Aquat. Sci. 68 (2006) 142–153
1015-1621/06/020142-12
DOI 10.1007/s00027-006-0818-5

Aquatic Sciences

Research Article

Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models?

Darren L. Bade[1,3,*], Michael L. Pace[2], Jonathan J. Cole[2] and Stephen R. Carpenter[1]

[1] Center for Limnology, University of Wisconsin-Madison, USA
[2] Institute of Ecosystem Studies, Millbrook, NY, USA
[3] Present address: Institute of Ecosystem Studies, PO Box AB (65 Sharon Turnpike), Millbrook, NY 12545, USA

Received: 30 June 2005; revised manuscript accepted: 7 December 2005

Abstract. Differential fractionation of inorganic carbon stable isotopes during photosynthesis is an important cause of variability in algal carbon isotope signatures. Several physiological models have been proposed to explain algal photosynthetic fractionation factors (ε_p). These models generally consider CO_2 concentration, growth rate, or cell morphometry and have been supported by empirical evidence from laboratory cultures. Here, we explore the applicability of these models to a broad range of lakes with mixed phytoplankton communities. Understanding this fractionation is necessary for using carbon stable isotopes for studies ranging from food webs to paleolimnology. In our largest comparative study, values of δ^{13}C-POC ranged from –35.1‰ to –21.3‰. Using several methods to obtain an algal isotopic signature, we found high variability in fractionation among lakes. There was no relationship between ε_p and one of the most important predictors in existing models, pCO_2. A whole-lake inorganic ^{13}C addition was used to create distinct algal isotope signatures to aid in examining ε_p. Measurements and a statistical model from the isotope addition revealed that algal fractionation was often low (0 – 15‰).

Key words. Photosynthetic fractionation; carbon stable isotopes; algae; particulate organic carbon; lakes.

Introduction

Fractionation of carbon isotopes during photosynthesis is a key parameter for understanding organic carbon isotope signatures in aquatic ecosystems. Models of algal photosynthetic fractionation have served as components of some paleolimnological and aquatic food web studies. Paleolimnological studies benefit from fractionation models, because with the models and with measurement of the isotope signatures of sedimented algal material it may be possible to reconstruct past levels of productivity or CO_2 concentrations (Oana and Deevey, 1960; Hollander and McKenzie, 1991; Schelske and Hodell, 1995; Meyers and Lallier-Verges, 1999). Aquatic food web studies utilize models of photosynthetic fractionation to determine the isotope signature of phytoplankton, one important base of aquatic food webs (Karlsson et al., 2003; Pace et al., 2004).

During photosynthesis, plants preferentially acquire the lighter carbon isotope, ^{12}C. Consequently, plant organic matter has a lighter isotope ratio than the source inorganic carbon. Photosynthetic fractionation of carbon isotopes can occur at the diffusion, dissolution and carboxylation steps. For land plants, differences among photosynthetic pathways (C_3, C_4 and CAM) result in unique isotopic signatures among different types of plants, and water use efficiency may cause some varia-

* Corresponding author phone: +1 845 677 5343;
fax: +1 845 677 5976; e-mail: BadeD@ecostudies.org
Published Online First: May 30, 2006

tion within types (Lajtha and Michener, 1994). For C_3 photosynthesis (a common pathway of terrestrial and aquatic photosynthesis) most of the fractionation occurs during carboxylation of CO_2, and for the enzyme RUBISCO this fractionation may be near –25‰ to –28‰ in algae (Goericke and Fry, 1994; Popp et al., 1998). If diffusion of CO_2 into the cell becomes rate limiting (i.e. if CO_2 concentration is low), then the fractionation during carboxylation will become minimal. Diffusion of CO_2 is slower in water than in air, thus the decline from the maximal fractionation is observed frequently in aquatic plants, especially benthic algae (Finlay et al., 1999). Early observations of the photosynthetic fractionation factor (ε_p) in algae found a relationship between external CO_2 concentrations and ε_p (Rau et al., 1989). Later, workers recognized the possibility of an inverse relationship between ε_p and $\mu/[CO_2]$, where μ is the growth rate of the algae (Francois et al., 1993; Laws et al., 1995). Cell geometry also explained some of the species-specific differences observed in the ε_p versus $\mu/[CO_2]$ relationship (Popp et al., 1998).

Other factors also affect the photosynthetic fractionation by algae. Some algae have the ability to use HCO_3^- in addition to CO_2 (Raven, 1970). Since HCO_3^- has a greater affinity for ^{13}C, the apparent fractionation ($\varepsilon_p = \delta^{13}CO_2$-$\delta^{13}$algae) will be smaller. However, present models are not able to discern whether CO_2 or HCO_3^- is the source of inorganic carbon, and whether it is taken up passively or actively (Laws et al., 1997; Keller and Morel, 1999). The presence of a carbon concentrating mechanism has been proposed for some algae, and this mechanism has been shown to reduce fractionation (Sharkey and Berry, 1985). Finally different photosynthetic pathways in land plants (C_3, C_4, and CAM) fractionate carbon distinctively. Analogously, carboxylation by enzymes other than RUBISCO within aquatic algae could lead to differences in the degree of fractionation observed (Falkowski, 1991).

Although models of photosynthetic fractionation have seldom been tested in natural freshwater environments (Yoshioka, 1997; Finlay, 2004), their use has been relatively widespread in aquatic ecology. However, there is some indication that these models may not be appropriate in all freshwater situations since fractionation appears to be less than would be predicted based on these models (Cole et al., 2002; Pace et al., 2004). Also, it is increasingly recognized that for marine algae these models are not applicable to all species, and that differing growth limitation may also influence ε_p (Rau et al., 1989; Burkhardt et al., 1999a, b).

This study examines estimates of ε_p in freshwater lakes. Our goal is to determine if the general patterns found in laboratory estimates of ε_p apply to freshwater ecosystems with diverse and mixed algal species. Specifically we investigate if CO_2 or CO_2 and growth rate are suitable for predicting ε_p among lakes. We examine ε_p based on isotopic measurements of dissolved inorganic carbon (DIC), particulate organic carbon (POC) and size-separated POC, across a gradient of lakes. We made similar measurements during an experimental inorganic carbon isotope manipulation in two lakes to assess temporal variability in ε_p. Although mechanisms represented in laboratory models are important, our results indicate there are many complicating factors that prevent general use of these existing models for phytoplankton communities in lakes. Investigators should instead consider measuring algal carbon isotopes directly.

