

Correlates of elemental-isotopic composition of stream fishes: the importance of land-use, species identity and body size

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The isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and stoichiometric (C:N:P) compositions of four fish species (Family Centrarchidae: *Lepomis auritus*, *Lepomis cyanellus*; Family Cyprinidae: *Nocomis leptocephalus*, *Semotilus atromaculatus*) were examined across four North Carolina Piedmont streams arrayed along an urbanization gradient. Both isotopic and stoichiometric composition of fishes appeared to track changes occurring in basal resource availability. Values of $\delta^{13}\text{C}$ of basal resources and consumers were more enriched at the most urbanized streams. Similarly, basal resources and consumers were $\delta^{15}\text{N}$ -enriched at more urbanized streams. Basal resource stoichiometry varied across streams, with periphyton being the most variable. Primary consumers stoichiometry also differed across streams. Intraspecific variation in fish stoichiometry correlated with the degree of urbanization, as the two cyprinids had higher N content and *L. cyanellus* had higher P content in more urbanized streams, probably due to enrichment of basal resources. Intrinsic factors, specifically species identity and body size also affected stoichiometric variation. Phosphorus (P) content increased significantly with body size in centrarchids, but not in cyprinids. These results suggest that although species identity and body size are important predictors of elemental stoichiometry, the complex nature of altered urban streams may yield imbalances in the elemental composition of consumers *via* their food resources.

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Key words: basal resources; Centrarchidae; Cyprinidae; nutrient dynamics; urbanization; urban–rural gradient.

INTRODUCTION

Multiple interacting factors affect the structure and function of ecological communities (*e.g.* primary production, interspecific interactions, climate and biogeography; Gido & Jackson, 2010; Olden *et al.*, 2010; Vellend, 2010). Fish assemblages in different environments appear to be structured by regional (*i.e.* fluvial geomorphology, dispersal) and local processes (*i.e.* limnological and structural characteristics of the habitat and ecological interactions; Wang *et al.*, 2006; Montaña & Winemiller, 2010; Montaña *et al.*, 2014). In urban catchments, the structure of fish assemblages results from in-stream conditions including flow regimes and variability in nutrient loading, which

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can alter the elemental and isotopic composition of primary production sources and other components of the food web (O'Brien & Wehr, 2010; Winemiller *et al.*, 2011).

Previous studies have used ecological stoichiometry (ES) as an important framework to examine the transfer, flow and movement of nutrients in ecological systems (Sterner & Elser, 2002; Hessen *et al.*, 2013) and to examine the effects of anthropogenic activities on stream biota (O'Brien & Wehr, 2010; Tsoi *et al.*, 2011; Milanovich *et al.*, 2014). This field of ES focuses on understanding the relative balance of key chemical elements (C:N:P) between consumers and their food resources (Hessen, 1997; Sterner & Elser, 2002). ES theory proposes that consumers, while considering their specific identities, maintain relatively constant stoichiometry by regulating their whole-body elemental composition (Sterner & Elser, 2002). Imbalances in a consumer's elemental requirements may constrain their growth and reproduction (Sterner & Elser, 2002; Vanni *et al.*, 2002). These imbalances can propagate up to other trophic levels and organizational hierarchies (Hessen, 1997) and consequently affect a variety of ecological, physiological and biogeochemical processes (Sterner & Elser, 2002; Vanni *et al.*, 2002; El-Sabaawi *et al.*, 2012).

Stable isotope analysis (SIA) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is another complementary technique that has been used extensively to examine food-web structure across aquatic ecosystems as they reveal variation in food-web structure in relation to anthropogenic inputs (Peterson, 1999; Anderson & Cabana, 2005). For instance, anthropogenic changes, such as urbanization or agriculture can influence enrichment of $\delta^{15}\text{N}$ in basal production sources (*e.g.* seston, algae, periphyton) consequently affecting the trophic structure of consumers living in urbanized streams (Ulseth & Hershey, 2005; Winemiller *et al.*, 2011). Thus, significant changes in carbon sources ($\delta^{13}\text{C}$) have been observed in fish inhabiting urbanized *versus* less urbanized streams (Eitzmann & Paukert, 2010). Given the usefulness of stable-isotope analysis, it is important to identify those environmental factors that can influence the isotopic composition of organisms in aquatic ecosystems.

Species identity and body size are important determinants of organism elemental composition (Pilati & Vanni, 2007; Torres & Vanni, 2007). Stoichiometric theory predicts that the ratio of C:P decreases with body size because of a greater allocation of P-rich bone tissues in organisms with large body size. Fishes in the family Centrarchidae and Percidae have particularly higher P content compared with fishes in the family Cyprinidae suggesting that the low P content of cyprinids is related to their relatively softer bodies and lack of dense bones (Sterner & George, 2000). Comparisons of species among different families of freshwater fishes emphasized the importance of body size driving overall organismal stoichiometry (Vanni *et al.*, 2002; Hendrixson *et al.*, 2007; McIntyre & Flecker, 2010). In Venezuelan Piedmont streams, the N:P ratio of excreted nutrients in 47 fish species increased as a function of body size, with large-bodied fishes excreting relatively more N than P compared with small-bodied fish (Vanni *et al.*, 2002; McIntyre & Flecker, 2010).

Anthropogenic activities are major drivers that affect the availability of key elements (*e.g.* N and P) in natural ecosystems (Vitousek *et al.*, 1997; Peñuelas *et al.*, 2013). Anthropogenic runoff, such as inorganic fertilizers, human sewage and detergents, can alter nutrient quality and quantity because they contain high concentrations of limiting nutrients (Seitzinger *et al.*, 2005). Moreover, anthropogenic changes in land cover (*e.g.* increased impervious surface) can alter flow regimes and enhance nutrient inputs due to increased runoff entering these aquatic systems (Walsh *et al.*, 2005; O'Brien & Wehr,

2010; Tsoi *et al.*, 2011; Mehler *et al.*, 2013). In highly urbanized streams, extrinsic factors such as sewage inputs and altered soils contain a higher concentration of soluble reactive phosphorus (SRP) and nitrates, which increase nutrient loading of N and P and ultimately increase periphyton biomass (O'Brien & Wehr, 2010). Downs *et al.* (2016) observed high variation in algal stoichiometry (C:P) as a response to light and nutrient (*i.e.* P) availability which, in turn, affected the elemental stoichiometry and growth of bluegill *Lepomis macrochirus* Rafinesque 1819.

