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Comparisons of stable isotope (C, H, N) signatures for revealing organic matter sources and trophic relationships in headwater streams

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Abstract

We compared the efficacy of carbon, hydrogen, and nitrogen stable isotopes in identifying the resources used by insect consumers in headwater streams of southern California. We also compared gut contents to consumer stable isotope ratios and mixing model estimates of resource contributions to predator diet. Stable hydrogen isotope ratios (as $\delta^2\text{H}$) of algivores were well separated from ratios for detritivores, whereas relationships between stable carbon (as $\delta^{13}\text{C}$) and nitrogen (as $\delta^{15}\text{N}$) ratios of consumers and their expected diets were weaker and more ambiguous. $\delta^2\text{H}$ values of primary consumers more strongly reflected the proportions of their gut contents consisting of algae than $\delta^{13}\text{C}$ values. The proportions of algivorous prey in predator gut contents increased with mixing model estimates of algivore contributions to predator diet using $\delta^2\text{H}$ but not $\delta^{13}\text{C}$ values. Our findings support the use of hydrogen isotope ratios in food web studies of streams in southern California and their potential use in assessing the effects of anthropogenic and natural disturbance on basal resource contributions to food webs that might otherwise not be identified using carbon isotope ratios.

20 Introduction

21 Terrestrial organic matter and stream algae are recognized as the two most important
22 sources of primary production fueling stream food webs (Cummins 1974; Finlay 2001;
23 Doucett et al. 2007; Caraco et al. 2010). Although the general importance of terrestrial
24 plant and stream algal production to stream communities is well known, quantifying the
25 relative contributions of these basal resources to food webs is challenging (e.g. Cummins
26 1979; France 1996; Doucett et al. 1996). Consumer nutrition inferred from gut contents is
27 invaluable in describing recent diets (Cross et al. 2013; Rosi-Marshall et al. 2016);
28 however, the digestibility and assimilation of different foods may vary, and consumer diets
29 can change over time, even within the same taxon, making inferences about consumer
30 nutritional support from only gut content analyses problematic (Chapman and Demory
31 1963; Moore 1977; Mihuc and Minshall 1995; Lancaster et al. 2005; Jardine et al. 2014).

32 Stable isotope analysis can provide additional and complementary insights into the
33 contributions of terrestrial plant and stream algal production to stream food webs.
34 Generally, isotope ratios of carbon are used to identify organic carbon sources (Finlay
35 2001; McNeely et al. 2007; Ishikawa et al. 2012), whereas isotope ratios of nitrogen are
36 used to assist in this evaluation and to identify trophic levels or positions (Vander Zanden
37 and Rasmussen 1999; Post 2002; Anderson and Cabana 2007). Stable carbon isotope
38 ratios provide a time-integrated signature of dietary support provided by different carbon
39 sources and possess an advantage over nitrogen isotopes in that trophic discrimination, the
40 enrichment of the consumer relative to the resource in the heavy isotope (^{13}C or ^{15}N), is
41 smaller for $\delta^{13}\text{C}$ values and, therefore, a better direct indicator linking prey and consumers
42 across trophic levels (Peterson and Fry 1987; Post 2002). Mixing models using isotope

data can quantify the relative contribution of different resources to consumer nutrition (Parnell et al. 2010; Layman et al. 2012).

The use of carbon isotope ratios to identify basal resource contributions to aquatic food webs may be limited in some cases by insufficient separation in carbon isotope end-member values (typically $< 1.0\text{--}2.0\text{‰}$, Jardine et al. 2009; Skinner et al. 2016) for terrestrial detritus and algal basal resources. Insufficient separation in end-member values makes descriptions of food web structure using isotope biplots or quantitative mixing models challenging (France 1996; Doucett et al. 1996; Finlay 2001; McNeely et al. 2006; Doucett et al. 2007; Ishikawa et al. 2012). Although the carbon isotope ratios of leaf detritus, the principal allochthonous basal resource in stream food webs, vary over a relatively narrow range (Finlay 2001), the carbon isotope ratios of autochthonous algal biomass can vary greatly (reviewed in Ishikawa et al. 2012). Variation in the carbon isotope ratios of algae has been related to variation in the availability and isotopic signatures of aqueous CO_2 and dissolved inorganic C (DIC), and to factors that affect the fractionation of carbon isotopes ($^{13}\text{C}/^{12}\text{C}$) during algal uptake, including algal productivity and water current velocity (Doucett et al. 1996; Finlay et al. 1999; Finlay 2004; Ishikawa et al. 2012).

Stable isotope ratios of hydrogen (as $\delta^2\text{H}$) may provide a complementary or alternative tracer for use in food web studies (Estep and Dabrowski 1980; Macko et al. 1983). Recent investigations have suggested that isotope ratios of hydrogen provide more consistent and clear end-member separation of terrestrial and algal basal resources than isotope ratios of carbon in stream, river, and lake environments (Doucett et al. 2007; Finlay et al. 2010; Cole et al. 2011; Karlsson et al. 2011). Distinct isotopic separation between terrestrial and

algal sources are achieved largely because of the fractionation of hydrogen isotopes during evapotranspiration of water from the leaves of terrestrial plants, causing isotopic enrichment of the remaining leaf water, which is assimilated into plant biomass during photosynthesis (Smith and Epstein 1970).

Although the use of hydrogen isotope ratios to trace allochthonous and autochthonous sources of production in aquatic food webs appears promising (Cole et al. 2006; 2011; Doucett et al. 2007; Finlay et al. 2010; Wilkinson et al. 2013; 2015; Vander Zanden et al. 2016), their effectiveness as a tracer may vary with study system. For example, Jardine et al. (2009) reported less overlap of basal resource end-member values with carbon than hydrogen isotope ratios in streams in eastern Canada. In addition, there are concerns about the effects of dietary water, as well as trophic and tissue discrimination, on the hydrogen isotope values of consumers (Macko et al. 1983; Solomon et al. 2009; Peters et al. 2012; Soto et al. 2013; Wilkinson et al. 2015; Vander Zanden et al. 2016). Relatively few studies have compared the use of carbon and hydrogen stable isotope ratios as tracers in streams or, importantly, examined the assumption that hydrogen isotope signatures reflect consumer diets.

Preliminary data suggested that carbon isotope ratios could not resolve sources of terrestrial versus aquatic production supporting food webs in the small headwater streams of southern California (subwatershed areas $< 13 \text{ km}^2$), necessitating the evaluation of an alternative tracer for use in these systems. The streams of southern California are located in a semi-arid Mediterranean climate and subject to natural (e.g., floods, drought) and anthropogenic (e.g., fire, contaminants, exotic species, dams, diversions) disturbances that

affect the structure of stream communities and food webs (Cooper et al. 2013; Verkaik et al. 2013; Cooper et al. 2015).

