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Patterns and controls of lotic algal stable carbon isotope ratios

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Abstract

Spatial and temporal variations in stable carbon isotope ratios (i.e., $\delta^{13}C$) of primary producers are common but poorly understood features of isotopic characterizations of aquatic food webs. I investigated factors that control $\delta^{13}C$ of algae (concentration and $\delta^{13}C$ of inorganic carbon, algal fractionation, and growth rates) in riffle habitats across a gradient in stream size and productivity in northern California. There was considerable seasonal and spatial variation in $\delta^{13}C$ of the green alga *Cladophora glomerata,* microalgal-influenced epilithic biofilms, and their herbivores. Algal and herbivore $\delta^{13}C$ were depleted in ^{13}C in small, unproductive tributary streams (−44‰ to −30‰) compared with more productive sites downstream (−31‰ to −23‰). The majority of variation in algal $\delta^{13}C$ of *Cladophora* and epilithic biofilms was determined by dissolved CO_2 (CO_{2aq}) via effects on $\delta^{13}C$ of CO_{2aq} and photosynthetic fractionation. In contrast, two other taxa (the cyanobacterium *Nostoc pruniforme* and the red alga *Lemanea* sp.) showed little variation in $\delta^{13}C$ or fractionation in response to varied inorganic carbon availability because of their distinct modes of inorganic carbon acquisition. Although variation in algal $\delta^{13}C$ might complicate use of $\delta^{13}C$ to resolve consumer diet sources under some circumstances, better understanding of such variation should improve the use of $\delta^{13}C$ techniques in aquatic food web studies.

Measurements of stable carbon isotope ratios ($^{13}C/^{12}C$ or $\delta^{13}C$) are increasingly useful tools for population, food web, and ecosystem ecology. In fresh water, plant $\delta^{13}C$ values are highly variable and thus useful tracers of plant carbon use (e.g., Raven et al. 1982; Keeley and Sandquist 1992) and energy flow in food webs (Fry and Sherr 1984; Rounick and Winterbourn 1986). Although $\delta^{13}C$ measurements are now commonly employed in lotic ecological studies, the extent to which variation in algal $\delta^{13}C$ reflects differences in inorganic carbon sources, plant physiology, or environmental factors is largely unknown. Lacking this knowledge, a priori assessment of the potential for quantitative use of $\delta^{13}C$ is not possible, and management of variability that could confound such applications is difficult (Boon and Bunn 1994). Improved understanding of the sources and scales of variation in algal $\delta^{13}C$, however, could allow more effective and efficient use of $\delta^{13}C$ as a tracer of food web interactions.

For C_3 plants that acquire CO_2 via passive diffusion, $\delta^{13}C$ are generally determined by variation in the isotope ratio of the inorganic carbon source and the amount of fractionation during carbon uptake and assimilation as described by Farquhar et al. (1982).

$$\text{Plant } \delta^{13}C = \delta^{13}C_{CO_2} - a - (b - a)c_i/c_e \qquad (1)$$

$\delta^{13}C_{CO_2}$ is the isotope ratio of CO_2, a is discrimination against ^{13}C during diffusion of CO_2, b is discrimination by Rubisco, c_i is intercellular CO_2 concentration, and c_e is external CO_2 concentration. For terrestrial C_3 plants, $\delta^{13}C_{CO_2}$, a, and c_e are relatively constant in temperate watersheds. As a result, plant $\delta^{13}C$ values are mainly determined by c_i, internal CO_2, which is strongly influenced by plant growth rates and water use (Farquhar et al. 1982). Terrestrial plant $\delta^{13}C$ for C_3 taxa range between −34‰ and −22‰, but a great majority of values fall between −29‰ and −25‰ (Rounick and Winterbourn 1986). Perhaps because the aquatic detrital pool integrates terrestrial plant $\delta^{13}C$ through time and space, particulate terrestrial detritus $\delta^{13}C$ in temperate stream ecosystems has a well-constrained mean value of -28.2 ± 0.2‰ (±SE; Finlay 2001).

By contrast, potential strong influences on autotrophic $\delta^{13}C$ are much more diverse in aquatic ecosystems, resulting in a wide range of observed algal $\delta^{13}C$. For example, $\delta^{13}C$ of dissolved inorganic carbon (DIC) in freshwaters can range from −26‰ up to 0‰ (Mook and Tan 1991), and aquatic plants can use two forms of DIC (CO_2 and HCO_3^-). Under equilibrium conditions, these two carbon species have different $\delta^{13}C$, with dissolved CO_2 (hereafter CO_{2aq}) consistently ^{13}C-depleted relative to HCO_3^- by 7–10‰, depending on temperature (Mook et al. 1974). Furthermore, diffusional effects on fractionation might be much more variable in aquatic ecosystems because the thickness of stagnant boundary layers around plant cells are affected by water velocity (Keeley and Sandquist 1992; Hecky and Hesslein 1995; Finlay et al. 1999). Finally, environmental CO_{2aq} concentrations (hereafter $[CO_{2aq}]$) are far more variable in aquatic than in terrestrial ecosystems, and internal CO_2 is expected to be at least as variable for algae as for terrestrial vegetation. Thus,

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unlike terrestrial plants, algal $\delta^{13}C$ could be strongly affected by multiple factors in aquatic environments.

The controls of algal $\delta^{13}C$ are comparatively well understood only for marine phytoplankton in environments in which $[CO_{2aq}]$ and $\delta^{13}C$ of DIC exhibit a relatively restricted range of variation compared with freshwater ecosystems. Recent research shows that lotic algal $\delta^{13}C$ values are highly variable (Finlay 2001; Zah et al. 2001; Finlay et al. 2002; McCutchan and Lewis 2002), but the relative importance of the many potential influences on lotic algal $\delta^{13}C$ has rarely been examined within a single site (*but see* Raven et al. 1982; MacLeod and Barton 1998). Even fewer studies have examined controls of algal $\delta^{13}C$ through time or across the typical range of environmental conditions found in lotic ecosystems.

Despite the lack of direct study, there is some evidence to suggest that algal productivity could play an important overall role in determining algal $\delta^{13}C$. As discussed previously, algal growth rates can strongly influence fractionation, and algal CO_2 uptake can influence stream $[CO_{2aq}]$ and $\delta^{13}C$ of DIC (McKenzie 1985; Dawson et al. 2001; Finlay 2003). A general pattern of increasing algal and herbivore $\delta^{13}C$ with watershed area in temperate streams and rivers (Finlay 2001) is consistent with decreasing fractionation as a result of increasing rates of algal photosynthesis or decreasing $[CO_{2aq}]$, both of which occur over stream size gradients in forested watersheds (Lamberti and Steinman 1997; Jones and Mulholland 1998; Finlay 2003). However, data for few of the variables that can influence algal $\delta^{13}C$ in streams ($[CO_{2aq}]$, $\delta^{13}C$ of DIC, algal photosynthesis rates and biomass, and water velocity) are available in studies that report algal $\delta^{13}C$, so the relative importance of such influences on algal $\delta^{13}C$ cannot be directly evaluated. The purpose of this study was to determine the primary controls of lotic algal $\delta^{13}C$ by examining the role of $\delta^{13}C$ of DIC and fractionation on algal $\delta^{13}C$ across a gradient of stream size and productivity typical of small streams to midsized rivers.

Methods

Site description and sampling design—Most study sites were located in or near the Angelo Coast Range Reserve in the forested headwaters of the South Fork (SF) Eel River in Mendocino County, California, USA (39°44′N, 123°39′W). Almost all precipitation to the watershed falls as rain between October and May. Discharge declines after winter floods to stable summer baseflows. The larger streams and rivers have wide channels and sunlit streambeds and are highly productive during summer baseflows. During the baseflow period, dissolved organic carbon concentrations (1–3 mg L^{-1}) and turbidity are low and water clarity high (Finlay 2003; pers. obs.). The riparian forest is largely composed of evergreen species such as Douglas fir, redwoods, and bay laurel (*Pseudotsuga menziesii, Sequoia sempervirens,* and *Umbellularia californica,* respectively). As a consequence, there is very little seasonal change in canopy cover at the study sites.

Potential variables influencing algal $\delta^{13}C$ were measured monthly during summer and bimonthly in winter for up to 2 yr at the SF Eel River and five tributary stream and river sites (i.e., monitoring sites) that varied in size. These sites ranged from heavily shaded headwater streams to unshaded, productive reaches of the SF Eel River, Elder Creek, and Ten Mile Creek near the town of Branscomb, California. To examine a greater range of stream productivity and inorganic carbon chemistry, a larger number of streams in the Eel River watershed and other rivers in northern California were surveyed during mid-summer in 1998 and 1999 (i.e., survey sites) in addition to the monitoring sites. Most of the survey sites were tributaries of the SF Eel River along a 15-km length of river near Branscomb, but several other larger rivers were also sampled (the Middle Fork Eel, Trinity, and Klamath Rivers). In 1998, sampling encompassed a wide range of river sizes. In 1999, sampling efforts concentrated on headwater streams with highly variable $[CO_{2aq}]$. Some sites from 1998 were resampled to assess between-year variability.

