

# The river continuum concept predicts prey assemblage structure for an insectivorous fish along a temperate riverscape

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**Abstract:** The river continuum concept (RCC) provides a framework for processes structuring lotic ecosystems by synthesizing sources and transport of C in streams. Considerable attention, refinement, and testing of the RCC has occurred since its inception >35 y ago, but few investigators have tested its predictions by explicitly linking consumer groups. We assessed insect assemblage structure in the diet of a broadly distributed insectivorous fish (*Cottus caroliniae*) in the Roaring River continuum of Tennessee to test 3 predictions from the RCC: 1) longitudinal change in relative biomass of insect functional feeding groups (FFGs) including decrease for shredders, increase for collectors, intermediate maximum for grazers, and consistency for predators; 2) maximum taxonomic diversity at stream orders 3 to 5; and 3) temporal turnover in taxonomic composition across 1 y. We found that relative biomass of insect FFGs consumed by *C. caroliniae* broadly matched predictions from the RCC. Maximum taxonomic diversity assessed at the family rank occurred at stream order 4 where diel and annual water temperature fluctuations were greatest, and monthly prey assemblages followed a sequence of turnover and a return to starting conditions across 1 y. Our novel approach illustrates proof of concept that RCC tenets are integrated into the diet of at least 1 higher-level consumer and, therefore, transcend assemblage boundaries in regulating the longitudinal (up- to downstream) and vertical (multiple consumer groups) flow of C in streams.

**Key words:** stream ecosystems, river zonation, river continuum concept, community ecology, predator–prey interactions, riverine landscapes

Advancing the field of ecology requires empirical application of theoretical concepts that describe the structuring of ecosystems. For example, the trophic–dynamic concept in ecology described by Lindeman (1942) was initially criticized because it lacked empirical support (Cook 1977) but is now regarded as a central theory in ecology and is still used to generate testable hypotheses (Dodds 2009, Layman et al. 2015). A parallel example of theoretical advancement in aquatic ecology was the pioneering work of Vannote et al. (1980) that established linkages between the distribution of energy production and insect feeding groups in streams ranging from small headwaters to large rivers (i.e., the river continuum concept [RCC]). The RCC posits that longitudinal (i.e., from up- to downstream) changes in canopy cover, subsidy deposition, energy production, and stream size govern aquatic communities and ecosystem

structure in streams (Vannote et al. 1980). The RCC was initially criticized because of insufficient empirical support and questionable transferability among biomes (Winterbourn et al. 1981, Barmuta and Lake 1982, Ward and Stanford 1983, Dudgeon 1984, Statzner and Higler 1985), although empirical support did emerge (Hawkins and Sedell 1981, Minshall et al. 1983, Grubaugh et al. 1997). Central tenets of the RCC are still used to generate testable predictions regarding aquatic invertebrate assemblage structure (e.g., Rosi-Marshall and Wallace 2002, Tomanova et al. 2007), and the resulting empirical support has contributed to designation of the RCC as a general theory in ecology (Dodds 2009). However, despite general acceptance of RCC ‘theory’ as a framework structuring aquatic invertebrate assemblages, virtually no tests have been based on direct linkages between invertebrates and their fish preda-

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tors. This situation is surprising given that fishes are typically the most nutrient-rich and dominant consumer group in streams (McIntyre and Flecker 2010).

Upward continuation of RCC assemblage structuring mechanisms from invertebrates to fishes is the primary pathway for C flow in stream ecosystems. The relative roles of bottom-up vs top-down control of assemblage and ecosystem structuring have long been debated (Power 1992), but little question exists that trophic interactions contribute to stream fish assemblage structuring (Giam and Olden 2016). Previous tests of the RCC documented strong trophic relationships between primary producers and invertebrate consumers and showed that invertebrate functional feeding groups (FFGs) vary along the river continuum (Rosi-Marshall and Wallace 2002, Tomanova et al. 2007). However, no consideration was given to if, or how, invertebrate assemblages were reflected in higher-level consumers, such as fishes. Vannote et al. (1980) included fishes in their original conceptual diagram of the RCC, but fishes received little consideration other than reference to dominance by insectivores in headwater streams and occurrence of planktivores in large rivers. Li et al. (1987) and Bayley and Li (1992) highlighted the potential for the RCC to predict fish-assemblage functional traits, such as increases in planktivores and herbivores downstream, and these predictions were later tested and largely confirmed globally, including streams in France (Oberdorff et al. 1993), the USA (Goldstein and Meador 2004), Bolivia (Pouilly et al. 2006), Gabon (Ibanez et al. 2007), and Brazil (Wolff et al. 2013). Previous investigators collectively illustrated that invertebrate and fish assemblages covary along stream size gradients, but none rigorously tested for mechanistic linkages between these assemblages. Given that fishes are integrated within the ecosystems they inhabit (Vanni 2010), detection of RCC-based patterns in the prey assemblages consumed by insectivorous fishes might provide multiconsumer-group proof of concept for the RCC. Insectivorous fishes occur consistently along entire river continua (Goldstein and Meador 2004), so RCC predictions might be tested by holding the identity of the insectivorous fish constant and assessing prey assemblage structure across streams of varying size.

At least 3 RCC predictions for invertebrate assemblage structure can be tested by using prey assemblages consumed by an insectivorous fish. According to the RCC, headwaters (stream orders 1–3; Strahler 1957) are primarily heterotrophic because canopy cover prevents major primary production and input of detritus in the form of coarse particulate organic matter (CPOM; particles >1 mm) is extensive (Vannote et al. 1980). Further downstream (orders 4–6), river systems change to primarily autotrophic because of increased input of fine particulate organic matter (FPOM; 50  $\mu\text{m}$ –1 mm) and increased primary production where canopy cover is sparse. However, primary production de-

clines where large rivers (orders 7+) become turbid and sunlight does not penetrate to the benthos. These longitudinal patterns influence assemblage structure of invertebrates based on the FFGs, including shredders, collectors, grazers, and predators, described by Cummins (1973). Thus, 1 testable prediction of the RCC involves the spatial structure of insect assemblages as measured by FFG designations. Shredders are predicted to show high relative biomass in headwater streams, but to decline in a downstream direction because of longitudinal reductions in CPOM; collectors are predicted to increase in larger order streams where FPOM is more prevalent; grazers are predicted to increase in mid-sized streams where canopy cover first attenuates; and predators are predicted to maintain consistent relative biomass throughout a river continuum because of consistent availability of prey items (Cummins 1974). A 2<sup>nd</sup> testable prediction of the RCC is that insect taxonomic diversity is greatest at intermediate stream orders (3–5), where water temperature variability is greatest (Vannote et al. 1980). A 3<sup>rd</sup> testable prediction of the RCC is that local assemblages will undergo temporal sequences of turnover in which different taxa contribute most to assemblage composition across an annual cycle. This prediction hinges on dynamic seasonal variation in abiotic factors that govern the manner in which biota use available energy and is absent among stream ecosystems with stable abiotic regimes (Statzner and Higler 1985). Though these predictions and their tests in stream ecosystems are not novel (Rosi-Marshall and Wallace 2002, Tomanova et al. 2007), use of fish prey assemblages to test the extent to which each of these predictions are integrated into a higher-level consumer group is an innovative approach to advancing ecological theory.

