

^{13}C dynamics in benthic algae: Effects of light, phosphorus, and biomass development

W. R. Hill¹ and S. E. Fanta

Illinois Natural History Survey, 1816 S. Oak Street, Champaign, Illinois 61820

B. J. Roberts²

Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831

Abstract

We performed three experiments in indoor streams and one experiment in a natural stream to investigate the effects of growth factors on $\delta^{13}\text{C}$ levels in benthic microalgae. In the indoor streams, algae grown under conditions of high light and high phosphorus had $\delta^{13}\text{C}$ values that were 16‰ higher than those in algae grown under conditions of low light and low phosphorus. Light effects were much stronger than phosphorus effects. The effects of both factors increased in strength as algal biomass accrued, and by the end of the experiments, algal $\delta^{13}\text{C}$ and biomass were highly correlated. In the natural stream, algae exposed to direct sunlight were enriched 15‰ over shaded algae, corroborating the strong effect of light in the indoor streams. Growth factors such as light and nutrients probably reduce discrimination against ^{13}C (raising $\delta^{13}\text{C}$ values) in benthic microalgae by causing CO_2 depletion both within individual cells and within the assemblage matrix. However, because the most marked fractionation occurred in older and thicker assemblages, CO_2 depletion within the assemblage matrix appeared to be more important than depletion within individual cells. In the absence of carbon-concentrating mechanisms, elevated $\delta^{13}\text{C}$ suggests that inorganic carbon may limit the growth of benthic algae. The extensive range of $\delta^{13}\text{C}$ values (−14‰ to −36‰) created by light and nutrient manipulations in this study easily encompassed the mean $\delta^{13}\text{C}$ values of both C_3 and C_4 terrestrial plants, indicating the challenge aquatic ecologists face in identifying carbon sources for higher trophic levels when light and nutrient conditions vary.

Factors influencing the relative abundances of stable carbon isotopes in primary producers are of considerable interest to ecologists because of the widespread use of these isotopes to identify food sources for higher trophic levels. In terrestrial plants, the relative abundance of ^{13}C appears to be determined primarily by the type of carbon fixation, with $\delta^{13}\text{C}$ values of C_3 plants clustering around −28‰ and $\delta^{13}\text{C}$ values of C_4 plants clustering around −14‰ (Rounick and Winterbourn 1986). In aquatic primary producers, the variability in $\delta^{13}\text{C}$ values is quite large, ranging from −10‰ to −50‰. The range of $\delta^{13}\text{C}$ in algae easily encompasses mean values for both C_3 and C_4 plants (e.g., Fry and Sherr 1984; Rounick and Winterbourn 1986; France 1995). This

large variability reflects the great variety of environmental factors that affect carbon isotope dynamics in aquatic ecosystems (Finlay 2004).

Much of the variability in algal $\delta^{13}\text{C}$ has been attributed to the range of concentrations of aqueous CO_2 in natural waters. This is because discrimination against ^{13}C by carboxylating enzymes like Rubisco is strongly affected by the availability of CO_2 . Whereas the concentration of CO_2 available to terrestrial plants from the atmosphere is narrowly constrained, $[\text{CO}_{2(\text{aq})}]$ is not, especially in freshwaters. Aqueous CO_2 concentrations are highly influenced by pH, temperature, photosynthetic carbon uptake, and respiratory processes in aquatic ecosystems and their surrounding catchments (e.g., Jones and Mulholland 1998; Finlay 2004). The relatively slow rate of $\text{CO}_{2(\text{aq})}$ diffusion in water adds to the variability in $\text{CO}_{2(\text{aq})}$ availability to aquatic primary producers: diffusion gradients created by boundary layers in aquatic ecosystems retard the movement of new $\text{CO}_{2(\text{aq})}$ to actively photosynthesizing algal cells. The depth of these layers is strongly affected by turbulence, so that benthic algae in high-flow environments are much more depleted in ^{13}C than benthic algae in low-flow environments (e.g., Finlay et al. 1999; Trudeau and Rasmussen 2003). The sensitivity of algal $\delta^{13}\text{C}$ to $[\text{CO}_{2(\text{aq})}]$ has encouraged attempts to estimate paleo- CO_2 environments from the $\delta^{13}\text{C}$ of ancient sediments (e.g., Popp et al. 1997).

Algal $\delta^{13}\text{C}$ is affected by internal factors as well as by external CO_2 (Fry and Wainright 1991; Rau et al. 1992). Cells that photosynthesize at high rates deplete internal CO_2 , causing a substantial reduction in the inherent −29‰ discrimination against ^{13}C by Rubisco (Goericke et al. 1994). Several investigators have combined the potential

¹ Corresponding author (wrhill@uiuc.edu).

² Present address: Louisiana Universities Marine Consortium, Defelice Center, 8124 Highway 56, Chauvin, Louisiana 70344.

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effects of growth (μ) and $[\text{CO}_{2(\text{aq})}]$, predicting that phytoplankton $\delta^{13}\text{C}$ would be a function of $\mu: [\text{CO}_{2(\text{aq})}]$ (Francois et al. 1993; Goericke et al. 1994; Laws et al. 1995). Using the $\mu: [\text{CO}_{2(\text{aq})}]$ ratio and knowledge of $\text{CO}_{2(\text{aq})}$, Laws et al. (1995) were able to use $\delta^{13}\text{C}$ data to calculate the growth rate of equatorial Pacific phytoplankton.

The effect of growth and factors that affect growth on the $\delta^{13}\text{C}$ of benthic algae, however, is poorly quantified. Most of the research on benthic algae in streams has focused on the effects of water velocity or external carbon concentrations (e.g., Finlay et al. 1999; Trudeau and Rasmussen 2003; Singer et al. 2005). Finlay (2004) did report diminished ^{13}C fractionation by benthic algae in more productive streams but emphasized the external effects of growth on streamwater $[\text{CO}_{2(\text{aq})}]$. In one of the few studies to look at the effect of a growth factor on the stable isotope composition of benthic microalgae, MacLeod and Barton (1998) reported that light caused a detectable, albeit small (2–3‰), increase in the $\delta^{13}\text{C}$ of stream periphyton, and attributed the increase to photosynthetically diminished internal $[\text{CO}_2]$. Chlorophyll standing stock has been positively correlated with $\delta^{13}\text{C}$ in lotic algae (Hill and Middleton 2006; Rasmussen and Trudeau 2007), consistent with predicted growth effects. However, it is unclear whether the cause of the correlation between $\delta^{13}\text{C}$ and chlorophyll was a direct effect of growth or whether it was an indirect effect, mediated through increases in pH and diffusional gradients accompanying a vertical expansion of the algal matrix.