At small stream sites where $[CO_{2aq}]$ often varied considerably over short distances (as little as 10–20 m; Finlay unpubl. data), sampling for epilithic algal $\delta^{13}C$ was conducted within 10 m of the point of stream chemistry sampling. At larger sites, where $[CO_{2aq}]$ was much less spatially variable, sampling was conducted within at least 300 m of the stream chemistry sampling site.

Samples for stream chemistry were collected from well-mixed stream water during early afternoon. Methods for sample collection and measurement of stream water pH, conductivity, $[CO_{2aq}]$, DIC concentration, $\delta^{13}C$ of DIC, HCO_3^-, and CO_{2aq} are described in Finlay (2003). $\delta^{13}C$ of DIC was measured in 1997 and 1998, but not in 1999.

As noted, boundary layer thickness can be an important factor, influencing algal carbon supply and stable carbon isotope ratios. Water velocity can affect boundary layer thickness, and thus might influence algal $\delta^{13}C$ in rivers (e.g., Finlay et al. 1999). For this study, I attempted to hold such boundary layer effects constant by restricting investigation to riffle habitats (i.e., habitats dominated by turbulent flow) in each stream or river. Chutes with very fast laminar flow, a rare riffle habitat, were avoided. Average water velocities were measured in riffles along multiple cross-channel transects at 0.6 of total depth at each point with a Marsh McBirney (model 2000) flowmeter.

Algal and epilithon stable carbon isotope ratios—The study focused on microalgae within epilithic films in riffles, the dominant growth form in most streams and small rivers, and to a lesser extent, the filamentous chlorophyte *Cladophora glomerata,* the filamentous rhodophyte *Lemanea* sp., and the cyanobacterium *Nostoc pruniforme*. Epilithic microalgae grew in thin biofilms that were heavily grazed by invertebrates at all sites. Epilithic biofilms included a diverse flora of diatoms dominated by *Melosira* spp. and *Cymbella* spp. in shaded streams and *Achnanthes minutissima, Cocconeis* spp., and *Epithemia* spp. in more open, canopied sites (J. Marks pers. comm.), as well as chlorophytes (often *Cladophora*), bacteria, and unidentifiable material. *Cladophora* occurred in dense clusters of fine filaments. *Lemanea* occurred in tufts of thick filaments in only the fastest flowing areas of riffles during the spring and early summer. *Nostoc* occurred in gelatinous balls and "ears" formed by midge larvae in slower flowing areas of riffles and in pools. The latter two species are inedible to most invertebrate species present.

In the larger sites (those watersheds >10 km^2), sampling for epilithic and macroalgal $\delta^{13}C$ was conducted by compositing subsamples within a given riffle and by collecting multiple samples from adjacent riffles. For each stream or river, microalgae and macroalgae were sampled in at least two riffle sites, usually within several hundred meters from the point of water chemistry sampling.

At these sites, samples for microalgae were collected by removing epilithic biofilm material from cobbles with a wire brush. All cyanobacteria and algal filaments longer than 0.5 cm were avoided during collection of microalgae. Microscopic examination showed that the majority of identifiable material was diatoms (Finlay pers. obs.). However, a significant fraction of these samples was amorphous material that could not be identified. Density separation of algal material from this matrix (Hamilton and Lewis 1992) was not possible because of the dominance of diatoms in the samples. As a consequence, samples for microalgae might have contained some terrestrial organic carbon present as detritus or heterotrophic bacteria. These samples are thus referred to generally as epilithon, whereas the algal component of such samples are referred to as epilithic microalgae.

Because microalgal $\delta^{13}C$ values were relatively difficult to measure, herbivore $\delta^{13}C$ measurements were sometimes used to infer microalgal $\delta^{13}C$. This approach was reasonable at open, canopied sites because of strong relationships between herbivore (collector and scraper functional feeding groups) and epilithic algal $\delta^{13}C$ in open, canopied study sites in the watershed (Finlay et al. 1999, 2002). The relationship between herbivore and epilithic algal $\delta^{13}C$ was assessed with additional samples of algae and herbivores in this study. There were no within-riffle differences between scraper and collector $\delta^{13}C$ in open, canopied streams (Finlay unpubl.), so averages of all herbivores were used to estimate algal $\delta^{13}C$ in these sites when direct measurements were not made.

In closed canopied headwater streams (i.e., drainage area of <10 km^2), large samples of epilithon with a high microalgal content could not be reliably obtained for analyses. In such streams, microalgal $\delta^{13}C$ values were estimated from $\delta^{13}C$ of several scraper taxa (usually *Glossosoma penitum, Neophylax splendens,* and *N. rickeri*). Scraper data were used for two reasons. First, scrapers rely strongly on microalgae. In a literature review, Finlay (2001) found that scraper $\delta^{13}C$ closely tracked variation in epilithic algal $\delta^{13}C$, even in small streams. Second, in contrast to open, canopied rivers, scraper $\delta^{13}C$ in the small streams were significantly lower than collector $\delta^{13}C$ sampled from the same location (Finlay unpubl.), suggesting greater reliance on algae by scrapers compared with collectors. Further justification and potential limitations of this approach are described in Finlay (2001) and discussed further in this paper. When herbivores were used to infer microalgal $\delta^{13}C$, 5–30 individuals were collected from epilithic surfaces in riffles.

Algae, epilithon, and invertebrate samples were processed and analyzed as described in Finlay et al. (1999, 2002). Briefly, epilithon samples were filtered onto precombusted GFF filters and dried after removing invertebrates. Macroalgal samples were sorted to remove invertebrates and detritus, rinsed, and dried. For invertebrates, guts were dissected and discarded after collection, and samples were dried. Filters containing epilithon were analyzed whole or subsampled. Macroalgal and invertebrate samples were ground to a fine powder after drying. Samples were not acid washed because the carbonate content of soils and surface waters in the region is low, and tests showed no significant effect of acid treatment. Approximately 20% of samples were analyzed in duplicate, and the average standard deviations for $\delta^{13}C$ analyses were 0.12‰, 0.18‰, and 0.11‰ for 1997, 1998, and 1999, respectively.

Means for riffle epilithon, algae, and herbivores for individual stream and river sites were calculated by averaging data from adjacent subsites where physical and chemical conditions were similar. In small streams with steep gradients in [CO_{2aq}], only data for samples collected near (i.e., within 20 m) the site of water chemistry sampling were used in analyses. Within larger streams and rivers (i.e., a watershed area of >10 km^2), between-riffle variation in chemistry and algal $\delta^{13}C$ was low. At these sites, means were calculated by averaging data for all riffles sampled.

Algal biomass and productivity—At each site, samples for epilithic algal biomass were collected from several riffles by removing algae from known areas of cobbles with a wire brush. Subsamples were filtered onto GFC filters in the lab. Total chlorophyll *a* concentration was determined fluorometrically following extraction of filters in 90% acetone.

Algal productivity was inferred from measurements of canopy cover and light levels in 1998 because there is a robust relationship between irradiance and algal production in streams (Lamberti and Steinman 1997). Canopy cover was measured with a spherical densitometer (Lemmon 1956). In 1999, direct measurements of microalgal primary production were made during midsummer to assess the relationship between light levels and photosynthesis rates at a subset of nine study sites. Photosynthesis was estimated from changes in dissolved oxygen in recirculating chambers containing river cobbles following methods of Bowden et al. (1992). Cobbles were incubated in either Fox Creek (low light conditions) or the SF Eel River (high light conditions). Light levels were further adjusted to ambient midday levels with shade cloth. Gross primary production and community respiration were estimated from changes in dissolved oxygen measured with a YSI model 95 dissolved oxygen meter during midday and nighttime. Photosynthetically active radiation (PAR) was measured with a LiCor underwater quantum sensor.

Fractionation calculations and statistical analyses—Algal discrimination against $\delta^{13}C$ during assimilation of inorganic carbon, or photosynthetic fractionation (ε), was calculated relative to $\delta^{13}C$ of DIC, HCO_3^-, or CO_{2aq} as determined by the form of carbon used by each taxon according to Freeman and Hayes (1992).

$$\varepsilon\ (‰) = \frac{\delta^{13}C_{\text{inorganic carbon}} - \delta^{13}C_{\text{algae}}}{1 + \delta^{13}C_{\text{algae}}/1{,}000} \qquad (2)$$

Table 1. Site descriptions from monitoring and survey sites. Bold type indicates sites sampled seasonally (monitoring sites). Temperature data are from samples collected on one day in midsummer 1998. Sampling at survey sites took 2 weeks to complete, and there was no rainfall during the sampling period. The two smallest sites in terms of watershed area were sampled at headwater springs where water emerged from the ground.