We used prey assemblages (i.e., all taxa consumed at a location at a given time) ingested by a broadly distributed insectivorous fish to test predictions derived from the RCC. *Cottus caroliniae* is a benthic fish that occupies stream orders 1 to 7 throughout the southeastern USA (Etnier and Starnes 1993) and exhibits broad diet niche breadth with little apparent prey selectivity (Phillips and Kilambi 1996). Recent works suggest *C. caroliniae* is an ideal candidate species for assessing the ecology of local ecosystems because populations use habitat similarly across streams of contrasting size (Gebhard et al. 2017) and exhibit restricted movement in which most individuals remain within a focal habitat (Wells et al. 2017). We collected specimens from sites distributed across stream orders 2 to 5 during 12 months and identified invertebrate prey to FFG and family. We predicted that: 1) prey FFGs would follow predictions outlined in the RCC, 2) local prey assemblage taxonomic diversity would be greatest at intermediate stream sizes, and 3) prey assemblages would follow a temporal sequence of turnover across an annual cycle. This approach allows us to assess whether and to what extent theoretical

predictions for aquatic invertebrate assemblage structure are reflected in the diet of a higher-level consumer. Confirmation that insect prey assemblages are regulated by the central tenets of the RCC would support a well-known theory describing flow of C, whereas lack of RCC pattern would support either specialized feeding by *C. carolinae* or contributions by untested mechanisms.

## METHODS

### Study area

We conducted our study at 4 sites in the Roaring River watershed in the Cumberland River basin in north-central Tennessee, USA (Fig. 1). We selected sites to represent a gradient in stream size ranging from order 2 through 5 (Strahler 1957). Sites were: Little Creek (LC; 2<sup>nd</sup> order; lat 36°11'40.5"N, long 85°32'38.4"W), West Blackburn

Fork (WB; 3<sup>rd</sup> order; lat 36°13'20.9"W, long 85°34'26.0"W), Blackburn Fork (BB; 4<sup>th</sup> order; lat 36°17'46.9"N, long 85°33'40.4"W), and Roaring River (RR; 5<sup>th</sup> order; lat 36°21'03.5"N, long 85°33'53.9"W). We classified streams by order based on stream-segment attributes from the National Hydrography Dataset (NHD; USGS 2012) and recorded hourly water temperature and depths at each site with a HOBO Model U20L Water Level Logger (Onset Computer Corporation, Bourne, Massachusetts). We measured stream discharge twice per month from April 2015 to March 2016 with the methods described by Turnipseed and Sauer (2010). At the completion of the study (April 2016), we measured channel width (m; as defined by Davies-Colley 1997) and canopy openness (measured with a concave densitometer facing upstream) at 10 transects distributed longitudinally at each site to summarize spatial variation in abiotic variables (Table 1).

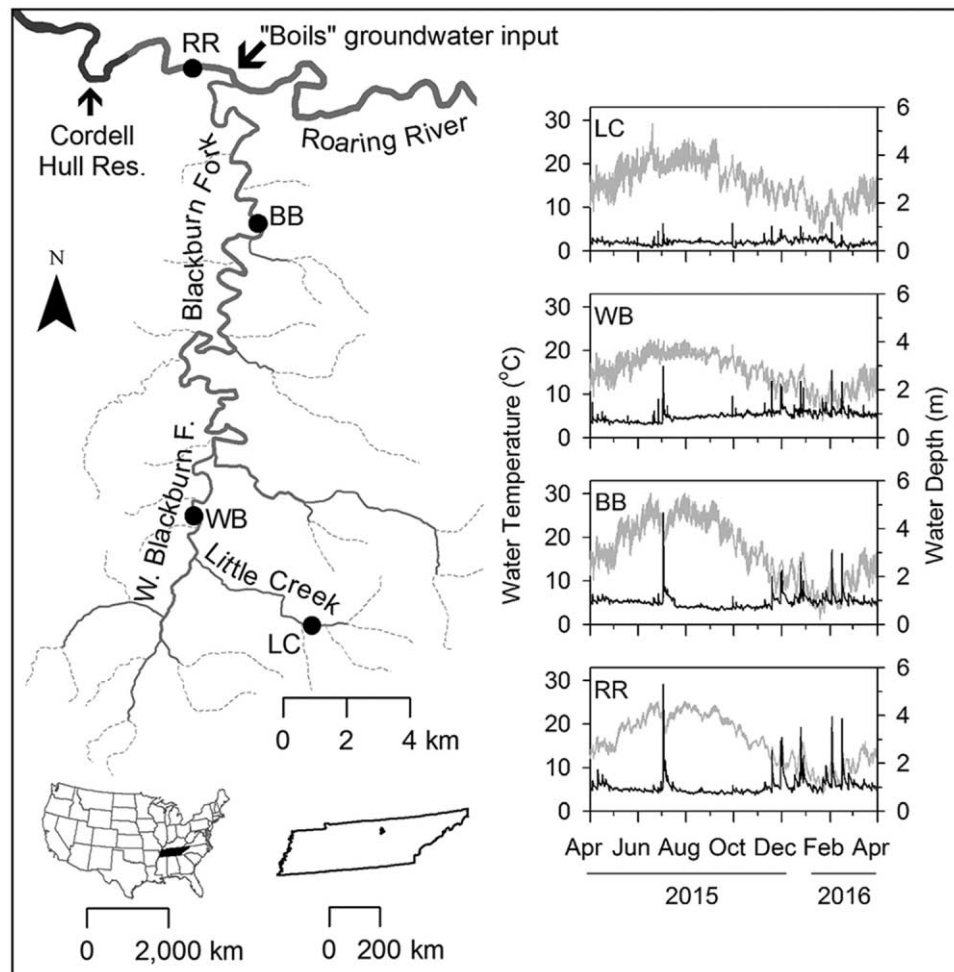


Figure 1. Map of study area showing distribution of sampling sites along the Roaring River continuum in north-central Tennessee, USA, including Little Creek (LC, order = 2), West Blackburn Fork (WB, order = 3), Blackburn Fork (BB, order = 4), and Roaring River (RR, order = 5). Hourly water temperature (gray lines) and depth (black lines) fluctuations over the course of the study are shown for each site.

Table 1. Sampling site name, code, stream order (SO; Strahler 1957), length of stream sampled, area of stream sampled, mean and range density of *Cottus carolinae*, mean active channel width (across 10 transects), mean and range discharge, mean and range water temperature, and canopy openness.