Methods

Total POC comparative study

Two separate comparative studies were conducted. The first involved collection of whole water POC for isotope analysis and the second involved collection of specific size fractions of POC for isotope analysis. For the first study, surface waters (1m depth) were sampled in 32 temperate lakes in the Northern Highland region of northern Wisconsin and the Upper Peninsula of Michigan during the summer of 2000. The lake water was pre-filtered through 153-µm mesh to remove large zooplankton, and POC was collected on pre-combusted 25-mm Whatman GF/F filters and dried at 60 °C for at least 48 h. Filters were fumed with HCl prior to analysis to remove inorganic carbon. The University of Alaska-Fairbanks Stable Isotope Facility conducted C isotopic analysis of the POC using a Carlo Erba Elemental Analyzer, a Finnigan MAT Conflo II/III interface with a Delta+ mass spectrometer. Samples for ^{13}C-DIC (dissolved inorganic carbon) were collected in 1 L amber glass bottles and preserved at pH < 2 with 1 ml 10 N H_2SO_4. The samples were stored in the dark until they were sent to the Marine Biological Laboratory, Woods Hole, MA for isotope analysis. Other limnological sampling of these lakes is given by Bade et al. (2004). Of interest for this study are the partial pressure of CO_2 (pCO_2), and Chl. *a*. CO_2 partial pressures were measured with the headspace equilibration technique (Cole et al., 1994). Chl. *a* was collected on 47-mm Whatman GF/F filters, frozen, and extracted with methanol; concentrations were measured fluorometrically with corrections for pheopigments (Marker et al., 1980). Chl. *a* samples were not pre-filtered through 153-µm mesh, unlike the POC samples. Several lakes were sampled multiple times throughout the summer. These were considered independent samples in statistical analysis.

Sestonic POC is a mixture of material, including algae and terrestrial detritus (del Giorgio and France, 1996; Hessen et al., 2003). We calculated the signature of the algal portion by using a two end-member mixing model of the form:

$$\delta^{13}\text{algae} = (\delta^{13}\text{POC}[\text{POC}] - \delta^{13}\text{terr}[\text{terr}]) / [\text{algae}]. \quad (1)$$

The mass of the algal component, [algae], was calculated by using the measured amount of Chl. *a* and multiplying by an assumed algal-C:Chl ratio of 40 (by mass). The remainder of the mass of POC, [POC], was considered to be terrestrial detritus, [terr] = [POC] – [algae]. The non-algal component was assumed to have an isotope signature of –28‰, similar to that of C3 terrestrial vegetation (Lajtha and Michener, 1994), which dominates the region. Our own measurements of terrestrial vegetation near some of the lakes had a mean (±95 % C.I.) signature of –29.1 ± 1.2‰ (n = 10; unpublished data). Additionally, from a survey of dissolved organic carbon (DOC) isotope signatures in these same lakes we determined a mean signature of –26.8 ± 0.4 (n = 36; Bade, 2004). Algal-C:Chl ratios are variable in nature (Leavitt and Carpenter, 1990), so a range of values (25 and 100) was examined to assess the sensitivity of our results to the assumed algal-C:Chl ratio. At an algal-C:Chl ratio of 100 there were some cases in which the amount of POC that was algal was greater than 100%. For these samples we assumed that 100 % of the POC was algal. Although the amount of Chl. *a* present on the POC samples could have been slightly overestimated because Chl. *a* samples were not pre-filtered through a 153-µm mesh, the difference should be negligible. Also, other components (e.g. bacteria, dead algae, etc.) could influence the isotope signature of POC, but we had no means to further isolate these.

The photosynthetic fractionation factor was approximated as

$$\begin{aligned}\varepsilon_p &= (\delta^{13}CO_2 - \delta^{13}\text{algae}) / [1 + (\delta^{13}\text{algae} / 1000)] \\ &\approx \delta^{13}CO_2 - \delta^{13}\text{algae}.\end{aligned} \quad (2)$$

Throughout, the δ^{13}C-CO_2 was calculated from δ^{13}C-DIC, DIC concentration, pH or pCO_2, and temperature using carbonate equilibrium constants (Stumm and Morgan, 1996) and associated equilibrium fractionation factors (Mook et al., 1974).

Size-separated POC comparative study

In the second comparative study, we attempted to physically separate algae from POC by filtering water through consecutively smaller sized Nitex mesh with the goal of obtaining pure, or nearly so, phytoplankton samples. Zohary et al. (1994) employed a similar method in their study of Lake Kinneret. We sampled the surface water (1 m) of three lakes in the summer of 2002 and 16 lakes in the summer of 2003. For most samples, water was first prefiltered through 153-µm mesh to remove most large zooplankton and then through 65-µm mesh removing most rotifers, but sometimes large or filamentous algae were also removed. The water was then filtered through 35-µm and then 10-µm mesh sizes. In a few samples, mesh sizes of 45-µm and 20-µm were also used. The material collected on the mesh was examined qualitatively under a microscope for the presence of non-algal material immediately after collection, or refrigerated and examined within one day. The size fractions that were chosen for isotope analyses consisted mostly of material that could be identified as algal, and only a small proportion of unidentifiable amorphous material. Samples were rejected if they contained only a small amount of algal material relative to the amount of water that was filtered, or if they had a large amount of material that could not be identified as algal. The filtered material, if it was identified as being a reasonably clean algal sample, was then collected on GF/F filters for Chl. *a* and carbon isotope measurements. As well, measurements of Chl. *a* were made on the filtrate of each size mesh to determine the amount Chl. *a* that was captured by any particular size mesh. For a subset of six lakes, samples were collected from all size fractions to examine the potential for difference in POC isotope signatures as a function of size. Since there is no means to show that these samples were indeed purely phytoplankton, we will refer to them as size-separated POC (SS_POC).

Samples for DIC isotope analysis in the comparative study of SS_POC were collected in 60-ml serum vials, acidified to pH < 2 with 10N H_2SO_4 and sealed with butyl rubber septa and aluminum crimp caps. Samples were analyzed by the University of Waterloo Environmental Isotope Laboratory using a Micromass Isochrome GC-C-IRMS. All other analysis was similar to the first comparative study.

Whole-lake ^{13}C additions

$NaH^{13}CO_2$ was added to Tuesday and Peter Lakes in the summer of 2002. These lakes are small softwater systems in the Upper Peninsula of Michigan (Carpenter and Kitchell, 1993). We made daily additions of 250 mmoles and 590 mmoles of $NaH^{13}CO_3$ (>98 % purity; Isotec) over 35 days in Tuesday and Peter Lakes, respectively. Samples for isotope analysis were collected for DIC, POC and separated algae as outlined above. DIC isotope samples were sent to the University of Waterloo Environmental Isotope Laboratory. Other methods were similar to those described above. In addition, Peter Lake received nutrient amendments to increase primary productivity. H_3PO_4 (0.69 mmol P m^{-2}) and NH_4NO_3 (18.9 mmols N m^{-2}) were added initially on 3 June and then daily additions of 0.11 mmols P m^{-2} and 2.7 mmol N m^{-2} were made from 10 June to 25 August.