Stream or river	Watershed area (km²)	Temperature (°C)	Canopy cover (%)	Total chlorophyll *a* (μg cm⁻²)
Sugar (left fork)	0.4	12.3	98.2	—
Sugar (right fork)	0.4	12.3	98.2	—
Sugar	0.8	12.8	98.2	2.3
McKinley	1.0	15.3	98.1	0.8
Skunk	1.4	13.3	97.0	0.8
Dark Canyon	1.7	16.3	98.4	1.3
Barnwell	1.8	15.8	98.7	—
Fox	2.6	17.1	97.5	0.9
Deer	3.0	16.3	98.7	1.1
Redwood	7.8	15.8	92.0	2.2
Jack of Hearts	10.2	15.2	88.4	1.1
Elk	10.3	20.7	70	3.0
Elder	17.0	18.8	86.1	1.9
Rattlesnake	57.9	22.1	52.3	2.1
South Fork Eel	130.0	23.5	39.4	1.6
Ten Mile	180.0	24.4	6.9	2.1
Middle Fork Eel	1,907.2	27.0	0	0.2
Trinity	7,303.7	22.1	0	1.2
Klamath	21,696.0	23.1	0	—

Fractionation estimates for epilithic microalgae might have been biased by two factors. First, fractionation for epilithic microalgae was calculated relative to $\delta^{13}C$ of CO_{2aq} assuming that microalgae used CO_{2aq} as their primary inorganic carbon source. Fractionation calculated for HCO_3^-, rather than CO_{2aq} use by all taxa, would result in values 7‰ to 10‰ higher than for CO_{2aq}. Second, as discussed earlier, epilithon and herbivore $\delta^{13}C$ might not have always adequately represented algal $\delta^{13}C$ because of the inclusion or assimilation of terrestrial detritus (−27‰). Therefore, fractionation could be underestimated when algal $\delta^{13}C$ values were lower than terrestrial $\delta^{13}C$ and overestimated when algal $\delta^{13}C$ values were higher than terrestrial $\delta^{13}C$.

Linear regression models were used to analyze the relationship of microalgal $\delta^{13}C$ with $\delta^{13}C$ of CO_{2aq} and algal fractionation with $[CO_{2aq}]$ (i.e., $1/[CO_{2aq}]$). To examine the degree that $[CO_{2aq}]$ explained overall variation in algal $\delta^{13}C$, the relationship between algal $\delta^{13}C$ and $[CO_{2aq}]$ also was analyzed. Slopes for regression models were analyzed with *t*-tests ($P < 0.05$).

Results

Range of environmental variables—Study sites ranged from small shaded streams with low rates of microalgal photosynthesis and high $[CO_{2aq}]$ to larger, open, canopied streams and rivers with higher rates of microalgal photosynthesis rates and low $[CO_{2aq}]$ (Figs. 1, 5B; Table 1). During the winter and spring months, when epilithic algal photo-

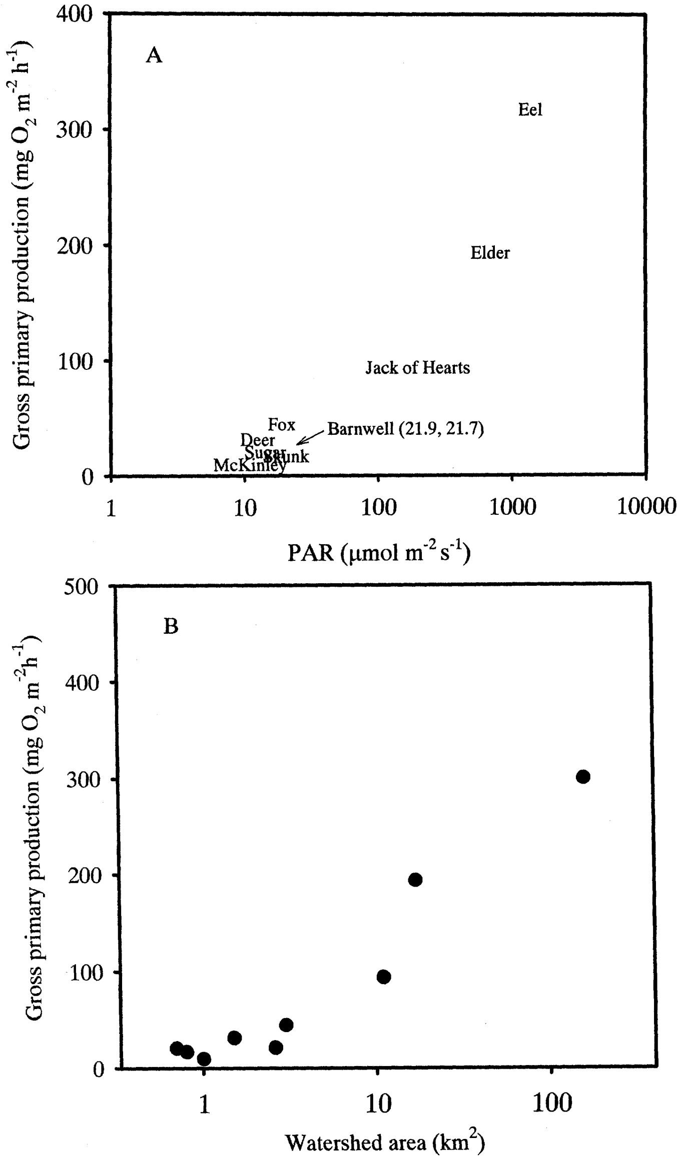

Fig. 1. Relationship between (A) watershed area and gross primary production and (B) PAR and gross primary production for epilithic algae in midsummer 1999. Each photosynthesis value represents the mean of two chamber measurements, except for the South Fork Eel River, where $n = 4$.

synthesis was probably low because of reduced water temperatures and irradiance and high turbidity, $[CO_{2aq}]$ was supersaturated with respect to atmospheric levels at all sites, with little variation between rivers and streams. Thus the range of environmental variables did not encompass a complete matrix of CO_{2aq} availability and algal productivity (i.e., no sites with high productivity and high $[CO_{2aq}]$ were sampled) but, rather, represented typical conditions found in temperate forested watersheds.

Algal biomass and photosynthesis—Microalgal biomass showed no clear pattern with increasing stream size and light levels (Table 1), perhaps as a result of heavy invertebrate grazing pressure at all sites (e.g., Lamberti and Resh 1983). Filamentous algae were abundant in some areas of larger

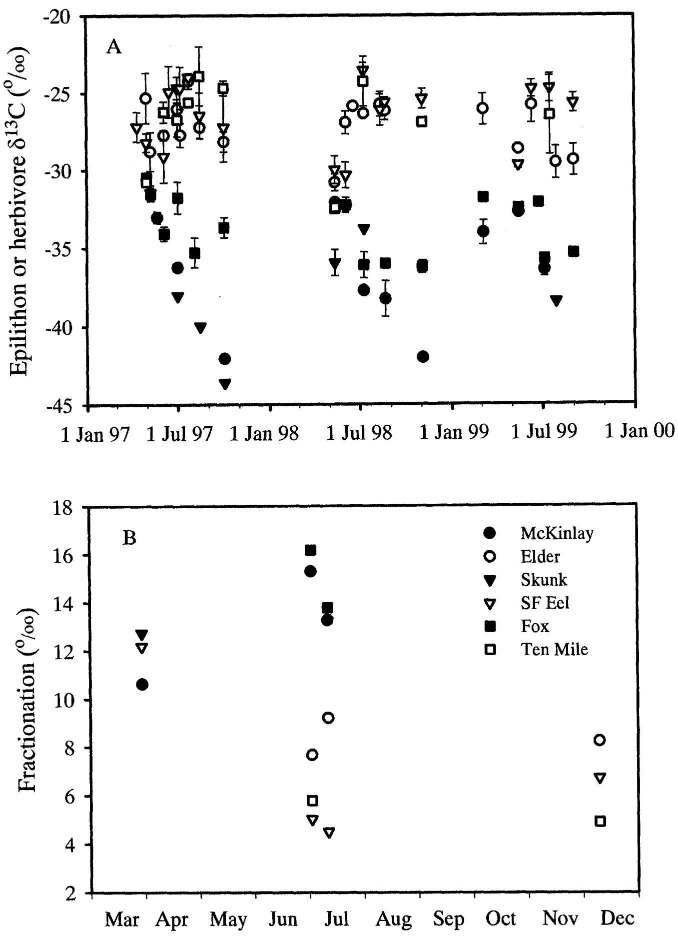

Fig. 2. Seasonal patterns in (A) riffle microalgal $\delta^{13}C$ and (B) fractionation relative to $\delta^{13}C$ of CO_2 at the six monitoring sites in the SF Eel Watershed from July 1997 through September 1999. Filled symbols represent the three shaded tributary streams, and open symbols represent the larger, more open, canopied sites. Error bars are ±SE.

