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APPLIED ISSUES

Reach-scale geomorphology affects organic matter and consumer $\delta^{13}C$ in a forested Piedmont stream

D. M. WALTERS,* K. M. FRITZ* AND D. L. PHILLIPS†
*U.S. Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH, U.S.A.
†U.S. Environmental Protection Agency, National Health & Environmental Effects Research Laboratory, Corvallis, OR, U.S.A.

SUMMARY

1. We investigated the spatial (longitudinal position and reach geomorphology) and seasonal (spring and autumn) influences on the variation of $\delta^{13}C$ among organic matter sources and consumers in a forested Piedmont river, South Carolina, U.S.A.
2. Six sites were sampled along a continuum and varied in basin area from approximately 30 to 300 km^2. Sites fell into two geomorphic categories (i) high-gradient, rock bed ('rock') or (ii) low-gradient, sand bed ('sand') sites.
3. Variation in $\delta^{13}C$ was more strongly related to reach geomorphology than longitudinal position. $\delta^{13}C$ of biofilm and consumers was consistently enriched at rock sites. Leaf litter (i.e. coarse particulate organic matter, CPOM) $\delta^{13}C$ did not vary with bed type. There was significant $\delta^{13}C$ enrichment at rock sites for biofilm, seston, fine benthic organic matter (FBOM), and eight of nine consumer trophic guilds (e.g. grazing invertebrates, insectivorous fishes). $\delta^{13}C$ of biofilm and four trophic guilds was also positively correlated with drainage area, but the magnitude of enrichment was less than between bed types.
4. $\delta^{13}C$ was generally enriched in spring, but this varied among organic matter types, consumers, and by bed type. CPOM and seston were enriched in spring, FBOM was enriched in autumn, and biofilm showed no trend. Five consumer guilds were enriched in spring, and only one fish guild, generalised carnivores, showed enrichment of muscle tissue in autumn.
5. Consumer $\delta^{13}C$ enrichment at rock sites suggests greater reliance on algal carbon than for consumers at sand sites, but we also found $\delta^{13}C$ enrichment of biofilm at rock sites. Thus, differences in consumer $\delta^{13}C$ between bed types could be related to (i) increased consumption of biofilm at rock compared with sand sites, or (ii) consumption of biofilm at rock sites that is enriched relative to biofilm at sand sites or (iii) both mechanisms.
6. $\delta^{13}C$ signatures in local food webs appear to respond to processes operating at multiple spatial scales. Overall downstream enrichment of biofilm and consumers was disrupted by strong local effects related to bed morphology. These results suggest that human alteration of channel habitat will have corresponding effects on stream food webs, as assessed by changes in $\delta^{13}C$.

Keywords: biofilm, food web, sediment, stable isotopes, stream gradient

Introduction

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been increasingly used over the last two decades to identify the trophic basis of stream food

Correspondence: David Walters, US EPA, National Exposure Research Laboratory, 26 W. MLK Blvd., Cincinnati, OH 45268, U.S.A. E-mail: walters.davidm@epa.gov

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webs (France, 1994; Finlay, 2001; Schindler & Lubetkin, 2004). A common goal of this research is to identify the relative contribution of allochthonous leaf litter (hereafter 'detritus') and autochthonous benthic algae (hereafter 'biofilm'). Spatial variation in consumer $\delta^{13}C$ has been used to support the hypothesis that biofilm is more important to downstream food webs (Doucett *et al.*, 1996b; Finlay, 2001), a central prediction of the river continuum concept (Vannote *et al.*, 1980), and to infer human-induced changes in organic matter utilisation in stream food webs (Rounick & Winterbourn, 1986; England & Rosemond, 2004). However, food web characterisation using stable isotopes depends upon distinct isotopic signatures of biofilm and detritus (France, 1994; Doucett *et al.*, 1996a; Finlay, 2001).

Stream biofilm and detritus often have similar $\delta^{13}C$ values, hampering attempts to quantify their relative inputs to food webs. A meta-analysis by France (1994) showed that leaf litter $\delta^{13}C$ was remarkably consistent among studies, but that biofilm $\delta^{13}C$ was highly variable and enveloped the range of litter. Thus, the use of $\delta^{13}C$ to quantify resource use by consumers is site-specific and is dependent upon the biofilm $\delta^{13}C$ signal (Doucett *et al.*, 1996a). This observed spatial variation in biofilm $\delta^{13}C$ is poorly understood, but biofilm and herbivore $\delta^{13}C$ have been shown to increase with catchment area (Finlay, 2001). A subsequent study attributed downstream enrichment of algal $\delta^{13}C$ to longitudinal variation in stream productivity and dissolved CO_2 availability (Finlay, 2004). However, small-scale variation in habitat may also influence the $\delta^{13}C$ signal of algae and consumers. For example, Finlay, Power & Cabana (1999) found a negative correlation between water velocity and herbivore $\delta^{13}C$ (which reflected algal $\delta^{13}C$). They attributed these effects to changes in dissolved CO_2 related to current velocity and showed that effects were strongest in pools, where CO_2 availability was low relative to photosynthetic rates.

We investigated longitudinal and reach-scale variation in organic matter and consumer $\delta^{13}C$ in wadeable (e.g. approximately 30–300 km^2 basin area) streams. Biofilm and herbivore $\delta^{13}C$ are highly variable in streams of this size range and overlap $\delta^{13}C$ values of conditioned leaves (Finlay, 2001). The sites we studied had starkly contrasting morphologies and were dominated by either rock (primarily bedrock with some boulder and large cobble) or sand channels. We focused on these channel bed types because their biofilm communities are known to differ in terms of biomass, resistance to disturbance, and assemblage structure and function. Epipsammic (biofilm on soft sediments) biomass is often lower than epilithic (biofilm on rock) biomass (Tett *et al.*, 1978; Biggs, 2000) and is less frequently available to consumers as it is more prone to scour during small, frequent flood events (Tett *et al.*, 1978). Epixylic biofilm (biofilm on wood) may also be an important resource to consumers at sand sites, where wood is the primary stable substratum for biofilm production (Couch & Meyer, 1992). Epixylic and epipsammic communities are more heterotrophic than epilithic communities (Couch & Meyer, 1992; Hudson, Roff & Burnison, 1992; Tank & Winterbourn, 1996; Sabater, Gregory & Sedell, 1998) and consequently may have a $\delta^{13}C$ signal more similar to allochthonous litter than would epilithic biofilm. Values from the literature for stream episammon and epixylon $\delta^{13}C$ are scarce. Mulholland *et al.* (2000) reported epixylon $\delta^{13}C$ of −26‰ for a single sample from a first-order stream in Tennessee (U.S.A.). This value approximates that of leaf litter (approximately −27‰) reported in the meta-analysis of France (1994).

Based upon these observed differences in biofilm availability and ecology, we expected that consumers at rock sites would utilise more algal carbon than those at sand sites. We predicted consumer $\delta^{13}C$ would be enriched at rock sites as epilithic biofilm $\delta^{13}C$ is usually enriched compared with leaf litter (and presumably epixylon) in large streams (Finlay, 2001). Alternatively, a consistent pattern of downstream enrichment of consumer $\delta^{13}C$ would indicate that local morphology is less important than longitudinal position in determining algal carbon inputs to food webs (Vannote *et al.*, 1980; Rounick & Winterbourn, 1986; Finlay, 2001). Seasonal shifts in isotopic signals are also an important consideration, particularly where biofilm and leaf litter $\delta^{13}C$ values are similar. We compared spring and autumn signals to investigate seasonal variation in $\delta^{13}C$ of organic matter and stream consumers. Finally, we explored the implications of spatial and seasonal $\delta^{13}C$ variability for determining resource use by stream consumers.

