

Dissolved carbon dioxide concentration controls baseline stable carbon isotope signatures of a lake food web

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Abstract

Temporal variation in the baseline stable carbon isotope ($\delta^{13}\text{C}$) value of a well-studied, productive lake was examined over a 26-yr period using archived samples of the herbivorous zooplankter *Daphnia galeata* as a proxy because of its phytoplankton diet. The baseline $\delta^{13}\text{C}$ value was strongly correlated with pH and the concentration of dissolved carbon dioxide in the lake as well as with the $\delta^{13}\text{C}$ value of a predatory zooplankter. An isotopic fractionation model incorporating algal physiology (the growth rate, surface area, and carbon content of the main phytoplankton species) and the dissolved carbon dioxide concentration in the lake was used to predict, successfully, the baseline $\delta^{13}\text{C}$ values of the lake over the study period. In aquatic ecosystems where the concentration of dissolved carbon dioxide is temporally variable, the baseline $\delta^{13}\text{C}$ value can be more clearly defined by taking the concentration of CO_2 into account. This approach will allow food web relationships to be quantified more accurately.

The rapidly increasing use of stable isotopes as an ecological tool has enabled researchers to examine food web structure, carbon fluxes, and migration at the ecosystem scale more easily (Grey 2006; Michener and Lathja 2007). In order to characterize food web structure and quantitatively assess trophic positions of organisms both within and among ecosystems, there is a need for not only adequate estimates of trophic fractionation between a consumer and its putative prey but also a reliable measure of an isotopic baseline, which is generally assumed to be static (Woodland et al. 2012b). In some ecosystems, strong seasonal variation in the isotopic baseline can occur, especially in productive lakes (Harrod and Grey 2006; Gu et al. 2011). In particular, the stable carbon isotope values ($\delta^{13}\text{C}$) of the phytoplankton and periphyton that form the base of aquatic food webs have been shown to be highly variable, most notably over the summer when sampling effort is typically greatest (Finlay 2004; Gu et al. 2011).

Primary consumers, such as bivalves and snails, have been used as isotopic baselines to overcome the strong seasonal variability in the isotopic composition of primary producers since these organisms are long lived and so can integrate or smooth the isotopic variation of their food source (Cabana and Rasmussen 1996). However, the isotopic composition of these large primary consumers can also show substantial seasonal shifts that may introduce significant errors when they are used as baselines in isotopic mixing models (Woodland et al. 2012a). Another attempt to address the variation in the isotopic composition of primary producers has been to incorporate changes into isotopic mixing models that allow for a dynamic baseline (Woodland et al. 2012b). This approach is promising, but it requires multiple measurements of isotopic baseline values of the ecosystem to assess the rate and magnitude of change. Thus, the ability to predict variations in the isotopic baseline of a lake using an easily measurable proxy would enable such modeling

approaches to be used more widely and could potentially reduce their error.

Lakes are frequently out of equilibrium with the atmosphere (Cole et al. 1994), and productive lakes can experience substantial drawdown of CO_2 concentrations during periods of high productivity in the summer (Maberly 1996). During these times, continued carbon uptake by phytoplankton depends on carbon concentrating mechanisms (CCMs), many of which rely on active uptake of bicarbonate (Raven et al. 2011). Since bicarbonate is less depleted in ^{13}C than CO_2 (Mook et al. 1974), uptake of these different forms of inorganic carbon can alter the organic $\delta^{13}\text{C}$ value (Maberly et al. 1992). Alternatively, phytoplankton may use CCMs to concentrate CO_2 as a preferred carbon source but with reduced isotopic fractionation associated with its fixation during photosynthesis (Fogel and Cifuentes 1993). Both of these mechanisms will produce organic carbon that is less depleted than that where uptake relies on passive diffusion of CO_2 .

In both lakes and rivers, the $\delta^{13}\text{C}$ values of phytoplankton and periphyton have been shown to correlate strongly with the concentration of dissolved carbon dioxide ($[\text{CO}_{2(\text{aq})}]$) (Finlay 2004; Gu et al. 2011). However, although models have been developed to use $[\text{CO}_{2(\text{aq})}]$ to predict isotopic fractionation during photosynthesis in marine phytoplankton (Cassar et al. 2006; Hopkinson et al. 2011), relatively few studies have attempted to model the $\delta^{13}\text{C}$ value of freshwater phytoplankton within a lake (but see Yoshioka 1997). In addition, the work that has been done has included data over short time scales (1–2 yr) or comparisons across multiple lakes (Karlsson et al. 2003; Bade et al. 2006) and in some cases has failed to include lakes in which $[\text{CO}_{2(\text{aq})}]$ is below a threshold for the activation of CCMs that affect isotopic fractionation during photosynthesis (approximately $< 10 \mu\text{mol L}^{-1}$; Fogel and Cifuentes 1993; Lehmann et al. 2004).

The herbivorous, nonselective grazing zooplankton (*Daphnia* spp.) has been shown to reflect, consistently and

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accurately, the δ¹³C value of its phytoplankton diet (Marty and Planas 2008) and is thus a reliable proxy for the pelagic baseline δ¹³C value (δ¹³C_{baseline}) of a lake ecosystem (Matthews and Mazumder 2003). In addition, preserved samples of zooplankton are suitable for stable isotope analysis (Feuchtmayr and Grey 2003; Syväranta et al. 2008), and they can provide a valuable means of assessing the response of an ecosystem to a major perturbation (Maguire and Grey 2006; Grey et al. 2009). Therefore, analysis of the δ¹³C value of preserved *Daphnia* spp. samples over a long period with substantial fluctuations in the [CO_{2(aq)}] of a lake ecosystem would allow the hypothesis that variation in δ¹³C_{baseline} of a lake is driven by changes in [CO_{2(aq)}] to be tested.

The purpose of this study was to examine if the [CO_{2(aq)}] in a productive lake could be used to model δ¹³C_{baseline} for the ecosystem. In addition, this work sought to test the ability of an isotopic fractionation model based on algal physiology to predict δ¹³C_{baseline} in order to explain the correlations between [CO_{2(aq)}] and δ¹³C_{baseline} that have been observed in lake ecosystems.

Methods

Study site and field sampling—Windermere is England's largest lake and is located in the English Lake District at 54°22'N, 2°57'W. It is one of the best-studied lakes in the world with long-term records extending back to the 1930s. Details of its characteristics can be found in Talling (1999) and Reynolds and Irish (2000). Zooplankton was collected at two deep, well-established sampling sites in Windermere. In the North Basin of Windermere, zooplankton was collected with vertical tows from a depth of 40 m in approximately 60 m of water using a 120-μm-mesh net (30-cm diameter). In the South Basin, zooplankton was collected with vertical tows of the entire water column (approximately 40 m) using the same type of net. Zooplankton was immediately killed with a small amount of 70% ethanol and later transferred into vials and stored in 4% formaldehyde (for samples from the North Basin) or in 70% ethanol (for samples from the South Basin).

Samples from the North Basin were chosen from a sample archive at approximately monthly intervals from May to September for the following years: 1985, 1987, 1989, 1992, 1993, 1994, 2000, 2001, 2002, 2003, 2004, 2009, and 2010. In addition, monthly samples from January to December were selected in 1986 to include instances of higher [CO_{2(aq)}] during the winter. Samples from the South Basin were taken at approximately monthly intervals from May to September for 2006 and 2010. For a few samples, there were insufficient numbers of particular zooplankton species for stable isotope analysis.