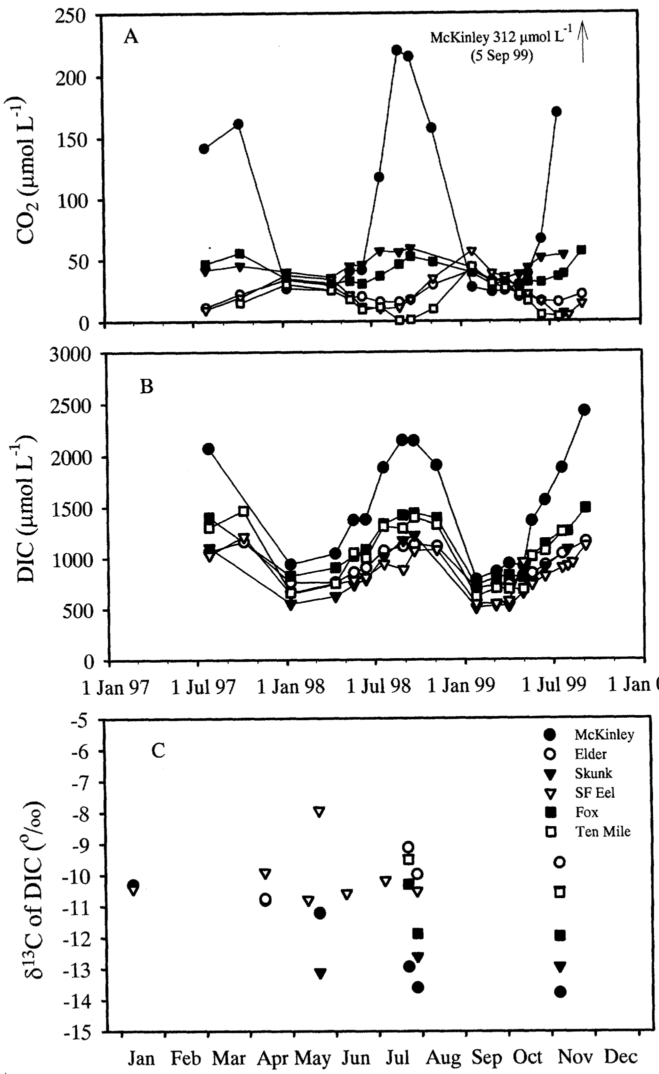

Fig. 3. Seasonal patterns in (A) $[CO_{2aq}]$, (B) DIC concentration, and (C) $\delta^{13}C$ of DIC at the six monitoring sites. Each point is the mean of duplicate samples. More data were available for $\delta^{13}C$ of DIC than for $\delta^{13}C$ of CO_2, so only DIC data are shown in panel C.

rivers (Power 1992; Finlay unpubl. data), but abundance was not quantified at most sites.

Canopy cover decreased and photosynthesis increased with stream size during midsummer (Table 1; Fig. 1A). The pattern in photosynthesis arose because of the strong effects of light on microalgal photosynthesis (Fig. 1B).

Microalgal and herbivore relationships—The relationship between epilithon and herbivore $\delta^{13}C$ in open, canopied rivers was linear and highly significant (slope = 0.96 ± 0.05, $P < 0.001$, $n = 16$, $r^2 = 0.96$). The mean difference between epilithon and herbivore $\delta^{13}C$ was −0.34 ± 0.17‰ (±SE). Most data were from the SF Eel River ($n = 9$) and Elder Creek ($n = 4$), but samples from Ten Mile ($n = 2$) and Jack of Hearts Creeks ($n = 1$) were also included.

Temporal patterns—Riffle epilithon and herbivore $\delta^{13}C$ were characterized by distinct seasonal patterns in shaded tributary streams compared with larger, more open, canopied monitoring sites. Epilithon and herbivore $\delta^{13}C$ were most similar among sites during springtime (Fig. 2A), when water temperature, $[CO_{2aq}]$, and $\delta^{13}C$ of DIC were most similar (Fig. 3A,C; temperature data not shown). Epilithon $\delta^{13}C$ decreased during summer and fall baseflows in the three smallest sites to a minimum of −44‰ (Fig. 2A). In contrast, epilithon $\delta^{13}C$ increased up to −23‰ as summer progressed in the three larger sites. Similar contrasts between small tributary streams and larger sites were observed for $[CO_{2aq}]$ and $\delta^{13}C$ of DIC. $[CO_{2aq}]$ increased in smaller streams but decreased in larger sites as summer progressed (Fig. 3A). With one exception (Fox Creek in July 1998), $\delta^{13}C$ of DIC decreased for most small streams as summer progressed while clear seasonal trends were absent at the larger sites.

As observed for CO_{2aq}, photosynthetic fractionation by epilithic algae was characterized by distinct seasonal patterns in the smallest tributary streams compared with the three larger sites. Fractionation was most similar among sites in spring but increased as summer baseflows progressed in the small streams and decreased in the larger sites (Fig. 2B).

Macroalgae showed taxon-specific temporal patterns in

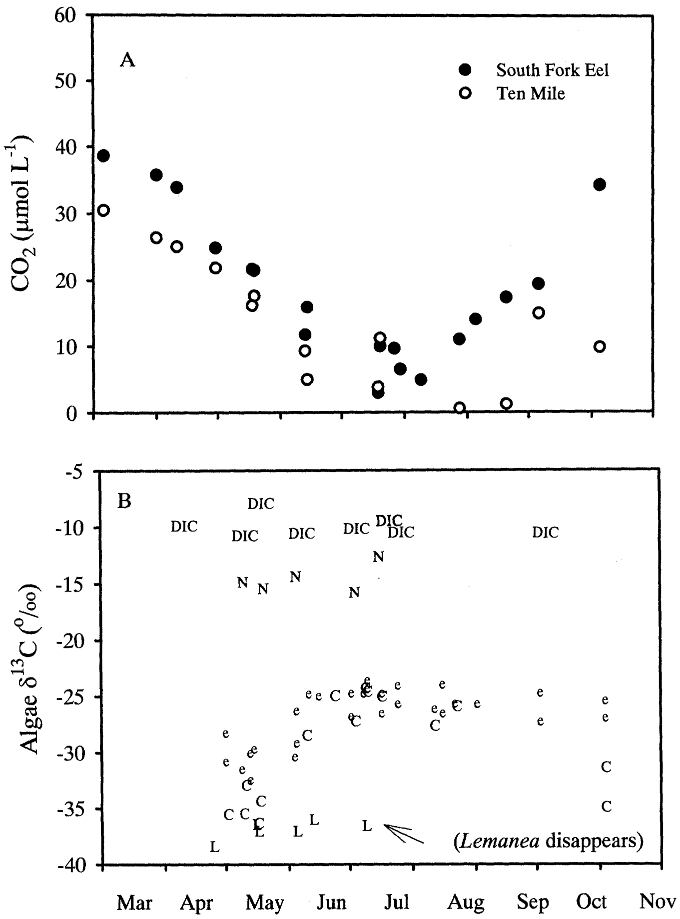

Fig. 4. Seasonal patterns in (A) [CO_{2aq}] and (B) DIC for *Nostoc pruniforme* (N), *Cladophora glomerata* (C), and *Lemanea* sp. (L) $\delta^{13}C$ for the SF Eel River and Ten Mile Creek from 1997 to 1999. Data for epilithic microalgae (e) at these sites (Fig. 2A) is also shown for comparison. $\delta^{13}C$ values are not distinguished by river because there were no clear differences in $\delta^{13}C$ between sites.