In this study, we examine the effect of growth factors on the stable carbon isotope composition of benthic microalgae. Three experiments were performed in which light and phosphorus were simultaneously manipulated in flow-through indoor streams. Because of the potential effect of biomass accrual on algal $\delta^{13}\text{C}$, we examined the effect of light and phosphorus at three times during algal assemblage development in each experiment. An additional field experiment was performed in a natural stream in which light levels were manipulated with artificial shading. We show dramatic effects of growth-stimulating factors on algal $\delta^{13}\text{C}$, and demonstrate that $\delta^{13}\text{C}$ dynamics are linked to assemblage development.

Methods

Indoor stream experimental design—Phosphorus and light were manipulated in three experiments at the Oak Ridge National Laboratory indoor stream facility. The streams in this facility are 22 m long and 0.3 m wide, U-shaped, and supplied with water from First Creek, a first-order, spring-fed stream. First Creek water is moderately alkaline (pH \approx 8 and alkalinity \approx 1–3 meq L^{-1}) and contains relatively low concentrations of dissolved nutrients (dissolved phosphorus \approx 3–6 $\mu\text{g L}^{-1}$ and dissolved nitrogen \approx 100 $\mu\text{g L}^{-1}$) (Loar 1994). The inflow of First Creek water into each stream was maintained at 0.3 L s^{-1} in this study. Mean water depth in the streams was 2 cm. Illumination was provided by metal halide lamps (400 W) positioned approximately 1 m above the streams; a light

dark period of 14:10 was maintained throughout each experiment with automatic timers. Substrata in the streams consisted of continuous mats of unglazed square ceramic tiles. Individual tiles served as periphyton sampling units; each tile was $2.4 \times 2.4 \times 0.6$ cm. Cobbles from First Creek were placed at the heads of the streams to provide a source of microalgae for the streams, but the unfiltered First Creek water flowing into the streams undoubtedly contained additional microalgal colonists as well. The dates of the three experiments were 21 January 2006–09 February 2006, 11 February 2006–28 February 2006, and 09 March 2006–22 March 2006.

Six different phosphorus levels were applied to six streams in each of the three experiments. Stock solutions of dissolved Na_2HPO_4 were pumped from carboys at the heads of the streams with peristaltic pumps at a rate calculated to achieve specific target concentrations in the streams. Target concentrations of soluble reactive phosphorus (SRP) were 6, 12, 25, 75, 150, and 300 $\mu\text{g L}^{-1}$ in the first two experiments and 6, 12, 25, 50, 75, and 150 $\mu\text{g L}^{-1}$ in the third experiment. The lowest concentration was always that of inflowing First Creek water (zero Na_2HPO_4 in the stock solution), which was estimated at 6 $\mu\text{g L}^{-1}$ SRP. Dissolved NaNO_3 was included in the stock solutions to reduce the potential for nitrogen limitation of algal growth; it was added in an amount calculated to result in a nitrogen concentration \geq 300 $\mu\text{g L}^{-1}$ in the streams.

A gradient of light intensity ranging from photosaturating to highly shaded was established in each stream in all three experiments. There were five treatment levels in the first two experiments, designated as “+,” “0,” “1,” “2,” and “3.” The “+” treatment was created by mounting a 300-W halogen work light close (ca. 40 cm) to the tile substrata, producing irradiances of ca. 375 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The “0” treatment was the irradiance supplied by the overhead lamps, approximately 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The “1,” “2,” and “3” treatments were created by shading 1-m-long sections of the streams with 1, 2, or 3 layers of nylon window screening; these layers created irradiances of approximately 60, 40, and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The third experiment had an additional light treatment level (“4”) created by 4 layers of window screening that created an irradiance of approximately 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. All light treatment sections were located in the lower half of the streams to allow for complete mixing of nutrient stock solutions by the time the flowing water reached these sections. The longitudinal order of light treatments within these downstream sections was randomly assigned in each stream. Each treatment section was approximately 1 m long and was separated from adjacent sections by approximately 1 m.

Indoor stream sampling and analysis—Sampling in each experiment began at the first sign of color on the tiles, 3–4 d after the application of phosphorus and light treatments. Samples of both dissolved nutrients and periphyton were collected at midmorning every 2 d, approximately 3 h after the lights were turned on. Water samples for nutrient analysis were collected at the halfway point in each stream, at the bend in the channel. Samples were filtered through

Table 1. Treatment conditions for the three experiments. SRP=soluble reactive phosphorus; light=photosynthetically active radiation. Each value is the mean±SD of repeated measurements made over time; n=7 or 8 for SRP, n=7 or 8 for light.

	SRP (µg L ⁻¹)		Light (µmol photons m ⁻² s ⁻¹)					
	Target	Actual	+	0	1	2	3	4
Experiment 1								
	6	4±2	399±26	106±3	70±2	33±3	17±2	—
	12	10±1	377±20	113±3	61±3	24±1	18±1	—
	25	24±2	395±24	87±3	60±3	27±2	14±1	—
	75	76±6	398±21	108±3	56±1	42±2	20±1	—
	150	173±10	375±22	94±4	51±1	30±2	22±1	—
	300	329±44	357±32	91±5	50±1	32±1	19±2	—
Experiment 2								
	6	6±2	398±38	112±3	53±2	34±3	20±1	—
	12	12±1	362±46	102±5	56±3	31±2	17±3	—
	25	24±3	343±33	130±5	59±3	30±1	18±3	—
	75	68±2	388±26	87±3	62±4	38±2	23±1	—
	150	178±34	337±35	113±17	57±14	27±2	20±2	—
	300	292±27	374±24	108±2	62±1	43±2	19±3	—
Experiment 3								
	6	6±2	365±30	132±4	60±2	48±2	19±2	9±1
	12	12±2	352±24	91±5	73±5	37±1	20±2	10±1
	25	26±2	377±41	104±3	60±2	36±4	20±1	8±0
	50	52±5	397±17	109±4	67±1	38±2	23±3	11±1
	75	66±10	345±34	96±7	78±2	45±2	24±3	12±1
	150	134±11	387±19	120±4	77±2	46±1	22±3	11±2
Mean			374	106	62	36	20	10