All samples were thoroughly rinsed with deionized water, and typically 50–200 individuals of the herbivorous zooplankton *Daphnia galeata* (Sars) and the predatory zooplankton *Cyclops strenuus abyssorum* (Sars) were sorted and placed into microcentrifuge tubes. These were oven dried at 60°C for 24–48 h prior to stable isotope analysis. The calcium concentration and alkalinity of Windermere are low and thus preclude the need for acid treatment of the

zooplankton to remove possible carbonate precipitates. Additional zooplankton samples were collected in 2005 from the same sampling sites in both basins with vertical tows of the entire water column using the same types of net. However, the zooplankton was retained in ambient lake water (generally for 2–4 h but never more than 24 h) to allow for gut clearance sensu Feuchtmayr and Grey (2003) before being transferred onto a nylon mesh and frozen for storage. After thawing, the same zooplankton species were sorted into microcentrifuge tubes, refrozen and then lyophilized at –55°C prior to stable isotope analysis.

Physical and chemical analyses—The temperature, alkalinity, and pH of the North and South Basins of the lake were measured weekly from 1985 to 1991 and biweekly from 1992 to 2010. Alkalinity was determined by Gran titration (Mackereth et al. 1978), and pH was determined using a calibrated pH electrode corrected for differences in temperature between the lake and the water sample. The concentration of dissolved carbon dioxide (free CO₂) was calculated from the pH, alkalinity, and temperature of the lake (Mackereth et al. 1978). For comparisons with the measured zooplankton δ¹³C values as well as inputs for the isotopic fractionation model, the [CO_{2(aq)}] and pH measurements were averaged (geometric mean) between those made on the same date as the δ¹³C value and the measurements taken 2 weeks previously. This is referred to as the biweekly average and was calculated to account for a slower isotopic turnover in zooplankton relative to phytoplankton. The relationships between zooplankton δ¹³C values and the [CO_{2(aq)}] and pH measurements made on the same date were also examined for comparison. In addition, the results were compared with the relationships of the δ¹³C values of particulate organic matter (POM) and [CO_{2(aq)}] in Windermere and four other lakes from the literature.

Stable isotope analysis—Approximately 0.6 mg of dried zooplankton was weighed into tin capsules, then combusted in a Flash Elemental Analyzer (1112 series; Thermo-Finnigan) coupled to a Finnigan DeltaPlus isotope ratio mass spectrometer (Thermo-Finnigan), apart from the samples from 2005 when a Costech Elemental Combustion System 4010 was used. The instruments were run in continuous flow mode, allowing simultaneous measurement of stable carbon isotope values and atomic C:N ratios based on calibration with a urea or acetanilide standard. The weight percentages of carbon and nitrogen were converted to atomic C:N ratios. Stable carbon isotope values are reported using the delta notation in per mil (‰) relative to Vienna Pee Dee Belemnite. The accuracy and precision of measurement was independently verified using the National Institute of Standards and Technology sucrose standard (RM 8542). Precision of the instruments (± 0.2‰) was verified by repeated analysis of a laboratory standard (fish muscle) that was interspersed regularly throughout the sample runs. The δ¹³C values of all of the zooplankton samples were corrected for their varying lipid content based on their atomic C:N ratios (Smyntek et al. 2007).

Isotopic fractionation model—An isotopic fractionation model based on algal physiology (Cassar et al. 2006) was used to predict $\delta^{13}\text{C}_{\text{baseline}}$ in the lake as a function of $[\text{CO}_{2(\text{aq})}]$. The isotopic fractionation caused by uptake and photosynthesis of $\text{CO}_{2(\text{aq})}$ (ε_p), assuming active transport of $\text{CO}_{2(\text{aq})}$ into the algal cell, was calculated as

$$\varepsilon_p = \left(\frac{\delta^{13}\text{C}_{\text{CO}_{2(\text{aq})}} + 10^3}{\delta^{13}\text{C}_{\text{baseline}} + 10^3} - 1 \right) \times 10^3 \quad (1)$$

$$= \varepsilon_t + (\varepsilon_{\text{fix}} - \varepsilon_t) \times \left(\frac{\text{PCi}}{\text{PCi} + \mu\text{C}} \right) \left(\frac{\text{P}'\text{Cc}}{\text{P}'\text{Cc} + \mu\text{C}} \right)$$

where $\delta^{13}\text{C}_{\text{CO}_{2(\text{aq})}}$ is the $\delta^{13}\text{C}$ value of the dissolved carbon dioxide in the lake (‰), $\delta^{13}\text{C}_{\text{baseline}}$ is the $\delta^{13}\text{C}$ value of *D. galeata* (‰), ε_t is the isotopic fractionation associated with diffusion and active transport = 1‰, ε_{fix} is the isotopic fractionation associated with enzymatic carboxylation = 27‰, P is the permeability of the plasmalemma to $\text{CO}_{2(\text{aq})}$ ($\text{L d}^{-1} \text{ cell}^{-1}$), Ci is $[\text{CO}_{2(\text{aq})}]$ inside the algal cell and is assumed be equal to $[\text{CO}_{2(\text{aq})}]$ in the lake ($\mu\text{mol L}^{-1}$), μ is the growth rate of the algal cell (d^{-1}), C is the carbon content of the cell ($\mu\text{mol C cell}^{-1}$), P' is the permeability of the chloroplast membrane to $\text{CO}_{2(\text{aq})}$ ($\text{L d}^{-1} \text{ cell}^{-1}$), and Cc is $[\text{CO}_{2(\text{aq})}]$ inside the chloroplast ($\mu\text{mol L}^{-1}$) calculated from this cubic equation:

$$\text{Cc}^3 + \left(\frac{\text{C}\mu}{2\text{P}'} - [\text{CO}_{2(\text{aq})}] \right) \times \text{Cc}^2 - \chi^2 \times \left(\frac{\text{C}\mu}{2\text{P}'} \right) = 0 \quad (2)$$

where χ describes the ratio of the energetic costs associated with the maintenance of Rubisco and the active transport of inorganic carbon across the chloroplast membrane (Cassar et al. 2006).

Biovolume data on the phytoplankton community in the North Basin of Windermere (S. C. Maberly unpubl. data) from the years analyzed between 1985 and 2003 were used to calculate several parameters for the model. A mean carbon content per cell (C; $3.33 \pm 0.75 \times 10^{-4} \mu\text{mol C cell}^{-1}$) of the algal community was calculated from literature values of the carbon content of algal cells, colonies, or filaments of the 15 most abundant algal species by biovolume on each sampling date. Similarly, a mean surface area was calculated from these biovolume data using literature values in order to estimate the permeability of the plasmalemma to CO_2 for the algal community (P; $2.55 \pm 0.65 \times 10^{-5} \text{ L d}^{-1} \text{ cell}^{-1}$). This was done by multiplying the mean surface area by $5 \times 10^{-5} \text{ m s}^{-1}$, an average permeability of the plasmalemma to CO_2 (Cassar et al. 2006). Values for P' ($0.05 \times \text{P}$) and χ ($223 \mu\text{mol L}^{-1}$) were taken from the model results of Cassar et al. (2006). Finally, a cellular growth rate (μ) representative of the temperature, light, and nutrient conditions as well as the phytoplankton community composition of Windermere was chosen as 0.23 d^{-1} based on an optimization of the model's fit with the observed data. This is a cell replication rate rather than a rate of increase in the total abundance of the algal community, and it is similar to reconstructed replication rates for algal species in a small lake that drains into Windermere as well as modeled rates for algal species in Windermere (see Reynolds 2006, table 5.4).

The modeled values of ε_p were used in conjunction with Eq. 1 and the mean $\delta^{13}\text{C}_{\text{CO}_{2(\text{aq})}}$ of the lake to calculate $\delta^{13}\text{C}_{\text{baseline}}$. The mean $\delta^{13}\text{C}_{\text{CO}_{2(\text{aq})}}$ ($-14.6 \pm 1.4\text{‰}$) was calculated from six measurements of the $\delta^{13}\text{C}$ values of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) taken in the North and South Basins of Windermere between April and October 2005 (S. C. Maberly unpubl. data) and from temperature data using the approach of Mook et al. (1974).