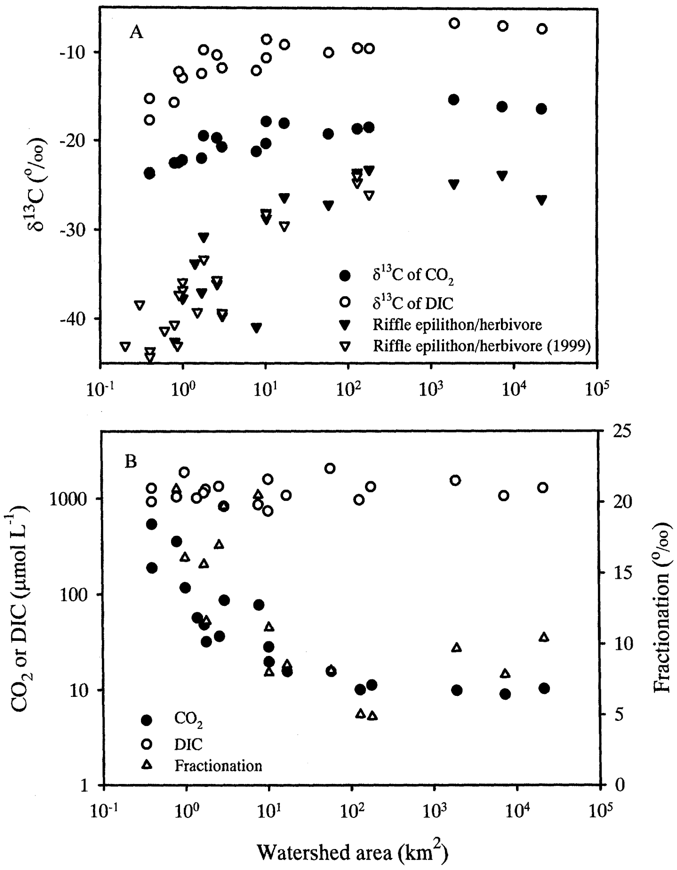

Fig. 5. Midsummer longitudinal patterns in (A) $\delta^{13}C$ of DIC and CO_2 in 1998 and microalgal $\delta^{13}C$ in 1998 and 1999, and (B) riffle microalgal fractionation, [CO_{2aq}] and DIC concentration at survey sites in 1998.

$\delta^{13}C$. Patterns of $\delta^{13}C$ of *Cladophora* resembled those of microalgae in SF Eel River and Ten Mile Creek by increasing as [CO_{2aq}] declined in summer (Fig. 4A,B). In contrast, $\delta^{13}C$ of *Nostoc* and *Lemanea* showed no temporal trends or response to seasonal changes in availability of inorganic carbon or environmental conditions. *Lemanea* $\delta^{13}C$ values were highly ^{13}C-depleted relative to all taxa except *Cladophora* in the spring, where *Nostoc* $\delta^{13}C$ values were highly ^{13}C-enriched relative to all taxa (Fig. 4A).

DIC $\delta^{13}C$ showed little temporal variation in Ten Mile Creek and the SF Eel River (Fig. 4A), indicating that the observed seasonal patterns in *Cladophora* and epilithon $\delta^{13}C$ were a result of changes in fractionation in response to variable CO_{2aq} availability at these sites. Fractionation was calculated relative to the mean value of $\delta^{13}C$ of DIC, HCO_3^-, or CO_{2aq} (−9.9‰, −9.6‰, and −19.5‰ respectively) depending on the form of inorganic carbon used by each taxon. Fractionation by *Cladophora* was calculated relative to both $\delta^{13}C$ of CO_{2aq} (minimum 4.9‰, maximum 17.5‰; Fig. 7B) and HCO_3^- (minimum 15.0‰, maximum 27.7‰, data not shown) because this taxon can use both CO_{2aq} and HCO_3^- (Raven et al. 1982). Similarly, fractionation for epilithic microalgae was calculated relative to $\delta^{13}C$ of CO_{2aq} (minimum 4.1‰, maximum 13.3‰) and HCO_3^- (minimum 14.2‰, maximum 23.5‰, data not shown) because HCO_3^- use by some of the microalgal assemblage was possible. Fractionation by *Lemanea* (18.1‰) was calculated relative to $\delta^{13}C$ of CO_{2aq} because this taxon can only use CO_{2aq} (Raven et al. 1982). Fractionation by *Nostoc* (4.9‰) was calculated relative to $\delta^{13}C$ of DIC because of active concentration of DIC (Goericke et al. 1994).

Spatial patterns—During summer baseflow conditions in 1998 and 1999, epilithon and herbivore $\delta^{13}C$ increased with watershed area across the gradient in stream size and productivity examined. Riffle epilithon and herbivore $\delta^{13}C$ increased from −44‰ in small headwater streams up to −23‰ in open, canopied rivers (Fig. 5A). The inferred increase in epilithic microalgae $\delta^{13}C$ with stream size was greatest in small headwater streams (watershed area of 0.5–15 km²) with steep gradients in $\delta^{13}C$ of DIC and [CO_{2aq}] (Fig. 5A,B). Epilithic microalgal fractionation decreased with stream size from 22‰ in the smallest streams up to 7‰ at downstream sites (Fig. 5B).

In addition to inorganic carbon concentration and $\delta^{13}C$, fractionation could have been affected by differences in water velocities among sites during midsummer. Average water

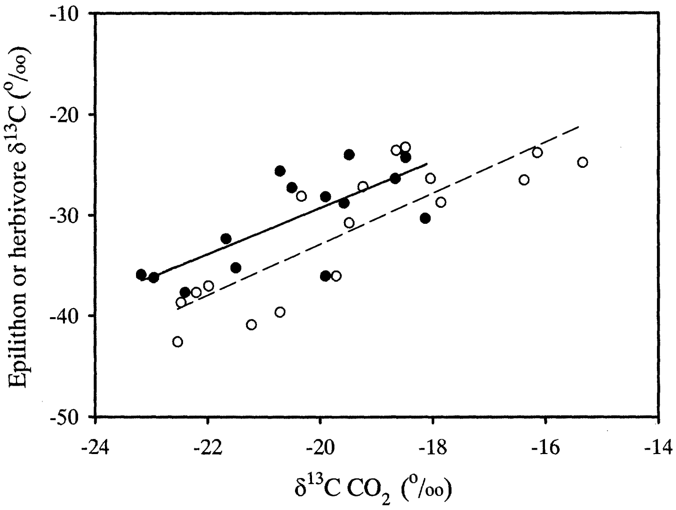

Fig. 6. Relationship between $\delta^{13}C$ of CO_2 and microalgal $\delta^{13}C$ for monitoring sites (closed circles and solid regression line; $Y = 16.4 + 2.3X$, $n = 15$, $P = 0.001$, $r^2 = 0.53$ for a linear model) and survey sites (open circles and dashed regression line; $Y = 18 + 2.5X$, $n = 17$, $P < 0.001$, $r^2 = 0.69$).

velocities increased with stream size from 0.35 m s^{-1} in small streams, 0.6 m s^{-1} in Ten Mile Creek and the SF Eel River, and around 1.0 m s^{-1} in the three largest sites. Previous research has shown a positive influence of water velocity on algal fractionation (Finlay et al. 1999). Thus, higher water velocities in downstream sites relative to tributaries suggest that the actual downstream trend of decreasing fractionation with stream size might have been stronger than shown in Fig. 5A if water velocity was held constant across sites.

Influences on algal $\delta^{13}C$—Riffle algal $\delta^{13}C$ of the most common groups were influenced by two main factors. First, spatial and temporal variation in $\delta^{13}C$ of CO_2 explained a high proportion of variance in epilithic microalgal $\delta^{13}C$ for both monitoring and survey sites (Fig. 6). Second, *Cladophora* and epilithic microalgal fractionation was strongly influenced by $[CO_{2aq}]$ (Fig. 7A–C). Inclusion of microalgal growth rates into analyses of $[CO_{2aq}]$ effects on fractionation explained less variation than CO_{2aq} availability alone (*see Fig. 7*), suggesting that $[CO_{2aq}]$ was the primary driver of fractionation across sites. However, effects of microalgal growth rates on fractionation were difficult to assess with limited measurements of photosynthesis in open, canopied sites where fractionation effects could be expected to be more important.

