

# Using stable isotopes to test for trophic niche partitioning: a case study with stream salamanders and fish

ADAM J. SEPULVEDA\*, WINSOR H. LOWE<sup>†</sup> AND PETER P. MARRA<sup>‡</sup>

\*US Geological Survey, Northern Rocky Mountain Science Center, Bozeman, MT, U.S.A.

<sup>†</sup>Division of Biological Sciences, University of Montana, Missoula, MT, U.S.A.

<sup>‡</sup>Smithsonian Conservation Biology Institute, Migratory Bird Center, National Zoological Park, Washington DC, U.S.A.

## SUMMARY

1. Stream salamanders and fish often co-occur even though fish prey on and outcompete salamanders. However, the mechanisms that allow palatable salamanders to coexist with fish are unknown.
2. We tested mechanisms in the field that promote coexistence between Idaho giant salamanders (*Dicamptodon aterrimus*) and stream salmonid fishes in headwater streams. Previous research in this system indicated that salamander dispersal did not promote coexistence with fish. We tested the hypothesis that *D. aterrimus* shift their diet when they occur with fish, facilitating coexistence through local niche partitioning.
3. We used nitrogen and carbon stable isotopes to describe the trophic niche of *D. aterrimus* and fish in three co-occurring populations of salamanders and fish and three populations of salamanders without fish. We used two approaches to quantify trophic niche partitioning with stable isotopes: 95% kernel density estimators and isotopic mixing models.
4. We found that salamanders and fish were generalists that consumed aquatic invertebrates primarily, but both species were also cannibalistic and predatory on one another. We also found no support for trophic niche partitioning as a coexistence mechanism because there were no differences in the trophic niche metrics among salamander populations with and without fish.
5. Although we did not identify mechanisms that facilitate salamander and fish coexistence, our empirical data and use of novel approaches to describe the trophic niche did yield important insights on the role of predator–prey interactions and cannibalism as alternative coexistence mechanisms. In addition, we found that 95% kernel estimators are a simple and robust method to describe population-level measure of trophic structure.

*Keywords:* coexistence, *Dicamptodon aterrimus*, diet, headwater stream, stable isotope

## Introduction

Stream-dwelling salamanders and fish often co-occur even though fish prey on and outcompete salamanders (Petranka, 1983; Resetarits, 1991; Barr & Babbitt, 2007), but the mechanisms allowing palatable salamanders to coexist with fish are unknown. Inferences about local mechanisms that promote coexistence of salamanders and fish have been based largely on experimental additions of fish into fishless reaches and mesocosms with naïve salamanders

(Resetarits, 1991, 1995; Storfer & Sih, 1998; Barr & Babbitt, 2007). These studies have shown that salamanders can reduce negative interactions with fish by using different habitats and altering behaviour. However, these strategies have large fitness costs: salamanders that alter their habitats and behaviour in the presence of fish have lower prey consumption, lower growth rates, decreased survival and lower abundances (Resetarits, 1995; Barr & Babbitt, 2007). These costs suggest that stable coexistence involves other, previously unidentified mechanisms.

Correspondence: Adam J. Sepulveda, U.S. Geological Survey, Northern Rocky Mountain Science Center, 2327 University Way, Suite 2, Bozeman, MT 59715, U.S.A. E-mail: asepulveda@usgs.gov

Stream salamanders and fish are size-selective predators that share many of the same prey resources. Smaller juveniles feed on invertebrates and larger adults feed on smaller vertebrates and invertebrates (Werner & Gilliam, 1984; Parker, 1994). When diet overlap between dominant and subordinate competitors is large and there is a shared resource that is limiting, niche-based competition theory predicts that the subordinate competitor will coexist only if it alters its diet. This niche shift often results in trophic niche contraction because the subordinate competitor forages on fewer prey species (Roughgarden, 1972; Pianka, 1974). Furthermore, the presence of a dominant competitor that is also a predator may alter the subordinate competitor's behaviour and further exacerbate niche shifts and contractions (Lima, 1998; Barr & Babbitt, 2007).

In this study, we tested the mechanisms in the field that promote coexistence between Idaho giant salamanders (*Dicamptodon aterrimus*, Cope) and salmonid fishes (*Oncorhynchus* sp.) in headwater streams. Across taxa, interspecific interactions are influenced by resource availability and use at the local-scale, but landscape-scale dispersal can alter the outcome of the local interactions by changing the demographic and evolutionary dynamics of populations (Bohonak, 1999; Lowe, 2003). Past work suggests that dispersal does not promote coexistence in our study system. Specifically, Sepulveda & Lowe (2011) tested whether salamander populations from upstream reaches without fish maintain or supplement populations in downstream reaches with fish. They found that *D. aterrimus* populations with and without fish were not influenced demographically by dispersal, were stable ( $\lambda \geq 1$ ) and had similar mean body conditions and growth rates.

Salamander coexistence with fish may be promoted by local niche partitioning, such that salamanders shift their diet when they co-occur with fish. We expected that food resources would be limiting in the streams where *D. aterrimus* and fish coexist. The diet of *D. aterrimus* has not been described previously, but other *Dicamptodon* species that occur in similar habitats feed primarily on stream insects (e.g. Parker, 1994). Moreover, the shaded headwater streams that drain granitic soils found throughout our study region in Idaho have low productivity, especially after the decline of anadromous salmon and their contribution of marine-derived nutrients (Kohler & Taki, 2010). Like fish, *D. aterrimus* are confined primarily to stream channels because all larvae and many adults are strictly aquatic. Thus, food resources that remain outside of the stream are not available to fish or salamanders.

Here, we used staple isotopes of carbon (C) and nitrogen (N) to test the hypothesis that *D. aterrimus* (hereafter referred to as salamanders) alter their trophic niche when they occur with fish, facilitating coexistence through local niche partitioning. Stable isotopes are a common alternative for studying trophic niches because they integrate feeding over time and space (e.g. Bearhop *et al.*, 2004). To test whether trophic niche shifts promote coexistence, we compared the diet composition, niche size and niche overlap among salamander populations that occur naturally with fish, salamander populations that do not occur with fish and fish populations that occur with salamanders. We predicted that (i) salamander diet composition would differ in streams with and without fish, (ii) salamander trophic niche size would be greater in streams without fish and (iii) trophic niche overlap would be greater among salamander populations without fish. We also predicted that (iv) trophic niche overlap between salamanders and fish would be greater where they do not co-occur.