Statistical analyses—Statistical analyses were performed using Microsoft Excel or MiniTab 16 software, and residuals were checked for normality and heteroscedasticity. Data that were not normally distributed or that had unequal variances were subject to a Box-Cox transformation prior to statistical tests. Statistical significance was defined at a level of $\alpha = 0.05$ unless stated otherwise.

Results

Fluctuations in lake chemistry and $\delta^{13}\text{C}_{\text{baseline}}$ —The pH and dissolved carbon dioxide concentration exhibited high seasonal (May–September) and interannual variation in the North and South Basins of Windermere, while the alkalinity varied to a much lesser extent (Table 1). The concentration of dissolved carbon dioxide ($[\text{CO}_{2(\text{aq})}]$) varied by nearly three orders of magnitude from 0.1 to $98.4 \mu\text{mol L}^{-1}$ (Table 1). Over the summer (May–September), $[\text{CO}_{2(\text{aq})}]$ was typically highest in early May or late September with minima in July or August (Fig. 1A). The summer means (\pm SD) ranged from $8.3 \pm 9.9 \mu\text{mol L}^{-1}$ to $29.9 \pm 10.9 \mu\text{mol L}^{-1}$ for different years.

The $\delta^{13}\text{C}$ values of *Daphnia* ($\delta^{13}\text{C}_{\text{Daph}}$) and *Cyclops* ($\delta^{13}\text{C}_{\text{Cycl}}$) varied widely over the study period, particularly for *Daphnia*, which, for example, changed by up to 12.5‰ over the summer and became enriched in ^{13}C by 7.4‰ in a period of only 4 weeks during 2003. Values of $\delta^{13}\text{C}_{\text{Daph}}$ were more variable than $\delta^{13}\text{C}_{\text{Cycl}}$ in both basins (Fig. 1; Table 1). $\delta^{13}\text{C}_{\text{Daph}}$ and $\delta^{13}\text{C}_{\text{Cycl}}$ rapidly increased when $[\text{CO}_{2(\text{aq})}]$ dropped below $10 \mu\text{mol L}^{-1}$, with a mean increase of 5.7‰ and 3.4‰, respectively, compared with those where $[\text{CO}_{2(\text{aq})}] \geq 10 \mu\text{mol L}^{-1}$. In both basins, $\delta^{13}\text{C}_{\text{Daph}}$ for samples collected when $[\text{CO}_{2(\text{aq})}] < 10 \mu\text{mol L}^{-1}$ were significantly enriched in ^{13}C relative to those collected when $[\text{CO}_{2(\text{aq})}] \geq 10 \mu\text{mol L}^{-1}$, as were $\delta^{13}\text{C}_{\text{Cycl}}$ in the North Basin (Table 1). There were no significant long-term trends in $\delta^{13}\text{C}_{\text{Daph}}$ or $\delta^{13}\text{C}_{\text{Cycl}}$ over the time period studied, although there were substantial interannual differences of up to 4.7‰ and 4.4‰ between summer means for $\delta^{13}\text{C}_{\text{Daph}}$ and $\delta^{13}\text{C}_{\text{Cycl}}$, respectively. For the years in which monthly $\delta^{13}\text{C}_{\text{Daph}}$ values were available from May to September, there were no significant differences between years (one-way ANOVA, $F = 1.53$, $\text{df} = 44$, $p = 0.182$), but there were significant differences in the standard deviations between the years (Bartlett's test, $\chi^2 = 17.00$, $\text{df} = 44$, $p = 0.030$).

$\delta^{13}\text{C}_{\text{Daph}}$ was significantly correlated with the biweekly average $[\text{CO}_{2(\text{aq})}]$ and pH values of the lake (Fig. 2A; Table 2). $\delta^{13}\text{C}_{\text{Cycl}}$ was also significantly correlated with the biweekly average pH and $[\text{CO}_{2(\text{aq})}]$ in the lake, although these relationships were weaker than those for $\delta^{13}\text{C}_{\text{Daph}}$ (Table 2). Correlations between $\delta^{13}\text{C}_{\text{Daph}}$ and the $[\text{CO}_{2(\text{aq})}]$

Table 1. Mean (standard deviation), range, and number of samples analyzed for parameters from the North and South Basins of Windermere. Mean δ¹³C values of *Daphnia* (δ¹³C_{Daph}) and *Cyclops* (δ¹³C_{Cycl}) within each basin for [CO_{2(aq)}] above and below the 10-μmol L⁻¹ threshold are denoted by a different letter (A, B, C) if they were significantly different from each other (one-way ANOVA, Tukey's family error rate; North Basin: $F_{154} = 40.54$, $p < 0.001$; South Basin: $F_{27} = 5.01$, $p = 0.008$).

Parameter	North Basin	Range	<i>n</i>	South Basin	Range	<i>n</i>
pH (units)	7.58 (0.53)	6.78–9.65	233	7.95 (0.61)	7.30–9.51	33
Alkalinity (mmol L ⁻¹)	0.228 (0.032)	0.160–0.333	233	0.296 (0.013)	0.276–0.318	33
Surface temperature (°C)	14.2 (4.2)	3.0–23.1	233	15.7 (4.4)	5.6–24.1	33
[CO _{2(aq)}] (μmol L ⁻¹)	23.2 (17.0)	0.1–98.4	233	14.8 (11.8)	0.1–44.1	33
δ ¹³ C _{Daph} (‰)	-29.1 (3.0)	-33.7 to -20.1	77	-25.3 (3.6)	-31.0 to -18.1	13
δ ¹³ C _{Daph} (‰) for [CO _{2(aq)}] < 10 μmol L ⁻¹	-24.8 (3.2) ^A	-33.7 to -20.1	15	-23.4 (3.2) ^A	-28.0 to -18.1	8
δ ¹³ C _{Daph} (‰) for [CO _{2(aq)}] ≥ 10 μmol L ⁻¹	-30.1 (1.8) ^B	-33.1 to -24.2	62	-28.2 (2.0) ^B	-31.0 to -25.5	5
δ ¹³ C _{Cycl} (‰)	-27.9 (2.3)	-32.7 to -22.6	78	-25.0 (2.3)	-28.4 to -20.8	15
δ ¹³ C _{Cycl} (‰) for [CO _{2(aq)}] < 10 μmol L ⁻¹	-25.3 (2.0) ^A	-29.8 to -22.6	15	-24.2 (2.2) ^A	-28.1 to -20.8	9
δ ¹³ C _{Cycl} (‰) for [CO _{2(aq)}] ≥ 10 μmol L ⁻¹	-28.5 (1.9) ^C	-32.7 to -23.5	63	-26.0 (1.8) ^{A,B}	-28.4 to -23.7	6

or pH values measured on the same date were slightly weaker than the same comparisons with the biweekly average values and less responsive at lower [CO_{2(aq)}] (Table 2), but they were not examined in detail since δ¹³C_{Daph} may not reflect rapid fluctuations in [CO_{2(aq)}] or pH values because of the time required for tissue turnover in *Daphnia* (see Discussion). Correlations between the δ¹³C value of particulate organic matter (δ¹³C_{POM}) and [CO_{2(aq)}] obtained from the literature for Windermere and several other productive lakes were also strong and significant (Table 2).

