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Southern Two-Lined Salamander Diets in Urban and Forested Streams in Western Georgia

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Abstract - Streams are heavily affected by watershed urbanization as increased stormwater runoff changes their physical and chemical composition. Benthic macroinvertebrate species richness has been consistently shown to decline with urbanization. Conversely, biomass of macroinvertebrates can increase with urban development. We examined the effect of such shifts in macroinvertebrate assemblages on the diet of larval *Eurycea cirrigera* (Southern Two-lined Salamanders). Salamanders have been documented to decrease in diversity in urban habitats; however, Southern Two-lined Salamander larvae which persist in urban streams (at lower densities) tend to grow larger than larvae in forested streams. Diet may play a role in these diversity and growth trends. We examined prey consumed by larval salamanders during spring, summer, and winter seasons across urban and forested watersheds. Prey diversity in salamander digestive tracts peaked during summer. We found Chironomidae (Diptera) larvae to be the most common prey item, followed by Ostracoda. Gastropoda were a common prey item during summer, which may be indicative of nutrient requirements of premetamorphic larvae. Overall, we observed minor differences in larval diet between urban and forested watersheds. A previous study within these same watersheds found that larvae in urban watersheds grew larger than those in forested watersheds, and the authors suggested prey availability may have contributed to that finding. The diet data we present here do not support such a hypothesis.

Introduction

Urbanization alters biomass, diversity, and species richness of biota occupying formerly undeveloped habitats (Czech and Krausman 1997, Klein 1979, Paul and Meyer 2001, Stratford and Robinson 2005). Streams are especially influenced by watershed urbanization, as increased impervious surfaces (e.g., roads, roofs) cause increased overland flow, which can lead to extreme physical alteration of instream habitats (Galster et al. 2008, Walsh et al. 2005). Ecologists have repeatedly shown a decline in species richness of stream macroinvertebrates following watershed urbanization (Klein 1979, Paul and Meyer 2001, Walsh et al. 2005). Recently, Helms et al. (2009) documented a similar decline in species richness of macroinvertebrates; however, they recorded an overall increase in biomass 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 *Eurycea cirrigera* Green (Southern Two-lined Salamander) larvae, and also may contribute to a loss of other amphibian species (Barrett and

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Guyer 2008). 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 decreased biomass and density of larval *Eurycea wilderae* Dunn (Blue Ridge Two-lined Salamander) as a result of diet shifts caused by experimental detritus removal.

Many benthic predators actively select particular prey based on nutritional content (Schaefer et al. 2008). If, because of decreased invertebrate species richness, preferred salamander prey disappears with urbanization, then the loss of an important resource base may translate to negative effects on salamander larvae. Conversely, stream-dwelling salamander larvae have been recorded to consume a wide variety of prey items (Burton 1976, Caldwell and Houtcooper 1973, Petranka 1984). If salamanders do not discriminate among available prey, then an increase in invertebrate biomass associated with urbanization, which was observed by Helms et al. (2009), could result in ample resources for the salamander larvae that are able to persist in urban streams. Barrett et al. (2010) documented higher growth rates in Two-lined Salamander larvae from urban watersheds relative to forested ones, which is consistent with the hypothesis of prey as a non-limiting resource for this species in urban streams.

To determine effects of urbanization on larval salamander diet, we quantified dietary compositional shifts for Southern Two-lined Salamander larvae seasonally and across land-cover categories for streams in forested, suburban, and urban watersheds. 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 topic that warrants increased study (Faeth et al. 2005).

Study Area

We examined the diet of larval Southern Two-lined Salamanders in nine second- or third-order streams in western Georgia, all within the larger Chattahoochee River Basin (Fig. 1). To evaluate larval diets in urban habitats, we selected three streams within Columbus, GA (Bradley Creek [BR], Cooper Creek [BU2], and Roaring Branch [RB]). These sites were heavily urbanized, with at least 25% of the land cover in the watershed as impervious surface (mean = 32%, range = 25–40%). For comparison, we also selected three streams (Blanton Creek [BLN], Cline's Branch [MO], and Turntime Branch [MU3]) within forested watersheds (Lockaby et al. 2005) approximately 30 km north of Columbus (Meriwether County). We refer to these streams as reference streams because they retain forested borders that approximate the ancestral landscape. These sites had a minimum of 75% (mean = 79%, range: 76–81%) of the total watershed as forested area (evergreen + deciduous forest), and no more than 1% of the total watershed land-cover as impervious surface. 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 (developing streams), a rapidly developing suburban area adjacent to Columbus (Schley Creek [SB1], Standing Boy Creek Tributary [SB2], and Standing Boy Creek [SB4]). The watersheds for these streams all had relatively low impervious surface cover within the individual watersheds (mean = 3%, range = 2–3%); however, this level of development relative