Across a wide spatial and temporal range, epilithic microalgal and *Cladophora* $\delta^{13}C$ values were well explained by a single variable, $[CO_{2aq}]$, which explained 76% of observed variation in microalgal $\delta^{13}C$ for monitoring sites (Fig. 8A), 80% for *Cladophora* in the SF Eel River (Fig. 8B), and 90% for survey sites during summer baseflow conditions (Fig. 8C). The strong influence of $[CO_{2aq}]$ on algal $\delta^{13}C$ arose from the effect of $[CO_{2aq}]$ on algal fractionation, as described above, and the ^{13}C depletion of CO_{2aq} derived from respiration relative to other sources of DIC (*see* Finlay 2003). Var-

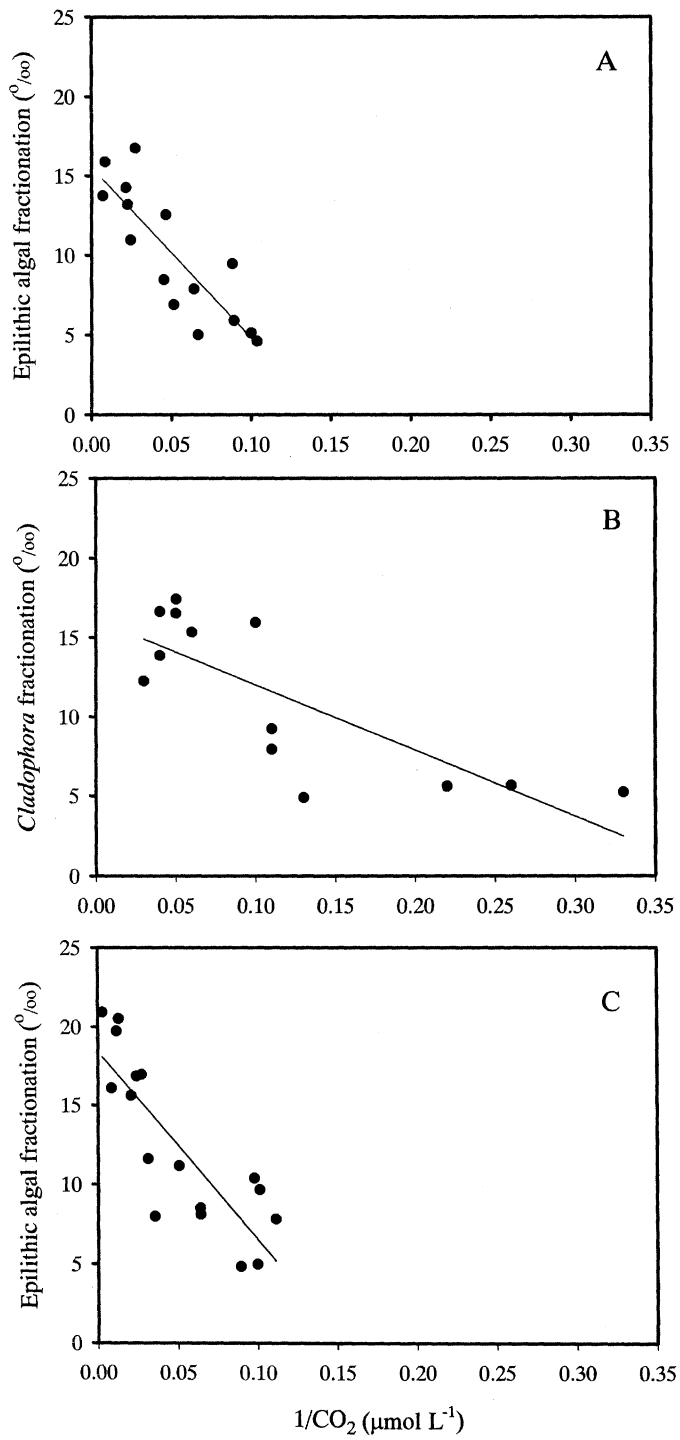

Fig. 7. Relationship between CO_{2aq} availability (i.e., $1/[CO_{2aq}]$) and algal fractionation for (A) epilithic microalgae at monitoring sites ($Y = 15.6 - 107.4X$, $P < 0.001$, $n = 15$, $r^2 = 0.71$) and (B) *Cladophora* at Ten Mile Creek and SF Eel River (data combined, $Y = 16.2 - 41.4X$, $P = 0.001$, $n = 13$, $r^2 = 0.59$) and (C) epilithic microalgae at survey sites ($Y = 18.4 - 119X$, $P < 0.001$, $n = 17$, $r^2 = 0.67$) during midsummer 1998. For panels B and C, a polynomial model provided a better fit to the data ($r^2 = 0.66$ and 0.80, respectively) than linear models. For panel C, regression analyses of a model that included algal growth rates with the use of data from Fig. 1 explained less variation in fractionation ($n = 9$, $r^2 = 0.49$) than CO_{2aq} availability alone, but few data were available from sites with high photosynthesis rates.

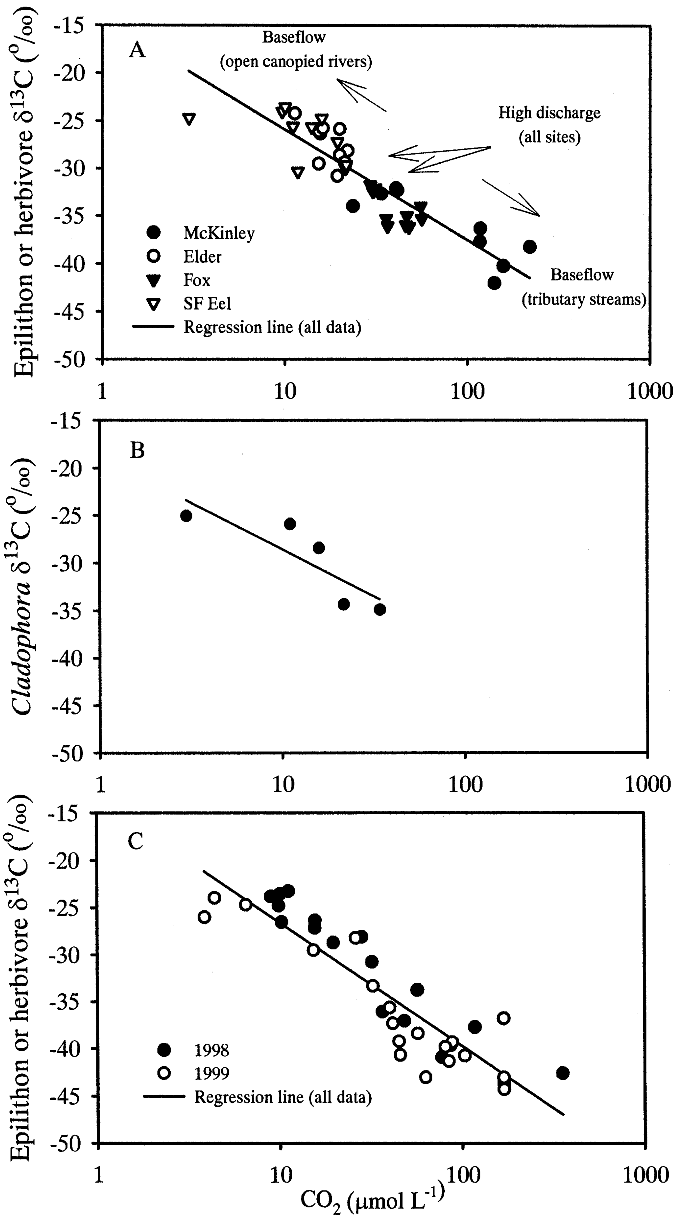

Fig. 8. Relationship between log [CO_{2aq}] and riffle algal $\delta^{13}C$ for (A) monitoring sites (B) *Cladophora* in SF Eel River, and (C) survey sites. For panel A, the regression relationship for a linear model including data for all sites was $Y = -14.3 - 11.6X$, $n = 41$, $P < 0.001$, $r^2 = 0.80$. Relationships between log [CO_{2aq}] and riffle algal $\delta^{13}C$ within sites were weaker for Elder Creek ($P < 0.060$, $r^2 = 0.30$) and the SF Eel River ($P = 0.049$, $r^2 = 0.33$, data not log transformed) than for McKinley ($P = 0.004$, $r^2 = 0.67$) or Fox Creeks ($P = 0.01$, $r^2 = 0.46$). For panel B, the relationship is shown with log-transformed [CO_{2aq}] for comparison to panels A and C. However, a regression model that used untransformed data explained more variation in algal $\delta^{13}C$ ($Y = -23.5 - 0.34X$, $n = 5$, $P = 0.026$, $r^2 = 0.80$) than the model that used transformed data ($r^2 = 0.64$). For panel C, the regression relationship for a linear model including all data was $Y = -13.4 - 13.3X$, $n = 38$, $P < 0.001$, $r^2 = 0.82$. Regression relationships were similar between 1998 ($Y = -11.1 - 13.9X$, $P < 0.001$, $n = 18$, $r^2 = 0.85$) and 1999 ($Y = -16.9 - 11.9X$, $P < 0.001$, $n = 20$, $r^2 = 0.82$).

