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Diet composition of two larval headwater stream salamanders and spatial distribution of prey

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SUMMARY

1. Interspecific overlap in resource use can determine the degree to which different presumed guild members have distinct or similar effects on ecosystem processes or responses to environmental change. Many headwater streams of North America support multiple species of larval salamanders commonly defined as a single guild that can influence macroinvertebrate communities and nutrient dynamics and are sensitive to stream alteration.

2. We explored macroinvertebrate distributions in conjunction with stable isotope and gut content analyses of salamanders to examine similarities in diet between two sympatric larval salamander species (*Desmognathus quadramaculatus* and *Eurycea cirrigera*) in four headwater streams. We determined the degree to which larval salamanders used similar prey functional feeding groups (FFGs) and taxa and determined the primary source habitat (pools versus riffles) of prey.

3. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) suggest the two salamander species occupied similar trophic positions and individual-based stable isotope mixing models indicated similar use of macroinvertebrate predators and filterers by both species. Diet analyses were generally consistent with stable isotope results in identifying prey FFGs that composed the largest biomass of salamander diets. However, despite similarities in diet at the resolution of FFGs, there was little overlap in the specific taxa consumed by the two salamander species: 52 prey taxa were consumed over all samples, with only 16 taxa in common. Further, only five prey taxa were common in dominating diet biomass of both species; there was more overlap in taxa in terms of diet abundance.

4. We assessed patterns in benthic macroinvertebrate biomass and compared the biomass of taxa that were in pools versus riffles to the biomass and abundance of taxa in salamander diets. Total macroinvertebrate biomass was generally higher in pools; however, the majority of salamander prey biomass was from riffle habitats, a trend that was stronger for *D. quadramaculatus* than for *E. cirrigera*. There was greater similarity in taxa comprising diet by abundance with the majority of prey items originating from pools.

5. The larval salamander species used similar prey FFGs but differed significantly in specific prey taxa. We hypothesise that species differences in diets were most likely a function of differences in larval size and microhabitat use. Consequently, the treatment of larval salamanders as a guild is probably inadequate for predicting the effect of larval salamander diversity on some stream processes, and species may differ in how they respond to factors affecting prey assemblages.

Keywords: food web, headwater stream, mixing model, salamander, stable isotope

Introduction

Predatory vertebrates play important roles in regulating communities and ecosystem processes in freshwater ecosystems. These vertebrates affect the composition and abundance of macroinvertebrate communities and affect temporal and spatial dynamics of energy flow and nutrient dynamics (Carpenter, Cottingham & Schindler, 1992; Vanni, 2002; Regester, Lips & Whiles, 2006; Small, Helton & Kazanci, 2009). For systems with diverse

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predator guilds, it is important to understand the degree to which guild members, defined through utilisation of similar resources (Root, 1967), overlap in resource use. Such information is important for understanding community dynamics (Simberloff & Dayan, 1991), predicting the resilience of ecosystem processes to species losses (Walker, 1992; Schwartz *et al.*, 2000), and for determining whether all members of a guild will respond similar to environmental change (Simberloff & Dayan, 1991). For guild members that are taxonomically closely related, it is often assumed that responses of one species to resource shifts are generalisable to all closely related members of the guild (Simberloff & Dayan, 1991). These assumptions may oversimplify the response of guild members to changes in prey availability or system perturbations.

In headwater streams of eastern and north-western North America, salamanders are often the most abundant vertebrate predators of stream macroinvertebrates. The larvae of salamanders can affect macroinvertebrate composition and abundance (Keitzer & Goforth, 2013a) as well as stream nutrient dynamics (Keitzer & Goforth, 2013b; Milanovich, Maerz & Rosemond, 2015). Salamander assemblages have been characterised extensively as guilds (Davic, 1983; Hairston, 1987; Davic & Welsh, 2004), and indeed, some environmental stressors appear to have consistent effects among sympatric species. For example, Johnson *et al.* (2006) and Bumpers *et al.* (in press) have shown size or growth rate of two larval salamander species, *Eurycea wilderae* and *Desmognathus quadramaculatus*, increased similarly in response to experimental nutrient enrichments. Therefore, consistent with the guild designation, the response of one larval salamander species to resource availability appears generalisable to other members of the guild. However, descriptions of their diets indicate that larval salamanders use taxa representing multiple trophic levels and invertebrate guilds [functional feeding groups (FFGs)] and that there is high variability in prey use within and among species, generally linked to consumer size (Burton, 1976; Davic, 1983, 1991; Hairston, 1987; Johnson & Wallace, 2005). Recent experimental work has demonstrated species-specific effects of *E. wilderae* and *D. quadramaculatus* on stream macroinvertebrate assemblages due to differential consumption of FFGs (Keitzer & Goforth, 2013a). Diet comparisons suggest distinct prey use by larval salamander species; thus, studies of *E. wilderae* reported a dominance of Chironomidae and copepod prey (Johnson & Wallace, 2005; Barrett *et al.*, 2012), while *D. quadramaculatus* diets are more diverse and include a greater proportion of larger-bodied prey (Davic, 1991). Bumpers (2014) found shifts in larval *D. quadramaculatus* but not in larval *E. wilderae* diets in response to nutrient enrichment. These studies suggest important differences in resource use among larval salamander species, and the need for finer-resolution diet studies to inform our understanding of resource use in larval salamander guilds.

