

## Changes in carbon stable isotope ratios during periphyton development

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### Abstract

Stable isotopes are widely used to infer trophic relationships with little attention paid to temporal variability at the base of the food web. We examined changes in the carbon-stable isotope composition during periphyton development, sampling periphyton that accumulated on ceramic tiles at four stream sites over a 2-month period. Periphyton <sup>13</sup>C rose and fell in general concordance with rising and falling biomass at all four sites, resulting in significant correlations between periphyton  $\delta^{13}\text{C}$  and chlorophyll *a* (Chl *a*). Mean  $\delta^{13}\text{C}$  values at one site rose from  $-26\text{‰}$  to  $-20\text{‰}$  in 2 weeks, falling back to  $-24\text{‰}$  the next week after a large scouring spate. Periphyton <sup>13</sup>C also underwent a smaller, longer-term increase that correlated with a gradual rise in stream temperature. Multiple regression analysis with both Chl *a* and temperature as independent variables accounted for up to 88% of the temporal variability in  $\delta^{13}\text{C}$ , with Chl *a* the largest source of variability. Water velocity, measured on each sampling occasion, was unrelated to temporal changes in <sup>13</sup>C. Depletion of inorganic carbon within the periphyton matrix is the probable cause of increasing <sup>13</sup>C in periphyton as biomass develops. Rising  $\delta^{13}\text{C}$  values during periphyton biomass development suggest the possibility of carbon-limited periphyton growth, even in alkaline waters. The strong link between biomass and periphyton <sup>13</sup>C helps explain the large range of  $\delta^{13}\text{C}$  reported for periphyton in streams, where temporal and spatial variability in periphyton biomass are notorious.

Stable isotope analysis is a powerful technique of trophic analysis that has become almost commonplace in the study of terrestrial and aquatic food webs. Inherent differences in carbon acquisition by different types of photoautotrophs are manifested in differing ratios of <sup>12</sup>C and <sup>13</sup>C, allowing for the identification of food sources in higher trophic levels. Unfortunately, application of stable isotope analysis to identify food sources of higher trophic levels in streams has been plagued by highly variable <sup>13</sup>C signatures in periphyton, the main source of autochthonous carbon in most lotic ecosystems (France 1995*a*). The range of <sup>13</sup>C values of periphyton in any particular stream often overlaps that of terrestrial detritus, making it difficult to resolve the relative contributions of these two major food sources to the nutrition of herbivores and carnivores.

Much of the variability in periphyton carbon isotope ratios has been attributed to the variable nature of current in streams. Current affects carbon isotope ratios by reducing the thickness of the boundary layer of still water through which inorganic carbon molecules (e.g., CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>) must diffuse before being utilized by aquatic phototrophs. Diffusion is a relatively slow process, and when it limits the supply rate of inorganic carbon to photosynthesizing cells, these cells utilize isotopically heavy

carbon molecules that they normally discriminate against. As a consequence, plants in moving water tend to be enriched in <sup>13</sup>C relative to plants in still water (France 1995*b*). A number of studies have now reported substantial and predictable effects of current on periphyton carbon isotope ratios (MacLeod and Barton 1998, Finlay et al. 1999, Trudeau and Rasmussen 2003). These predictable effects have been exploited to compare riffles and pools as carbon sources for primary and secondary consumers (Finlay et al. 1999). Nonetheless, substantial variability in the carbon-stable isotope ratios of periphyton is not explained by current (e.g., Trudeau and Rasmussen 2003). Some of this variability is due to isotopic differences in the inorganic carbon sources of periphyton in different streams (Finlay 2001), and some may be due to light or nutrient effects on carbon uptake rates (MacLeod and Barton 1998).

An additional and largely unexplored source of stable carbon isotope variability in periphyton is the natural variability in periphyton thickness. Periphyton assemblages range from thin films one or a few cells deep to multilayered assemblages hundreds of cells deep. Within thicker periphyton, active photosynthesis, combined with diffusion-limited movement of new inorganic carbon from the water to cells within the periphyton matrix, is likely to cause the depletion of inorganic carbon within the matrix. Evidence for the photosynthetic depletion of carbon can be found in pH microprofiles that show increasing pH with depth in illuminated periphyton (Jorgensen et al. 1983). Algal cells photosynthesizing within areas of carbon depletion in thick periphyton are likely to become enriched in <sup>13</sup>C because of the scarcity of CO<sub>2</sub> and forms of HCO<sub>3</sub><sup>-</sup> that are isotopically light and normally subject to

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Table 1. Site characteristics. Nitrate and total phosphorus values are means of samples taken quarterly in 2003; pH values are the mean of weekly values obtained during this study.

	East Fork 23	East Fork 13	East Fork 13 shaded	Brushy Fork
Nitrate ( $\mu\text{g L}^{-1}$ )	1,780	1,350	1,350	570
Total P ( $\mu\text{g L}^{-1}$ )	117	131	131	98
pH	8.5	8.4	8.5	8.0
Shade	None	None	Heavy	Moderate
Dominant algal species	<i>Diatoma vulgare</i>	<i>Gomphonema olivaceum</i>	<i>Gomphonema olivaceum</i>	<i>Gomphonema olivaceum</i>

discrimination by photosynthetic enzymes. Thin periphyton, with less total photosynthesis and a larger proportion of cells close to the water/periphyton interface, should have fewer carbon constraints and therefore a lower proportion of  $^{13}\text{C}$ .

In this study, we investigated the relation between biomass development and carbon isotope composition in stream periphyton. We used biomass (e.g.,  $\mu\text{g cm}^{-2}$ ) as a proxy for periphyton thickness because biomass accrual in periphyton essentially occurs vertically. Developmental sequences of stream periphyton were used to test the hypothesis that increases in periphyton biomass are associated with increases in  $^{13}\text{C}$  content. Periphyton-scouring spates reset the biomass development cycle twice during the study, allowing us to parcel out the effects of biomass and time-associated variables such as temperature. The results have significant implications for the methodology of food web analysis and suggest that inorganic carbon limits maximum photosynthetic rates in periphyton.