Predictions from an isotopic fractionation model—The isotopic fractionation model derived all its parameters from field observations, biovolume calculations, and literature values, with the exception of growth rate, which was selected to optimize the model's fit with the observed data. The mean isotopic fractionation (ε_p) that occurred during CO_{2(aq)} uptake and photosynthesis was calculated as 14.2 ± 3.7‰ using the fractionation model and [CO_{2(aq)}], which agreed well with the observed value of 14.4 ± 3.5‰. The modeled ε_p values gradually increased with [CO_{2(aq)}] from 13.8‰ to 18.1‰ for [CO_{2(aq)}] ≥ 10 μmol L⁻¹ with a mean of 15.9 ± 1.0‰ (Fig. 2B). They rapidly decreased below this concentration, dropping as low as 2.2‰ for [CO_{2(aq)}] = 0.2 μmol L⁻¹. Model-predicted δ¹³C_{baseline} values were not significantly different from observed values both above and below the 10 μmol L⁻¹ threshold (paired *t*-tests, $t = 0.118$, $df = 66$, $p = 0.906$, and $t = 1.353$, $df = 22$, $p = 0.190$, for [CO_{2(aq)}] ≥ 10 μmol L⁻¹ and [CO_{2(aq)}] < 10 μmol L⁻¹, respectively) as well as over the entire range of [CO_{2(aq)}] (paired *t*-test, $t = 1.019$, $df = 89$, $p = 0.311$). Predicted δ¹³C_{baseline} values were also significantly correlated with the observed δ¹³C_{baseline} values (Fig. 3; Table 2), and the slope and the y-intercept were not significantly different from 1 and 0, respectively (*t*-tests, $t = 1.895$, $df = 89$, $p = 0.062$, and $t = 1.76$, $df = 89$, $p = 0.081$, for the slope and the y-intercept, respectively).

Since we had only limited measurements of δ¹³CO_{2(aq)}, we checked how sensitive the model fit was to this parameter. Varying the value of δ¹³CO_{2(aq)} by ± 1.0‰ had differing effects on the fit of the model to the observed values on either

side of the [CO_{2(aq)}] threshold. If δ¹³CO_{2(aq)} was enriched in ¹³C by 1.0‰, the model-predicted values for both [CO_{2(aq)}] < 10 μmol L⁻¹ and [CO_{2(aq)}] ≥ 10 μmol L⁻¹ were significantly different from the observed values (paired *t*-tests, $t = 2.958$, $df = 22$, $p = 0.007$, and $t = 5.616$, $df = 66$, $p < 0.001$, for [CO_{2(aq)}] < 10 μmol L⁻¹ and [CO_{2(aq)}] ≥ 10 μmol L⁻¹, respectively). In contrast, if δ¹³CO_{2(aq)} was depleted in ¹³C by 1.0‰, the model-predicted values for [CO_{2(aq)}] < 10 μmol L⁻¹ were not significantly different from the observed values (paired *t*-test, $t = 0.242$, $df = 66$, $p = 0.810$) but were for [CO_{2(aq)}] ≥ 10 μmol L⁻¹ (paired *t*-test, $t = 5.606$, $df = 22$, $p < 0.001$).

The model was also sensitive to changes in the cellular growth rate (μ) of the phytoplankton. Since this is difficult to estimate in a lake, we checked the sensitivity of the model to variation in this parameter. When μ (0.23 d⁻¹) was increased or decreased by ± 0.10 d⁻¹, predicted δ¹³C_{baseline} values for [CO_{2(aq)}] ≥ 10 μmol L⁻¹ were significantly different from observed values (paired *t*-tests, $t = 16.39$, $df = 66$, $p < 0.001$, and $t = 11.31$, $df = 66$, $p < 0.001$, for μ = 0.13 d⁻¹ and μ = 0.33 d⁻¹, respectively). Similarly, predicted δ¹³C_{baseline} values were also significantly different from observed values for [CO_{2(aq)}] < 10 μmol L⁻¹ when μ was increased or decreased by ± 0.10 d⁻¹ (paired *t*-tests, $t = 3.26$, $df = 22$, $p = 0.004$, and $t = 4.68$, $df = 22$, $p < 0.001$, for μ = 0.13 d⁻¹ and μ = 0.33 d⁻¹, respectively). However, the fit between modeled and observed δ¹³C_{baseline} values for samples where [CO_{2(aq)}] < 2 μmol L⁻¹ improved at the lower growth rate, and using μ = 0.23 ± 0.1 d⁻¹ in the model gave results that encompassed 87% of the observed values (Fig. 3).

Although δ¹³C_{Cycl} did not change as rapidly or to the same extent as δ¹³C_{Daph}, it did follow similar trends with the patterns in [CO_{2(aq)}] and pH (Table 2). There was also a significant correlation between δ¹³C_{Cycl} and δ¹³C_{Daph} over both the basins (Fig. 4; Table 2).

Discussion

Using archived samples collected over a 26-yr period, rapid fluctuations in δ¹³C_{baseline} of the pelagic zone were shown to correlate strongly with changes in the [CO_{2(aq)}] in

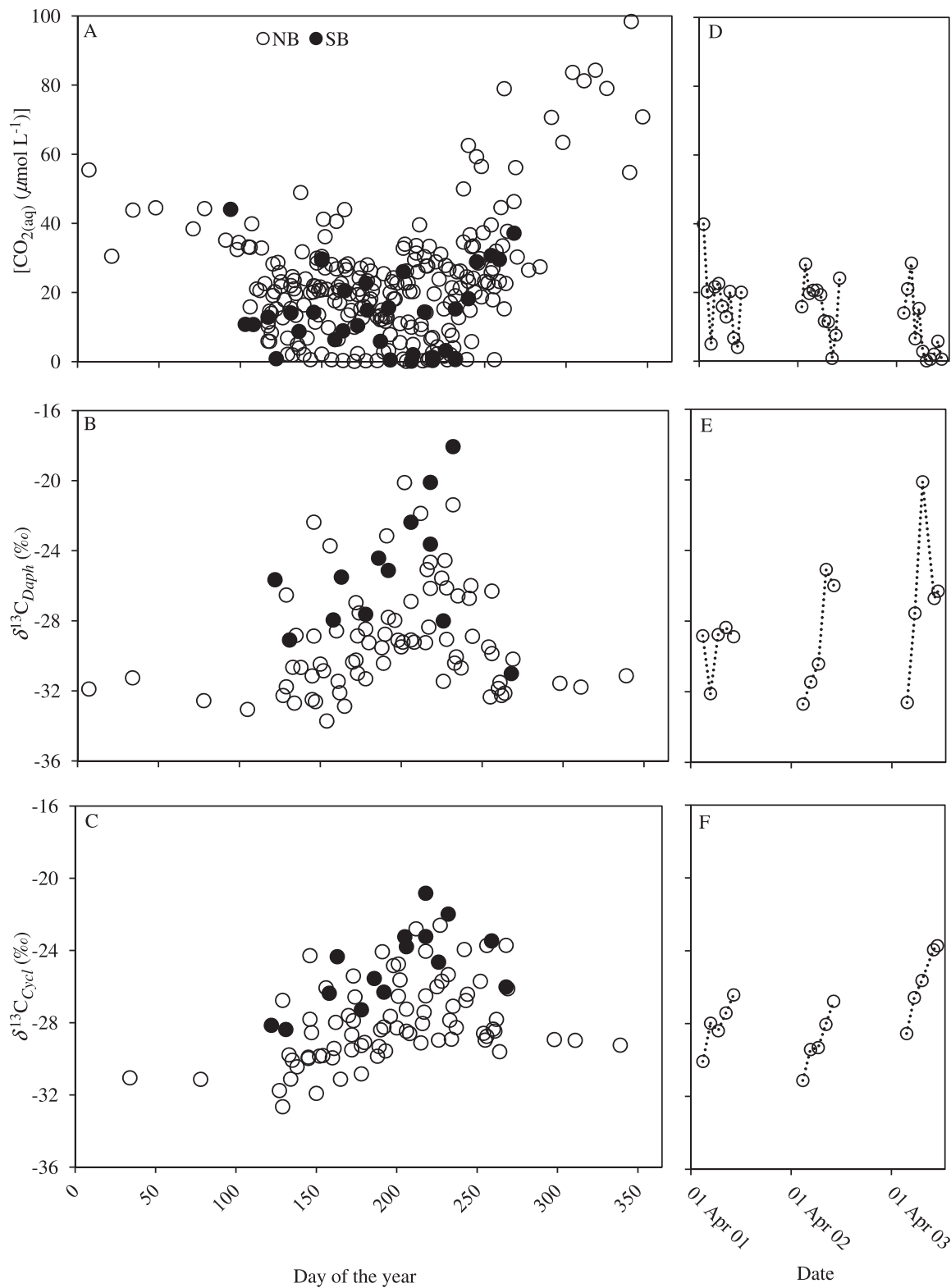


Fig. 1. Seasonal fluctuations in (A) $[\text{CO}_{2(\text{aq})}]$, (B) $\delta^{13}\text{C}$ values for *Daphnia* (taken as $\delta^{13}\text{C}_{\text{baseline}}$), and (C) $\delta^{13}\text{C}$ values for *Cyclops* in the North (NB) and South (SB) Basins of Windermere for selected years from 1985 to 2010 and changes in (D) $[\text{CO}_{2(\text{aq})}]$, (E) $\delta^{13}\text{C}$ values for *Daphnia*, and (F) $\delta^{13}\text{C}$ values for *Cyclops* in the North Basin of Windermere from May to September for 2001–2003.