In this study, we provide new information on the stable carbon, hydrogen, and nitrogen isotope ratios of primary and secondary insect consumers in the streams of semi-arid southern California. We explore the hypothesis that stable isotope ratios of hydrogen provide more distinct separation in isotopic end-member values between terrestrial detritus and stream algal-based resources than carbon or nitrogen isotope ratios, and thus are more useful in identifying the relative contributions of these resources to the food webs of southern California streams. Because of potential overlap in the carbon isotope signatures of terrestrial detritus and stream algae, we also hypothesized that hydrogen isotope ratios would provide better agreement with recent diets, as determined from gut content analyses, than carbon isotope ratios for both primary consumers and predators. Because the isotope ratios of consumer tissues are assumed to reflect those of their diets integrated over time, tissue values may not necessarily reflect the isotopic signatures of recently consumed food items. Nevertheless, a concordance between isotope ratios and diets assessed from gut content analysis can provide ancillary support that isotope ratios reflect dietary sources (Peterson 1999). Although gut content data have been compared to data on stable isotope ratios of carbon and nitrogen (e.g. Mihuc and Toetz 1994; Lancaster et al. 2005; McNeely et al. 2007), no studies, to our knowledge, have explored these relationships for hydrogen isotope ratios.

109 **Materials and Methods**

110 *Study streams*

111 We measured the stable carbon, nitrogen, and hydrogen isotope ratios of basal resources
112 and a diverse array of invertebrate consumers in nine streams. The study streams drain
113 independent subwatersheds, ranging in area from ~3 to 13 km², on the south side of the
114 Santa Ynez Mountains (Santa Barbara County, California, USA), a steep, coastal mountain
115 chain separated from the Pacific Ocean by a narrow coastal plain. The Santa Ynez
116 Mountains have a Mediterranean climate, with average annual rainfall ranging from 45
117 cm/year at sea level to 100 cm/year in the mountains, with nearly all rain falling between
118 November and March. The drainage basins for our sites largely occurred above developed
119 areas (< 4% urban and < 6 % agricultural). Wetted channel width at base flow averaged ~2
120 to 5 m in our study streams and early summer water velocity ranged from 0.02 to 0.10 m/s
121 in pools and from 0.06 to 0.36 m/s in riffles. NO₃⁻-N concentrations at the time that
122 samples were collected for isotope analysis ranged from 5 to 184 µM across streams, with
123 the highest value from Mission Creek.

124 Riparian canopy cover at our study sites ranged from 27 to 98%. Riparian vegetation
125 was dominated by alder (*Alnus rhombifolia*), California laurel (*Umbellularia californica*),
126 coast live oak (*Quercus agrifolia*), willow (*Salix* spp.), and California sycamore (*Platanus*
127 *racemosa*). The riparian vegetation bordering two of our study streams in larger (~10 km²)
128 subwatersheds (Mission and San Antonio Creeks) had burned approximately one year prior
129 to our sampling, whereas upland, but not riparian vegetation, burned in the catchment of
130 another study site (Rattlesnake Creek). Our stream sites represented a subset of sites

sampled in a larger study that explored the effects of wildfire on stream community structure and food webs (see Cooper et al. 2015, for a more detailed description).

Sampling design. Basal resources and consumers

We collected samples of basal resources (conditioned leaves, algae, fine particulate organic matter) and invertebrates from the major functional feeding groups for stable carbon, nitrogen, and hydrogen isotope analysis, as well as invertebrates for gut content analyses, from pool and riffle habitats separately in 9 streams in June, 2010, approximately 2 months after the last rains of the wet season.

Conditioned leaves of *Alnus rhombifolia*, *Umbellularia californica*, *Quercus agrifolia*, *Salix* spp., and/or *Platanus racemosa* were collected from stream bottoms with Surber samplers at each site, and a subset was subjected to stable isotope analysis. Macroalgae (primarily *Cladophora*) were collected by hand from hard substrata (cobbles, boulders, bedrock). Benthic microalgae, although sampled, were not used in our analysis because samples contained substantial amounts of detritus based on microscopic examination. Previous studies have found that microalgae were more enriched in deuterium (^2H) than *Cladophora* (Finlay et al. 2010) and “filamentous algae” (Doucett et al. 2007), but all algae were well separated isotopically from stream leaf litter. Fine particulate organic matter (FPOM) was sampled by coring soft substrata (silt, sand, gravel, pebbles) to a depth of 0.8 cm with the barrel of a syringe sampler (Davies and Gee 1993). After agitation, elutriation, and mixing, suspended FPOM in subsamples was filtered through GF/C filters. Because FPOM samples from riffles did not contain sufficient quantities of organic matter for hydrogen isotope analysis, we used FPOM isotope values from pools to represent FPOM values for both pool and riffle habitats. Macroinvertebrates for isotope analysis

154 were collected at each stream site using Surber samplers (250 μm mesh) and aquatic D-
155 nets (1 mm mesh). For all basal resources and invertebrates, samples were taken from 5
156 pools and 5 riffles at each site, samples were combined by habitat (pools vs. riffles) and
157 thoroughly mixed, and representative subsamples were taken from these amalgamated
158 samples.

159 All samples taken for stable isotope and gut content analysis were stored frozen at
160 -20°C . The guts of individuals of common primary and secondary consumer taxa, with the
161 exception of chironomids, were removed and preserved in vials containing either formalin
162 (primary consumers) or ethanol (predators). Invertebrate specimens without guts or entire
163 chironomids were used for stable isotope analysis. Specimens of each taxon were not
164 always represented in samples from each stream.

165 *Sample preparation and isotope analysis*

166 In the laboratory, subsamples of leaves and macroalgae were thawed and rinsed in de-
167 ionized water to remove adhering material. FPOM was removed wet from GF/C filters.
168 Samples of leaves, macroalgae, FPOM, and insects were dried in new glass scintillation
169 vials without caps at 60°C and ground (or gently crushed for some small insect specimens)
170 to a fine powder using mortar and pestle. The ground material was divided in half, with
171 one portion analyzed for hydrogen isotopes and the other half analyzed for carbon and
172 nitrogen isotopes. The number of insect specimens processed varied by taxon depending
173 on availability and individual size, with 10 to 100 individuals of each taxon from pools
174 versus riffles at each site combined to obtain sufficient material for analysis. Lipids were
175 not removed from samples prior to analysis, similar to earlier studies (Doucett et al. 2007,
176 Finlay et al. 2010, Cole et al. 2011). The wide separation in hydrogen isotope ratios

between detritivores and detritus, on one hand, and algae and algivores, on the other, suggested that variation in lipid content would not be an important driver of hydrogen isotope patterns in our study (see also Wilkinson et al. 2015). This assumption was further supported by the congruence between gut contents and isotope mixing model estimates of resource contributions to predator diets (see Results). However, because of smaller differences in carbon isotope ratios between resources and predators, we applied the correction for lipids recommended by Skinner et al. (2016) based on C:N ratios (generally 4.0-4.5) to data used in mixing model analyses (see below).

Isotopic analysis of ground samples or subsamples (typically ~0.5 mg for hydrogen, ~1 mg for carbon and nitrogen) was conducted by the Facility for Isotope Mass Spectrometry (FIRMS, <http://ccb.ucr.edu/firms.html>) at the University of California, Riverside using a Thermo-Chemical Elemental Analyzer interfaced to a Thermo-Finnigan Delta-V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific Corp., Bremen, Germany). Hydrogen isotope ratios are expressed as $\delta^2\text{H}$. The $\delta^2\text{H}$ of non-exchangeable hydrogen in all samples was measured using the comparative equilibration method (Wassenaar and Hobson 2003, Kelly et al. 2009) with the isotopic values normalized to the Vienna Standard Mean Ocean Water (VSMOW) - Standard Light Antarctic Precipitation (SLAP) scale. The following standards were equilibrated with the samples and included in each analytical run: Caribou Hoof Standard ($\delta^2\text{H} = -197\text{‰}$), Kudu Horn Standard ($\delta^2\text{H} = -54\text{‰}$), and Spectrum Chemical keratin ($\delta^2\text{H} = -121\text{‰}$) (Flockhart et al. 2013). The natural abundances of carbon and nitrogen isotopes are expressed relative to the Pee Dee Belemnite (PDB) standard for carbon and atmospheric N_2 for nitrogen. Variation in isotope values between replicate portions of the same ground sample averaged 3.4‰ , 0.1

$‰$, and $0.2 ‰$ for $\delta^2\text{H}$ ($n=10$), and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($n=15$), respectively. Dividing these precision estimates by the differences in mean stable isotope ratios between algivores and detritivores in Table 1 produces values of 3.5%, 3.0%, and 8.7% for hydrogen, carbon, and nitrogen signatures in pools and 4.0%, 2.9%, and 7.7% for hydrogen, carbon, and nitrogen signatures in riffles. Thus, although the absolute precision value was highest for hydrogen isotope ratios, relative precision for hydrogen isotopes was comparable to or better than the relative precision estimates for carbon and nitrogen isotope ratios given the measured differences in mean stable isotope ratios between algivores and detritivores in Table 1.

Gut content analyses

We examined the gut contents of primary consumers and predators from our study sites to explore relationships between consumer isotope values and recently consumed food items. Gut contents of selected primary consumer taxa (*Lepidostoma*, *Paraleptophlebia*, *Baetis*, *Callibaetis*, *Centroptilum*, *Diphetera*, *Leucrocuta*, *Eubrianax*, *Simulium*) processed for isotope analysis (see above) were homogenized and a subsample was removed to a microscope slide for the identification, measurement, and enumeration of food items under a compound microscope at 400 X magnification. Counts were converted to biovolumes using known geometric formulae for algal taxa and fungi (Hillebrand et al. 1999). In addition, the slide area occupied by leaf fragments or amorphous detritus was measured and this was converted to a biovolume by multiplying the area by the measured average thickness ($18.8 \mu\text{m}$) of detrital particles.

We also examined the gut contents of predatory invertebrate taxa from pools and riffles. The gut contents of individual predatory taxa were placed in a small dish and examined under a dissecting microscope, with any prey body parts (mandibles, claws, tarsi, labra,

heads) being removed and placed in a drop of 100% glycerol on a microscope slide. Any body parts were identified to the lowest practical taxonomic level (usually genus) under a compound microscope by comparing them to photographs and illustrations of body parts taken from known taxa. The number of each taxon per gut sample was calculated as the rounded-up integer of the number of claws/6, tarsi/6, mandibles/2, heads, opercula, or labra (whichever was greatest). Because left and right mayfly mandibles were distinguishable, the number of mayfly nymphs per gut was determined by the number of left mandibles, right mandibles, or other body parts (as calculated above), whichever was greatest.

Statistical analysis

We used general linear models to test for significant separation in stable isotope ratios (expressed as $\delta^2\text{H}$, $\delta^{13}\text{C}$, or $\delta^{15}\text{N}$) among categories of resources and primary consumers (categories = conditioned leaves, FPOM, *Cladophora*, detritivores, algivores). The detritivore category included two common taxa presumed to rely primarily on terrestrial detritus and for which gut content data were available, the shredder caddisfly *Lepidostoma* and collector mayfly *Paraleptophlebia*. Many Chironominae/Orthocladiinae taxa are also considered detritivorous (Armitage et al. 1997), but the enriched nitrogen isotope ratios of chironomids collected in one creek (Mission) with elevated nitrate levels (see Results) suggested that this group could be using appreciable amounts of algae at some times or places. As a consequence, the Chironominae/Orthocladiinae isotope results are presented in Figure 1, but they were not considered resource specialists on leaves or algae and were not included in the specialist primary consumer and predator mixing model analyses (see below). The algivore category included common taxa for which gut content data were available, i.e., mayflies of the Baetidae (*Baetis*, *Callibaetis*, *Centroptilum*) and larvae of

the beetle *Eubrianax*. Streams were treated as replicates in analyses, with pools and riffles analyzed separately because differences in current velocity and other factors between these habitats could influence stable isotope ratios (Finlay et al. 1999; 2010).

Preliminary review of the isotope data suggested that values varied with the subwatershed area of each stream site, similar to Finlay et al.'s (2010) results. Therefore, we included subwatershed area as a covariate in the analysis. When the linear model showed no significant interaction effects of resource or consumer category with subwatershed area, we used the Sidak test to identify significant differences in isotope values between categories (e.g., leaves vs. algae, detritivores vs. algivores) for both pools and riffles at a mean subwatershed area of $\sim 8 \text{ km}^2$. The Sidak test adjusts *p*-values in multiple pairwise comparisons to reduce Type I error (Day and Quinn 1989). If the interaction between category and subwatershed was significant, we evaluated differences between resource and primary consumer categories at two covariate values (5 and 10 km^2 basin areas), representing “small” and “large” subwatersheds, respectively.

We tested for an effect of habitat (pool vs. riffle) on the isotope ratios of algivores (*Baetis*, *Eubrianax*) and detritivores (*Lepidostoma*, *Paraleptophlebia*) that were collected in both habitats using a paired t-test (pairing habitats for each taxon by stream). We used regression analyses to explore relationships between stable isotope ratios and gut contents. Proportion data were arcsine square root transformed prior to analysis, producing similar results as logit transformations. Statistical analyses were performed using SigmaPlot 13 (Systat Software, Inc, 2008) and SPSS 22 (IBM Corporation, 2013).

Comparison of predator stable isotope ratios to predator gut contents

To estimate the proportional contribution of algivorous prey to predator diets using hydrogen and carbon stable isotope ratios, we used the Stable Isotope Analysis in R (SIAR), a commonly applied statistical package that computes dietary contributions of resources to a consumer using a Bayesian framework (Parnell et al. 2010). The SIAR model includes variability in source, consumer, and trophic enrichment (TEF) in iterative model fittings to generate probability estimates of source proportions to consumer diets. We used the “siarsolo” routine in SIAR because we did not have within-stream estimates of variation in isotope ratio or dietary composition values for predators (one value per prey taxon or group per stream).

For the algivore end-member in the SIAR model, we used the averaged $\delta^2\text{H}$ or $\delta^{13}\text{C}$ values (and standard deviation) for individual algivorous taxa (*Baetis*, *Callibaetis*, *Centroptilum*, *Eubrianax*) as replicates from each stream. Based on the high proportion of algae in their gut contents (see Results), the isotope values of these taxa also served as proxies for the isotope values of microalgae (as in Vander Zanden and Rasmussen 2001; Finlay 2001a; 2004; Post 2002), which could not be determined directly because periphyton samples contained considerable amounts of terrestrial detritus that could not be removed by silicon centrifugation or other methods prior to isotope analysis. For the detritivore end-member, we used the average $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values of the common shredder *Lepidostoma*, and collector *Paraleptophlebia*, which had isotope values similar to those of conditioned leaves and gut contents comprised almost entirely of detrital particles (see Table 1, Fig. 1).

For carbon isotopes, we used a trophic enrichment factor (TEF) of $0.4 \pm 1.3\text{‰}$ from prey to predator (Post 2002), whereas the lack of consensus regarding trophic enrichment

for hydrogen isotopes (Vander Zanden et. al. 2016) precluded the inclusion of a TEF value for hydrogen isotopes. Proportional algivore contributions to predator diets were modeled separately for hydrogen and carbon isotope ratios and compared with proportions of algivore prey in predator gut contents. For $\delta^2\text{H}$, solutions of the SIAR model for algivore contributions to predator diet were highly correlated with the solutions of a single isotope two-source mixing model (Fry 2006) ($R^2 = 0.95$, $p < 0.001$, SIAR algivore proportion = $0.75 * (\text{two-source algivore proportion}) + 0.12$), but the outputs of SIAR and single isotope mixing models were not related when $\delta^{13}\text{C}$ values were used, because of greater overlap in prey end-member values for $\delta^{13}\text{C}$ than $\delta^2\text{H}$ (discussed below).

We also explored how incorporating environmental water into the SIAR model affected our conclusions by varying the hydrogen stable isotope ratios of stream water ($\delta^2\text{H}_{\text{H}_2\text{O}}$) and the proportion of consumer tissue consisting of stream water hydrogen (ω) (Solomon et al 2009 ; Wilkinson et al. 2015). $\delta^2\text{H}_{\text{H}_2\text{O}}$ values for our study streams were not available. Given the proximity and similar elevations of our study streams (Klose et al. 2015), we expected that $\delta^2\text{H}_{\text{H}_2\text{O}}$ values would be similar among streams, as supported by estimates of precipitation $\delta^2\text{H}_{\text{H}_2\text{O}}$ based on the geo-referenced locations of study sites and the online tool [Waterisotopes.org](https://www.waterisotopes.org) (Bowen et al. 2005; Bowen 2008). Estimated annual precipitation $\delta^2\text{H}_{\text{H}_2\text{O}}$ values were similar across our study area (-53 to -48‰ , $\bar{X} = -49\text{‰}$) and comparable to $\delta^2\text{H}_{\text{H}_2\text{O}}$ values reported for dry season flows measured in eight streams with undeveloped watersheds in the Santa Monica Mountains, approximately 100 km south (Hibbs et al. 2011).

Some information is available on possible ω values for the shredder *Lepidostoma* (0.12) and scrapers (0.06) from Finlay et al. (2010) in Wilkinson et al. (2015). Modifying Wilkinson et al. (2015), we adjusted the detritivore and algivore $\delta^2\text{H}$ end-member values for each stream prior to mixing model calculations by adjusting for the contribution of environmental water (ω_1) as follows: $\delta\text{D}_{\text{adjusted end-member}} = (\delta\text{D}_{\text{end-member}} - (\omega_1 * \delta^2\text{H}_{\text{H}_2\text{O}}))/(1 - \omega_1)$. Because environmental water contributions also change with trophic level, we also adjusted $\delta^2\text{H}$ values for each predator ($\delta^2\text{H}_{\text{predator}}$) as follows (modified from Wilkinson et al. 2015): $\delta^2\text{H}_{\text{adjusted predator}} = (\delta^2\text{H}_{\text{predator}} - (\omega_2 * (\delta^2\text{H}_{\text{H}_2\text{O}})))/(1 - \omega_2)$, where $\omega_2 = 1 - (1 - \omega_1)^2$. We compared unadjusted mixing model results to results adjusted for three values of environmental water $\delta^2\text{H}$ (-45, -50, -55‰) reported by Hibbs et al. (2011) for dry season flows and suggested from precipitation values in Waterisotopes.org, and for two levels of ω_1 , i.e., 0.1, representative of shredders and scrapers, and 0.2, suggested as a default value where ω is unknown (Wilkinson et al. 2015). Proportional contributions of algal-based resources to predator diet were then calculated using the SIAR mixing model with algivore and detritivore end-member and predator hydrogen and carbon stable isotope ratios.

Results

Isotope values of basal resources and specialist primary consumers

Across all streams, $\delta^2\text{H}$ values of conditioned leaves, FPOM, and detritivores were enriched on average by $\sim 100\text{‰}$ in pools and $\sim 80\text{‰}$ in riffles compared to values for *Cladophora* and algivores (Table 1, Fig. 1 a, b). $\delta^2\text{H}$ values of algivores, but not of *Cladophora* ($p > 0.8$), declined significantly with subwatershed area in pools and riffles

(pools, $p < 0.002$, $R^2 = 0.76$; riffles, $p = 0.015$, $R^2 = 0.60$, $n = 9$). $\delta^2\text{H}$ values of detritivores were not correlated with subwatershed area ($p > 0.5$).

In both pools and riffles, $\delta^2\text{H}$ values of detritivores were significantly enriched relative to values for algivores in accordance with differences in the $\delta^2\text{H}$ values of their expected food sources (pools: resource-consumer category, $p < 0.001$, $F_{4,28} = 16.3$, category x subwatershed area, $p = 0.001$, $F_{4,28} = 5.9$, Fig. 1 a; riffles: category, $p < 0.001$, $F_{4,26} = 10.4$, category x subwatershed area, $p = 0.13$, $F_{4,26} = 2.0$; Sidak *post hoc* test results in Table 2, Fig. 1 b). $\delta^2\text{H}$ values of detritivores were not significantly different from those for conditioned leaves and FPOM (Table 2). $\delta^2\text{H}$ values of algivores were not significantly different from values for *Cladophora* in pools, but were enriched relative to this alga in riffles (Table 2).

In both pools and riffles, $\delta^{13}\text{C}$ values also differed among basal resources, detritivores, and algivores (pools, resource-consumer category, $p = 0.001$, $F_{4,32} = 6.6$, category x subwatershed area, $p = 0.06$, $F_{4,32} = 2.5$, Table 2, Fig. 1 c; riffles, category, $p = 0.05$, $F_{4,28} = 2.7$, category x sub-watershed area $p = 0.65$, $F_{4,28} = 0.6$, Table 2, Fig. 1 d). In contrast to the results for $\delta^2\text{H}$, there was no significant difference in $\delta^{13}\text{C}$ values between algivores and detritivores or leaves in pools or riffles (Table 2, Fig. 1c). In both pools and riffles, there were no significant differences in $\delta^{15}\text{N}$ values among resource-consumer categories, and no category x subwatershed area interaction effects (pools: category, $p = 0.36$, $F_{4,31} = 1.1$, category x sub-watershed, $p = 0.51$, $F_{4,31} = 0.8$, Table 2, Fig. 1e; riffles, category $p = 0.70$, $F_{4,27} = 0.5$, category x sub-watershed, $p = 0.51$, $F_{4,27} = 0.8$, Table 2, Fig. 1f). Algivores and Chironominae/Orthocladiinae in both pools and riffles were enriched in ^{15}N

in Mission Creek relative to other creeks, which was associated with the appreciable ^{15}N enrichment of algae at this site (Figs. 1e, f).

There was no difference between pool and riffle habitats in the isotope values ($\delta^2\text{H}$, $\delta^{13}\text{C}$, or $\delta^{15}\text{N}$) of detritivores (*Lepidostoma*, *Paraleptophlebia*) or algivores (*Baetis*, *Eubrianax*) (p 's > 0.1, $n = 9$, paired t-tests).

Relationship between isotope ratios and gut contents of primary consumers

$\delta^2\text{H}$ values of primary consumers from both pools and riffles were negatively correlated with the proportions of gut contents consisting of algae (Figs. 2 a, b). As expected, the gut contents of the shredder *Lepidostoma* and collector *Paraleptophlebia* were comprised almost entirely of detrital particles (pools: $99.9 \pm 0.1\%$, $n = 9$; riffles: $95.5 \pm 2.5\%$, $n = 4$, $\bar{x} \pm 1\text{SE}$), consistent with their enriched $\delta^2\text{H}$ values (Figs 2 a, b). Algae comprised a much higher proportion of the gut contents (pools: $70 \pm 3\%$, $n = 11$; riffles: $40 \pm 3\%$, $n = 10$) of algivores (*Baetis*, *Callibaetis*, *Centroptilum*, *Eubrianax*), consistent with their more depleted $\delta^2\text{H}$ values. $\delta^{13}\text{C}$ values of primary consumers from pools also were negatively correlated with the proportions of their gut contents consisting of algae, but the relationship was weaker than that for $\delta^2\text{H}$ (Fig. 2c), and not significant for consumers from riffles (Fig. 2d).

Trophic support of predators: comparison of isotope ratios

$\delta^2\text{H}$ values of common predator taxa declined with subwatershed area (pools, $p < 0.001$, $R^2 = 0.80$; riffles, $p = 0.04$, $R^2 = 0.62$, $n = 9$, Figs. 3a, b). This analysis excluded the beetle *Stictotarsus*, which appeared to be a specialist predator on algivores based on consistently depleted $\delta^2\text{H}$ values. In the smallest streams, $\delta^2\text{H}$ values of predators in pools were

generally similar to values for detritivores and well separated from algivore values suggesting the primary use of detritivorous prey in these streams (Fig. 3a). However, the dramatically lower values of the damselfly, *Archilestes*, in two larger streams with elevated nitrate concentrations, and where the riparian vegetation had burned in a wildfire (Mission and San Antonio Creeks), suggested greater use of algivorous prey at those sites (Fig. 3a, see also Cooper et al. 2015). These patterns were less evident for predators sampled from riffles. Notably, the stoneflies *Isoperla* and *Calineura* tended to have depleted $\delta^2\text{H}$ values in the four smaller streams where they occurred, suggesting a greater use of algivorous prey by these stonefly predators than by other predators at those sites (Fig. 3b).

There were no relationships between $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of predator taxa from pools or riffles and subwatershed area (p 's > 0.1 , Figs. 3c - f). There was inconsistent separation among streams between detritivores and algivores in $\delta^{13}\text{C}$ (range of separation, +0.5 to -7.7‰) and $\delta^{15}\text{N}$ (+0.1 to +7.2) values, although most predator $\delta^{13}\text{C}$ values were similar to detritivore values in the three smallest streams (Fig. 3 c-f). Predators were more enriched in ^{15}N than detritivores (Figs. 3e, f vs. 1e, f), even in small streams, reflecting the trophic enrichment from prey to predator expected with N isotopes (Vander Zanden and Rasmussen 2001; Post 2002). However, $\delta^{15}\text{N}$ values of predators in Mission and San Antonio Creeks also were enriched relative to other streams (Figs. 3e, f), suggesting the consumption of algivores feeding on microalgae enriched in ^{15}N at these sites.

Trophic support of predators: mixing model estimates vs. gut contents

There was no difference between pools and riffles in the relationship between either $\delta^2\text{H}$ or $\delta^{13}\text{C}$ values of predators and the proportions of algivorous prey in their guts (all habitat and habitat X proportion of gut contents comprised of algivores effects were not

significant, $p > 0.2$), so data from pools and riffles were analyzed and presented together (Figs. 4a, c). $\delta^2\text{H}$ values of predators were negatively correlated with the proportions of algivorous prey in their guts ($p < 0.001$, Fig. 4a) and there was a positive correlation between the percent algal-based contributions to predator diet, as estimated using the SIAR model, and the proportions of their gut contents consisting of algivorous prey ($p < 0.001$, Fig. 4b).

Including the possible effects of environmental water on prey end-member and predator $\delta^2\text{H}$ values prior to mixing model calculations increased estimates of algal-based prey resource use relative to uncorrected values by 6 to 16%, depending on the ω and $\delta^2\text{H}_{\text{H}_2\text{O}}$ values used (Table 3). Increasing environmental water content (ω) from 0.1 to 0.2 had a greater effect on mixing model estimates than decreasing $\delta^2\text{H}_{\text{H}_2\text{O}}$ values from -45 to -55‰ (Table 3). Despite the possible effects of environmental water on mixing model results, the positive relationship between model estimates of algal-based contributions to predator tissue support and the proportions of algivores in gut contents remained highly significant across all combinations of ω and $\delta^2\text{H}_{\text{H}_2\text{O}}$ values (p 's < 0.001 , shown only for $\omega = 0.2$, $\delta^2\text{H}_{\text{H}_2\text{O}} = -55$ ‰ in Fig. 4b).

There was a weak relationship between predator $\delta^{13}\text{C}$ values and the proportions of their gut contents comprised of algivorous prey ($p = 0.001$, $R^2 = 0.19$, Fig. 4c). However, there was no relationship between SIAR model estimates of algivore-based carbon contributions to predator diets and the proportions of algivorous prey in predator guts ($p = 0.5$, Fig. 4d).

Discussion

Hydrogen stable isotopes ($\delta^2\text{H}$) more consistently and clearly distinguished algae and algivores from conditioned terrestrial leaves, FPOM, and detritivores in our headwater streams than did carbon isotopes ($\delta^{13}\text{C}$), with an average $\delta^2\text{H}$ separation of $> 80\text{‰}$ between algal and detrital resources and consumers across habitats and streams. The correspondence between the $\delta^2\text{H}$ values of specialist primary consumers and their expected diets in our small streams is congruent with comparisons of $\delta^2\text{H}$ values between consumers and their assumed food sources in streams in northern California (Finlay et al. 2010). In that study, average differences in $\delta^2\text{H}$ values between algivorous invertebrates and algae, and between detritivorous invertebrates and terrestrial detritus, were much smaller ($< 5\text{‰}$) than the differences between these two feeding groups and food sources ($> 60\text{‰}$).

There also was remarkably good agreement between our results and those of Finlay et al. (2010) for the average $\delta^2\text{H}$ values of conditioned leaves (-110‰ for our data vs. -109‰ for Finlay et al., 2010), detritivores (pools, -108 vs. -105‰), *Cladophora* (riffles, -219 vs. -225‰) and algivores (riffles, -189 vs. -175‰), despite differences in the riparian vegetation, consumer taxa, and hydrological and other conditions between our study sites. The close correspondence and wide separation in $\delta^2\text{H}$ values between specialist consumers and their food resources in our study and that of Finlay et al. (2010) suggest that the effects of trophic and tissue fractionation, and $\delta^2\text{H}$ values of environmental water, on body tissue $\delta^2\text{H}$ values (Solomon et al. 2009; Wilkinson et al. 2015) were similar between our sites and those of Findlay et al. (2010).

In contrast, Jardine *et al.* (2009), working in streams in New Brunswick, Canada, reported a smaller overall mean difference and more variation in the separation of $\delta^2\text{H}$

values in stream “biofilm” versus leaves (alder) (mean difference $\pm 1\text{SD}$ of 39.2 ± 31.2 ‰, all 31 sites, from Table 1, range +15.9 to -109 ‰) than found in our study and that of Finlay et al. (2010). Furthermore, the $\delta^2\text{H}$ values for leaves and biofilm overlapped at more sites than $\delta^{13}\text{C}$ values, precluding the application of mixing models using $\delta^2\text{H}$ data because of insufficient $\delta^2\text{H}$ end-member separation (as based on criteria of non-overlapping standard deviations around mean values). They suggested that local factors, such as the chemistry and $\delta^2\text{H}$ values of the water body, and perhaps consumer lipid content, affected the relative effectiveness of carbon and hydrogen isotope ratios in tracing food web pathways at their sites.

Although the separation in $\delta^2\text{H}$ values between algivore and detritivore taxa was distinct in our study streams, $\delta^2\text{H}$ values of algivores declined with increasing subwatershed area and with the loss of canopy cover owing to wildfire in two of the larger sub-watersheds (Mission, San Antonio, Cooper et al. 2015). Finlay et al. (2010) also reported decreasing $\delta^2\text{H}$ values with increasing catchment area for filamentous green algae, diatoms, and herbivorous caddisflies in northern California streams. One possible explanation for this pattern is that the microalgae consumed by algivores were more enriched in $\delta^2\text{H}$ in the smaller than larger streams. Finlay et al. (2010) proposed that such enrichment could arise through the greater fractionation of hydrogen isotopes during photosynthesis or higher algal lipid concentrations, which are depleted in deuterium (^2H), in small than large streams. However, in our study, $\delta^2\text{H}$ values of the filamentous macroalga *Cladophora* did not decrease with subwatershed area, which is inconsistent with this explanation.

Another possibility is that algivores consumed more detritus in small than large streams congruent with McNeely et al.'s (2006) results showing increasing algal biomass, and increasing proportions of algae in the guts of some taxa, with increases in watershed area. This possibility is also supported by studies showing flexibility in primary consumer diets, even within functional groups, including scrapers or grazers, generally considered to be largely algivorous, along stream or river continua (Rosi-Marshall et al. 2016; Collins et al. 2016). Until more information is available, the use of algivore $\delta^2\text{H}$ values as proxies for the microalgal end-member in mixing models needs to consider that the $\delta^2\text{H}$ values of microalgae may vary among streams and that algivores may derive increased nutritional support from terrestrial detritus (versus algae) in small streams. Although some shredder or collector taxa have been reported to consume large amounts of algae in other systems (Rosi-Marshall et al. 2016), the detritivore taxa representing the terrestrial end-member in our study were distinctly detritivorous across streams, as evidenced by enriched $\delta^2\text{H}$ values that were widely separated from algivore values, and gut contents consisting almost entirely of detrital particles.

In contrast to $\delta^2\text{H}$, $\delta^{13}\text{C}$ values of basal resources, algivores, and detritivores were not consistently well separated in all streams. This lack of consistent separation in $\delta^{13}\text{C}$ values between basal resources (algae, CPOM, FPOM) has limited the utility of carbon isotopes in tracing the sources and fates of organic matter in freshwater ecosystems and has been attributed to variation in the $\delta^{13}\text{C}$ carbon values of algae rather than terrestrial detritus (France 1996; Doucett et al. 1996; Finlay 2001; McNeely et al. 2006; Doucett et al. 2007; Ishikawa et al. 2012). $\delta^{13}\text{C}$ carbon values of terrestrial detritus vary over a relatively narrow range (e.g., conditioned leaves, -29.0 ± 0.3 , our study, Table 1; -28.2 ± 0.2 ($\pm 1\text{SE}$) in

Finlay 2001), whereas $\delta^{13}\text{C}$ carbon values of algae and algivores can vary widely with factors that affect the fractionation of carbon isotopes during the uptake of CO_2 and DIC by algae, such as algal productivity and aqueous CO_2 availability (Finlay et al. 2001).

Using nitrogen isotopes to trace trophic pathways also requires sufficient and reliable separation in resource end-member values. Nitrogen isotope ratios can be highly variable in time and space owing to variation in the isotopic signature and availability of dissolved inorganic N (DIN) assimilated by producers, changes in the $\delta^{15}\text{N}$ values of detritus during decomposition by microbes, and variation in producer $\delta^{15}\text{N}$ values resulting from anthropogenic DIN inputs (Peterson 1999; Post 2002; Moore et al. 2014). In this study, stable nitrogen isotope values were not consistently or clearly different between different resource and primary consumer categories. As a consequence, $\delta^{15}\text{N}$ values were not useful in identifying basal resource contributions to consumers in our study streams.

Predators in Mission and San Antonio Creeks, however, were enriched in ^{15}N relative to other streams, suggesting the consumption of algivores feeding on microalgae with enriched $\delta^{15}\text{N}$ values, engendered by increased light and nutrient levels following a wildfire the year before (Cooper et al. 2015). Interestingly, there also was some ^{15}N enrichment of the detritivores, *Lepidostoma* and *Paraleptophlebia* ($\sim +2\text{‰}$) in these two streams. $\delta^{15}\text{N}$ values of conditioned leaves were not enriched, suggesting that these detritivores were deriving some nutritional support from microbial cells that had incorporated DIN (Hamilton et al. 2004). Variation in the $\delta^{15}\text{N}$ values of predators across streams emphasizes the importance of calculating increments in ^{15}N values from prey to predators for each stream independently to assess the trophic level of individual taxa (Vander Zanden and Rasmussen 1999; Post 2002), and also illustrates the potential use of

512 $\delta^{15}\text{N}$ to trace nutrient enrichment effects through the food web (McClelland et al. 2014;
513 Moore et al. 2014).

514 There was better agreement (higher R^2 values) between $\delta^2\text{H}$ values, than $\delta^{13}\text{C}$ values,
515 and recent consumer diets. One likely explanation for this finding is the more consistent
516 and greater separation of $\delta^2\text{H}$ than $\delta^{13}\text{C}$ values between algal and terrestrial basal resources
517 across streams. The better congruence between $\delta^2\text{H}$ than $\delta^{13}\text{C}$ signatures and recent diets
518 also could occur because of differences in isotope turnover times, such as hydrogen
519 isotopes turning over more rapidly than carbon isotopes, as has been found for some
520 vertebrate tissues (Wolf et al. 2012), thus better reflecting recently-consumed food items.
521 The concordance between consumer $\delta^2\text{H}$ values and gut contents supports conclusions
522 regarding the relative contributions of algae and terrestrial detritus to primary consumer
523 diets and nutrition, but the time frame over which $\delta^2\text{H}$ values integrate past consumption
524 are unknown (e.g. Jardine et al. 2005; McNeely et al. 2007; Li and Dudgeon 2008; Araujo
525 et al. 2014).

526 The efficacy of hydrogen isotope ratios in delineating food webs in our streams was
527 further supported by the significant correlation between algivore contributions to predator
528 nutrition, estimated using a commonly applied Bayesian mixing model, and the
529 proportions of algivorous prey in predator gut contents, a relationship that was unaffected
530 by adjusting measured resource and predator $\delta^2\text{H}$ values for the possible effects of
531 environmental water. The variable and smaller separation of carbon isotope ratios between
532 algivores and detritivores resulted in a lack of agreement between estimates of algivore
533 contributions to predator diet using the mixing model and predator gut contents, and there
534 was no pattern of carbon isotope ratios with subwatershed area to indicate better separation

of end-members in larger catchments (Findlay et al. 2010). As a consequence, carbon isotope signatures were not a consistent indicator of resource use by consumers in our streams and not reliable as a complementary tracer. Our results indicate that individual streams need to be evaluated *a priori* to determine whether sufficient separation exists in resource end-member values before specific stable isotopes are used to delineate food web structure.

In conclusion, although the importance of allochthonous terrestrial organic matter subsidies and autochthonous algal production to the food webs of streams is widely recognized, the ability of stable carbon isotope ratios to quantify the relative contributions of these resources to higher trophic levels has been limited in many cases. Our findings support the use of hydrogen isotope ratios to trace the sources and fates of organic matter in the food webs of small headwater streams of southern California, and probably other streams in arid and semi-arid climates and/or during drought (Doucett et al. 2007). Our results support the use of hydrogen isotope ratios in delineating food web structure along longitudinal river continua (Finlay et al. 2010; Collins et al. 2016) and in evaluating food web responses to human activities and natural disturbances that alter riparian shading and inputs, and algal production, such as land use changes (logging, livestock grazing, agricultural and urban development), wildfires, and floods (Kiffney 2008; Wootton 2012; Cooper et al. 2013; Cooper et al. 2015), producing more consistent and clearer results than those based on carbon isotope ratios.

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Figure Legends

- Figure 1. $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values of basal resources and specialist algivore and detritivore taxa from pool (left) and riffle (right) habitats of the study streams. Streams arranged, left to right, from smallest to largest subwatershed areas (in parentheses, km^2). Mean values for detritivores and algivores from pools and riffles computed across streams are shown as dashed lines (from Table 1). Leaf and FPOM values from pools only.
- Figure 2. Relationships between a-b) $\delta^2\text{H}$ and c-d) $\delta^{13}\text{C}$ tissue values and the proportion of algae in guts (algal biovolumes/algal + detrital biovolumes, arcsine square-root transformed) for known detritivores ($\triangle$: *Lepidostoma*, *Paraleptophlebia*), algivores ($\circ$: *Baetis*, *Callibaetis*, *Eubrianax*, *Centroptilum*), and other primary consumer taxa with variable diets ($\square$: *Leucrocuta*, *Dipheter*, *Simulium*) from pool (left) and riffle (right) habitats. Linear regression equations (for significant relationships), coefficients of determination (R^2), associated p -values, and sample sizes are also shown.
- Figure 3. $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values of predator taxa from pool (left) and riffle (right) habitats of the study streams. Streams arranged as in Figure 1. Values for detritivores (open bar) and algivores (black dash) are shown for each stream where data were available.
- Figure 4. Relationships between the proportions of predator gut contents composed of algivorous prey (algivorous prey/algivorous + other prey, arcsine square-root transformed) and a) $\delta^2\text{H}$ values of predator taxa, b) SIAR mixing model

estimates ($\pm 1SD$) of algivore contributions to predator nutrition based on δ^2H values of predator, algivore, and detritivore tissues for each stream (Figs. 1, 3): solid points, solid line = unadjusted values, open points, dashed line = example with values adjusted for environmental water (Table 3), setting $\omega_1 = 0.2$, $\delta^2H_{H_2O} = -55\text{‰}$, c) $\delta^{13}C$ values of predator taxa, and d) SIAR mixing model estimates of algivore contributions to predator nutrition based on $\delta^{13}C$ values of predator, algivore, and detritivore tissues for each stream. Regression lines were fit to data from pools and riffles combined. Regression equations, coefficients of determination, associated p-values, and sample sizes are also shown for significant relationships.

Table 1. Mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$ values ($\pm 1\text{SE}$) of basal resources, algivores, and detritivores in Figure 1 computed across study streams. Sample size (number of streams for each isotope value) in parentheses.

Habitat	Category	Taxon	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^2\text{H}$ (‰)
Pools	Algivores	<i>Baetis</i>	-31.8 ± 1.0 (9)	2.3 ± 0.9 (9)	-209 ± 8 (9)
		<i>Callibaetis</i>	-32.6 ± 1.2 (4)	3.7 ± 2.5 (4)	-228 ± 10 (4)
		<i>Eubrianax</i>	-30.9 ± 1.3 (5)	1.9 ± 1.7 (5)	-192 ± 7 (5)
		<i>Centroptilum</i>	-30.8 ± 1.2 (5)	3.2 ± 1.8 (5)	-216 ± 11 (7)
		Mean	-31.5	2.8	-211
	Detritivores	<i>Paraleptophlebia</i>	-29.1 ± 0.7 (7)	0.9 ± 0.2 (7)	-122 ± 10 (7)
		<i>Lepidostoma</i>	-27.9 ± 0.3 (8)	-0.4 ± 0.6 (8)	-105 ± 5 (8)
		Mean	-28.5	0.5	-114
	Macroalgae	<i>Cladophora</i>	-34.3 ± 2.3 (6)	1.2 ± 1.8 (6)	-217 ± 9 (6)
	Conditioned leaves	Various	-29.0 ± 0.3 (9)	-2.1 ± 0.4 (9)	-110 ± 4 (9)
	FPOM		-26.4 ± 0.7 (9)	1.3 ± 0.5 (9)	-95 ± 4 (5)
Riffles	Algivores	<i>Baetis</i>	-31.7 ± 1.2 (7)	3.2 ± 1.3 (7)	-190 ± 9 (8)
		<i>Eubrianax</i>	-32.8 ± 2.5 (3)	2.5 ± 3.1 (3)	-187 ± 2 (3)
		Mean	-32.3	2.9	-189
	Detritivores	<i>Paraleptophlebia</i>	-29.4 ± 1.2 (4)	1.0 ± 0.6 (4)	-115 ± 6 (5)
		<i>Lepidostoma</i>	-27.7 ± 0.3 (3)	-0.4 ± 0.3 (3)	-92 ± 1 (3)
		Mean	-28.6	0.3	-104
	Macroalgae	<i>Cladophora</i>	-34.6 ± 1.4 (6)	0.3 ± 1.1 (6)	-219 ± 8 (6)

Table 2. Significance (*p* -value) of Sidak multiple pairwise tests comparing the stable isotope signatures ($\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of basal resources and specialist primary consumers from pool and riffle habitats. *Category x subwatershed interaction effect significant (*p* < 0.05) in general linear model resulting in Sidak tests evaluated at subwatershed areas of 5 and 10 km², with *p* values provided for both subwatershed areas only if different. Significant differences (*p* < 0.05) identified in pairwise tests are in boldface. + Result from pairwise test not consistent with known trophic relationships.

Pools					Riffles				
$\delta^2\text{H}^*$	<i>Cladophora</i>	Algivores	Detritivores	FPOM	$\delta^2\text{H}$	<i>Cladophora</i>	Algivores	Detritivores	FPOM
<i>Cladophora</i>					<i>Cladophora</i>				
Algivores	0.1, 1				Algivores	0.006⁺			
Detritivores	0.001	0.001			Detritivores	0.001	0.001		
FPOM	0.001	0.001	1, 0.2		FPOM	0.001	0.001	0.5	
Leaves	0.001	0.001	0.5, 0.8	0.6, 0.9	Leaves	0.001	0.001	1	0.5
$\delta^{13}\text{C}^*$					$\delta^{13}\text{C}$				
<i>Cladophora</i>					<i>Cladophora</i>				
Algivores	0.1				Algivores	0.7			
Detritivores	0.001	0.1 ⁺			Detritivores	0.02	0.4 ⁺		
FPOM	0.001	0.001	0.3		FPOM	0.001	0.001	0.2	
Leaves	0.001	0.2 ⁺	1	0.3	Leaves	0.001	0.06 ⁺	1	0.3
$\delta^{15}\text{N}$					$\delta^{15}\text{N}$				
<i>Cladophora</i>					<i>Cladophora</i>				
Algivores	0.9				Algivores	0.6			
Detritivores	1 ⁺	1 ⁺			Detritivores	0.4 ⁺	1 ⁺		
FPOM	1 ⁺	0.8 ⁺	1		FPOM	1 ⁺	1 ⁺	0.8	
Leaves	0.6 ⁺	1 ⁺	0.6	0.8	Leaves	0.5 ⁺	0.005	0.005⁺	0.05⁺

Table 3. Percentage difference in algal-based contributions to predator diet among SIAR mixing model estimates using measured algivore, detritivore and predator end-member values compared to values adjusted for environmental water using the different ω and $\delta^2\text{H}_{\text{H}_2\text{O}}$ values listed below ($\bar{x} \pm 1$ SE, $n = 47$ predator values).

$\delta^2\text{H}_{\text{H}_2\text{O}}/_{\text{‰}}$	ω	
	0.10	0.20
-45	7.3 $\pm$ 0.8	16.1 $\pm$ 1.6
-50	6.8 $\pm$ 0.8	15.2 $\pm$ 1.6
-55	6.3 $\pm$ 0.8	9.2 $\pm$ 1.4