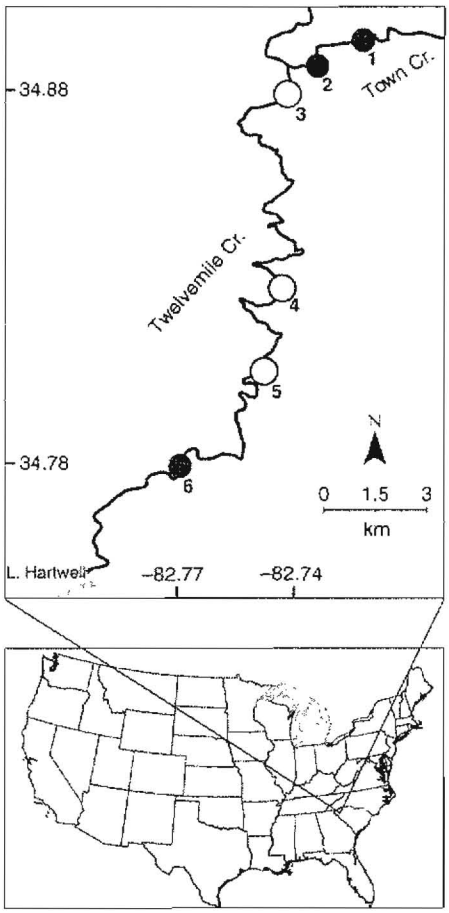

Fig. 1 Rock (solid circles) and sand (open circles) sites sampled in the Twelvemile Creek drainage, SC, U.S.A.

Methods

Study area

Twelvemile Creek is near Clemson, SC, in the south-eastern U.S.A. and flows into Lake Hartwell, a reservoir on the Savannah River (Fig. 1). Most of the drainage lies in the Piedmont physiographic province, with some headwaters in the Blue Ridge. Twelvemile Creek (catchment area approximately 350 km^2) has a mean annual discharge of 5.5 $m^3 s^{-1}$ (U.S. Geologic Survey, 2006). The dominant land cover types are forest (54%), followed by pasture (21%) and urban lands (13%).

We sampled two sites in Town Creek, a tributary, and four sites along Twelvemile Creek (Fig. 1). Sampling was conducted over 100 m of stream at each site, except for fishes which were sampled over 150 m to obtain adequate sample sizes. Sites were sampled in May (spring) and November (autumn) in 2003 and 2004. Four sites (1, 2, 3 and 6) were sampled in spring and autumn, whereas two (4 and 5) were sampled only in spring. Sites were selected to assess both longitudinal and reach-scale patterns in $\delta^{13}C$ values. Catchment area increased by an order of magnitude from 35 to 337 km^2, and mean width increased fourfold from 9 to 37 m between sites 1 and 6 (Table 1). Sites fell into two distinctive geomorphic categories based on gradient and bed texture: low-gradient, sand-bed sites and high-gradient, rocky bed sites (hereafter 'sand' and 'rock' sites, respectively). Rock sites included the two farthest upstream and the most downstream site sampled, while sand sites occupied the middle portion of the study area (Fig. 1).

Geomorphic sampling

Reach-scale geomorphology was measured in spring 2004 during baseflow conditions. The channel gradient in the thalweg was measured using a hand-held laser level and stadia rod. Bed particle size, depth and the occurrence of coarse woody debris (CWD, i.e. wood >10 cm diameter) were recorded at 34 locations spaced at 3-m intervals along the thalweg at each site. We assessed visually a 50-cm^2 patch of stream bed at each location and recorded the modal particle class (Walters *et al.*, 2003) based upon the Wentworth particle scale (Gordon, McMahon & Finlayson, 1992). These 34 observations were used to calculate the percentage cover of rock (particles >64 mm) or sand and gravel (particles <64 mm) for each site. CWD was recorded as 'present' if it occurred anywhere within the wetted channel at each sample interval. These observations were used to calculate the percentage occurrence of CWD for each site. Wetted width and percentage canopy cover were measured at the ends and the middle of the site ($n = 3$ locations). Canopy cover was measured in the thalweg using a convex spherical densitometer held 30 cm above the water surface. Canopy cover at each thalweg point was calculated as a mean of four observations made facing up- and downstream and both banks.

Food web sample collection, preparation and processing

We collected conditioned leaves (i.e. coarse particulate organic matter, CPOM), seston, fine benthic organic matter (FBOM), biofilm, macroinvertebrates and fish.

Table 1 Geomorphic characteristics of Twelvemile Creek (South Carolina, U.S.A.) study sites

	Site						Bed type	
	1	**2**	3	4	5	**6**	Rock	Sand
Basin area (km^2)	35.2	37.1	135.8	219.9	269.9	337.1	136.5 (100.3)	208.5 (39.1)
Gradient (%)	0.8	1.5	0.3	0.2	0.5	2.9	1.74 (0.62)	0.33 (0.09)
Sand and gravel (%)	8.8	26.5	100	97.1	64.7	5.9	13.7 (6.4)	87.3 (11.3)
Cobble, boulder and bedrock (%)	91.2	73.5	0	2.9	35.3	94.1	86.3 (6.4)	12.2 (11.1)
CWD (%)	5.9	0	47.1	61.8	73.5	0	2.0 (2.0)	60.8 (7.7)
Canopy cover (%)	70.3 (4.9)	83.4 (10.3)	43.6 (2.6)	60.7 (9.7)	56 (5.8)	7.5 (2.1)	53.7 (23.4)	53.4 (5.1)
Depth (m)	0.34 (0.02)	0.36 (0.03)	0.6 (0.03)	0.57 (0.02)	0.57 (0.03)	0.6 (0.03)	0.43 (0.08)	0.58 (.01)

Values shown are mean (1SE). Sites in bold were designated as 'rock' sites (Fig. 1). Site mean values were averaged to calculate mean values for sand and rock sites.

Three replicates of organic matter and consumers were collected at each sampling event whenever possible. Replicate samples from spring 2004 were mistakenly pooled prior to stable isotope analysis, so $n = 1$ replicate per site for all samples collected that season. Samples were prepared for isotopic analysis by freeze-drying and then grinding to a fine powder using a ball mill. Samples were combusted to CO_2 and N_2 and analysed in a Carlo Erba NA 1500 CHN analyser (Carlo Erba Instrumentazione, Milan, Italy) connected to a Finnigan Delta C isotope ratio mass spectrometer (Thermo Electron, Waltham, MA, U.S.A.) to determine $\delta^{13}C$ and $\delta^{15}N$. Reference standards for $\delta^{13}C$ and $\delta^{15}N$ were PeeDee Belemnite Carbonate and atmospheric N_2, respectively. Reproducibility was monitored using bovine liver (National Institute of Standards and Technology No. 1577b), and precision was better than 0.2‰ (1SD).