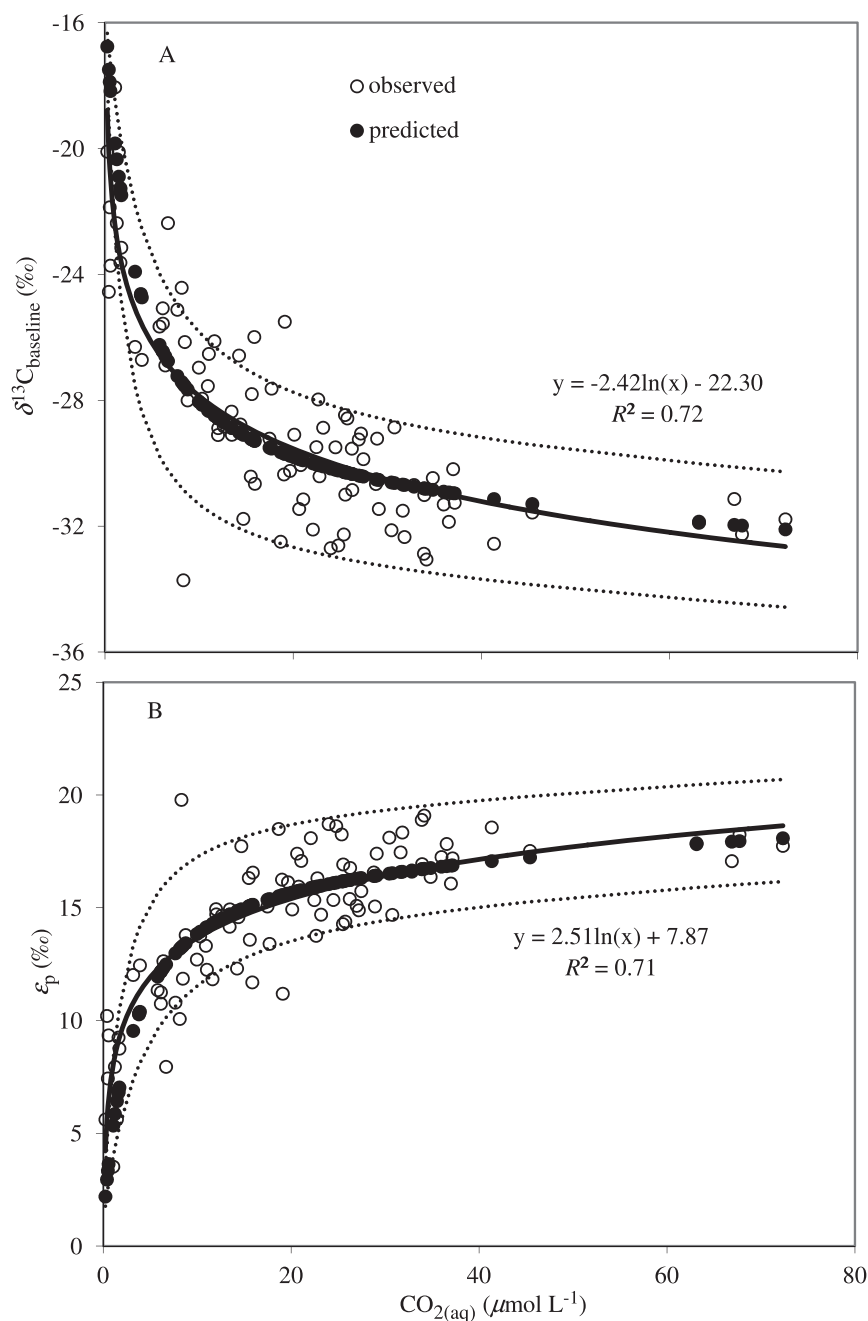


Fig. 2. Observed (open circles) and model-predicted (filled circles) (A) $\delta^{13}\text{C}_{\text{baseline}}$ and (B) isotopic fractionation due to photosynthesis (ϵ_p) derived from *Daphnia* as a function of the biweekly average $[\text{CO}_{2(\text{aq})}]$ in the North and South Basins of Windermere. The logarithmic equation and R^2 value are for the best-fit regression line (solid line) for the observed values. The upper and lower dotted lines represent an increase and a decrease in the algal growth rate (0.23 d^{-1}) by 0.10 d^{-1} , respectively.

a productive lake. This relationship is supported by an isotopic fractionation model based on algal physiology and represents an opportunity to use a relatively easily measured parameter, $[\text{CO}_{2(\text{aq})}]$, to supplement or extend direct $\delta^{13}\text{C}_{\text{baseline}}$ measurements of lake ecosystems. This may allow $\delta^{13}\text{C}_{\text{baseline}}$ data from lakes to be interpreted more accurately and enable researchers to incorporate

greater detail on temporal variation into models for calculating food web metrics.

Consequences of variability in $[\text{CO}_{2(\text{aq})}]$ for $\delta^{13}\text{C}_{\text{baseline}}$ —The magnitude and rate of change in the $\delta^{13}\text{C}$ value of *Daphnia* (henceforth $\delta^{13}\text{C}_{\text{baseline}}$) over the sampling period was often large and variable, but it consistently followed

Table 2. Statistical results of regression analyses for $\delta^{13}\text{C}_{Daph}$, $\delta^{13}\text{C}_{Cycl}$, and $\delta^{13}\text{C}_{POM}$ as a function of the biweekly average and current pH or $[\text{CO}_2(\text{aq})]$, model-predicted $\delta^{13}\text{C}_{\text{baseline}}$ as a function of observed $\delta^{13}\text{C}_{\text{baseline}}$, and $\delta^{13}\text{C}_{Cycl}$ as a function of $\delta^{13}\text{C}_{Daph}$. Regressions with $[\text{CO}_2(\text{aq})]$ are logarithmic, and those marked with an asterisk use the pH or $[\text{CO}_2(\text{aq})]$ measured on the same date as the $\delta^{13}\text{C}$ values. Literature data from other lakes (Grasmere, Esthwaite Water, Lake Wauberg, and Mohonk Lake) are included, and regressions marked with a 1, 2, or 3 are from Smyntek (2006), Gu et al. (2006), and Herczeg (1987), respectively.