Site	Code	SO	Length (m)	Area (m <sup>2</sup> )	Density (fish/100 m <sup>2</sup> )	Width (m)	Discharge (m <sup>3</sup> /s)	Temperature (°C)	Canopy (%)
Little Creek	LC	2	353	1060	2.26 (0.94–4.72)	5.9	0.07 (0.001–0.18)	15.5 (3.9–29.3)	23
West Blackburn Fork	WB	3	227	680	3.66 (2.06–5.73)	16.1	0.56 (0.12–1.57)	15.1 (3.9–22.6)	30
Blackburn Fork	BB	4	106	385	2.25 (0.25–4.71)	19.9	1.87 (0.49–6.86)	16.8 (1.1–30.1)	98
Roaring River	RR	5	55	320	8.69 (2.2–20.37)	52.9	5.44 (1.33–11.49)	16.1 (3.9–25.2)	93

### Data collection

We made monthly collections of *C. carolinae* from each site from April 2015 through March 2016 with a combination of seining and backpack electrofishing. At sites LC and WB, we repeatedly sampled all habitats in defined stream reaches with single-pass backpack electrofishing (125 V, direct current) with 1 shocking unit and 2 netters (3.2-mm mesh). At sites BB and RR, we repeatedly sampled portions of riffle geomorphic units with a seine (4.5 × 1.8 m, 3.2-mm mesh) set facing upstream and a single backpack shocking unit positioned 3 m upstream of the seine, and shocked with a single, downstream pass so that fishes drifted into the seine (Gebhard and Perkin 2017). Because of known diel activity patterns of *C. carolinae* (Gebhard et al. 2017), we reversed the order of visits to sites (up- to downstream vs down- to upstream) during sampling to ensure diel (i.e., morning vs afternoon) effects caused by sampling were minimized. We tracked the area sampled and density of sculpin at each site (Table 1) and targeted a minimum of 10 individuals (ind) site<sup>-1</sup> mo<sup>-1</sup>. We anesthetized retained fishes with a lethal dose of MS-222 (80 mg/L) before preservation in 10% neutrally buffered formalin solution. In the laboratory, we removed stomachs by using dissection scissors to make incisions at the sphincters of the esophagus and intestine. We removed stomach contents and placed them in an empty Petri dish with a minimal amount of water to avoid desiccation during sorting, and we identified taxa with the aid of aquatic insect keys (Smith 2001, Merritt et al. 2008). We initially identified specimens to the lowest practical rank, generally genus or species except for insect parts that could be identified only to order, and then ‘scaled back’ resolution so that assemblage composition could be analyzed consistently across the riverscape. Family rank produced the most consistent pool of prey assemblage members across all sites and seasons. Once stomach contents were sorted, we blotted each taxonomic group with a paper towel to remove excess water before weighing them to the nearest 0.1 mg with an electronic balance.

### Statistical analyses

We tested for variation in *C. carolinae* size structure across sites and in prey assemblage diversity across fish

sizes and sites to ensure that pairwise comparisons in prey assemblages were not confounded by changes in fish size structure. We first tested for sitewise differences in fish lengths with bootstrap Kolmogorov–Smirnov (KS) tests from the *ks.boot* function in the *Matching* package in R (Sekhon 2011). We illustrated patterns as length–frequency histograms (bin size = 5 mm). Next, we estimated prey diversity for each fish based on the number of aquatic insect families consumed and tested for differences across fish sizes and sites with a generalized additive mixed model (GAMM). A GAMM was more appropriate than a general least-squares regression model because we sampled sites repeatedly (i.e., assumption of independence violated), residuals were heterogeneous (i.e., assumption of homoscedasticity violated), the error distribution was nonnormal (i.e., assumption of normality violated), and the potential relationship was not linear. We fit a GAMM with prey diversity as the response variable, total length as a smoothed predictor variable, site as a fixed factorial variable, and month as a random factorial variable with the *gamm* function in the *gamm4* package in R (Wood and Scheipl 2016).

Our first prediction was that relative biomass of FFGs would follow patterns described during the initial conception of the RCC (Vannote et al. 1980). We assigned each taxon identified from *C. carolinae* stomach samples to 1 of 4 FFGs (shredders, collectors, scrapers [grazers], and predators) based on existing literature (Hawkins and Sedell 1981, Smith 2001, Merritt et al. 2008). We excluded any fishes, noninsect invertebrates, parasites, organic (e.g., detritus), or inorganic (e.g., rocks) matter present in stomach samples. We elected to use ‘hard classifications’ in which each family was assigned a single FFG based on consensus among multiple literature sources listing primary trophic roles, but we acknowledge that FFG designations are sensitive to taxonomic resolution and that other FFG designations within family ranks are possible (Tomanova et al. 2007). We then summed biomass values for each FFG across all 12 mo for each site and calculated the relative proportion of each FFG. We tested all pairwise site comparisons of biomass distributions with the *multinomial.test* function from the *EMS* package in R. We used Monte Carlo (MC) simulations with 100,000 trials of 1,000,000 samples

(Menzel 2013). We report Pearson's  $\chi^2$  values for pairwise comparisons and the MC  $p$ -value. We also qualitatively compared the relative dominance of FFGs along the Roaring River continuum with expectations described by Vannote et al. (1980).

Our 2<sup>nd</sup> and 3<sup>rd</sup> predictions were that prey diversity would reach a maximum at intermediate stream sizes, and local prey assemblages would follow a temporal sequence of turnover. We constructed diet assemblages from stomach contents of individual *C. carolinae* that were expended during sampling, so we used nonmetric multidimensional scaling (NMDS) to test for differences in prey assemblages at the site level with individual stomachs as observational units. We first ran an NMDS analysis with all sites combined (i.e., global analysis) and used a permuted multivariate analysis of variance (PERMANOVA) to test for significant differences among sites and months using the *metaMDS* and *adonis* functions from the *vegan* package in R (Oksanen et al. 2016). We used the output from the global analysis to illustrate spatiotemporal separation of insect prey assemblages in multivariate space for the entire Roaring River continuum by plotting mean axis scores by month at each site and weighting symbol sizes according to the average number of families consumed during a month. The global analysis revealed significant and interactive differences among sites and months, so we next ran independent NMDS analyses for each site. For all NMDS analyses, we used occurrence data (i.e., presence/absence) for aquatic insect families to construct Jaccard dissimilarity distances among stomach samples. We measured site-specific diet diversity to test our 2<sup>nd</sup> prediction based on a generalized linear mixed model (GLMM). A GLMM was more appropriate than a repeated-measures analysis of variance (rmANOVA) because error distributions were nonnormal. We fit a GLMM with the number of aquatic insect families consumed as the response variable, site as a factorial fixed effect, month as a factorial random effect, and used a Poisson error distribution with the *glmer* function from the *lme4* package in R (Bates et al. 2015). To test our 3<sup>rd</sup> prediction, we assessed temporal turnover in prey assemblages at local sites with site-specific NMDS outputs and the *simper* function from the *vegan* package. We used a temporal sequence of pairwise comparisons (e.g., January–February, February–March, etc.) to assess which families had the highest contribution to dissimilarity between months and, thus, to prey assemblage turnover across the year. We conducted all analyses in R (version 3.2.3; R Project for Statistical Computing, Vienna, Austria).

## RESULTS

We collected and dissected 507 *C. carolinae*, and excluded 16 from analyses because of empty gut tracks (analysis  $n = 491$ ). Final sample sizes included 129 from LC (April 2015–March 2016; sample sizes:  $n = 9, 10, 8, 9,$

13, 10, 12, 10, 12, 13, 11, 12), 134 from WB ( $n = 11, 10, 7, 12, 12, 14, 11, 11, 13, 10, 11, 12$ ), 90 from BB ( $n = 10, 10, 6, 11, 4, 10, 4, 10, 4, 9, 12, 0$ ), and 138 from RR ( $n = 11, 10, 12, 16, 14, 12, 10, 9, 12, 10, 11, 11$ ). Fish sizes ranged from 39 to 135 mm among sites (Fig. 2A–D), and size distributions were similar in all pairwise comparisons with the exception of WB and BB (KS test,  $D = 0.19$ ,  $p = 0.04$ ; Table 2). We detected no relationship between the number of aquatic insect families consumed and the size of a fish (GAMM,  $F = 1.12$ ,  $p = 0.3$ ,  $n = 491$ ,  $R^2 = 0.02$ ).