Photosynthetic fractionation (ε_p) in the experimental lakes was evaluated by two methods. The first was by examining SS_POC (same methods as above) and the instantaneous measurements of $\delta^{13}C_{SS\_POC}$ and δ^{13}C-CO_2 as in equation 2. The other method was a univariate statistical model that attempted to account for a terrestrial

component of the POC as well as a lag of previously produced algal C. Pace et al. (2004) utilized this model in previous whole-lake ^{13}C additions. The model structure is:

$$\delta^{13}POC = (1-w)[(1-m)(\delta^{13}CO_2 - \varepsilon_p)_t + m(\delta^{13}CO_2 - \varepsilon_p)_{t-u} + w(-28)] \quad (3)$$

where w is the proportion of terrestrial material, m is the proportion of carbon formed u days prior to t. The parameters w, m, u and ε_p were fitted by least squares and parameter uncertainty estimated by bootstrapping as in Pace et al. (2004). In the case of Peter Lake, CO_2 concentration was drawn down to low levels because of increased primary production, and bicarbonate uptake seemed likely. In order to model the changes in Peter Lake from nominal conditions that were similar to Tuesday Lake (sufficient CO_2) to conditions where CO_2 was exceedingly low, we modeled ε_p values as an inverse function of CO_2. So for Peter Lake,

$$\varepsilon_p = \varphi[CO_2], \quad (4)$$

where the parameter φ was fitted. Since DIC was almost exclusively HCO_3^- in Peter Lake for much of the summer, and both would have essentially the same isotope signature when CO_2 is low, ε_p was calculated with respect to $\delta^{13}C$-DIC as opposed to $\delta^{13}C$-CO_2, such that

$$\delta^{13}POC = (1-w)[(1-m)(\delta^{13}DIC - \varepsilon_p)_t + m(\delta^{13}DIC - \varepsilon_p)_{t-u} + w(-28)]. \quad (5)$$

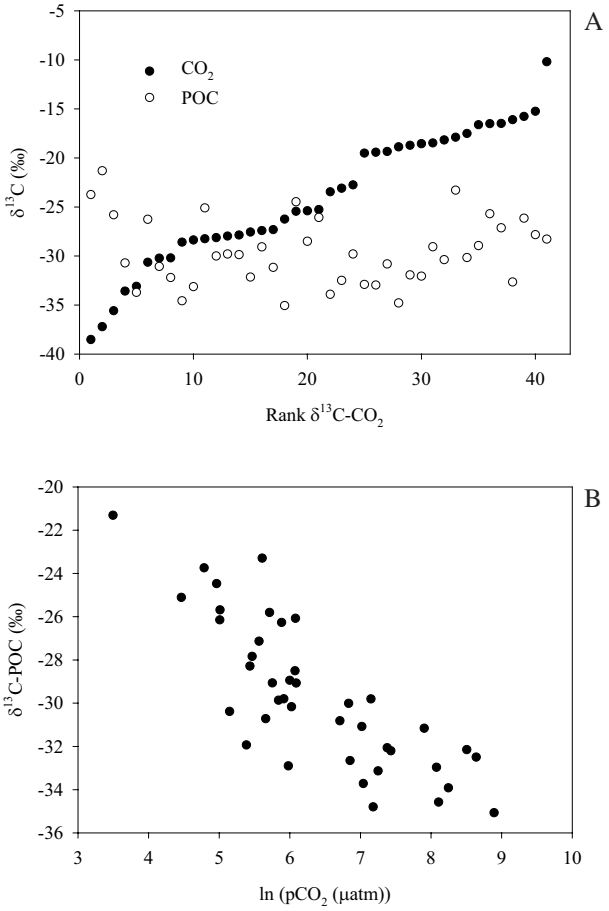

Figure 1. a) $\delta^{13}C$-CO_2 and $\delta^{13}C$-POC plotted versus the rank of $\delta^{13}C$-CO_2. The rank starts with the lowest $\delta^{13}C$-CO_2 value and increments to the highest $\delta^{13}C$-CO_2 value. b) Correlation of $\delta^{13}C$-POC and the natural log of the partial pressure of CO_2 (µatm). The correlation was significant ($r = -0.81$; $p < 0.01$; $n = 41$). No correlations were found with temperature, DIC, pH, Chl. *a*, or POC concentration ($p > 0.05$).

Results

Comparative studies

From the 32 lakes surveyed in the Northern Highland Lake District, $\delta^{13}C$-CO_2 varied by nearly 30‰, while $\delta^{13}C$-POC only varied by about 15‰ (Fig. 1a). The range of pCO_2 in the lakes was 33–7280 µatm and POC isotope signatures were inversely related to pCO_2 (Fig. 1b). In most cases $\delta^{13}C$-POC was lower than $\delta^{13}C$-CO_2, which, if POC is assumed to be of algal origin, is expected due to preferential uptake of ^{12}C. It should be noted however that POC is not likely to be entirely of algal origin (del Giorgio and France, 1996; Pel et al., 2003) and that several factors (e.g., $\delta^{13}C$-CO_2, ε_p, and the amount of terrestrial detritus) establish the isotope signature of POC; therefore the correlation shown in figure 1b cannot be assumed as evidence for a relationship between ε_p and CO_2 concentration.

In several lakes (Snipe, Peter, Diamond (days 201 and 229), Bog Pot, Cranberry (day 165), and Crystal) $\delta^{13}C$-POC was higher than $\delta^{13}C$-CO_2 (Fig. 1a). All these lakes have low DIC concentrations (<55 µmol/L) and some of the lowest $\delta^{13}C$-CO_2 values. Peter Lake had high pH (9.04) owing to high levels of productivity. The others had ranges of pH from circumneutral to acidic (7.42–4.83). We omitted these six samples, for which $\delta^{13}C$-POC was higher than $\delta^{13}C$-CO_2 from further results, but discuss them later.

