survival to metamorphosis in urban waters to do so. If factors associated with growth lead to larger size at metamorphosis for larvae that manage to survive in urban areas, then one component of overall fitness might offset effects of another, allowing persistence of two-lined salamanders in urban habitats. Conversely, if factors accompanying urbanization decrease larval growth, then this factor and survivorship might both reduce fitness in urban habitats.

We used data from nine streams representing a gradient of urbanization to assess growth in larval salamanders. Our primary interest was to determine whether or not growth reinforced survivorship in reducing fitness in urban streams. Secondarily, we evaluated specific mechanisms that could alter growth by testing four select hypotheses derived from the literature (Table 5-1). First, we predicted a positive correlation between temperature and growth and we anticipated temperatures to be highest in urban streams. Second, we predicted that the lower survivorship in urban streams would allow for increased growth of hatchlings in a density-dependent manner. Third, we predicted that growth would vary with prey availability, which increases with urbanization. Finally, we evaluated the hypothesis that increased predator density in urban streams could decrease growth by reducing foraging. We use data from fish density surveys and data on foraging success to test this hypothesis.

METHODS

We collected *Eurycea cirrigera* larvae from nine 2nd- or 3rd-order streams in western Georgia, USA (Table 5-2). The streams and their surrounding watersheds were categorized as either reference, urban, or developing. There were three streams in each of the categories. These streams were categorized based on a 1-m resolution land cover analysis of the entire watershed for each stream. Reference sites were those sites with at least 75 % of the overall land cover as forest, the urban land cover category contained at least 25 % of the watershed as impervious surface, and finally the developing category referred to streams selected to represent watersheds with recent low-density residential development (average impervious surface = 3%). These developing sites were situated in Harris County, Georgia, which is one of the fastest growing counties in the United States (Lockaby et al. 2005). Detailed methods of the spatial analysis and quantitative justification for land use catergories are detailed in Lockaby et al. (2005) and Barrett and Guyer (2008), respectively.

Salamander and environmental sampling

Animals were collected from each stream during density surveys conducted along five, 15-m transects distributed in a stratified random manner along a 500 m stream reach (Chapter 3). Captured animals were measured (snout-vent length and total length) and weighed prior to release. These surveys were conducted at five regularly spaced intervals throughout the duration of both the 2006 and 2007 cohort. Sampling of a cohort of larvae began in spring (April), just after they hatched from eggs, and continued through summer (July), fall (October), winter (January), and the following spring (April of the next year, when larvae were of pre-metamorphic size). During the spring and summer sampling periods representatives of two cohorts often were captured. We were able to separate individuals into respective cohorts for analyses by visual inspection of SVL histograms (Bruce 1995). During each season, we attempted to capture, euthanize, and preserve at least five individuals from each stream for gut content analysis.

We collected temperature data from January 2007 through June 2008 using OnSet Hobo H8 temperature data loggers (Onset Computer Corporation Bourne, MA). One logger was placed in each of the study streams. The loggers were enclosed in a waterproof plastic case and submerged 5 – 8 cm under the water's surface in each stream. We programmed loggers to take a temperature recording once every hour.

Because resource availability and predator presence also are known to impact salamander growth, we measured both aquatic invertebrate density and the density of fishes in the family Centrarchidae, which are most likely the dominant predators of two-lined salamander larvae (Hecnar an M'Closkey 1997). We quantified benthic macroinvertebrates and fishes in 3 pools and 3 runs per stream reach during summer (September), winter (February), and spring (April-May) samples during 2003 and 2004. For macroinvertebrates, we used a Surber sampler (250-μm mesh; 0.093 m^2 sampling area), consolidating multiple Surber collections in each habitat (3 for pools, 4 for runs), resulting in a 0.27- and 0.36-m^2 sample from each pool and run, respectively (1.89 m^2 total area sampled for each study reach per stream and season). We identified macroinvertebrates to least inclusive possible taxonomic level, generally genus. We sampled fishes in each habitat to depletion with block nets, a backpack electroshocker

(Smith-Root LR-24) and seines. We identified and measured total length (TL) of all fishes captured and returned them near the point of collection, except for voucher specimens of each species, which were deposited in the Auburn University Museum Fish Collection.

Gut content surveys

To determine the relative foraging success of larvae among streams and land cover categories, we examined the gut contents of two to five individuals from each stream during the summer 2006 and spring and summer of 2007. Larvae were euthanized shortly after capture (typically within 5 hours) with MS-222 and then frozen until they could be examined. To determine gut contents of specimens, we made a sagittal incision along the ventral midline of each individual and subsequently opened the digestive tract so that contents could be removed by flushing with 70% ethanol (Bardwell et al. 2007). We sorted prey items under a dissecting scope, counted individuals, and identified them to the least inclusive taxonomic level possible (typically order).

Data Analysis

Our primary interest was in determining whether or not larvae differed in size among land cover categories. To evaluate this, we compared mean SVL of larvae from the three land cover categories within seasons for each of two cohorts (2006 and 2007). We included land cover categories in comparisons only when a given category contained at least five individuals. Comparisons were made using two-sample *t*-tests or one-way ANOVA as appropriate.

To evaluate the effect of the factors hypothesized to influence growth we used linear regression. For several analyses described below we use mean change in SVL between hatch and summer as a response variable to approximate growth. This time period represents the period with the greatest mean difference in SVL for any two consecutive seasons (Figure 5-1). We assume this change in SVL represents growth, and refer to it as hatchling growth; however, we consider other alternative explanations for this change in size in the Discussion. We examined the impact of temperature on growth using the 2007 cohort. We plotted hatchling growth against the median temperature for that same time period. We also hypothesized an effect of competition on growth. Specifically, we tested for an effect of inter-cohort competition by examining the regression of hatchling growth against the density of pre-metamorphic individuals from the 2006 cohort (as measured during the spring of 2007; see Chapter 3 for methods regarding density sampling).

To evaluate the impact of either top-down or bottom-up factors on growth, we coupled hatchling growth data from 2006 and 2007 with survey data on aquatic invertebrate and centrarchid density from the same streams. Resource availability (bottom-up effect) was evaluated by regressing hatchling growth against log aquatic invertebrate density for each stream. Similarly, we plotted hatchling growth against predator density to assess top-down regulation of larval salamander growth. We also evaluated the relationship, for each stream, between the number of prey items in salamander guts and the density of centrarchids to further evaluate the hypothesis that predators influence foraging behavior.