We used two stable isotope approaches to quantify niche size and overlap. First, we built from Layman *et al.*'s (2007a) approach and used 95% fixed kernel density estimates of the areas occupied by salamander and fish populations in C and N isotope bi-plot space to quantify the trophic niche. Second, we applied Newsome *et al.* (2007) and Decottignies *et al.*'s (2007) approach and used mixing models to transform isotope values into dietary proportions of different prey sources. We then used these proportions in common metrics from community ecology to quantify the trophic niche (e.g. Krebs, 1999). We used both approaches because they provide different insights into the types of resources consumed by salamanders and fish.

## Methods

### Study system

*Dicamptodon aterrimus* is a large salamander ( $\leq 220$  mm snout-vent length) found in streams and rivers in the Rocky Mountains of northern and central Idaho and western Montana (Stebbins, 2003). This species exhibits facultative paedomorphosis, a polymorphism that results in the coexistence of gilled, fully aquatic adults and terrestrial metamorphic adults in the same populations. Aquatic individuals have broad habitat requirements within streams and frequently occur with fish (Sepulveda & Lowe, 2009). Movement and gene flow within and between stream reaches is common (Mullen *et al.*, 2010), but population growth in stream reaches with and

without fish is a function of local survival and recruitment, not dispersal (Sepulveda & Lowe, 2011). The diet of this salamander has not been described previously.

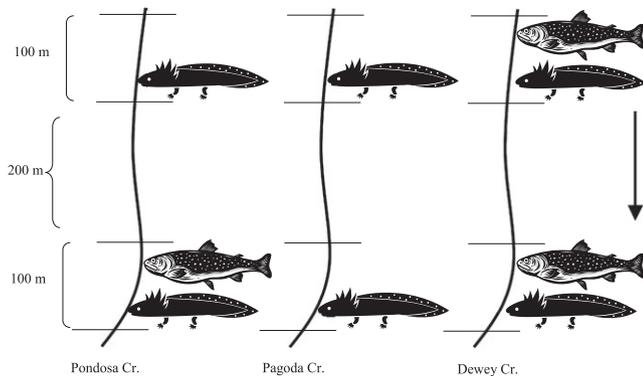
We conducted this study in three headwater streams (i.e. first and second order) in the Lochsa River sub-catchment in eastern Idaho, U.S.A. Pondosa Creek and Pagoda Creek are within the Fish Creek drainage. Dewey Creek is within the Post Office Creek drainage, which drains into the Lochsa River 30 km upstream from the mouth of Fish Creek. Within each stream, we sampled two 100-m reaches separated by 200 m of stream length. The lower reach began 25 m upstream of the confluence with another stream. The lower and upper reaches of all sampled streams had salamanders, but fish presence varied because of natural barriers that prevented upstream fish movement (Fig. 1). The lower reach of Pondosa Creek had fish, but the upper reach was fishless. In Pagoda Creek, neither reach had fish. In Dewey Creek, both reaches had fish. Fish species included steelhead and rainbow trout (*O. mykiss* Walbaum) and westslope cutthroat trout (*O. clarkii lewisi* Pratt & Graham). Because of

their proximity and similar geology, the six stream reaches had similar physical habitat (Table 1; Sepulveda & Lowe, 2009). This study design provided three co-occurring populations of salamanders and fish and three populations of salamanders without fish. The unit of replication was the stream reach, assuming that reaches in the same stream were independent of one another with regard to salamander and fish diets. Below we describe how we tested the assumption of independence of reach-scale stable isotope values in each stream.

#### Stable isotope sampling

To describe salamander and fish diet, niche size and niche overlap within each stream reach, we used stable isotopes of C ( $^{13}\text{C}:^{12}\text{C}$ , expressed as  $\delta^{13}\text{C}$ ) and N ( $^{15}\text{N}:^{14}\text{N}$ , expressed as  $\delta^{15}\text{N}$ ).  $\delta^{13}\text{C}$  provides information about the production base (aquatic or terrestrial) of the food web, and  $\delta^{15}\text{N}$  indicates the trophic position of an organism (Cabana & Rasmussen, 1996). Used together,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  reflect temporally integrated data on an individual's trophic niche, which is difficult to compile with stomach data alone (Sepulveda *et al.*, 2009).

Within each 100-m reach, we captured salamanders and fish using a backpack electroshocker. Stable isotope samples were collected from a small piece of tail clipped from each salamander (Simon, Benfield & Macko, 2003) and from white muscle below the dorsal fin of each fish (Sepulveda *et al.*, 2009). We sampled larval and aquatic adult salamanders, but not terrestrial adult salamanders because they rarely occur in the stream with fish and are therefore not relevant to this study. Because we wanted to quantify the trophic niche of salamanders and fish that overlapped in space and time, we sampled only fish that measured less than 200 mm (total length). Fish larger than 200 mm were likely to be migratory and have foraged downstream of our study reach (e.g. Downs, White & Shepard, 1997). To prevent duplicate sampling, we individually marked all sampled salamanders and fish with fluorescent elastomer (Northwest Marine Technologies,



**Fig. 1** Schematic representation of the sampling design used to determine the niche overlap and niche size among populations of *Dicamptodon aterrimus* and fish in headwater streams of the Lochsa River sub-catchment in Idaho. Survey reaches were 100 m long and separated by 200 m of stream channel. The arrows indicate the direction of flow.

**Table 1** Physical characteristics of the lower and upper reaches of Pondosa, Pagoda and Dewey Creek in the Lochsa River sub-catchment, Idaho, in the summer of 2007. Please see Sepulveda & Lowe (2009) for methods

Stream	Reach	Elevation (m)	Temperature °C	Conductivity $\mu\text{S cm}^{-1}$	Wetted width (m)	Depth (cm)	% Canopy cover
Pondosa	Low	732	13.3	33.7	1.56	5.5	95
	Up	796	14.0	32.7	1.63	7.0	69
Pagoda	Low	760	13.1	26.1	1.18	7.2	90
	Up	774	12.8	26.0	1.08	4.5	93
Dewey	Low	909	14.0	36.8	2.53	8.0	85
	Up	939	13.9	41.9	2.27	9.2	83

Shaw Island, Washington DC, U.S.A.). To test whether salamander and fish diets remained relatively invariant over time (Bearhop *et al.*, 2004), we sampled individuals randomly in each stream reach in July and August 2007 and July 2008.

To quantify the contribution of major prey sources to salamander and fish diets, we collected stable isotope samples from aquatic invertebrates, tailed frog larvae (*Ascaphus montanus* Myers), salamanders, salmonid fish and terrestrial invertebrates in July and August of 2007. To collect aquatic invertebrates, we took four random kicknet samples. Invertebrates were sorted to genus and classified into functional feeding groups (Merritt & Cummins, 1996). Isotope analyses were performed on composite samples of whole invertebrates for each category. We captured *A. montanus* using a backpack electroshocker and collected individual tail clips for isotope analysis. Terrestrial invertebrates were sampled from within 2 m of the stream's wetted width using pitfall traps, leaf litter sifters and sweep net sampling. We performed isotope analysis on composite samples of whole invertebrates. All samples were stored on ice in the field and then frozen until processed.