Particulate organic carbon cannot be assumed to be entirely of algal origin and in addition is composed of terrestrial detritus and other seston, including bacteria. After correcting for the amount of POC that is not algal by assuming algal-C:Chl ratios, the photosynthetic fractionation factor (ε_p) ranges from approximately 0 to 40‰ with an average ε_p approximately 13‰ (Fig. 2a). Some values of ε_p were not greatly affected by varying the assumed algal-C:Chl, while others ranged greater than 30‰. Those with the greatest range in signatures were the samples that had estimated algal signatures (at the nominal algal-C:Chl ratio of 40) that were most different

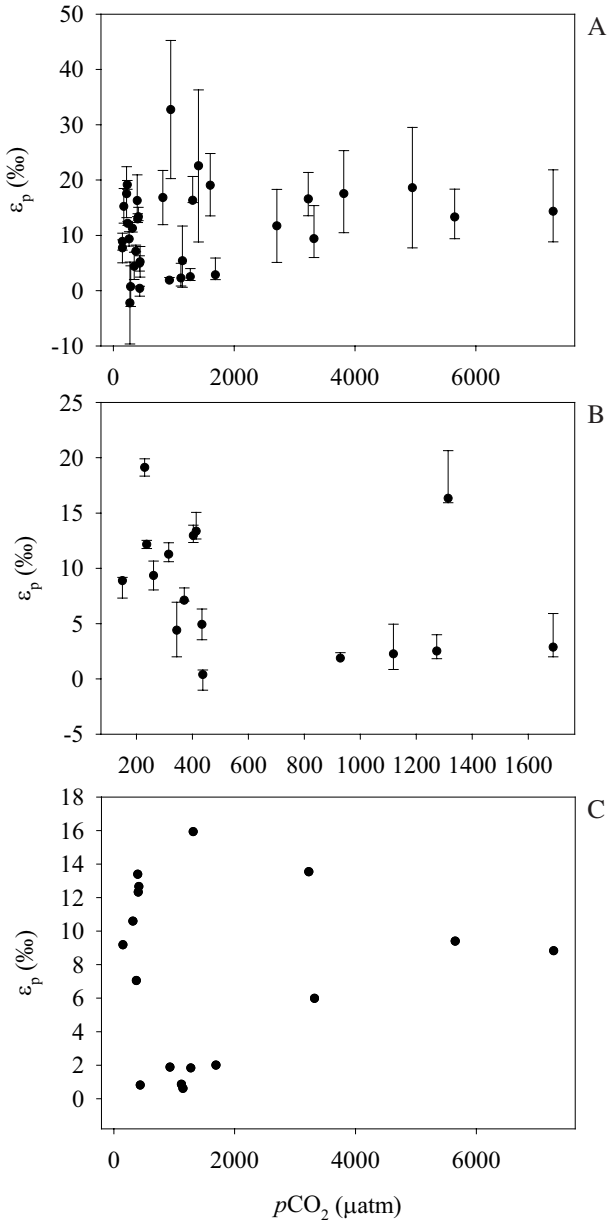

Figure 2. Plots of ε_p versus pCO$_2$ for survey lakes. a) Data points are ε_p calculated assuming C:Chl = 40. Error bars indicate a range of ε_p values due to differences in assumed C:Chl from 25 to 100. b) Lakes from Figure 2a with ε_p ranges less than 5‰ in magnitude. c) Lakes with C:Chl ratios of POC <80. In c, ε_p is calculated as $\delta^{13}CO_2$ - δ^{13}POC, because in these cases we assume that POC consists mostly of algae. Lakes with positive ε_p values are omitted from this graph.

from the terrestrial signature of –28‰. This is because variations in the proportion of algal and terrestrial material will have little impact on the resulting signature if the estimated algal isotope signature is near the terrestrial signature. An incorrect estimate of the algal-C:Chl ratio can cause ε_p to either be over- or underestimated depending on the signature of algal material relative to the terrestrial end-member. We also considered that our estimate of the terrestrial carbon isotope ratio could vary. Our measurements of DOC and terrestrial vegetation suggest that it is probably constrained well between –27‰ and –29‰ (see methods), the range over which we tested. At the nominal algal-C:Chl ratio of 40, ε_p of a single sample could range from nearly 0 to 7.6, increasing as the proportion of terrestrial material increased. Therefore the influence of the assumed terrestrial signature varies depending on the assumed algal-C:Chl ratio.

The lakes with algae that had little variation in their isotopic signature were examined more closely because there should be greater certainty in the estimate of the algal signature, regardless of the assumed algal-C:Chl ratio. Values of ε_p in lakes with ε_p that ranged less than 5‰ were plotted against pCO$_2$ (Fig. 2b). In addition, ε_p values in lakes with POC samples that had POC:Chl ratios less than 80, suggesting a dominance of algal carbon, were also plotted against pCO$_2$ (Fig. 2c). In Figure 2c, ε_p was determined directly as $\delta^{13}CO_2$–δ^{13}POC, since the POC:Chl suggests that the POC was mostly algal. In either plot it is difficult to discern any pattern in fractionation in relation to changes in pCO$_2$ despite the fact that pCO$_2$ is the main correlate implicated in models of algal photosynthetic fractionation.

Since gross primary production (GPP) was measured in a subset of lakes in this comparative study using continuous, in situ oxygen measurements (Hanson et al., 2003), it was possible to consider the combined effects of CO$_2$ concentration and growth rate and compare these to existing models of species-specific photosynthetic fractionation given by Popp et al. (1998). In the subset of lakes with GPP measurements, the data do not follow any particular model (data not shown; Bade, 2004). The models of the four species studied by Popp et al. (1998) bracket most of the data points, potentially suggesting that a mix of appropriate species-specific models might provide useful information on algal isotopic signatures.

Calculated ε_p for the survey of size-separated POC has a range of values from –8.8 to 14.9‰ and the average of all lakes and samples is 7.3‰ (Table 2). From the six lakes in which we examined multiple size fractions, there is no pattern that suggests a larger algal component at a particular size. Generally, the isotope signatures from the largest size fraction appear as a mix of the signatures from the smaller size fractions. The smallest size fraction also tended to be more similar to a terrestrial signature than the intermediate size fractions, although the isotopic differentiation between different size samples was sometimes small and very near a terrestrial signature. With this data there is also no apparent trend in either $\delta^{13}C_{SS\_POC}$ or ε_p with pCO$_2$ (Bade, 2004).

Carbon isotope addition experiment

Figure 3 (a and b) show the time course of DIC, CO$_2$ and Chl. *a* in Tuesday and Peter Lake. The nutrient addition

in Peter Lake caused a large increase in Chl. *a* for a short period in the first part of July and a sustained peak near the end of July. There was a concomitant decrease in DIC and CO_2 during these periods. During the periods of high Chl. *a*, pH increased substantially to levels approaching 9.5. In Tuesday Lake there was no nutrient addition and Chl. *a* concentrations were more stable and averaged 6.8 µg L^{-1}. Chl. *a* concentrations were slightly higher early in the summer in Tuesday Lake, and DIC was also slightly lower during this period.