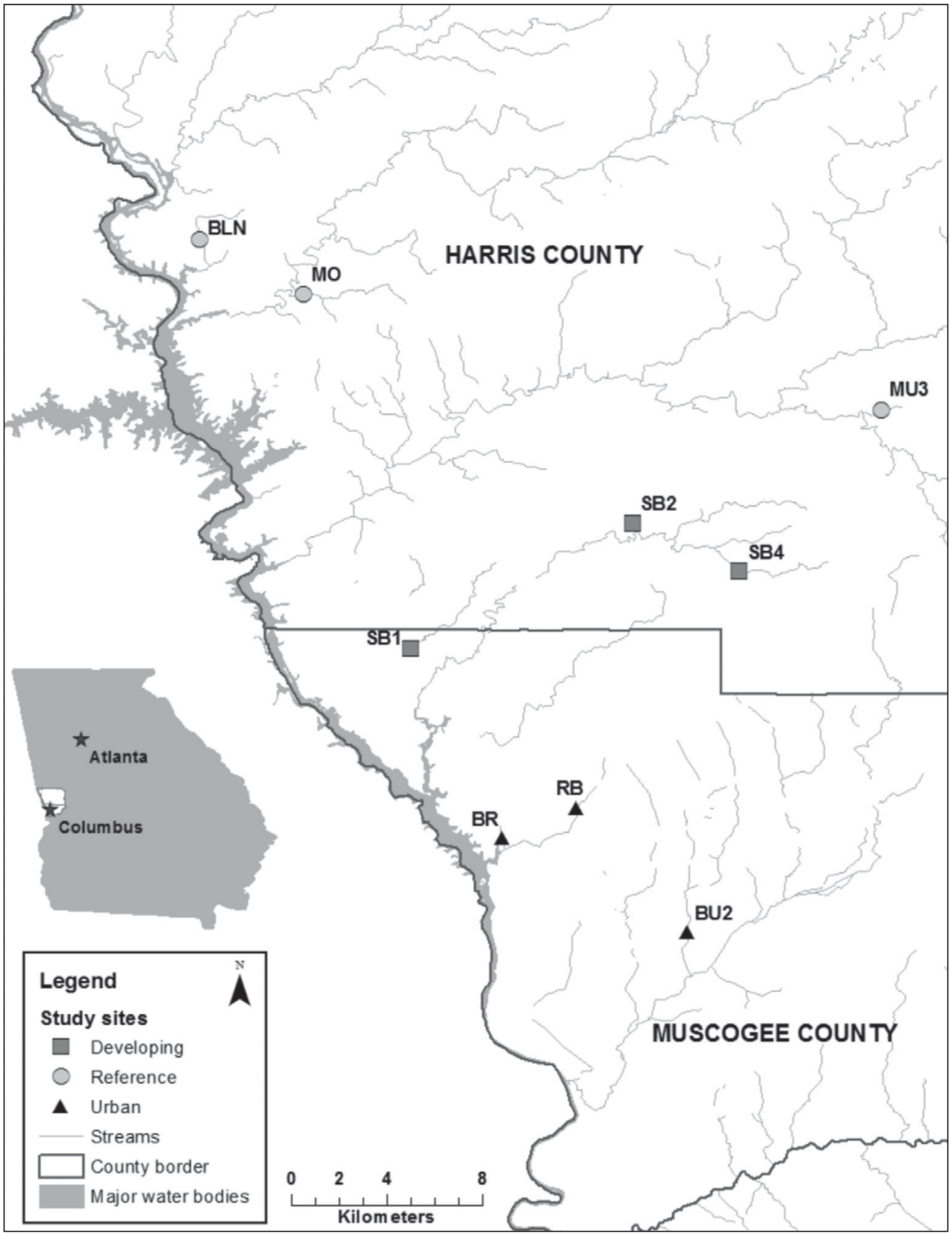

Figure 1. Location of study sites and associated waterways located within the Chattahoochee River Basin of western Georgia. Sites were divided into three different land-cover categories (see Study Area for descriptions). The white area in west central portion of the inset map depicts the location of the two counties shown in the main map.

to forested watersheds appears to be sufficient enough that the biological character of these streams has been altered (Barrett and Guyer 2008). The qualitative land-cover category delineations were supported by a principal components analysis described in Barrett and Guyer (2008).