Aquatic insects occurred in 76.4 to 90.8% of prey assemblages across sites, including 8 orders and 19 families (Table 3). Ephemeroptera occurred most frequently (42.1–57.1% across sites), followed by Trichoptera (21.8–45.1%), Diptera (14.3–34.6%), Plecoptera (7.7–15.7%), and Coleoptera (1.4–6.4%). Occurrences of aquatic insect families were highest for Heptageniidae (12.1–38.7%), Hydropsychidae (12.8–31.9%), Chironomidae (7.9–20.3%), and Baetidae (5.0–13.2%). Family-rank classifications of FFG included 10 predators, 4 collectors, 3 shredders, and 2 grazers. Although not included in analyses, terrestrial insect prey items

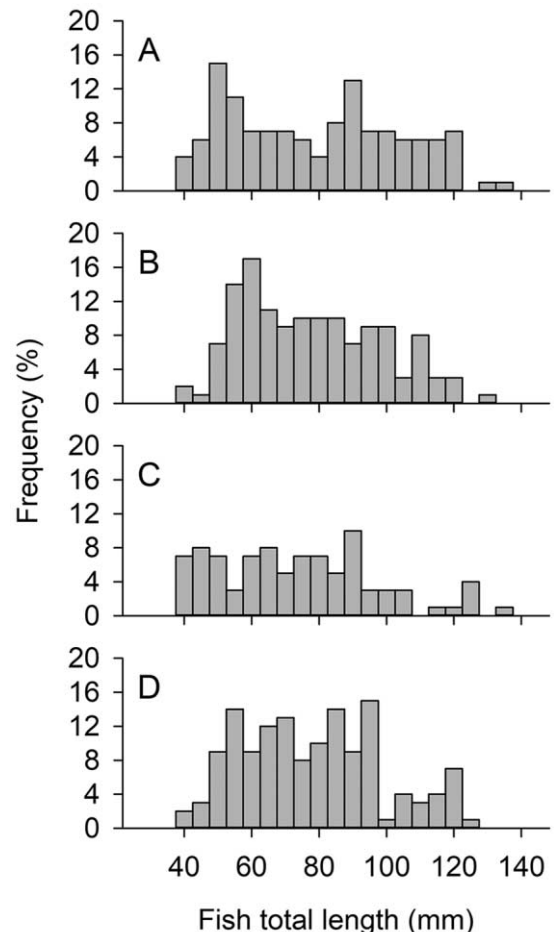


Figure 2. Length–frequency histograms for *Cottus carolinae* total lengths at Little Creek (A), West Blackburn Fork (B), Blackburn Fork (C), and Roaring River (D).

Table 2. Pairwise site comparisons of fish length–frequency distributions using bootstrap Kolmogorov–Smirnov tests. *D*-values are shown in the upper right and *p*-values are shown in the lower left. Bold indicates significantly different relationships.

Site	LC	WB	BB	RR
LC	–	0.13	0.16	0.12
WB	0.25	–	<b>0.19</b>	0.06
BB	0.12	<b>0.04</b>	–	0.15
RR	0.31	0.98	0.17	–

were consumed at 3 of the 4 sites but did not exceed 2.3% occurrence, salamanders were consumed at 1 site, fish were consumed at all 4 sites and ranged from 2.1 to 15.7% occurrence, plant matter was rarely ingested at all sites (0.7–4.5% occurrence), and rocks occurred in 3.3–14.3% of individuals across sites. Unidentifiable insect parts and organic matter (i.e., detritus) occurred in 38.6 to 57.4% of individuals.

Relative biomass of the 4 FFGs differed among sites ( $\chi^2 > 16.33$ ,  $p < 0.01$  across all pairwise comparisons) as streams transitioned from 2<sup>nd</sup> to 5<sup>th</sup> order (Fig. 3A–D). Shredder relative biomass was greatest at LC (20%) and WB (23%), but decreased downstream at BB (8%) and RR (15%). Collectors maintained consistent relative biomass in headwater sites including LC (20%) and WB (21%), but increased at BB (67%) and RR (40%). Grazer relative biomass was intermediate at LC (31%), highest at WB (47%), lowest at BB (11%), and intermediate at RR (29%). Predator relative biomass showed no clear pattern in across sites, including LC (28%), WB (9%), BB (14%), and RR (16%).

The taxonomic composition of *C. carolinae* prey assemblages differed spatially and temporally and was most diverse at BB. The global NMDS analysis of prey assemblage structure showed a significant site  $\times$  month interaction (PERMANOVA,  $PseudoF_{32,444} = 3.14$ ,  $p < 0.001$ ,  $R^2 = 0.15$ ). All prey assemblage samples combined showed overlap among sites in multivariate space (Fig. 4A). Family scores ranged from most negative for Hydropsychidae and Psephenidae along NMDS 1 to most positive for Corydalidae along NMDS 1, and from most negative for Aeshnidae and Athericidae along NMDS 2 to most positive for Perlidae, Calopterygidae, and Ceratopogonidae along NMDS 2 (Fig. 4B). Prey assemblage structures showed less overlap among sites when temporal trajectories were overlaid and symbols were weighted by the average number of families consumed (Fig. 4C–F). Parameter estimates from the GLMM showed that a higher number of aquatic insect families was consumed at BB than at other sites (Fig. 5).

Site-specific prey assemblages followed temporal sequences in turnover of aquatic insect families throughout the year. Assemblage composition significantly differed among months at LC (PERMANOVA,  $PseudoF_{11,117} = 6.32$ ,  $p = 0.001$ ,  $R^2 = 0.37$ ), WB (PERMANOVA,

$PseudoF_{11,122} = 2.08$ ,  $p = 0.001$ ,  $R^2 = 0.16$ ), BB (PERMANOVA,  $PseudoF_{10,79} = 3.14$ ,  $p = 0.001$ ,  $R^2 = 0.28$ ), and RR (PERMANOVA,  $PseudoF_{11,126} = 5.16$ ,  $p = 0.001$ ,  $R^2 = 0.31$ ). Proportional contributions to monthly differences in prey assemblage structure at LC were greatest for Perlidae, Heptageniidae, and Chironomidae in spring, Hydropsychidae and Chironomidae in summer, Psephenidae in autumn, and Heptageniidae in winter (Fig. 6A). At WB, Heptageniidae contributed most to monthly turnover in prey assemblages, but Chironomidae, Hydropsychidae, Baetidae, and Isonychiidae contributed to subtler turnover (Fig. 6B). At BB, Isonychiidae, Hydropsychidae, Carambidae, and Chironomidae contributed most to turnover (Fig. 6C), whereas at RR, Isonychiidae, Hydropsychidae, Heptageniidae, Corydalidae, and Leuctridae contributed most to turnover (Fig. 6D).