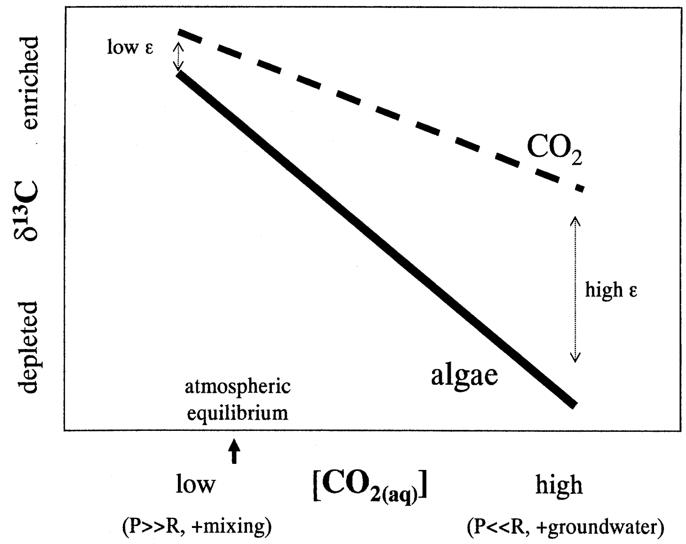

Fig. 9. Conceptual model of the role of CO_{2aq} in controlling algal $\delta^{13}C$ in lotic ecosystems for species that acquire CO_{2aq} and HCO_3^- without the use of active DIC concentrating mechanisms. The dynamics of CO_{2aq} that determine algal $\delta^{13}C$ are complex, involving multiple physical and biogeochemical processes. Briefly, algal $\delta^{13}C$ decrease with [CO_{2aq}] because of negative effects of respiratory CO_{2aq} on $\delta^{13}C$ of CO_{2aq} and positive effects on fractionation, ε. Several processes important in determining CO_{2aq} concentration and $\delta^{13}C$ are represented on the *X*-axis. "P" refers to net ecosystem primary production, "R" is ecosystem respiration, "mixing" is equilibration of atmospheric CO_{2aq} with stream DIC, and "groundwater" is addition of groundwater charged with CO_{2aq} derived from heterotrophic respiration. Nonlinear relationships between algal $\delta^{13}C$ and [CO_{2aq}] might be present at extremes in CO_{2aq} values according to Eq. 1 and as suggested by Fig. 8C, but the linear form is shown here for simplicity.

iation in microalgal $\delta^{13}C$ might have been dampened by the influence of terrestrial detritus in epilithon and herbivore samples, so the influence of [CO_{2aq}] on microalgal $\delta^{13}C$ observed here can be considered to be a conservative estimate of such effects.

Discussion

Algal $\delta^{13}C$ of common taxa were strongly related to availability of CO_{2aq} across an environmental gradient typical of lotic ecosystems in small to midsized temperate watersheds. The influence of [CO_{2aq}] on algal $\delta^{13}C$ shown here is well known for ocean phytoplankton (Kroopnick 1985; Laws et al. 1995; Lynch-Stieglitz et al. 1995; Fry 1996; Rau et al. 1996) but has not previously been examined in lotic ecosystems. The robust relationship between [CO_{2aq}] and algal $\delta^{13}C$ and its underlying mechanisms has been integrated in a conceptual model (Fig. 9) that considers the effects of source and concentration of CO_{2aq} on $\delta^{13}C$ of CO_2 and fractionation. In the model, the source of CO_{2aq} in excess of atmospheric concentrations is assumed to be heterotrophic respiration, so that increasing CO_{2aq} decreases $\delta^{13}C$ of inorganic carbon. Thus, the negative relationship between algal $\delta^{13}C$ and [CO_{2aq}] arises through the combined effects of decreasing $\delta^{13}C$ of DIC and increasing photosynthetic fractionation across a gradient in CO_{2aq} availability (Fig. 9). The quantitative nature of these relationships is likely to vary greatly among watersheds because of a wide range of observed $\delta^{13}C$ DIC values in rivers (Mook and Tan 1991) and variation in bicarbonate concentrations, which in part determine the influence of [CO_{2aq}] on $\delta^{13}C$ of dissolved inorganic carbon (Mook et al. 1974). The model, explored in further detail below, is thus intended to provide a framework for understanding the primary controls of variation in algal $\delta^{13}C$ in streams.

The role of inorganic carbon $\delta^{13}C$—Spatial and temporal variation in $\delta^{13}C$ of CO_2 played an important role in determining microalgal $\delta^{13}C$ in the watershed. The role of weathering reactions in determining $\delta^{13}C$ of DIC in streams is often emphasized (Mook and Tan 1991), but related research in the SF Eel watershed shows that stream water CO_{2aq} dynamics play an equally important role in controlling DIC $\delta^{13}C$ values (Finlay 2003). Stream water [CO_{2aq}] is negatively related to $\delta^{13}C$ of DIC in this system because conditions of high [CO_{2aq}] arise from inputs of CO_2 derived from heterotrophic respiration of ^{13}C-depleted terrestrial organic matter, whereas low [CO_{2aq}], from turbulent mixing and algal uptake, is associated with inputs of CO_2 from the atmosphere that are enriched in ^{13}C (Mook and Tan 1991; Keough et al. 1998; Finlay 2003).

As a consequence, respiratory sources of CO_{2aq} were important to microalgae in headwater streams, as indicated by ^{13}C-depleted $\delta^{13}C$ of CO_2, epilithon, and herbivore $\delta^{13}C$. In contrast, larger, more productive downstream sites showed increasing contributions of atmospheric CO_2 to algae, as observed in lake ecosystems (Schindler et al. 1972, 1997). Such changes in inorganic carbon sources for freshwater autotrophs with ecosystem productivity likely arise because the dominance of heterotrophic versus autotrophic processes determines, in part, the source of inorganic carbon in streams and rivers (Schindler et al. 1997; Finlay 2003). Patterns in inorganic carbon sources might also exist with ecosystem size in rivers and lakes because of increasing residence times and decreasing groundwater inputs with stream drainage area or lake volume, leading to greater mixing of atmospheric CO_2 relative to declining heterotrophic CO_2 inputs.

Controls of algal fractionation—Variation in fractionation was the second major influence on microalgal and *Cladophora* $\delta^{13}C$ (Fig. 9). The positive effect of [CO_{2aq}] on fractionation resulted in high values for many small streams and low values in all larger, more productive downstream sites, particularly during the summer months. Fractionation values only approached values typical of terrestrial C_3 plants ($\geq$20‰) at extremely high levels of [CO_{2aq}] in the least productive streams (Figs. 1, 5B, 7B), further demonstrating the important role of diffusive limitation on fractionation of carbon isotopes by aquatic algae relative to temperate terrestrial vegetation.

The patterns in epilithic algal and *Cladophora* fractionation also suggest strong physical or physiological consequences of CO_{2aq} availability on inorganic carbon acquisition by algae. Low [CO_{2aq}] could reduce fractionation through several specific mechanisms. First, if algae acquired CO_{2aq} via passive diffusion, then low [CO_{2aq}] could lead to diffusion-limited transport of CO_{2aq} and reduced discrimination against ^{13}C (Keeley and Sandquist 1992; Rau et al. 1996). Although I was unable to rigorously test for agreement with a diffusive model of CO_2 uptake in this study, the suggestion of nonlinearities in the relationships between CO_{2aq} availability and fractionation (Fig. 7B,C) is inconsistent with a passive diffusion model at downstream, more productive, sites (Rau et al. 1996).

An alternative explanation is increased use of HCO_3^- by algae in epilithic biofilms at low [CO_{2aq}] (Smith and Walker 1980; Sharkey and Berry 1985). Exclusive use of HCO_3^- by epilithic algae would increase fractionation estimates by 7–10‰ over values estimated for CO_{2aq}. Although HCO_3^- use would yield improbably high values in small tributary streams (i.e., 23–30‰) during midsummer, fractionation relative to HCO_3^- would yield plausible values at larger sites (i.e., 15 to 18‰).

A third hypothesis is that increased enzymatic affinity for CO_{2aq} with decreasing CO_{2aq} availability could also reduce fractionation as [CO_{2aq}] declined (Sharkey and Berry 1985; Peterson et al. 1993). Finally, increased use of active transport and concentration of DIC at low [CO_{2aq}] could also decrease fractionation. However, as seen for *Nostoc* (Fig. 4B), use of such mechanisms discriminates very little against ^{13}C, resulting in similar DIC and plant $\delta^{13}C$ values (Goericke et al. 1994). Epilithic algal fractionation data are inconsistent with the use of this method of carbon acquisition (Fig. 7A,C). However, our poor understanding of inorganic carbon use by the wide array of algal taxa present in freshwaters films prevents further resolution of the first three hypotheses at this time.

Consistently low rates of algal photosynthesis from heavy shading in all headwater streams indicated an unimportant role for variation in growth rates in influencing fractionation in small streams. Such effects were expected to be greater at more productive downstream sites (*see* MacLeod and Barton 1998) but could not be rigorously examined here because of limited measurements of photosynthesis at open, canopied sites. However, [CO_{2aq}] explained less seasonal variability in epilithic algal $\delta^{13}C$ within sites (Fig. 8A) than between sites during baseflow periods (Fig. 8C), suggesting that growth rates or other factors such as changes in species composition in epilithic biofilms might have been important during the spring and fall.

