

Depth-specific variation in carbon isotopes demonstrates resource partitioning among the littoral zoobenthos

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SUMMARY

1. Benthic animals readily consume benthic algae, phytoplankton and terrestrial organic matter and are themselves a major component of fish diets. However, the effect of variation in resource availability on resource use by littoral macroinvertebrates remains poorly resolved.
2. Using stable isotopes of carbon, we quantified depth-specific resource use by zoobenthic functional feeding groups in five lakes in northern Wisconsin. The littoral zoobenthos was collected from soft sediments at several depths in conjunction with samples of bulk periphyton (top 5 mm of sediment and biofilm) and measurements of benthic algal primary productivity.
3. Periphyton $\delta^{13}\text{C}$ was positively correlated with depth-specific benthic algal primary productivity, but grazer $\delta^{13}\text{C}$ was consistently higher than that of the periphyton.
4. The $\delta^{13}\text{C}$ of infaunal collectors and predators was tightly correlated with, and nearly identical to, bulk periphyton $\delta^{13}\text{C}$ (collectors: R^2 , 0.88; slope, 0.97; $P < 0.0001$; predators: R^2 , 0.78; slope, 0.88; $P < 0.0001$). Reliance of collectors and predators on benthic algal carbon varied between 43 and 100%, depending on whether grazers or bulk periphyton was used as the benthic algal end-member.
5. Despite the apparent homogeneity of the sediments, benthic grazers and collectors partitioned resources in a consistent way in our study lakes, indicating either selective ingestion or assimilation of different components of the biofilm.

Keywords: food webs, invertebrates, littoral, periphyton, stable isotopes

Introduction

Freshwater food webs are sustained by many carbon sources. It has long been accepted that stream food webs are supported by both terrestrial carbon and in-stream periphyton production (Vannote *et al.*, 1980; Wallace & Webster, 1996; Finlay, 2001). For lakes, there has been a historical focus on the primacy of phytoplankton, although more recently there has been an emerging consensus that phytoplankton, benthic algae and terrestrial organic carbon together form the energetic basis of lake food webs (Vadeboncoeur, Vander Zanden & Lodge, 2002; Reynolds, 2008; Karlsson *et al.*, 2009; Cole *et al.*, 2011; Vander Zanden, Vadeboncoeur & Chandra, 2011). The littoral zoobenthos is a key group in this paradigm of lentic food-web structure because littoral animals

readily consume benthic, planktonic and terrestrial carbon sources (Hecky & Hesslein, 1995; Vadeboncoeur *et al.*, 2003; Solomon *et al.*, 2008, 2011; Bartels *et al.*, 2012). Littoral macroinvertebrates are a primary link between these various basal resources and fish (Vander Zanden & Vadeboncoeur, 2002; Vander Zanden *et al.*, 2011).

Research in lakes has emphasised the role of fish in integrating energy pathways through their consumption of zoobenthos (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002). In contrast, in stream research, the zoobenthos is considered key to understanding how diverse basal resources are incorporated into secondary production (Benke & Huryn, 2010). The availability of light at the sediment–water interface and the proximity, timing and rate of input of terrestrial

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carbon to streams interact to provide a mosaic of resources that are differentially exploited by specialised trophic guilds within the lotic zoobenthic community. However, lake ecologists have paid less attention to how the within- and among-lake variation in basal resource composition affects resource use by the zoobenthos (Sierszen, Peterson & Scharold, 2006). The degree to which benthic algae are incorporated into primary consumers may depend upon both the quantity of benthic algae available (Sierszen *et al.*, 2006; Kobayashi *et al.*, 2011) and the relative quality of basal resources (Brett *et al.*, 2009). Within lakes, the quantity of benthic algae relative to phytoplankton declines with depth because light strongly limits periphyton productivity (Vadeboncoeur *et al.*, 2003, 2008).

Periphyton consists of benthic algae, bacteria and fungi growing on submerged surfaces. In this study, we focus on the benthic algal component of the periphyton as a resource for the zoobenthos. Stable carbon isotope ratios provide a measure of the reliance on periphyton in lakes because diffusion gradients across the sediment–water interface cause benthic algae to be enriched in ^{13}C relative to the phytoplankton, and there is little difference between prey and consumer $\delta^{13}\text{C}$ (Hecky & Hesslein, 1995). Comprehensive syntheses of stable carbon isotope data demonstrate that many lake-dwelling fish species are isotopically enriched with ^{13}C , indicating reliance on periphyton via their consumption of benthic invertebrates (Vander Zanden *et al.*, 2011). However, inferences regarding benthic energy pathways are often derived from relatively coarse models. There has been limited assessment of how spatial variation in benthic primary productivity affects resource use by consumers or, secondarily, how gradients in depth and primary productivity affect the appropriate isotopic end-member for isotopic mixing models (Hill, Fanta & Roberts, 2008).

We used stable isotopes of carbon to explore the following questions: (i) To what extent does the zoobenthos on soft unvegetated sediments rely on carbon fixed by benthic algae? (ii) Is within- and among-lake spatial variation in the use of benthic algal carbon by the zoobenthos correlated with variation in benthic algal productivity? (iii) How does end-member selection affect estimates of the use of benthic algal carbon by consumers?

Methods

Study sites

We studied five north-temperate lakes in Wisconsin, U.S.A. (Table 1). The littoral zone of each lake is com-

posed primarily of soft sediments of varying organic content, although areas of cobble and macrophytes are present in three of the lakes. Little Rock Lake sediments have the highest organic content (Table 1) where the substratum is composed of loose, amorphous organic conglomerates rich in fungal hyphae and vascular plant detritus. At the opposite extreme, Crystal Lake has sandy sediments, a thin algal biofilm and extremely low quantities of fine particulate organic matter. Sediment organic matter in Sparkling Lake is largely composed of a well-developed (*c.* 1 cm thick), stable periphyton community, rich in pennate diatoms, that overlies sandy sediments. Trout Lake and Big Muskellunge Lake sediments are sandy, with moderately thick periphyton mats. Periphyton structure changed as a function of depth in all lakes. Periphyton formed a thin crust on sand near the shoreline at depths <1 m. Periphyton communities were up to 1 cm thick at depths between 2 and 4 m changing into well defined, but thin (*c.* 2 mm) cyanobacterial mats around 8 m depth and deeper.