Regression	R^2	F	p	df
$\delta^{13}\text{C}_{Daph}$ vs. pH	0.74	255.04	<0.001	89
$\delta^{13}\text{C}_{Daph}$ vs. pH*	0.67	177.68	<0.001	89
$\delta^{13}\text{C}_{Daph}$ vs. $[\text{CO}_2(\text{aq})]$	0.72	221.01	<0.001	89
$\delta^{13}\text{C}_{Daph}$ vs. $[\text{CO}_2(\text{aq})]^*$	0.67	178.77	<0.001	89
$\delta^{13}\text{C}_{Cycl}$ vs. pH	0.47	81.00	<0.001	92
$\delta^{13}\text{C}_{Cycl}$ vs. pH*	0.34	47.13	<0.001	92
$\delta^{13}\text{C}_{Cycl}$ vs. $[\text{CO}_2(\text{aq})]$	0.44	70.38	<0.001	92
$\delta^{13}\text{C}_{Cycl}$ vs. $[\text{CO}_2(\text{aq})]^*$	0.32	42.28	<0.001	92
Predicted vs. observed $\delta^{13}\text{C}_{\text{baseline}}$	0.70	209.19	<0.001	89
$\delta^{13}\text{C}_{Cycl}$ vs. $\delta^{13}\text{C}_{Daph}$	0.62	139.87	<0.001	86
Windermere $\delta^{13}\text{C}_{POM}$ vs. $[\text{CO}_2(\text{aq})]^*.1$	0.69	17.40	0.003	9
Grasmere $\delta^{13}\text{C}_{POM}$ vs. $[\text{CO}_2(\text{aq})]^*.1$	0.99	513.97	<0.001	4
Esthwaite Water $\delta^{13}\text{C}_{POM}$ vs. $[\text{CO}_2(\text{aq})]^*.1$	0.69	22.11	0.001	11
Lake Wauberg $\delta^{13}\text{C}_{POM}$ vs. $[\text{CO}_2(\text{aq})]^*.2$	0.65	—	<0.05	23
Mohonk Lake $\delta^{13}\text{C}_{POM}$ vs. $[\text{CO}_2(\text{aq})]^*.3$	0.68	8.46	0.044	5

the patterns in $[\text{CO}_2(\text{aq})]$ even though measurements were made only every 2 wk for most of the period of study. The concentration of $\text{CO}_2(\text{aq})$ can vary rapidly (Maberly 1996), and it is possible that if this is measured and taken into account, then the effect of CO_2 on $\delta^{13}\text{C}_{\text{baseline}}$ could be estimated more accurately. Nevertheless, the $10 \mu\text{mol L}^{-1}$ threshold for $[\text{CO}_2(\text{aq})]$ that has been previously noted as the level below which phytoplankton adapted to low $[\text{CO}_2(\text{aq})]$ may begin to concentrate dissolved inorganic carbon (Fogel and Cifuentes 1993; Lehman et al. 2004) appeared to be very appropriate for Windermere. For dates in which generally $[\text{CO}_2(\text{aq})] \geq 10 \mu\text{mol L}^{-1}$, $\delta^{13}\text{C}_{\text{baseline}}$ was approximately $30\text{‰} \pm 2\text{‰}$, which is typical for a lake of Windermere's latitude and productivity (Gu et al. 2011). However, when $[\text{CO}_2(\text{aq})]$ decreased below this $10 \mu\text{mol L}^{-1}$ threshold, $\delta^{13}\text{C}_{\text{baseline}}$ increased rapidly, and the interannual variability in $\delta^{13}\text{C}_{\text{baseline}}$ was substantial.

The large seasonal fluctuations in the $[\text{CO}_2(\text{aq})]$ observed in Windermere are a common feature in many lake ecosystems caused by periods of rapid growth and uptake of $\text{CO}_2(\text{aq})$ by phytoplankton (Maberly 1996). Cyanobacteria in particular are known to possess effective CCMs (Price 2011), to flourish at low $[\text{CO}_2(\text{aq})]$ in some systems (Shapiro 1997), and to possess relatively enriched $\delta^{13}\text{C}$ values (Vuorio et al. 2006). Cyanobacteria frequently dominate the phytoplankton community in Windermere during the summer (Reynolds and Irish 2000) and so may be partly responsible for increases in $\delta^{13}\text{C}_{\text{baseline}}$ in the summer because of their ability to initiate and maintain low $[\text{CO}_2(\text{aq})]$ in lakes

(Shapiro 1997). Although daphnids cannot ingest large forms of some phytoplankton, particularly cyanobacteria, they are capable of feeding on the smaller, decomposing pieces (Gulati et al. 2001) as well as the bacteria that decompose them (Thouvenot et al. 1999), so they will still reflect $\delta^{13}\text{C}_{\text{baseline}}$ during cyanobacterial blooms. The strong correlations between $\delta^{13}\text{C}_{\text{baseline}}$ and $[\text{CO}_2(\text{aq})]$ observed in other lakes during summer periods dominated by cyanobacteria (Herczeg 1987; Gu et al. 2006; Smyntek 2006) also support the possibility that cyanobacteria can cause increases in $\delta^{13}\text{C}_{\text{baseline}}$ and may indicate that a similar relationship could be observed in other lake systems that experience cyanobacterial blooms.

According to Mook et al. (1974), there is a very small discrimination between gaseous CO_2 and dissolved CO_2 in solution (dissolved CO_2 is -1.1‰ more depleted at 15°C and not very variable with temperature). Assuming that atmospheric CO_2 has a $\delta^{13}\text{C}$ value of about -8‰ (Verburg 2007), dissolved CO_2 produced by invasion of atmospheric CO_2 will have a $\delta^{13}\text{C}$ value of about -9‰ . In 2005 (four occasions), the $\delta^{13}\text{C}$ value of dissolved CO_2 in the two basins of Windermere ranged from about -18‰ in January to -13‰ in July. The values in July are consistent with a possible influx of less ^{13}C -depleted atmospheric CO_2 but potentially also from Rayleigh fractionation resulting from discrimination against ^{13}C during photosynthesis. However, the magnitude of the seasonal change in $\delta^{13}\text{C}$ of dissolved CO_2 , about 5‰ , is much less than the seasonal change in $\delta^{13}\text{C}$ of the baseline organic carbon (up to 12.5‰) so influx of atmospheric CO_2 cannot explain all of the observed variation.

Evaluation of algal isotopic fractionation model to predict $\delta^{13}\text{C}_{\text{baseline}}$ —Previous studies have found a strong relationship between the $\delta^{13}\text{C}$ values of particulate organic matter ($\delta^{13}\text{C}_{\text{POM}}$) and $[\text{CO}_2(\text{aq})]$ in both lakes (Gu et al. 2006, 2010) and streams (Finlay 2004). In addition, others have found a strong relationship between $\delta^{13}\text{C}_{\text{POM}}$ and $\delta^{13}\text{C}$ values of zooplankton (Marty and Planas 2008) and (indirectly) between the $\delta^{13}\text{C}$ values of zooplankton and the $[\text{CO}_2(\text{aq})]$ across a number of lake systems (Mohamed and Taylor 2009). These studies, in conjunction with the current results, may point toward a basic underlying mechanism affecting $\delta^{13}\text{C}_{\text{baseline}}$ that is related to the discrimination against inorganic carbon by phytoplankton communities at different $[\text{CO}_2(\text{aq})]$. This can be explored through the evaluation of the relationship between $\delta^{13}\text{C}_{\text{baseline}}$ and $[\text{CO}_2(\text{aq})]$ using a theoretical model of isotopic fractionation based on algal physiology.