## DISCUSSION

Our study provides empirical evidence for linkages between stream gradients and their ecological properties. We found that the Roaring River continuum was characterized by increasing stream size, flow variability, and canopy openness among sampling sites in a downstream direction. Across this gradient, *C. carolinae* size structure and size-dependent prey consumption were largely consistent, but spatiotemporal trajectories in prey functional and taxonomic assemblages were not. Investigators have found that *C. carolinae* population dynamics are consistent across the same hydrologic gradient studied here (Gebhard and Perkin 2017), habitats used in the Roaring River are consistent with habitats used across the species' range (Gebhard et al. 2017), and the species is unlikely to move great distances beyond focal habitats (Wells et al. 2017). We leveraged these consistencies in *C. carolinae* ecology across stream gradients to test a general theory in stream ecology, the RCC. We predicted invertebrate prey assemblages consumed by *C. carolinae* would support 3 tenets of the RCC, including: 1) longitudinal organization of invertebrate FFGs, 2) peak taxonomic diversity at an intermediate stream size, and 3) temporal turnover in taxonomic contributions to diet dissimilarity throughout the year. We found that FFGs consumed by *C. carolinae* were consistent with expectations of longitudinal change derived from the RCC, particularly for shredders and collectors. Furthermore, the maximum diversity of prey items was consumed at BB, an intermediate site. Last, multivariate analysis of spatiotemporal patterns in assemblage change highlighted temporal turnover in prey assemblages through time. Although limited in their scale and extent, these findings provide proof of concept that RCC mechanisms regulating invertebrate assemblages are integrated within a higher-level consumer group across axes of space and time.

The longitudinal zonation of stream invertebrate FFGs is an artifact of spatial fluxes in the interactions between

Table 3. Taxon frequency of occurrence (mean % by mass) in prey assemblages consumed by *Cottus caroliniae* along the Roaring River continuum. Codes used in ordinations and functional feeding groups (FFGs) are given for each aquatic invertebrate family, including collectors (C), shredders (S), grazers (G), and predators (P). Locations for sites Little Creek (LC), West Blackburn Fork (WB), Blackburn Fork (BB), and Roaring River (RR) are given in Fig. 1.

Taxon	Code	FFG	LC	WB	BB	RR
Aquatic invertebrates	–	–	82.0 (49.2)	90.8 (49.4)	86.8 (41.2)	76.4 (24.5)
Coleoptera	–	–	3.8 (0.2)	6.4 (0.8)	3.3 (0.5)	1.4 (0.1)
Hydrophilidae	Hydroph	P	0.7 (<0.1)	0 (0)	1.1 (<0.1)	0 (0)
Psephenidae	Pseph	G	1.5 (0.1)	6.4 (0.8)	2.2 (0.4)	1.4 (0.1)
Decapoda	–	–	9.8 (21.6)	11.3 (24.4)	2.2 (2.4)	2.9 (3.9)
Diptera	–	–	34.6 (0.4)	23.4 (0.1)	22.0 (0.1)	14.3 (<0.1)
Ceratopogonidae	Cerat	P	0 (0)	0 (0)	1.1 (<0.1)	0 (0)
Chironomidae	Chiro	P	20.3 (0.2)	14.2 (0.1)	15.4 (<0.1)	7.9 (<0.1)
Athericidae	Ather	P	0 (0)	0 (0)	0 (0)	0.7 (<0.1)
Tipulidae	Tipul	S	5.3 (<0.1)	5 (<0.1)	4.4 (<0.1)	0 (0)
Ephemeroptera	–	–	42.1 (8.3)	56.7 (8.3)	57.1 (8.7)	48.6 (7.7)
Baetidae	Baeti	C	11.3 (0.4)	10 (0.3)	13.2 (0.3)	5.0 (0.1)
Ephemerellidae	Ephem	C	0.8 (0.1)	1.4 (0.2)	4.4 (0.5)	2.9 (0.3)
Heptageniidae	Hepta	G	23.3 (7.6)	38.7 (6.4)	12.1 (1.2)	20.0 (4.2)
Isonychiidae	Ison	C	0.8 (<0.1)	8.5 (0.7)	31.9 (6.2)	19.3 (2.0)
Gastropoda	–	–	0.8 (0.1)	0 (0)	0 (0)	0 (0)
Isopoda	–	–	1.5 (0.2)	0 (0)	0 (0)	0 (0)
Lepidoptera	–	–	0 (0)	4.3 (0.4)	6.6 (<0.1)	0 (0)
Carambidae	Cram	S	0 (0)	4.3 (0.4)	6.6 (<0.1)	0 (0)
Megaloptera	–	–	0.8 (<0.1)	0 (0)	7.7 (6.6)	7.9 (8.2)
Corydalidae	Coryd	P	0 (0)	0 (0)	6.6 (6.6)	5.7 (8.0)
Nematoda	–	–	6.8 (<0.1)	1.4 (<0.1)	0 (0)	1.4 (<0.1)
Odonata	–	–	4.5 (1.1)	0.7 (0.4)	2.2 (5.4)	0 (0)
Aeshnidae	Aesh	P	0 (0)	0 (0)	1.1 (2.5)	0 (0)
Calopterygidae	Calop	P	3.8 (<0.1)	0.7 (0.4)	1.1 (<0.1)	0 (0)
Gomphidae	Gomp	P	0 (0)	0 (0)	1.1 (0.9)	0 (0)
Libellulidae	Libel	P	0 (0)	0 (0)	1.1 (2.0)	0 (0)
Plecoptera	–	–	11.3 (1.5)	9.9 (0.2)	7.7 (0.1)	15.7 (1.4)
Leuctridae	Leuct	S	1.5 (<0.1)	0.7 (<0.1)	1.1 (<0.1)	12.1 (1.3)
Perlidae	Perli	P	9.8 (1.4)	3.5 (0.2)	1.1 (<0.1)	2.1 (0.1)
Trichoptera	–	–	21.8 (1.1)	32.6 (0.9)	45.1 (2.8)	26.4 (0.6)
Hydropsychidae	Hydrops	C	12.8 (0.8)	13.5 (0.6)	31.9 (2.0)	17.1 (0.5)
Oligochaeta	–	–	1.5 (7.2)	1.4 (2.3)	1.1 (5.6)	0 (0)
Terrestrial	–	–	2.3 (<0.1)	2.1 (<0.1)	0 (0)	0.7 (<0.1)
Salamander	–	–	0.8 (1.3)	1.4 (11.5)	0 (0)	0 (0)
Fish	–	–	11.9 (38)	2.1 (25.3)	9.9 (52.4)	15.7 (73.2)
Detritus	–	–	53.4 (10.4)	57.4 (12.1)	47.3 (6.1)	38.6 (2.3)
Plant Matter	–	–	4.5 (1)	2.1 (<0.1)	1.1 (0.2)	0.7 (<0.1)
Rocks	–	–	14.3 (1.1)	9.2 (1.6)	3.3 (0.2)	5.0 (<0.1)

terrestrial and stream ecosystems. The RCC posits headwater streams with large amounts of canopy cover should be primarily heterotrophic and characterized by large inputs of detritus and consequently abundant instream CPOM (Vannote et al. 1980). In these headwater streams, shredder

FFGs that are critical for breakdown of CPOM are predicted to out-number other groups, such as collectors that process FPOM and grazers that target autochthonous production. As streams increase in size in a downstream direction, canopies open, autochthonous production increases,