Coarse particulate organic matter from the channel was collected by hand, gently rinsed to remove FBOM and insects, shredded and mixed. Subsamples were taken from this shredded mixture. All sites were bordered by riparian forest buffer ranging from approximately 5 m to >100 m in width. Bottomland hardwood tree species typical of the Piedmont (e.g. *Betula nigra* L., *Carpinus caroliniana* Walt., *Liquidambar styraciflua* L., *Lirodendron tulipifera* L., *Platanus occidentalis* L. and multiple species of *Quercus*) were common at all sites. FBOM was separated from surface sediment collected from multiple depositional areas throughout the site (Lazorchak, Klemm & Peck, 1998). Sediments were thoroughly mixed and then elutriated several times to remove the coarse fraction. The fine fraction was sieved through 250 μm mesh, then filtered onto precombusted glass fibre filters (0.7 μm) for analysis. Seston was collected using 48 and 202 μm mesh bongo nets (McGowan & Brown, 1966). Bongo nets are two identical nets (except for mesh size) arranged side by side on a metal frame and are typically used for collecting plankton in marine and lentic systems. The nets were deployed in the water column just upstream of the sample site for 1–2 h. Collected material was mixed, elutriated, sieved and filtered as described above for FBOM. Collected seston ranged in size from 48–250 μm.

Biofilm was collected from cobbles and boulders at rock sites and from woody debris at sand sites. We selectively sampled these substrata because: (i) rocks and wood were the dominant stable substrata at rock and sand sites, respectively, (ii) rocks were completely absent from one sand site, and (iii) the few rocks available at the sand sites were sparsely colonised by the macrophyte *Podostemum ceratophyllum* Michx. Rocks were gently washed to remove macroinvertebrates, and then scrubbed with a wire brush into a collection pan. Cobbles colonised by *P. ceratophyllum* were excluded. Wood was gently scrubbed by hand and rinsed into the collection pan using stream water. Sampling was limited to hard pieces of conditioned wood to minimise possible contamination by bark or soft wood. C : N ratios from processed samples indicated that wood contamination was minimal. The C : N ratios were 7.2 for epilithic biofilm from rock sites and 10.2 for epixylic biofilm from sand sites, compared with a C : N of 177.3 for CWD. The biofilm slurry was filtered onto precombusted glass fibre filters (0.7 μm). Filters were inspected in the field and small macroinvertebrates (e.g. Chironomidae) were removed.

Multiple individuals of each consumer taxa were usually compiled into a single composite sample for

stable isotope analysis in order to minimize within population variance (Lancaster & Waldron, 2001). Individuals of a similar size or cohort were included in composites. Samples were wrapped in foil, placed on ice and frozen at the end of each day. Macroinvertebrates were collected with a variety of methods including kick-nets in riffles, bank sampling with D-nets, and picking wood by hand. Macroinvertebrates were sorted in the field and identified to family (in some cases genus; see Table 2). They were kept in unfiltered stream water for one to several hours before packaging to clear their guts. All macroinvertebrates were analysed whole except for crayfish and clams. Tail muscle tissue was dissected from crayfish, and the entire soft body tissue of clams was removed for analysis. Fish were collected using backpack electrofisher, seine and dipnet. Samples were partially thawed in the laboratory and skinless dorsal muscle tissue was dissected for analysis. To avoid potentially confounding effects of ontogeny on isotopic values, we selected adult fish of similar size (i.e. <10% variation in length) for inclusion in a replicate.

Data analysis

We focused these analyses on $\delta^{13}C$ to infer differences in carbon flow among sites. $\delta^{15}N$ was used secondarily to infer trophic relationships among organic matter and consumers. Spatial and seasonal patterns were identified from scatter plots of organic matter and consumer $\delta^{13}C$ in rock versus sand sites in spring versus autumn. We pooled data from both years for this analysis because all consumers were not collected at each site and sampling event and because preliminary analysis with consumers grouped by feeding guild showed that yearly variation was minor compared with seasonal and site variability.

Analysis of covariance (ANCOVA) was used to test for spatial and seasonal differences in organic matter and consumer $\delta^{13}C$. The ANCOVA models included bed type, season, bed type-by-season interaction, and year, with drainage area as a covariate. Over the six sites (three rock, three sand), two seasons (autumn, spring), and two years (2003, 2004), $\delta^{13}C$ was measured in 180 samples of four organic matter sources, 720 samples of macroinvertebrates, and 436 samples of fish. Consumer taxa were combined into trophic guilds prior to ANCOVA. Macroinvertebrates were assigned to six trophic guilds (shredder, grazer, collector-gatherer, filter feeder, omnivore and predator) based on functional feeding groups and diet summaries (Pennak, 1989; Merritt & Cummins, 1996). Fish were assigned to three guilds (omnivore, insectivore and generalised carnivore) based on diet information from regional texts (Etnier & Starnes, 1993; Jenkins & Burkhead, 1994; Boschung & Mayden, 2003).

Carbon stable isotopes are only useful to infer organic matter inputs to consumers under two conditions. First, organic matter sources must show isotopic separation (Peterson & Fry, 1987). Secondly, consumer $\delta^{13}C$ values must fall within the range observed for organic matter sources, after accounting for trophic fractionation (Post, 2002). Preliminary plots of $\delta^{13}C$ and $\delta^{15}N$ data indicated that these conditions were met at rock, but not at sand sites. To describe these patterns better, we compared the ranges of organic matter and consumer $\delta^{13}C$ between bed types. Organic matter range ($\Delta\delta^{13}C$) was calculated as the mean difference between CPOM and biofilm observed at each site and season. This value measures isotopic separation between the organic matter sources. Only CPOM and biofilm are included in this analysis as these are the primary basal resources for stream consumers. Consumers were first grouped into 'macroinvertebrate' and 'fish' categories for simplicity. $\Delta\delta^{13}C$ was calculated as the mean difference between the largest and smallest values observed for macroinvertebrates and fish at each site and season.

Results

Geomorphology

Sand and rock sites had starkly contrasting geomorphologies (Table 1). Sand sites were low gradient (mean = 0.33) and averaged 87% coverage of sand and gravel. Their beds were unstable, and we observed widespread movement of surface sediments during baseflow conditions. The channels were 'U-shaped' with homogenous, less turbulent flows and little backwater habitat to support stable mats of epipsammic biofilm. The primary stable substrate supporting biofilm growth at sand sites was CWD, which was more common at sand than rock sites (Table 1). Channel beds at rock sites were higher gradient (mean = 1.74%) and composed mostly of bedrock, boulder and cobbles (mean channel bed coverage = 86%). The combination of stable

Table 2 Summary of $\delta^{13}C$ values for organic matter and consumers sampled from rock and sand sites in Twelvemile Creek system during spring and autumn 2003 and 2004