The experimental addition of inorganic ^{13}C in Peter and Tuesday lakes substantially changed the $\delta^{13}C$-DIC and therefore $\delta^{13}C$-CO_2 (Fig. 4a, b). The uptake of this labeled inorganic carbon by algae resulted in noticeable changes in the $\delta^{13}C$-POC and $\delta^{13}C$ of the size-separated POC (Fig. 4a, b). In Tuesday Lake, the physically separated material is considerably enriched in ^{13}C compared with the total POC samples (Fig. 4a). The total POC samples are closer to the signature of terrestrial material than the size-separated POC. The large divergence between total POC samples and size-separated POC (up to ~20‰) displays that at least in the example of Tuesday lake, our method of size separation produces a sample that has lost much of the terrestrial material. The percentage of ter-

Table 1. Characteristics of POC and values needed to calculate ε_p using assumed algal-C:Chl ratios

Lake	Day of year (2000)	δ CO_2 ‰	δ POC ‰	Tot. Chl *a* (µg L^{-1})	POC (µg L^{-1})	POC:Chl (mass)
Allequash	173	–18.5	–29.1	7.97	524	66
Allequash	200	–16.5	–27.1	8.35	830	99
Allequash	228	–18.2	–30.4	12.60	1146	91
Big Musky	230	–17.9	–23.3	4.48	470	105
Bog Pot	210	–33.6	–30.7	37.52	3482	93
Bolger	214	–23.5	–33.9	13.78	1211	88
Brown	196	–16.6	–28.9	13.21	888	67
Crampton	181	–27.4	–29.1	3.48	605	174
Cranberry	165	–28.2	–25.1	30.51	2167	71
Cranberry	195	–25.3	–26.1	16.99	826	49
Cranberry	237	–30.2	–31.1	20.24	1181	58
Crystal	200	–25.4	–24.5	2.66	352	133
Diamond	174	–25.4	–28.5	1.73	324	187
Diamond	201	–35.6	–25.8	3.06	453	148
Diamond	229	–30.6	–26.3	2.81	509	181
East Long	235	–30.2	–32.2	14.68	709	48
Helmet	221	–27.3	–31.2	3.46	484	140
Hiawatha	217	–22.8	–29.8	13.62	567	42
Hummingbird	164	–28.4	–33.1	20.45	3656	179
Hummingbird	187	–26.2	–35.1	15.36	1093	71
Hummingbird	230	–28.6	–34.6	23.61	1435	61
Kickapoo	192	–19.4	–33.0	14.34	925	64
Little Arbor Vitae	208	–16.5	–25.7	56.90	2566	45
Mary	220	–28.0	–29.8	25.11	1383	55
Morris	186	–18.6	–32.1	7.72	730	95
Morris	210	–18.9	–34.8	23.38	990	42
Musky	207	–18.7	–31.9	18.41	1541	84
North Gate Bog	206	–27.6	–32.2	2.65	465	176
Palmer	227	–19.5	–32.9	12.20	777	64
Paul	166	–19.3	–30.8	2.81	326	116
Peter	236	–37.2	–21.3	30.18	2536	84
Plum	193	–15.8	–26.2	10.31	1000	97
Reddington	206	–23.1	–32.5	17.60	1318	75
Snipe	207	–38.5	–23.7	12.13	1684	139
Sparkling	227	–15.2	–27.8	2.35	326	139
Tenderfoot	195	–17.5	–30.2	17.31	917	53
Trout	202	–10.2	–28.3	2.98	569	191
Trout Bog	200	–28.1	–30.0	38.83	1214	31
Tuesday	171	–27.9	–29.9	14.29	1311	92
Ward	210	–16.1	–32.7	5.80	1041	179
West Long	234	–33.1	–33.7	7.63	561	73

restrial detritus in the POC samples, estimated from the size-separated POC isotope signature, averaged 63 %. By contrast in Peter Lake, the physically separated material has similar signatures to that of the POC (Fig. 4b). Thus in highly productive lakes such as Peter Lake, most POC may be of algal origin, while in lakes of lower productivity similar to Tuesday Lake, much of the POC is not of algal origin.

Photosynthetic fractionation in Tuesday Lake, determined from instantaneous measurements of $\delta^{13}C_{SS\_POC}$ and $\delta^{13}C\text{-}CO_2$, ranged from approximately 2‰ to 15‰, and averaged 8.5 ± 3.9‰ (±1 S.D.) (Table 3). No relationship existed between the variation in ε_p and pCO_2 within Tuesday Lake for these measurements. Most of the phytoplankton samples from Tuesday Lake represent the 20–45 µm size range, although the first sample date is drawn from the 10–20 µm size range. If not all terrestrial material was removed due to the size separation procedure, the resultant ε_p values during the isotope addition would be even smaller, as the terrestrial material should be biasing the size-separated POC to more negative values. The average amount of Chl. *a* that was retained for all these samples was 24 ± 6 %. The remaining proportion of Chl. *a* was retained by larger size filters or passed through the particular size filter used for collection. Most of these samples had a POC:Chl ratio near 120 ± 65 (Table 3). The POC:Chl ratio of all non-separated POC (total POC) samples averaged 173 ± 110 (n = 15). The most

Table 2. Lake chemistry and characteristics of physically separated algae. Asterisks (*) denote lakes in which pCO_2 was determined by pH, DIC and temperature. Samples from the size fraction "<153" are the same as POC samples from other aspects of this study (i.e., all POC samples were prefiltered with 153-µm mesh). The material sampled from Trout Bog on 19-Aug-03 was *Gonyostomum,* which was picked by hand.