Isotopic and stoichiometric changes of in-stream biota inhabiting urban streams has received less attention compared with streams in forested or agricultural catchments (O'Brien & Wehr, 2010; Tsoi *et al.*, 2011; Milanovich *et al.*, 2014). Globally, the rate of urbanization is increasing (Alig *et al.*, 2004; White *et al.*, 2009) and understanding the response of biodiversity to increasingly urbanized habitats has important implications to the understanding of ecosystem structure and function. The aim of this study is to examine the extrinsic (stream type) and intrinsic (species identity, body size) correlates that may drive variation in isotopic and elemental composition of fish consumers in four streams in the Piedmont ecoregion of North Carolina, U.S.A. Streams were arrayed along an urbanization gradient that provided an ideal system to address questions relating landscape changes to ecological stoichiometry. Because an increase in impervious surface cover can alter runoff and consequently have negative effects on in-stream nutrient dynamics, in-stream biota and the elemental composition of other food-web components, this study addressed the following questions: is there a relationship between basal resources, consumer isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and degree of urbanization; does consumer elemental composition vary as a function of species identity and body size? To address these questions, different food-web components were sampled and focused on four species of fish that were common in most streams, but varied in respect to their life histories, trophic ecology and species identity. Consequently, they may exhibit elemental composition plasticity in response to local stream conditions.

MATERIALS AND METHODS

STUDY SYSTEM AND STUDY ORGANISMS

This study was conducted in the North Carolina Piedmont ecoregion, characterized by rolling foothills leading up to the mountainous region of the state. Streams were selected near Raleigh, North Carolina, one of the fastest growing cities in the United States (Kennen *et al.*, 2005). Impervious surface cover was used as a proxy for the degree of localized urbanization and was assessed based on analysis of 2011 Landsat (www.landsat.gsfc.nasa.gov) imagery (30 × 30 m cell resolution, 1 km and 5 km buffers). The four streams surveyed were arrayed along a gradient of increasing urbanization: Newlight Creek (least urbanized), Poplar Creek (moderately urbanized), House Creek (highly urbanized) and Pigeon House Branch (the most urbanized) (Fig. 1 and Table I).

Comparisons were made among four fish species from two higher taxonomic groups that occurred in most streams: two cyprinids, creek chub *Semotilus atromaculatus* (Mitchill 1818) and bluehead chub *Nocomis leptocephalus* (Girard 1856) and two centrarchids, green sunfish *Lepomis cyanellus* Rafinesque 1819 and redbreast sunfish *Lepomis auritus* (L. 1758) (sampling at House Creek did not yield any *L. auritus*). The species within each family share similarities in functional traits associated with feeding, but differ in other aspects of their life history (*e.g.* reproduction, growth) that could affect their stoichiometric composition. The two cyprinid species are elongate-bodied, soft-rayed fishes, with generalized omnivorous diets that

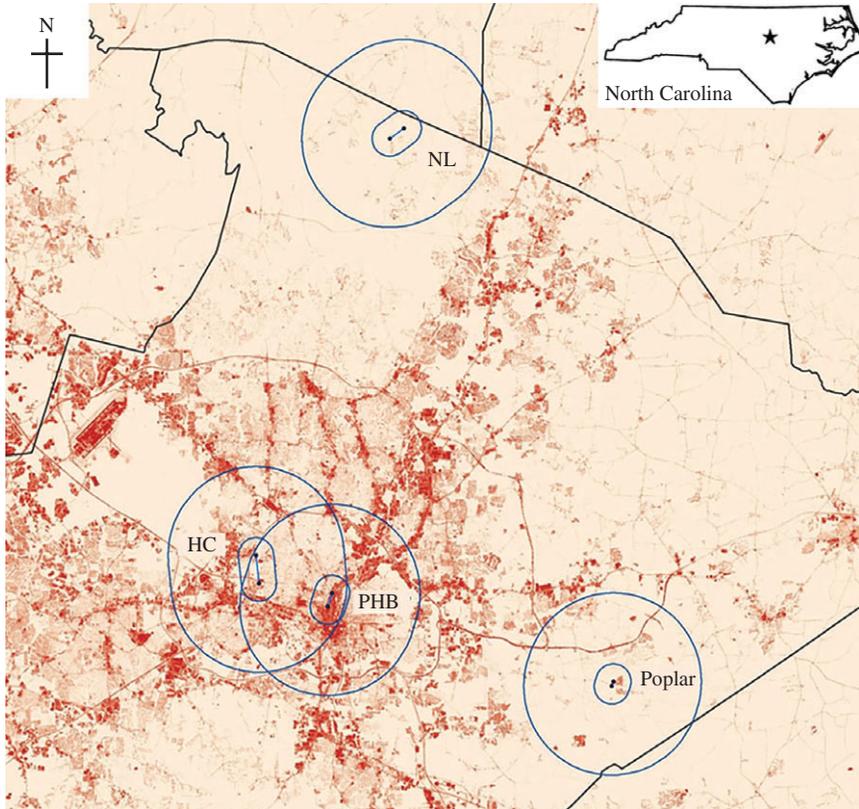


FIG. 1. Map displaying the four Piedmont stream reaches sampled (●—●) in Raleigh, North Carolina, U.S.A. and the surrounding area. Small (○, 1 km buffers) and large (○, 5 km buffers) labelled stretches around each stream summarize the impervious surface cover. The values in the image range from 0% (□) to 100% (■) impervious cover. The streams are arrayed along a gradient of increasing impervious cover: NL, Newlight Creek; Poplar Creek; HC, House Creek; PHB, Pigeon House Branch.

include plant material, invertebrates and small fishes (Kennen *et al.*, 2005). The two centrarchid species both have heavily ossified craniums and internal skeletons, with generalized carnivorous diets that include zooplankton and invertebrates as juveniles, while as adults, their diet can be expanded to small fishes and snails (Montaña & Winemiller, 2013). During ontogeny, the two cyprinids undergo a nearly six-fold increase in body size [juvenile to adult maximum total length (L_T), *S. atromaculatus* 50–303 mm and *N. leptocephalus* 50–260 mm] whereas the two centrarchids increase in body size approximately 10 fold (juvenile to adult maximum L_T , *L. cyanellus* 30–310 mm and *L. auritus* 32–305 mm).