We dried samples for 48 h at 65 °C and then vacuum freeze-dried them for 4 h. All samples were ground to a fine powder and packed in 4 × 6 mm tin capsules (Costech Analytical Technologies, Valencia, CA, U.S.A.).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured using a Thermo Scientific Delta V Advantage mass spectrometer coupled with a Costech ECS 4010 elemental analyzer via a ConFlo IV gas interface at the Smithsonian Institution OUSS/MCI Stable Isotope Mass Spectrometry Facility (Suitland, MD, U.S.A.). We reported all stable isotope values in the  $\delta$  notation where  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N} = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$ , where R is  $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$ . The global standard for  $\delta^{13}\text{C}$  is Vienna PeeDee Belemnite and for  $\delta^{15}\text{N}$  is atmospheric nitrogen. All runs included a set of standards for every 10 samples. Standards include USGS40 (L-glutamic acid), USGS41 (L-glutamic acid) and Costech acetanilide. The reproducibility of standards during individual runs was no greater than  $\pm 0.2\text{‰}$  ( $1\sigma$ ) for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and all sample data were therefore considered to have an associated error of  $\pm 0.2\text{‰}$ . The reproducibility of replicate salamander ( $n = 20$ ) and fish ( $n = 5$ ) samples ran throughout this analysis was  $0.25\text{‰}$  ( $1\sigma$ ) for  $\delta^{13}\text{C}$  and  $0.19\text{‰}$  ( $1\sigma$ ) for  $\delta^{15}\text{N}$ . We used individual  $\delta$ -values to estimate reach averages for salamanders without fish, salamanders with fish and fish with salamanders.

We plotted the atomic C:N ratios (moles C/moles N) of all samples to check for outliers. Atomic ratios that

exceeded three standard deviations of the population reach mean were not used in analyses. To test whether salamander and fish stable isotope values remained relatively invariant over time, we used analysis of variance (ANOVA) to determine whether the atomic C:N ratio varied by sampling date (July 2007, August 2007 and August 2008). To test the assumption that reaches in the same stream were independent of one another with regard to salamander and fish diets, we used ANOVA to test for independence of reach-scale  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in each stream. We used JMP 8 (SAS Institute, Carey, NC, U.S.A.) for all statistical analyses.

### Diet description

To describe salamander and fish diets, we used ISOSOURCE 1.3 software (Phillips & Gregg, 2003). We assigned potential prey sources into six categories: aquatic primary consumers (shredders and grazers), aquatic secondary consumers (collector-gathers and predators), terrestrial invertebrates, *A. montanus* tadpoles, salmonid juveniles (<60 mm total length) and salamander juveniles (<60 mm snout to vent length). We used multiple discriminant function analyses to test whether the assignment of individuals into these six prey categories were isotopically distinct for each stream reach. Because we found that  $\geq 90\%$  of the samples were reclassified correctly, we used these six prey categories for all diet analyses. We corrected prey isotope values for trophic enrichment using the most widely accepted values of 1 and  $3.4\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively (Post, 2002). We used the means of prey isotope values from each stream reach to estimate the proportional contribution of each prey source to individual salamander and fish consumers. The source increment was set at 1%. Tolerance was initially set at  $0.01\text{‰}$  but we incrementally increased the tolerance value by  $0.05\text{‰}$  up to a maximum of  $0.5\text{‰}$  if the mixture isotope values were outside the polygon delineated by the six prey sources.

Following Phillips & Gregg (2003), we recorded the range (1st–99th percentile) and mean feasible dietary contributions of each prey source for each consumer entered into ISOSOURCE simulations. Low maxima (99th percentile) from ISOSOURCE simulations indicate that a prey source can be rejected as important, while relatively high minima indicate that the source may be important (Phillips & Gregg, 2003; Benstead *et al.*, 2006). We used ANOVA with Tukey's honest significance distance (HSD) *post hoc* tests and Kruskal–Wallis with nonparametric multiple comparisons with unequal sample size *post hoc* tests (Zar, 1996) to compare the mean 1st percentile and

mean 99th percentile of each prey source for reaches with salamanders without fish, salamanders with fish and fish with salamanders.

#### *Trophic niche analysis*

We used two stable isotope approaches to quantify salamander and fish trophic niche size and overlap. First, we used 95% fixed kernel density estimates of a population's trophic niche in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plot space (Layman *et al.*, 2007a). Second, we used isotopic mixing models that transform  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values into dietary proportions of different prey sources (e.g. Decottignies *et al.*, 2007; Newsome *et al.*, 2007). We quantified trophic niche size for populations of salamanders without fish, salamanders with fish and fish with salamanders. We then conducted pairwise comparisons of trophic niche overlap (i) between salamander populations without fish and with fish, (ii) between salamander populations without fish and fish populations and (iii) between salamander populations with fish and fish populations. To test the assumption that salamander diets were not influenced by habitat differences among our reaches, we also compared niche overlap among populations of salamanders without fish.

#### *Fixed kernel density estimates*

To estimate trophic niche area and overlap, we used 95% fixed kernel density estimates of the areas occupied by salamander and fish populations in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space. Previous studies have used minimum convex polygons of  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space to estimate trophic niche size (Layman *et al.*, 2007a,b). However, tests of minimum convex polygons to estimate home range size show that they are sensitive to sample size and influenced by outliers (i.e. individuals or species with extreme positions on either the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  axis; Laundre & Keller, 1984; Seaman *et al.*, 1999; Harris *et al.*, 1990; Layman *et al.*, 2007a). Kernel-based estimators are robust to small sample sizes and are less sensitive to outliers, while still being able to consider outliers as part of the overall distribution (Kernohan, Gitzen & Millsbaugh, 2001).

Using  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space to estimate trophic niche size, like kernel estimators, depends on the variability of individual diets and the amount of isotopic variation among food sources. For example, a population composed of individuals that specialise on isotopically different food will have a larger niche area than a population composed of individual generalists (Bearhop *et al.*, 2004; Newsome

*et al.*, 2007). Because different feeding pathways may lead to the same position of two or more species in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space, the use of kernel estimators are most informative in systems when distinct feeding niches are reflected by different positions of individuals in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space (Layman *et al.*, 2007a). However,  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space approaches have few assumptions and require relatively few samples because only isotopic data on the focal taxa are needed.