Lake	Date	pCO_2 (µatm)	DIC (µmol)	pH	$\delta^{13}C$ DIC (‰)	$\delta^{13}C$ CO_2 (‰)	$\delta^{13}C$ algae (‰)	ε_p (‰)	% Chl. retained	size fraction (µm)	C:Chl
Paul	28-Jun-02	1226	96	n/a	–15.2	–20.2	–30.1	9.9	41	45<x<153	52
East Long	12-Jul-02	595	39	n/a	–21.7	–25.9	–26.0	0.1	8	45<x<64	46
Hummingbird	5-Aug-02	422	29	n/a	–26.3	–30.8	–22.1	–8.8	43	45<x<64	54
Peter	09-Jun-03	822	137	6.59	–11.4	–17.3	–26.5	9.3	n/a	20<x<45	95
Paul	10-Jun-03	750	90	6.31	–16.2	–20.5	–32.1	11.6	n/a	45<x<153	n/a
West	11-Jun-03	916	62	5.93	–22.5	–24.9	–31.9	6.9	n/a	45<x<153	n/a
Tenderfoot	17-Jun-03	1248*	814	7.60	–10.6	–19.2	–26.0	6.8	3	20<x<45	n/a
Crampton	18-Jun-03	543	35	5.79	–22.8	–24.7	–27.0	2.4	16	10<x<35	227
Tender Bog	24-Jun-03	7370*	301	4.21	–27.3	–27.3	–31.2	3.8	27	35<x<64	47
Tenderbog	24-Jun-03	7370*	301	4.21	–27.3	–27.3	–27.4	0.1	20	10<x<35	118
Brown	25-Jun-03	273*	1072	8.40	–11.4	–20.3	–31.4	11.1	20	10<x<35	n/a
Morris	14-Jul-03	692	842	7.47	–10.5	–19.0	–29.0	10.0	12	10<x<35	141
Palmer	15-Jul-03	744	872	7.10	–12.9	–20.7	–30.1	9.3	22	10<x<35	78
Trout Lake	11-Aug-03	233	880	7.81	– 5.2	–14.1	–28.1	14.0	0	<153	220
Trout Lake	11-Aug-03	233	880	7.81	– 5.2	–14.1	–26.0	11.9	7	35<x<64	268
Trout Lake	11-Aug-03	233	880	7.81	– 5.2	–14.1	–27.1	12.9	15	10<x<35	262
Trout Lake	11-Aug-03	233	880	7.81	– 5.2	–14.1	–27.9	13.8	n/a	<10	271
Allequash	12-Aug-03	178	843	8.13	–17.3	–26.1	–27.1	1.0	2	<153	134
Allequash	12-Aug-03	178	843	8.13	–17.3	–26.1	–28.1	2.0	11	35<x<64	82
Allequash	12-Aug-03	178	843	8.13	–17.3	–26.1	–27.2	1.1	25	10<x<35	94
Allequash	12-Aug-03	178	843	8.13	–17.3	–26.1	–27.1	1.0	n/a	<10	167
Big Musky	14-Aug-03	184	437	7.68	– 5.3	–13.9	–25.9	12.0	7	<153	734
Big Musky	14-Aug-03	184	437	7.68	– 5.3	–13.9	–24.9	11.0	12	10<x<35	692
Big Musky	14-Aug-03	184	437	7.68	– 5.3	–13.9	–26.1	12.2	n/a	<10	752
Trout Bog	19-Aug-03	705	34	4.53	–22.6	–22.7	–28.3	5.6	n/a	*Gonyostomum*	n/a
North Gate Bog	20-Aug-03	2530	100	3.89	–27.2	–27.2	–27.7	0.5	8	<153	186
North Gate Bog	20-Aug-03	2530	100	3.89	–27.2	–27.2	–26.5	–0.7	5	35<x<64	1282
North Gate Bog	20-Aug-03	2530	100	3.89	–27.2	–27.2	–24.8	–2.5	0	10<x<35	793
North Gate Bog	20-Aug-03	2530	100	3.89	–27.2	–27.2	–29.5	2.3	n/a	<10	144
Hiawatha	22-Aug-03	765	396	6.91	–10.6	–17.6	–30.6	13.0	1	<153	84
Hiawatha	22-Aug-03	765	396	6.91	–10.6	–17.6	–32.5	14.9	19	35<x<64	68
Hiawatha	22-Aug-03	765	396	6.91	–10.6	–17.6	–28.3	10.7	16	10<x<35	109
Hiawatha	22-Aug-03	765	396	6.91	–10.6	–17.6	–29.4	11.7	n/a	<10	73
Fence	24-Aug-03	222	768	7.94	– 3.9	–12.8	–25.8	12.9	5	<153	105
Fence	24-Aug-03	222	768	7.94	– 3.9	–12.8	–22.3	9.4	11	35<x<64	78
Fence	24-Aug-03	222	768	7.94	– 3.9	–12.8	–25.7	12.8	18	10<x<35	111
Fence	24-Aug-03	222	768	7.94	– 3.9	–12.8	–26.2	13.3	n/a	<10	109

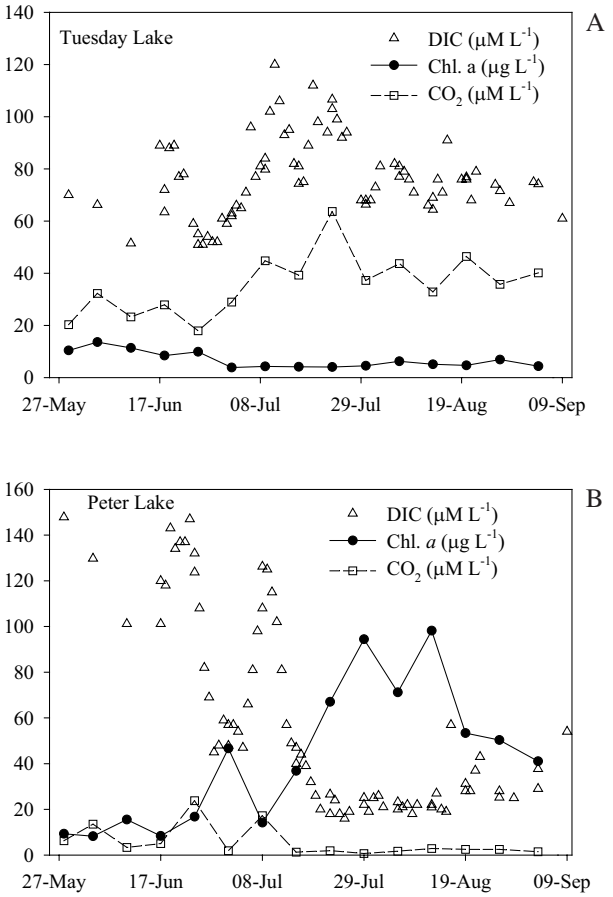

Figure 3. DIC, CO_2, and Chl. *a* during the summer 2002 for a) Tuesday Lake and b) Peter Lake. DIC was measured on a daily basis after the fourth week of sampling. These samples were collected several hours before the regular weekly sampling.

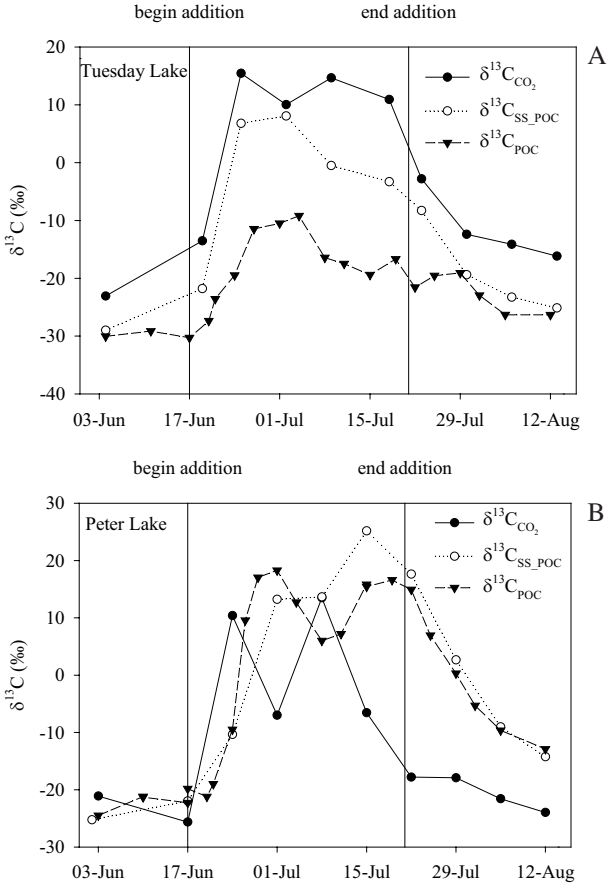

Figure 4. Time course of $\delta^{13}CO_2$, $\delta^{13}C_{SS\_POC}$ and $\delta^{13}C_{POC}$ for a) Tuesday Lake and b) Peter Lake.

abundant algal species observed were generally armored dinoflagellates (*Peridinium* spp.).