RESULTS

The 2006 cohort of larvae provided a more complete data set for evaluating size differences between land cover categories, as the number of captures was far lower in all land cover categories during the fall, winter and pre-metamorphic spring samples of the 2007 cohort. In 2006 and 2007, there was no significant size difference among land cover categories for the newly emerged (spring) hatchlings (Fig. 5-1). By the following summer (4 months after the initial measurements), urban larvae for both cohorts and larvae for developing sites for the 2006 cohort had achieved larger sizes than larvae at reference sites (ANOVA, $p < 0.0005$ for both comparisons; Fig. 5-1). Larvae from urban and developing sites continued to be larger than those from reference streams during fall samples of both cohorts (ANOVA, $p \leq 0.004$ for both cohorts), but this difference vanished during winter samples of 2006 and 2007. By the following spring, pre-metamorphic larvae of the 2006 cohort were larger for urban sites than reference sites (*t*-test, $p = 0.003$; Fig. 5-1). Larval capture success was too low in 2007 to compare sizes among land cover categories during the pre-metamorphic stage.

Linear regressions indicated none of the evaluated factors were correlated with larval growth (Table 5-1). Of our *a priori* hypotheses, the regression of temperature on growth (heat-island effect) had the strongest support (Table 5-1); however, the failure of one temperature logger resulted in low statistical power. The test for an effect of centrarchid density on growth had the strongest statistical support, but was in the opposite direction of our *a priori* hypothesis.

DISCUSSION

We observed that hatchling larvae of two-lined salamanders from urban, developing, and reference streams did not differ in size. However, larvae in developing and urban habitats quickly attained larger SVL than their reference counterparts. Except for a possible amelioration of this difference during winter months, this size advantage appeared to remain in larvae at metamorphosis. These observations provided a foundation for evaluating existing hypotheses regarding factors that influence the growth of amphibians (and more generally, all ectotherms) in the context of an urban-forested gradient.

Increased growth of two-lined salamander larvae from developing and urban watersheds may serve to partially offset lower survivorship known to occur in these same habitats during the larval phase (Chapter 3). An increase in spate frequency and magnitude that accompanies urbanization likely washes many developing larvae from the stream (Chapter 3, 4). Those that do survive appear to grow faster. This could allow larvae to undergo metamorphosis earlier and/or at larger sizes, which would presumably provide a fitness benefit during the terrestrial phase of the life cycle (Semlitsch et al. 1988, Beck and Congdon 1999).

We investigated relationships between salamander growth and four major abiotic and biotic factors (Table 5-1) in an attempt to determine mechanisms driving observed patterns. While none of the factors we evaluated had a statistically significant relationship with change in SVL, we believe our results provide data that can help guide similar studies in the future. First, despite the fact that perceived predation risk has been found to reduce foraging and/or activity rates of larval amphibians in experimental trials (Laurila et al. 2006, Currens et al. 2007), we found no evidence to support the hypothesis that Centrarchidae density would negatively influence growth of two-lined salamander larvae. In fact, the slope between hatchling growth and predatory fish density was positive, and the relationship between the amount of food in larval stomachs versus fish density was only weakly negative (and not statistically significant). We do not believe further tests of this hypothesis in an urban-rural context would be fruitful; however, below we outline an alternative explanation for the observed size distribution that is driven by predation. Second, from previous work (Helms 2008), we knew prey availability increased with urbanization and hypothesized this would increase larval growth in these streams relative to reference environments. The direction of the relationship was consistent with our prediction, but a greater number of streams will need to be evaluated before this trend can be verified and be confidently attributed to the forested-urban gradient. Third, we found only very weak support for the prediction that inter-cohort competition would decrease growth of hatchling larvae. This prediction was based on the fact that hatchlings emerge in the presence of, and potentially compete with, larvae from the previous year's cohort that have yet to undergo metamorphosis. Because survivorship of larvae is low in urban streams (Chapter 3), hatchlings in these streams are released from inter-cohort competitive pressures. We were only able to assess this hypothesis with data from a single year, and the overall trend was consistent with our hypothesis; however, the relationship was not statistically significant. We would suggest that this hypothesis cannot confidently be rejected. Finally, we hypothesized that the heat-island effect, elevated water temperatures from runoff, and decreased riparian vegetation in urban habitats would result in higher larval growth relative to cooler, reference environments (Pluhowski 1970, Oke 1995, Paul and Meyer 2001). This relationship was the most consistent with our a priori predictions, and the hottest environments were in fact the urban stream systems (Figure 5-2).

If the heat-island effect is driving growth differences among larvae in reference and urban streams, this biological signal, which could be considered beneficial for two-lined salamanders (i.e., they achieve larger size at metamorphosis or emerge into the terrestrial environment quicker than conspecifics in other habitats), may be detrimental to other species. Previous studies (Price et al. 2006, Barrett and Guyer 2008) noted a decline in amphibian species richness and/or species abundances with urbanization. While there are many factors that contribute to such declines, the low thermal tolerances of some stream amphibians may explain the loss of some species from streams subject to urbanization (Bury 2008). Plethodontids likely evolved in cool, mountain stream-type habitats (Wilder and Dunn 1920, Beachy and Bruce 1992), and as such, these species may not be able to cope with the hotter temperatures, and more open canopies that accompany urbanized stream channels (Barrett and Guyer 2008).

It is possible that the differences in size observed among land use categories is not related to differences in growth, but instead relates directly to differential survivorship. We hypothesize two mechanisms by which differential survivorship could occur. First, in Chapter 3 it was noted that an increase in magnitude and frequency of flood events in urban streams lowers survivorship of salamander larvae in urban habitats relative to their reference stream counterparts. If flood events selectively wash the smallest larvae downstream, than the overall effect on seasonal SVL measures could produce the distribution of data we observed. Second, fish are gape-limited predators, and as such, may generate the size patterns we observed. Fish are known to actively select prey based in part on a trade-off between handling time and energetic reward (Mittelbach 1981). During the spring newly hatched fish are denser in the urban streams (Helms 2008). As a result, it is possible that the dense assemblage of gape-limited predators could be actively selecting the smallest hatchlings in urban streams.

As with any biological response as complicated as growth, there are undoubtedly myriad factors influencing it, and identifying which factors are most important in the context of urbanization will take a very large-scale effort. Our study offers a foundation upon which larger-scale studies can build and test hypotheses that will be the ones most likely to offer insights regarding demography shifts in amphibians (i.e., growth and metamorphosis). Understanding which environmental changes in an urbanized environment translate to demographic shifts in stream-breeding amphibians would allow for a more focused, species-specific approach to amphibian conservation (Hamer and McDonnell 2008).