Benthic stable isotopes

We collected the majority of the invertebrates for isotope analysis during summer monitoring (2005–07) of zoobenthic biomass at 0.5, 2, 4, 8, 10 m in each lake (Butkas, Vadeboncoeur & Vander Zanden, 2011). We supplemented these data with simultaneous collections of zoobenthos and periphyton biofilms: on several dates, a scuba diver collected periphyton at 1-m depth intervals with a syringe corer and immediately collected zoobenthos from the area using a D-net. We also collected foot tissue of unionid mussels at nine depths in the littoral zone of Sparkling and Trout lakes. Zooplankton was sampled using epilimnetic vertical plankton net tows (243- μm mesh) five times from each lake during summer of 2007.

Benthic invertebrates from each sampling event and depth were identified to the lowest practical taxonomic level. This was often family, although many insects were identified to genus (Table 2). Several individuals were pooled to generate a single isotope sample for each taxon. Molluscs and crayfish were removed from their shells, and only body tissue was analysed for ^{13}C . Extreme care was taken to avoid inclusion of any shell fragments in samples. Each taxon was assigned to one of the following functional feeding groups (FFG): grazer, collector, predator or filter feeder based on the literature (Shapas & Hilsenhoff, 1976; Cummins & Klug, 1979; Merritt & Cummins, 1996) (Table 2). Filter feeders included pelagic zooplankton and unionid mussels. We

Table 1 Lake physical characteristics and summer average water chemistry: dissolved organic carbon (DOC), chlorophyll-a (Chl), total phosphorus (TP), $\delta^{13}\text{C}$ of the periphyton biofilm (Biofilm $\delta^{13}\text{C} \pm \text{SE}$), sediment organic content ($\pm \text{SE}$) and average midday periphyton primary productivity

Lake	Area [†] (ha)	Mean Depth [†] (m)	Littoral Area (%)	DOC [†] (mg L ⁻¹)	Chl [†] (µg L ⁻¹)	TP [†] (µg L ⁻¹)	Biofilm $\delta^{13}\text{C}$ (‰)	Sediment Organic Content (mg C g ⁻¹)	Benthic primary productivity (mg C m ⁻² h ⁻¹)
Little Rock	8.1	3.1	100	5.2	7.9	33.7	-29.7 ± 0.9	241 ± 18.2	12.5
Crystal	36.7	10.4	91	2.4	1.8	8.7	-21.7 ± 1.0	10.1 ± 1.3	19.0
Sparkling	64	10.9	68	3.3	1.6	7.1	-22.4 ± 0.5	52.0 ± 8.1	36.2
Big Muskellunge	396.3	7.5	83	3.8	2.9	17.5	-16.1 ± 0.6	27.1 ± 2.6	23.1
Trout	1607	14.6	43	2.8	3.1	13.5	-23.3 ± 0.5	54.7 ± 19.0	24.5

[†]Data obtained from LTER database (unmarked data from are from this study).

Table 2 Zoobenthic invertebrate taxa included in the study and their fractional reliance on carbon fixed by periphyton. Functional feeding groups were assigned based on Merritt & Cummins (1996) and Cummins & Klug (1979)

Functional Feeding Group	Taxon	Lakes	Mean $\delta^{13}\text{C} \pm \text{SE}$ (n)	Mean reliance on periphyton $\pm \text{SE}$ (n)
Grazers	<i>Hyalella</i>	BM, TL	-16.40 ± 1.27 (11)	0.93 ± 0.06
	<i>Ephemera</i>	SL, TL	-22.02 ± 0.85 (7)	0.61 ± 0.06
	<i>Choroterpes</i>	BM	-15.83 ± 0.98 (2)	0.43 ± 0.23
	<i>Stenonema femoratum</i>	BM, SL, TL	-16.03 ± 0.98 (10)	0.99 ± 0.01
	<i>Physidae</i>	BM	-11.79 ± 1.28 (4)	0.96 ± 0.04
	<i>Planorbidae</i>	SL	-14.71 ± 0.48 (13)	1.00 ± 0.00
Collectors	<i>Orconectes propinquus</i>	BM	-14.39 ± 0.28 (6)	0.81 ± 0.07
	<i>Orconectes rusticus</i>	SL, TL	-19.0 ± 70.36 (12)	0.87 ± 0.04
	<i>Caenis</i>	BM, TL	-20.77 ± 1.44(3)	0.46 ± 0.29
	<i>Eurylophella</i>	BM	-13.82 ± 1.56 (2)	0.75 ± 0.25
	<i>Hexagenia</i>	BM, SL, TL	-19.72 ± 1.07 (21)	0.52 ± 0.08
	<i>Caecidotea</i>	CL, SL	-20.32 ± 0.45 (5)	0.89 ± 0.10
	<i>Oligochaeta</i>	All Lakes	-20.92 ± 1.04 (20)	0.70 ± 0.08
	<i>Limnephilus</i>	LRL, SL	-27.26 ± 0.53 (4)	0.56 ± 0.26
	<i>Oecetis</i>	BM, SL	-18.31 ± 2.32 (5)	0.52 ± 0.18
	<i>Turbellaria</i>	BM	-14.79 ± 0.07 (2)	0.61 ± 0.04
Predators	<i>Gomphidae</i>	BM, CL, SL, TL	-21.33 ± 0.88 (20)	0.52 ± 0.07
	<i>Hagenius brevistylus</i>	SL	-17.69 ± 0.75 (2)	0.91 ± 0.06
	<i>Libellulidae/Corduliidae</i>	LRL, SL	-25.06 ± 2.30 (6)	0.80 ± 0.16
	<i>Macromia</i>	BM, SL	-17.50 ± 1.33 (4)	0.61 ± 0.12
	<i>Tanypodinae</i>	BM, SL, TL	-20.85 ± 1.55 (13)	0.58 ± 0.11
	<i>Tabanidae</i>	CL	-19.57 ± 0.22 (2)	1.00 ± 0.00
	<i>Hirudinea</i>	LRL	-26.74 ± 0.33 (3)	1.00 ± 0.00
	<i>Sialis</i>	BM, CL, SL,	-21.64 ± 0.86 (11)	0.57 ± 0.10
	<i>Coenagrionidae</i>	SL	-17.71 ± 0.81 (3)	0.67 ± 0.18
Filterers	<i>Unionidae</i>	SL, TL	-29.66 ± 0.46 (14)	0.03 ± 0.02
	Zooplankton	All Lakes	-26.92 ± 2.22 (36)	0.01 ± 0.01