Here, we use stable isotopic analyses in conjunction with high-resolution taxonomic identification of gut contents to compare the contributions of specific macroinvertebrate taxa and their defined FFGs to the diets of larval blackbelly salamanders (*D. quadramaculatus*; hereafter *Dq*) and southern two-lined salamanders (*Eurycea cirrigera*; hereafter *Ec*). Because stream macroinvertebrates may seek particular habitat patches (Palmer *et al.*, 2000), we also measured available prey biomass and abundance to determine whether larval salamander prey may be biased to a specific in-stream habitat (pools versus riffles). Larval *Eurycea* spp. and *Dq* are the two most abundant species and account for the majority of larval salamander biomass in southern Appalachian headwater streams (Milanovich *et al.*, 2015). *Dq* has a 24- to 48-month larval period and is larger than *Ec*, which has a 12- to 14-month larval period, and *Dq* is reported to occur in faster flowing portions of streams and to consume a variety of small and large taxa (Davic, 1991; Mills, 1996). In contrast, *Ec* is reported to primarily occur within leaf packs and fine detritus in slower velocity stream habitats and to feed predominantly on smaller taxa, particularly Chironomidae and copepods (Petranka, 1998; Muenz *et al.*, 2008). We note, however, that although these genera and other salamander species are typically classified as a guild, few studies have previously quantified and compared prey use among salamander species concurrently in the same streams (but see Bumpers, 2014).

Methods

Study sites

Study sites were within the Dawson Forest Wildlife Management Area and Potts Mountain, located in the Blue Ridge region of north Georgia near Jasper, Georgia, USA (34°44′N 844°22′W). We studied four streams in forested catchments, all of which are second-order, fishless headwater tributaries of the Etowah River with catchment sizes ranging from 0.6 to 1.05 km^2. The streams were typical of cold-water, Appalachian mountain streams with coarse cobble and gravel bed material. Catchments were relatively intact with closed canopies

Table 1 Mean (±SD) site characteristics of the four headwater streams investigated. Mean active channel width, mean pebble size, and % pool and % riffle were determined February–April 2008. Nutrient concentrations (NO_3-N, NH_4-N, PO_4-P), total nitrogen (TN) and total phosphorus (TP) were sampled monthly from July 2008 to February 2009 (means and SD of monthly means $n = 7$ per stream, each month mean was typically based on $n = 10$ water samples)

Stream	Active channel width (m)	Pebble size (mm)	NO_3-N ($\mu g\ L^{-1}$)	NH_4-N ($\mu g\ L^{-1}$)	PO_4-P ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	% Pool	% Riffle
1	1.92 (0.49)	62 (99)	7.0 (8.0)	44.3 (38.0)	0.9 (2.0)	186.2 (332.0)	10.2 (13.0)	59.51	40.49
2	2.99 (0.73)	57 (77)	11.0 (9.0)	71.0 (111.0)	3.6 (3.0)	180.0 (190.0)	10.9 (9.0)	65.75	34.25
3	1.77 (0.45)	33 (53)	10.5 (14.0)	38.1 (45.0)	0.2 (0.0)	130.0 (35.0)	9.8 (12.0)	50.13	49.87
4	2.03 (0.36)	45 (69)	18.6 (9.0)	36.4 (32.0)	1.1 (3.0)	236.7 (98.0)	9.5 (10.0)	50	50

and little to no development. All study reaches were 100 m. Nutrient concentrations were low and similar among streams (mean TN < 240 $\mu g\ L^{-1}$, mean TP < 11 $\mu g\ L^{-1}$; Table 1). The representation of pool and riffle habitats in the study streams was reasonably consistent (pool 66–50%, riffle 50–34%; Table 1).

Estimation of macroinvertebrate taxonomic composition and biomass

Macroinvertebrate taxonomic composition and biomass were estimated in autumn (November 2008), spring (April 2009) and summer (July 2009) in both pools and riffles ($n = 5$ of each per season per stream). Riffles were sampled using a Surber sampler (250-μm mesh, 0.09-m^2 sampling area) and hand scrubbing substratums within the sampler for three minutes. Pools were sampled with a stovepipe corer (0.04 m^2). The top 10 cm of sediment from the corer was removed and elutriated in the field by rinsing through a 250-μm sieve (Roy *et al.*, 2003). Samples were returned to the laboratory on ice and preserved in 70% ethanol. All large (>1 mm) and small (<1 mm) macroinvertebrates were handpicked using a dissecting microscope at 10× magnification. In rare instances, small macroinvertebrates were subsampled after being handpicked using a Folsom plankton splitter (McEwen, Johnson & Folsom, 1954). Macroinvertebrate insect larvae were identified to genus using standard taxonomic keys (Merritt, Cummins & Berg, 2007). Chironomidae were identified as Tanypodinae or non-Tanypodinae, and non-insects were identified to order or higher (e.g. oligochaetes, nematodes, copepods). All macroinvertebrates were measured to the nearest millimetre and population-level macroinvertebrate biomass was determined as AFDM using literature-based length–mass regressions (Benke *et al.*, 1999; J. B. Wallace, unpubl. data). Macroinvertebrates were assigned as FFGs based on morpho-behavioural food acquisition (Merritt *et al.*, 2007) or identified as terrestrial. For each macroinvertebrate FFG, we tested for season and microhabitat differences of macroinvertebrate FFG biomass using 2-way ANOVA in R (version 2.10.1; R Development Core Team, 2010)