	Rock		Sand	
	Spring	Autumn	Spring	Autumn
Organic matter sources				
CPOM	−29.35 (0.25) 12	−30.14 (0.46) 18	−29.63 (0.19) 12	−29.94 (0.12) 6
Fine benthic organic matter	−27.12 (0.09) 9	−26.86 (0.47) 18	−27.46 (0.08) 12	−27.03 (0.1) 6
Biofilm	−25.62 (0.56) 10	−25.52 (1.11) 18	−27.74 (0.18) 12	−28.47 (0.2) 6
Seston	−27.31 (0.14) 9	−27.59 (0.57) 15	−27.29 (0.15) 11	−28.18 (0.12) 6
Macroinvertebrates				
Antocha (D, p)	−27.46 (0.45) 7	–	–	–
Argia (O, p)	−25.79 (0.19) 4	–	–	–
Baetidae (E, gr)	−28.26 (0.67) 13	−28.38 (1.37) 18	−32.13 (0.24) 6	−32.75 (0.42) 3
Boyeria (O, p)	−26.91 (0.28) 3	−27.43 (0.46) 9	−28.68 (0.35) 12	−29.67 (0.59) 6
Baetisca (E, cg)	–	–	–	−34.85 (0.12) 4
Caenis (D, cg)	–	–	−28.32 (0.4) 3	–
Calopteryx (O, p)	−27.08 (0.3) 8	−27 (0.12) 9	−28.05 (0.62) 3	−29.68 (0.2) 6
Cambarus spp. (De, o)	−25.79 (0.09) 10	−25.76 (0.44) 16	−26.01 (−0.13) 13	−26.13 (0.12) 7
Corbicula fluminea Muller (V, ff)	−27.87 (0.43) 8	−28.51 (0.88) 15	−28.83 (0.42) 12	−29.15 (0.28) 6
Corydalidae (M, p)	−26.78 (0.27) 12	−26.98 (0.7) 13	−28.65 (0.58) 10	−30.93 (0.88) 3
Dineutus (C, p)	–	–	−26.81 1	−28.34 (0.31) 4
Elimia proxima Say (N, gr)	−25.13 (2.01) 6	−25.2 1	–	–
Elmidae (C, gr)	−28.84 (0.44) 7	–	−29.76 (0.39) 12	–
Ephemerellidae (E, cg)	−27.76 (0.7) 4	–	−29.41 (0.41) 10	−33.99 (0.48) 3
Gomphidae (O, p)	−26.9 (0.4) 6	−27.24 (0.45) 6	−27.5 (0.39) 5	−27.2 (0.49) 3
Hexagenia (E, cg)	−28.26 1	–	−27.6 1	–
Hydropsychidae (T, ff)	−27.9 (0.22) 13	−28.27 (0.47) 27	−28.65 (0.11) 9	−30.15 (0.12) 9
Isonychia (E, ff)	−26.99 (1.21) 6	−29.84 (0.63) 6	−29.22 (0.18) 10	−31.08 1
Leuctra (P, shr)	–	−27.28 (0.06) 2	–	−28.99 (0.18) 6
Macromia (O, p)	−27.82 (0.2) 6	–	−28.43 (0.57) 5	−30 (0.31) 6
Nectopsyche (T, shr)	−26.2 1	–	−27.93 (0.1) 2	–
Nontanypodinae Chironomidae (D, cg)	−27.35 (0.33) 12	−28.06 (3.27) 13	−26.68 (0.98) 12	−27.9 (0.47) 4
Oligochaeta (cg)	−25.95 (0.23) 12	–	−27.92 (0.89) 6	–
Perlidae (P, p)	−26.68 (0.12) 13	−27.85 (0.36) 6	−28.12 (0.17) 34	−30.32 (0.21) 9
Perlodidae (P, p)	−27.05 (0.2) 17	−28.85 (0.73) 6	−28.28 (0.41) 8	–
Pteronarcys (P, shr)	−27.06 1	−28.01 (0.8) 7	−28.92 (0.59) 11	−28.71 (0.25) 5
Pycnopsyche (T, shr)	−26.34 (0.25) 4	–	−27.05 (0.57) 4	–
Rhyacophila (T, p)	−25.99 1	–	–	–
Simulium (D, ff)	−26.8 (0.24) 12	−28.86 (0.78) 10	−28.62 (0.24) 6	−29.47 (0.06) 3
Stenonema (E, gr)	−26.66 (0.33) 10	−27.63 (0.49) 21	−29.74 (0.19) 12	−31.24 (0.28) 9
Tanypodinae Chironomidae (D, p)	−25.52 (0.32) 3	–	−27.22 (0.76) 2	–
Tipula (D, shr)	−26.11 (0.22) 7	−28.48 (0.51) 12	−27.63 (0.62) 5	−28.56 (0.27) 6
Fishes				
Ameiurus brunneus Jordan (I, o-f)	−24.36 1	−25.9 1	−28.99 1	–
A. natalis Lesueur (I, o-f)	−24.97 (0.44) 2	−25.27 1	−28.02 1	–
A. nebulosis Lesueur (I, o-f)	–	−23.89 (1.89) 8	−26.41 1	–
A. platycephalus Girard (I, o-f)	−24.74 (0.32) 7	−25.59 (0.6) 9	−27.51 (1.46) 2	−26.22 (0.68) 2
Cyprinella nivea Cope (C, i)	−25.19 (0.31) 3	−25.13 (0.68) 6	−25.43 (0.35) 6	−25.98 1
Etheostoma inscriptum Jordan & Brayton (P, i)	−25.62 (0.3) 12	−26.13 (0.55) 18	−25.84 (0.45) 8	−30.06 1
Gambusia affinis Baird & Girard (Po, i)	–	–	–	−24.94 1
Hybopsis rubrifrons Jordan (C, i)	−25.5 (0.73) 11	−25.18 (0.98) 18	−26.48 (0.31) 12	−27.29 (0.27) 2
Hypentelium nigricans Lesueur (*Ca, i*)	−24.87 (0.41) 11	−24.77 (1.68) 17	−26.5 (0.47) 12	−27.79 (0.62) 6
Lepomis auritus Linnaeus (Ce, i)	−25.45 (0.32) 10	−24.8 (0.37) 10	−25.83 (0.26) 11	−26.45 (0.26) 3
L. cyanellus Rafinesque (Ce, gc)	−25.93 (0.4) 5	−24.46 (1.66) 5	−27.2 (0.48) 4	−21.4 (1.56) 4
L. gulosis Cuvier (Ce, gc)	−25.84 1	–	–	–
L. macrochirus Rafinesque (Ce, i)	−25.77 (0.75) 10	−25.12 (0.57) 5	−25.87 (0.57) 10	−27.91 (0.95) 2
Micropterus coosae Hubbs & Bailey (Ce, gc)	−25.89 1	−23.86 1	–	–

Table 2 (*Continued*)

	Rock		Sand	
	Spring	Autumn	Spring	Autumn
M. punctulatus Rafinesque (Ce, gc)	–	–	−29.74 1	–
M. salmoides Lacepède (Ce, gc)	−26.26 (0.43) 3	−24.35 (0.87) 4	−25.07 1	–
Moxostoma anisurum Rafinesque (Ca, i)	−24.83 1	−24.65 1	–	–
Notropis hudsonius Clinton (C, i)	–	–	–	−25.27 (0.24) 3
N. lutippinis Jordan & Brayton (C, o-f)	−24.65 (0.22) 12	−25.31 (0.37) 18	−25.34 (0.21) 12	−26.23 (0.24) 5
Nocomis leptocephalis Girard (C, o-f)	−25.06 (0.24) 12	−25.34 (0.5) 18	−25.61 (0.22) 12	−25.29 (0.25) 6
Noturus insignis Richardson (I, i)	−25.83 (0.03) 3	−25.96 (0.78) 12	−27.55 (0.2) 2	−30.66 1
Percina nigrofasciata Agassiz (P, i)	−25.62 (0.4) 12	−25.63 (0.58) 16	−27.55 (0.48) 10	−30.03 (0.24) 3
Scartomyzon rupiscartes Jordan & Jenkins (Ca, i)	−24.02 1	–	–	–
Semotilus atromaculatus Mitchill (C, o-f)	−24.19 1	−24.86 (0.28) 3	–	–