precombusted Whatman GFF filters and frozen at -20°C. SRP was later analyzed with the ascorbic acid-molybdate method (APHA 2005). Periphyton was collected by carefully removing one tile from each light-treatment section in each stream and placing it in an individually marked plastic Petri dish, which was frozen at -85°C. Photosynthetically active radiation was measured with a quantum sensor at the specific location of each tile collected. Periphyton on the tiles was later harvested by brushing the surface of thawed tiles and rinsing the brushed material with deionized water into a slurry that was then filtered onto precombusted and preweighed Whatman GFF filters. The filters were dried at 60°C for at least 24 h before being weighed for the calculation of dry mass.

Filters from three sampling dates in each experiment, representing early, middle, and later developmental stages, were then cut into half or quarter sections to provide an appropriate carbon mass for stable isotope analysis. These sections were fumed with concentrated HCl for 6 h to eliminate carbonates that might have been included in the periphyton (Harris et al. 2001). The filter sections were sent to the University of California-Davis Stable Isotope Facility for carbon stable isotope analysis. Unidentified samples of standard reference material (tomato leaves, National Institute of Standards and Technology Standard Reference Materials 1573a) were submitted along with the experimental samples for quality control purposes.

Tiles were also collected and brushed at the end of the experiments for the microscopic examination of algal assemblage structure. These samples were preserved in Lugol's solution until they were examined at 1,000× magnification with an inverted microscope. At least

500 cells were enumerated in each sample. Diatoms were identified to species with the help of subsamples that were cleared with hot H₂O₂ and mounted in Naphrax.

Water velocity, temperature, pH, and alkalinity were measured during the study to provide experimental context. Water velocity was estimated at the beginning and end of the experiments by timing the downstream movement of NaCl injections. Water velocity estimates ranged from 11 cm s⁻¹ to 13 cm s⁻¹ at the beginning and from 7 cm s⁻¹ to 10 cm s⁻¹ at the end. Temperature was measured daily at the outlet of each stream in each experiment. The range of water temperatures during the experiments was small: 11–13°C, 10–12°C, and 13–15°C for experiments 1, 2, and 3, respectively. Differences between streams were <1°C. A

Table 2. Algal assemblages in the laboratory stream experiments. Values are mean±SE of relative biovolumes (%) of the top 10 contributors to total algal biovolume. The 10 taxa constituted >95% of algal biovolume in each experiment.

	Experiment		
	1	2	3
<i>Melosira varians</i>	31.5±2.6	47.9±2.7	38.5±2.8
<i>Gomphonema truncatum</i>	34.4±2.1	19.4±1.6	36.0±2.6
<i>Fragilaria rumpens</i>	8.3±0.6	10.8±0.6	6.0±0.4
<i>Achnanthydium minutissima</i>	5.2±0.5	4.1±0.4	5.9±0.6
<i>Synedra ulna</i>	0.8±0.3	8.0±0.9	6.2±1.0
<i>Nitzschia</i> spp.	8.5±0.8	1.0±0.2	1.1±0.2
<i>Synedra acus</i>	3.0±0.5	2.6±0.5	0.6±0.1
<i>Cymbella cymbiformis</i>	2.4±0.4	1.0±0.2	1.3±0.2
<i>Stephanocyclus meneghiniana</i>	1.2±0.3	2.0±0.3	0.9±0.2
<i>Gomphonema parvulum</i>	0.9±0.2	1.1±0.1	0.8±0.1

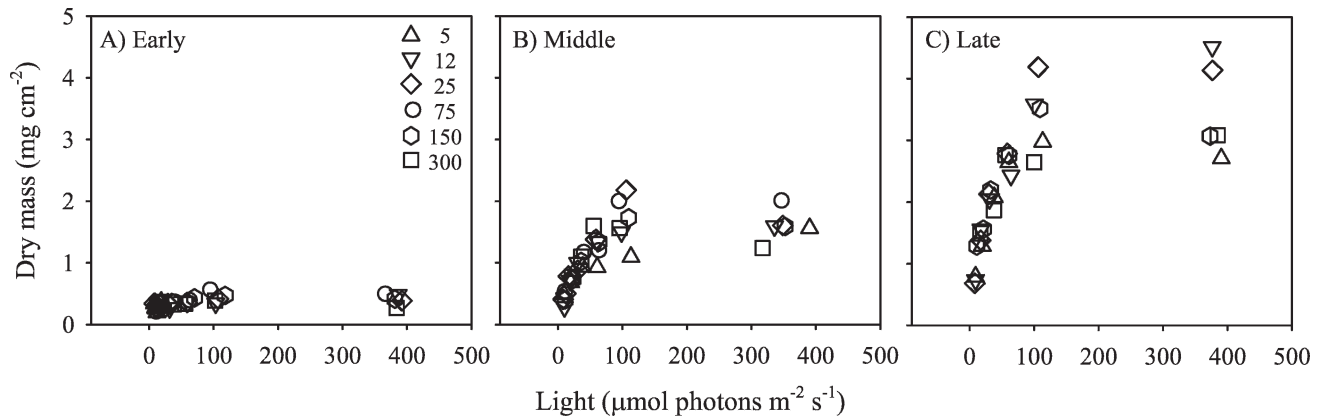


Fig. 1. Algal dry mass vs. light at three stages of assemblage development: (A) early, (B) middle, and (C) late. Data points represent the mean dry mass plotted against the mean light level from three experiments; error bars were omitted for clarity. Symbol legend numbers refer to nominal SRP concentrations ($\mu\text{g L}^{-1}$).

handheld pH meter was used to measure pH daily at the outlet of each stream in the first experiment. The range of pH in this experiment was 7.9–8.4. Measurements of pH were not recorded in following experiments because the meter malfunctioned. Alkalinity was measured in water samples taken at the outlets of the streams at the end of each experiment: mean alkalinity of the six streams was 1.54, 1.46, and 1.64 meq L^{-1} for experiments 1, 2, and 3, respectively.