STREAM SURVEYS

Streams were surveyed four times each, between April 2014 and June 2014. The streams were surveyed in a reach of $c.1.6$ km in length. During each survey, water samples, basal resources (*e.g.* filamentous algae, periphyton, leaf litter and moss), two primary consumers (snails *Campeloma floridense* and earthworms Oligochaeta: Lumbricidae) and the four target fish species were collected. A YSI meter (www.ysi.com) was used to measure water temperature ($n = 3$), conductivity ($n = 3$) and dissolved oxygen ($n = 3$) at each site per visit. Turbidity was measured with a Hatch 2100 portable turbidimeter (www.hach.com) and pH with a portable

TABLE I. Mean (\pm S.D., $n = 3$) values of physicochemical variables in four Piedmont streams in North Carolina, U.S.A. Streams are ordered along the gradient of increasing urbanization. Significant differences among sites ($P < 0.05$) are in bold

Stream properties	Newlight	Poplar	House Creek	Pigeon House Branch
Location	36° 1'. 875' N, 78° 35'. 719' W 0.7	35° 44'. 868' N, 78° 27'. 958' W 4.6	35° 48'. 042' N, 78° 41'. 254' W 19.8	35° 47'. 742' N, 78° 38'. 490' W 43.3
Impervious surface cover (%) at 1 km buffer and at 5 km buffer	1.2	2.7	17.4	23.8
pH	6.6 (\pm 0.1)	6.3 (\pm 0.0)	8.9 (\pm 1.2)	7.20 (\pm 0.4)
Temperature ($^{\circ}$ C)	14.7 (\pm 3.1)	15.3 (\pm 4.5)	20.7 (\pm 1.9)	17.9 (\pm 3.5)
Conductivity (μ S cm $^{-1}$)	75.0 (\pm 7.1)	114.3 (\pm 14.8)	129.0 (\pm 10.0)	217.9 (\pm 6.4)
Turbidity (NTU)	6.5 (\pm 2.1)	8.1 (\pm 4.8)	1.6 (\pm 0.4)	4.03 (\pm 0.5)
Dissolved oxygen (mg l $^{-1}$)	10.6 (\pm 0.4)	10.3 (\pm 3.5)	7.5 (\pm 0.8)	11.4 (\pm 0.8)
Ammonium-nitrogen (NH $_4$ -N, mg l $^{-1}$)	0.005 (\pm 0.01)	0.02 (\pm 0.01)	0.01 (\pm 0.01)	0.04 (\pm 0.1)
Soluble reactive phosphorous (SRP, mg l $^{-1}$)	0.01 (\pm 0.01)	0.2 (\pm 0.1)	0.03 (\pm 0.01)	0.04 (\pm 0.01)
Nitrate + nitrite (NO $_3$ -N + NO $_2$ -N, mg l $^{-1}$)	0.2 (\pm 0.0)	1.2 (\pm 0.4)	1.2 (\pm 0.6)	1.3 (\pm 0.7)

pH meter. Water samples ($n = 3$ per stream per visit) were collected for analysis of dissolved nutrients [ammonium-nitrogen ($\text{NH}_4\text{-N}$), nitrite + nitrate-nitrogen ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$) and soluble reactive phosphorous (SRP)]. Water samples were filtered through combusted $0.7 \mu\text{m}$ glass microfibre filters (Whatman GF/F; Sigma-Aldrich; www.sigmaaldrich.com) at each site. All samples were stored in 20 ml acid washed (10% HCl) scintillation vials, kept on ice in a cooler, transported to North Carolina State University (NCSU) and stored at -20°C until analysed. Snails and earthworms were used as primary consumers because they generally have isotopic signatures similar to that of periphyton and leaf litter (Post, 2002; Najera-Hillman *et al.*, 2009). Also, they were common items in the stomach content of fish species collected in most streams (C.G. Montaña unpubl. data).

For filamentous algae and submerged moss (Bryophyta), samples were collected by hand. Periphyton was sampled by scrubbing two to three small rocks using a toothbrush and rinsing with distilled water until no obvious biomass remained. Snails and earthworms were collected by hand. Basal sources and primary consumers were rinsed with deionized water and dried at 65°C for 48–72 h. Fishes were collected using a LR-20B Smith-Root electrofishing backpack (www.smith-root.com) and dip nets. Fishes were euthanised *via* whole body immersion in MS-222 and stored on ice until returned to the laboratory. Fishes were measured (L_T , mm), dissected and had their guts removed and dried at 65°C for several days until a constant dry mass (g) was achieved. In total, 34 basal resource samples, 16 primary consumer samples and 207 individual fishes were examined for body elemental composition (C,N,P) (*S. atromaculatus* $n = 65$, *N. leptocephalus* $n = 45$, *L. cyanellus* $n = 60$ and *L. auritus* $n = 37$). Basal resource, primary consumer and fish samples were ground to fine powder using a coffee grinder and mortar and pestle. Sub-samples (1.5 mg) of basal sources and consumers were analysed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N at Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, U.S.A. The analytical precision for the stable-isotope measurements was ± 0.1 for $\delta^{13}\text{C}$ and ± 0.2 for $\delta^{15}\text{N}$. Phosphorus content was estimated in basal sources ($c. 20 \text{ mg}$) and consumers ($c. 10 \text{ mg}$) by combusting the dried samples in a muffle furnace (500°C for 6 h) and digesting the samples in a reagent acid solution (nitric acid and hydrochloric acid) (Fourqurean & Ziemann, 1992). The concentration of dissolved P in the digested solution was measured using the molybdate-blue method (Parson *et al.*, 1984). Bone meal (NIST # 1486) was used as internal standard reference and the efficiency of P extraction was typically $>95\%$.