To estimate trophic niche size, we first standardised salamander and fish  $\delta$ -values using aquatic snails sampled within each reach as an isotopic baseline (e.g. Post, 2002). This allowed for better comparisons of trophic niche size among different stream reaches. We then plotted the relative positions of salamander and fish of these adjusted  $\delta$ -values in  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot space using ARCMAP 9.2 (ESRI, Redlands, CA, U.S.A.). We described trophic niche size using 95% fixed kernel density estimators with least-squares cross-validation using the Home Range Tools extension for ARCGIS, Version 1.1 (<http://flash.lakeheadu.ca/~arodgers/hre/>; Rodgers *et al.*, 2007). We used the area of the 95% kernel as our measure of trophic niche size.

To quantify niche overlap, we used estimates of the trophic niche size calculated for each population. We calculated niche overlap ( $O$ ) as:

$$O_{12} = 2 \left( \frac{A_{12}}{A_1 + A_2} \right)$$

where  $O_{12}$  is the niche overlap between populations 1 and 2,  $A_1$  and  $A_2$  are the areas for populations 1 and 2 calculated from the 95% fixed kernel density estimates and  $A_{12}$  is the area of overlap between populations 1 and 2. Because estimates of overlap were not normally distributed (even after various transformations), we used Kruskal-Wallis tests to test whether proportional niche overlap differed among the four pairwise comparisons.

#### *Mixing-model estimates*

We used ISOSOURCE mixing models to transform isotope values into dietary proportions of different isotopic prey sources. We then transformed dietary proportions into common metrics to describe trophic niche area and overlap. Unlike kernel estimators, these metrics are independent of the absolute value of isotopic signatures and are easier to compare among studies (Newsome *et al.*, 2007). However, isotopic mixing models make assumptions about trophic enrichment and require intensive sampling of the focal taxa and all potential prey sources.

We used mean feasible dietary contributions of each prey source from ISOSOURCE mixing models to estimate trophic niche size and niche overlap. To estimate trophic niche size, we used Levins' standardised measure of niche breadth (Levins, 1968; Krebs, 1999),

$$B_A = \frac{B - 1}{n - 1}$$

$$B = \frac{1}{\sum_1^n p_j^2}$$

where  $B_A$  is Levins' standardised niche breadth,  $B$  is Levins' measure of niche breadth,  $n$  is the number of possible source types and  $p_j$  is the fraction of food category  $j$  in the diet (mean of ISOSOURCE simulations were used because  $p_j$  must sum to 1). To correct for body size effects on trophic niche size, we used residuals from  $B_A$  regressed against size in all comparisons.

Trophic niche overlap was estimated using Pianka's measure (Pianka, 1974; Krebs, 1999),

$$O_{jk} = \frac{\sum_i^n p_{ij} p_{ik}}{\sqrt{\sum_i^n p_{ij}^2 \sum_i^n p_{ik}^2}}$$

where  $O_{jk}$  is Pianka's measure of niche overlap between population  $j$  and  $k$ , and  $p_{ij}$  and  $p_{ik}$  are the proportions of food source  $i$  (mean of ISOSOURCE simulations) of the total sources used by population  $j$  and  $k$ . Means of ISOSOURCE simulations were used rather than minima because food source proportions must sum to 1. Pianka's measure of niche overlap ranges from no overlap ( $O_{jk} = 0$ ) to complete overlap ( $O_{jk} = 1$ ). These data were normally distributed, so we used ANOVA and Tukey's HSD tests to compare Pianka's measure of niche overlap.

## Results

We sampled 239 salamanders and 30 fish across all stream reaches in 2007 and 2008 (Table 2). Fifteen salamanders and three fish samples were not included in the analysis because their C:N molar weight exceeded three standard deviations of the population reach means. Mean  $\delta^{13}\text{C}$  values ranged from  $-33.10$  in terrestrial invertebrates to  $-22.67$  in salamanders (Table 3). Mean  $\delta^{15}\text{N}$  values ranged from  $-0.39$  in terrestrial invertebrates to  $7.58$  in fish (Table 3). The atomic ratios of  $\delta^{13}\text{C} : \delta^{15}\text{N}$  were time invariant for salamanders (2-way ANOVA:  $F_{9,224} = 1.18$ ,  $P = 0.31$ ) and fish ( $F_{9,29} = 0.57$ ,  $P = 0.67$ ), so we pooled data across all sampling dates. We also found support for our assumption that diet within a stream was independent of reach-scale  $\delta$ -values in each stream – salamander atomic ratio differed by reach nested within stream

**Table 2** The number ( $n$ ) and length range (mm; SVL is snout-vent length and TL is total length) of *Dicamptodon aterrimus* salamanders and fish sampled in the lower and upper reaches of Pondosa, Pagoda and Dewey Creek in the Lochsa River sub-catchment, Idaho, in the summers of 2007 and 2008. A dash (—) indicates that no fish were present in a sampled reach

	Pondosa		Pagoda		Dewey	
	Lower	Upper	Lower	Upper	Lower	Upper
Salamander						
$n$	55	34	46	42	22	25
SVL	45–131	65–120	48–131	34–133	25–125	27–106
Fish						
$n$	8	—	—	—	10	9
TL	55–145	—	—	—	45–143	50–131

(ANOVA:  $F_{5,279} = 2.35$ ,  $P = 0.04$ ), but not by stream ( $F_{3,279} = 1.10$ ,  $P = 0.35$ ).

## Diet

We found that salamander and fish individuals had high interindividual diet variability (Fig. 2) and that all salamander and fish populations were generalists (Fig. 3). Minima and maxima dietary contributions from ISOSOURCE simulations ranged from 0 to 97% within each population, but this variability was not correlated with salamander or fish size ( $R < 0.05$ ). In all salamander populations, maxima dietary contributions from each source were  $>15\%$ , suggesting that salamanders consumed all prey sources. However, we could reject terrestrial invertebrates as an important prey source for fish because the maximum was *c.* 0%. We also found that salamanders were cannibalistic and consumed juvenile fish and that fish consumed other fish and salamander larvae.

Although both salamanders and fish ate a variety of prey, we did find differences in mean minima and maxima dietary contributions among salamanders without fish, salamanders with fish and fish (Fig. 3). The minimum and maximum dietary contributions of terrestrial invertebrates to salamanders without fish were greater than the contribution to salamanders with fish and to fish (minimum, Kruskal–Wallis:  $\chi^2 = 6.54$ , d.f. = 2,  $P = 0.04$ ; maximum, ANOVA:  $F_{2,6} = 11.23$ ,  $P = 0.009$ ). The minimum contribution of aquatic non-predators to salamanders with fish was greater than the contribution to fish, but the maximum contribution did not differ (minimum, ANOVA:  $F_{2,6} = 4.93$ ,  $P = 0.05$ ; maximum, ANOVA:  $F_{2,6} = 4.11$ ,  $P = 0.08$ ). For all other prey sources, dietary contributions did not differ among populations.