Indoor stream data analysis—Phosphorus treatments were not replicated in individual experiments because of the limited number of streams. Data from all three experiments were therefore combined for analyses of variance (ANOVAs). A mixed model ANOVA (SAS Proc Mixed) tested the overall effects of phosphorus, light, and the phosphorus \times light interaction on algal biomass and ^{13}C . Stream and the experiment \times phosphorus interaction were specified as random effects in this model. Data from light treatment 4 in the third experiment were excluded from ANOVA and from the calculation of means for specific phosphorus concentrations because this light treatment did not occur in the first two experiments. Data from the 50 $\mu\text{g L}^{-1}$ treatment stream in the third experiment were excluded from both graphical and statistical analyses for the same reason. The mass specific growth rate of the benthic microalgal assemblages was calculated as $\mu = (\ln d_f - \ln d_i)/t$, where d_f and d_i = final and initial dry mass, respectively, and t = days.

Algal ^{13}C vs. light in East Fork Poplar Creek—Algal samples from an in situ shading experiment (Hill and Larsen 2005) were used to examine the effect of light on algal ^{13}C in a natural stream. In this experiment, eight sheets of clear Plexiglas ($60 \times 40 \text{ cm}^2$) were suspended above the water surface at an unshaded site in upper East Fork Poplar Creek, a third-order stream in Oak Ridge, Tennessee (Adams et al. 2002). Four of the sheets were covered with dark screens that reduced light on the underlying stream to approximately 5% of ambient. Clay paving bricks placed under the sheets on the stream bottom served as substrata for the colonization and growth of benthic algae. The bricks were left in the stream for 4 weeks. During this period, light was measured under each experimental unit with an underwater quantum sensor held at brick level. Light measurements were made at approximately midday on 5 separate d; mean irradiances for the shaded and unshaded algal assemblages were 32 and 670 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Mean water velocity, measured just above the bricks with an electromagnetic flowmeter, was 24 cm s^{-1} . Algal biomass on the bricks was harvested at the end of 4 weeks by brushing, and it was prepared for stable carbon isotope analysis as described for the indoor stream experiments. Samples from three of the shaded and three of the unshaded bricks were submitted for analysis. Algal assemblages on the shaded bricks were composed primarily of small diatoms, whereas those on the unshaded bricks were primarily chlorophytes (Hill and Larsen 2005). Mean growth rates of the shaded and unshaded assemblages were 0.08 and

Table 3. F values from the analysis of variance of phosphorus and light effects on algal dry mass and algal ^{13}C .

	df	log (dry mass)			Algal ^{13}C		
		Early	Middle	Late	Early	Middle	Late
Phosphorus	5	0.4	1.2	4.4**	1.7	4.0**	6.8***
Light	4	6.0**	27***	83***	2.9*	23***	92***
Phosphorus \times light	20	1.2	0.8	1.4	1.6	0.6	1.1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