Salamander diet analyses

Gut contents were identified and quantified for *Dq* ($n = 45$; 23 in spring, 22 in summer) and *Ec* ($n = 43$; 21 in spring, 22 in summer). Salamanders were collected at night, when larvae were most active, using an aquarium dip net (1-mm mesh) and headlamp while turning over rocks and leaf litter in the stream (Johnson & Wallace, 2005). Salamanders were immediately euthanised with a 0.5% solution of tricaine methanesulfonate (MS-222), neutral pH buffered with sodium bicarbonate. Immediately after euthanasia, salamanders were rinsed with deionised water, and tails were removed, transferred to vials and placed immediately on ice for stable isotope analysis (Milanovich & Maerz, 2012). The remaining body was preserved in Kahle's solution in the field to preserve gut contents (Stehr, 1987). In the laboratory, each animal's snout–vent length (SVL: from the tip of the snout to the posterior portion of the vent) was measured to the nearest millimetre and then, guts were removed under a dissecting microscope and the gut contents teased out. Macroinvertebrate prey were identified to genus when possible except for Chironomidae, which were identified as either non-Tanypodinae or Tanypodinae, and non-insect taxa (e.g. nematodes, copepods) and terrestrial taxa were identified to order or higher (Merritt *et al.*, 2007). All prey items were counted to determine prey abundance and measured to the nearest millimetre using an ocular micrometre (Johnson & Wallace, 2005) to determine prey biomass. Prey biomass (AFDM) was estimated using length–mass or head width–mass regressions based on genera or in some cases family (Sample *et al.*, 1993; Benke *et al.*, 1999; Sabo, Bastow & Power, 2002). Proportion of diet was

determined based on biomass and abundance. A small number of terrestrial invertebrates were found in larval *Dq* guts only ($n = 5$ ants, $n = 2$ Coleoptera of 198 prey items), but their percentages were not included in further analyses to keep our data focussed on aquatic prey resources.

To help determine whether salamanders were utilising prey in pool versus riffle habitats, we assessed whether taxa were dominant in pool or riffle habitat from our stream benthic samples. Specifically, we determined whether more than 75% of total biomass of a given taxon was associated with pool or riffle habitat and identified them as either 'pool dominants' or 'riffle dominants' accordingly (some taxa were not dominant in either habitat and were identified as 'both'). We then determined which taxa were dominant in salamander diets by biomass by identifying those taxa that made up more than 1.5% of gut biomass (as an arbitrary 'highly utilised prey' reference point). We also determined which taxa were dominant in salamander diets by abundance by identifying those taxa that made up more than 5% of gut abundance. The source habitat (based on benthic samples) of dominant taxa contributing to both diet biomass and diet abundance was then assessed.

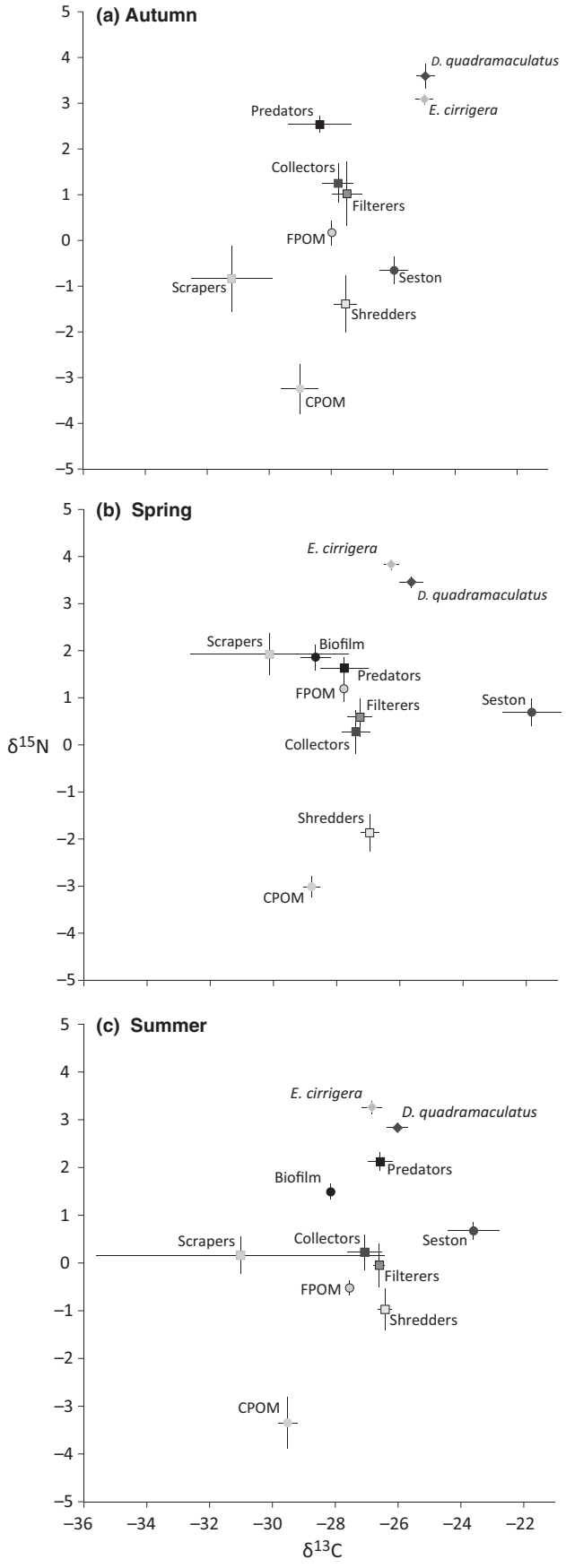
Stable isotope analyses of basal resources, macroinvertebrates and salamanders