DATA ANALYSES

Biplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of basal resources and consumers were used to depict patterns of isotopic variation across streams. One-way analysis of variance (ANOVA) was used to examine differences in isotopic and elemental composition of consumers within and among streams. When data did not meet assumptions of normality and homogeneity of variance, a non-parametric Kruskal-Wallis (K-W) Test was performed followed by a Mann-Whitney pair-wise comparison test. Analysis of covariance (ANCOVA) was performed to examine the relative influence of stream site identity (categorical variable) and L_T (continuous variable) on whole-body P content on consumer stoichiometry. Linear regression analyses were performed to determine the relationship between L_T and body P content of each consumer. Statistical analyses were performed in JMP Pro_11.2. (SAS Institute, Cary, NC).

RESULTS

STREAM WATER QUALITY

Conductivity, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ concentrations were significantly higher at the most urbanized stream (Pigeon House Branch; ANOVA, $F_{3,8} = 4.3$, $P < 0.05$); whereas SRP was significant higher in Poplar Creek ($F_{3,12} = 4.1$, $P < 0.05$) (Table I). Newlight Creek, the least urbanized stream, had the lowest nutrient values compared with the other streams (Table I).

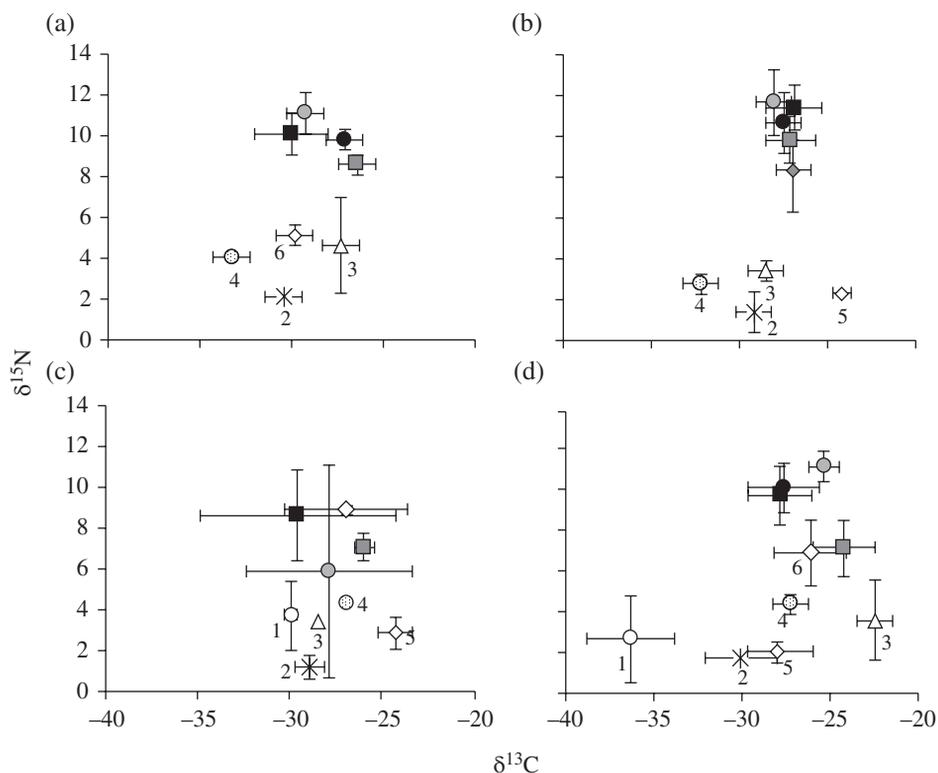


FIG. 2. Biplots of mean \pm S.D. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Semotilus atromaculatus* (□), *Nocomis leptocephalus* (■), *Lepomis cyanellus* (○), *Lepomis auritus* (●), basal resources (1, algae; 2, leaf litter; 3, periphyton; 4, moss), and two primary consumers (5, earthworm; 6, snail) collected across four Piedmont streams located in or near Raleigh, North Carolina, U.S.A.: (a) Newlight Creek, (b) Poplar Creek, (c) House Creek and (d) Pigeon House Branch.

ISOTOPIC COMPOSITION OF BASAL RESOURCES AND CONSUMERS

The $\delta^{13}\text{C}$ of moss and periphyton varied significantly across streams (K-W test, $H = 8.21$, $P < 0.05$) and was more enriched at Pigeon House Branch (Fig. 2). The $\delta^{13}\text{C}$ of the two primary consumers did not vary significantly across streams ($H = 4.21$, $P > 0.05$; Fig. 2). Composition of $\delta^{15}\text{N}$ varied significantly across streams for both basal resources ($H = 7.53$, $P < 0.05$) and primary consumers ($H = 4.5$, $P < 0.05$). Pair-wise comparisons suggested high ^{15}N -enrichment of moss, periphyton and snails at the two more urbanized streams, Pigeon House Branch and House Creek, respectively.

Fish $\delta^{13}\text{C}$ values differed significantly across streams ($H = 51.59$, $P < 0.01$; Fig. 2). Fishes at Pigeon House Branch had $\delta^{13}\text{C}$ -enriched values when compared with fishes at other streams (Fig. 2). Significant differences were also found in $\delta^{15}\text{N}$ across streams ($H = 68.78$, $P < 0.01$; Fig. 2). Fishes at Poplar, House Creek and Pigeon House Creek were $\delta^{15}\text{N}$ enriched when compared with Newlight Creek. Values of $\delta^{15}\text{N}$ for *L. auritus* did not differ among streams.

ELEMENTAL STOICHIOMETRY OF BASAL RESOURCES AND CONSUMERS

Basal resources had variable stoichiometry and differed across streams (Table II). Mean values of %C, %N, %P, N:P and C:P were significantly higher ($H = 22.94$, $P < 0.01$) for periphyton at Pigeon House Branch, the most urbanized stream. Mean %P content of algae also increased at Pigeon House Branch (Table II). Snails %C, %P and C:N was higher at Pigeon House Creek, whereas means of %C, %N and N:P of earthworms were highest at Poplar Creek, the moderately urbanized stream (Table II).