Methods

Southern Two-lined Salamander larvae were captured for gut content analysis during four seasons. The captures from summer (July 2006 and 2007) and fall (November 2006 and October 2007) were combined for this analysis, as this period of warm temperatures likely represents the peak of salamander foraging, and we refer to them as our summer sample. We also captured larvae during winter (January 2007) and spring (April 2007). Upon capture, individuals were euthanized in 0.04% unbuffered MS 222 solution and then preserved by freezing until examined for gut contents in the laboratory.

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 lowest possible taxonomic level (typically order).

We compared prey composition among land-cover categories and seasons using a Fisher's exact test (FET) in Program R (Version 2.13.0). This test is appropriate for determining whether or not an association exists between categorical variables, and it is particularly suited for situations where some of the expected frequencies are very small (i.e., less than five; Crawley 2007). In short, the test was used to determine if the number of prey observed in one category (e.g., land cover) depended upon another category (i.e., taxa). A lack of independence between categories implies a shift in prey composition as a function of either site or season.

To evaluate prey composition shifts, we combined data from streams within land-cover categories and used prey taxa categories that had at least five occurrences across land-cover categories in a given season (Table 1). This procedure resulted in the inclusion of the following prey groups: Coleoptera, Diptera, Gastropoda, Ostracoda, and Other (a combination of taxa too infrequent to analyze separately). Because of the complications involved in evaluating a three-way interaction between taxon, season, and land-cover category, and because we a priori expect variations in diet across seasons, we focused more detailed analyses within seasons to evaluate shifts in taxon composition across land-cover categories. For these analyses, we first evaluated the FET for a table including all taxa meeting our minimum requirement of at least 5 observations across land-cover categories. If the test was significant ($P < 0.05$), then we dropped from the table the taxon that appeared to contribute most to the lack of independence in counts between land-cover category and taxa. This decision was made based on a qualitative assessment of the data, and was done because there is no formal post hoc pairwise test available for categorical data with small expected frequencies. After removing a taxon, we then performed the test again on the reduced taxa set, and continued this process until the test was no longer significant. A lack of statistical significance implied that counts of taxa did not depend on land-cover category (i.e., there were no diet shifts observed across land-cover categories for those groups included in the test). In addition to this analysis, we compared taxonomic richness across seasons and land-cover categories with a goodness of fit (GOF) test, and we calculated Shannon diversity index (H') for diets in each of the land-cover categories in each season. The Shannon diversity index is often used as a measure of diet breadth (Levins 1968, Pianka 1986).

Results

We captured a total of 145 Southern Two-lined Salamander larvae across all seasons and land-cover categories (Table 1). Twelve individuals were found with either no food in their guts, or contained no identifiable prey. Among all prey taxa, Diptera larvae consistently made up the largest proportion of larval diets (Table 2). The FET on a table of season x land-cover category x taxa revealed that counts within a particular invertebrate taxon varied as a function of season and land-cover category ($P < 0.0001$). As described in the Methods, we made no attempt to investigate this table further, and focused instead on the within-

Table 1. Number of prey items (expressed as a sum per taxa) found in the diet of Southern Two-lined Salamander larvae in nine streams in western Gerogia. The number of digestive tracts examined for each stream is represented below the stream name in parentheses.

	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	-	-	-	1	-	1	-	1	-
Trichoptera	-	1	-	1	10	-	-	-	2
Unidentified	2	4	7	4	7	-	1	9	3
Empty gut	1	-	3	-	3	-	2	3	-

season analyses. The analysis of spring prey items included Diptera, Ostracoda, and Other. The FET on the 3 x 3 table (all spring taxa and the three land-cover categories) was significant (*P* < 0.0001). We then eliminated Ostracoda from the table (as this group was encountered in the diets of larvae from developing streams in much higher proportion than in reference and urban streams; Table 2). The test of a table with only Diptera and Other was not significant (FET: *P* = 0.70). Within all land-cover categories during summer, we observed a notable increase in the proportion of Gastropoda (snails, primarily Physidae and Planorbidae) within larval diets (Table 2). Analysis of summer data included Diptera, Ostracoda, Coleoptera, Gastropoda, and Other. No combination of taxa resulted in a non-significant FET; therefore, we concluded that counts for all taxa groups showed a lack of independence with the land-cover category variable (FET: *P* < 0.02 for all tests; Table 2). In summer, we observed significantly fewer Diptera in larvae captured in the urban land-cover category relative to the other two land-cover groups. Developing sites had larvae with higher counts of Other invertebrates relative to larvae in reference and urban streams, while reference sites had significantly lower counts for Ostracoda relative to the other two land-cover categories. Coleoptera constituted 14% of the summer diet for larvae found in urban streams, but made up <5% of larval diets in the other land-cover categories. Finally, the winter analysis included Diptera, Coleoptera, and Other. The FET was significant with all three groups included in the analysis (*P* = 0.05); however, when the combined taxonomic group of Other was removed, there was no evidence that counts of Diptera or Coleoptera varied across land-cover categories.