BM, Big Muskellunge Lake; CL, Crystal Lake; LRL, Little Rock Lake; SL, Sparkling Lake; TL, Trout Lake.

classified amphipods and gastropods as grazers. Some Ephemeropterans (Choroterpes and Heptageniidae) were also categorised as grazers based on published gut content studies of these taxa in Wisconsin (Shapas & Hilsenhoff, 1976). Snails were the most abundant grazer in three of the lakes, but were uncommon in Little Rock Lake and Crystal Lake, probably due to low calcium

and conductivity (Lodge *et al.*, 1987). In Little Rock Lake, the benthic invertebrate community was dominated by limnephilid caddisflies, which have many diatoms in their guts (Shapas & Hilsenhoff, 1976) and exert strong top-down control on periphyton productivity (Y. Vadeboncoeur, unpubl. data). We classified limnephilid caddisflies as grazers, although they are typically treated as

shredders. Collectors included oligochaetes, crayfish, chironomids and some caddisflies and mayflies (Table 2). Predators included odonates, megaloptera, tanytopid chironomids and leeches.

Little Rock Lake was sampled at a single transect twice in 2005. The remaining lakes were each sampled at least twice in a single summer at a minimum of two transects. We analysed over 700 benthic invertebrate samples for isotope signatures. For each collection depth, we averaged the $\delta^{13}\text{C}$ data from the different transects to yield a depth-specific $\delta^{13}\text{C}$ for each combination of lake, date and taxon. Averaging within depths across transects reduced the number of observations to 409.

Periphyton was collected at 0.5 m and also between 1- and 10-m depths at 1-m intervals at two to four transects in each lake. A scuba diver used a modified 20-cc syringe as a plunge corer to collect intact sediment cores. For isotope analysis, we retained the surface algal layer of each core (c. 5 mm), which we identified visually as the photosynthetically active layer based on an obvious green biofilm. The study lakes are soft water, low alkalinity lakes (Table 1) without visible carbonate accumulation on benthic surfaces. We tested for the presence of carbonate in periphyton by adding drops of 1 N HCl to wet samples. This did not yield CO_2 , and therefore, we did not fume or acidify periphyton samples. Periphyton samples were immediately frozen, then lyophilised (VirTis[®] Model 6K, Gardiner, NY, USA) for up to 48 h and stored in the freezer.

Stable isotope analysis was conducted at the Stable Isotope Facility at UC-Davis on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). Replicates were run on 8% per cent of the samples to determine analytical error.

Benthic primary productivity

Between 2005 and 2007, we measured benthic algal primary productivity three to seven times during the summer at three to four depths in the littoral zone of each study lake. Scuba divers used sealable clear acrylic cores to collect intact sediment cores (Vadeboncoeur *et al.*, 2006; Vander Zanden *et al.*, 2006). We used Winkler titrations to measure the change in oxygen within chambers relative to initial water column oxygen concentrations (Carignan, Blais & Vis, 1998). At each depth, a scuba diver collected intact sediment cores (5.2 cm diameter, c. 10 cm of sediment plus 15 cm of overlying water) in clear ($N = 6$) and opaque ($N = 2$) acrylic tubes.

Cores were sealed at the bottom with a nylon plug and at the top with a clear or opaque plastic lid. We covered the light cores with up to four layers of neutral density shade cloth to create a light gradient. We incubated cores *in situ* for 2 h at midday and generated photosynthesis–irradiance (P-I) curves for each depth (Jassby & Platt, 1976). We calculated average light-saturated productivity (P_{max}) and the light intensity at onset of saturation (I_k) for each date and depth. We used these field values to estimate average seasonal P_{max} and I_k as a continuous function of depth using the Loess function in the statistical package R. We measured light with a Li-Cor underwater sensor at 1-m depth intervals every 2 weeks throughout the summer and calculated an average summer light attenuation coefficient (k_d). Total daily irradiance was measured at a nearby airport. We calculated midday productivity rates using average summer noon time surface irradiance, k_d , and the modelled change in P_{max} and I_k at 1-m depth intervals (Vadeboncoeur *et al.*, 2008). This approach accounts for changes in light with depth and yields realised productivity rates at noon ($\text{mg C m}^{-2} \text{ h}^{-1}$) at each depth. The use of midday productivity, as opposed to daily productivity, does not affect comparisons among depths, and we prefer this approach because it facilitates among-lake comparisons of productivity rates across latitudes and seasons.

Phytoplankton productivity was estimated using contemporaneous North Temperate Lakes Long-Term Ecological Research (NTL-LTER) data for three of the lakes (<http://lter.limnology.wisc.edu>) and our own comparable measurements in Little Rock Lake and Big Muskegon Lake. We estimated total autotrophic production (phytoplankton + periphyton) on the sediments and in the water column above each 1-m depth interval. Using phytoplankton PI curves, irradiance at noon and k_d , we estimated phytoplankton productivity at 0.5-m depth intervals. We summed productivity over the water column to calculate phytoplankton productivity m^{-2} and added area-specific periphyton productivity (described above) to obtain total integrated production at a given depth.

Data analysis

We used ANOVA to test whether the primary consumers that feed on periphyton (grazers and collectors) had significantly different $\delta^{13}\text{C}$ than filter feeders. For this analysis only, we averaged values from all grazers and collectors collected between 0 and 8 m, creating an average value for each functional feeding group for each

sample date. This made our spatially explicit zoobenthic samples comparable to the composite vertical epilimnion tows for zooplankton, although it substantially reduced the sample size for zoobenthos. We used a Dunnett's one-sided comparison to test whether the $\delta^{13}\text{C}$ of each of the benthic primary consumers was significantly greater than the zooplankton $\delta^{13}\text{C}$ in each lake.