**Table 3** The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $\pm 95\%$  C.I.) of the six prey sources used in ISOSOURCE simulations in the lower and upper reaches of Pondosa, Pagoda and Dewey Creek. A dash (—) indicates that no fish were present in a sampled reach

	Pondosa		Pagoda		Dewey	
	Lower	Upper	Lower	Upper	Lower	Upper
Aquatic 1° consumer						
$\delta^{13}\text{C}$	-26.44 (0.60)	-27.13 (0.24)	-25.82 (0.76)	-26.74 (0.81)	-27.00 (0.02)	-27.00 (0.54)
$\delta^{15}\text{N}$	1.95 (0.66)	0.87 (0.85)	0.34 (0.50)	0.89 (0.44)	-0.28 (0.11)	0.01 (0.14)
Aquatic 2° consumer						
$\delta^{13}\text{C}$	-25.67 (0.63)	-25.41 (1.40)	-23.08 (0.34)	-25.24 (0.55)	-23.75 (0.37)	-26.20 (0.37)
$\delta^{15}\text{N}$	4.16 (0.68)	1.81 (1.24)	3.30 (0.28)	4.25 (0.40)	2.88 (0.21)	3.52 (0.34)
Terrestrial invertebrate						
$\delta^{13}\text{C}$	-31.01 (0.39)	-30.20 (1.37)	-31.18 (0.94)	-30.12 (0.57)	-31.17 (0.50)	-33.10 (1.95)
$\delta^{15}\text{N}$	0.93 (0.68)	0.30 (0.95)	0.85 (0.63)	0.45 (0.52)	0.19 (0.60)	-0.39 (0.26)
<i>Ascapthus montanus</i>						
$\delta^{13}\text{C}$	-27.55 (0.57)	-27.84 (0.57)	-25.51 (0.87)	-26.38 (0.92)	-27.53 (1.36)	-26.80 (1.55)
$\delta^{15}\text{N}$	3.13 (0.10)	1.00 (0.13)	2.39 (0.23)	1.61 (0.06)	1.81 (0.16)	1.79 (0.39)
<i>Dicamptodon aterrimus</i>						
<i>n</i>	4	6	7	13	8	6
$\delta^{13}\text{C}$	-26.33 (0.19)	-25.94 (0.32)	-22.67 (0.54)	-25.68 (0.22)	-25.61 (0.90)	-26.58 (0.44)
$\delta^{15}\text{N}$	3.93 (0.28)	2.76 (0.25)	3.87 (0.24)	3.87 (0.22)	3.21 (0.35)	3.49 (0.70)
Fish						
<i>n</i>	6	—	—	—	4	5
$\delta^{13}\text{C}$	-24.49 (0.36)	—	—	—	-24.55 (1.91)	-25.48 (0.45)
$\delta^{15}\text{N}$	7.58 (0.38)	—	—	—	2.46 (1.76)	4.77 (0.39)

### Fixed kernel density estimates

We found no difference in the 95% kernel density estimates of trophic niche size among populations of salamanders without fish, salamanders with fish and fish (Fig. 4; ANOVA:  $F_{2,6} = 0.48$ ,  $P = 0.64$ ). We also found no difference in the 95% kernel density estimates of niche overlap across all pairwise population comparisons (Fig. 5; Kruskal–Wallis:  $\chi^2 = 5.19$ , d.f. = 3,  $P = 0.16$ ).

### Mixing-model estimates

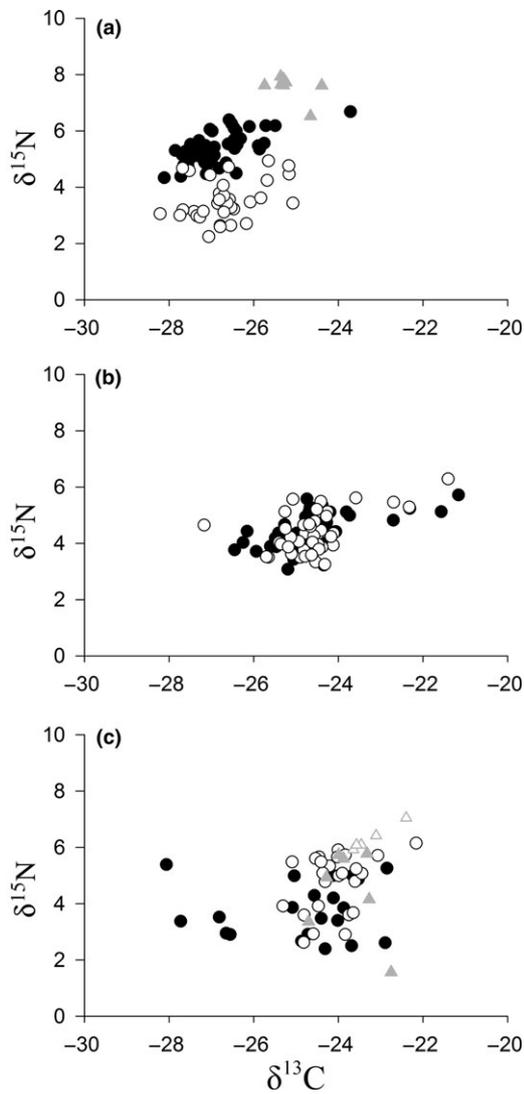
Levins' standardised niche breadth did not differ among salamanders without fish, salamanders with fish and fish (Fig. 4;  $F_{2,6} = 1.51$ ,  $P = 0.30$ ). However, there was a significant difference using Pianka's measure of niche overlap (Fig. 5; ANOVA:  $F_{3,26} = 4.92$ ,  $P = 0.008$ ). Niche overlap between populations of fish and salamanders without fish was smaller than niche overlap in the other three pairwise comparisons.