## Methods

**Study sites**—All four locations used in this study were shallow, second- to third-order sites in east Tennessee streams. Three of the four sites were located in East Fork Poplar Creek (EFPC), a moderately eutrophic stream that originates from springs in the Department of Energy's Y-12 National Security Complex in Oak Ridge ( $35^{\circ}59'\text{N}$ ,  $84^{\circ}15'\text{W}$ ). The most upstream site, EF23, was located 23.4 km upstream of the confluence of EFPC with Poplar Creek. The other two EFPC sites, EF13 and EF13-SH, were located 13 km upstream of the confluence with Poplar Creek; EF13 was approximately 100 m upstream of EF13-SH. The fourth site, BF, was located in Brushy Fork, a tributary of Poplar Creek approximately 7 km north of EFPC.

Relatively high concentrations of nitrogen and phosphorus characterize EFPC (Table 1). Nutrient concentrations in BF are somewhat lower than those in EFPC. Both EFPC and BF are moderately alkaline, with pH near 8 and alkalinities 2–3 meq  $\text{L}^{-1}$  (Hinzman 1993). Both EF23 and EF13 are unshaded sites with little riparian vegetation development. A black tarp was stretched over the stream at EF13-SH to provide artificial shading; light levels at the stream surface (30 cm below the tarp) were approximately 10% of ambient levels, as measured with a quantum sensor. The BF site was partly shaded by the trunks and branches of streamside trees.

**Substrata deployment and periphyton collection**—Un-glazed ceramic tiles were used as substrata for periphyton colonization and development. Four groups of 24 tiles apiece were attached with silicon glue to four bricks and deployed at each site on 3 January 2004. Bricks were placed in riffle-run sections at each site and secured to the streambed with steel reinforcing bars driven 20 cm into the bed. Each brick was considered the experimental unit (replicate) in this study.

After a 16-d colonization and development period, 1–4 tiles and their attached periphyton were collected from each brick at approximately weekly intervals for the next 2 months. More tiles were collected when periphyton biomass was low to ensure sufficient material for analysis. Tiles were removed from the bricks underwater and transported to the laboratory in water-filled containers. Care was taken to avoid dislodging periphyton during the collection process. Because the substrata were located in areas of rapidly flowing water, the periphyton was strongly attached to the tiles and not easily disturbed. Periphyton was brushed from the tiles in the laboratory, using distilled water to rinse the brushed periphyton from the tiles. Aliquots of the resulting periphyton slurry were filtered onto two pre-ashed glass fiber filters (Whatman GFF): one filter was used for chlorophyll *a* (Chl *a*) analysis, the other for dry mass and carbon isotope analysis. An aliquot of the periphyton slurry was also preserved in Lugol's solution for the microscopic examination of periphyton taxa.

**Periphyton biomass and  $^{13}\text{C}$** —All four filters reserved for Chl *a* from each site and date were extracted with dimethyl sulfoxide and quantified spectrophotometrically (Palumbo et al. 1987). Corrections were made for pheopigments. All four filters reserved for dry mass and carbon isotope analysis were dried at  $60^{\circ}\text{C}$  and weighed. Two to three of these filters from each site and date were fumed in HCl to remove carbonates (Harris et al. 2001). These filters were then submitted to the University of California-Davis Stable Isotope Laboratory for  $^{13}\text{C}$  analysis. Unidentified samples of standard reference material (tomato leaves, NIST SRM 1573a) were submitted along with the experimental samples for quality-control purposes.

**Periphyton taxonomic composition**—Algae in preserved samples were identified and counted at  $\times 400$  magnification with an inverted microscope. Diatom taxa were identified to species level with the help of subsamples that were acid-cleaned, mounted on glass slides with Naphrax, and

examined at  $\times 1000$  with an oil immersion objective. Soft taxa (e.g., chlorophytes and cyanophytes) were identified to genus. At least 500 cells were counted per sample. Duplicate samples (from different bricks) were examined for each date for each site, excepting BF, for which only single samples per date were examined. Counts were converted to biovolumes by estimating the cell volume of each taxon with measured cell dimensions and standard geometric formulae (Hillebrand et al. 1999).

**Environmental measurements**—Temperature, pH, and water velocity were measured weekly beginning 1 week after deployment of the substrata at all the sites. Temperature and pH were measured with a Horiba U-10 water quality checker. Current velocity at the front edge of each brick was measured with a Global water flow probe model FP101.

## Results

**Temporal patterns in abiotic parameters and periphyton biomass**—Water temperature gradually increased at all sites over the course of the study after an initial stable period (Fig. 1). The pH of the water at all sites remained near 8 with only small variations over time. Current velocity measured at the bricks was more variable, differing by as much as 40% between dates at EF13 and EF13-SH. Current velocity exceeded  $0.5 \text{ m s}^{-1}$  on all dates.

Periphyton biomass (Chl *a*) increased logarithmically during the first few weeks until a storm and resulting spate on 06 Feb 2004 scoured periphyton back to low levels (Fig. 2). Periphyton biomass underwent a second period of rapid growth, peaking just before a second major storm and spate occurred on 06 Mar 2004. Biomass accrual differed among sites, with periphyton at EF23 and EF13 reaching substantially higher peak biomasses than periphyton at EF13-SH and BF. Periphyton Chl *a* per unit area was highly correlated (Spearman's  $\rho > 0.89$ ,  $p < 0.001$ ) with dry mass and carbon mass per unit area (data not shown).

**Periphyton assemblage composition**—Periphyton assemblages were relatively simple and stable through much of February, becoming slightly more complex by the end of the study (Fig. 3). Diatoms dominated assemblage composition, contributing  $>95\%$  of biovolume at all sites. *Diatoma vulgare* was the most important contributor to biovolume at EF23, whereas *Gomphonema olivaceum* was the most important contributor at the other three sites.

**Periphyton  $^{13}\text{C}$** —The stable carbon isotope composition of periphyton varied significantly over time (Fig. 4). Periphyton at all sites rapidly became more enriched in  $^{13}\text{C}$  as development proceeded (e.g., rising from  $-26\%$  to  $-20\%$  in 2 weeks at EF23), then it became more depleted (or stabilized in the case of EF13) after the spate of 06 Feb. Periphyton  $^{13}\text{C}$  increased again after the spate, reaching a second, higher peak in March.