Fish stoichiometry differed across streams (Fig. 3). For cyprinids, %N and N:P of *S. atromaculatus* showed high variation at Pigeon House Branch [Fig. 3(a), (d)], but no variation was observed for %P. *Notropis leptocephalus* had significantly lower %N and %P at House Creek and Poplar, respectively [Fig. 3(a), (b)]. High values of C:N and N:P ratios were also observed on *N. leptocephalus* from House Creek and Poplar creek. For centrarchids, %N, %P, N:P and C:N of *L. auritus* remained relatively constant across streams [ANOVA, $F_{2,37} = 4.2$, $P > 0.05$; Fig. 3(a)–(d)]. *Lepomis cyanellus* had significantly lower body %N and N:P at the two more urbanized streams, Pigeon House Branch ($F_{3,55} = 11.5$, $P < 0.01$) and House Creek [$F_{3,55} = 17.1$, $P < 0.01$; Fig. 3(b), (d)]. %P of *L. cyanellus* was highest at Pigeon House Branch [$F_{3,55} = 11.5$, $P < 0.01$; Fig. 3(b)] and C:N ratios was highest at House Creek [$F_{3,55} = 26.4$, $P < 0.001$; Fig. 3(c)]. Overall, species differed in their elemental composition (Fig. 4). *Semotilus atromaculatus* had significant higher values of %C content (ANOVA, $F_{3,201} = 14.5$, $P < 0.01$) and %N content ($F_{3,201} = 15.0$, $P < 0.01$), whereas the two species of *Lepomis* had the highest values of %P content compared with the two cyprinids ($F_{3,201} = 47.8$, $P < 0.001$).

Body size was positively correlated with whole-body %P content for the two *Lepomis* species and this pattern was consistent across all streams (Fig. 5 and Table III). For the two cyprinids, body size alone was a poor predictor for whole-body %P content (Table III). Stream identity and its interaction with body size was a good predictor of whole-body %P content of *N. leptocephalus* (Table III). Whole body %P content of *S. atromaculatus* was not affected by stream identity or body size (Table III).

DISCUSSION

Isotopic and elemental composition of basal resources and consumers varied along an urbanization gradient suggesting that such variation might reflect changes in stream properties. Values of $\delta^{13}\text{C}$ of periphyton and moss were more enriched at the most urbanized streams (House Creek and Pigeon House Branch) and $\delta^{13}\text{C}$ of primary consumers and fish track closely with these values of basal resources at the same streams. Instream conditions including current speed, productivity, dissolved organic carbon and dissolved inorganic carbon, are known to affect carbon composition of primary producers in lotic systems (Boon & Bunn, 1994; Epstein *et al.*, 2016). Increased urbanization can cause shifts in the organic carbon quantity, sources and quality; consequently, affecting all food-web components. The $\delta^{15}\text{N}$ signature of basal resources did not vary across streams. Snail $\delta^{15}\text{N}$ was however, more enriched at the most urbanized streams (Pigeon House Branch) and fish $\delta^{15}\text{N}$ was more enriched at Poplar Creek, which is a stream with moderate urban development and intact riparian forest, but it has some of the worst water quality in the catchment, primarily due to high pollution discharges

TABLE II. Mean (\pm S.D.) of elemental composition (%C, %N, %P) and stoichiometric ratios (N:P, C:P, C:N) for basal resources and primary consumers across four Piedmont streams in North Carolina, U.S.A.

Basal resources	%C	%N	%P	N:P	C:P	C:N
Periphyton						
Newlight Creek	6.1 (\pm 2.1)	0.1 (\pm 1.1)	0.10 (\pm 0.02)	1.0 (\pm 0.1)	30.5 (\pm 1.0)	55.0 (\pm 3.2)
Poplar Creek	2.1 (\pm 0.5)	0.2 (\pm 0.5)	0.11 (\pm 0.01)	1.8 (\pm 1.2)	19.1 (\pm 0.2)	10.5 (\pm 0.4)
House Creek	4.4 (\pm 1.5)	0.8 (\pm 1.2)	0.21 (\pm 0.01)	3.8 (\pm 1.1)	21.0 (\pm 0.2)	5.5 (\pm 1.1)
Pigeon House Branch	7.8 (\pm 1.2)	1.9 (\pm 2.0)	0.20 (\pm 0.03)	9.5 (\pm 1.8)	39.0 (\pm 2.2)	4.1 (\pm 2.1)
Moss						
Newlight Creek	30.4 (\pm 0.2)	1.7 (\pm 0.2)	0.11 (\pm 0.0)	22.3 (\pm 0.1)	266.3 (\pm 0.1)	17.8 (\pm 0.3)
Poplar Creek	39.7 (\pm 0.2)	2.1 (\pm 0.3)	0.21 (\pm 0.01)	10.1 (\pm 0.2)	189.0 (\pm 0.2)	18.9 (\pm 0.1)
House Creek	30.9 (\pm 0.1)	2.0 (\pm 0.1)	0.20 (\pm 0.03)	11.4 (\pm 0.1)	152.0 (\pm 0.1)	14.5 (\pm 0.1)
Pigeon House Branch	26.0 (\pm 3.8)	2.1 (\pm 0.4)	0.27 (\pm 0.02)	12.7 (\pm 0.9)	96.2 (\pm 0.1)	12.3 (\pm 4.4)
Algae						
Newlight Creek*			0.13 (\pm 0.0)			
Poplar Creek*			0.22 (\pm 0.02)			
House Creek	18.3 (\pm 0.2)	2.5 (\pm 0.02)	0.29 (\pm 0.0)	8.9 (\pm 0.4)	64.5 (\pm 3.2)	7.2 (\pm 0.01)
Pigeon House Branch	36.6 (\pm 0.3)	3.1 (\pm 0.01)	0.30 (\pm 0.1)	10.5 (\pm 2.6)	121.9 (\pm 3.2)	11.5 (\pm 0.1)
Primary consumers						
Snail						
Newlight Creek	24.6 (\pm 4.6)	6.7 (\pm 0.2)	0.5 (\pm 0.0)	11.9 (\pm 1.0)	49.2 (\pm 2.1)	3.6 (\pm 0.4)
Poplar Creek	34.8 (\pm 0.5)	9.3 (\pm 2.0)	0.9 (\pm 0.01)	10.2 (\pm 1.2)	43.1 (\pm 1.0)	3.7 (\pm 0.5)
House Creek	33.0 (\pm 0.1)	9.1 (\pm 4.6)	0.6 (\pm 0.1)	15.1 (\pm 0.2)	55.1 (\pm 4.6)	3.6 (\pm 0.5)
Pigeon House Branch	43.4 (\pm 1.4)	9.1 (\pm 2.3)	1.5 (\pm 0.2)	6.0 (\pm 1.5)	29.0 (\pm 0.02)	4.7 (\pm 2.1)
Earthworm						
Newlight Creek#						
Poplar Creek	33.4 (\pm 0.5)	7.5 (\pm 0.9)	0.4 (\pm 0.02)	19.7 (\pm 0.1)	86.8 (\pm 0.9)	4.4 (\pm 2.1)
House Creek	30.1 (\pm 1.4)	6.5 (\pm 4.0)	0.4 (\pm 0.02)	15.1 (\pm 0.1)	70.0 (\pm 1.3)	4.6 (\pm 4.4)
Pigeon House Branch	29.9 (\pm 1.0)	6.4 (2.1)	0.5 (\pm 0.1)	10.8 (\pm 1.7)	50.6 (\pm 8.2)	4.6 (\pm 0.1)