In Peter Lake, the instantaneous measurement of fractionation, with respect to $\delta^{13}C$-CO_2, was in many cases negative, as was also the case when considering the isotope signature of DIC as the source of inorganic carbon (Table 3). The size fractions collected in Peter Lake were the same as in Tuesday Lake. The POC:Chl ratios of the size-separated material in Peter Lake (64 ± 43) were much lower than in Tuesday Lake (Table 3). For non-separated POC in Peter Lake, the mean POC:Chl ratio was 58 ± 33. The proportion of Chl. *a* that was extracted was small in the first four sample periods (2.5–16 %) but increased considerably in the last sampling periods ranging from 34–73 %. In the later sampling periods *Staurastrum* and a filamentous bluegreen algae (probably *Anabaena*) were the dominant algae, although *Staurastrum* was present throughout the summer.

The statistical model of Tuesday Lake revealed ε_p to be 8.34 ± 2.20‰, in close agreement with the instantaneous measurements. The other fitted parameters were the proportion of terrestrial carbon, w = 0.59 ± 0.045, the proportion of carbon form u days prior, m = 0.44 ± 0.273, and the lag, u = 5 ± 3.27 days. The residual standard deviation for the model was 2.78‰ and R^2 = 0.81.

Recall that in the Peter Lake statistical analysis, the parameter φ was used to account for the effects of the extreme drawdown of CO_2 caused by the nutrient addition. Thus, in Peter Lake we expected the system to change from nominal fractionation, similar to conditions in the other lakes, to a situation where fractionation would be dramatically reduced under extremely low CO_2. The parameter, φ was 1.57 ± 0.42. From φ, we calculated ε_p (with respect to $\delta^{13}C$-DIC) for corresponding dates when POC was collected (Fig. 5). For much of the season, ε_p was near 0‰ as CO_2 concentrations were low and bicarbonate uptake was likely. The proportion of terrestrial material was small (w = 0 ± 0.007), and a large proportion of the POC was algae produced in the recent past (m = 0.83 ± 0.116, and u = 8 ± 1.20 days). The model for Peter Lake had a residual standard deviation of 5.75‰ and R^2 = 0.93.

Table 3. Photosynthetic fractionation factors (ε_p) determined by physical separation of algae in the isotope addition experiments and characteristics of the physically separated algae. For Peter Lake the ε_p values in parenthesis are taken with respect to δ^{13}C-DIC as opposed to δ^{13}C-CO_2.

Date	ε_p	C:Chl	Size fraction (μm)
Tuesday Lake			
6/4/2002	5.93	290.08	10<x<20
6/19/2002	8.27	n/a	20<x<45
6/25/2002	8.68	82.89	20<x<45
7/2/2002	1.99	93.23	20<x<45
7/9/2002	15.19	106.73	20<x<45
7/18/2002	14.24	101.98	20<x<45
7/23/2002	5.51	92.55	20<x<45
7/30/2002	7.01	118.39	20<x<45
8/6/2002	9.16	97.52	20<x<45
8/13/2002	8.97	95.60	20<x<45
Peter Lake			
6/3/2002	5.20 (12.83)	173.71	10<x<20
6/17/2002	–3.88 (5.44)	83.45	20<x<45
6/24/2002	21.71 (28.43)	72.21	20<x<45
7/1/2002	–20.54 (–11.78)	58.28	20<x<45
7/8/2002	0.45 (7.63)	64.57	20<x<45
7/15/2002	–32.01 (–23.11)	47.06	20<x<45
7/22/2002	–36.07 (–27.25)	36.10	20<x<45
7/29/2002	–20.80 (–11.95)	45.45	20<x<45
8/5/2002	–13.21 (–4.12)	32.67	20<x<45
8/12/2002	–11.13 (–2.14)	25.18	20<x<45

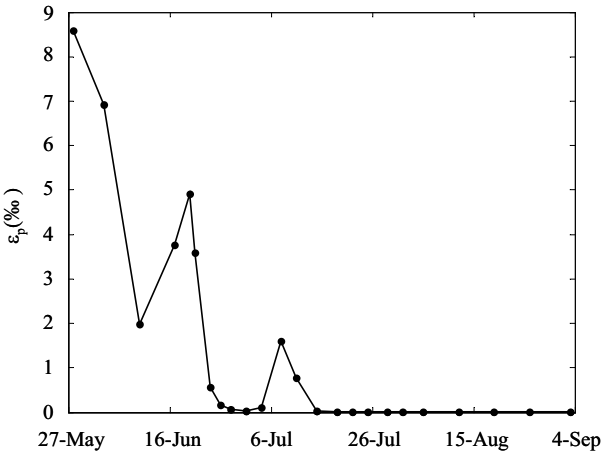

Figure 5. Values of ε_p in Peter Lake through time estimated from the univariate statistical model. These values are calculated from the fitted value φ (1.57) and CO_2 concentration estimated from DIC and pH.

We tested the model for sensitivity to the assumed terrestrial signature over a range of –27‰ to –29‰. In Peter Lake there was no change in the value of φ, since terrestrial material was essentially nil. In Tuesday Lake, the value of ε_p ranged from 9.8 to 6.4 over the respective range in assumed terrestrial signature. The assumed terrestrial signature had no effect on the other model parameters.

Discussion

Many studies, most taking place in laboratory settings, have shown that aqueous pCO_2 is an important variable explaining differences in observed ε_p. Even in studies that consider growth rate, pCO_2 alone still explains a large portion of the variation (e.g., Laws et al., 1997). Our data show that other factors besides pCO_2 must drive the variation in ε_p observed among lakes. Although Figure 1b suggests some correlation between ^{13}C-POC and pCO_2, ε_p is only one of several factors that determine ^{13}C-POC. After estimating the algal isotope signature by accounting for non-algal material, or physically separating representative algal portions, we do not observe the general patterns of increasing photosynthetic fractionation with increases in pCO_2. In addition, for many of our observations the fractionation is generally below the maximum fractionation between 20‰ to 30‰ (Goericke et al., 1994), even in lakes with high pCO_2. The lack of conformity between our results and previous laboratory studies is likely due to the presence of differing algal communities among lakes. Pel et al. (2003) discovered phytoplankton taxa differing by 6–10‰ within the same sample. Therefore one model may be satisfactory for a single species, but an assemblage of taxa within a given lake will present a mixed result that appears patternless when many lakes are compared. In addition seasonal succession of algal species may also make using models of algal fractionation difficult to employ for aquatic studies (e.g., Zohary et al., 1994).