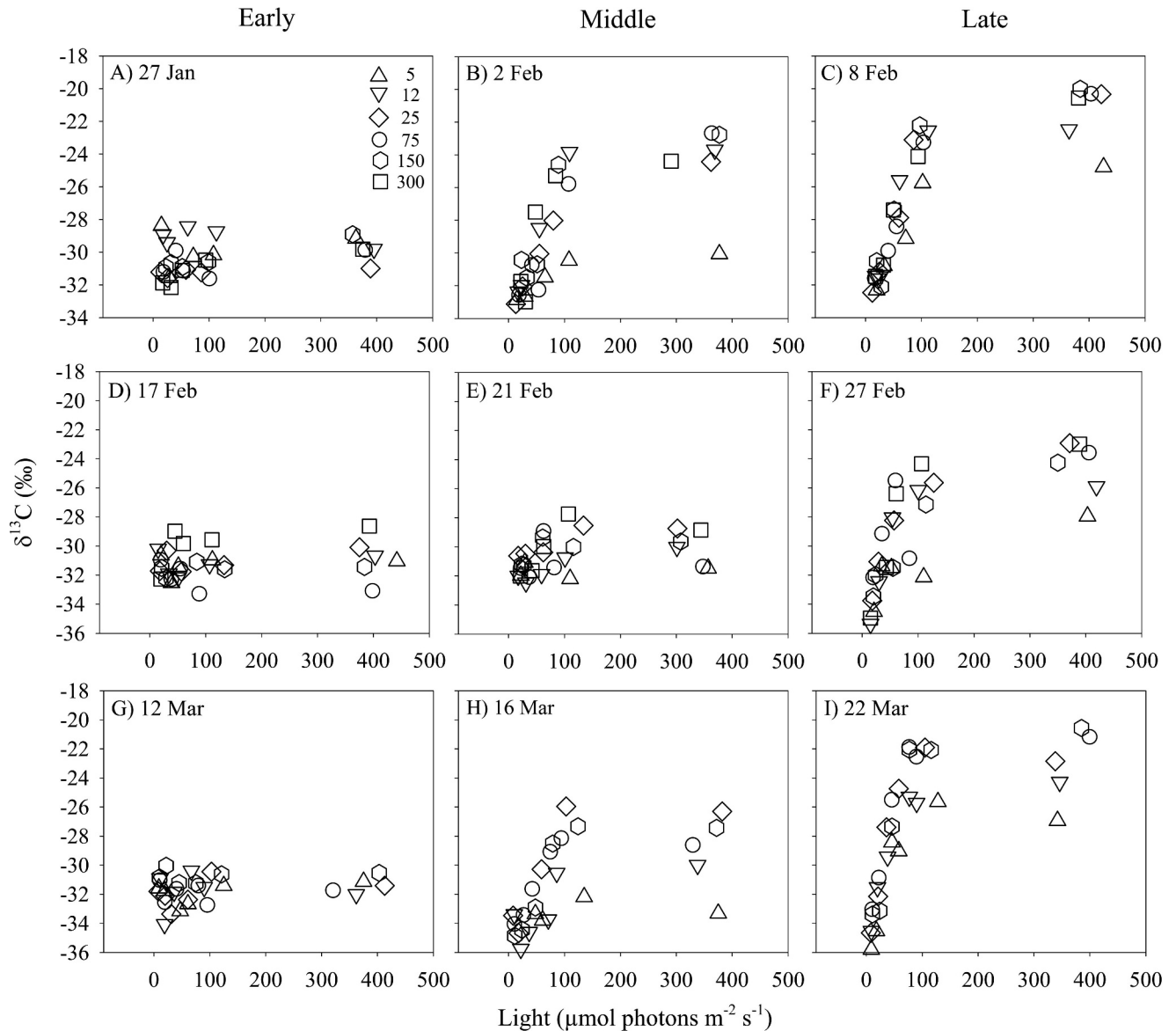


Fig. 2. Algal $\delta^{13}\text{C}$ vs. light at three stages of assemblage development (early, middle, and late). Each panel row represents an individual experiment. Experiment 1 on (A) 27 Jan, (B) 02 Feb, and (C) 08 Feb. Experiment 2 on (D) 17 Feb, (E) 21 Feb, and (F) 27 Feb. Experiment 3 on (G) 12 Mar, (H) 16 Mar, and (I) 22 Mar. Symbols represent different nominal SRP concentrations ($\mu\text{g L}^{-1}$).

0.19 d^{-1} , and mean biomasses of the shaded and unshaded assemblages were 0.55 and $1.22 \text{ mg ash-free dry mass cm}^{-2}$ (Hill and Larsen 2005).

Results

Indoor stream treatments—Strong gradients in both phosphorus and light were maintained in all three experiments. Mean concentrations of SRP measurements made every 2 d during the experiments were close to targeted concentrations, and light measurements (also made every other day) demonstrated the efficacy of the light manipulations (Table 1). Differences in light levels between streams were primarily caused by variability in lamp output. Despite this variability, a consistent experimental gradient of light was effected in each stream.

Indoor stream algal assemblages—Diatoms dominated the algal assemblages that developed in the experimental streams, constituting 99% of algal biovolume. Two large species, *Gomphonema truncatum* and *Melosira varians*, were consistently the major contributors (66–75%) to biovolume at the end of all three experiments (Table 2). Although samples for quantitative analysis were not taken before the ends of the experiments, qualitative microscopic examination of earlier stages of development revealed that the assemblages contained the same suite of diatoms as seen at the ends, but overstory species such as *M. varians* appeared to be less abundant in early stages.

Light and phosphorus effects in indoor streams—Treatment effects on both algal biomass and $\delta^{13}\text{C}$ depended on developmental stage. Light effects on biomass accumula-