After testing for differences among zoobenthic and zooplanktonic primary consumers, we returned to the spatially discrete data and calculated the average $\delta^{13}\text{C}$ of each benthic functional feeding group. A mean for each depth \times date combination was calculated by averaging the $\delta^{13}\text{C}$ values for all of the taxa that contributed to the functional feeding group. Analysis of covariance (ANCOVA; SAS version 9.2, PROC GLM, SAS Institute Inc., Cary, NC, USA) tested for significant effects of depth and functional feeding group on the $\delta^{13}\text{C}$ of benthic consumers. ANCOVA was also used to test the effect of benthic primary productivity and lake on the $\delta^{13}\text{C}$ of periphyton and $\delta^{13}\text{C}$ benthic grazers.

To determine reliance on benthic algal carbon for each taxon, we developed two end-member mixing models for each depth. Consumer reliance on benthic algal carbon (B_{FRAC}) was calculated for each taxon at each depth using the following equation:

$$B_{\text{FRAC}} = \frac{\text{Pel}_{\text{END}} - \text{Con}_{\delta^{13}\text{C}}}{\text{Pel}_{\text{END}} - \text{Ben}_{\text{END}}} \quad (1)$$

where Pel_{END} is the mean planktonic $\delta^{13}\text{C}$ end-member (zooplankton), $\text{Con}_{\delta^{13}\text{C}}$ is the depth-specific consumer $\delta^{13}\text{C}$, and Ben_{END} is the depth-specific benthic $\delta^{13}\text{C}$ end-member. We calculated B_{FRAC} using two different benthic end-members – known grazers on periphyton or the periphyton biofilm itself. For calculations that used grazers as the benthic algal end-member, we used a carbon trophic fractionation of 0.4‰ for each increase in trophic level (Vander Zanden & Rasmussen, 2001; Post, 2002), but did not correct the signature of collectors because they are on the same trophic level as the grazer end-member. When the periphyton end-member was used, we applied a 0.4‰ trophic fractionation to each trophic step for all groups, assigning grazers and collectors to the second, and predators to the third, trophic level. We also calculated B_{FRAC} , assuming that terrestrial organic carbon, but not phytoplankton was the non-periphyton end-member in Eq. 1. For terrestrial organic carbon, we assumed $\delta^{13}\text{C}$ to be -28‰ , based on data from nearby Crampton Lake (Pace *et al.*, 2007; Cole *et al.*, 2011). Differences in mixing models outputs as a result of using different end-members (i.e. periphyton or grazer $\delta^{13}\text{C}$) were assessed with paired t-tests. This approach yields a range of possible

values for the importance of periphyton to the zoobenthos, but does not quantify the relative importance of terrestrial organic matter versus phytoplankton.

Results

The zoobenthos was consistently enriched in ^{13}C relative to zooplankton. With the exception of collectors in Big Muskellunge Lake, the $\delta^{13}\text{C}$ of benthic primary consumers (grazers and collectors) was significantly ($P < 0.05$) greater than that of zooplankton in all lakes (Table 3). Mean zooplankton $\delta^{13}\text{C}$ ranged from -18.5‰ in Big Muskellunge to -32‰ in Little Rock Lake (Fig. 1). Unionid mussels from Trout and Sparkling lakes had similar isotopic values to those of zooplankton (Fig. 1). The $\delta^{13}\text{C}$ of mussels did not decline with depth but other zoobenthic FFG, especially collectors and predators, became more depleted with depth (Fig. 1; Table 4). Benthic grazers had the most positive $\delta^{13}\text{C}$, which is consistent with these taxa selectively consuming benthic algae. Amphipods and snails had the most positive $\delta^{13}\text{C}$ among grazers at any depth (Table 2). There were significant effects of both depth and functional feeding group on the $\delta^{13}\text{C}$ of the zoobenthos (Table 4).

The contribution of benthic algae to total autotrophic production decreased with depth (Fig. 2). Phytoplankton and benthic algae made approximately equal contributions to the total areal primary production between 3 and 7 m depths, depending on the lake.

For Sparkling, Big Muskellunge and Trout lakes, there was a significant positive relationship between areal benthic primary productivity and periphyton $\delta^{13}\text{C}$ (Fig. 3a; ANCOVA, $F_{4,34} = 52.04$, $P < 0.0001$), and areal benthic primary productivity and grazer $\delta^{13}\text{C}$ (Fig. 3b; ANCOVA, $F_{3,18} = 33.75$, $P < 0.0001$).

Table 3 ANOVA comparing average epilimnetic (0–8 m) $\delta^{13}\text{C}$ of primary consumer functional feeding groups (FFG): filter feeding zooplankton, benthic grazers and benthic collectors. A Dunnett's one-sided comparison was used to test whether the benthic primary consumers had a higher $\delta^{13}\text{C}$ than zooplankton

Lake	Factor	d.f.	F	P	Dunnett's one-tailed test	
					Grazers	Collectors
Little Rock	FFG	2,6	13.27	0.0063	*	*
Crystal	FFG	2,6	28.25	0.0009	*	*
Sparkling	FFG	2,14	28.33	0.001	*	*
Big Muskellunge	FFG	2,6	4.82	0.056	*	NS
Trout	FFG	2,10	19.05	0.004	*	*

*Dunnett's significance: $P < 0.05$.

NS, not significant.