Samples for stable isotopes were collected for a variety of basal food resources including seston, fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM) and biofilm, as well as macroinvertebrate and salamander taxa. Within 2 weeks of macroinvertebrate biomass sampling, isotopic analysis sampling for all food-web components was conducted. For data analyses, streams were used as replicates and means of sample types were determined by each stream and season. Basal resource samples consisted of seston ($n = 2$), FPOM (<1 mm, >0.7 μm; $n = 5$), CPOM (>1 mm; $n = 3$), and biofilm (5 hard substratum – rock, 5 soft substratum – sand; $n = 10$), for a total of 20 samples per stream per date. Seston samples were collected upstream and downstream of the 100-m sampling reach (3 L each) prior to any stream sampling. FPOM was obtained by disturbing the top 1 cm of streambed at five haphazardly selected locations per stream and sifting organic matter through a 1-mm sieve. Samples were then filtered onto pre-ashed glass fibre filters (0.7 μm), oven-dried at 60 °C, scraped from filters and ground. CPOM samples consisted of grab samples of leaf litter of terrestrial origin collected at three haphazardly selected locations per stream that were subsequently oven-dried at 60 °C and ground. Biofilm samples were collected along the study reach selecting either rock or sand substratum using a Loeb sampler (modified after Loeb, 1981). Both biofilm slurry and seston samples were returned to the laboratory and lyophilised prior to analyses.

For each stream and sampling date, we collected on average 12 numerically dominant macroinvertebrate taxa for stable isotope analysis per stream per season (for a total of 144 macroinvertebrate isotopic samples) that were then averaged to obtain FFG values. Specimens were fresh-frozen, and guts were later removed before being dried and ground for stable isotope analysis. Macroinvertebrate isotope samples ranged from 1 to 10 individuals per sample depending on size and rarity, with some instances of Chironomidae (separate Tanypodinae and non-Tanypodinae) up to 180 individuals per sample. Salamander tails were dried at 60 °C and homogenised. Samples were then analysed by combusting in a Carlo Erba (Milan, Italy) NA 1500 CHN analyser coupled to a Finnigan Delta C mass spectrometer (Thermo Electron Corp., Waltham, MA, U.S.A.) as a continuous flow system.

Analysis of salamander dependence on prey FFG using isotope mixing models

To predict FFG prey composition for individual larval salamanders based on stable isotope values, we used an individual hierarchical Bayesian mixing model designed by Semmens *et al.* (2009) that incorporates multiple sources of uncertainty including variance in source isotope values (Moore & Semmens, 2008). This modelling approach is valuable in that it both estimates the composition of individual salamander diet and also the variation in diet among particular portions of the population (Semmens *et al.*, 2009). We used the model to estimate variability in diet composition among both spatial (stream) and temporal scales (season). The proportional contribution of each prey FFG to salamander isotopic composition was evaluated using MixSIR run in the program R (R 2.10.1; R Development Core Team, 2010) and JAGS (Semmens *et al.*, 2009). To assess the variation in trophic relationships among salamander species among seasons, individual salamander isotopes were analysed for each season and average per cent contribution of each macroinvertebrate FFG was determined for each salamander species. Individual isotopic values of macroinvertebrates were combined to obtain average FFG prey values for each season. We used isotopic trophic fractionation values for $\delta^{13}C$ and $\delta^{15}N$ of



1.67 ± 0.12 and 2.43 ± 0.25, respectively. These values are lower than 'standard' values assumed in most isotope studies (Post, 2002) but were based on published values for taxonomically relevant species (e.g. amphibians; Whiles *et al*., 2006; Schiesari, Werner & Kling, 2009) and on values derived from feeding experiments of treefrog tadpoles (J. C. Maerz, unpubl. data). Using uninformed priors, the MixSIR model ran for 8×10^4 iterations, resulting in convergence on posterior source contributions of the different FFG prey items of the diet of individual salamander species (Moore & Semmens, 2008). Results of the model are presented as mean and standard deviation. Because *Dq* exhibited a significant negative relationship between $\delta^{15}N$ and body size (SVL) from hatching up to 20 mm SVL that we believe reflects a maternal signature (Trice, 2011), only individuals larger than 20 mm SVL were averaged from the mixing model results and for bi-plots.