The pattern of biomass development was very similar to that of periphyton  $^{13}\text{C}$  content, exhibiting a pattern of

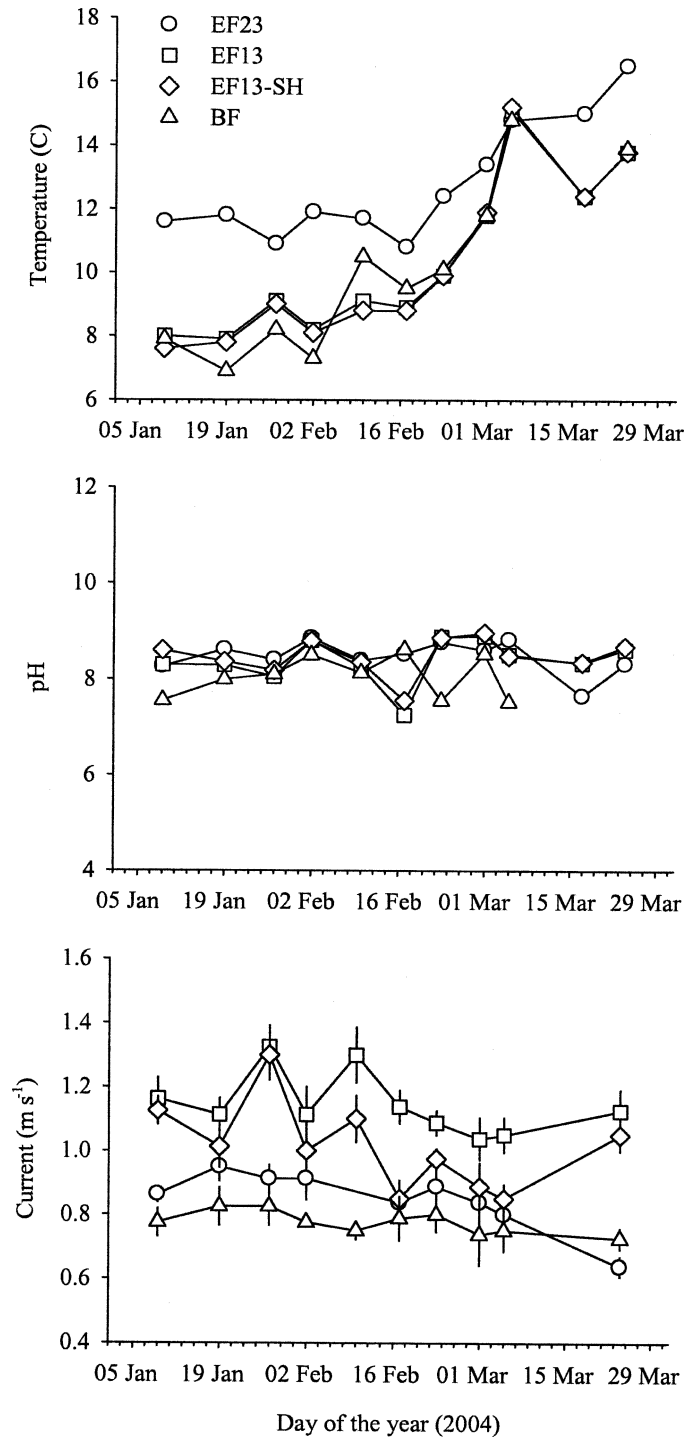


Fig. 1. Trends in temperature, pH, and current during periphyton development. Temperature and pH values represent single measurements at each site. Current values represent the mean of four measurements taken at the location of the four sampling blocks at each site; error bars are standard errors.

peaks and troughs generally concordant with  $^{13}\text{C}$  (Fig. 4). Scatter plots of individual  $^{13}\text{C}$  and chlorophyll observations confirm the association between these two parameters (Fig. 5). Because the relation between  $^{13}\text{C}$  and periphyton biomass was not assumed to be linear, nonparametric

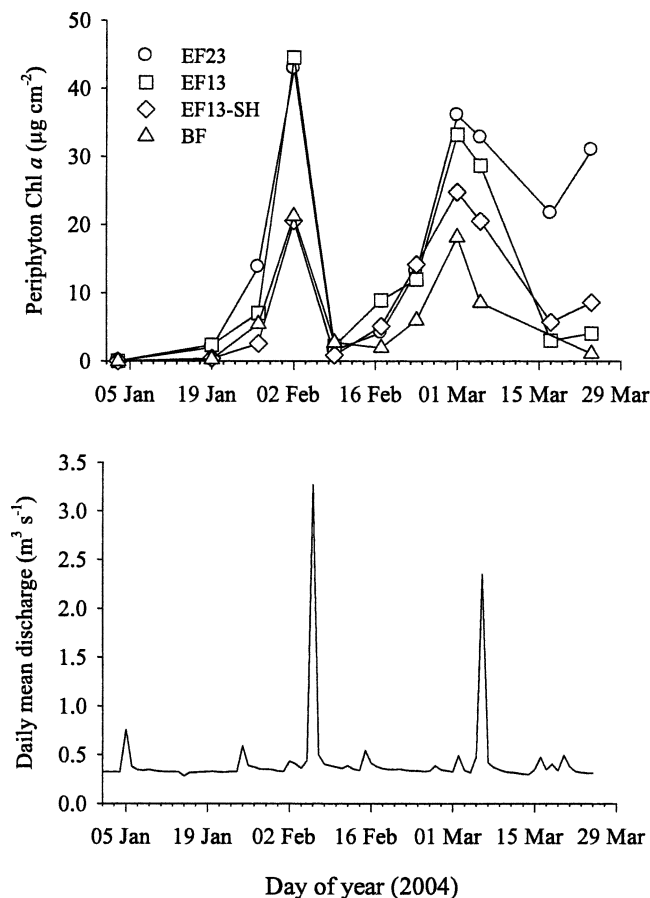


Fig. 2. Temporal changes in periphyton biomass and stream discharge during periphyton development. Each symbol represents the mean Chl *a* density on four replicate tiles at each site; error bars omitted for clarity. Mean daily discharge is for site EF23. Peak discharge rates on 06 February and 06 March resulted from storms that dropped 8.8 and 6.2 cm of rain, respectively.

correlation analysis was performed. The relation was significant ( $p < 0.05$ ) for one site, and highly significant ( $p < 0.01$ ) for the other three sites.