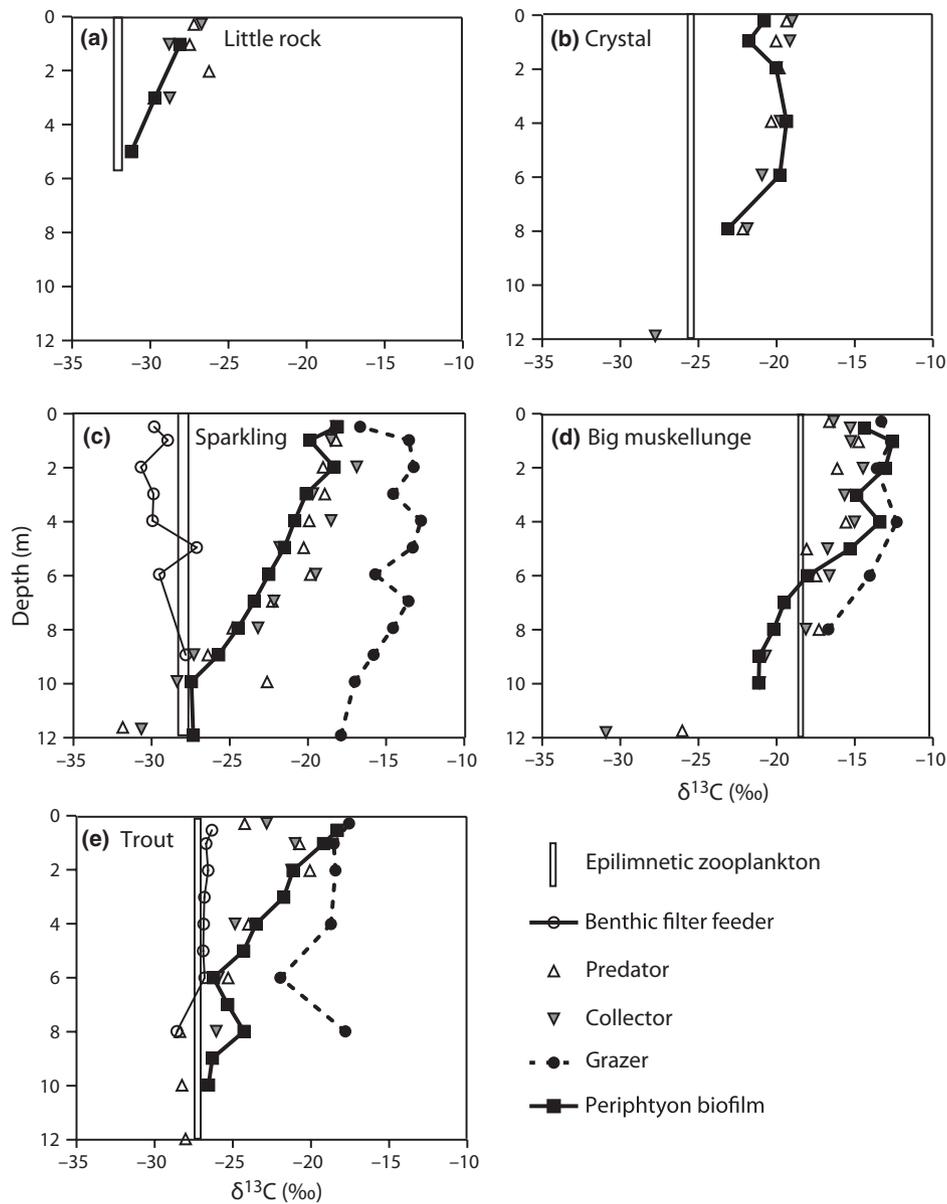


Fig. 1 Stable carbon isotope values of benthic invertebrate functional feeding groups and sediment biofilm as a function of depth. Vertical bars indicate zooplankton $\delta^{13}\text{C}$, which is representative of carbon derived from phytoplankton and/or terrestrial carbon. The width of the bar indicates 1 standard error. Lakes are ordered from smallest (Little Rock Lake) to largest (Trout Lake).

Grazers were enriched in ^{13}C relative to bulk periphyton biofilm, with an offset of 0–10‰ (Fig. 4a). The offset was greatest for Sparkling Lake, which also had the thickest biofilm. The $\delta^{13}\text{C}$ of collectors was strongly correlated with that of periphyton with a near 1 : 1 relationship (Fig. 4b; $F_{3,20} = 25.83$, $P < 0.0001$, $R^2 = 0.88$). Predator $\delta^{13}\text{C}$ was similarly correlated with periphyton $\delta^{13}\text{C}$ (Fig. 4c; $F_{3,20} = 28.02$, $P < 0.0001$, $R^2 = 0.78$).

Use of either periphyton or grazers as the benthic end-member gave similar estimates of collector and predator B_{FRAC} in Big Muskellunge Lake (Table 5). In

contrast, in Trout and Sparkling lakes, the B_{FRAC} for collectors and predators was significantly higher using the periphyton end-member compared with the grazer end-member (t -test, $t_{61} = -3.6282$, $P = 0.001$). Using the periphyton end-member, mean B_{FRAC} was 94 and 75% in Crystal and Little Rock lakes, respectively. When we substituted the terrestrial $\delta^{13}\text{C}$ (–28‰) for the phytoplankton end-member, reliance of zoobenthos on periphyton increased relative to the mixing model based on zooplankton in Trout and Big Muskellunge lakes, but not Sparkling Lake (Table 5).

Table 4 General linear model analysis of the effects of depth (z , continuous) and functional feeding group (FFG, categorical) on $\delta^{13}\text{C}$ of zoobenthic grazers, collectors and predators. Statistical tests are presented for Type I Sum of Squares

Lake	Source	d.f.	F	P
Little Rock	z	1	5.28	0.04
	FFG	2	0.27	0.77
Crystal	z	1	62.19	0.0001
	FFG	2	3.92	0.04
Sparkling	z	1	26.32	0.0001
	FFG	2	48.53	0.0001
Big Muskellunge	z	1	31.33	0.0001
	FFG	2	7.90	0.0012
Trout	z	1	1.51	0.2285
	FFG	2	14.01	0.001

Discussion

Two strong patterns emerge from the spatially detailed analysis of zoobenthic isotopic signatures. First, the littoral zoobenthos is energetically dependent on benthic algal production. Second, the zoobenthos partitions resources in consistent ways, both within and among lakes. In spite of the differences in community composition among our study lakes, the information derived from stable isotope mixing models and the functional feeding categories was largely congruent. The $\delta^{13}\text{C}$ of filter feeding mussels reflected the planktonic signal and did not vary with depth. Grazers had the most positive $\delta^{13}\text{C}$ suggesting specialisation on benthic algae, and grazer $\delta^{13}\text{C}$ was correlated with benthic primary productivity. Collector and predator $\delta^{13}\text{C}$ declined with depth and was strongly correlated with the $\delta^{13}\text{C}$ of bulk periphy-

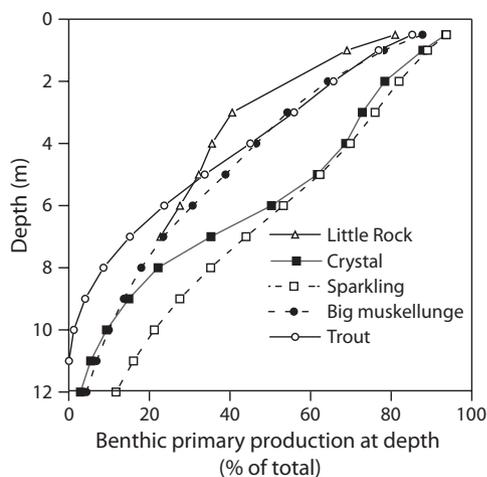


Fig. 2 Contribution of periphyton to total primary production (periphyton + phytoplankton) as a function of lake depth. For each depth, total primary production is the sum of periphyton production (mg m^{-2}) at depth and phytoplankton production integrated over the water column [$\text{PPR}(\text{mg m}^{-3}) \text{ depth}(\text{m})$].

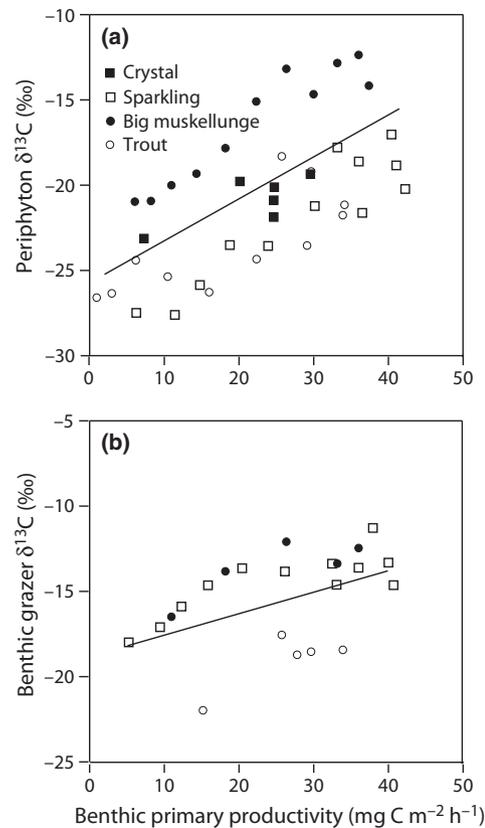


Fig. 3 Relationship between benthic primary productivity and the $\delta^{13}\text{C}$ of periphyton and grazers. Each point represents a different depth. Benthic primary productivity rates are based on the light availability at depth at midday. The line in each graph is derived from an overall regression that does not factor in the effect of lake. (a) Relationship between periphyton $\delta^{13}\text{C}$ and benthic primary productivity (ANCOVA: $F_{4,34} = 52.03$, $P < 0.0001$). Both productivity and lake are significant ($P < 0.0001$), but there is no difference in slope among lakes ($P = 0.081$). (b) Relationship between grazer $\delta^{13}\text{C}$ and benthic primary productivity ($F_{3,18} = 33.75$, $P < 0.0001$). There is no difference in slope among lakes ($P = 0.62$).

ton. These patterns indicate a zoobenthic community whose structure and resource use is closely tied to resource availability, and one in which periphyton is a critical resource.

Filter feeding mussels and zooplankton had the most negative $\delta^{13}\text{C}$, generally similar to that of terrestrial detritus (-28‰). We cannot distinguish between phytoplankton and terrestrial carbon (Schiff *et al.*, 1990; Cole *et al.*, 2002), perhaps with the exception of Big Muskellunge, where zooplankton was surprisingly enriched (-18.5‰).

Grazer $\delta^{13}\text{C}$ was correlated with benthic algal productivity particularly deeper in the littoral zone (Fig. 3b), but grazers were rare or absent from the zoobenthos below 5% of surface light in most lakes. These patterns indicate that epibenthic grazers rely heavily on benthic

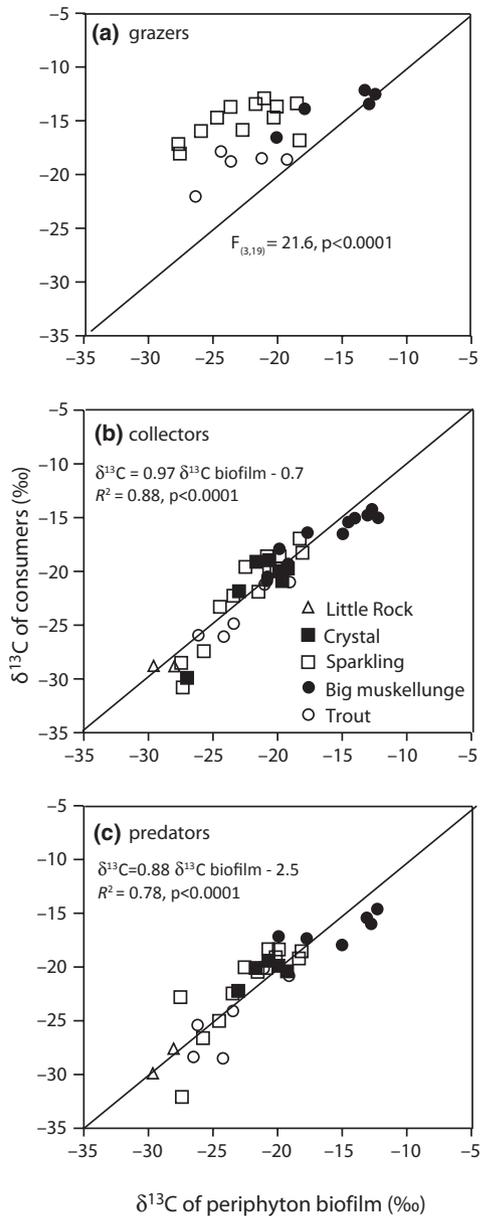


Fig. 4 Relationship between periphyton biofilm $\delta^{13}\text{C}$ and benthic macroinvertebrate $\delta^{13}\text{C}$ from five north-temperate lakes. The equation on each graph is for the overall regression without lake as a factor. The diagonal line is the 1 : 1 relationship. Comparisons are for three functional feeding groups: (a) grazers, (b) collectors and (c) predators.