*Insufficient samples for analysis of stable-isotope ratios (C and N).

#Newlight Creek did not yield any earthworm consumers.

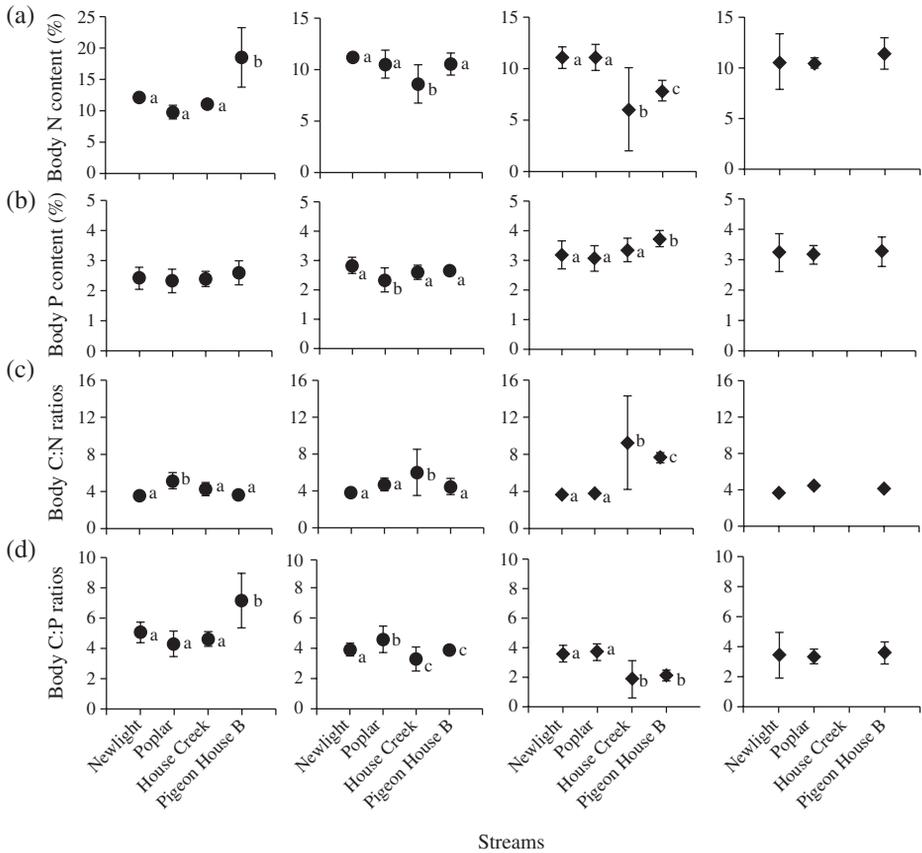


FIG. 3. Mean \pm S.D. nutrient content for fish consumers, *Semotilus atromaculatus*, *Nocomis leptocephalus*, *Lepomis cyanellus* and *Lepomis auritus*, in four Piedmont streams in Raleigh, North Carolina, U.S.A. Streams on the x-axis are ordered along a gradient of increasing urbanization. Row (a) nitrogen and row (b) phosphorus as percentage of body mass; row (c) C:N and row (d) N:P are molar ratios respectively. Significant differences are designated by different lower-case letters ($P < 0.05$). N.B. *L. auritus* was not collected in House Creek. ●, Family Cyprinidae; ◆, Family Centrarchidae.

(NCEEP, 2011). This stream also had the highest values of nitrites and nitrates and SRP in the water, suggesting that N-enrichment of aquatic food-web components is probably linked to anthropogenic sources in the catchment. Lenat & Crawford (1994) reported high concentration of nutrients and available dissolved nitrogen in streams running urban and agricultural sites in the Piedmont of North Carolina; such conditions were associated with low richness and low biomass of fish species and aquatic macro-invertebrates collected in these sites when compared with forested streams.