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Table 5 - 1. Hypotheses relating to factors effecting increased salamander growth in urbanized watersheds, and results from linear regressions used to evaluate each hypothesis.

Hypothesis	**Predictor variable** **Response variable**	**N**	**Slope**	r^2	p
Elevated temperatures in urban environments leads to increased growth in these habitats	Median temperature (Apr - Jul 2007) Hatchling growth (2007 cohort)	5	0.87	0.53	0.17
Low survivorship of larvae in urban streams results in less inter-cohort competition for hatchlings emerging during the spring	06 cohort density of pre-metamorphs Hatchling growth (2007 cohort)	7	-0.43	0.31	0.19
Increased prey availability increases growth of hatchlings	Aquatic invertebrate density Hatchling growth (2006 cohort)	7	5.14	0.25	0.31
Increased prey availability increases growth of hatchlings	Aquatic invertebrate density Hatchling growth (2007 cohort)	6	1.98	0.22	0.29
High predator density reduces larval foraging rate, which results in slower growth	Centrarchidae density Hatchling growth (2006)	7	8.27	0.57	0.08
High predator density reduces larval foraging rate, which results in slower growth	Centrarchidae density Hatchling growth (2007)	6	2.38	0.35	0.16
High predator density reduces larval foraging rate, which results in less food consumed	Centrarchidae density Number of prey items in stomach	9	-1.43	0.04	0.60

Table 5 - 2. Land cover and physical characteristics of study watersheds. IS = % impervious surface cover, Forest = % total forest cover, and LU/LC = dominant land cover in watershed (defined in Methods).

Site	**Watershed size (km^2)**	**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	2	13	81	Forest
MU3	10.4	2	15	78	Forest
BU1	25.5	40	23	34	Urban
BU2	24.7	25	25	46	Urban
RB	3.7	30	27	39	Urban

Figure 5 - 1. Mean (± SE) larval two-lined salamander size comparisons within land cover categories by season for (a) 2006 and (b) 2007 cohorts. The first set of bars labeled Hatch on the x-axis represent the newly hatched individuals sampled in April of the given cohort year. Letters above the bars represent significant differences from two-sample t-tests or a one-way ANOVA followed by Tukey's pair-wise comparison, as appropriate. Sample sizes are listed inside the bars. We did not plot the means for or statistically compare larvae from land cover categories with fewer than five captures within a given season.

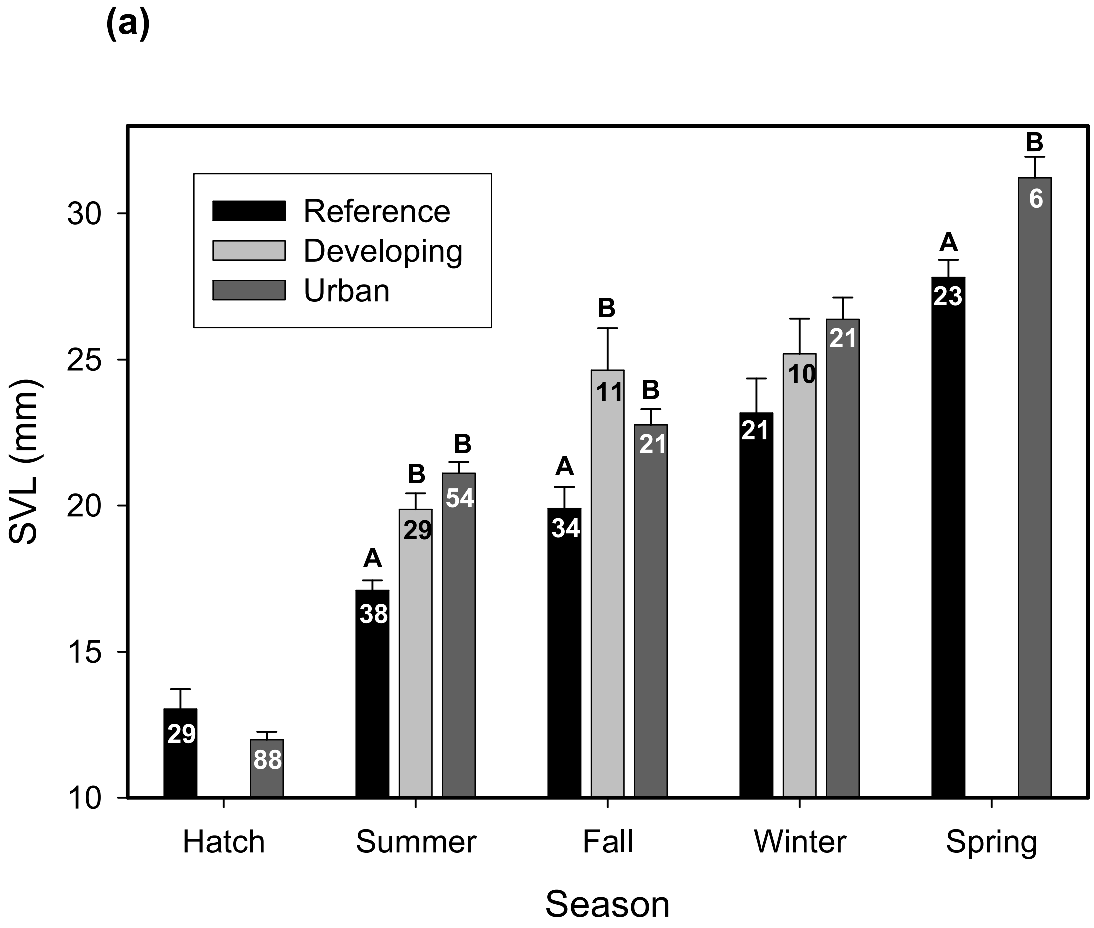
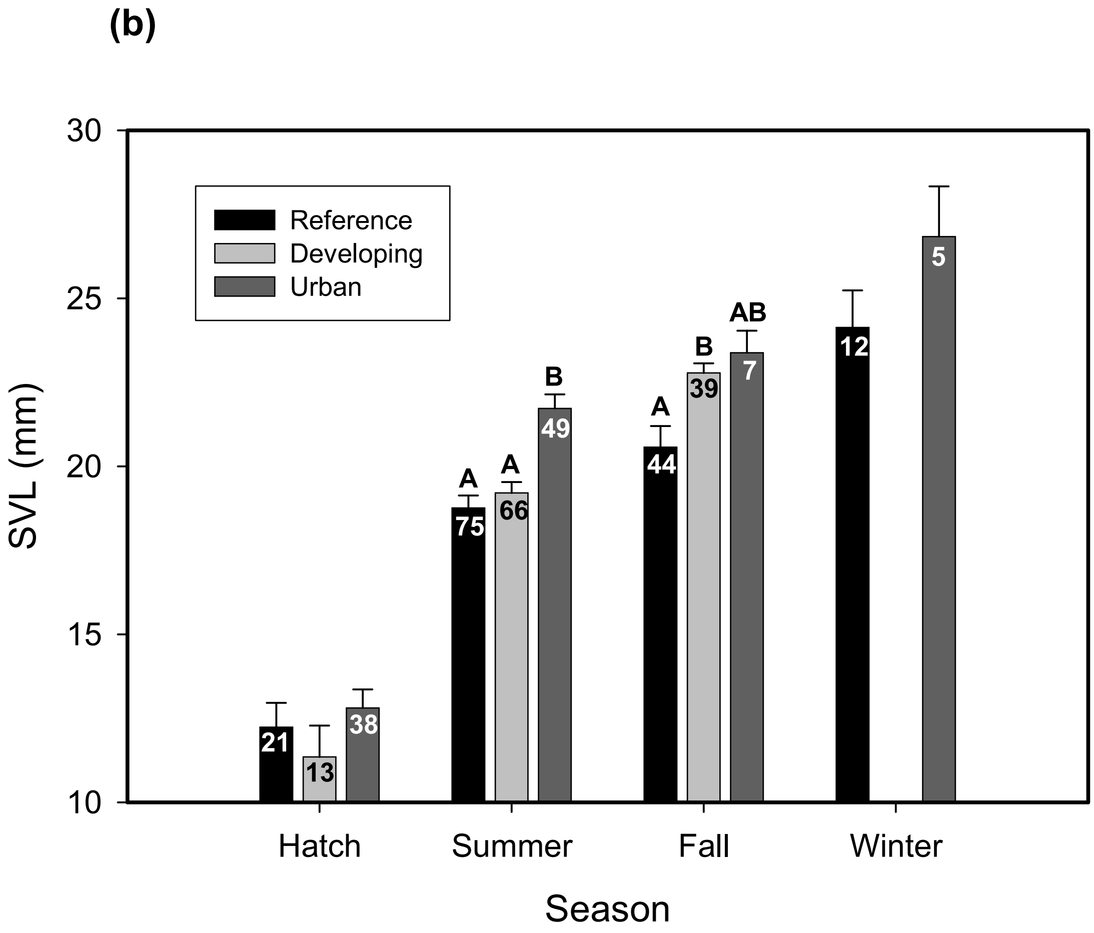


Figure 5-1


(b)
Reference
Developing
Urban
SVL (mm)
30
25
20
15
10
Hatch
Summer
Fall
Winter
Season
21
13
38
A
A
B
75
66
49
A
B
AB
44
39
7
12
5

CHAPTER 6. TWO-LINED SALAMANDER DIETS IN URBAN AND FORESTED STREAMS IN WESTERN GEORGIA, USA

ABSTRACT

Stream systems are heavily impacted by urbanization as increased runoff changes physical and chemical composition of stream habitats. Biotic response to these changes is especially consistent in benthic macroinvertebrates, where species richness often declines with urbanization. Conversely, biomass of macroinvertebrates can increase with urban development. We examined the effect of these shifts in the macroinvertebrate assemblage on the diet of larval two-lined salamanders. We examined prey consumed by salamanders during spring, summer, and winter seasons across an urban – forest gradient. We also evaluated evidence for prey selection or avoidance in these habitats. We found dipteran larvae to be the most common prey item, followed by ostracods. Snails were a common prey item during summer. Prey diversity in salamander digestive tracts peaked during summer, while in-steam diversity was highest during spring. Overall, we observed minor differences in diet along the urban – forest gradient; however, these differences are not a likely explanation for differential growth differences observed during a separate study. The value of our observations lies in the foundation they provide for future studies examining shifts in trophic dynamics that may occur with urbanization.

INTRODUCTION

Urbanization impacts biomass, diversity, and species richness of formerly undeveloped habitats (Klein 1979, Czech and Krausman 1997, Paul and Meyer 2001, Stratford and Robinson 2005). Streams are especially impacted by urbanization, as increased impervious surfaces (e.g., roads, roofs) create increased runoff, which leads to extreme physical alteration of in-stream habitats (Walsh et al. 2005, Galster et al. 2008). Ecologists have repeatedly shown a decline in species richness of stream macroinvertebrates following urbanization of a watershed (Klein 1979, Paul and Meyer 2001 Walsh et al. 2005). Recently, Helms (2008) documented a similar decline in species richness of macroinvertebrates; however he recorded an overall increase in biomass (secondary production) of stream invertebrates with urbanization.

Like macroinvertebrates, species richness of stream-breeding salamanders declines with urbanization (Barrett and Guyer 2008, Hamer and McDonnell 2008). The altered hydrology that accompanies urban development has been linked to a decline in density of two-lined salamander larvae, and may contribute to the loss of species as well (Chapter 3). A shift in trophic dynamics of urban communities is also likely to be important in explaining species richness and abundance of top consumers in urban habitats (Faeth et al. 2005). For example, Johnson and Wallace (2005) demonstrated a shift in diet of a larval salamander, *Eurycea wilderae*, with experimental detritus removal. These shifts translated to a decrease in biomass and density of the salamander.

Many predators actively select particular prey based on nutritional content (Schaefer et al. 2008). If, because of decreased invertebrate species richness, preferred salamander prey disappear with urbanization, then bottom-up effects may be evident in salamander species. Conversely, stream-dwelling salamander larvae have been recorded to consume a wide variety of prey items (Caldwell and Houtcooper 1973, Burton 1976, Petranka 1984). If salamanders do not discriminate among available prey, then an increase in secondary production with urbanization, which was observed by Helms (2008), could be a boon for salamander larvae able to persist in urban streams.

To determine effects of urbanization on larval salamander diet, we describe dietary compositional shifts for two-lined salamander larvae (*Eurycea cirrigera*) both seasonally and across land cover categories for streams in forested, developing, and urban watersheds. Because we examine larvae from the same streams surveyed for macroinvertebrates by Helms (2008), we are able to assess larval prey selection across season and land cover categories. Results from this analysis will contribute to our ability to examine shifts in community interactions with urbanization. This area of urban ecology has received little attention in stream systems; however, analyses from other community types suggest it is a field worth of study (Faeth 2005).