Values shown are mean (1 SE) *n*.
Macroinvertebrates: C, Coleoptera; D, Diptera; De, Decapoda; E, Ephemeroptera; M, Megaloptera; N, Neotaenioglossa, O, Odonata; P, Plecoptera; T, Trichoptera; V, Veneroida; cg, collector gatherer; ff, filter feeder; gr, grazer; o, omnivore; p, predator; shr, shredder.
Fishes: C, Cyprinidae; Ca, Catostomidae; Ce, Centrarchidae; I, Ictaluridae; P, Percidae; Po, Poeciliidae; gc, generalised carnivore; i, insectivore; o-f, omnivore fish.

substratum and shallow water resulted in a near continuous surface for biofilm growth. Mean canopy cover was similar between the bed types, but was more variable among rock sites.

Spatial patterns in $\delta^{13}C$ values

Organic matter and consumers were consistently enriched in $\delta^{13}C$ at rock compared with sand sites (Table 2; Fig. 2). An exception was CPOM, which demonstrated little spatial variation among sites or between bed types. Biofilm was consistently enriched compared with CPOM at both bed types and in both seasons. Consumer enrichment at rock sites was consistent between seasons (Fig. 2a,b).

Bed type was consistently the strongest factor explaining variation in $\delta^{13}C$ among organic matter and trophic guilds, but drainage area was a secondary predictor (Table 3). Bed type showed a significant effect in 11 of 13 models whereas drainage area was

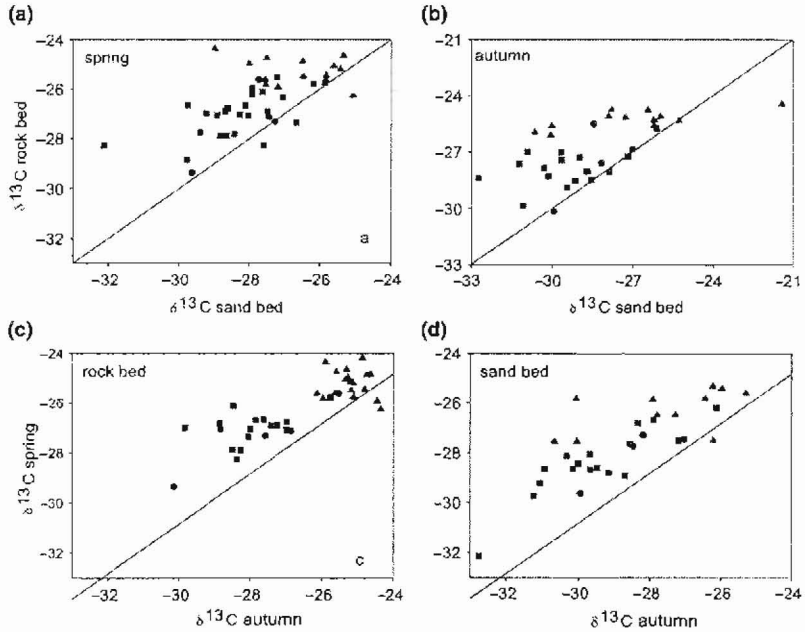

Fig. 2 Scatterplots showing shifts in $\delta^{13}C$ in relation to stream bed morphology and season. The solid line represents the 1 : 1 relationship. $\delta^{13}C$ in sand versus rock sites in spring (a, $n = 6$ sites) and autumn (b, $n = 3$ rock and 1 sand sites). $\delta^{13}C$ in spring versus autumn for rock (c, $n = 3$ sites) and sand sites (d, $n = 1$ site). Samples are: ●, organic matter; ■, macroinvertebrates; ▲, fishes.

Table 3 Results of ANCOVA for $\delta^{13}C$ of organic matter sources and trophic guilds

	n	Drainage area	Bed type	Season	Bed* season	Year	$\delta^{13}C_{site6} - \delta^{13}C_{site1}$ (‰)	$\delta^{13}C_{rock} - \delta^{13}C_{sand}$ (‰)
Organic matter source								
CPOM	48	NS	NS	*	NS	NS	(0.26)	(0.08)
FBOM	45	NS	*	**	NS	*	(0.21)	0.26
Biofilm	46	***	***	NS	NS	NS	2.08	2.71
Seston	41	**	*	**	*	NS	0.68	0.37
Trophic guild – invertebrates								
Collector/gatherer	85	*	***	**	*	NS	1.85	2.68
Filter feeder	153	**	***	***	NS	*	−0.65	1.24
Grazer	118	*	***	NS	NS	NS	0.95	3.33
Omnivore	45	NS	*	NS	NS	*	(0.11)	0.33
Predator	246	NS	***	***	**	***	(0.09)	1.80
Shredder	73	NS	***	***	*	NS	(−0.16)	1.18
Trophic guild – fish								
Generalised carnivore	30	NS	NS	***	**	NS	(0.75)	(−0.81)
Insectivore	244	***	***	*	NS	NS	1.55	1.73
Omnivore	162	NS	***	NS	NS	NS	(0.18)	0.89

Bed type (rock, sand), season (autumn, spring), and year (2003, 2004) are categorical variables, drainage area is a covariate, and bed*season is the interaction term.

The final two columns show the total magnitude of the drainage area effect [estimated $\delta^{13}C$ difference between sites 6 (furthest downstream) and 1 (furthest upstream), and the bed type effect (estimated $\delta^{13}C$ difference between rock and sand sites)]. Values shown in parentheses were not significantly different from 0.

NS $P > 0.05$ (not significant for effect tests), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

significant in six cases. The $\delta^{13}C$ enrichment related to bed type was greater than that related to drainage area in models where either effect was significant, except for seston. The model for biofilm illustrates this common pattern of enrichment related to bed type and drainage area (Fig. 3). Biofilm was enriched at