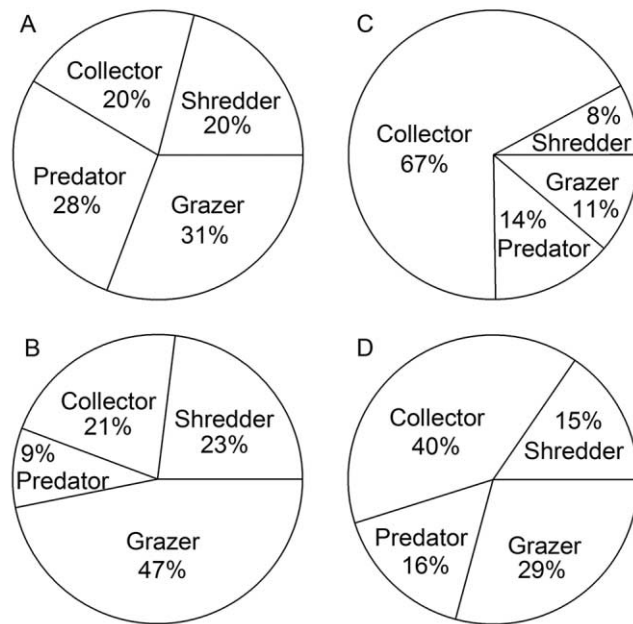


Figure 3. Relative biomass (g; wet mass) of aquatic insect functional feeding groups in prey communities consumed by *Cottus caroliniae* collected along the Roaring River continuum from Little Creek (A), West Blackburn Fork (B), Blackburn Fork (C), and Roaring River (D).

and dominance by CPOM is replaced by FPOM. Consistent with these habitat shifts along a stream continuum is the expectation that dominance by shredder FFGs will be replaced by dominance by collectors and grazers (Cummins 1973, 1974). We found that insect prey assemblages consumed by *C. caroliniae* contained higher relative biomass of shredders at up- (LC: 20%, WB: 23%) than downstream sites (BB: 8%, RR: 15%). Furthermore, relative biomass of collectors increased from up- (LC: 20%, WB: 21%) to downstream sites (BB: 67%, RR: 40%). These patterns match those predicted by Vannote et al. (1980) and reflect observations made during more contemporary tests of the RCC based on stream invertebrate assemblages sampled in the USA (Rosi-Marshall and Wallace 2002) and Bolivia (Tomanova et al. 2007). A major exception to our first prediction was that grazer biomass peaked at stream order 3 (WB) rather than 5 as predicted by Vannote et al. (1980). This inconsistency might be related to the relativity of gradient lengths and 'sliding scale' of shifts from heterotrophy to autotrophy in streams as determined by canopy openness (Minshall 1978, Dodds et al. 2012). Our data show the greatest shift in canopy openness between WB and BB, which is apparently an artifact of a 2010 flood that resulted in loss of large riparian trees and a shift from primarily closed to open canopies at BB and RR (M. Thurman, Tennessee Wildlife Resources Agency, personal communication). Consequently, the strongest shift in canopy cover in the Roaring River con-

tinuum was between orders 3 (WB, 30% open) and 4 (BB, 98% open), and this threshold change (as opposed to continual change) corresponded with a large change in grazer vs collector FFG dominance. If canopy openness were the primary regulator of grazer biomass, then the largest grazer biomass should have occurred at BB rather than WB. This pattern suggests that other mechanisms operated to regulate either the number of grazers available for consumption by *C. caroliniae* (i.e., extrinsic factors) or foraging behavior and prey selection by *C. caroliniae* (i.e., intrinsic factors) at the BB site. For example, local habitat influences on aquatic invertebrate abundance and the flood-affected habitats at BB (i.e., few pools, deposited substrates) might have extrinsically affected the abundance of grazers available for consumption (Grubaugh et al. 1997). Alternatively, the flood-affected habitats, specifically lack of deeper pools, might have limited diel foraging transitions from riffles to pools by *C. caroliniae* and thereby intrinsically altered prey selection or encounter rate (Greenberg and Holtzman 1987, Gebhard et al. 2017). We sampled only prey assemblages (instead of assemblages not consumed) and cannot test which of these causes is the case. Nevertheless, organization of local habitats can cause divergences from RCC-based predictions (Thorp and Delong 1994) and cannot be excluded as a potential cause of our unexpected observations.

Patterns in taxonomic diversity among prey assemblages consumed by *C. caroliniae* were shaped by scale-dependent spatial and temporal factors. The RCC predicts that medium-sized streams (3<sup>rd</sup>–5<sup>th</sup> order) with maximum fluctuations in temperature should exhibit the greatest levels of taxonomic diversity (Vannote et al. 1980). We found that taxonomic diversity measured at the family rank and among insects consumed by *C. caroliniae* was greatest at BB (order 4), the same site with the greatest fluctuation in water temperature. Statzner and Higgler (1985) suggested that a limitation of the RCC is that only diel fluctuations in temperature were considered by Vannote et al. (1980), and if annual amplitudes of temperature are overlaid, then the maximum variation is unlikely to occur at mid-reaches (orders 3–5). In our study, the maximum fluctuations in diel and annual temperatures did indeed occur at the same mid-reach site (BB; see Fig. 1 inset), and this observation lends credit to theoretical expectations from the RCC. We did not test for a mechanistic relationship between temperature fluctuation and diversity, but previous investigators have documented a linear relationship between maximum taxonomic diversity in aquatic insect assemblages measured at the family rank and maximum stream temperature (Jacobsen et al. 1997). Despite consistencies with the RCC, our use of family- rather than species-rank taxonomy means that any patterns in  $\beta$  diversity across these ranks confounded our findings. This limitation potentially was emphasized by the inherently degraded nature of ingested and partly digested prey removed