Within our comparative studies we must consider whether our techniques or assumptions lead us to the conclusion that other factors besides CO_2 are important for predicting ε_p among lakes. The results shown in Figure 2a demonstrate the consequences of our assumption on algal-C:Chl ratios. The large range in potential ε_p values makes it difficult to draw a strong conclusion. However, the results in Figure 2b are less influenced by our assumptions. Figure 2b perhaps contains the strongest evidence that patterns of ε_p and CO_2 do not exist when comparing among lakes.

Our second comparative study, which relies on separating different size-fractions of POC, also has potential weaknesses. Our main concern is that these samples are not completely algal material as indicated by the C:Chl ratios for some samples. Nonetheless, we found encour-

agement from the results of the isotope addition experiment in Tuesday Lake. The size-separated POC samples in Tuesday Lake showed very distinct signatures indicating that the method had excluded a significant contribution from detritus that had a terrestrial signature.

The inorganic carbon isotope additions provided a unique opportunity to create changes in algal signatures that allowed estimates of ε_p, both by direct separation of algae and by using a statistical model. Previously, Pace et al. (2004) used this statistical model to determine ε_p in Paul Lake ($\varepsilon_p = 11.5 \pm 0.90$) and Peter Lake ($\varepsilon_p = 11.4 \pm 1.25$) for a similar experiment conducted in 2001. An inorganic ^{13}C addition reported by Cole et al. (2002) found ε_p to be 5.4‰ in East Long Lake, using a carbon flow model. In all these examples, excluding Peter Lake in 2002, CO_2 concentrations were at or above atmospheric saturation, yet the magnitude of ε_p was low.

Tuesday Lake is a slightly acid lake, with pCO_2 generally at or above atmospheric equilibrium. Productivity levels were low to moderate. Particulate organic carbon from this lake appears to have a large terrestrial component as suggested by the difference observed in isotope signatures between the physically separated algae and the POC. This is corroborated by the results of the statistical model that showed nearly 60% of the POC to be of terrestrial origin. Instantaneous measurements and statistical results both found that ε_p was near 8‰. The low ε_p values might be explained by the large abundance of *Peridinium* spp. *Peridinium* are heavily armored and it is possible that diffusion may be more limiting in these species thus reducing the preferential uptake of ^{12}C. Zohary et al. (1994) found that *Peridinium gatunense* was more enriched in ^{13}C than most other phytoplankton in Lake Kinneret, suggesting that photosynthetic fractionation is reduced in this genus. Also, some dinoflagellate species are known to be heterotrophic (Graham and Wilcox, 2000), so their carbon isotope signature would be influenced by the carbon they ingest.

Based on instantaneous measurements, photosynthetic fractionation was anomalous in Peter Lake during much of the experiment. The added nutrients increased productivity and reduced pCO_2 to very low levels. POC overwhelmingly consisted of algal material, as suggested by the statistical model and the similarity between the isotope signatures of the size-separated POC and total POC. Therefore the presence of terrestrial detritus was unlikely the cause for any uncharacteristic fractionation. Bicarbonate uptake and the presence of cyanobacteria may have influenced observed ε_p (Goericke et al., 1994). The positive fractionation might be explained by a large amount of residual algae that had slow carbon biomass turnover rates relative to the change in ^{13}C-CO_2. This fact can be seen in the results of the statistical model, showing that over 80% of the POC consisted of algae produced in the recent past (7–9 days).

Although the instantaneous measurements and statistical results for ε_p are in agreement in Tuesday Lake, the lack of agreement in Peter Lake suggests that instantaneous measurement of ^{13}C-CO_2 and ^{13}C-POC may not accurately represent ε_p when ^{13}C-POC or ^{13}C-CO_2 are highly dynamic. This may be one reasonable explanation for the positive values of ε_p observed in some lakes or might explain the lack of relationships overall. Lakes not amended with inorganic ^{13}C will not experience such large dynamics in ^{13}C-CO_2 as observed in Tuesday or Peter lakes. However, several mechanisms could lead to a divergence between current POC isotope signatures and ^{13}C-CO_2. A brief period of high productivity could create a large pool of algal POC with slow turnover. Similarly high productivity can lead to depleted CO_2 and non-linear shifts in ^{13}C-DIC through chemically enhanced diffusion (Bade and Cole, 2006).

Although models of photosynthetic fractionation have been constructed from laboratory studies (e.g., Laws et al., 1995) there are limited examples of field observations in freshwater ecosystems that are closely congruent with the results from these models (Hollander and McKenzie, 1991; Yoshioka, 1997). Finlay (2004) showed that in certain stream periphyton taxa a large proportion of variation in the ^{13}C was explained by CO_2 concentration, while in other taxa there was little response to variation in CO_2. A lack of correlation between ε_p and CO_2 was also noted for algae in Monterey Bay (Rau et al., 2001). Falkowski (1991) found large interspecific variability (over 20‰) in 13 species of marine algae grown under similar conditions, which was attributed to differences in the capacity for β-carboxylation pathways as opposed to direct incorporation into ribulose 1,5-bisphosphate. Additionally, differential allocation of photosynthetic products (e.g., lipids, polysaccharides, and proteins) among species could lead to isotopic differences among species (e.g., Pel et al., 2003). Other species-specific differences, such as cell volume and surface area, have been accounted for in some models (Popp et al., 1998). Carbon concentrating mechanisms or active uptake of inorganic carbon can also lead to patterns of fractionation that are not linearly related to CO_2 (Sharkey and Berry, 1985; Keller and Morel, 1999). Finally, factors such as limiting nutrients or light regime may influence photosynthetic fractionation to a greater extent than CO_2 or growth rate (Burkhardt et al., 1999a, b).

Single models for accurately predicting inter-lake patterns of fractionation do not appear to exist at the present time or are confounded by carbon isotope dynamics that are not easily accounted for. For paleolimnological studies, we support the suggestion by Brenner et al. (1999) that because of the complexities involved it is difficult to construct a transfer function that directly relates changes in organic sediment ^{13}C to changes in productivity. For food web studies, a more thorough measure

of algal isotope signatures, beyond just ^{13}C-POC, may be needed. These measurements may require isotopic analysis of algal specific biomarkers (Bidigare et al., 1991) or other means of physical separation of algal cells (Hamilton et al., 2005). However, as more precise methods of measuring algal isotope signatures in situ become available (e.g., Pel et al., 2003), there may be potential to create models that accurately estimate photosynthetic fractionation for natural populations of phytoplankton.

Acknowledgments

We thank Mathew Van de Bogert, R. Adam Ray, Crystal Fankhauser, and Carl Johnson for assistance in the field and laboratory. Dr. Gary Belovsky and Dr. