

# Tracing energy flow in stream food webs using stable isotopes of hydrogen

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## SUMMARY

1. Use of the natural ratios of carbon and nitrogen stable isotopes as tracers of trophic interactions has some clear advantages over alternative methods for food web analyses, yet is limited to situations where organic materials of interest have adequate isotopic separation between potential sources. This constrains the use of natural abundance stable isotope approaches to a subset of ecosystems with biogeochemical conditions favourable to source separation.

2. Recent studies suggest that stable hydrogen isotopes ( $\delta D$ ) could provide a robust tracer to distinguish contributions of aquatic and terrestrial production in food webs, but variation in  $\delta D$  of consumers and their organic food sources are poorly known. To explore the utility of the stable hydrogen isotope approach, we examined variation in  $\delta D$  in stream food webs in a forested catchment where variation in  $\delta^{13}C$  has been described previously.

3. Although algal  $\delta D$  varied by taxa and, to a small degree, between sites, we found consistent and clear separation (by an average of 67‰) from terrestrial carbon sources. Environmental conditions known to affect algal  $\delta^{13}C$ , such as water velocity and stream productivity did not greatly influence algal  $\delta D$ , and there was no evidence of seasonal variation. In contrast, algal  $\delta^{13}C$  was strongly affected by environmental factors both within and across sites, was seasonally variable at all sites, and partially overlapped with terrestrial  $\delta^{13}C$  in all streams with catchment areas larger than 10 km<sup>2</sup>.

4. While knowledge of isotopic exchange with water and trophic fractionation of  $\delta D$  for aquatic consumers is limited, consistent source separation in streams suggests that  $\delta D$  may provide a complementary food web tracer to  $\delta^{13}C$  in aquatic food webs. Lack of significant seasonal or spatial variation in  $\delta D$  is a distinct advantage over  $\delta^{13}C$  for applications in many aquatic ecosystems.

*Keywords:* energy flow, food webs, stable isotopes, subsidies, trophic interactions

## Introduction

Knowledge of the sources and fluxes of energy and nutrients through food webs is integral to understanding the productivity and diversity of consumers and essential to any effort to understand their ecological interactions. The sources of organic matter that fuel aquatic consumers are highly diverse in space and time, a feature that has presented

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substantial challenges to understanding food web dynamics. Stable isotopes are increasingly used to trace energy flow and trophic structure in food webs because they integrate across spatial and temporal scales and thus provide important information that is not otherwise accessible without very large expenditures of research time and resources (Peterson & Fry, 1987).

Most natural abundance isotope studies of diet sources use stable isotopes of carbon (i.e.  $\delta^{13}\text{C}$ ). Tracing of sources using  $\delta^{13}\text{C}$  in aquatic ecosystems relies on isotopic differences between potential organic matter sources that arise from variation within or among ecosystems in plant  $\delta^{13}\text{C}$ , usually because of effects of inorganic carbon  $\delta^{13}\text{C}$  and fractionation of inorganic carbon  $\delta^{13}\text{C}$  during photosynthesis (Peterson & Fry, 1987). After carbon is fixed by plants, there are minor changes associated with aerobic decomposition and trophic transfer so that  $\delta^{13}\text{C}$  is an effective tracer of diet sources (Vander Zanden & Rasmussen, 2001). While environmental variation or physiological differences among plants create useful separation between source  $\delta^{13}\text{C}$  in some streams and lakes (e.g. Finlay, 2001; Grey, Jones & Sleep, 2001), there are others where source  $\delta^{13}\text{C}$  overlap occurs during some or all of the year. Terrestrial plants show a somewhat limited range of  $\delta^{13}\text{C}$  values such that variation in algal  $\delta^{13}\text{C}$  largely determines the degree of overlap between allochthonous and autochthonous sources in freshwaters (Finlay & Kendall, 2007). Algal  $\delta^{13}\text{C}$  can have a range that exceeds 15‰ within a site because of variation in water flow (France, 1995; Hecky & Hesslein, 1995; Trudeau & Rasmussen, 2003; Singer *et al.*, 2005), biomass (Hill & Middleton, 2006), dissolved  $\text{CO}_2$  concentrations and  $\delta^{13}\text{C}$  of dissolved inorganic carbon (Finlay, 2004). In aquatic ecosystems where environmental conditions are highly dynamic, tracing of energy flow with  $\delta^{13}\text{C}$  may not be possible or may require a high intensity sampling effort.

Greater knowledge of environmental effects on variation in  $\delta^{13}\text{C}$  values of sources and recognition of the inherent complexity of many food webs suggests a need for additional tracers of energy flow pathways in aquatic ecosystems. Use of multiple tracers greatly increases the ability to resolve organic matter sources in food webs and ecosystems (Phillips & Gregg, 2003). The stable isotope tracer often used to complement  $\delta^{13}\text{C}$  in natural abundance studies of aquatic ecosystems is  $\delta^{15}\text{N}$ . While most commonly

used to measure trophic position,  $\delta^{15}\text{N}$  can provide a complementary source tracer to  $\delta^{13}\text{C}$  in some settings, such as at the marine–freshwater interface, and in highly eutrophic ecosystems where source differences are large (e.g. Delong & Thorp, 2006). Such applications are limited, however, by variable trophic fractionation by primary consumers (e.g. Goedkoop, Akerblom & Demandt, 2006) and temporal changes in organic matter  $\delta^{15}\text{N}$  because of variation in  $\delta^{15}\text{N}$  of dissolved inorganic nitrogen and microbial immobilisation of N onto organic matter (Finlay & Kendall, 2007). Sulphur stable isotopes ( $\delta^{34}\text{S}$ ) are increasingly used to complement  $\delta^{13}\text{C}$  analyses in organic matter source tracing. A recent review found that  $\delta^{34}\text{S}$  distinguished organic matter sources in coastal marine ecosystems better than  $\delta^{15}\text{N}$  (Connolly *et al.*, 2003). Within freshwater ecosystems, however, the sparse data available indicate relatively little difference between major organic matter sources in  $\delta^{34}\text{S}$  in most ecosystems (Finlay & Kendall, 2007).

Hydrogen stable isotopes (i.e.  $^2\text{H}$  and  $^1\text{H}$ , deuterium : hydrogen,  $\delta\text{D}$ , or  $\delta^2\text{H}$ ) in organic materials are variable in the environment and could provide an alternative or complementary natural abundance isotopic tracer to those of carbon, nitrogen and sulphur.  $\delta\text{D}$  is increasingly used to track movement and migrations of animals (Hobson, 1999; Bowen, Wassenaar & Hobson, 2005) but has not been widely used to distinguish organic matter sources and energy flow pathways in food webs. Such use was considered decades ago (Smith & Epstein, 1970; Estep & Dabrowski, 1980) but was not widely adopted because of methodological limitations and concern about unexplained variability in  $\delta\text{D}$  (see Doucett *et al.*, 2007).

Recent advances in stable hydrogen isotope methods have triggered new interest in  $\delta\text{D}$  as a tracer in freshwater ecosystems.  $\delta\text{D}$  has been most commonly analysed in specific compounds rather than bulk organic materials because of variation among biological tissues and methodological concerns. Improved analytical techniques (Wassenaar & Hobson, 2003) have, however, addressed some of the methodological issues associated with analyses of bulk tissues. Further, a recent study suggests that effects of trophic fractionation and exchange of isotopes between organism and water are relatively small and may be predictable (Solomon *et al.*, 2009). Finally, a study in rivers of the southwestern U.S.A. demonstrated a large isotopic separation between non-exchangeable

$\delta D$  in terrestrial and aquatic sources of organic matter and their consumers (Doucett *et al.*, 2007). Together, these advances suggest the potential of  $\delta D$  as an effective tool to analyse energy flow in aquatic food webs.

There is, however, little information to determine the general efficacy of source separation using  $\delta D$ . In plants, stable isotope ratios are determined by the  $\delta$  value of inorganic nutrients and fractionation during photosynthetic uptake. Inorganic hydrogen incorporated into plant biomass is derived from water. While  $\delta D$  of precipitation may be highly variable over short timescales, ground and surface water are more temporally integrated and show more dampened seasonal signals in many catchments (Kendall & Coplen, 2001), suggesting that effects of variation in source water  $\delta D$  on autotroph  $\delta D$  may be minimal or could be predictable from catchment hydrology. Less is known about fractionation of hydrogen stable isotopes during uptake and assimilation. Terrestrial and aquatic plants tend to be isotopically distinct for  $\delta D$  because of the kinetic fractionation associated with evaporative enrichment of  $^2H$  in water within leaves of terrestrial plants. Water enriched in  $^2H$  is assimilated into terrestrial plant biomass during photosynthesis (Smith & Ziegler, 1990), a process that does not affect aquatic plants. In one study of streams of arid catchments, this process resulted in  $\delta D$  differences between terrestrial and aquatic plants of 52–167‰, with an average difference of about 100‰ (Doucett *et al.*, 2007).

Apart from a small number of early studies (e.g. Smith & Epstein, 1970) and work of Doucett *et al.* (2007), it is not well known how variable terrestrial and aquatic plant  $\delta D$  values are under typical environmental conditions in freshwaters, how distinct potential organic matter sources are, and, overall, how well measurements of  $\delta D$  in bulk organic materials perform as food web tracers. To examine the potential for source separation in aquatic food webs, we examined  $\delta D$  in resources and invertebrate consumers in streams and rivers of a temperate-forested catchment. The goal of our study was to compare the efficacy of terrestrial versus algal source separation with  $\delta D$  and  $\delta^{13}C$  in lotic ecosystems across gradients of environmental conditions known to affect algal  $\delta^{13}C$ . We specifically examined effects of water velocity, longitudinal environmental gradients and seasonal variation on  $\delta D$  source separation within these streams.

## Methods

### General approach

The primary study sites were in the South Fork of the Eel River catchment in the Coast Range of Mendocino County, CA. Previous studies of stream food webs at this site have identified the controls over  $\delta^{13}C$  of resources and invertebrate consumers (Finlay, Power & Cabana, 1999; Finlay, Khandwala & Power, 2002; McNeely, Clinton & Erbe, 2006; McNeely, Finlay & Power, 2007). Here, we examined  $\delta D$  source separation through a combination of  $\delta D$  measurements of archived samples previously analysed for  $\delta^{13}C$  and reported earlier, and collection and analyses of  $\delta D$  in new samples. Sampling focused on intensive seasonal and spatial sampling within a small number of streams, complemented with investigations during summer base flows across a broader gradient of stream size. Variation in stream size is defined by catchment area in this study. Catchment area is strongly correlated with channel width and depth and provides a more robust metric than stream order for comparing environmental conditions among sites.

The archived samples were collected during 1997–99 and 2003 and were stored dry in glass vials in the dark after analysis for  $\delta^{13}C$ . The new samples were collected during 2005–07 at some sites sampled previously, using similar methods. These samples were not analysed for  $\delta^{13}C$ .

### Site description

The Eel River catchment has dry, warm summers and cool wet winters with very little snowfall. The riparian forest is largely composed of evergreen species such as Douglas fir, redwood and bay laurel [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*, *Sequoia sempervirens* (D. Don) Endl., and *Umbellularia californica* (Hook. & Arn.) Nutt., respectively]. As a consequence, there is little seasonal change in canopy cover at the study sites. Streams in the catchment are mostly steep, with cobble and boulder-dominated step-pool channel morphology. During the summer base flow period, dissolved organic carbon concentrations are low (0.2–1.5 mg L<sup>-1</sup>) and water clarity is high (Finlay, 2003). Midsummer algal production in small tributary streams is low but increases with stream size and light levels (Finlay, 2004). Dissolved nutrient levels are very low throughout the catchment; dissolved CO<sub>2</sub>

concentrations decrease with stream size because of degassing and algal uptake (Finlay, 2003). Algal biomass is also low on cobble surfaces because of low light and high herbivore densities (Finlay, 2004; McNeely *et al.*, 2006).

#### Sample collection

Algal samples (including filamentous diatoms) were collected by hand into clean glass vials or plastic bags. Samples were picked clean of visible detrital material and rinsed with filtered water. Epilithic diatoms were scraped from stones using a toothbrush and rinsed into clean centrifuge tubes. Subsamples were examined under the microscope to confirm that the material was mostly diatoms, and the rest of the sample was centrifuged at 111 g to form a pellet of organic material, which was transferred to a glass vial and dried at 60 °C. To characterise  $\delta D$  of terrestrial detritus, we collected stream-conditioned deciduous leaves from eight sites. Most data were generated from samples collected during 1997–99, but collections made in 2005 and 2006 were included to provide additional data. Data for  $\delta^{13}C$  are reported by Finlay (2001) and McNeely *et al.* (2006).

Most invertebrates were sampled at mid- to late instar phases. Samples of 3–30 larvae were composited at each site (or at each point for water velocity analyses). Gut contents were dissected and discarded; invertebrate samples were dried at 60 °C in glass vials and stored until analysed as described below. Data for  $\delta^{13}C$  are reported by Finlay (2001, 2004), Finlay *et al.* (1999, 2002) or were previously unpublished but collected in similar manner to the published data, as described below.

Water samples for oxygen and hydrogen stable isotopes analyses were collected to help understand sources of variation in autotroph  $\delta D$ . In 1998, only water  $\delta^{18}O$  was analysed; on all other dates, both isotopes were analysed to establish relationship between water  $\delta^{18}O$  and  $\delta D$ . Samples were collected without aeration and transferred to glass scintillation vials with tightly sealed cone caps and stored under refrigeration prior to analysis.

#### Water velocity effects

To assess water velocity effects on algal  $\delta D$ , we analysed samples of 13 herbivorous invertebrates,

including mayflies and grazing caddisflies, collected during midsummer 1998 and 1999 across a gradient in water velocity (0–0.8 m s<sup>-1</sup>) in the South Fork Eel River. Previous research showed these invertebrates had primarily algal diets (Finlay, 2001; McNeely *et al.*, 2006), and  $\delta^{13}C$  of algae and consumers was negatively related to water velocity (Finlay *et al.*, 2002). Water velocity was measured with a Marsh McBirney (Model 2000; Hach Inc., Loveland, CO, U.S.A.) acoustic flow meter 5 cm above the cobble substrate. Previously reported data for herbivore  $\delta^{13}C$  in Finlay *et al.* (2002) were used to compare with new measurements of  $\delta D$ .

#### Longitudinal patterns

Environmental conditions change substantially throughout river networks, and many of these changes (light, productivity, groundwater inputs) are known to affect  $\delta^{13}C$  of allochthonous and autochthonous resources through spatial differences in isotope ratios of inorganic elements used by plants and microbes and fractionation during uptake (Finlay & Kendall, 2007). We examined the sensitivity of  $\delta D$  to environmental changes across sites in the South Fork Eel river basin and several nearby streams during summer base flow. We sampled common algae, including *Cladophora glomerata* (L.) (Kütz.), *Nostoc parmeloides* (Kütz.), *Lemanea* sp. and epilithic diatoms, conditioned leaves and consumers with diets known to be either highly linked to terrestrial detritus (the shredders *Lepidostoma* sp. and *Psychoglypha* sp.) or dominated by algae (several trichopteran herbivores including *Glossosoma penitum* (Banks), *Glossosoma califica* (Denning), *Neophylax splendens* (Denning) and *Neophylax rickeri* (Milne) (Finlay, 2004; McNeely *et al.*, 2006). To assess the influence of water  $\delta D$  on spatial variation in algal and consumer  $\delta D$ , we measured water  $\delta D$  and  $\delta^{18}O$  at five to seven sites in the catchment during midsummer 1998 and 2005. We report water  $\delta^{18}O$  for dates where analyses of  $\delta D$  were not available since  $\delta^{18}O$  is well correlated with  $\delta D$  (Gat, 1996). Sites were sampled in the South Fork Eel River catchment ranging from very small headwater streams to the River at the Angelo Coast Range Reserve and Ten Mile Creek at its confluence with the South Fork Eel River. Samples for algae and herbivores were collected from the Trinity and Klamath Rivers to the north of the Eel River to extend the range of stream size and productivity examined.

### Temporal patterns

To assess temporal variation in response to environmental variation in allochthonous and autochthonous diet sources, we analysed  $\delta D$  of a common macroalga, *Cladophora* in the South Fork Eel River and obligate detritivores (shredders mostly of the genus *Lepidostoma*) in Fox Creek, a small and heavily shaded headwater stream. *Cladophora* was selected to examine variation in autochthonous sources because it is conspicuous and easy to collect throughout the year in the South Fork Eel River, unlike other algae and herbivorous invertebrates. Data for  $\delta^{13}C$  of *Cladophora* were previously reported by Finlay (2004). Water  $\delta D$  was analysed to assess its contribution to temporal variation in *Cladophora*  $\delta D$ . Water samples were collected at the same sites in the South Fork Eel River to where algae and invertebrates were sampled, but during a later period (2005–07). These data provide a characterisation of the temporal variation in water  $\delta D$  which we expect to be similar to the period of examination of algal  $\delta D$  (see Table 2). Data for *Lepidostoma*  $\delta^{13}C$  had not been reported previously for all dates; these data were generated using similar methods as for other samples. Analyses of  $\delta^{13}C$  of shredders in small forested streams such as Fox Creek have shown no contribution of algae to shredder diets (Finlay, 2001) suggesting that shredders should be indicative of terrestrial organic matter sources to food webs.

### Stable isotope analyses

Analyses of  $\delta^{13}C$  were performed at the Center for Stable Isotope Biogeochemistry at University of California, Berkeley using a Carlo Erba Elemental Analyser (CE Instruments, Milan, Italy) interfaced with a Europa 20–20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, U.K.). Further details of analyses are reported by Finlay (2001) and Finlay *et al.* (1999). Hydrogen stable isotopes of solid organic materials were analysed at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University (Flagstaff, AZ, U.S.A.) using a Thermo-Chemical Elemental Analyser interfaced to a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo Fisher Scientific Corp., Bremen, Germany). Organic  $\delta D$  values (for the non-exchangeable H portion of each sample) were equilibrated for isotope exchange and

normalised using similar procedures and standards as those found in Wassenaar & Hobson (2003) and Doucett *et al.* (2007). All  $\delta D$  values are expressed in per mil (‰) notation relative to Vienna Standard Mean Ocean Water (VSMOW). Precision on several internal organic standards was always better than  $\pm 3.0\text{‰}$  (1 SD). In most cases,  $\delta^{13}C$  and  $\delta D$  analyses of organic samples were performed on a subsample of an individual sample. Only  $\delta^{13}C$  data from samples also analysed for  $\delta D$  are presented; some  $\delta D$  samples were not analysed for  $\delta^{13}C$ . Water samples were analysed for  $\delta^{18}O$  and  $\delta D$  using a Los Gatos Research DLT-100 liquid-water isotope analyser (Los Gatos Research, Inc., Mountain View, CA, U.S.A.). All water data were normalised on the VSMOW-SLAP (Standard Light Antarctic Precipitation) scale using calibrated internal standards. Precision on laboratory water standards was always better than  $\pm 0.2\text{‰}$  and  $\pm 0.6\text{‰}$  (1 SD), for  $\delta^{18}O$  and  $\delta D$ , respectively. Relationships between isotopic values and water velocity and stream size were assessed using linear regression. Slopes for regression models were analysed with *t*-tests ( $P < 0.05$ ).

## Results

### Water velocity effects

Analyses of  $\delta D$  on samples collected across water velocity gradients for several adjacent pool-riffle sequences in the South Fork Eel River showed no consistent trend with flow rate but showed a wide range of  $\delta D$  values (i.e.  $-210\text{‰}$  to  $-163\text{‰}$ ) (Fig. 1). The large  $\delta D$  overall difference between water (i.e.  $-45\text{‰}$ )

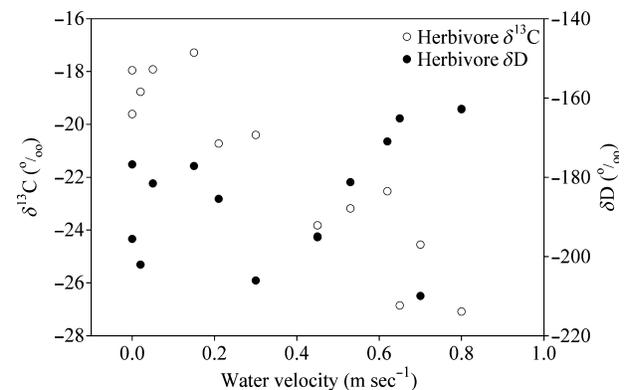


Fig. 1 Relationship between water velocity and stable carbon and hydrogen isotope ratios of herbivorous invertebrates in the South Fork Eel River.

and algae and herbivores was consistent with a large isotopic fractionation associated with assimilation of hydrogen into algae during photosynthesis (Smith & Ziegler, 1990). Fractionation did not appear to be influenced predictably by factors associated with water velocity, which can include influences on algal metabolism, nutrient uptake and biomass. In contrast to  $\delta D$ ,  $\delta^{13}C$  values for the same samples, reported previously by Finlay *et al.* (2002), were strongly influenced by water velocity ( $r^2 = 0.87$ ,  $P < 0.001$ ).

### Longitudinal patterns

$\delta D$  of terrestrial detritus and its most closely associated consumer, invertebrates of the shredder functional feeding group, showed no consistent pattern of variation in the South Fork Eel River catchment during midsummer base flow conditions.  $\delta D$  values for conditioned leaves were similar to those of shredders and were much higher (i.e. more enriched, by almost 70‰) than those observed for diatoms and herbivores at these sites (Table 1, Fig. 2). Algae and herbivores always had lower (i.e. more depleted)  $\delta D$  compared to terrestrial organic matter, but herbivores showed a significant decrease in  $\delta D$  with increasing stream size ( $r^2 = 0.63$ ,  $P < 0.001$ ; Fig. 2). Filamentous green algae were consistently depleted relative to herbivorous caddisflies and diatoms (Fig. 2, Table 1), but the trend of decreasing  $\delta D$  with catchment area was similar ( $r^2 = 0.85$ ,  $P < 0.001$ ). Stream temperature, light and algal productivity

rates all increased, and  $CO_2$  concentrations decreased along the stream size gradient; together, these changes led to increasing  $\delta^{13}C$  values downstream (Fig. 2; Finlay, 2001, 2004).

We expected no longitudinal pattern in terrestrial  $\delta D$  within the South Fork Eel River catchment, since terrestrial vegetation is dominated by  $C_3$  plants with  $\delta^{13}C$  values that show a restricted range (Fig. 2), indicating similar levels of water availability and drought stress throughout the region. Little is known about controls of fractionation of  $\delta D$  by aquatic plants, but there is no *a priori* reason to expect that fractionation would change with productivity. The observed decrease in algal and herbivore  $\delta D$  with stream size was therefore surprising, especially considering the lack of spatial variation in water  $\delta D$  (Table 2) and the lack of temporal variation in algal  $\delta D$  (see below).

### Temporal variation

No significant change for  $\delta D$  of a common autotroph (i.e. *Cladophora*) and consumers of detrital organic matter (primarily *Lepidostoma* sp.) was observed over a wide range of dates at two streams in the South Fork Eel River catchment ( $P > 0.05$  for both linear regression analyses). In contrast, a strong seasonal pattern in  $\delta^{13}C$  was observed for *Cladophora* (Fig. 3). As expected, shredder  $\delta^{13}C$  did not vary temporally ( $P > 0.05$ ), consistent with the terrestrial diets of these consumers in small headwater streams (Finlay, 2001).

**Table 1** Comparison of allochthonous and autochthonous resources and consumer  $\delta D$  for invertebrate functional groups with well-characterised diet sources (i.e. leaf litter and shredders, and diatoms and herbivores). Sites are listed in order of increasing stream size as defined by catchment area

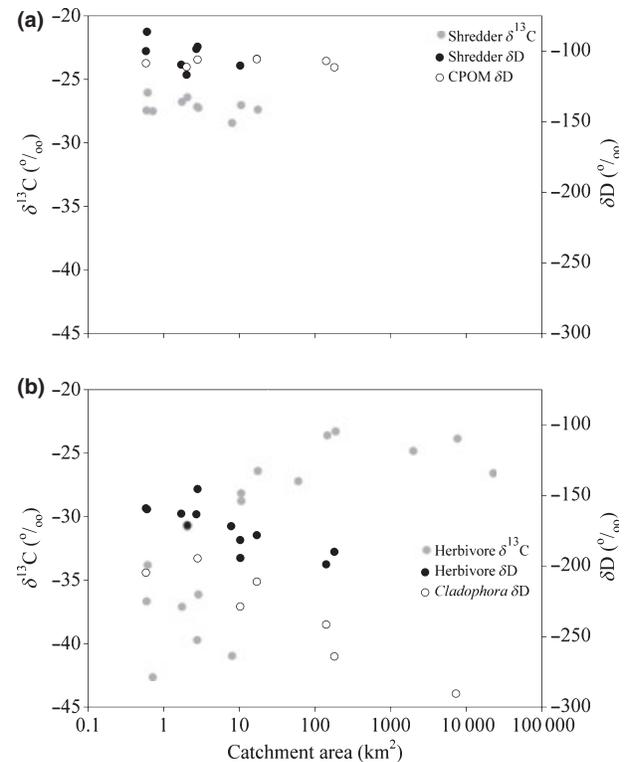
Site (catchment area, km <sup>2</sup> )	%‰ ± SE (n)						
	Allochthonous		Autochthonous				
	Leaves	Shredders	Diatoms	Herbivores	<i>Cladophora</i>	<i>Lemanea</i>	<i>Nostoc</i>
McKinley (0.6)	-108.6 ± 2.7 (3)	-100.0		-156.5 ± 5 (3)	-204.9 ± 5 (5)		
Barnwell (2.1)	-111.3	-108.2 ± 9 (2)		-170.5 ± 10 (2)			-196.2
Fox (2.8)	-106.0 ± 0.5 (2)	-100.0 ± 3 (10)	-145.8 ± 4 (2)	-145.5	-194.7 ± 15 (2)		-218.0 ± 1 (3)
Jack Hearts (10)		-110.2	-185.2 ± 3 (3)	-181.7 ± 6 (5)	-228.8 ± 5 (5)		
Elder (17)	-105.7 ± 2.9 (3)	-105.5	-189.6 ± 4 (11)	-178.2 ± 3 (8)	-213.9 ± 4 (9)	-136.0	-183.5 ± 15 (2)
South Fork Eel (150)	-107.2 ± 3.3 (3)	-140.0 ± 8 (9)*		-198.9 ± 4 (3)	-241.6 ± 5 (9)	-138.1	-225.8 ± 5 (3)
Ten Mile (160)	-111.5			-190.0	-264.2 ± 9 (2)	-149.1 ± 3 (2)	-213.6 ± 6 (2)
Average (±SD)	-108.4 ± 2.5	-104.8 ± 5	-173.5 ± 24	-174.5 ± 19	-224.6 ± 26	-141.0 ± 7	-207.4 ± 17

\*Indicates that shredders at this site relied on both terrestrial and algal food sources (see Finlay *et al.*, 2002 and Fig. 4); this point was not included in the overall average.

**Table 2** Stable oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta\text{D}$ ) isotope ratios of stream water in the South Fork Eel River catchment. Comparisons across sites were made during summer base flow in 1998 ( $\delta^{18}\text{O}$  only) and 2005, and temporal comparisons were made for the South Fork Eel River over an annual cycle during 2006–07

Site	Date	$\delta\text{D}$	$\delta^{18}\text{O}$
Spatial comparisons			
Barnwell	August/September 2005	-47.2	-7.6
Fox	August/September 2005	-48.4	-7.4
Jack of Hearts	August/September 2005	-45.7	-7.2
Elder	August/September 2005	-48.1	-7.7
South Fork Eel	August/September 2005	-46.3	-7.3
Sugar	July 1998	-7.5	-7.5
Fox	July 1998	-7.8	-7.8
Elder	July 1998	-7.9	-7.9
South Fork Eel	July 1998	-7.4	-7.4
Ten Mile	July 1998	-7.3	-7.3
Temporal comparisons			
South Fork Eel	15 June 2006	-45.8	-6.9
South Fork Eel	29 June 2006	-45.3	-7.2
South Fork Eel	24 July 2006	-44.5	-7.1
South Fork Eel	28 July 2006	-43.7	-6.8
South Fork Eel	31 July 2006	-44.4	-7.0
South Fork Eel	23 August 2006	-45.1	-6.6
South Fork Eel	4 September 2006	-42.8	-6.1
South Fork Eel	16 October 2006	-43.9	-6.7
South Fork Eel	24 November 2006	-41.3	-6.3
South Fork Eel	24 January 2007	-48.9	-7.3
South Fork Eel	4 March 2007	-46.7	-7.3
South Fork Eel	18 April 2007	-50.1	-7.2
South Fork Eel	28 May 2007	-49.1	-7.2

Although water  $\delta\text{D}$  did not vary temporally during the spring and summer (Table 2), other environmental conditions that could potentially affect isotopic fractionation of  $\delta\text{D}$  did change substantially. In the South Fork Eel River, for example, temperature was low during spring and autumn compared to summer peak values of up to 23 °C (Finlay *et al.*, 2002). Dissolved  $\text{CO}_2$  showed the opposite pattern, with higher availability in spring and autumn and low concentrations during summer (Finlay, 2003). The South Fork Eel River is canyon-bound and turbid in winter, indicating strong seasonal variation in light availability and algal productivity. These environmental changes led to seasonal cycles of variation in  $\delta^{13}\text{C}$  but, surprisingly, not for  $\delta\text{D}$  of the alga examined (Finlay, 2004; Fig. 3), suggesting that fractionation of  $\delta\text{D}$  by algae is relatively insensitive to temporal variation in environmental conditions. Similarly, no temporal variation was observed for shredders, indicating little effect of seasonal drought associated with warm, dry summers on terrestrial  $\delta\text{D}$  values.

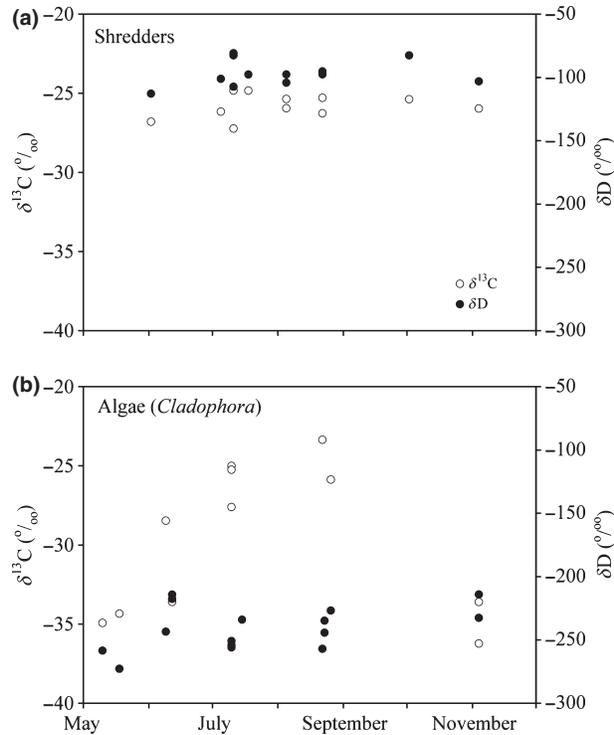


**Fig. 2** Spatial patterns of (a)  $\delta\text{D}$  of terrestrial detritus and of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  for shredders in pools and (b) *Cladophora* and herbivores in riffles of streams in the South Fork Eel River catchment and the Trinity and Klamath Rivers in Northern California. For (a), shredder data for the South Fork Eel River were not included because this group consumes both algae and terrestrial detritus at this site (see Fig. 4).

#### Comparison of resources and invertebrate consumer $\delta\text{D}$

Comparisons of consumer–resource relationships for  $\delta\text{D}$  of two consumer groups with close affiliation for specific diet sources showed close correspondence between diet and body tissue  $\delta\text{D}$  (Table 1). Shredders had slightly enriched  $\delta\text{D}$  compared to leaf litter (0–8‰ higher), and a similar pattern was evident for trichopteran herbivores and diatoms (Table 1). The small differences in  $\delta\text{D}$  between these diet sources and their specific consumers indicate minor effects of trophic fractionation and isotope exchange with stream water on body  $\delta\text{D}$ , consistent with a recent review of trophic fractionation data (Solomon *et al.*, 2009).

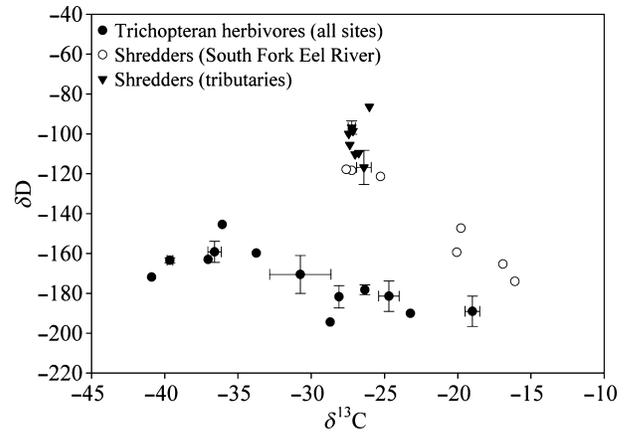
Comparisons of  $\delta\text{D}$  for autotrophs across several sites in the South Fork Eel River catchment showed substantial variation among taxa, suggesting species-specific differences in fractionation of  $\delta\text{D}$  during



**Fig. 3** Temporal patterns of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  for (a) shredders in a small shaded tributary stream and (b) *Cladophora* in the South Fork Eel River.

incorporation of water hydrogen into algal biomass. Among algal taxa, average  $\delta\text{D}$  for *Lemanea* were most enriched ( $-141\text{‰}$ ), followed by diatoms ( $-174\text{‰}$ ), *Nostoc* ( $-207\text{‰}$ ) and *Cladophora* ( $-225\text{‰}$ ). Diatoms are the only one of these taxa edible to most stream consumers.

A dual isotope plot summarises patterns of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  for shredders and herbivores during mid-summer (Fig. 4). These consumer groups showed distinct  $\delta\text{D}$  values, with some overlap of  $\delta^{13}\text{C}$  values in the larger streams and rivers. In the South Fork Eel River, shredders are omnivorous, consuming both terrestrial detritus and algae in varying amounts (Finlay *et al.*, 2002), and so individual samples from August 1997 for shredders at this site are shown to illustrate the utility of  $\delta\text{D}$  for diet tracing. South Fork Eel River shredders spanned a range of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values from those consistent with consumption of terrestrial detritus to values enriched in  $^{13}\text{C}$  and depleted in  $^2\text{H}$  that indicate near-complete reliance on algae growing in pool habitats with very slow water velocity where algal  $\delta^{13}\text{C}$  is highly enriched (Finlay *et al.*, 2002).



**Fig. 4** Dual isotope plot of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  for primary consumers of terrestrial detritus (shredders) and algae (herbivores) during summer in the South Fork Eel catchment. Except for shredders in the South Fork Eel River, each data point represents mean values for all samples collected at an individual site in the catchment ranging from small tributary streams to large streams and rivers during the summers of 1997–98. Data for shredders in the South Fork Eel River represent individual samples collected in August 1997 and are shown for comparison to herbivore and shredder data for other sites. All herbivore data represent consumers in riffles except for the highest  $\delta^{13}\text{C}$  value, which is from the average for herbivores in pools of the South Fork Eel River using data from Fig. 1.

**Discussion**

Stable isotopes of hydrogen in organic materials derived from terrestrial and algal sources appear to be free of some of the characteristics that limit source tracing of food webs using  $\delta^{13}\text{C}$  in streams and lakes. In this study, terrestrial and aquatic sources of organic matter were always isotopically distinct for  $\delta\text{D}$ . Across a broad environmental gradient ranging from small, shaded headwater streams to unshaded, productive rivers,  $\delta\text{D}$  of diatoms and their herbivores, and obligate consumers of terrestrial detritus, were separated consistently by at least  $45\text{‰}$  to well above  $100\text{‰}$  (Table 1, Fig. 4). The low values for algal  $\delta\text{D}$  compared to terrestrial plants arise from the large fractionation associated with assimilation of hydrogen into organic matter and the lack of influence from evaporative enrichment that affects terrestrial plants (Dawson *et al.*, 2002).

Importantly, autotrophic and allochthonous organic matter sources were separated by  $\delta\text{D}$  at sites where  $\delta^{13}\text{C}$  values overlapped (i.e.  $\delta^{13}\text{C}$  values of  $-29\text{‰}$  to  $-24\text{‰}$ , observed commonly in larger streams). Over-

lap between terrestrial and autotroph  $\delta^{13}\text{C}$  arose through both spatial and temporal environmental changes. Spatially, effects of water velocity on algal  $\delta^{13}\text{C}$  produced a wide range of  $\delta^{13}\text{C}$  values (i.e. up to 15‰) for diatoms within sites in larger streams (Finlay *et al.*, 1999, 2002). The negative response of algal and herbivore  $\delta^{13}\text{C}$  to increasing water velocity results from higher fractionation with greater availability of  $\text{CO}_2$  via delivery by advective transport, with low apparent fractionation in stagnant water where diffusive transport of  $\text{CO}_2$  dominates (Finlay *et al.*, 1999, 2002). Across sites, downstream increases in productivity and decreases in dissolved  $\text{CO}_2$  led to greater frequency of  $\delta^{13}\text{C}$  overlap for riffle algae and terrestrial detritus as stream size increased (Finlay, 2001). Temporal variation in dissolved inorganic carbon  $\delta^{13}\text{C}$  and environmental conditions that influence isotopic fractionation commonly leads to seasonal changes in algal  $\delta^{13}\text{C}$ . Strong seasonal changes can lead to periods of isotopic overlap between terrestrial and autochthonous sources (e.g. Finlay *et al.*, 2002; Hill & Middleton, 2006). Temporal variation in  $\delta^{13}\text{C}$  is especially problematic for source separation in situations where environmental conditions affecting algal  $\delta^{13}\text{C}$  are frequently changing so that consumers are not fully equilibrated with their food resources (Singer *et al.*, 2005; Hill & Middleton, 2006).

Because of consistent separation in  $\delta\text{D}$ , a two-source isotope mixing model could be employed using  $\delta\text{D}$  to quantitatively estimate diet source contributions to consumers throughout the catchment. In contrast, a mixing model using  $\delta^{13}\text{C}$  could not always be used unambiguously without additional information from gut content analyses to eliminate potential sources from the model (Benstead *et al.*, 2006). For other forested catchments,  $\delta^{13}\text{C}$  overlap for organic matter sources most often occurs in larger streams and rivers, and under eutrophic or hydrologically variable conditions (Finlay & Kendall, 2007), suggesting that  $\delta\text{D}$  may be very useful in such settings. A combination of  $\delta^{13}\text{C}$ ,  $\delta\text{D}$  and  $\delta^{15}\text{N}$  tracing in two, three or four-source mixing models may provide a robust method of characterising consumer diet sources in streams and lakes.

Although we found some influence of local (i.e. within catchment) environmental conditions on  $\delta\text{D}$ , autotrophic  $\delta\text{D}$  values were generally less variable spatially and temporally than  $\delta^{13}\text{C}$ . We found no effect of water velocity on herbivore  $\delta\text{D}$ , and temporal

changes in stream environmental conditions did not affect  $\delta\text{D}$  of a common alga. Longitudinal changes in  $\delta\text{D}$  of algae and herbivores were observed, suggesting that, as for other common stable isotope tracers, site-specific sampling is necessary to trace energy flow through food webs using  $\delta\text{D}$ . However, the large separation between allochthonous and autochthonous  $\delta\text{D}$  persisted throughout the range of streams examined. As with  $\delta^{13}\text{C}$ ,  $\delta\text{D}$  of terrestrial detritus was constrained both spatially and temporally in the South Fork catchment of the Eel River. As a consequence, there was consistent  $\delta\text{D}$  separation of terrestrial and algal organic matter sources to food webs.

Despite increasing evidence for the utility of  $\delta\text{D}$  in food web studies, several remaining issues must be thoroughly explored to fully assess the efficacy of  $\delta\text{D}$  as a tracer in aquatic food webs. First and foremost, very little is known about effects of trophic fractionation and equilibration of consumers with environmental water on tissue  $\delta\text{D}$ . This study and the results of Doucett *et al.* (2007) show a strong association between invertebrates and their diets in natural settings. Similarly, Solomon *et al.* (2009) found very small effects of trophic fractionation and modest but variable effects of environmental water on consumer  $\delta\text{D}$ . In contrast, Whitley, Johnson & Martinez (2006) suggested that  $\delta\text{D}$  of fish was primarily determined by water  $\delta\text{D}$ . There is a clear need for more controlled experiments that vary both diet and water  $\delta\text{D}$  to fully assess the relative contribution of each to consumer  $\delta\text{D}$ .

Some other results of this study also warrant further exploration. While effects of landscape position on  $\delta\text{D}$  were not as strong as observed for  $\delta^{13}\text{C}$ , significant trends of decreasing  $\delta\text{D}$  with stream size were observed. Stream size is correlated with many factors, including light, water temperature and algal production in forested catchments. Water  $\delta\text{D}$  did not vary among sites, and at this point, the mechanisms that drive the longitudinal pattern in the catchment are unknown.

The downstream decrease in algal  $\delta\text{D}$  could arise from at least two mechanisms. First, increased fractionation or increased lipid content may lead to lower  $\delta\text{D}$  with increasing stream size. Increasing fractionation does not seem likely because algal photosynthesis rates increased with stream size, a process likely to decrease fractionation. Lipids are known to be depleted in deuterium relative to other tissues within organisms (Sessions *et al.*, 1999), and it is

plausible that increased light availability leads to greater contribution of isotopically light lipids to both algae and herbivores. Direct measurements of lipid concentrations across the productivity gradient in future work could address this hypothesis.

Alternatively, the pattern could arise from greater contamination of algal samples by terrestrial organic carbon in headwater streams compared to larger, downstream sites. This explanation seems unlikely, given the extremely low dissolved organic carbon and particulate organic carbon concentrations in headwater streams (0.2–0.5 and 0.1–0.5 mg L<sup>-1</sup> respectively; J. Finlay, unpubl. data). Furthermore, if the pattern of *Cladophora*  $\delta$ D with stream size was related to the presence of terrestrial carbon in algae, then a large percentage of algal biomass must have been actually composed of terrestrially derived organic matter. Visual examination showed no conspicuous presence of detritus in algal samples (J. Finlay, pers. comm), suggesting that terrestrial detritus was not the cause of patterns in algal  $\delta$ D.

Significant differences between autotrophs were also observed, and these differences could complicate the use of  $\delta$ D in some cases. While many macroscopic aquatic autotrophs are inedible to aquatic herbivores, there are some ecosystems where macroalgae are edible to some consumers and others where several microscopic algal forms are common in addition to diatoms. Although these concerns are not specific to  $\delta$ D and have been shown to effect studies involving  $\delta^{13}\text{C}$ , use of isotopic mixing models incorporating either  $\delta$ D or  $\delta^{13}\text{C}$  to describe food webs will certainly benefit from the use of additional information from gut content analyses and alternate tracers. Finally, intensive measurements of herbivore  $\delta$ D at one site during midsummer showed substantial variability (Fig. 1). High intrasite variation has been observed previously in aquatic ecosystems (Smith & Epstein, 1970) and may be related to variation in lipid content of algae and consumers (Sessions *et al.*, 1999), differences in algal species composition or the presence of terrestrial detritus. Although data for other sites with two to five samples (each representing a composite of 3–20 invertebrate larvae) did not show excessive intrasite variability, the sensitivity to sampling intensity and effort must be thoroughly investigated in future studies.

In summary, our analyses indicate that  $\delta$ D provides a useful tracer of autochthonous and allochthonous organic matter in freshwaters because of large isotopic

separation, restricted spatial variation and little to no temporal variation. Further studies of trophic fractionation, differences between algal taxa or growth forms and of basins with more temporal variation in water  $\delta$ D are necessary to fully evaluate the performance of this tracer. However, results of this study, combined with one over a larger geographic area (Doucett *et al.*, 2007), suggest that  $\delta$ D can complement  $\delta^{13}\text{C}$  and, in some cases, provide a better tracer of organic matter sources to aquatic food webs.

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