The potential influence of current and temperature on periphyton  $^{13}\text{C}$  was explored through correlation and multiple regression. When the data from all sites were combined, there was no significant overall relation between current velocity and periphyton  $^{13}\text{C}$  (Fig. 6). However, there was a highly significant overall relation between temperature and periphyton  $^{13}\text{C}$ . Multiple regression of periphyton  $^{13}\text{C}$  versus Chl *a*, current velocity, temperature, and pH, using the entire data set, revealed significant additive effects of Chl *a* and temperature, but the analysis was weakened by heteroscedasticity, despite repeated transformations of all the variables.

Multiple regression analysis was then used on a site-by-site basis to determine if current velocity or temperature was linked to periphyton  $^{13}\text{C}$ . First, individual periphyton  $^{13}\text{C}$  values were regressed against log-transformed Chl *a* and current velocity data. Because multiple observations were available for each sampling date and site, the number of

observations in the site-specific regressions was 15–18. Chl *a* accounted for a significant portion of  $^{13}\text{C}$  variability at each site, but current velocity was not a significant variable in the multiple regression at any site (Table 2). The regressions accounted for 30–57% of the variation in periphyton  $^{13}\text{C}$ .

Mean  $^{13}\text{C}$  per sampling date was then regressed against log-transformed mean Chl *a* and temperature at each site. Sample size ( $n = 8$ –10 per regression) was limited to the number of sampling dates because there was only one value from each site on each date. Chl *a* accounted for a significant portion of  $^{13}\text{C}$  variance at each site, as expected. However, temperature also accounted for a significant ( $p < 0.05$ ) portion of the unexplained variance at two of the sites (EF13 and EF13-SH), and temperature explained a marginally significant ( $p = 0.10$ ) portion of the variance at a third site (EF23) (Table 3). Temperature was not a significant variable in the regression of the data from the BF site. As judged by the marginal sum of squares, temperature was less important than Chl *a* in accounting for periphyton  $^{13}\text{C}$ , except in the regression of site EF13-SH data where the marginal sums of squares of the two variables were equal. Biomass (Chl *a*) and temperature together accounted for up to 88% of the temporal variation in periphyton  $^{13}\text{C}$ . Inclusion of pH as an independent variable did not result in a significant improvement in the regression for any site.

## Discussion

Several variables are expected to affect the stable carbon isotope composition of periphyton. These variables include the composition of the dissolved inorganic carbon (DIC) available to periphyton, water velocity, periphyton taxonomic composition, growth rate, and, as we postulate in this paper, periphyton biomass (thickness). Changes in any of these variables over time could theoretically have contributed to the temporal pattern of periphyton  $\delta^{13}\text{C}$  observed in this study. Periphyton  $\delta^{13}\text{C}$  is strongly related to both the  $\delta^{13}\text{C}$  of total DIC and the concentration of  $\text{CO}_2$  in stream water (Finlay 2001); systematic changes in these parameters could be related to hydrological conditions (e.g., time elapsed since the last spate) in EFPC and BF, and could have paralleled the dynamics of periphyton biomass accrual that were highly correlated with periphyton  $\delta^{13}\text{C}$ . We did not measure the concentration or stable carbon isotope composition of DIC during this study, and therefore cannot exclude temporal changes in the DIC as a contributing factor for temporal changes in periphyton  $\delta^{13}\text{C}$ . However, it does seem unlikely that the changes in the concentration of  $\text{CO}_2$  could account for the temporal variability of periphyton  $\delta^{13}\text{C}$ . Streamwater pH, which is highly correlated with the relative abundance of  $\text{CO}_2$ , varied little during the study, and it was not significantly correlated with periphyton  $\delta^{13}\text{C}$ .

Water velocity has been strongly linked to the stable carbon isotope composition of periphyton in both laboratory and field studies (e.g., Finlay et al. 1999; Trudeau and Rasmussen 2003). In this study, it accounted for insignificant amounts of the temporal variability in periph-