Table 2. Proportion of prey items found in the diet of Two-Lined Salamanders across seasons in nine west Georgia streams. Values were calculated by combining data from streams within land cover categories (Ref = Reference, Dev = Developing, Urb = Urban).

	Spring			Summer			Winter		
Taxon	Ref	Dev	Urb	Ref	Dev	Urb	Ref	Dev	Urb
Acari	-	-	-	0.01	0.02	0.02	-	-	-
Amphipoda	-	-	-	0.01	-	-	0.01	-	-
Cladocera	0.01	-	-	-	0.01	-	0.13	-	-
Coleoptera	0.02	0.02	0.10	0.02	0.01	0.14	0.03	0.04	0.12
Collembola	-	-	-	0.01	-	-	-	0.01	-
Copepoda	0.05	0.03	0.05	0.01	0.01	-	0.06	0.06	0.06
Diptera	0.78	0.29	0.52	0.74	0.62	0.47	0.71	0.72	0.67
Ephemeroptera	-	-	-	-	0.01	0.01	-	0.01	-
Gastropoda	-	-	-	0.02	0.04	0.13	-	-	-
Hemiptera	-	-	-	-	0.01	-	-	-	-
Hymenoptera	-	-	-	0.01	0.01	0.01	-	0.01	-
Lepidoptera	0.01	-	-	-	0.03	-	0.01	-	-
Megaloptera	-	-	-	0.01	-	-	-	-	-
Nematoda	-	-	0.05	0.01	-	-	-	-	-
Odonata	0.01	-	-	-	0.01	0.01	-	-	-
Ostracoda	0.07	0.62	0.05	0.03	0.13	0.13	0.04	0.06	0.03
Plecoptera	-	-	-	-	0.01	0.01	-	-	-
Trichoptera	-	-	-	0.01	0.07	0.01	-	-	-
Uidentified	0.04	0.01	0.19	0.01	0.03	0.05	0.01	0.07	0.06