METHODS

We examined diet of larval two-lined salamanders in nine streams in western Georgia, USA. To evaluate larval diets in urban habitats we selected three streams within Columbus, GA. For comparison, we also selected three streams within forested watersheds (Lockaby et al. 2005) approximately 30 km north of Columbus (Meriwether Co.), which we refer to as reference streams because they retain forested borders that characterize the ancestral landscape. Finally, to determine if watersheds subjected to small amounts of very recent development contained larvae with altered diets, we examined larvae from three streams within Harris County, a rapidly developing suburban area adjacent to Columbus (i.e., developing streams).

Data on macroinvertebrate availability were obtained from Helms (2008). Macroinvertebrates were sampled from the three streams above during summer (August and September 2003), winter (January and February 2004), and spring (April and May 2004), with the exception of the urban stream BR, which was only sampled during spring. We used a Surber sampler (250-µm mesh; 0.093 m^2 sampling area), consolidating multiple Surber collections in each habitat (3 for pools, 4 for runs), resulting in a 0.27- and 0.36-m^2 sample from each pool and run, respectively (1.89 m^2 total area sampled for each study reach per stream and season). We identified macroinvertebrates to least inclusive taxonomic level (generally genus) but use order- (or higher) level data in our analyses.

Salamanders were captured for gut content analysis during four seasons (rather than three as above). The captures from summer (July 2006 and 2007) and fall (November 2006 and October 2007) lie on either side of the summer macroinvertebrate sampling, so we combined these samples for this analysis and refer to them as our summer sample. We also captured larvae during winter (January 2007) and spring (April 2007). Upon capture, individuals were sacrificed (0.04% unbuffered MS 222 solution), and preserved by freezing until they could be examined for gut contents in the lab.

To identify prey items consumed by salamanders, we made a sagittal incision along the ventral midline of each individual and subsequently opened the digestive tract so that contents could be removed by flushing with 70% ethanol (Bardwell et al. 2007). We sorted prey items under a dissecting scope, counted individuals, and identified them to the least inclusive taxonomic level possible (typically order).

We examined prey composition and taxa richness among land cover categories and seasons using a Goodness-of-Fit test. To evaluate prey composition we combined data from streams and used prey taxa categories that had at least five occurrences in each land cover category and season (Appendix 6 – A). This resulted in a comparison of the following prey groups: Coleoptera, Diptera, Gastropoda, Ostracoda, Other, and Unidentified. We also calculated Shannon diversity index for land cover categories in each season. The Shannon diversity index is often used as a measure of niche breadth (Levins 1968, Pianka 1986), and was calculated as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i ,$$

where S is the total number of species present in the sample and p_i is the proportion of individuals in the total sample belonging to species *i*. Finally, we used Strauss' index of electivity (Strauss 1979) and the Vanderploeg and Scavia (1979) relativized index of electivity to evaluate prey selection by larval salamanders. We used two different indices because each has strengths and weaknesses, and no one electivity index has emerged as the best index (Lechowicz 1982). The Strauss index (L) calculates the selection or avoidance of particular prey items by a predator using the equation

$$L = r_i - p_i,$$

where r_i and p_i are the relative abundances (proportions) of prey item *i* in the digestive tract and habitat, respectively. The index is linear (any change in food availability or utilization will have the same effect on the index, regardless of levels of availability or utilization), which is a desirable property of any electivity index (Lechowicz 1982). The Vanderploeg and Scavia relativized index (E_i*) calculates electivity as

$$E_i^* = [W_i - (1/n)]/[W_i + (1/n)],$$

where $W_i = (r_i/p_i)/\Sigma r_i/p_i$ (r_i and p_i defined as above) and n = the number of prey items in the gut. Ei* was the index recommended by Lechowicz (1982) among a comparison of several available indices; however, it does have some properties, such as non-linearity, that make it undesirable. E_i* could only be calculated for items that were found to be both available and consumed by larvae; therefore, its values often differ dramatically from Strauss' index. Both indices are bounded between -1 and 1, and negative values of an index represent prey items that are consumed proportionately less than their abundance in the habitat, whereas positive values represent the opposite. Zero values indicate prey items consumed in the same proportion as their abundance (i.e., no selection for the prey item). The electivity data must be viewed cautiously, as we assumed prey availability within a season does not differ dramatically among years. The data on macroinvertebrates used to calculate electivities were collected two to three years prior to the salamander collection dates. Any perceived prey selection or avoidance may instead result from strong inter-annual variation in prey availability.

RESULTS

We captured a total of 147 larvae across all seasons and land cover categories (Appendix 6-A). A total of thirteen individuals were found with either no food in their guts, or nothing that was identifiable as a prey item. Seasonal diets of two-lined salamanders differed significantly among land cover categories (GOF test, df = 10, $p < 0.0001$). Among all prey taxa, dipteran larvae consistently made up the largest proportion of larval diets (Fig. 6-1). The exception to this observation was at developing sites during spring, where ostracods comprised greater than 60 % of observed prey items (Fig. 6-1A). Within all land cover categories during summer, we observed a notable increase in the proportion of Gastropoda (snails, primarily in the families Physidae and Planorbidae) within larval diets (Figure 6-1C).

Niche breadth as measured by the Shannon index was highest in urban habitats during spring and summer seasons and lowest in reference streams; however, during winter niche breadth was lowest in urban streams and highest in reference streams (Fig. 6 – 2A). Taxonomic richness (primarily assessed at the order level) showed high variability among land cover categories, but was significantly higher in all categories during summer (GOF test, df = 2, p = 0.007; Fig. 6 – 2B).

Strauss' electivity index revealed little selection for most prey items in reference streams (i.e., all values were near zero; Table 6 – 1), with the exception of dipteran larvae, which salamanders selected during all seasons. Positive selection for dipteran larvae (primarily Chironomidae) was observed in all land cover categories except for developing sites in spring, during which we observed avoidance of this prey item among two-lined salamander larvae. During spring, salamander larvae in developing sites exhibited strong selection of ostrocods (Table 6 – 1). In winter, salamanders in developing sites exhibited negative selection of cladocerans (Table 6 – 1). Salamanders in urban streams exhibited very strong positive selection of dipterans in winter, and moderate selection of coleopteran larvae in all season (Table 6 – 1). The index indicated negative selection for trichopteran larvae in all seasons, while snails were positively selected by larvae in all land cover categories during the summer (Table 6 – 1).