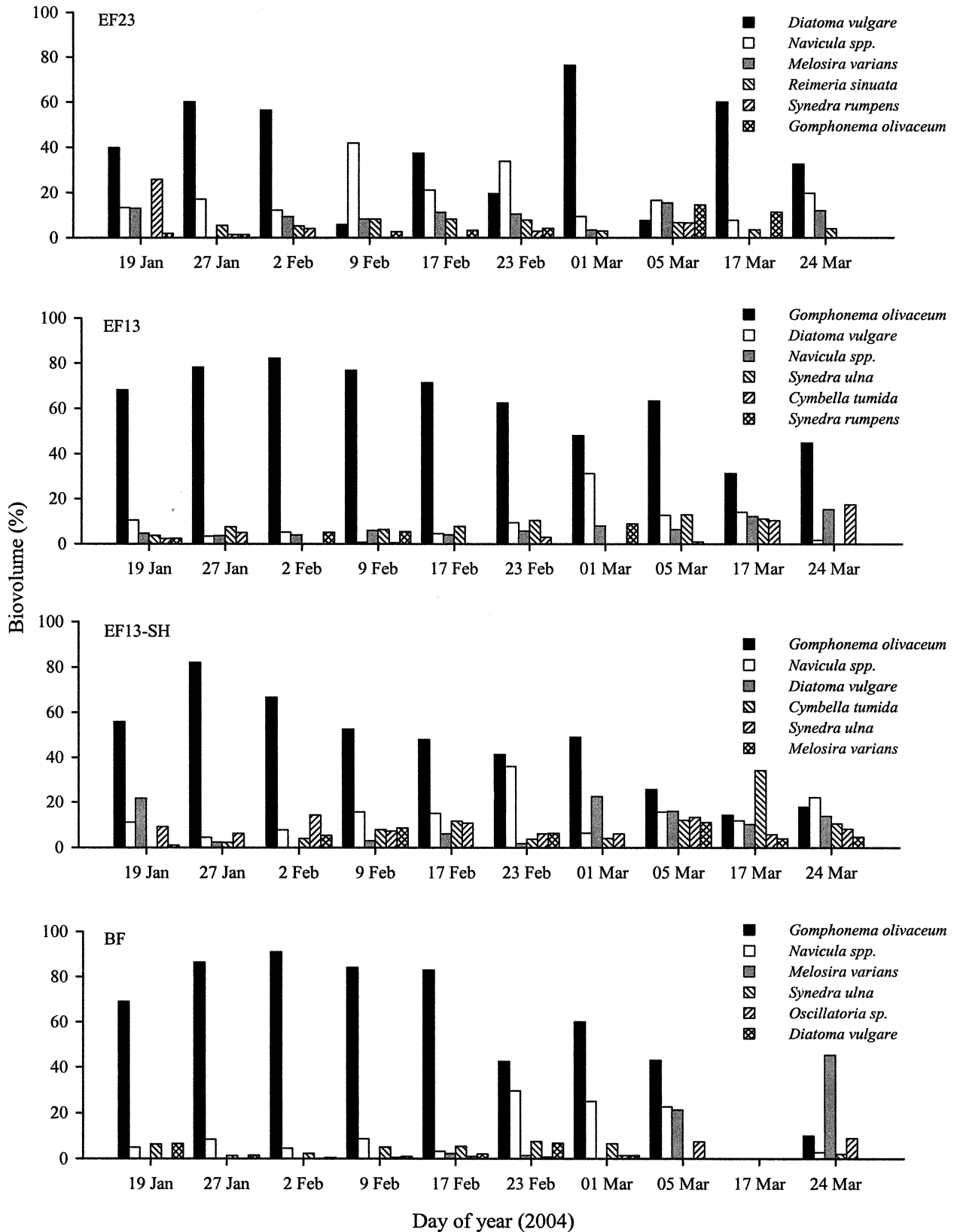


Fig. 3. Periphyton assemblage composition. Only the top six contributors to biovolume are shown; these six taxa accounted for >80% of total biovolume at each site. Each bar represents the mean of two replicates, except the bars for Brushy Fork, which represent individual samples.

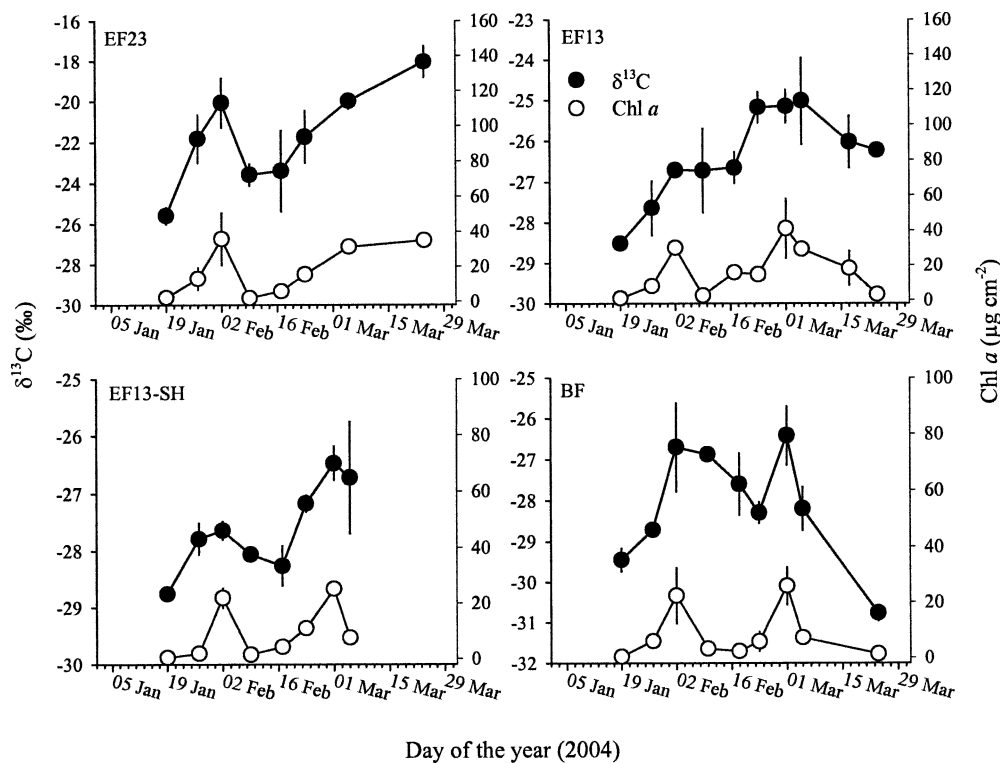


Fig. 4. Temporal patterns of periphyton  $^{13}\text{C}$  and Chl *a*. Each data point represents the mean of 2–3 observations; error bars represent the range of individual values.