photosynthesis and may selectively consume or assimilate benthic algae. Grazers were consistently more isotopically enriched than bulk periphyton (Fig. 4a), which may be due to grazers avoiding detritus in the periphyton mat. Alternatively, grazers may consume the most rapidly growing algal cells at the surface of the structurally complex benthic algal biofilm. Controlled mesocosm experiments demonstrate that increased periphyton productivity leads to higher $\delta^{13}\text{C}$ at spatial scales of

Table 5 Mean reliance of collectors on benthic algal carbon ($\pm\text{SE}$) calculated using three different mixing models. Either the grazer $\delta^{13}\text{C}$ or bulk periphyton $\delta^{13}\text{C}$ was used as the benthic end-member, and zooplankton was used as an index of phytoplankton $\delta^{13}\text{C}$. Benthic algal reliance was also calculated using grazer $\delta^{13}\text{C}$ as the benthic end-member and terrestrial carbon $\delta^{13}\text{C}$ as the non-benthic resource. There were not enough grazers sampled in Crystal Lake and Little Rock Lake to complete an analysis with depth-specific grazer end-members

Lake	Fractional reliance of collectors on benthic algae		
	End-members		
	Benthic: Grazer Non-benthic: Zooplankton	Benthic: Periphyton Non-benthic: Zooplankton	Benthic: Grazer Non-benthic: Terrestrial
Little Rock	–	0.75 \pm 0.25	–
Crystal	–	0.95 \pm 0.04	–
Sparkling	0.69 \pm 0.06	0.99 \pm 0.01	0.65 \pm 0.06
Big muskellunge	0.43 \pm 0.10	0.54 \pm 0.11	0.86 \pm 0.02
Trout	0.62 \pm 0.08	0.94 \pm 0.03	0.67 \pm 0.07

centimetres (Hill *et al.*, 2008), while ^{15}N tracer experiments have demonstrated that caddisflies, mayflies and snails selectively graze on the rapidly growing cells at the surface of the periphyton mat (Rezanka & Hershey, 2003).

Collectors were depleted in ^{13}C relative to grazers, demonstrating resource partitioning despite the superficial appearance of homogeneity of the sediment habitat. Mixing models revealed substantial use of benthic algal carbon by collectors, ranging from 43% to nearly 100% (Table 5). Collector $\delta^{13}\text{C}$ was nearly identical to bulk periphyton (Fig. 4b). Collectors may be non-selective, ingesting and assimilating terrestrial organic matter, phytoplankton detritus and benthic algae. In Crystal Lake, periphyton was our only benthic end-member because we analysed only a few grazers for ^{13}C . The reliance of collectors on periphyton reflects the limited resource pool available to consumers in Crystal Lake (Fig. 4). Predator $\delta^{13}\text{C}$ was slightly more negative than that of collectors deeper in the lakes. Direct predation on planktonic organisms would lead to this pattern. Odonates and megalopterans were the dominant invertebrate predators in the study lakes, and the former are known to couple littoral and planktonic food chains through consumption of zooplankton (Burks, Jeppesen & Lodge, 2001).

One explanation for the decrease in bulk periphyton $\delta^{13}\text{C}$ with depth (Fig. 1) is that particulate detritus from the terrestrial ecosystem is a substantial component of the organic matter in sediments and that sediment

focussing moved this material downslope. The organic content of sediments tended to increase with depth in all lakes (data not shown), but only the sediments of Little Rock Lake were composed substantially of vascular plant detritus. Given the extremely sparse macrophyte cover in Little Rock Lake, vascular plant material was probably of terrestrial origin. Amorphous aggregates derived from terrestrial carbon can be incorporated into lake sediments through flocculation of dissolved organic carbon (DOC) (Brett *et al.*, 2012), but the relatively low DOC concentrations in the study lakes (Table 1) argue against flocculation as a dominant flux of organic carbon to the sediments (Lick, Lick & Ziegler, 1992; von Wachenfeldt & Tranvik, 2008; von Wachenfeldt *et al.*, 2008; Brett *et al.*, 2012). Although we did not attempt to assess quantitatively the origin of sediment organic matter, it was certainly composed of both autochthonous and allochthonous organic carbon and we cannot rule out sediment focussing of terrestrial detritus as a driver of the decrease in periphyton $\delta^{13}\text{C}$ with depth.

Decreases in the $\delta^{13}\text{C}$ of benthic consumers with depth similar to those seen in this study have previously been attributed to the increasing importance of phytoplankton detritus in the diet of zoobenthos as a function of depth (Chandra *et al.*, 2005; Sierszen *et al.*, 2006). Superficially, our data appear to be consistent with this explanation, especially given the concordance between increases in total water column phytoplankton productivity with depth and the monotonic decline in biofilm $\delta^{13}\text{C}$ in Big Muskellunge and Trout lakes. However, several well-established processes argue against accepting that there are large phytoplankton contributions to sediment organic matter. Zooplankton can graze 2–21% of the phytoplankton biomass per day (Cyr & Pace, 1992), and labile phytoplankton carbon is rapidly remineralised in the water column (Kalff, 2001; Wetzel, 2001). Deposition rates of phytoplankton detritus are low (0.5–20% per day) as a result of grazing by zooplankton and bacterial mineralisation (Kalff, 2001). Thus, the flux of detrital phytoplankton to the sediments is a small fraction of the phytoplankton production (Fig. 2), such that phytoplankton detritus cannot account for the convergence of the periphyton and filter feeder $\delta^{13}\text{C}$ observed in the metalimnion of the lakes. A tracer addition in a nearby lake supports this perspective: there was little or no influence of experimentally enriched phytoplankton on sediment periphyton $\delta^{13}\text{C}$ (Solomon *et al.*, 2008). Finally, our non-quantitative microscopic examination of the periphyton revealed sediment algal communities composed of benthic pennate diatoms,

attached filamentous algae and benthic filamentous cyanobacteria. Planktonic algae were rarely observed.