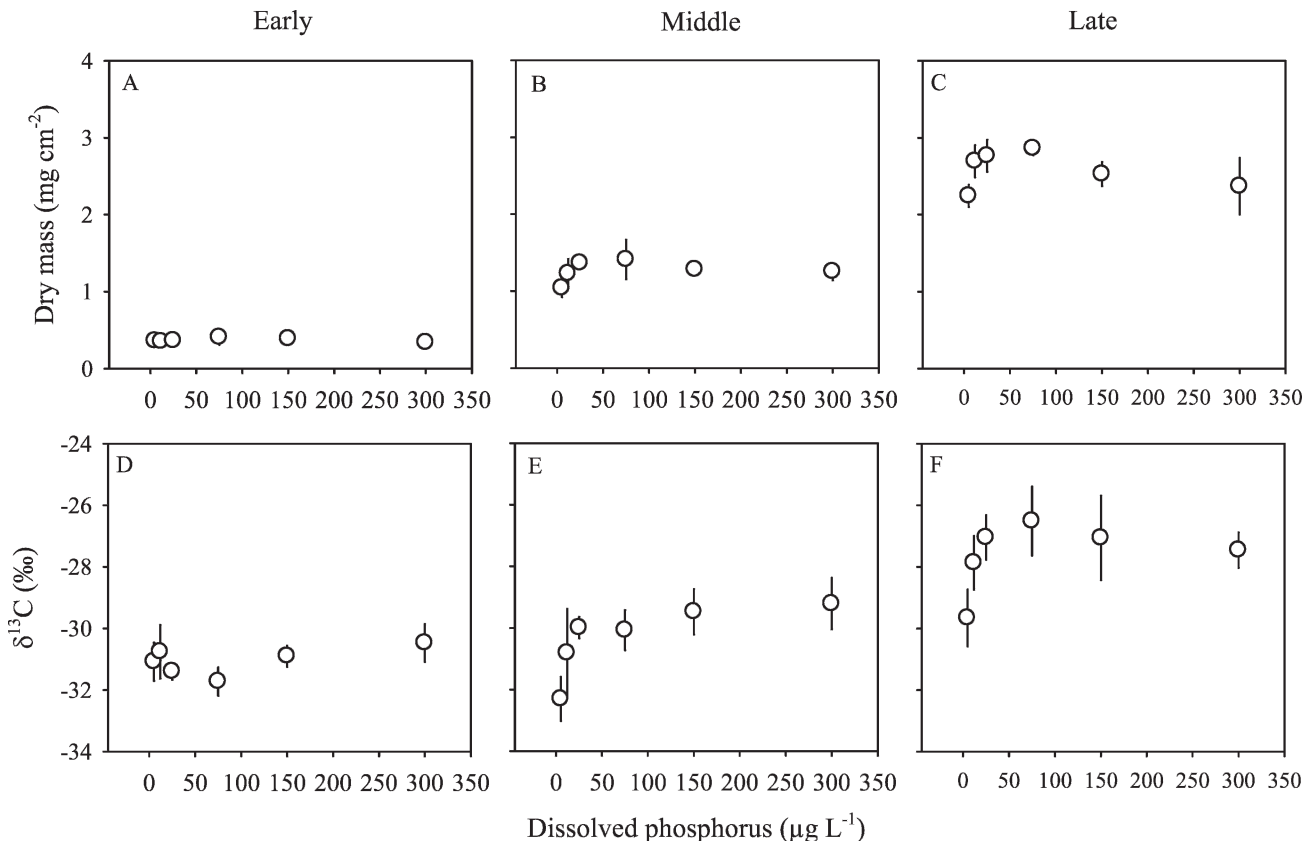


Fig. 3. Algal dry mass and $\delta^{13}\text{C}$ vs. phosphorus concentration at three stages of assemblage development. Dry mass at (A) early, (B) middle, and (C) late stages of development. Algal $\delta^{13}\text{C}$ at (D) early, (E) middle, and (F) late stages of development. Each point represents the mean \pm SE ($n = 3$) of the mean values from three experiments; mean values from each experiment were the average of all light treatments, excluding light treatment 4. Phosphorus concentration is the nominal SRP concentration.

tion were evident in all stages, but they became progressively more distinct as the benthic algal community developed (Fig. 1, Table 3). The relationship between light and biomass accumulation in later stages was hyperbolic, resembling a photosynthesis-irradiance curve. There was no clear relationship between $\delta^{13}\text{C}$ and light in the early stages of any of the three experiments, but a hyperbolic relationship was clearly evident by the later stages in each experiment (Fig. 2). The range of $\delta^{13}\text{C}$ increased dramatically during development, rising from 4‰ in the early stages to 16‰ in later stages. Both the lowest (-36‰) and the highest (-20‰) $\delta^{13}\text{C}$ values were found in later stages. Within any single stream, algal $\delta^{13}\text{C}$ spanned a range of 12‰ in a distance of a few meters or less. Light effects depended to some extent on phosphorus concentration: the highest $\delta^{13}\text{C}$ values occurred in algae growing at high irradiances in streams with $\text{SRP} \geq 25 \mu\text{g L}^{-1}$ (Fig. 2). Algae growing in streams with 5 and $12 \mu\text{g L}^{-1}$ SRP consistently had the lowest $\delta^{13}\text{C}$ values at the higher light levels.

Phosphorus effects on biomass accumulation (averaged over all light treatments) appeared to increase in later stages, though the effect of phosphorus in all stages was relatively small (Fig. 3). Phosphorus effects on biomass were statistically significant only for the later stages of development (Table 3). Phosphorus effects on algal $\delta^{13}\text{C}$

were more pronounced than the effects on biomass, but the effects on $\delta^{13}\text{C}$ were also dependent on developmental stage. Algal $\delta^{13}\text{C}$ appeared to be unaffected by phosphorus in early stages, where the variation in mean $\delta^{13}\text{C}$ values (averaged over all light levels) was limited to 1‰ (Fig. 3, Table 3). By the middle stages of development, a hyperbolic relationship between phosphorus concentration and algal $\delta^{13}\text{C}$ was evident, and the range in $\delta^{13}\text{C}$ increased to 3‰. A similar relationship was apparent in the late stage of development, though the $\delta^{13}\text{C}$ values were 3‰ more positive for any particular SRP concentration. In both middle and late stages, algal $\delta^{13}\text{C}$ increased with increasing phosphorus concentration up to $25 \mu\text{g L}^{-1}$ SRP, where the relationship began to plateau.

Algal $\delta^{13}\text{C}$ vs. growth and biomass in indoor streams—The similarities in the responses of biomass accrual and algal $\delta^{13}\text{C}$ to light and phosphorus manipulations suggested that the growth and biomass would be highly correlated to $\delta^{13}\text{C}$. This was confirmed by plotting $\delta^{13}\text{C}$ of the last developmental stage (third sampling date) vs. growth rate and biomass (Fig. 4). Nonparametric correlation analysis confirmed a highly significant relationship between algal $\delta^{13}\text{C}$ and the two parameters, but the relationship was tighter and the correlation coefficient was stronger for algal $\delta^{13}\text{C}$ and biomass.

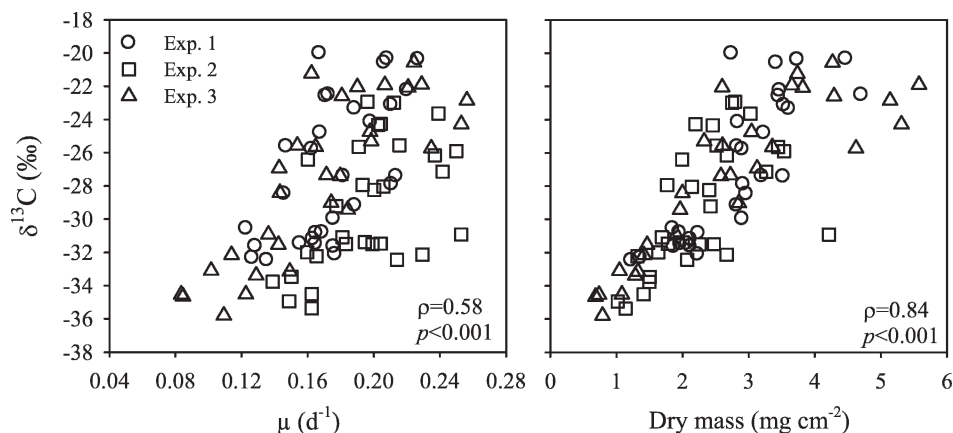


Fig. 4. Algal $\delta^{13}\text{C}$ vs. growth and biomass for the later stages of assemblage development. Spearman's nonparametric correlation coefficients (ρ) are shown.

Algal ¹³C vs. light in East Fork Poplar Creek—Very large differences in $\delta^{13}\text{C}$ were caused by light manipulations in East Fork Poplar Creek, consistent with the results of the laboratory stream experiments. Unshaded algae had $\delta^{13}\text{C}$ values that were as much as 15‰ more positive than those in shaded algae (Fig. 5).

Discussion

This study leaves little doubt that growth factors can have dramatic effects on the carbon stable isotope composition of benthic microalgae. At the end of each of the three indoor stream experiments, the combined effects of light and nutrients produced an overall $\delta^{13}\text{C}$ range of 16‰, and within any single stream, light manipulation was responsible for $\delta^{13}\text{C}$ differences as large as 12‰. These differences were achieved over relatively small spatial scales (as little as 1 m separated low- and high-light treatments) and short time periods (≤ 10 d). Results from the field experiment corroborated the strong effect of light observed in the indoor streams: unshaded, fast-growing algae in East Fork Poplar Creek had $\delta^{13}\text{C}$ values that were 14–15‰ more enriched than those of shaded, slow-growing algae. The range of $\delta^{13}\text{C}$ values produced by the manipulations of light and phosphorus in this study are comparable to those found in phytoplankton studies in which both growth and inorganic carbon concentrations were varied (e.g., Laws et al. 1995).

The effects of light and phosphorus on algal $\delta^{13}\text{C}$ were generally independent of any potential photosynthetic depletion of streamwater dissolved inorganic carbon (DIC). Elevated rates of carbon fixation by light- or nutrient-stimulated algae are known from laboratory and field studies to reduce $[\text{CO}_{2(\text{aq})}]$ available to phytoplankton, causing ^{13}C enrichment (e.g., Fry 1996). Enriched algal $\delta^{13}\text{C}$ values at open, more productive sites in streams have also been attributed to the photosynthetic depletion of $[\text{CO}_{2(\text{aq})}]$ (Finlay 2004), but depletion of $[\text{CO}_{2(\text{aq})}]$ in bulk stream water would have been responsible for little, if any, of the algal $\delta^{13}\text{C}$ enrichment in this study. The full range of light treatments in the indoor streams (which were responsible for most of the algal $\delta^{13}\text{C}$ effects) was randomly

applied within each stream, and in East Fork Poplar Creek, both shaded and unshaded algae experienced the same stream water (each block of treatments was oriented perpendicular to the current).

Light and phosphorus effects on algal $\delta^{13}\text{C}$ did not appear to be caused by large changes in algal assemblage composition. Diatoms dominated assemblages in all the laboratory stream experiments, and the same suite of species occurred in all treatments. Light manipulations did substantially alter the assemblage composition of benthic microalgae in East Fork Poplar Creek, so inherent differences between the $\delta^{13}\text{C}$ signatures of diatoms (shaded treatments) and chlorophytes (unshaded treatments) could have contributed to differences in $\delta^{13}\text{C}$ created by the two treatments. Nonetheless, the similarity in the direction and magnitude of the light effects on algal $\delta^{13}\text{C}$ in the field experiment and in the laboratory experiments are striking and suggest a common physiological mechanism.

The major influence of light and nutrients on algal $\delta^{13}\text{C}$ in this study appeared to be through the stimulation of growth. At the scale of individual cells, high rates of growth deplete intracellular inorganic carbon faster than it can be replenished by diffusion, reducing the ratio of internal carbon to external carbon ($C_i:C_e$) (Laws et al. 1995, 1997). Discrimination against ^{13}C by Rubisco is theoretically proportional to this ratio (Farquhar et al. 1982), so fast-growing algal cells that reduce C_i relative to C_e are expected to be enriched in ^{13}C . Photosynthesis by cells within the three-dimensional matrix of even a modestly thick benthic assemblage, combined with diffusion-limited movement of new inorganic carbon from the water to these cells, is likely to cause the depletion of inorganic carbon in the interstitial water within the assemblage and in the boundary layer outside of the assemblage (Jørgensen et al. 1983; Jones et al. 2000). Because $[\text{CO}_{2(\text{aq})}]$ concentrations are highly sensitive to pH, algal cells photosynthesizing within thicker benthic assemblages are likely to experience a shortage of CO_2 , and therefore have more positive $\delta^{13}\text{C}$ values. Thinner assemblages, with less total photosynthesis and a larger proportion of cells close to the water-algae interface, should have fewer carbon constraints and therefore more

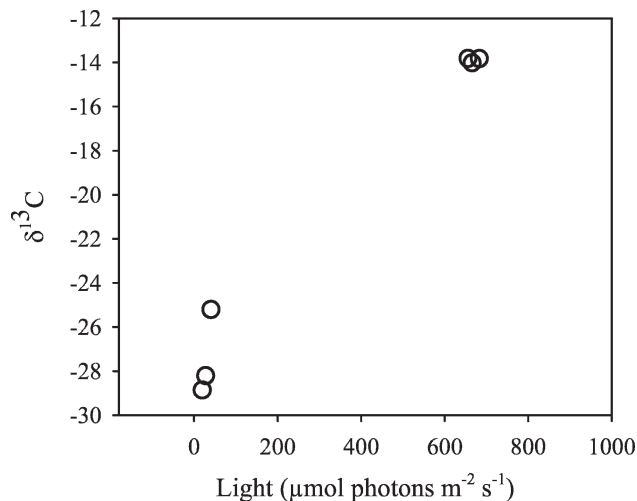


Fig. 5. Algal $\delta^{13}\text{C}$ vs. light in East Fork Poplar Creek. Light levels were the mean of measurements made with an underwater quantum sensor at midday on 5 separate d.

negative $\delta^{13}\text{C}$ (Hill and Middleton 2006). It seems likely that the microhabitat within thicker and faster-growing assemblages compounds the effect of growth factors such as light and nutrients on individual cells. Benthic algal biomass may be a better predictor of $\delta^{13}\text{C}$ than growth per se because it can be related to both growth and the DIC availability to individual cells within the algal matrix. Algal biomass may then serve as the benthic equivalent of the $\mu:\text{CO}_{2(\text{aq})}$ ratio used for phytoplankton.