Vanderploeg and Scavia's relativized index values differed greatly from those calculated from Strauss' index (Table 6 – 1). For example, the index indicated an avoidance of diptera in most cases, whereas Strauss' index suggested the opposite trend. Index values indicated salamander larvae in reference streams avoided most prey items, with the exception of one taxa in each season that was found to be disproportionately selected (Table 6-1). These selected prey items were rare according to stream samples, but their occurrence (even in low frequency) as larval prey greatly impact the index. As with Strauss' index, larvae in developing streams were found to select for ostracods. Larvae in urban streams selected for diptera in spring and winter, but strongly avoided this taxon during summer, during which time they showed a strong preference for snails.

DISCUSSION

The main differences in prey composition among land cover categories were not from the presence or absence of a given prey item, but rather the proportions in which they were consumed. For example, ostracods appeared to be especially important in developing streams, particularly during spring. Gastropods and coleopteran larvae were consumed more in urban streams than in any other category. Finally, number of rarely occurring taxa (items only observed a few times) was greater in reference streams than in the other two categories.

While we observed some shifts in the diet across land cover categories and seasons, other aspects showed consistency. Diptera (primarily in the family Chironomidae) was the main prey taxon of larvae in nearly all streams and seasons. This finding is consistent with several other foraging studies on larval *Eurycea* (Caldwell and Houtcooper 1973, Burton 1976, Petranka 1984, Johnson and Wallace 2005, Muenz et al. 2008). Ostracoda was the next most abundant prey taxon (and the most abundant during spring at developing sites). In previous studies this taxon was either not observed in the guts of other larval *Eurycea* (Burton 1976, Johnson and Wallace 2005), or was observed with few occurrences (Caldwell and Houtcooper 1973, Petranka 1984, Muenz et al. 2008). Plecoptera larvae, which were found to be important in the diet of two-lined salamanders studied by Caldwell and Houtcooper (1973) were not predominant prey in the organisms we examined or in those examined in pasture and forested habitats by Munenz et al. (2008). Finally, we found that larvae in all land cover categories avoided trichopterans during all seasons. Taxa richness of prey consumed and dietary niche breadth of salamander larvae were both found to increase in summer across all land cover categories. This result does not correspond to the period of greatest macroinvertebrate diversity, which was found to be during spring (Helms 2008). The greater diversity of prey items consumed by salamanders during summer may represent an attempt to consume as much biomass as possible during warmer months when growth rates are high (Chapter 5).

Snails were one prey group occurring only during summer that contributed to the high species richness, and they were found in all land cover categories. The only other study with a seasonal component during which gastropods were observed as prey for two-lined salamanders also recorded the presence of snails in the diet during summer (Caldwell and Houtcooper 1973). Many of the larvae we captured during the summer were pre-metamorphic. In tadpoles, calcium deposits increase dramatically during the pre-metamorphic stage (McDonald et al. 1984). Presumably the increase occurs due to calcification of the skeleton as larvae prepare for increased skeletal demands associated with terrestrial life. It is possible that larval two-lined salamanders consume snails, which have extensive calcium deposits in the shell, for similar reasons.

In general, the strong selection we observed for multiple taxa (and avoidance of others) suggests that *Eurycea* larvae are not complete generalist predator, as has been suggested (Petranka 1984). Strauss' electivity analysis showed selection for Diptera (except developing sites during spring), and avoidance of Trichoptera in all seasons. Results from Vanderploeg and Scavia's relativized index corroborated the avoidance of trichopterans; however, the index indicated an avoidance of dipterans in most seasons. Coleoptea appeared to be marginally selected for in urban streams in all seasons. Selection of dipteran larvae is consistent with observations of Muenz et al. (2008). Because Strauss' electivity index was generally near zero in reference streams, but observed to fluctuate more in other habitats, it is possible that overall shifts in food web structure result with urban development. Nevertheless, measures of salamander growth in the same streams we surveyed for diet analysis revealed that salamanders in urban streams grow faster than those in reference streams. Thus, if food web structure does change because of urban development, the shift does not appear to impact salamander demography via growth rate. The major differences in index value we observed between the two electivity indices resulted from the extreme sensitivity of Vanderploeg and Scavia's relativized index to items that are rare in the stream but consumed, even in small amounts, by salamanders. As Lechowicz (1982) indicates, no one index satisfies all desirable critera for an electivity analysis, which is why we offered a composite description of two frequently used indices.

Our description of salamander diets provides the information necessary to begin constructing stream food webs in urban and reference habitats. As studies indicating a change in species richness or abundance of taxa with urbanization have accumulated, ecologists now must take the next step and examine multi-trophic interactions in reference and urban habitats (Faeth 2005, Helms 2008). Such an approach will increase our ability to understand how management strategies for one trophic level will cascade (up or down) to other trophic levels.

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Table 6 - 1. Strauss (1979) and Vanderploeg and Scavia (1979) electivities for prey items of by two-lined salamanders in reference, developing, and urban streams in western Georgia, USA. See text for description of indices.

			Spring			
	Reference		**Developing**		**Urban**	
Taxa	**L**	**Ei***	**L**	**Ei***	**L**	**Ei***
Cladocera	-0.07	-0.93	-0.04	--	--	-0.02
Coleoptera	0.01	-0.16	0.00	-0.48	--	0.10
Copepoda	0.05	--	0.03	--	--	0.05
Diptera	0.19	-0.40	-0.36	-0.79	0.29	0.09
Lepidoptera	0.01	--	0.00	--	--	0.00
Odonata	0.01	0.52	0.00	--	--	0.00
Ostracoda	0.03	-0.23	0.56	0.43	0.08	-0.01
			Summer			
Acari	-0.01	-0.90	0.01	-0.69	-0.08	--
Amphipoda	0.00	--	0.00	--	0.02	--
Cladocera	0.00	--	0.00	-0.83	0.00	--
Coleoptera	0.02	-0.33	-0.02	-0.94	-0.04	0.40
Collembola	0.00	--	0.00	--	0.13	--
Copepoda	0.01	--	0.01	--	0.00	--
Diptera	0.16	-0.63	0.05	-0.78	0.00	-0.96
Ephemeroptera	-0.15	--	-0.07	-0.98	0.06	-0.90
Gastropoda	0.02	--	0.04	--	0.00	0.64
Hemiptera	0.00	--	0.01	0.67	0.00	--
Hymenoptera	0.00	--	0.01	--	-0.01	--
Lepidoptera	0.00	--	0.03	--	0.00	--
Megaloptera	0.01	0.65	0.00	--	0.00	--
Nematoda	0.01	--	0.00	--	0.00	--
Odonata	-0.01	--	0.00	-0.66	0.00	-0.89
Ostracoda	0.02	-0.27	0.12	0.30	0.00	-0.96
Plecoptera	0.00	--	0.01	0.43	0.00	--
Trichoptera	-0.13	-0.99	-0.15	-0.93	0.01	-1.00
			Winter			
Amphipoda	0.01	--	0.00	--	0.00	--
Cladocera	0.11	-0.54	-0.32	--	0.00	--
Coleoptera	0.02	-0.74	0.03	0.43	0.12	--
Collembola	0.00	--	0.01	--	0.00	--
Copepoda	0.06	0.61	0.06	--	0.06	--
Diptera	0.11	-0.92	0.28	-0.18	0.42	0.77
Ephemeroptera	-0.03	--	0.00	-0.23	0.00	--
Hymenoptera	0.00	--	0.01	--	0.00	--
Lepidoptera	0.01	--	0.00	--	0.00	--
Ostracoda	0.03	-0.50	-0.08	-0.68	-0.09	-0.15