Results

Stable isotope signatures and mixing model results

Stable isotopic signatures in salamander tissues were consistent with a top trophic position in these stream food webs in regard to $\delta^{15}N$ and reliance on multiple potential food resources in regard to $\delta^{13}C$ (Fig. 1). Although mean values of $\delta^{15}N$ were roughly consistent across seasons, there was significant individual variation in these values (Trice, 2011). Salamander $\delta^{13}C$ values were intermediate among potential food resources in spring and summer but were less depleted than signa-

Fig. 1 $\delta^{13}C$ and $\delta^{15}N$ values of salamander species (*Desmognathus quadramaculatus* and *Eurycea cirrigera*), macroinvertebrate functional feeding groups (collectors, predators, scrapers, shredders and filterers) and basal resources (CPOM, seston, biofilm, leaf litter and FPOM). (a) Autumn 2008, (b) spring 2009, and (c) summer 2009. Mean (± SE) incorporates signatures over all streams for the appropriate category per season. CPOM is coarse particulate organic matter. FPOM denotes fine particulate organic matter. Biofilm was not sampled in autumn 2008. Collectors included *Dixa* (Diptera), *Hexagenia* (Ephemeroptera), non-Tanypodinae Chironomidae (Diptera) and Oligochaeta. Predators included *Aeshnidae* (Odonata), *Arigomphus* (Odonata), *Beloneuria* (Plecoptera), Ceratopogonidae (Diptera), *Cordulegaster* (Odonata), *Dicranota* (Diptera), *Gomphus* (Odonata), *Hexatoma* (Diptera), *Nigronia* (Megaloptera), *Rhyacophila* (Trichoptera), *Tabanus* (Diptera) and Tanypodinae (Diptera). Scrapers included *Eccoptura* (Plecoptera). Shredders included *Hydatophylax* (Trichoptera), *Lepidostoma* (Trichoptera), *Pycnopsyche* (Trichoptera), *Tallaperla* (Plecoptera) and *Tipula* (Diptera). Filterers included *Diplectrona* (Trichoptera), Simuliidae (Diptera) and *Parapsyche* (Trichoptera).

Table 2 Proportion of prey items contributing to carbon and nitrogen stable isotopes of salamander species seasonally (autumn, spring and summer), using MixSIR. Per cent gut content biomass of salamander species across all individuals is included for each prey item

		Desmognathus quadramaculatus		*Eurycea cirrigera*	
Season	FFG	% mixing model	% gut content	% mixing model	% gut content
Autumn	C	27.94 ± 38.01	–	30.44 ± 43.90	–
	P	22.90 ± 20.93	–	1.58 ± 9.38	–
	Sc	0.61 ± 4.70	–	0.54 ± 4.88	–
	Sh	4.99 ± 17.37	–	5.49 ± 19.93	–
	F	43.59 ± 40.27		61.94 ± 46.24	
Spring	C	5.51 ± 9.62	12.86	7.13 ± 8.97	32.46
	P	46.21 ± 15.89	49.03	29.79 ± 14.06	40.16
	Sc	6.09 ± 7.52	12.35	17.84 ± 10.70	0
	Sh	0.90 ± 1.43	4.59	1.32 ± 1.76	27.36
	F	41.29 ± 20.40	21.17	43.92 ± 16.78	0.01
Summer	C	10.51 ± 21.57	3.68	69.29 ± 41.55	38.15
	P	69.83 ± 25.34	24.28	15.50 ± 28.28	0.67
	Sc	8.72 ± 12.75	8.17	9.85 ± 21.90	46.99
	Sh	1.63 ± 6.55	1.86	1.55 ± 6.92	2.09
	F	9.33 ± 17.59	62.01	3.83 ± 12.38	12.10

C, collector; P, predator; Sc, scraper; Sh, shredder and F, filterer. Values are means from individual salamander isotopic model results for which there was significant variation. Mixing model results are listed as % ± SD.

tures of all potential macroinvertebrate prey mean values in autumn, perhaps owing to maternal signatures or early ontogenetic shifts in diet. In general, salamanders were supported ultimately by allochthonous resources in that FFG $\delta^{13}C$ signatures (and consideration of $\delta^{15}N$) aligned more with CPOM and FPOM than with other candidate resources. CPOM values were within the expected ranges of terrestrial-derived carbon (−28.7 to −29.5‰) and were more depleted in $\delta^{15}N$ compared to other food resources. Seston was typically less depleted in $\delta^{13}C$ than most of the macroinvertebrate FFGs. Biofilms had elevated $\delta^{15}N$ relative to macroinvertebrate FFGs, indicating that this was probably not a significant resource for prey taxa.

Mixing model estimates indicated similar patterns of macroinvertebrate FFG contributions to both larval salamander species (Table 2). Mixing model estimates of autumn, spring and summer samples all indicated a high contribution of macroinvertebrate predators to *Dq* diets. Estimates from autumn and spring samples also indicated a significant contribution of filterers to *Dq* diets comparable to contributions from predators, and estimates from summer samples indicated a modest 11% contribution of collectors and 9% scrapers to *Dq* diets. Estimates from autumn and summer samples indicated significant contributions of collectors to *Ec* diets. Estimates from spring samples indicated a contribution of predators, scrapers and filterers to larval *Ec* diets, and estimates from autumn samples indicated a modest 5% contribution of shredders to larval *Ec* diets with 62% attributed to filterers (Table 2).