The isotopic fractionation model based on physiological characteristics of the phytoplankton community of the lake was effective at predicting $\delta^{13}\text{C}_{\text{baseline}}$ for a wide range of $[\text{CO}_2(\text{aq})]$ over the 26-yr period. The agreement between the model-predicted and observed $\delta^{13}\text{C}_{\text{baseline}}$ was particularly strong when $[\text{CO}_2(\text{aq})] \geq 10 \mu\text{mol L}^{-1}$. For $[\text{CO}_2(\text{aq})] < 2 \mu\text{mol L}^{-1}$, the $\delta^{13}\text{C}_{\text{baseline}}$ values predicted by the model were slightly more enriched in ^{13}C than the observed values. This could be caused by a slower growth rate of the phytoplankton community during these times, particularly since slow-growing cyanobacteria were often dominant at

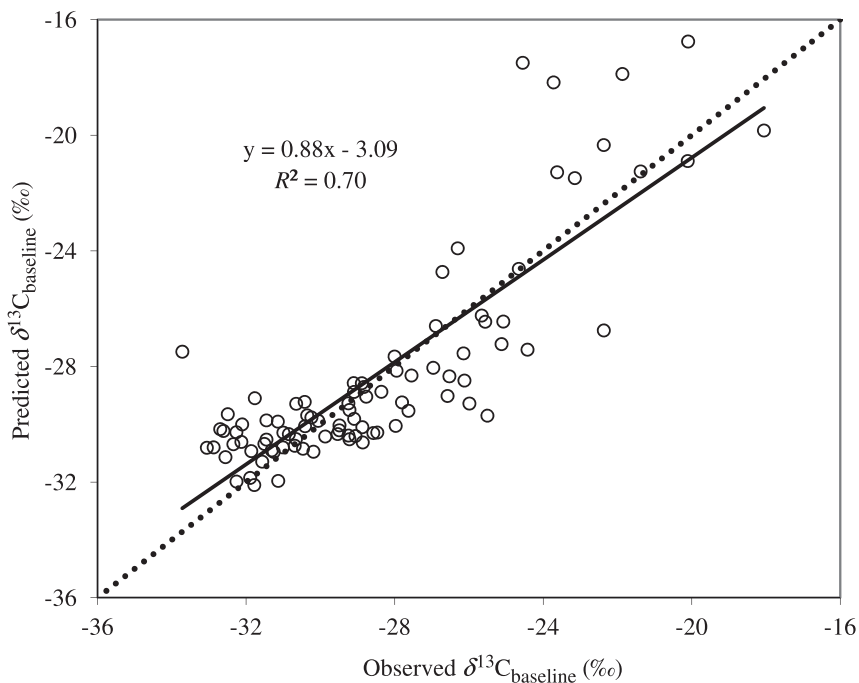


Fig. 3. Model-predicted vs. observed $\delta^{13}\text{C}_{\text{baseline}}$ in the North and South Basins of Windermere. The dotted line is the 1:1 line, while the solid line is the best-fit regression line.

these low $[\text{CO}_{2(\text{aq})}]$. Decreasing the growth rate in the model by 0.1 d^{-1} did make a substantial improvement to the fit between the observed and model-predicted values at these low $[\text{CO}_{2(\text{aq})}]$. In addition, the observed ($14.4 \pm 3.5\%$) and model-predicted ($14.2 \pm 3.7\%$) values of ε_p based on the measured $\delta^{13}\text{C}_{\text{baseline}}$ values and the average value of

$\delta^{13}\text{CO}_{2(\text{aq})}$ for the lake were consistent with a number of studies that have applied a variety of different approaches to evaluate the isotopic fractionation of $\text{CO}_{2(\text{aq})}$ by freshwater phytoplankton (Bade et al. 2006; Mohamed and Taylor 2009). This suggests that the theoretical assumptions of the isotopic fractionation model as well as

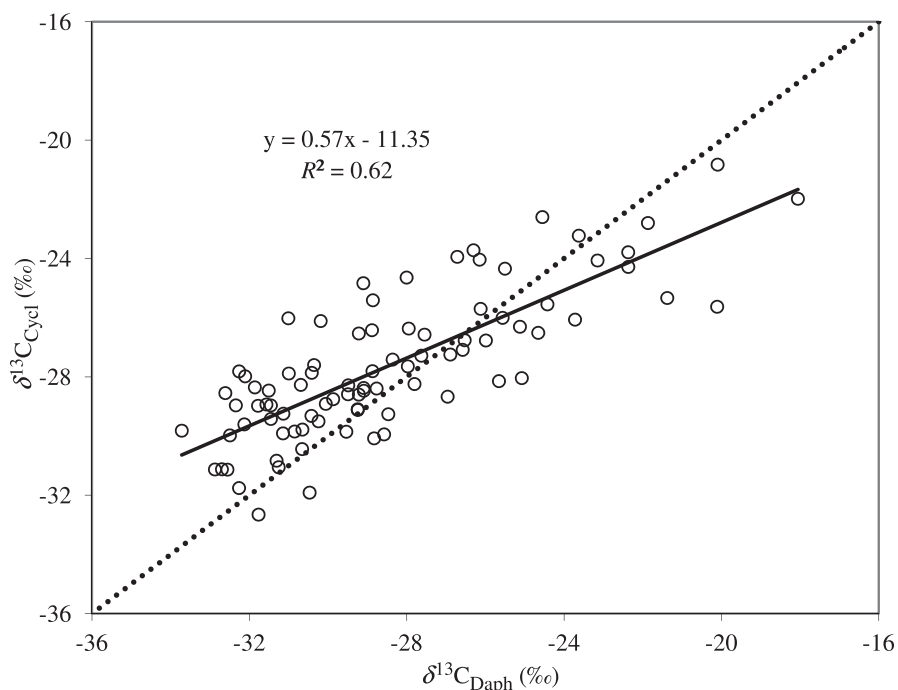


Fig. 4. The $\delta^{13}\text{C}$ values of *Cyclops* as a function of the $\delta^{13}\text{C}$ values of *Daphnia* in the North and South Basins of Windermere. The dotted line is the 1:1 line, while the solid line is the best-fit regression line.

the practical estimates that were made for some of its parameters were robust.

One might question the validity of applying an algal isotopic fractionation model developed for a small marine diatom to a seasonally variable freshwater phytoplankton community that can be dominated by cyanobacteria. However, the close fit between the model-predicted and observed $\delta^{13}\text{C}_{\text{baseline}}$ values suggests that the principal assumptions in the model may be valid at a basic level for the range of phytoplankton that comprises the algal community in Windermere. The main assumption for the model is that the phytoplankton obtain $\text{CO}_{2(\text{aq})}$ through active uptake via a carbon-concentrating mechanism and that the energetic requirements for this process are associated with the energetic costs to maintain a particular concentration of Rubisco within the chloroplast for carbon fixation. Although the dominant phytoplankton in the lake may have different physiological adaptations that allow them to cope with low $[\text{CO}_{2(\text{aq})}]$ in a manner that balances the energetic demands for the uptake and fixation of $\text{CO}_{2(\text{aq})}$, they ultimately may affect the isotopic composition of their own cellular organic composition in a similar fashion. The precise mechanisms involved in these processes are beyond the scope of this work. Nevertheless, the ability of the isotopic fractionation model to mimic the strong correlation between $[\text{CO}_{2(\text{aq})}]$ and $\delta^{13}\text{C}_{\text{baseline}}$ provides a firm basis for the use of $[\text{CO}_{2(\text{aq})}]$ as a proxy for $\delta^{13}\text{C}_{\text{baseline}}$.