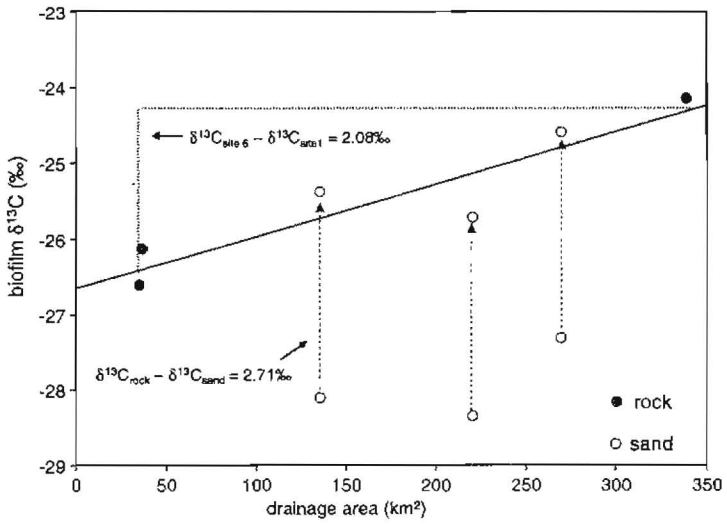

Fig. 3 Illustration of ANCOVA model showing biofilm as an example. Longitudinal position is indicated by increasing drainage area on the *x*-axis. Season and year did not have significant effects on biofilm $\delta^{13}C$, so for graphical simplicity site mean values are shown which average over seasons and years. Bed type had a significant effect ($P < 0.001$) on $\delta^{13}C$, with rock sites averaging 2.71‰ higher than sand sites (dashed vertical lines) when effects of the other factors in the model were controlled. Adjusting for this bed type effect (dashed lines), there was a significant linear relationship ($P < 0.001$) between $\delta^{13}C$ and drainage area, with a predicted 2.08‰ $\delta^{13}C$ enrichment (dotted vertical line) between the furthest upstream site (site 1) and the furthest downstream site (site 6).

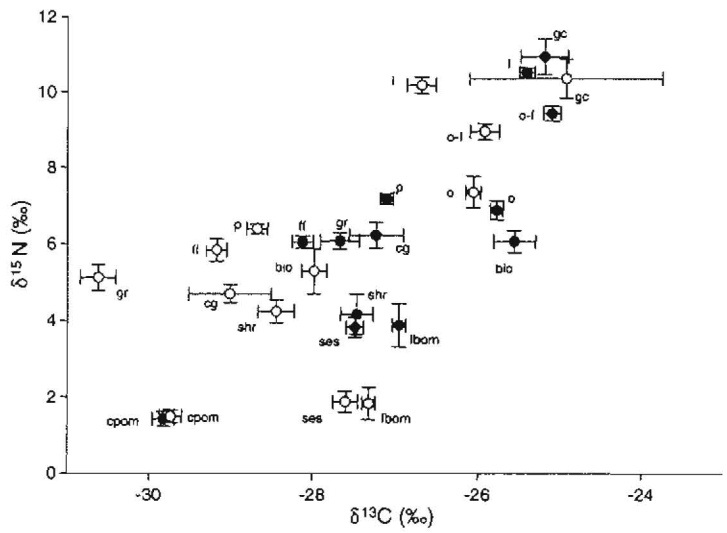

Fig. 4 Dual isotope plot of organic matter and consumer trophic guilds in sand (open circles, n = 535 samples) and rock streams (closed circles, n = 809 samples) for both seasons and years combined. Values are mean $\delta^{13}C$ versus $\delta^{15}N$ (‰ ± 1SE). Ses, seston; bio, biofilm; cg, collector gatherer; ff, filter feeder; gr, grazer; o, omnivore; p, predator; shr, shredder; gc, generalised carnivore; i, insectivore; o-f, omnivore-fish. Taxa in trophic guilds are listed in Table 2.

rock sites ($P < 0.001$) and with drainage area ($P < 0.001$), but the magnitude of enrichment was greater between bed type (2.71‰) than along the entire drainage area (2.08‰). Seston, FBOM and all consumer guilds were enriched at rock sites, except for generalised carnivores (which were infrequently collected). Filter feeders were the only guild to show significant downstream depletion. CPOM $\delta^{13}C$ was consistent regardless of bed type or longitudinal position.

We summarised mean $\delta^{13}C$ of organic matter and trophic guilds by bed type for both seasons to illustrate the effect of bed type identified by ANCOVA. Biofilm and all consumer guilds except generalised carnivores were enriched at rock sites compared with sand sites (Fig. 4). The enrichment of seston and FBOM, while significant, was small compared with the enrichment of biofilm. Biofilm and all consumers were enriched on average by 1.3‰ $\delta^{13}C$ at rock sites. No variable showed significant enrichment at sand sites.

We did not observe the expected trophic enrichment for $\delta^{13}C$ and $\delta^{15}N$ between biofilm and those macroinvertebrates that appeared to rely most heavily on biofilm as a food resource. Linear regression models indicated that biofilm $\delta^{13}C$ was significantly related to grazer $\delta^{13}C$ ($r^2 = 0.62$; $P = 0.006$; regression based on 10 mean $\delta^{13}C$ values calculated for each site and season) and weakly related to predator $\delta^{13}C$ ($r^2 = 0.39$; $P = 0.056$). However, both of these consumers were depleted by 1–2‰ $\delta^{13}C$ compared with biofilm and showed no enrichment in $\delta^{15}N$ among bed types (Fig. 4). Grazers were depleted in $\delta^{13}C$ compared with biofilm for all site-by-season comparisons with a maximum difference of 3.5‰. A similar comparison of $\delta^{15}N$ showed grazers were slightly enriched over biofilm in six of 10 cases, with a maximum enrichment of only 1.3‰. $\delta^{13}C$ of shredders, on the other hand, did follow the expected pattern of trophic enrichment compared with their putative food resource, CPOM. Shredders were enriched by 2.0‰ and 2.7‰ in $\delta^{13}C$ and $\delta^{15}N$, respectively, compared with CPOM (Fig. 4).

The range in organic matter (CPOM and biofilm) and consumer $\delta^{13}C$ differed by bed type. The difference between biofilm and CPOM $\delta^{13}C$ values was less at sand (1.8‰) than at rock sites (4.0‰, Table 4). Paradoxically, the range of consumer $\delta^{13}C$ was greater at sand sites. For example, macroinvertebrate mean $\Delta\delta^{13}C$ was 6.9‰ at sand sites compared with 4.3‰ at rock sites. This disparity is even larger when calculated as a ratio, $\Delta\delta^{13}C_{consumers} : \Delta\delta^{13}C_{organic\ matter}$ (Table 4). Macroinvertebrate $\Delta\delta^{13}C$ was fourfold higher and fish $\Delta\delta^{13}C$ was about twofold higher than organic matter $\Delta\delta^{13}C$ at sand sites. By comparison, these ratios were less than, or approximately equal to, one at rock sites.

Seasonal patterns in $\delta^{13}C$

Qualitative comparisons of autumn and spring samples revealed an overall trend towards spring enrichment for organic matter and consumers

Table 4 Range of $\delta^{13}C$ values for organic matter (CPOM and biofilm) and consumers (macroinvertebrates and fishes) at rock and sand sites

	Rock	Sand
Organic matter		
CPOM	−29.7	−29.7
Biofilm	−25.7	−27.9
$\Delta\delta^{13}C$	4.0	1.8
Macroinvertebrates		
Minimum	−29.4	−32.8
Maximum	−25.1	−25.9
$\Delta\delta^{13}C$	4.3	6.9
Fishes		
Minimum	−26.7	−29.3
Maximum	−23.9	−25.1
$\Delta\delta^{13}C$	2.8	4.2
$\Delta\delta^{13}C_{macroinvertebrates} : \Delta\delta^{13}C_{organic\ matter}$	1.1	3.8
$\Delta\delta^{13}C_{fishes} : \Delta\delta^{13}C_{organic\ matter}$	0.7	2.3

Values for CPOM and biofilm are mean values of mean values calculated for each site and season. Minimum and maximum values are mean values calculated for the most enriched (maximum) and depleted (minimum) consumer within each group (n = 4 and 6 observations at sand and rock sites, respectively, for organic matter and consumers). $\Delta\delta^{13}C$ is the difference between CPOM and biofilm and the difference between minimum and maximum values for each consumer group.

(Table 2, Fig. 2c,d). CPOM and seston were consistently enriched in spring compared with autumn, but biofilm was enriched in spring only at the sand site (Table 2). FBOM had the opposite response and was enriched in autumn, particularly at the sand site. Most macroinvertebrates were also enriched in spring. An exception was crayfish (*Cambarus* spp.), which showed autumn enrichment. Fish were enriched in spring at the sand site but not at rock sites (Fig. 2c,d).

Coarse particulate organic matter was enriched in spring (ANCOVA, $P = 0.027$) along with seston ($P = 0.009$; Table 3). Biofilm showed no seasonal effect, and FBOM was the only organic matter source enriched in autumn ($P = 0.009$). Trophic guilds with significant spring enrichment included shredders, collector gatherers, filter-feeders, predators and insectivorous fish. Only generalised carnivores were significantly enriched in autumn. Among dependent variables that demonstrated bed and season effects, a significant bed type-by-season interaction was documented for seston, collector gatherers, predators and shredders. The interactions indicated that $\delta^{13}C$ differences between bed types depended on season. However, the direction of response was always consistent, with enrichment at rock versus sand sites and in spring versus autumn.

Discussion

Spatial variation of $\delta^{13}C$ in stream food webs

Biofilm and consumer $\delta^{13}C$ usually exhibit a general pattern of downstream enrichment (Finlay, 2001, 2004). Downstream enrichment in biofilm $\delta^{13}C$ is mainly related to a downstream reduction in dissolved CO_2 concentration and photosynthetic fractionation rates coupled with downstream enrichment of $\delta^{13}C$ of CO_2 (Finlay, 2004). Downstream enrichment of consumers is often interpreted from the perspective that algal carbon is more important to downstream food webs as primary production increases (e.g. Doucett *et al.*, 1996b; Finlay, 2001), a central prediction of the river continuum concept (Vannote *et al.*, 1980). This pattern can be reversed in environments such as prairie and alpine ecosystems where stream headwaters flow through open meadow before entering a forested canopy (Zah *et al.*, 2001; McCutchan & Lewis, 2002; Dodds *et al.*, 2004). In either case, the exogenous forces of litter and light inputs are the presumed primary drivers of detritus and algal biomass and, consequently, variation in consumer $\delta^{13}C$ among sites (Rounick & Winterbourn, 1986). Our observation of downstream enrichment of biofilm and some trophic guilds partially supports these longitudinal predictions, but variation in $\delta^{13}C$ among trophic levels was more strongly related to local bed morphology. This suggests that endogenous factors related to physical habitat were the key determinant of isotopic signatures among trophic levels and outweighed longitudinal effects, as implied by the weaker relationship with drainage area. It is important to note that basin area among our sites increased from approximately 30–340 km^2, and that pronounced longitudinal enrichment of biofilm and consumers has been documented across this range of stream sizes (Finlay, 2001, 2004).

Local variation in stream slope created sites with drastically different bed morphologies and habitat for biofilm growth. High-slope sites had rocky beds with epilithic biofilm whereas low-slope sites were dominated by sand and CWD with epipsammic and epixylic biofilms. Biofilm and consumer $\delta^{13}C$ tracked these habitat differences, creating a pattern of sys-

tematic variation in food webs predicted by patch dynamic or discontinuity models of lotic ecosystems (Pringle *et al.*, 1988; Townsend, 1989; Poole, 2002). The systematic enrichment of consumer $\delta^{13}C$ at high-gradient, rock sites was similar to the findings of Huryn *et al.* (2002) for high-gradient, constrained and low-gradient, floodplain sites in New Zealand. Finlay *et al.* (1999) and Finlay, Khandwala & Power (2002) also documented changes in stream epilithon and consumer $\delta^{13}C$ related to current velocity differences between pool and riffle habitats, further illustrating the potential importance of local physical factors in among-site variation of organic matter and consumer $\delta^{13}C$. These findings, combined with our results, support the process domains concept of Montgomery (1999), which hypothesised that spatial variability in geomorphic processes controls the habitat and disturbance regimes influencing ecosystem dynamics.

Why did biofilm $\delta^{13}C$ depletion at sand sites override the expected pattern of downstream enrichment of biofilm $\delta^{13}C$ (Finlay, 2001, 2004)? Depletion in biofilm $\delta^{13}C$ at sand sites could be the result of differences in primary production and CO_2 availability between sand and rock sites. Increases in primary production are associated with lower photosynthetic fractionation of CO_2 and subsequent enrichment of biofilm $\delta^{13}C$ (Finlay, 2004). Thus, the pattern of biofilm $\delta^{13}C$ enrichment observed here could be attributed to higher primary production at rocky than at sand sites. Finlay *et al.* (1999) also showed that algal $\delta^{13}C$ was enriched in highly productive, low velocity (0.0–0.2 m s^{-1}) pool habitats compared with high velocity (<0.35 m s^{-1}) riffles. He attributed algal $\delta^{13}C$ enrichment in pools to a depleted CO_2 concentration in the boundary layer. Boundary layer effects are unlikely to explain biofilm $\delta^{13}C$ depletion at our sand sites for three reasons. First, we found enriched biofilm $\delta^{13}C$ at rock sites, which had higher mean current velocity than sand sites (D.M. Walters, unpublished data). Secondly, mean velocities from the biofilm sampling areas at sand and rock sites were 0.38 and 0.53 m s^{-1}, considerably higher than those associated with enriched algal carbon signals in the study of Finlay *et al.* (1999). Finally, low velocity (nearly stagnant), highly productive pools such as those sampled by Finlay *et al.* (1999) were absent from our study sites.

Another possible explanation for depletion of $\delta^{13}C$ at sand sites lies in the differences between the epixylic and epilithic biofilms at the sand and rock sites, respectively. Epixylic communities are more heterotrophic than epilithic communities (Tank & Winterbourn, 1996; Sabater *et al.*, 1998). The epixylon $\delta^{13}C$ signal was similar to that of CPOM, suggesting greater respiration of carbon derived from terrestrial detritus or use of the wood substratum itself as a carbon source (e.g. Sinsabaugh, Golladay & Linkins, 1991). Epipsammic biofilm, which we did not sample, is also more heterotrophic than epilithon, and sand habitats are important zones of bacterial processing of organic matter (Romani & Sabater, 2001).

Do food webs in rock and sand sites function differently? We expected that consumers at rock sites would utilise more algal carbon than those at sand sites, and that this increased consumption would lead to the enrichment of consumer $\delta^{13}C$. Our prediction that consumer $\delta^{13}C$ would be enriched at rock sites was supported, but we also found $\delta^{13}C$ enrichment of biofilm at rock sites. Thus, consumer enrichment at rock sites could be related to (i) increased primary production of algae at rock sites, corresponding with greater consumption of a food source that is enriched relative to alternative food sources, or (ii) consumption of biofilm at rock sites that is enriched relative to biofilm at sand sites or (iii) both mechanisms. We are unable to determine the mechanisms controlling $\delta^{13}C$ values of consumers without more detailed data on consumer diet, primary production, respiration, and organic matter inputs at sand and rock sites.