Figure 6 - 1. Top four (spring) or five prey items, by proportion, found in the digestive tract of two-lined salamander larvae inhabiting reference, developing, and urban streams in western, Georgia, USA.

(a)

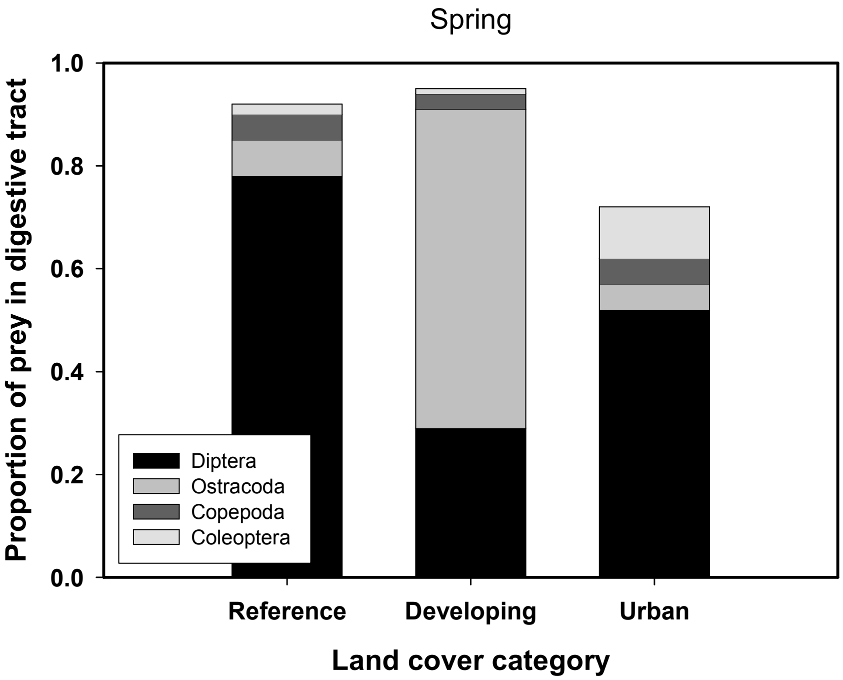
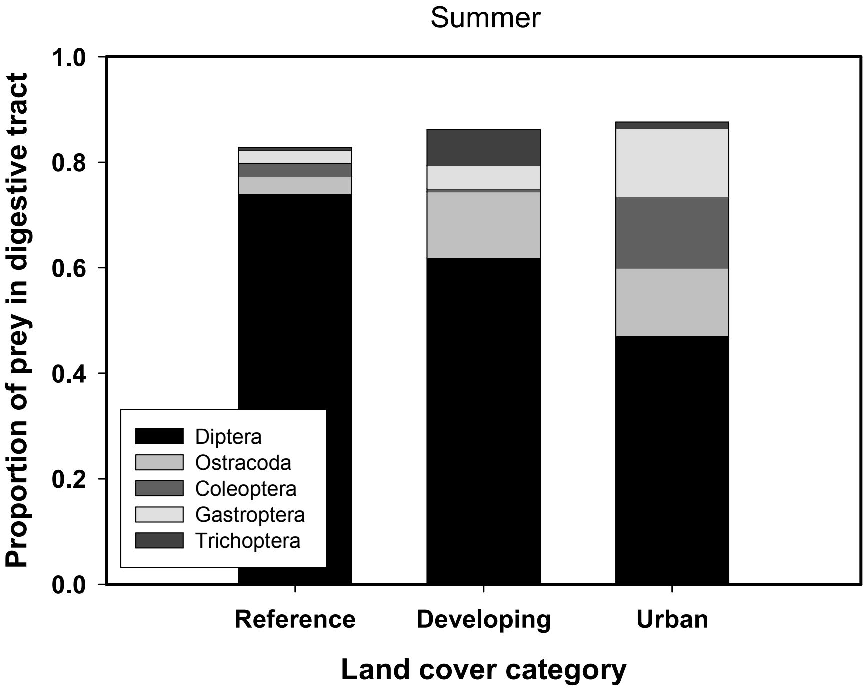


Figure 6 – 1

(b)


Summer
Proportion of prey in digestive tract
1.0
0.8
0.6
0.4
0.2
0.0
Diptera
Ostracoda
Coleoptera
Gastroptera
Trichoptera
Reference
Developing
Urban
Land cover category

(c)