Ascribing the changes in periphyton $\delta^{13}\text{C}$ with depth to either settling phytoplankton or terrestrial carbon is confounded by the potential for endogenous processes within the benthic algal community to generate the observed declines in periphyton $\delta^{13}\text{C}$. The use of $\delta^{13}\text{C}$ as an index of the reliance of consumers on periphyton depends on the lower, and more variable, discrimination against $^{13}\text{CO}_2$ by algae in periphyton relative to phytoplankton and terrestrial plants (Rounick, Winterbourn & Lyon, 1982; Hecky & Hesslein, 1995). Periphyton fractionation is negatively correlated with light availability and growth rate, and the depth-specific trends in periphyton $\delta^{13}\text{C}$ may reflect changes in periphyton productivity and thickness (Finlay, 2004; Hill & Middleton, 2006; Hill *et al.*, 2008). Periphyton $\delta^{13}\text{C}$ was positively related to periphyton productivity (Fig. 3a), and this can be explained by the effect of primary productivity rates on depletion of CO_2 in both the intracellular environment and the periphyton matrix (Hill *et al.*, 2008). High demand for inorganic carbon leads to less discrimination against ^{13}C at high values of light and productivity (Figs 1 & 3). The consistency of the relationship between benthic primary productivity and periphyton $\delta^{13}\text{C}$ across lakes (Fig. 3a) indicates a strong influence of benthic primary productivity on both periphyton and grazer $\delta^{13}\text{C}$.

Variation in benthic algal $\delta^{13}\text{C}$ might also be generated by gradients of light, pH and respired CO_2 at micrometre scales within the periphyton (Finlay, 2004). Light penetration into periphyton nears zero within 0.5–1 cm of the sediment surface (Kuhl & Jorgensen, 1994). The periphyton–sediment complex is an integration of highly productive algae growing at the surface, underlain by successively less productive layers of algae (Dodds, Biggs & Lowe, 1999; Martinez-Alonso *et al.*, 2004). This variation in productivity may produce a gradient of decreasing $\delta^{13}\text{C}$ between the surface and the deep layers of an algal biofilm. Bulk periphyton $\delta^{13}\text{C}$ was more negative than the $\delta^{13}\text{C}$ of grazers (Fig. 4a). Grazers may be selectively feeding on benthic algae and avoiding terrestrial detritus, or they may be specialising on the most superficial, and most productive, layer of a structured periphyton community.

We cannot distinguish unequivocally between changes in biofilm $\delta^{13}\text{C}$ with depth that are generated by unquantified contributions of phytoplankton or terrestrial carbon to periphyton and those that are generated by productivity gradients in periphyton with depth. Reliance on benthic algae by the zoobenthos is between 43 and 100%, the extreme estimates based on using epiben-

thic grazers or bulk periphyton, respectively, as benthic algal end-members. Using terrestrial carbon, rather than phytoplankton, as an end-member increases the likely importance of benthic algae to zoobenthos diets (Table 5). It should be noted that the lower estimates are conservative because none of our estimates are corrected for depth-specific production of the zoobenthos. Maximum zoobenthic production occurs at 2 m (Butkas *et al.*, 2011), where B_{FRAC} based on the grazer end-member is also maximal (Fig. 1).

Stream ecologists have used stable isotopes of nitrogen and carbon to demonstrate conclusively that grazing macroinvertebrates are energetically dependent upon benthic algal carbon across a wide range of terrestrial organic carbon loading and stream orders (Finlay, 2001, 2004; Rasmussen, 2010; Kobayashi *et al.*, 2011). Furthermore, collectors and invertebrate predators are also highly reliant on benthic algal carbon in streams, despite high terrestrial carbon inputs (Finlay, 2001; Lau, Leung & Dudgeon, 2009; Rasmussen, 2010; Kobayashi *et al.*, 2011). Many of these studies used longitudinal variation in periphyton $\delta^{13}C$ generated by variation in benthic algal productivity to demonstrate that reliance on benthic algal carbon occurs across a relatively invariant terrestrial detritus signal (Rasmussen, Trudeau & Morinville, 2009; Rasmussen, 2010). Our results are very similar to these stream studies. The variation in the isotopic signatures of the littoral zoobenthos has often been viewed as an impediment to delineating resource use in littoral food webs, but our data show remarkable consistency in resource use among benthic invertebrates when variation in primary producer isotopic signals is accounted for via high-resolution spatial sampling. Incorporating spatial variation in resource isotopic signatures into study design greatly improves our ability to establish patterns of resource use (Karube *et al.*, 2010).

The soft sediments of lakes provide a spatially variable and structured resource environment for the zoobenthos. There is a strong need for an empirical evaluation of the effects of productivity and pH gradients on isotopic profiles within sediment periphyton (Hill & Middleton, 2006). Epibenthic grazers, such as snails, and infaunal collectors, such as chironomids, may be exploiting completely different sources of carbon (e.g. benthic algae versus phytoplankton or terrestrial detritus). Alternatively, these two FFG may be spatially partitioning a heterogeneous benthic algal resource. Consumer reliance on benthic algal carbon will be underestimated if gradients in primary productivity with depth in the periphyton produce spatial gradients

of isotope enrichment (Finlay, 2004; Hill & Middleton, 2006).

Benthic invertebrates are a large component of fish diets and a major pathway for incorporation of littoral carbon into lake food webs (Vander Zanden & Vadeboncoeur, 2002; Weidel *et al.*, 2008). All the non-filter feeding zoobenthos in these north-temperate lakes relies heavily on carbon fixed by benthic algae. There is growing evidence that reductions in light can negatively impact zoobenthic and fish production by reducing periphyton productivity (Karlsson *et al.*, 2009; Premke *et al.*, 2010). This may be due to the high food quality of microalgae relative to terrestrial detritus (Brett *et al.*, 2009; Francis *et al.*, 2011) or the efficiency with which periphyton carbon flows through aquatic food webs (Vander Zanden *et al.*, 2006). Changing the availability of periphyton due to eutrophication and other impacts on the littoral zone will alter zoobenthic production, and those changes have the potential to propagate upward through lake food webs.

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