Nutrient enrichment in streams is known to increase N and P content in basal resources and invertebrates with associated changes in C:N:P ratios (Cross *et al.*, 2003; Feijó *et al.*, 2014). Results from this study support previous studies testing the effects of land cover on stream biota, where the nutrient content of consumers appears correlated with the local (*i.e.* stream) conditions (Tsoi *et al.*, 2011; Mehler *et al.*, 2013). Periphyton was the basal resource that appeared less homeostatic across

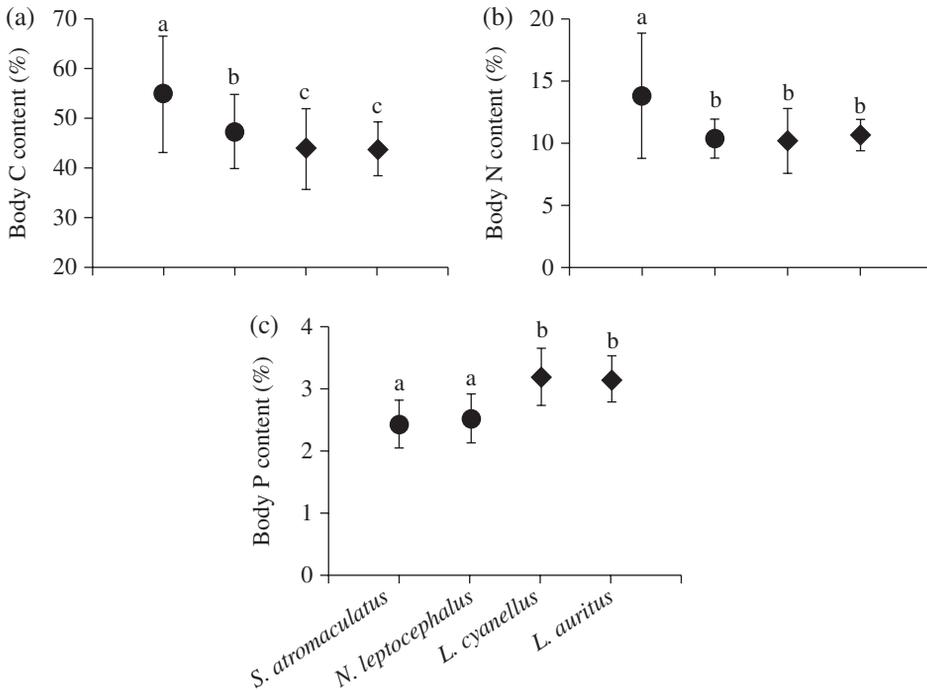


FIG. 4. Interspecific comparison of the mean \pm S.D. body elemental composition for four fish consumers: *Semotilus atromaculatus*, *Nocomis leptocephalus*, *Lepomis cyanellus* and *Lepomis auritus* pooled across four Piedmont streams in Raleigh, North Carolina, U.S.A. (a) Carbon, (b) nitrogen and (c) phosphorous as a percentage of body mass. Significant differences among species are designated by a different lower-case letters ($P < 0.05$). ●, Family Cyprinidae; ◆, Family Centrarchidae.

streams, with high values of %N and %P at the most urbanized streams. Increased %N and %P might provide greater nutrition to consumers that feed on periphyton and that require specific quantities of N and P for metabolism and growth. Snails were one of the two primary consumers sampled in this study and are likely to utilize periphyton. Herbivorous consumers are expected to have lower %N and %P content because plant material or algae often contain a lower amount of P than the food of predators (Fagan *et al.*, 2002). Considering that %P content of periphyton, moss and algae was higher at the most urbanized stream, snails, which are herbivorous, may have responded through an increase in %P and a reduction in N:P and C:P ratios. Experimentally, Stelzer & Lamberti (2002) found a positive correlation between P-enriched periphyton and increased growth of the stream-dwelling snail *Elimia flavescens*. Furthermore, consumption of elevated P content in primary consumers (*e.g.* snails) would have a significant effect on the amount of P moving through the food web; *e.g.* in snail-eating fish it is likely to alleviate these consumers from P limitation (Rosemond *et al.*, 2002). Further research is needed for understanding these nutrient imbalances in snails occupying streams under different land use. Increased values of N and P in periphyton in urban streams cannot be fully attributed to nutrient loads as this study did not measure benthic algae across all streams and high algal content in the mixed periphyton can lead to changes in C:N:P ratios in urbanized streams (O'Brien & Wehr, 2010). High

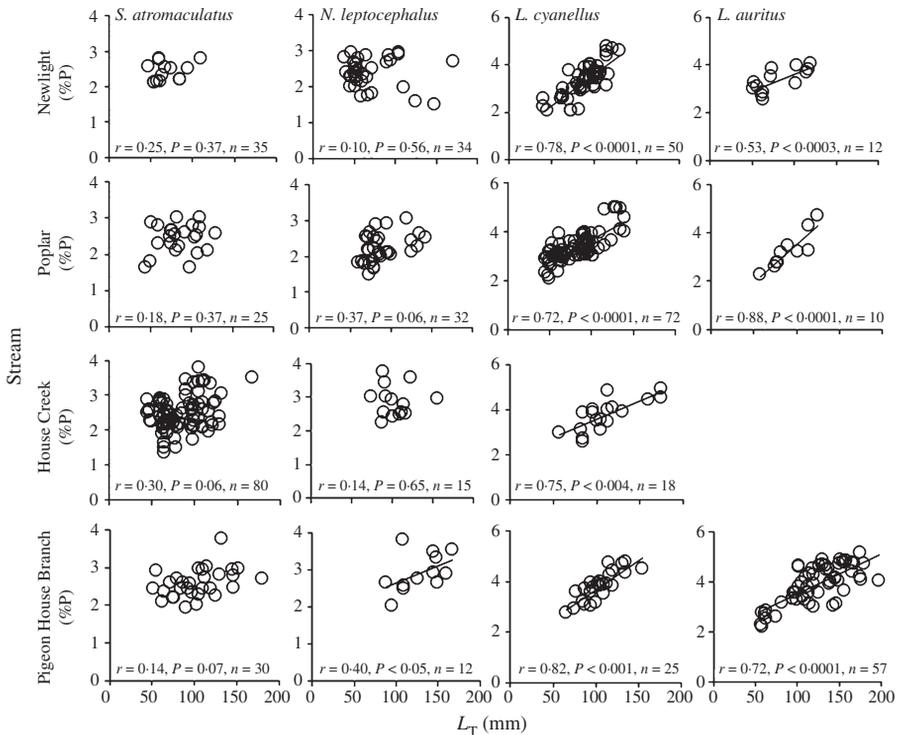


FIG. 5. Relationship between total length (L_T) and body %P content in *Semotilus atromaculatus*, *Nocomis leptocephalus*, *Lepomis cyanellus* and *Lepomis auritus* collected across four piedmont streams in Raleigh, North Carolina, U.S.A. Linear regressions were performed on individuals collected across a gradient of increasing urbanization. Significant relationships ($P < 0.05$) are designed by the presence of a trend line. N.B. *L. auritus* was not collected in House Creek.

values of SRP in the studied urban streams might however, have enhanced periphyton %N and %P. The elemental composition of primary consumers showed high variation in nutrient content and elemental ratios and seemed to track closely with changes in the main basal resources and increased with degree of urbanization. %P in snails and earthworms was higher at the most urbanized stream and consequently led to differences in N:P and C:N.