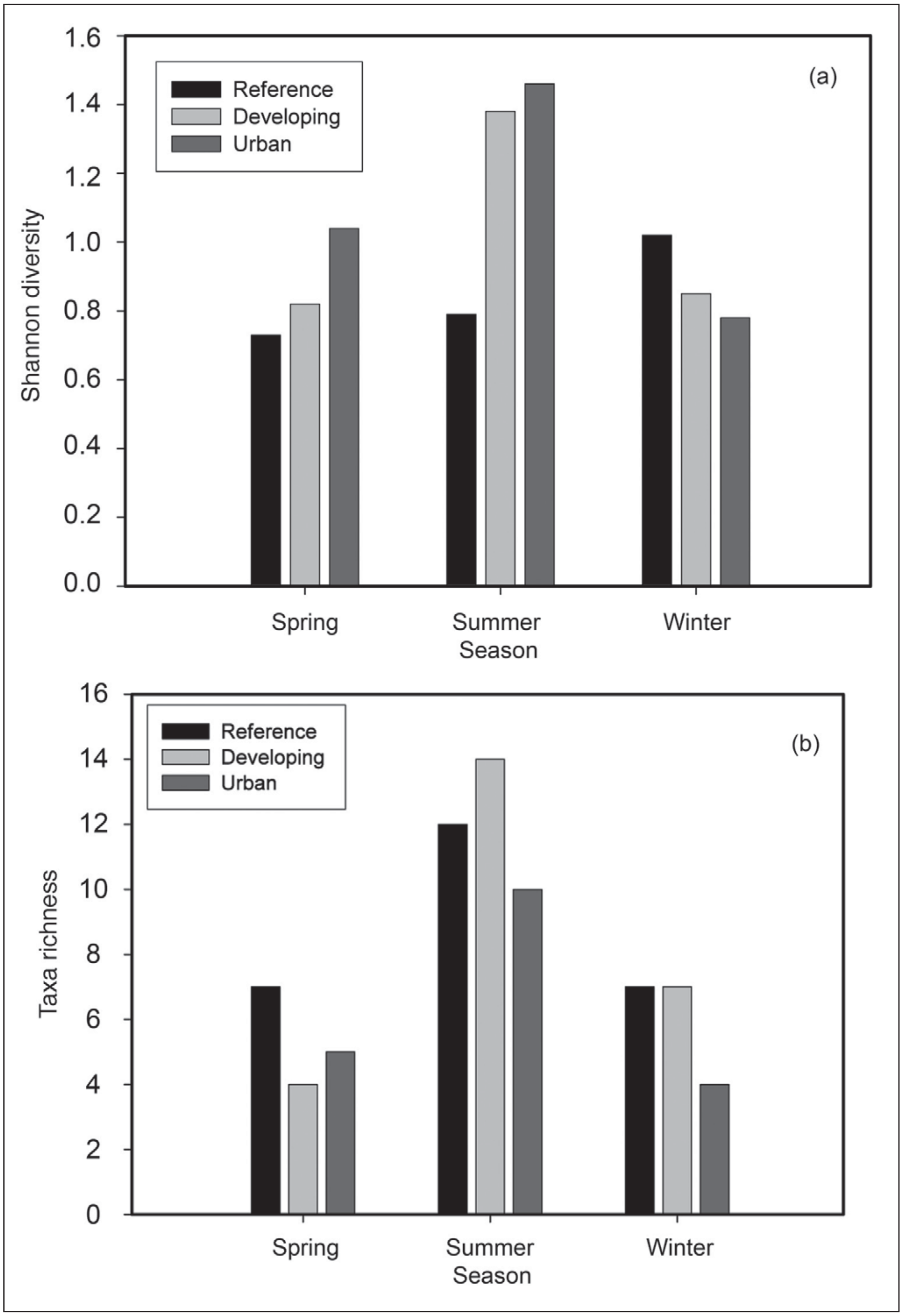

Figure 2. Shannon diversity (a) and taxa richness (b) for prey items found in the digestive tracts of Southern Two-lined Salamanders in reference (*n* = 47 larvae), developing (*n* = 48 larvae), and urban (*n* = 50 larvae) streams during three seasons in western Georgia. Larval sample sizes are the same as in Figure 1.

Counts for Other were far higher in larvae from reference streams than those from either developing or urban streams (Table 2).

Diet breadth, as measured by the Shannon index, was highest in urban and lowest in reference streams during spring and summer seasons; however, during winter, niche breadth was lowest in urban streams and highest in reference streams (Fig. 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. 2b).

Discussion

The main differences in prey composition of Southern Two-lined Salamanders 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, Ostracoda 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. Other aspects of larval diet composition showed some 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* (Burton 1976, Caldwell and Houtcooper 1973, Johnson and Wallace 2005, Muenz et al. 2008, Petranka 1984). 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, Muenz et al. 2008, Petranka 1984). Plecoptera larvae, which were important in the diet of Southern 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 Muenz et al. (2008). 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) for samples taken from these same study streams during 2004. The greater diversity of prey items consumed by salamanders during summer may represent a general increase in biomass consumption during warmer months when metabolic rates are likely increased and growth rates are high (Barrett et al. 2010).

Gastropoda 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 Southern 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 because of calcification of the skeleton as larvae prepare for increased skeletal demands associated with terrestrial life. It is possible that larval Southern Two-lined Salamanders consume snails, which have extensive calcium deposits in the shell, for similar reasons.

Previously, Barrett et al. (2010) documented increased growth rates of Two-lined Salamander larvae from urban streams relative to reference streams within the same western Georgia system we describe in this study. Several potential explanations for the observed growth differential were explored as part of that study, and one of them was a positive correlation between growth rate and invertebrate abundance (Helms 2008) within a stream. Little support for that relationship was found by Barrett et al. (2010). The relatively minor differences we observed in diet composition and overall invertebrate counts within larvae as part of this study further suggests that diet composition is not a suitable explanation for why Two-lined salamander larvae in these urban streams exhibit higher growth rates.

Our description of salamander diets provides the information necessary to begin constructing and comparing stream food webs in urban and forested habitats. Studies demonstrating a change in species richness or abundance of taxa with urbanization have accumulated rapidly, and sufficient information now exists to begin examining changes in multi-trophic interactions that result from urbanization (Faeth et al. 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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