The strengthening of light and phosphorus effects on algal $\delta^{13}\text{C}$ during assemblage development in the indoor stream experiments was consistent with the hypothesis that assemblage thickness influences $\delta^{13}\text{C}$ dynamics through microhabitat effects. The range of $\delta^{13}\text{C}$ expanded concurrently with increasing biomass, from 4‰ in the early stages of assemblage development to 16‰ in late stages, and maximum $\delta^{13}\text{C}$ values (–20‰) were always found at the end of each experiment. Interestingly, the lowest individual values (–36‰) were found not in early stages, but in middle and late stages (Fig. 2). It is plausible that these low values (at the lowest irradiances) occurred later in the experiments because of the results of fractionation by overall increases in photosynthetic biomass in the streams. As biomass increased and algae became progressively more enriched in the fully illuminated upstream sections, DIC in the water flowing down the indoor streams may have become progressively more depleted in ^{13}C , causing more negative $\delta^{13}\text{C}$ values for highly shaded, slow-growing algae.

There is considerable uncertainty about the specific forms of inorganic carbon utilized by freshwater algae. $\text{CO}_{2(\text{aq})}$ is assumed to be used preferentially when abundant because it diffuses easily through cell membranes and can be fixed without transformation by Rubisco. However, concentrations of $\text{CO}_{2(\text{aq})}$ are generally much lower than those of HCO_3^- in most waters, so the ability to use HCO_3^- is advantageous, particularly in the high pH (= low CO_2) microhabitats occupied by benthic microalgal cells. Direct transport and utilization of HCO_3^- would raise algal $\delta^{13}\text{C}$ because the $\delta^{13}\text{C}$ of HCO_3^- is approximately 0‰

whereas that of CO_2 is approximately –8‰ (Tortell and Morel 2002). Utilization of HCO_3^- could in theory account for part of the ^{13}C enrichment in faster-growing, higher-biomass assemblages (Hill and Middleton 2006), but the direct use of HCO_3^- by algae is not well documented (e.g., Laws et al. 1997) and would only account for part of the 16‰ enrichment we observed. High $\delta^{13}\text{C}$ values do not in themselves demonstrate HCO_3^- use (Morel and Reinfelder 1995).

Elevated $\delta^{13}\text{C}$ values suggest the possibility that shortages of inorganic carbon constrain the growth of benthic algal assemblages, particularly those that experience high irradiances and adequate nutrients. Although CO_2 -limited phytoplankton growth has been suggested (Riebesell et al. 1993), inorganic carbon limitation remains controversial. The existence of carbon-concentrating mechanisms (CCMs) by which cells accumulate relatively high intracellular concentrations of inorganic carbon could theoretically mitigate CO_2 scarcity (e.g., Tortell et al. 2006). CCMs include active transport of CO_2 , active transport of HCO_3^- , and extracellular carbonic anhydrase activity (Colman et al. 2002). It is not known whether any of the species present in our algal assemblages possess CCMs, nor are the costs of possessing and utilizing CCMs known. Few studies have directly tested for inorganic carbon limitation in benthic microalgae, but McIntire and Phinney (1965) did demonstrate increased productivity in stream periphyton when $[\text{CO}_{2(\text{aq})}]$ was experimentally increased, and Fairchild and Sherman (1992) showed that benthic algae in soft-water lakes respond positively to HCO_3^- amendments. These results and our elevated $\delta^{13}\text{C}$ values suggest that further investigations into the potential for inorganic carbon to limit growth would be useful. We believe that the crowded growth habit of benthic microalgae predisposes these important primary producers to inorganic carbon limitation.

This study demonstrated that growth factors such as light and nutrients can have very large influences on $\delta^{13}\text{C}$ under conditions in which external water velocity and DIC supply are essentially constant. These two factors are certainly not constant in streams, however, and they are likely to interact with growth factors to codetermine algal $\delta^{13}\text{C}$. In the case of water velocity, it is widely accepted that higher flow rates increase the supply of DIC to benthic algal assemblages by reducing diffusive boundary layers and relieving at least some of the carbon depletion occurring during photosynthesis (Finlay et al. 1999). We would expect growth effects to be lessened under higher flow rates. Lower flow rates are in turn likely to exacerbate carbon depletion within the assemblage matrix, so the greatest ^{13}C enrichment should occur when water velocity (or turbulence) is minimal and photosynthetic rates are high (e.g., when photons are abundant). It is somewhat difficult to place the results of our laboratory stream experiments within the context of studies that have investigated velocity effects on algal $\delta^{13}\text{C}$, because water in the laboratory streams was too shallow to use macroscale current meters that are standard in field studies. We did estimate bulk water velocity with solute injections (average velocity was approximately 10 cm s^{-1}), but these

measurements include peripheral and interstitial flowpaths and are likely to underestimate velocity obtained with a standard current meter. The large ¹³C fractionation in the East Fork Poplar Creek shading experiment occurred at meter-measured velocities >20 cm s⁻¹ and suggests that that growth effects are robust at moderate current velocities at least. Experiments in which growth rate, water velocity, and DIC vary simultaneously are needed to determine the relative effects of these factors and their potential interactions.

The potentially large influence of environmental factors that affect growth and algal ¹³C (light, nutrients, temperature, etc.) provides both opportunities and obstacles in the application of stable carbon isotope analysis to the study of benthic food webs. Finlay et al. (2002) were able to exploit the predictable variation in algal ¹³C caused by variation in water velocity to separate carbon contributions from riffles and pools in riverine food webs. A similar opportunity theoretically exists in habitats where light or nutrient sources are patchy. However, the broad range of algal ¹³C produced by different rates of growth should generally be problematic for stream ecologists trying to distinguish between autochthonous and allochthonous carbon sources for higher trophic levels. The range of algal δ¹³C values produced by growth variation in this study (-14‰ to -36‰) included the mean δ¹³C of both C₄ and C₃ terrestrial plants (-14‰ and -28‰, respectively). It would be relatively easy to mistakenly identify a consumer's food source as primarily allochthonous terrestrial matter if that consumer integrated algal ¹³C over a time period when light levels changed from saturating to limiting (i.e., during vernal canopy closure or leaf fall over woodland streams), particularly if terrestrial inputs changed during the same time period. Thorough sampling of benthic algae in both time and space would reduce the risk of misidentification. Continued research into stable isotope dynamics at the level of primary producers is key to the intelligent use of ¹³C in food web analysis.

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