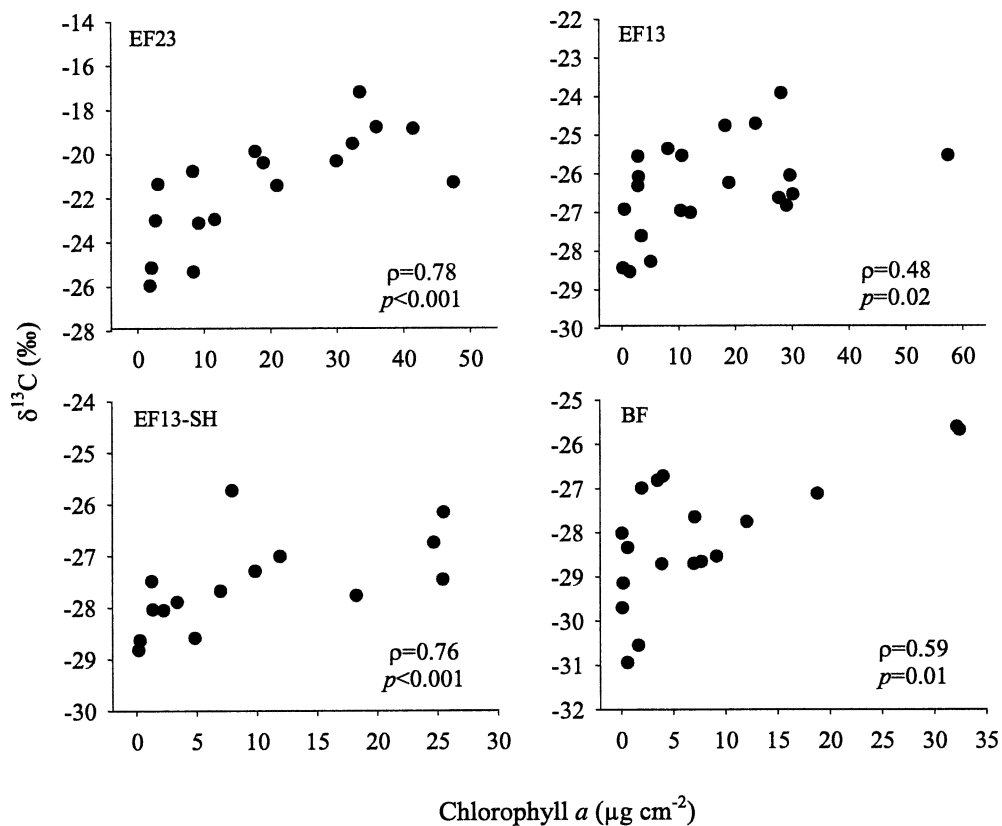


Fig. 5. Periphyton  $^{13}\text{C}$  versus biomass. Correlation coefficients ( $\rho$ ) are from Spearman's nonparametric correlation analysis.

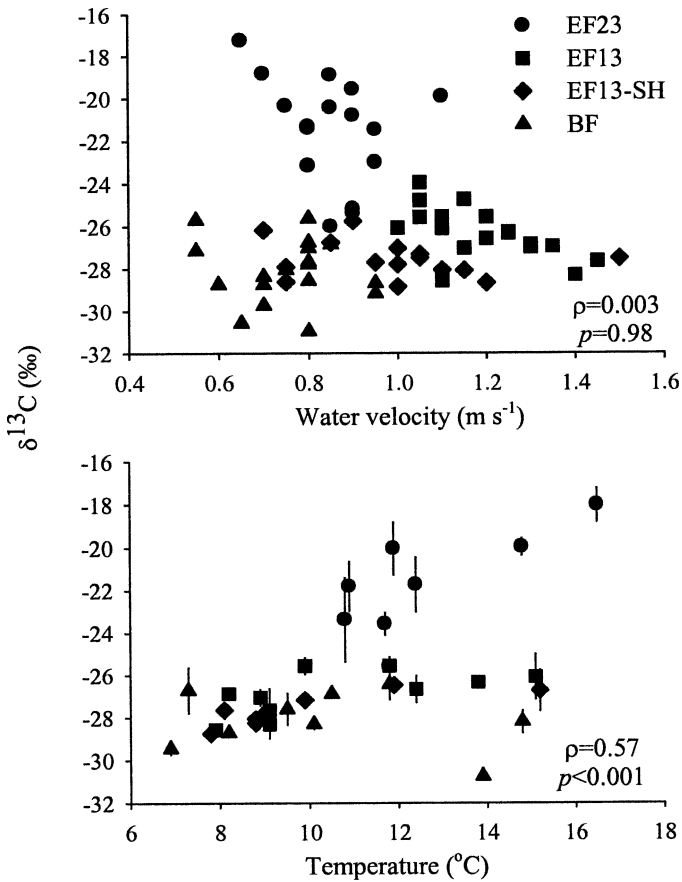


Fig. 6. Periphyton <sup>13</sup>C versus water velocity and temperature. Correlation coefficients ( $\rho$ ) are from Spearman's non-parametric correlation analysis.

yton <sup>13</sup>C in both univariate and multivariate statistical analyses (Fig. 6, Table 2). We are reluctant to conclude that water velocity is unimportant to periphyton stable carbon isotope composition in general because water velocity was not a manipulated variable in this study, and it was relatively high at all times, exceeding 0.5 m s<sup>-1</sup> at all sites. Water velocity may well have been a more important influence on periphyton <sup>13</sup>C if the velocities had dropped to lower levels during periphyton development. Water velocity may also have delayed effects on periphyton carbon-stable isotope composition: Singer et al. (2005) found that

instantaneous water velocities were less important to periphyton <sup>13</sup>C than flow events 3–4 weeks before sampling, ostensibly due to the carryover of scour-resistant algal cells at the base of the periphyton matrix after spates. However, the delayed effects of flow could at best have affected periphyton <sup>13</sup>C in the later part of this study, as the tile substrates were bare when deployed.

Changes in taxonomy or changes in growth rate were also unlikely to have been major factors influencing the temporal pattern of periphyton <sup>13</sup>C. Diatoms dominated periphyton algal assemblages at all sites and at all times, and the diatom species composition was stable during periods in which periphyton <sup>13</sup>C increased substantially. Biomass-specific growth and photosynthetic rates of periphyton are known to decrease with increasing biomass as the result of self-shading or other kinds of self-limitation (e.g., Boston and Hill 1991), so progressively slower growth rates would have been expected as periphyton biomass accumulated on the tiles. Because slower growth rates are associated with greater discrimination against <sup>13</sup>C (Laws et al. 1995), the most negative <sup>13</sup>C values would be expected for slower-growing, high-biomass periphyton. Instead, the opposite was observed: high-biomass periphyton had the highest <sup>13</sup>C values. This is not to suggest that growth rates are unimportant to periphyton <sup>13</sup>C, just that the effect of growth rates are likely to be constrained within the context of periphyton development and self-limitation.

Although we cannot rule out the potential contributions of the variables listed above, we feel that the best and simplest explanation for the temporal pattern of periphyton <sup>13</sup>C is that it was a direct effect of conditions created by the vertical accumulation (and loss) of periphyton biomass. A biomass effect on periphyton <sup>13</sup>C has been hypothesized previously (e.g., Singer et al. 2005), and decreased discrimination against <sup>13</sup>C by filamentous cyanophytes that clump and create localized conditions similar to those caused by vertically accumulating periphyton has been reported (Pardue et al. 1976), but this is the first stream study to show a significant relation between periphyton stable carbon isotope composition and biomass. Given the observed gradients of photosynthesis, oxygen production, and pH within periphyton matrices, it is logical to expect a relation between periphyton biomass and <sup>13</sup>C.

We hypothesize that the relation between periphyton biomass and stable isotope composition is the result of

Table 2. Multiple regression of periphyton <sup>13</sup>C versus biomass and current velocity. Dependent variable = individual <sup>13</sup>C observations; independent variables = log-transformed individual Chl *a* observations and individual current velocity measurements. Error values for the regression coefficients and standard errors of the estimates.

Site	<i>n</i>	Variable	Regression coefficients	<i>p</i>	<i>r</i> <sup>2</sup> (adj.)
East Fork 23	16	log Chl <i>a</i>	4.21±0.98	0.001	0.57
		current	-0.36±0.41	0.40	
East Fork 13	17	log Chl <i>a</i>	1.19±0.40	0.01	0.46
		current	-0.36±0.19	0.07	
East Fork 13—shaded	15	log Chl <i>a</i>	1.00±0.33	0.01	0.39
		current	0.03±0.10	0.74	
Brushy Fork	18	log Chl <i>a</i>	1.34±0.44	0.01	0.30
		current	0.07±0.27	0.79	

Table 3. Multiple regression results of periphyton  $^{13}\text{C}$  versus biomass and temperature. Mean  $^{13}\text{C}$  of periphyton per sampling date was used as the dependent variable; log-transformed mean Chl *a* and temperature were used as the independent variables. Error values for the regression coefficients are standard errors of the estimates.

Site	<i>n</i>	Variable	Regression coefficient	<i>p</i>	Marg. SS*	<i>r</i> <sup>2</sup> (adj.)
East Fork 23	8	log Chl <i>a</i>	3.42±0.74	0.01	15.0	0.88
		temperature	0.38±0.19	0.10	2.8	
East Fork 13	10	log Chl <i>a</i>	1.14±0.43	0.03	3.1	0.66
		temperature	0.23±0.09	0.04	2.7	
East Fork 13—shaded	8	log Chl <i>a</i>	0.68±0.19	0.02	1.1	0.86
		temperature	0.18±0.05	0.02	1.1	
Brushy Fork	9	log Chl <i>a</i>	1.72±0.60	0.03	8.8	0.46
		temperature	-0.16±0.13	0.29	1.4	

\*Marginal sum of squares.

progressively severe carbon limitation during biomass accrual. Within the crowded, three-dimensional matrix of algal cells and microheterotrophs that constitute periphyton, photosynthesis by algal cells depletes inorganic carbon in the water surrounding the cells. Carbon dioxide is undoubtedly depleted first, causing photosynthesizing cells to rely more heavily on bicarbonate as an inorganic carbon source. This shift in inorganic carbon source would account for at least some of the observed  $^{13}\text{C}$  enrichment in thicker periphyton, as bicarbonate  $\delta^{13}\text{C}$  is approximately 9‰ greater than that of  $\text{CO}_2$  (Hecky and Hesslein 1995; Fry 1996). The rest of the observed enrichment is likely to be the result of the disproportionate reduction of  $\text{H}^{12}\text{CO}_3^-$  in the inorganic carbon pool as photosynthesis proceeds. Photosynthetic enzymes discriminate against the heavy  $^{13}\text{C}$  isotope, so unless  $\text{H}^{12}\text{CO}_3^-$  is replaced faster than it is used,  $\text{H}^{13}\text{CO}_3^-$  will become a larger proportion of the inorganic carbon available for photosynthesis. As a consequence, the contribution of  $^{13}\text{C}$  to algal C will rise despite discrimination. Because replacement of inorganic carbon fixed by algal cells within the periphyton matrix will essentially be through the surface of the periphyton, thicker periphyton should be more carbon-limited than thin periphyton because of its greater biomass of photosynthesizing algal cells per unit surface area. Thicker periphyton also provides a longer diffusion distance for inorganic carbon to travel before reaching algal cells in the middle of the matrix, reducing the rate of carbon replenishment.

The above explanation of biomass effect implies a stratigraphy of carbon isotope composition in periphyton. Algal cells at or near the water/periphyton interface would be expected to be depleted in  $^{13}\text{C}$  because of their proximity to new inorganic carbon in the water column. Algal cells at progressively deeper levels within the periphyton matrix would be expected to become progressively enriched in  $^{13}\text{C}$  as the distance through which new inorganic carbon must travel increases. The vertical gradient of  $\delta^{13}\text{C}$  should mirror the pH gradient observed in profiles made with microelectrodes in which pH increases with increasing depth because of photosynthesis within the matrix and diffusion-limited transport of inorganic carbon (Jørgensen et al. 1983). Stratigraphy could have interesting implications for inferring carbon sources of higher trophic levels. Grazers such as collecting-gathering chironomids or mayflies that consume the outer layer of periphyton (e.g.,

Hill and Knight 1987) would be expected to be depleted in  $^{13}\text{C}$  compared to grazers such as some caddisflies and snails that consume deeper layers as well (e.g., Hill and Knight 1988).

Despite the strong correlation between periphyton  $\delta^{13}\text{C}$  and biomass, the temporal concordance between the two variables was not perfect. Periphyton  $\delta^{13}\text{C}$  values generally failed to decline as dramatically as did biomass after spates (Fig. 4). Postspate periphyton should retain at least some of the carbon isotope signature of prespate conditions; the influence of prespate carbon isotope ratios will depend on the length of time since the spate and the turnover rate of carbon in the periphyton. Because of the potential carryover of carbon fixed during high-biomass conditions, it was not surprising that periphyton  $\delta^{13}\text{C}$  values were higher than levels expected from prespate  $\delta^{13}\text{C}$ /biomass ratios.

Periphyton  $\delta^{13}\text{C}$  values appeared to undergo modest longer-term increases over the course of the study that were unrelated to changes in biomass. A potential causal agent for this increase was temperature, which rose gradually during the study and was uncorrelated with periphyton Chl *a* and dry mass ( $p > 0.10$ ). Temperature was significantly correlated with  $\delta^{13}\text{C}$  as a single variable (Fig. 6), and it was a significant covariate with biomass in site-specific multiple regressions of  $\delta^{13}\text{C}$  at three of the four sites, combining with biomass to account for up to 88% of the total variability in periphyton  $\delta^{13}\text{C}$  (Table 3). Temperature is expected to have a positive effect on periphyton  $\delta^{13}\text{C}$  values because: (1) it increases photosynthetic rates, thereby increasing the likelihood of carbon depletion and  $^{13}\text{C}$  utilization; (2) it reduces the equilibrium fractionation between  $\text{HCO}_3^-$  and  $\text{CO}_{2(\text{aq})}$ , thereby increasing  $\delta^{13}\text{C}$   $\text{CO}_{2(\text{aq})}$  up to 3‰ (Mook et al. 1974, Finlay 2001). However, it is not clear that increasing temperature was the major (or only) mechanism behind the gradual increases in periphyton  $\delta^{13}\text{C}$  over the course of the study. Light also would have increased from January to late March as a result of increasing daylength and sun angle, and could have contributed to increasing  $\delta^{13}\text{C}$  by increasing photosynthetic rates. Light was not monitored during this study.

Although periphyton  $^{13}\text{C}$  was significantly correlated with biomass at all sites, the strength of the relation did vary from site to site. The range of  $^{13}\text{C}$  variation was also

site-specific, with the smallest range occurring at EF13-SH (3‰) and the largest occurring at EF23 (8‰). Environmental conditions and periphyton assemblage composition differed between sites and undoubtedly contributed to site-to-site differences in periphyton  $^{13}\text{C}$ . Lower mean  $\delta^{13}\text{C}$  values and lower variability in these values at EF13-SH could have been due to light scarcity at this site; light is reported to influence periphyton stable carbon isotope composition (MacLeod and Barton 1998) and its scarcity may have suppressed both the mean and range of  $\delta^{13}\text{C}$ . Higher mean and range of  $\delta^{13}\text{C}$  values at EF23 could have been due to warmer temperatures, higher water velocities, or a different assemblage composition. However, ascribing site-to-site differences in  $^{13}\text{C}$  to specific causes is speculative without experimental control and replication. The important point is that periphyton  $^{13}\text{C}$  at all sites responded in the same general manner to variations in biomass.

Biomass development and water velocity are likely to interact in their influence on periphyton stable carbon isotope composition. Higher water velocities should mitigate some of the effect of biomass on periphyton  $^{13}\text{C}$  by increasing the flux of inorganic carbon into the periphyton matrix, counteracting some of the carbon depletion within thicker periphyton. If this is so, then the effects of biomass on periphyton  $^{13}\text{C}$  that we observed in this study are likely to be conservative as water velocity was relatively high at all times. Low velocities are likely to exacerbate carbon depletion within periphyton, increasing  $\delta^{13}\text{C}$  values already elevated in thicker periphyton. Unfortunately, water velocity and periphyton biomass often covary (Stevenson 1996), making it difficult to disentangle the effects of the two variables. Low periphyton biomass can result from high water velocities, and both low biomass and high velocity would be predicted to increase  $^{13}\text{C}$  in periphyton. Some of the effects attributed to water velocity in previous studies may in fact be due to an indirect effect of velocity on biomass. In any case, it would be prudent to report both biomass levels and water velocities in future studies using periphyton stable carbon isotopes.

One of the interesting implications of this study is that periphyton growth may be carbon-limited even in streams with moderately high levels of total inorganic carbon. Carbon limitation of periphyton biomass accrual has been demonstrated in soft-water lakes (Fairchild and Sherman 1992), but not in waters as alkaline as EFPC or BF. Under conditions of high light and nutrient excess, growth rates of marine phytoplankton appear to be limited by carbon dioxide supply (Riebesell et al. 1993). The notion that periphyton biomass accrual may be self-limited through biomass barriers to nutrient diffusion or through self-shading is not new (Hill and Boston 1991; Stevenson and Glover 1993), but self-limitation due to carbon depletion within the periphyton matrix has seldom been invoked as a constraint on periphyton development. Carbon limitation in alkaline waters may affect maximum biomass development more than accrual rate, and it may be demonstrable only after a threshold amount of periphyton biomass has accrued. Grazers, low light, low nutrients, or frequent spates can maintain periphyton biomass below threshold levels for carbon limitation in many instances, but

maximum biomass development in periphyton may ultimately be constrained by carbon limitation. Global increases in carbon dioxide may be responsible for larger and more frequent blooms of benthic algae, especially if increases in carbon dioxide are coupled with rising temperatures.

Temporal variability such as that demonstrated in this study complicates attempts to use stable carbon isotopes in food web analysis. The 6‰ range in periphyton  $\delta^{13}\text{C}$  that occurred in this study over a 3-week period at a single site included values representative of terrestrial detritus (approximately  $-26\text{‰}$  to  $-28\text{‰}$ ) and values that were clearly outside the typical range for terrestrial foliage. A one-time sampling for stable isotope analysis of food web structure could lead to very different conclusions about the carbon base of the food web depending on when sampling occurred. In streams such as EFPC, where temporal variability in periphyton biomass is large, sampling periphyton for stable carbon isotopes once or just a few times may seriously misrepresent the isotope ratios of food available to consumers.

Spatial variability in the carbon-stable isotope composition of periphyton and consumers should also result from the relation between biomass and periphyton  $\delta^{13}\text{C}$ . Within the continuum of a single lotic system, one would expect decreased fractionation of carbon (higher  $\delta^{13}\text{C}$  values) at downstream sites where primary production and periphyton biomass are less constrained by canopy cover (Vannote et al. 1980). Finlay (2001) reported decreasing  $^{13}\text{C}$  discrimination (higher  $\delta^{13}\text{C}$  values) in both periphyton and herbivores with increasing watershed area, attributing this relation to the depletion of  $\text{CO}_2$  in the bulk water by higher primary production occurring at downstream sites. If higher rates of primary production lead to higher levels of periphyton biomass, then  $\text{CO}_2$  depletion within periphyton matrix will exacerbate depletion of  $\text{CO}_2$  in the bulk water and cause further increases in  $\delta^{13}\text{C}$ . When comparing different lotic systems, those streams whose standing crop of periphyton biomass is limited by grazing or abiotic factors (e.g., light, nutrients, temperature) are likely to have periphyton and grazers with lower  $\delta^{13}\text{C}$  values. Productive streams that also experience frequent spates are expected to exhibit the most variable carbon-stable isotope composition, and frequent periphyton sampling is particularly recommended for stable isotope analyses of food webs in these streams.

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