Diet analyses

There were distinct differences in the taxonomic composition of *Dq* and *Ec* diets. Overall, 52 taxa were consumed by the larval salamanders in our samples, with only 16 taxa in common (see Table S2). *Dq* had a greater proportion of unique prey items compared with *Ec*, indicating greater potential diet breadth (27 taxa unique to *Dq* and 9 taxa unique to *Ec* diets) (Trice, 2011). These unique taxa found in diets were in a range of larvae size classes (see Table S2). To evaluate patterns in diet overlap between *Dq* and *Ec* over the dominant taxa consumed, we considered the degree of overlap for those prey taxa that we designated as 'highly utilised prey' by biomass. Only five of the 16 taxa in common comprised at least 1.5% of prey biomass for both species (Table 3). When combining both spring and summer diet data to investigate prey trends, predators and filterers comprised most of the diet of *Dq*, while collectors made up the highest percentage biomass in *Ec* diets, followed by scrapers and then predators. High per cent biomass taxa in *Dq* guts were the predator *Isoperla* (Plecoptera), filterer *Diplectrona* (Trichoptera) and collector *Paraleptophlebia* (Ephemeroptera), whereas the high per cent biomass taxa in *Ec* guts were predator Tanypodinae chironomids (Diptera) and collectors *Baetis* and *Paraleptophlebia* (Ephemeroptera). A scraper *Maccaffertium* (Ephemeroptera) and shredder *Tipula* (Diptera) also contributed significantly to gut contents of *Ec*.

Dq and *Ec* diets had some similarities when comparing prey taxa abundance. Although only a small portion of biomass, non-Tanypodinae chironomids were the most numerically dominant source of prey for both *Dq* and *Ec*. Other high abundance taxa in *Dq* guts were the collector *Paraleptophlebia* (Ephemeroptera), predator Tanypodinae chironomids and predator Ceratopogonidae (Diptera), all of which were also dominant in prey biomass (Table 3). With respect to *Ec* diets, the filterer Ostracoda, collectors Copepoda and *Paraleptophlebia* (Ephemeroptera) and predator Ceratopogonidae (Diptera) were high in abundance (Table 3 legend).

Dq had greater gut biomass and greater average number of prey items per gut compared with *Ec* (average gut biomass: 1.4 mg versus 0.55 mg AFDM; average

Table 3 Prey taxa comprising more than 1.5% biomass of *Desmognathus quadramaculatus* and *Eurycea cirrigera* gut contents (combining spring and summer)

D. quadramaculatus					*E. cirrigera*				
Prey taxa (only taxa > 1.5%)	*N*	Total biomass (mg AFDM)	% biomass (%AFDM/ total AFDM)	Habitat	Prey taxa (only taxa > 1.5%)	*N*	Total biomass (mg AFDM)	% biomass (%AFDM/ total AFDM)	Habitat
Aquatic Prey					Aquatic Prey				
Collectors (7.71%)					**Collectors (34.06%)**				
Paraleptophlebia sp.*	14	3.27	7.71	R	Ephemeroptera				
					Baetis sp.	3	3.44	14.50	R
Predators (35.99%)					*Paraleptophlebia* sp.*	13	3.43	14.45	R
Odonata					Diptera				
Calopteryx sp.	1	1.22	2.88	P	Non-Tanypodinae	36	1.21	5.11	P
Diptera									
Ceratopogonidae	10	1.78	4.20	P	**Predators (16.89%)**				
Tanypodinae*	14	1.13	2.67	B	Diptera				
Plecoptera					Tanypodinae*	4	4.01	16.89	B
Isoperla sp.	7	9.63	22.71	R					
Sweltsa sp.	1	1.50	3.54	B	**Scrapers (22.76%)**				
					Ephemeroptera				
Scrapers (9.25%)					*Maccaffertium* sp.*	1	2.48	10.43	R
Ephemeroptera					*Stenonema femoratum**	4	0.88	3.70	R
Maccaffertium sp.*	4	1.69	3.99	R	Trichoptera				
*Stenonema femoratum**	4	2.23	5.26	R	*Psilotreta* sp.	3	2.05	8.63	B
Filterers (33.72%)					**Shredders (11.67%)**				
Ephemeroptera					Diptera				
Isonychia sp.	1	2.18	5.14	NA	*Tipula* sp.	2	2.77	11.67	B
Trichoptera									
Diplectrona sp.*	5	8.14	19.20	R	**Filterers (5.55%)**				
Dolophilodes sp.	5	0.96	2.26	R	Trichoptera				
Wormaldia sp.	2	3.02	7.12	R	*Diplectrona* sp.*	3	1.32	5.55	R

N denotes the abundance found in all guts of each species. Total biomass is total biomass of a given prey taxon in the gut measured as mg AFDM. Per cent biomass is total biomass of that particular diet item divided by total diet biomass of all taxa multiplied by 100 for each salamander species. The percentages for different functional feeding groups here differ from those in Table 2 in that they integrate across both seasons when guts were sampled (spring and summer, only accounting for those taxa >1.5% in biomass). *Denotes prey taxa in common to both salamander species. Habitat associations of prey are designated as P = pool, R = riffle and B = both pool and riffle. P or R designations are denoted when >75% of biomass was found in a given habitat from benthic samples; B designation if percentages were <75%; NA for *Isonychia* sp., which was found in diet but not in benthic samples. Prey that dominated abundance of gut content (>5%) and their dominant habitat type for *Dq* were non-Tanypodinae chironomids (20.8%, P), *Paraleptophlebia* (7.3%, R), Tanypodinae chironomids (7.3%, B) and Ceratopogonidae (5.2%, P). These proportions and habitat for *Ec* were non-Tanypodinae chironomids (29%, P), Ostracoda (11.3%, P), *Paraleptophlebia* (10.5%, R), Ceratopogonidae (6.5%, P) and Copepoda (6.5%, P).

number of prey items per gut: 4.3 and 2.8, respectively, for *Dq* and *Ec*). A greater relative difference in biomass (2.54×) than abundance (1.54×) of prey utilised by *Dq* versus *Ec* reflects larger prey consumed by *Dq*.