## Discussion

Intraguild predation, defined as predator–prey interactions among consumers that are potential competitors (Holt & Polis, 1997), occurs between salamanders and fish. We used stable isotopes to infer trophic niches and found that salamanders consumed fish, fish consumed salaman-

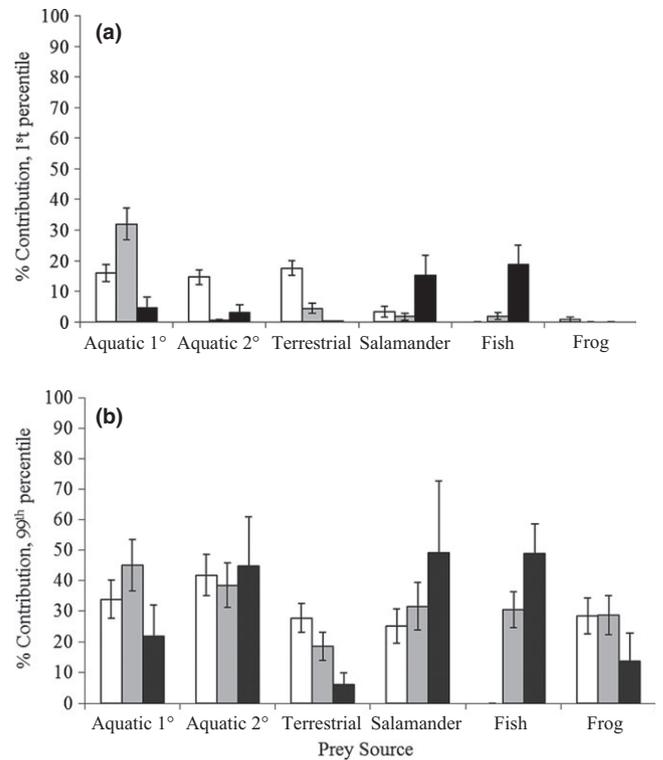
ders and both salamanders and fish consumed stream invertebrates. However, we found no evidence for trophic niche partitioning as a mechanism promoting salamander coexistence with fish. Salamanders and fish shared a common diet when sympatric and trophic niche size and overlap did not differ among populations of salamanders without fish and salamanders with fish. We did find limited evidence for diet differences and minimal trophic overlap when salamanders and fish occurred separately. Implications of our results are that shared food resources were not limited in stream reaches with and without fish or that partitioning occurred along another niche axis of resource use.

Salamanders and fish did not partition their trophic niches, but salamanders ate more terrestrial insects when alone than with fish (Fig. 3). This difference was not attributed to exploitative competition with fish because terrestrial invertebrates did not contribute to fish isotope values. Fish presence alone may have affected salamander feeding and behaviour. Because terrestrial insects fall into the stream, salamanders must be active above the substrata to capture terrestrial insects. This behaviour can increase predation risk from fish (Barr & Babbitt, 2007). Increased predation risk may have caused salamanders that occurred with fish to forage less often for terrestrial invertebrates and more often for benthic invertebrates. This explanation is supported by the increased dietary contribution of aquatic primary consumers to

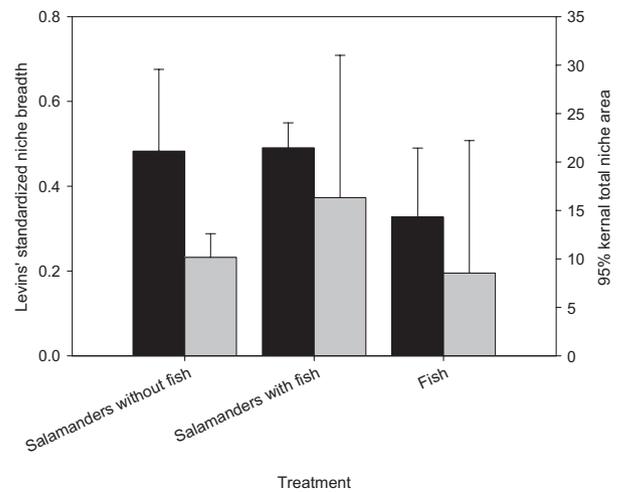


**Fig. 2**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for *Dicamptodon aterrimus* (circles) and fish (triangles) in the lower (filled symbols) and upper (open symbols) reaches of (a) Pondosa Creek, (b) Pagoda Creek and (c) Dewey Creek in 2007 and 2008.

salamanders with fish, which consisted primarily of benthic invertebrates. A previous study on stream salamanders (*Eurycea bislineata*) found that salamander activity on or above the substrata was greatly reduced in the presence of fish (Barr & Babbitt, 2007). Reduced energy intake and slower growth rates are often immediate costs of this antipredator behaviour, but we found no evidence of a cost in this study system. Body condition, growth rates and survival did not differ in salamander populations with and without fish (Sepulveda & Lowe, 2011), supporting the hypothesis that benthic invertebrates were not limiting in our study streams. We also cannot reject the hypothesis that site-specific habitat differences, rather than fish, drove differences in salamander diets because

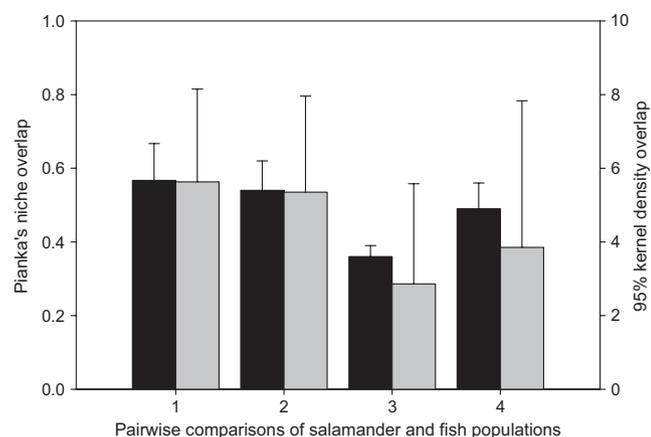


**Fig. 3** Mean per cent dietary contribution ( $\pm 95\%$  C.I.) of prey sources to *Dicamptodon aterrimus* without fish (white bars) *D. aterrimus* with fish (grey bars) and fish (dark bars) based on the 1st percentile minimum (a) and 99th percentile maximum (b) from ISOSOURCE simulations. Per cent contributions do not sum to 100% because these are minima and maxima.



**Fig. 4** Mean niche size ( $\pm 95\%$  C.I.) of *Dicamptodon aterrimus* without fish, *D. aterrimus* with fish and fish. Levin's standardised niche breadth (dark bars) was obtained from ISOSOURCE simulations, and total niche area (light bars) was obtained from 95% kernel density estimates.

trophic niche overlap among salamander populations without fish did not differ from the overlap between salamander populations with and without fish.