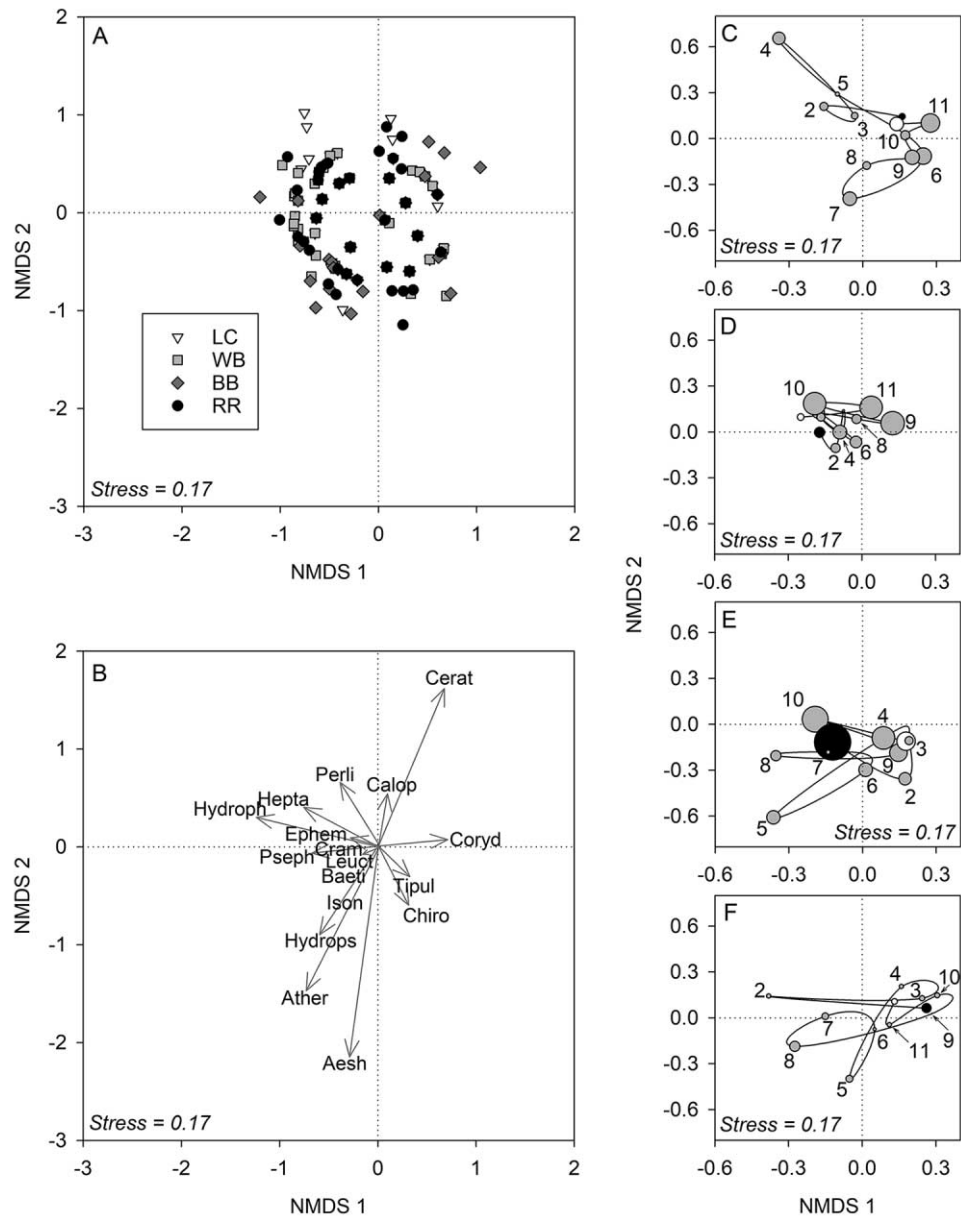


Figure 4. Nonmetric multidimensional scaling (NMDS) ordination of insect families consumed by *Cottus caroliniae* collected along the Roaring River continuum showing scores for all individuals across all sites (A) and for insect families (see Table 3 for abbreviations) (B) along NMDS 1 and 2, and temporal trajectories for monthly averages (1–12: Jan–Dec; January symbol is black; December symbol is white) shown as symbols sized in proportion to the number of insect families consumed at Little Creek (C), West Blackburn Fork (D), Blackburn Fork (E), and Roaring River (F). Length and angle of arrows in panel B show correlation of the relative abundance of insect families with the NMDS axes.

from the gut tract of fishes (Alexander and Perkin 2013). Furthermore, using fish diets to assess insect assemblage diversity might be confounded by prey selectivity of the focal fish (Goldstein and Meador 2004). This limitation highlights the utility of our focus on an opportunistic forager, such as *C. caroliniae* (Phillips and Kilambi 1996), that is unlikely to exhibit strong selectivity among the diversity of available prey items. That our findings align with the RCC despite these limitations suggests that concepts such

as functional redundancy within aquatic insect families (Poff et al. 2006, Boersma et al. 2014) enable us to detect patterns despite limited data resolution. Nevertheless, because we used relatively low taxonomic and identification resolutions, our work should be viewed as a coarse-scale test of RCC predictions.

Temporal turnover in prey assemblage composition consistent with RCC predictions was evident, but this evidence was not consistent across space. The RCC predicts that

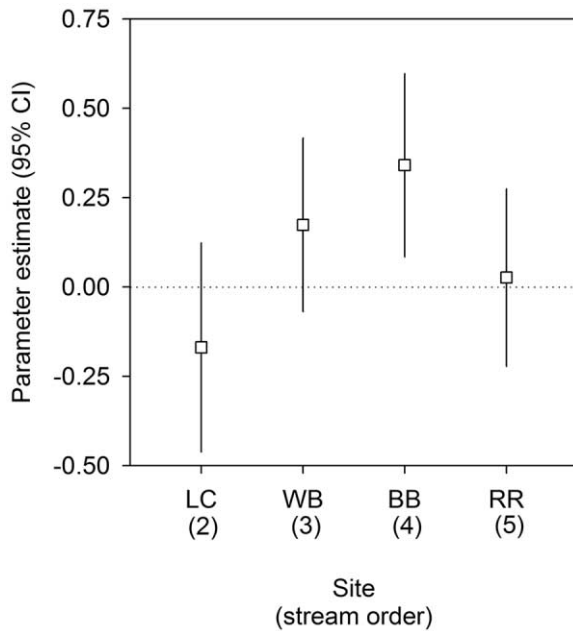


Figure 5. Site-specific parameter estimates (95% confidence interval [CI]) for a generalized linear mixed model fit to the number of insect families consumed by *Cottus caroliniae* at Little Creek (LC), West Blackburn Fork (WB), Blackburn Fork (BB), and Roaring River (RR). The 95% CI for BB does not overlap 0, illustrating significantly higher prey diversity at that site than at the others.

continuous replacements of species in local assemblages operates to maximize energy use in streams (Vannote et al. 1980). Essentially, as one species completes its life history, another rises to fill the void niche (Korhonen et al. 2010). We found that unique families contributed consistent levels of dissimilarity across the year at LC, BB, and RR. The exception was at WB, where Heptageniidae was the dominant contributor to assemblage variability year-round. Statzner and Higler (1985) criticized this tenet of the RCC and suggested that such patterns are limited to temperate biomes where annual fluctuations in temperature are most obvious. The site at which we observed deviation from RCC predictions was the site with the least obvious flux in annual temperature. The stability of water temperature at WB created a more stable ecosystem than would be predicted based on the position of the site within the river continuum, most likely because of groundwater inflow. The region of the Cumberland Plateau included in our study is characterized by percolation of water into shallow aquifers at upstream sites where water is stored and buffered from atmospheric conditions for short periods before being returned to stream channels (Mayfield 1986). The WB site is an area of groundwater return characterized by stabilized flow and water temperature regimes, and the Boils Wildlife Management Area near the RR site is another major

return (Gebhard and Perkin 2017). Both groundwater return sites had signatures of reduced variability in their annual (WB) and diel (RR) temperature profile plots (see Fig. 1). The observed increase in Heptageniidae abundance and year-round contribution to diet dissimilarity at WB might have been a consequence of these groundwater inflows. For example, Brown et al. (2007) found greater