Habitat-specific quantification of macroinvertebrate biomass in relation to salamander diets

Macroinvertebrate biomass was determined from a total of 102 021 macroinvertebrates collected from replicate pool and riffle habitats in the four study streams over three seasons. Total biomass varied markedly among seasons ($F_{2,119} = 4.12$, $P = 0.02$) (see Table S1). Total biomass was highest in pools in spring, being twice as high as riffles at that time and at least 3× higher than in other seasons (see Figure S1). At the level of FFGs, collectors comprised the majority of macroinvertebrate biomass but did not differ among habitats or seasons (see Figure S1b). Predator biomass also did not differ among habitats or seasons. Scrapers exhibited the highest biomass in spring and in riffles (see Figure S1d). Taxa comprising the majority of biomass (greater than 1.5%) in different FFGs were as follows: collector biomass was dominated by *Hexagenia* (Ephemeroptera), non-Tanypodinae (Diptera) and *Serratella* (Ephemeroptera); predator biomass was dominated by *Arigomphus* (Odonata), Ceratopogonidae (Diptera), *Cordulegaster* (Odonata) and *Hexatoma* (Diptera); filterer biomass was dominated by

Diplectrona (Trichoptera); scraper biomass was dominated by *Psilotreta* (Trichoptera); and shredder biomass was dominated by *Hydatophylax* (Trichoptera) and *Tipula* (Diptera) (A. E. Trice, unpubl. data).

Spatial distribution of prey important in diets

For both *Dq* and *Ec*, taxa that were dominant in diets by biomass were largely from riffle habitats. Seven of the 12 taxa that composed at least 1.5% of *Dq* prey biomass and five of nine taxa that composed at least 1.5% of *Ec* prey biomass were identified as riffle dominants (Table 3). Specifically, 75% of the prey taxa that constituted modest or significant amounts of salamander diet biomass were associated with riffle habitat. Only three taxa consumed by both species were pool dominants (some taxa were designated as not dominant in either habitat). *Isonychia* sp. was found in *Dq* diets but was not collected in our benthic samples. The dependence on riffles was a bit stronger for *Dq* than *Ec* (with 68% versus 49% of prey taxa being riffle dominants, respectively) (Table 3).

There was greater overlap between the two salamander species in terms of prey abundance, with some distinctions (Table 3). Dipterans (non-Tanypodinae chironomids and Ceratopogonidae) were pool dominants and comprised roughly 25–35% of diets based on abundance for both salamander species. *Paraleptophlebia*, a riffle dominant, was also abundant in both diets. Only 41% of *Dq*, but 57% of *Ec*, diet abundance could be accounted for by taxa comprising more than 5% abundance, consistent with a more diverse diet for *Dq*.

Discussion

Important distinctions were found between the two salamander species in diet composition and sources of prey. While there was similarity in prey utilisation evaluated at the FFG level (in terms of both stable isotope and diet analyses), there were clear distinctions in the unique prey taxa used. These differences were greater when evaluated in terms of taxon richness and biomass and less so for abundance. Our findings indicate that these sympatric species act as a guild in some respects but not others. Specifically, the two salamanders may respond similarly to large changes in the resource base of streams that affect production of prey in different FFGs, but they are predicted to affect populations of prey rather differently. In addition, the majority of prey biomass eaten by both species was derived from riffle habitats, but *Dq* derived relatively more biomass from riffles than *Ec*. Thus, shifts in in-stream habitat may be expected to affect the two salamander species differently.

To the degree that members of macroinvertebrate FFGs in allochthonous streams are likely to respond similar to shifts in resource availability, growth responses of larval salamander species should be roughly similar. Moreover, the consumptive effects of larval salamanders on ecosystem processes via the exploitation of particular FFGs may be similar among species despite the use of different specific prey taxa. For example, *Dq* and *E. wilderae* occur in similar abundance (Milanovich *et al.*, 2015), and *Dq* and *Ec* consumed similar amounts of filterer and collector FFG taxa in our study. Therefore, the two species may have similar top-down effects on processes mediated through those FFGs. However, our results and those of Keitzer & Goforth (2013a) indicate that ignoring species-specific diet differences would hamper the ability to predict effects of larval salamander diversity on stream processes. Keitzer & Goforth (2013a) found *Dq* and *E. wilderae* had species-specific, additive predatory effects on stream macroinvertebrate composition, which is consistent with our finding that the two larval salamander species generally consumed distinct taxa within FFGs. Although we cannot rule out prey selection as a mechanism for diet differences, we hypothesise that diet differences between the larval salamander species emerged largely as a result of different microhabitat preferences and larval size effects in terms of gape limitation. *Dq* are larger (range 13.7–37 SVL, median 21.5) than *Ec* (range 10.8–29.5 SVL, median 18.8), consume larger prey and derived most of their prey from riffle habitats in our study. Therefore, our findings and those of Keitzer & Goforth (2013a) support niche complementarity between *Dq* and larval *Eurycea* spp. and suggest that declines in larval salamander diversity would affect stream communities and processes differently despite similar use of macroinvertebrate FFGs among larval salamander species.

What prey sources and in-stream habitats support larval salamander production?