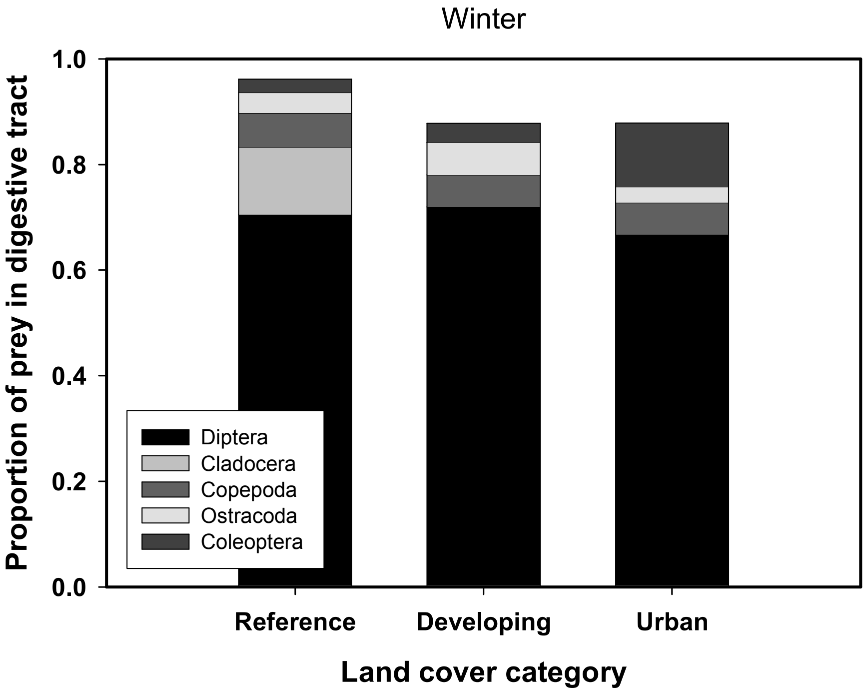
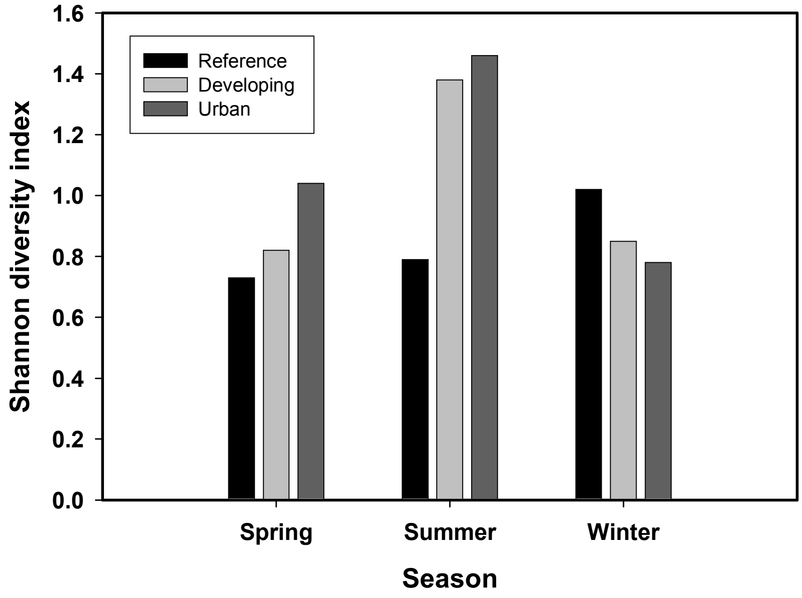
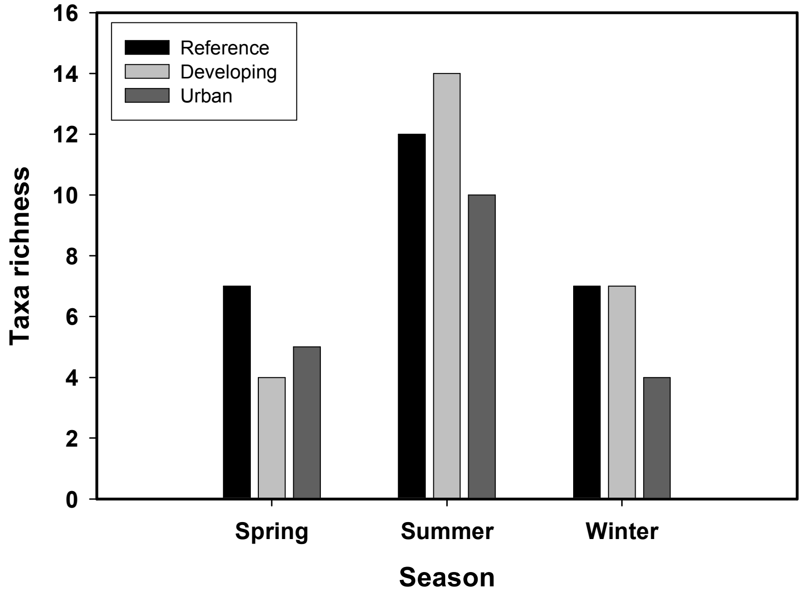

Winter
Proportion of prey in digestive tract
1.0
0.8
0.6
0.4
0.2
0.0
Diptera
Cladocera
Copepoda
Ostracoda
Coleoptera
Reference
Developing
Urban
Land cover category

Figure 6 - 2. Shannon diversity (A) and taxa richness (B) for prey items found in the digestive tracts of two-lined salamanders in reference, developing, and urban streams during three seasons in western Georgia, USA.

(a)



(b)



Appendix 6 – A. Number of prey items (expressed as a sum per taxa) found in the diet of two-lined salamander larvae in nine streams in western Gerogia, USA. N = the number of digestive tracts examined for each stream.

	Reference			**Developing**			**Urban**		
Taxon	**BLN (18)**	**MO (10)**	**MU3 (19)**	**SB1 (14)**	**SB2 (17)**	**SB4 (17)**	**BU2 (14)**	**BR (21)**	**RB (15)**
Acari	1	--	--	2	--	1	--	3	--
Amphipoda	2	--	--	--	--	--	--	--	--
Cladocera	10	--	1	--	--	1	--	--	--
Coleoptera	2	5	2	--	2	3	1	--	22
Collembola	1	--	--	--	--	1	--	--	--
Copepoda	6	3	5	--	--	9	--	3	--
Diptera	76	44	164	31	100	47	22	27	64
Ephemeroptera	--	--	--	--	--	2	--	1	--
Gastropoda	--	5	--	--	6	1	7	10	5
Hemiptera	--	--	--	--	--	1	--	--	--
Hymenoptera	1	--	--	1	1	1	--	1	1
Lepidoptera	1	--	1	3	1	--	--	--	--
Megaloptera	2	--	1	--	--	--	--	--	--
Nematoda	1	--	1	--	--	--	--	1	--
Odonata	--	1	--	1	--	--	1	--	--
Ostracoda	7	1	1	10	1	56	9	2	13
Plecoptera	0	--	--	1	--	1	--	1	--
Trichoptera	0	1	--	1	10	--	--	--	2
Unidentified	2	4	7	4	7	--	1	9	3
Empty gut	1	0	3	0	3	0	2	3	0