**Fig. 5** Mean niche overlap ( $\pm 95\%$  C.I.) for pairwise comparisons of (1) *Dicamptodon aterrimus* without fish  $\times$  *D. aterrimus* without fish, (2) *D. aterrimus* without fish  $\times$  *D. aterrimus* with fish, (3) *D. aterrimus* without fish  $\times$  fish and (4) *D. aterrimus* with fish  $\times$  fish. Pianka's measure of niche overlap (dark bars) was obtained from ISOSOURCE simulations, and proportional overlap (light bars) was obtained from 95% kernel density estimators. To standardise for differences in sample size across pairwise comparisons, we used jackknife resampling to calculate confidence intervals.

We also used two isotopic approaches to characterise trophic niches. We anticipated that kernel estimators and isotopic mixing models would provide different insights on salamander and fish trophic niche space because kernel estimators use all data to describe the niche whereas mixing models use means. We found only one difference – mixing-model estimates of niche overlap among fish populations and salamander populations without fish were smaller than niche overlap estimates among the other pairwise comparisons. This difference probably reflects the larger contribution of terrestrial invertebrates to salamander populations without fish and the near-zero contribution to fish. Except for this difference, kernel estimators and mixing-models estimates of trophic niche space were similar. These similarities raise the question: Are the two approaches redundant or complementary?

Kernel estimates require few assumptions and use all data to describe the niche, including the high interindividual variability that we found in all populations. Incorporating individual-level variation into population-level metrics provides a more complete and realistic description of a biological system (Bolnick *et al.*, 2002). In addition, more samples can be collected on the target species because prey sources do not have to be sampled. However, kernel estimates assume that distinct feeding niches are reflected by different positions of individuals in  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  bi-plot space (Layman *et al.*, 2007a). Because our focus was on the overall pattern of individuals within a population (i.e. the niche area) and not on the differences among individuals,

determining every factor affecting a single individual's isotope ratio was not essential (Layman *et al.*, 2007a). Kernel estimates are also more difficult to compare among different study systems because they are sensitive to the absolute value of isotopic signatures. We limited this weakness by standardising salamander and fish  $\delta$ -values with aquatic snails as an isotopic baseline (Post, 2002), but comparison of niche size against populations outside of our study system may be difficult.

Mixing models require that the interindividual variation in prey sources is collapsed into homogeneous means. In our study, we lumped prey sources into broad functional feeding groups to ensure that prey sources were isotopically distinct and because mixing models cannot accommodate a large number of sources. Mixing models also assume that all prey sources are sampled and that the correct trophic enrichment factor is used, but trophic enrichment can vary depending on physiological and environmental factors (McCutchan *et al.*, 2003). In this study, we used the widely accepted trophic enrichment factors of 1 and 3.4‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, but these values have not been validated for salamanders. Unlike kernel estimators, mixing models are independent of the absolute value of isotopic signatures and account for isotopic variation among consumer food sources. Therefore, mixing models may be more appropriate for comparing salamander and fish niche space among different stream reaches. Like Newsome *et al.* (2007), we suggest that the limitation and advantages of each method make them complementary and that kernel estimates provide a check on mixing-model assumptions. If kernel estimates of niche space corroborate mixing-model estimates, such as in our study, then this suggests that mixing-model assumptions were satisfied.

#### *Alternative mechanisms of coexistence*

It is likely that multiple mechanisms allow salamanders to coexist with competitively dominant fish and that the relative importance of the mechanisms vary in time and space (Reserits, 1995). However, our research on salamanders rejects several dominant hypotheses about coexistence. In this study, we found that salamanders did not shift their trophic niche in the presence of fish. In a previous study, Sepulveda & Lowe (2011) found no evidence to support the hypothesis that salamander source–sink dynamics promote coexistence. Sepulveda & Lowe (2009) also found no evidence for refuge in space within the stream because salamanders and fish co-occurred in the same habitat and habitat type was a poor predictor of salamander occurrence and density in streams with and without fish.

Our isotopic data suggest an additional mechanism that has received little attention in previous studies – cannibalism. We found that fish preyed upon smaller juvenile fish, many of which were likely to be conspecifics since westslope cutthroat trout were the most common fish species (A.J. Sepulveda, personal observation). In size-structured populations, cannibalism can act as a self-regulating process that intensifies negative intraspecific effects on population growth and reduces negative interspecific effects (Chesson, 2000; Rudolf, 2007). As a consequence, competition is relaxed between potential competitors and intraguild species that have overlapping niches can coexist (Polis, 1981; Polis & McCormick, 1987; Polis, Myers & Holt, 1989; Spence & Carcamo, 1991; Rudolf, 2008). In our system, fish cannibalism may be an important self-regulating process that promotes salamander × fish coexistence, but demographic analyses and experiments are needed to test this hypothesis.

Empirical tests of theoretical models for coexistence are limited by the difficulty of working in natural systems. As a result, theory has outpaced data. In this study, we advanced the conceptual framework proposed by Layman *et al.* (2007a) and Newsome *et al.* (2007) by demonstrating how novel quantitative metrics derived from stable isotopes can be used to test ecological theory. Using stable isotopes collected in the field, we found no support for trophic niche partitioning as a coexistence mechanism between intraguild prey and competitively dominant predators. However, this empirical data yielded important insights into cannibalism as an alternative mechanism that may promote coexistence. Population and community-wide metrics of resource use and niche space provide a powerful way to bridge the gap between theory and empirical data.

### Acknowledgments

This research was funded by grants from the Society for Northwestern Vertebrate Biology, Charlotte Martin Foundation, the Pacific Rivers Council, USGS Montana River Center and the University of Montana. We thank Christine France (Smithsonian OUSS/MCI Stable Isotope Mass Spectrometry Facility) for assistance with sample analysis. We thank Jeff Bates and Joe Hanlon for assisting with fieldwork. This manuscript benefited from the comments of Steve Corn, Megan Layhee, John Maron, Scott Mills, Mike Schwartz, Art Woods and Mike Young. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