Vertebrates appear to maintain a balance of their elemental composition within a small range (*i.e.* strict homeostasis) regardless of the elemental composition of their prey (Sterner & Elser, 2002). Nutrient enrichment due to anthropogenic nutrient loads may however, lead to substantial spatial variation in the elemental composition of stream food-web components (Small & Pringle, 2010). In stream ecosystems, predators can become enriched in P when fed with P-enriched prey (Cross *et al.*, 2003; Small & Pringle, 2010). Downs *et al.* (2016) suggested high value body P in *L. macrochirus* is a result of increased P in basal resources (*i.e.* algae). Fishes living in North Carolina urban streams appear to deviate from strict homeostasis, where both the elemental composition of predators and their potential food resources vary across streams. For instance, *Lepomis* species were P-enriched, with higher enrichment observed at the most urbanized stream.

TABLE III. Results of the analysis of covariance (ANCOVA) of interspecific variation in %P content of four fish consumers collected across four Piedmont streams in North Carolina, U.S.A.

Species	Variables	<i>F</i>	d.f.	<i>P</i>
<i>Semotilus atromaculatus</i>	Stream site	0.3	3138	>0.05
	L_T	0.01	1138	>0.05
	Stream site $\times L_T$	1.03	3138	>0.05
<i>Nocomis leptocephalus</i>	Stream site	4.97	3 84	<0.01
	L_T	2.98	184	>0.05
	Stream site $\times L_T$	3.35	384	<0.05
<i>Lepomis auritus</i>	Stream site	0.31	270	>0.05
	L_T	19.60	170	<0.001
	Stream site $\times L_T$	2.32	270	>0.05
<i>Lepomis cyanellus</i>	Stream site	1.30	3157	>0.05
	L_T	146	1157	<0.001
	Stream site $\times L_T$	1.76	3157	>0.05

Cyprinids were relatively lower in P compared with centrarchids, probably indicative of an increase of lipid storage in fish with rapid growth (Sterner & Elser, 2002). *Lepomis cyanellus* had high C:N ratios reflecting a low %N content on its food resources, whereas high N:P ratios in *S. atromaculatus* and *N. leptocephalus* at the most urbanized stream might reflect consumption of low %P content. Variation in the elemental ratios of fishes can however, be a consequence of greater variation in the quality of basal resources and primary consumers that fishes feed upon (Sterner & Elser, 2002; Downs *et al.*, 2016). Species identity has been cited as an important attribute driving elemental ratios differences in fish taxa (Sterner & George, 2000; Hendrixson *et al.*, 2007; McIntyre & Flecker, 2010). Cyprinids and centrarchids differ in their bony skeleton tissues. Cyprinids are soft-rayed fishes with incomplete ossified cranium and have less bone tissues compared with other fishes, which suggests that they might not require high quantities of P-rich RNA to maintain their body mass (Sterner & George, 2000). Centrarchids are laterally compressed spiny-rayed fishes with heavily ossified craniums (Hendrixson *et al.*, 2007) that require high quantities of P to maintain their body mass composed of bone (Sterner & George, 2000; Hendrixson *et al.*, 2007). The centrarchid *L. cyanellus* contained high P concentrations leading to a significant lower N:P than the two cyprinids. Current explanations for such a pattern in the functional link between degree of ossification and internal skeleton exhibited by centrarchids is consistent with existing data showing that body P content is a function of increasing body size in most vertebrates (Hendrixson *et al.*, 2007; McIntyre & Flecker, 2010).

When compared with *L. cyanellus*, the cyprinid *S. atromaculatus* had higher mean body N:P suggesting the low body P content relative to body N content and the high body P content of sunfishes (Sterner & George, 2000; McIntyre & Flecker, 2010). There was a positive and significant correlation between body size and %P in *Lepomis* species, but no trends were observed in cyprinids. Findings from this study are consistent with previous studies from Downs *et al.* (2016) with *L. macrochirus*, Pilati & Vanni (2007) with gizzard shad *Dorosoma cepedianum* (LeSueur 1818) and Boros *et al.* (2015) with fathead minnow *Pimephales promelas* Rafinesque 1820 and sheepshead minnow *Cyprinodon variegatus* Lacépède 1803], who found ontogenetic

variation in body stoichiometry to be driven by allocation of P to bone tissues. Davis & Boyd (1978) reported decreasing %N and increasing %P in *L. macrochirus* and largemouth bass *Micropterus salmoides* (Lacépède 1802) with increasing body size.

Urban streams are sensitive to rapid changes in land-use practices that alter in-stream physical and chemical characteristics including nutrient load. Changes in nutrient load can affect the elemental composition of primary producers and ultimately, the entire food web. Increased urban development affects natural processes and evolutionary dynamics and adaptations of species, consequently driven rapid evolutionary changes in traits related to ecosystem function (Alberti *et al.*, 2017). Fish stoichiometry, although in part affected by familial identity and body size, appeared to be influenced by the variability of nutrient enrichment of their food resources, a finding consistent with other studies that examined ecosystem properties on urban streams (O'Brien & Wehr, 2010; Tsoi *et al.*, 2011). Urban development is known to affect multiple stream facets including species composition, structure and function (Kennen *et al.*, 2005). Our results indicate the importance of considering multiple interacting factors to accurately assess the effects of urbanization on nutrient availability, consumers and subsequently food-web structure and function.

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