We found some overlap in dominant taxa in benthic samples and those in salamander diets, including *Diplectrona* (Trichoptera, filterer), Ceratopogonidae (Diptera, predator), non-Tanypodinae chironomids (Diptera, collector), *Psilotreta* (Trichoptera, scraper) and *Tipula* (Diptera, shredder). A comparison of diet and benthic samples showed that the 52 prey taxa identified in salamander diets represented 62% of the macroinvertebrate

species identified in benthic samples. Thus, larval salamander communities derive energy from much of the wide variety of stream macroinvertebrates. Such a diverse food base may help buffer these predators against natural or anthropogenic changes in prey composition.

Our results also show macroinvertebrate distributions were different between pool and riffle habitats. Pools tended to have a higher biomass of total macroinvertebrates, particularly during spring. However, the biomass of taxa common in larval salamander diets was greater in riffles. Additionally, riffles were important habitats for the biomass of scraper and shredder taxa that were consumed by larval salamanders. These patterns suggest that *Dq* and *Ec* rely more on the production of macroinvertebrates in riffles, despite generally higher biomass of macroinvertebrates in pools. With respect to abundance, non-Tanypodinae chironomids (Diptera, collector) were dominant in pool habitats and were numerically dominant in both *Dq* and *Ec* diets. *Paraleptophlebia* (Ephemeroptera, collector) was abundant in *Dq* diets and was prevalent in riffle habitats, whereas Ostracoda (filterer) was abundant within *Ec* diets and dominant in pools. Thus, abundance of prey for both salamanders was generally dominated by smaller pool-associated taxa, but biomass of prey consumed was dominated by larger riffle-associated taxa. These distinctions may simply be a function of encounter frequencies and time spent in the two habitats, or they may allow salamanders to use a variety of prey, as we observed, and help them meet their dietary needs. From an energetic perspective, however, salamanders were acquiring most of their carbon from taxa associated with riffle habitats.

How well do isotope mixing models agree with direct diet sampling?

It is important to note that while in general agreement, results from diet samples and stable isotope mixing models suggest different levels of importance for various FFGs; there were several potential sources of uncertainty that may affect our estimates from stable isotopes and diet samples. First, linking consumers to different resources depends on assumptions about stable isotope trophic fractionation, which can vary within and among consumer species and among prey types for the same consumer, and also depends on other factors such as whether the animal is undergoing rapid growth (Post, 2002; del Rio & Wolf, 2005). In the absence of known fractionation values for our focal taxa, we used the best available values reported for amphibians (J. C. Maerz, unpubl. data; Whiles *et al.*, 2006; Schiesari *et al.*, 2009). Based on measured stable isotope values for salamanders and prey, and direct information on salamander diets, our assumed fractionation values for stable isotopes appear reasonable and therefore probably are not responsible for discrepancies between diet and stable isotope estimates of diet contributions. Second, prey with similar stable isotope values can be difficult to distinguish, and Bayesian models will tend to converge on the prey species that is more variable. Collector, filterer and predatory macroinvertebrates had similar $\delta^{13}C$ values, but predators were more variable, which may explain why their contribution appears overestimated, in some cases, by mixing models. Third, the use of FFGs aggregates stable isotope values that may obscure relationships between predators and prey. For example, predatory macroinvertebrates varied significantly in stable isotope values by at least one trophic level, and some collectors and filterers can consume both FPOM and animal material. Fourth, not all of the disparities may be attributable to uncertainties associated with the use of stable isotopes. Diet samples are snapshots while stable isotopes are more integrative. Individuals in our samples were collected in close temporal proximity (a few nights), which would bias diet samples towards those prey most abundant or available during that brief time period. Also, there are differences between what an organism ingests and what actually contributes to the production of tissues. Prey may be assimilated differently depending on digestibility (e.g. amount of exoskeleton) and stoichiometry (Mihuc & Toetz, 1994), which would be reflected in stable isotope but not diet data. Therefore, we cannot defer to the results of one methodology and for now must focus on areas in which both approaches agree.

Implications of anthropogenic disturbance on food resources and effects of salamanders

Evidence from stable isotopes and diet analyses suggests that, ultimately, salamanders derived energy from prey supported by allochthonous detrital resources (collectors, filterers and shredders) and, to a lesser extent, autochthonous resources (scrapers). This reliance may make salamanders vulnerable to landscape activities that reduce energy flows from terrestrial ecosystems. Experimental and descriptive studies have highlighted the dependence of salamanders on detrital resources and shown negative impacts via abundance, biomass, growth or production related to reductions in detritus availability or riparian forest cover (Johnson & Wallace, 2005; Huntsman *et al.*, 2011; Cecala, 2012). In addition to

riparian forest loss, factors that affect size of prey (e.g. anthropogenic nutrient inputs (Davis *et al*., 2010) and temperature) may affect relative prey availability and selectivity by these gape-limited predators. Our results also suggest that processes that lead to reductions in riffle habitat, such as sedimentation, may have negative effects on prey availability for salamanders and that these effects might differ among species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Macroinvertebrate biomass in pool and riffle habitat.

Table S1. ANOVA results for total macroinvertebrate biomass and FFG biomass (collectors, predators, scrapers, shredders and filterers) testing differences in season, substratum and stream.

Table S2. Diet data from *Desmognathus quadramaculatus* (DQ) and *Eurycea cirrigera* (EC).

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