Consequences of rapid change in $\delta^{13}\text{C}_{\text{baseline}}$ for food web studies—The frequently large and rapid seasonal changes in $\delta^{13}\text{C}_{\text{baseline}}$ of the lake also reinforce the importance of averaging stable isotope data over an appropriate time interval in order to account for such variations at the base of the food web (O'Reilly and Hecky 2002; Woodland et al. 2012a). Failure to account for such large changes will introduce substantial bias into dietary mixing models. The difference in the response of the $\delta^{13}\text{C}$ values of the herbivorous *Daphnia* compared to the predatory *Cyclops* demonstrates this clearly. At high $[\text{CO}_{2(\text{aq})}]$, the $\delta^{13}\text{C}$ value of *Cyclops* is slightly enriched in ^{13}C relative to *Daphnia*, which is one of its major food sources. However, when the $\delta^{13}\text{C}$ value of *Daphnia* rapidly increases in response to the phytoplankton at low $[\text{CO}_{2(\text{aq})}]$, the $\delta^{13}\text{C}$ value of *Cyclops* responds more slowly because it has a lower carbon turnover rate. Thus, at times, *Cyclops* is substantially more depleted in ^{13}C relative to *Daphnia*. Such variation in the isotopic turnover between the herbivorous daphnids and predatory copepods has been reported (Grey 2000) and is likely to be associated with metabolic differences resulting from their distinct life history strategies (Smyntek et al. 2008). The use of $[\text{CO}_{2(\text{aq})}]$ as a proxy for such changes can potentially minimize or prevent misinterpretations of stable isotope data caused by rapid changes at the base of the food web. For example, we used this approach to assess the relative contributions of littoral and pelagic carbon sources to the diet of pike in Windermere. In 2003, the $\delta^{13}\text{C}$ value of pike in Windermere was -24.6 , and the $\delta^{13}\text{C}$ value of littoral macroinvertebrates was -20‰ (P. M. Smyntek unpubl. data). If these data are

used in a simple two-source mixing model with the mean $\delta^{13}\text{C}_{\text{Daphnia}}$ (representing pelagic carbon) measured on three sampling dates in May, July, and September 2003 (-26.3‰), the ratio of littoral to pelagic carbon in pike is approximately 1 : 3. However, substituting a single month, June for July, changes the mean $\delta^{13}\text{C}_{\text{Daphnia}}$ to -28.8‰ and results in a ratio of littoral to pelagic carbon of nearly 1 : 1. If modeled values of $\delta^{13}\text{C}_{\text{Daphnia}}$ calculated from $[\text{CO}_{2(\text{aq})}]$ for July and August are used with the measured values for May, June, and September, the mean $\delta^{13}\text{C}_{\text{Daphnia}}$ is -26.6‰ , and the ratio of littoral to pelagic carbon in pike is again approximately 1 : 3. This ratio is also consistent with data on the gut contents of pike from Windermere in 2003 (Winfield et al. 2012).

Slow-growing phytoplankton, especially cyanobacteria (Reynolds 2006), and rapid changes in water chemistry, especially $[\text{CO}_{2(\text{aq})}]$, pH, and $\delta^{13}\text{C}_{\text{CO}_2}$, make it difficult to use $\delta^{13}\text{C}_{\text{POM}}$ as a baseline. However, using an organism such as *Daphnia* spp. and considering $[\text{CO}_{2(\text{aq})}]$ prior to as well as on the sampling date of the $\delta^{13}\text{C}$ measurement can allow $\delta^{13}\text{C}_{\text{baseline}}$ to be modeled and interpreted more accurately. Daphnids turn over their carbon content in approximately 3–6 d, although this can vary depending on the carbon : phosphorus ratios of their body tissue and their food (He and Wang 2008). The strong relationship between the $\delta^{13}\text{C}$ values of *Daphnia* and the average of the current and previous biweekly $[\text{CO}_{2(\text{aq})}]$ measurement (thus an effective turnover time of 7 d) indicates that these zooplankton did rapidly respond to changes in the $\delta^{13}\text{C}$ values of their phytoplankton food source and thus serve as a reliable $\delta^{13}\text{C}_{\text{baseline}}$. Future use of $[\text{CO}_{2(\text{aq})}]$ as a proxy for $\delta^{13}\text{C}_{\text{baseline}}$ would benefit from regular monitoring of $[\text{CO}_{2(\text{aq})}]$ when possible.

Use of $[\text{CO}_{2(\text{aq})}]$ as a proxy for a zooplankton-derived $\delta^{13}\text{C}_{\text{baseline}}$ could complement other methods to establish an isotopic baseline in a lake, such as measurements of the $\delta^{13}\text{C}$ values of particulate organic matter or long-lived consumers (mussels and snails). It could provide a more accurate and integrated measure of the variability of a key parameter, $\delta^{13}\text{C}_{\text{baseline}}$, which is frequently used in mixing models to assess organism diet and carbon dynamics within lake ecosystems. It would be particularly useful if long-lived consumers were not available or easily accessible, such as in lakes that regularly experience seasonal hypoxia. In addition, this method could be helpful in examining changes in diet over short time scales, which could be relevant to evaluating the diets of organisms such as larval fish or zooplankton, which rapidly turn over their body carbon. It could also be of particular value to researchers conducting studies on many similar lakes within the same region or multiyear studies in a single-lake ecosystem. The $\delta^{13}\text{C}_{\text{baseline}}$ vs. $[\text{CO}_{2(\text{aq})}]$ relationship could be established in the first year to allow sampling efforts (for $\delta^{13}\text{C}_{\text{baseline}}$) to be reduced in subsequent years and replaced with simpler $[\text{CO}_{2(\text{aq})}]$ or pH measurements.

The ability to predict $\delta^{13}\text{C}_{\text{baseline}}$ of a lake ecosystem from a readily measured proxy such as $[\text{CO}_{2(\text{aq})}]$ could affect and improve the interpretation of stable isotope analysis as an ecological and biogeochemical tracing tool. For example, predictions of $\delta^{13}\text{C}_{\text{baseline}}$ could be

incorporated into models that include a dynamic isotopic baseline for more accurate determinations of aquatic food web structure (Woodland et al. 2012b). In addition, if the contributions of algal primary production to higher trophic levels can be better constrained using a modeling approach based on an easily measured proxy such as [CO_{2(aq)}] to account for variability in algal δ¹³C values, then the relative contributions of allochthonous and autochthonous production to lake food webs, which has been of much recent debate (Solomon et al. 2011), could be more readily investigated.

With the current results, intralake variation in δ¹³C_{baseline} and ε_p has been shown to be predicted reliably for a lake ecosystem over a long time period using [CO_{2(aq)}]. In order to make practical use of the relationship between [CO_{2(aq)}] and δ¹³C_{baseline} in productive lakes, we offer the following recommendations. Historical data on key biological phenomena in the lake (i.e., spring diatom bloom, peaks in daphnid abundance, and summer cyanobacterial blooms) should be examined and considered when planning the collection of samples for stable isotope analysis in order to capture a sufficient amount of the potential variation relevant to the ecological investigation. The [CO_{2(aq)}] of the lake should be also monitored weekly (or more frequently) for at least 1–2 weeks prior to collection of daphnid samples. [CO_{2(aq)}] should also be monitored between sampling dates when multiple sampling dates are required. If [CO_{2(aq)}] is consistently above the 10-μmol L⁻¹ threshold before and during the sampling period, variation in δ¹³C_{baseline} can generally be assumed to be unconnected to changes in algal fractionation of CO_{2(aq)}, and the measured δ¹³C_{baseline} are more likely to be representative of the sampling period. However, if [CO_{2(aq)}] frequently decreases below 10 μmol L⁻¹, a relationship between [CO_{2(aq)}] and δ¹³C_{baseline} should be derived to calculate a mean δ¹³C_{baseline} for the sampling period. The opportunity to model δ¹³C_{baseline} of lakes may enable more information to be gleaned from lakes where [CO_{2(aq)}] is monitored regularly, and it will also allow researchers to plan their sampling efforts more effectively to capture the amount of variation in the isotopic baseline that is representative of the lake system of interest.

Acknowledgments

We are grateful to the Freshwater Biological Association for access to archived samples prior to 1989. We also thank Ben James, Mitizi De Ville, Janice Fletcher, and Jack Kelly for help with sample collection and Mark Teece for advice regarding the stable isotope data from 2005. We appreciate the input of two reviewers whose comments substantially improved this manuscript. Funding for this work was provided by the Natural Environment Research Council (NERC) through the Ecology and Hydrology Funding Initiative, grant NE/H000208/1.

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Associate Editor: H. Maurice Valett

Received: 11 November 2011

Accepted: 14 May 2012

Amended: 23 May 2012