Macroalgae showed diverse $\delta^{13}C$ patterns in response to seasonal changes in [CO_{2aq}] and environmental conditions that appeared to be related to physiological differences between taxa. The most common taxon, *Cladophora,* showed similar seasonal trends to epilithic algae, but with lower $\delta^{13}C$ values in the spring and fall (Fig. 4B). This difference indicates either higher discrimination against $^{13}CO_2$ or lower use of HCO_3^- by *Cladophora* compared with epilithic algae when CO_{2aq} availability was greatest (Fig. 4B). Both mechanisms could arise from greater access to CO_{2aq} by long *Cladophora* filaments relative to the epilithic algal growth form. Lower $\delta^{13}C$ for chlorophytes compared with diatom biofilms has been widely noted in stream ecosystems (Rosenfeld and Roff 1992; Whitledge and Rabeni 1997; Evans-White et al. 2001).

In contrast to both epilithic algae and *Cladophora, Lemanea* and *Nostoc* were unresponsive to changes in inorganic carbon availability. As for *Cladophora, Lemanea* was ^{13}C-depleted relative to epilithon in the spring, but *Lemanea* $\delta^{13}C$ did not increase as $[CO_{2aq}]$ and water velocity decreased in the summer months. *Cladophora* grows in filament clusters and mats but is able to acquire inorganic carbon even when $[CO_{2aq}]$ is low with the use of HCO_3^- (Raven et al. 1982). However, *Lemanea,* like other freshwater rhodophytes and bryophytes, can only use CO_{2aq} as an inorganic carbon source (Raven et al. 1982). Furthermore, *Lemanea* has thick filaments, suggesting that low surface area to volume ratios of this macroalga could limit the effectiveness of passive diffusional CO_{2aq} transport when CO_{2aq} availability is low (Raven et al. 1982). The disappearance of *Lemanea* from the study sites in June might be related to its inability to use HCO_3^- for photosynthesis because $[CO_{2aq}]$ is less available (Fig. 4A) but could also be because of lower water velocities or higher temperatures or because of limitation by other nutrients.

Nostoc $\delta^{13}C$ were highly ^{13}C-enriched and showed no response to availability of inorganic carbon or environmental conditions. This contrast is likely a result of active concentration of DIC by this taxon, a process that results in low fractionation (Goericke et al. 1994). Consequently, *Nostoc* and *Lemanea* represent "extremes" in methods of inorganic carbon acquisition that produced distinct patterns in $\delta^{13}C$ compared with *Cladophora* or epilithic diatoms.

Algal $\delta^{13}C$ and the distinction of carbon sources in lotic food webs—Natural abundance stable isotope techniques have advantages over observational studies for understanding trophic dynamics in food webs (Rounick and Winterbourn 1986; Vander Zanden et al. 1997; Peterson 1999). In lotic ecosystems, and elsewhere, stable isotope analyses are increasingly used to distinguish carbon sources to food webs (Finlay 2001). Successful use of this approach is contingent on distinct $\delta^{13}C$ values of two potential carbon sources (France 1995, 1996; Doucett et al. 1996*a*; Finlay 2001) and adequate statistical characterizations of variability in populations of interest (Lancaster and Waldron 2001; Phillips and Gregg 2001). However, poor understanding of algal $\delta^{13}C$ has limited the ability of investigators to determine the efficacy of $\delta^{13}C$ as a tracer of carbon sources.

Assuming that the conceptual model presented in Fig. 9 is broadly applicable to stream ecosystems, simple direct or indirect measurements of CO_{2aq} availability (i.e., pCO_2 or pH and alkalinity) could be used to predict $\delta^{13}C$ values for common, edible, algal forms for the purposes of evaluation and design of natural abundance $\delta^{13}C$ techniques in food web studies or to help interpret spatial patterns and temporal trends in consumer $\delta^{13}C$. Furthermore, the influence of CO_{2aq} might provide insight into situations that favor or limit distinction of algal $\delta^{13}C$ from terrestrial detritus $\delta^{13}C$ (i.e., ca. $-27‰$ for C_3 plants) in temperate watersheds. Specifically, algal and terrestrial $\delta^{13}C$ might be most distinct when autotrophic production greatly exceeds or is greatly exceeded by in situ heterotrophic respiration or inputs of CO_{2aq} from groundwater. In strongly autotrophic ecosystems, limited CO_{2aq} supply relative to photosynthetic demand might increase algal $\delta^{13}C$ to values greater than $-27‰$ because of increased $\delta^{13}C$ of CO_2 from atmospheric invasion of CO_2 and reduced fractionation (Fig. 9). Conversely, in strongly heterotrophic ecosystems or those affected by groundwater inputs containing CO_{2aq} from plant root or soil microbial respiration, high concentrations of respiratory CO_{2aq} might decrease $\delta^{13}C$ of CO_2 and increase fractionation, reducing algal $\delta^{13}C$ below the range of terrestrial detritus $\delta^{13}C$.

Observations from temperate rivers (Finlay 2001) and lakes (e.g., Schindler et al. 1997) support the prediction that the balance between autotrophic and heterotrophic metabolism and groundwater inputs of CO_{2aq} broadly influence algal $\delta^{13}C$. However, the quantitative nature of such relationships can be modified by several factors. First, mixing with the atmosphere might reduce the influence of stream carbon cycling on algal $\delta^{13}C$. High discharge in steep streams enhances evasion of respiratory CO_2 and invasion of atmospheric CO_2 (Genereux and Hemond 1992). High water velocity would greatly decrease boundary layer limitation of CO_{2aq} supply to algae, and mixing of atmospheric CO_2 ($-8‰$) would provide a similar $\delta^{13}C$ of CO_2 as is available to terrestrial plants. Thus, high gas exchange rates with atmosphere should drive algal $\delta^{13}C$ toward those of terrestrial C_3 plants.

Second, variation in weathering processes could produce extreme values of $\delta^{13}C$ of DIC that could greatly alter the relationships between $[CO_{2aq}]$ and algal $\delta^{13}C$ shown in Fig. 8. Carbonate-rich or carbonate-free bedrock might yield ^{13}C-enriched or depleted values of $\delta^{13}C$ of DIC, respectively (Mook and Tan 1991; Kendall et al. 1992), that would deviate from typical values for temperate rivers (Mook and Tan 1991; Finlay 2003).

Although increased predictive understanding of variation in isotope values at the base of food webs will improve the use of $\delta^{13}C$ as a food web tracer, consideration of spatial and temporal variation in isotope values remains an important aspect of $\delta^{13}C$ techniques. Temporal variability in algal $\delta^{13}C$ could be considerable in streams when factors that influence algal $\delta^{13}C$ ($[CO_{2aq}]$, $\delta^{13}C$ of DIC, photosynthetic rates) are variable over short time periods. Changes in discharge could strongly influence all of these factors in small streams (e.g., Meyer et al. 1988; Stevenson 1990; Pinol and Avila 1992; Finlay 2003). Unless both algal $\delta^{13}C$ and tissue turnover times of consumers are well known, use of algal $\delta^{13}C$ to distinguish carbon sources to stream food webs might be most effective during periods of stable stream discharge.

Similarly, the spatial scale of trophic interactions must be considered when using natural abundance $\delta^{13}C$ measurements to distinguish carbon sources to lotic food webs. In food webs involving mobile predators, prey, or organic matter, spatial variability in algal $\delta^{13}C$ must be evaluated at the scale that the study organisms interact to effectively use $\delta^{13}C$ to trace organic matter sources. Factors that influence algal $\delta^{13}C$, such as $[CO_{2aq}]$, $\delta^{13}C$ of DIC, algal photosynthesis, and water velocity (Peterson et al. 1993; MacLeod and Barton 1998; Finlay et al. 1999; Finlay 2003), are often spatially variable in stream ecosystems.

Spatial and temporal variation in autotroph $\delta^{13}C$ in freshwater ecosystems is increasingly evident (Keeley and Sandquist 1992; Boon and Bunn 1994; France 1995; Doucett et al. 1996*b*; Finlay et al. 1999; Finlay 2001, this study; Zah et al. 2001; McCutchan and Lewis 2002), and it is clear that such variation must be addressed for successful use of $\delta^{13}C$ as a food web tracer. Although autotroph variation might preclude applications of $\delta^{13}C$ techniques at some scales or for some uses, pairing $\delta^{13}C$ measurements with other tracers and techniques, such as other isotopes, organism growth data, or tissue turnover measurements, might greatly increase the power of stable isotope data (Finlay 2001; Cloern et al. 2002; McCutchan and Lewis 2002). The strong causal relationship between biogeochemical variables and algal $\delta^{13}C$ shown here should refine the use of natural abundance stable carbon isotopes in analyses of sources and fluxes of carbon in food web studies.

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