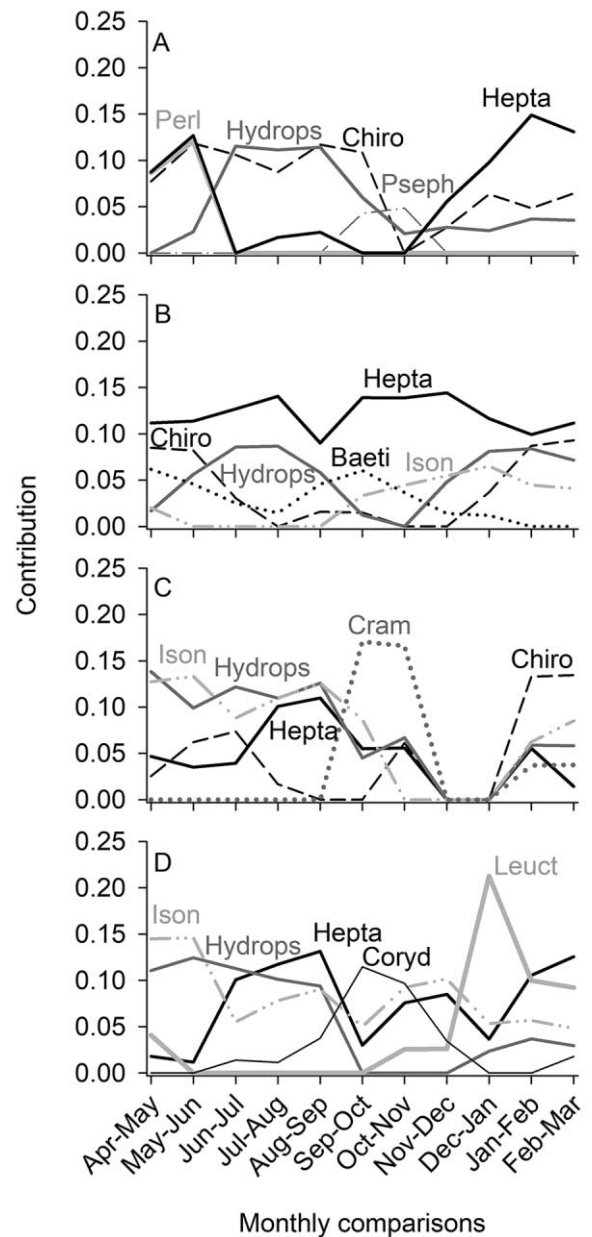


Figure 6. Temporal sequences in the proportional contribution of insect families to differences in prey assemblages consumed by *Cottus caroliniae* collected along the Roaring River continuum from Little Creek (A), West Blackburn Fork (B), Blackburn Fork (C), and Roaring River (D). Codes for insect families are given in Table 3.

abundance of Ephemeroptera taxa at sites with greater groundwater inputs and suggested that matches between physiochemical variables and invertebrate life histories were at least one mechanism for more stable invertebrate community composition. Greater stability (i.e., less variability) in prey assemblage composition at the WB site was apparent in the tight clustering in multivariate space relative to all other sites. Furthermore, despite dominance by a single family at this site, NMDS analyses illustrated annual variation in prey assemblages characterized by temporal flux and a return to trajectory starting point across all sites. These patterns reflect the concept of loose equilibrium in which community composition varies but remains within a confined boundary over time (May 1973, Matthews and Marsh-Matthews 2016) and suggests that although change at WB was subtle, evidence of annual turnover was present as predicted by the RCC.

Our coarse-scale tests and general confirmations of theoretical expectations from the RCC operated within a set of spatial and temporal bounds. For example, the scale of our study included a strong and uninterrupted hydrologic gradient, but such intact river continua are becoming increasingly rare because of stream habitat fragmentation on a global scale (Ward and Stanford 1983, Lehner et al. 2011). Extending the spatial extent of our study without encountering anthropogenic discontinuity would not have been possible because the Roaring and Cumberland river confluence is impounded by Cordell Hull Reservoir just downstream of our study area. Other RCC-interrupting mechanisms, including large changes in altitude (Tomano et al. 2007) and strong effects from agricultural land use (DeLong and Brusven 1998, Harding et al. 1999), are absent from our study system. Our study lacks replication among river continua because of the intensity of sampling and laboratory processing time. However, the Roaring River continuum is considered representative of others in the southeastern USA (Crumby et al. 1990). Furthermore, we conducted our study within a temperate biome, and applicability of the RCC is likely to be greatest in this biome (Statzner and Higler 1985), though adjustments to theoretical frameworks can be made for specific biomes (Dodds et al. 2015). For example, Thorp et al. (2006) developed the Riverine Ecosystem Synthesis (RES) to describe rivers as downstream arrays of hydrogeomorphic patches governed by climate, flow, and local geomorphic processes rather than as continua. The RES represents an amalgamation of portions of other riverine research frameworks, including the RCC, flood-pulse concept (Junk et al. 1989), and riverine productivity model (Thorp and DeLong 1994), and is capable of explaining deviations from predictions generated by any one of these frameworks. However, the RES has been criticized because of its inherent complexity and lack of testable hypotheses (Humphries et al. 2014). Despite previous criticisms and recent modifications to the

RCC, we found evidence of patterns predicted by the RCC in the Roaring River continuum. We hypothesize that flow pulses that propagate throughout the Roaring River basin provide longitudinal connectivity during high flows capable of reinforcing RCC-mechanisms in a manner consistent with the River Wave Concept (Humphries et al. 2014) because hydrologic connectivity is maintained during these periods (Pringle 2003). These pulses (waves) probably are strong enough and occur frequently enough to override local processes that promote deviation from community structuring mechanisms described by the RCC. Thus, predictable patterns in invertebrate assemblage composition persist and are integrated into fish diets.

Our work advances ecological theory by providing additional empirical support derived through a novel approach. Marquet et al. (2014) suggested that ecology should rely on 'efficient theories' characterized by foundations built on law-like postulates, mathematical expressions, and large numbers of predictions with as few free parameters as possible. Dodds (2009) proposed that the RCC 'theory' is built on principle laws such as, among many others, system openness (i.e., closed ecological systems do not exist), energy requirement (i.e., all organisms require energy for maintenance and reproduction), and nutrient cycling (i.e., all organisms must transform nutrients to operate). The mathematical foundations of the RCC are obvious, including ratios of productivity to respiration, FFG relative biomass, and relative taxonomic diversity (which can be measured at multiple taxonomic ranks using multiple formulae). The RCC contains multiple testable hypotheses based on central tenets for which support has been generated on a global scale. Our results suggest the RCC is, in fact, an efficient theory capable of expanding our understanding of aquatic ecology even >35 y after its conception and can be applied in multiconsumer-group frameworks to link longitudinal (up-to downstream) and vertical (multiple trophic levels) flow of C in streams.

## ACKNOWLEDGEMENTS

Author contributions: WJC, AEG, and JSP collected data in the field, WJC and AEG processed samples in the laboratory, JSP conducted statistical analyses, and all authors wrote portions of the manuscript.

This work was funded by the Department of Biology at Tennessee Technological University. Fish collections were approved by the Tennessee Wildlife Resources Agency (permit 1729 to JSP) and animal handling protocols were approved by the Tennessee Tech Institutional Animal Care and Use Committee (permit TTU-IACUC-14-15-001 to JSP). We thank C. Harty, T. Johnson, S. Johnston, E. Malone, R. Paine, J. Ridgway, Z. Tankersley, and J. Wellemeyer for assistance with field sampling and B. Cook for assistance with invertebrate identification. This manuscript was improved by comments from N. Knorp, C. Murray, D. Walker, the FTP journal club, and 2 anonymous referees.

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