### References

- Barr G.E. & Babbitt K.J. (2007) Trout affect the density, activity and feeding of a larval plethodontid salamander. *Freshwater Biology*, **52**, 1239–1248.
- Bearhop S., Adams C.E., Waldron S., Fuller R.A. & Macleod H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007–1012.
- Benstead J.P., March J.G., Fry B., Ewel K.C. & Pringle C.M. (2006) Testing IsoSource: stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology*, **87**, 326–333.
- Bohonak A.J. (1999) Dispersal, gene flow, and population structure. *The Quarterly Review of Biology*, **74**, 21.
- Bolnick D.I., Svanbäck R., Fordyce J.A., Yang L.H., Davis J.M., Hulsey C.D. *et al.* (2002) The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, **161**, 1–28.
- Cabana G. & Rasmussen J.B. (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, **93**, 10844.
- Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, **31**, 343–366.
- Decottignies P., Beninger P.G., Rincé Y., Robins R.J. & Riera P. (2007) Exploitation of natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas* and *Crepidula fornicata*. *Marine Ecology Progress Series*, **334**, 179–192.
- Downs C.C., White R.G. & Shepard B.B. (1997) Age at sexual maturity, sex ratio, fecundity, and longevity of isolated headwater populations of westslope cutthroat trout. *North American Journal of Fisheries Management*, **17**, 85–92.
- Harris S., Cresswell W.J., Forde P.G., Trehwella W.J., Woolard T. & Wray S. (1990) Home-range analysis using radio-tracking data – a review of problems and techniques particularly as applied to the study of mammals. *Mammal Review*, **20**, 97–123.
- Holt R.D. & Polis G.A. (1997) A theoretical framework for intraguild predation. *American Naturalist*, **149**, 745.
- Kernohan B.J., Gitzen R.A. & Millspaugh J.J. (2001) Analysis of animal space use and movements. In: *Radio Tracking and Animal Populations* (Eds J.J. Millspaugh & J.M. Marzluf), pp. 125–166. Academic Press, San Diego, CA, USA.
- Kohler A.E. & Taki D. (2010) Macroinvertebrate response to salmon carcass analogue treatments: exploring the relative influence of nutrient enrichment, stream foodweb, and environmental variables. *Journal of the North American Benthological Society*, **29**, 690–710.
- Krebs C.J. (1999) *Ecological Methodology*. Addison Wesley Longman Inc., Menlo Park, CA.
- Laundre J.W. & Keller B.L. (1984) Home-range size of coyotes: a critical review. *The Journal of Wildlife Management*, **48**, 127–139.

- Layman C.A., Arrington D.A., Montana C.G. & Post D.M. (2007a) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.
- Layman C.A., Quattrochi J.P., Peyer C.M., Allgeier J.E. & Suding K. (2007b) Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecology Letters*, **10**, 937.
- Levins R. (1968) *Evolution in Changing Environments*. Princeton University Press, Princeton, NJ.
- Lima S.L. (1998) Nonlethal effects in the ecology of predator-prey interactions. *BioScience*, **48**, 25–34.
- Lowe W. (2003) Linking dispersal to local population dynamics: a case study using a headwater salamander system. *Ecology*, **84**, 2145–2154.
- Mccutchan J.H., Lewis W.M., Kendall C. & Mcgrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.
- Merritt R.W. & Cummins K.W. (1996) *An Introduction to the Aquatic Insects of North America*. Kendall Hunt, Dubuque, IA.
- Mullen L.B., Woods H.A., Schwartz M.K., Sepulveda A.J. & Lowe W.H. (2010) Scale dependent genetic structure of the Idaho giant salamander (*Dicamptodon aterrimus*) in stream networks. *Molecular Ecology*, **19**, 898–909.
- Newsome S.D., Martinez Rio Del C., Bearhop S. & Phillips D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429–436.
- Parker M.S. (1994) Feeding ecology of stream-dwelling Pacific giant salamander larvae (*Dicamptodon tenebrosus*). *Copeia*, **1994**, 705–718.
- Petranka J.W. (1983) Fish predation: a factor affecting the spatial distribution of a stream-breeding salamander. *Copeia*, **1983**, 624–628.
- Phillips D.L. & Gregg J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–269.
- Pianka E.R. (1974) Niche overlap and diffuse competition. *Proceedings of the National Academy of Sciences USA*, **71**, 2141–2145.
- Polis G.A. (1981) The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics*, **12**, 225–251.
- Polis G.A. & McCormick S.J. (1987) Intraguild predation and competition among desert scorpions. *Ecology*, **68**, 332–343.
- Polis G.A., Myers C.A. & Holt R.D. (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297–330.
- Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Resetarits W.J. (1991) Ecological interactions among predators in experimental stream communities. *Ecology*, **72**, 1782–1793.
- Resetarits W.J. (1995) Competitive asymmetry and coexistence in size-structured populations of brook trout and spring salamanders. *Oikos*, **73**, 188–198.
- Rodgers A.R., Carr A.P., Beyer H.L., Smith L. & Kie J.G. (2007) *HRT: Home Range Tools for ArcGIS. Version 1.1*. Ontario Ministry of Natural Resources, Centre for Northern Forest Ecosystem Research, Thunder Bay, ON, Canada.
- Roughgarden J. (1972) Evolution of niche width. *The American Naturalist*, **106**, 683.
- Rudolf V.H.W. (2007) The interaction of cannibalism and omnivory: consequences for community dynamics. *Ecology*, **88**, 2697–2705.
- Rudolf V.H.W. (2008) Impact of cannibalism on predator-prey dynamics: size-structured interactions and apparent mutualism. *Ecology*, **89**, 1650–1660.
- Seaman D.E., Millsbaugh J.J., Kernohan B.J., Brundige G.C., Raedeke K.J. & Gitzen R.A. (1999) Effects of sample size on kernel home range estimates. *The Journal of Wildlife Management*, **63**, 739–747.
- Sepulveda A.J., Colyer W.T., Lowe W.H. & Vinson M.R. (2009) Using nitrogen stable isotopes to detect long-distance movement in a threatened cutthroat trout (*Oncorhynchus clarkii utah*). *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 672–682.
- Sepulveda A.J. & Lowe W.H. (2009) Local and landscape-scale influences on the occurrence and density of *Dicamptodon aterrimus*, the Idaho Giant Salamander. *Journal of Herpetology*, **43**, 469–484.
- Sepulveda A.J. & Lowe W.H. (2011) Coexistence in streams: do source-sink dynamics allow salamanders to persist with fish predators? *Oecologia*, **166**, 1043–1054.
- Simon K.S., Benfield E.F. & Macko S.A. (2003) Food web structure and the role of epilithic biofilms in cave streams. *Ecology*, **84**, 2395–2406.
- Spence J.R. & Carcamo H.A. (1991) Effects of cannibalism and intraguild predation on pondskaters (Gerridae). *Oikos*, **62**, 333–341.
- Stebbins R.C. (2003) *A Field Guide to Western Reptiles and Amphibians*. Houghton Mifflin Harcourt (HMH), Boston, MA.
- Storfer A. & Sih A. (1998) Gene flow and ineffective antipredator behavior in a stream-breeding salamander. *Evolution*, **52**, 558–565.
- Werner E.E. & Gilliam J.F. (1984) The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics*, **15**, 393–425.
- Zar J.H. (1996) *Biostatistical Analysis*. Prentice-Hall Inc., Upper Saddle River, NJ.

(Manuscript accepted 2 April 2012)