Seasonal variation in $\delta^{13}C$ in stream food webs

Surprisingly few studies have investigated temporal variation in lotic food webs (Woodward & Hildrew, 2002), even though inputs of organic matter sources to temperate forested streams show seasonal variation. Biofilm (i.e. periphyton) production in forested streams has been shown to peak in spring prior to canopy leaf-out (Bernhardt & Likens, 2004), and CPOM inputs peak in autumn. Consumption of these resources may shift seasonally as well, with consequent shifts in consumer $\delta^{13}C$. We found that biofilm was enriched over CPOM $\delta^{13}C$. We would expect spring enrichment of consumer $\delta^{13}C$ if they increase biofilm consumption in spring and if their tissues rapidly equilibrate to the isotopic signature of biofilm. Little work has been done on isotopic turnover rates of aquatic macroinvertebrates. However, diet switch experiments using terrestrial

insects have shown that carbon isotopic turnover generally occurs within a few days to a few weeks (Ostrom, Colunga-Garcia & Gage, 1997; Webb, Hedges & Simpson, 1998; Gratton & Forbes, 2006). Isotopic turnover should be particularly rapid if the invertebrates have high growth rates, as Fry & Arnold (1982) reported for brown shrimp *Penaeus aztecus* (Ives). We found spring enrichment of macroinvertebrate consumers, but could not attribute this to shifting resource use as CPOM $\delta^{13}C$ was also enriched in spring. Fish at rock sites did not track seasonal enrichment of macroinvertebrates, suggesting that the fish isotopic signature integrates food consumed over a longer period. However, fish at the sand site did show a trend towards spring enrichment. It is unclear why fish responded differently in the different bed types, and seasonal data from more sand sites are needed to determine the strength of this pattern.

Published studies of seasonal changes in $\delta^{13}C$ in stream consumers suggest that the seasonal response is highly variable among stream ecosystems. We observed spring enrichment of consumers, but Huryn *et al.* (2002) reported macroinvertebrate enrichment in autumn rather than spring in grassland streams, which they attributed to increased autumn consumption of algal carbon. Finlay *et al.* (2002) found consumer enrichment from spring to autumn in autotrophic streams in California, but Zah *et al.* (2001) reported consumer enrichment in summer versus winter at most sites they surveyed in an alpine system. Finlay (2004) found that small, unproductive streams were depleted in summer and autumn compared with spring whereas productive sites downstream showed the opposite response. He attributed these patterns to downstream changes in dissolved CO_2 and photosynthetic fractionation. Clearly, more mechanistic studies of seasonal shifts in $\delta^{13}C$ are needed to understand and predict seasonal changes in carbon flow among disparate lotic ecosystems.

Implications for modelling stream food webs using $\delta^{13}C$

The spatial patterns observed in $\delta^{13}C$ affect our ability to quantify organic matter inputs to stream food webs. Isotopic separation of CPOM and biofilm is the key factor in the successful use of stable isotopes to assess organic matter consumption in streams (Bunn *et al.*, 1989; France, 1994; Finlay, 2004). Bed morphology was related to isotopic separation of basal resources in our study. CPOM and biofilm $\delta^{13}C$ were similar ($\Delta\delta^{13}C$ approximately 1–2‰) at sand sites, but showed much greater separation at rock sites ($\Delta\delta^{13}C$ approximately 4‰). Furthermore, the range in $\delta^{13}C$ consumer values was up to four times that of organic matter sources at sand sites. From a modelling perspective, $\delta^{13}C$ values at sand sites have low signal (range of organic matter $\delta^{13}C$) and high noise (range of consumer $\delta^{13}C$), and thus are of little use for quantifying consumer resource use (Peterson & Fry, 1987).

Our results support a growing body of literature suggesting that the common assumption of constant fractionation rates for $\delta^{13}C$ should be applied with caution to stream consumers. Consumer isotopic signatures must be corrected for trophic fractionation to accurately model carbon flow through the food web (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001; Post, 2002). Average fractionation rates derived from meta-analyses are around 0.3–1.3‰ $\delta^{13}C$ (Post, 2002; McCutchan *et al.*, 2003), and these rates are commonly used to model carbon flow in aquatic systems (e.g. England & Rosemond, 2004). However, stream macroinvertebrates are often depleted in $\delta^{13}C$ relative to their putative food resources (e.g. Finlay *et al.*, 2002; Jardine *et al.*, 2005). Jardine *et al.* (2005) compared $\delta^{13}C$ of macroinvertebrates and gut items and found that five of seven functional feeding groups were depleted in $\delta^{13}C$ relative to their diet. We also found that grazers were depleted by 2–3‰ $\delta^{13}C$ over biofilm, even though regression analysis indicated that biofilm was their primary carbon source. This observed depletion could result from omnivory (e.g. consumption of allochthonous material by grazers) or selective assimilation of different heterotrophic, autotrophic or detrital elements in the biofilm. However, it remains troubling that these data do not support model assumptions at the primary step in the food web. In addition, constant fractionation rates have limited use where the range of consumer $\delta^{13}C$ is much larger than the presumed food resources, as we observed at sand sites. This pattern suggests that fractionation rates among consumers are highly variable, as prior meta-analyses have shown (Vander Zanden & Rasmussen, 2001; Post, 2002; McCutchan *et al.*, 2003), and that application of mean fractionation rates to a diverse assemblage of stream consumers could generate misleading results.

How significant were the shifts in $\delta^{13}C$ that we observed between rock and sand sites? Consider the

magnitude of change ($\Delta\delta^{13}C$) between bed types compared with expected trophic enrichment. Enrichment at rock sites compared with sand sites was 1.4‰ for macroinvertebrates analysed whole, fourfold higher than the expected trophic enrichment of 0.3‰ (McCutchan *et al.*, 2003). Fish muscle tissue at rock sites was enriched by 1.2‰ over that at sand sites, comparable with the expected trophic fractionation rate of 1.4‰ (McCutchan *et al.*, 2003). These comparisons show that the geomorphic $\Delta\delta^{13}C$ was just as large as, and in many cases much larger, than the expected trophic $\Delta\delta^{13}C$. Thus, although trophic enrichment of carbon is important, spatial effects must also be considered in order to assess resource utilisation accurately among sites.

In conclusion, we found that the physical template of the stream (*sensu* Southwood, 1977) influences the $\delta^{13}C$ signal of basal resources and consumers across trophic levels. This reach-scale response of food webs is probably an important source of the unexplained variation observed in the relationship between stream size and consumer $\delta^{13}C$ signal (Finlay, 2001). Our finding that reach morphology strongly affects consumer $\delta^{13}C$ has important implications for studies of anthropogenic alteration of stream food webs. Most studies have focused on human alteration of allochthonous light and litter inputs to streams and the indirect effects on consumer $\delta^{13}C$ (e.g. Rounick & Winterbourn, 1986; England & Rosemond, 2004). Anthropogenic changes in stream habitat, such as alteration of the channel bed via excessive sediment inputs, could also alter stream food webs or consumer $\delta^{13}C$, and deserve consideration in future studies.

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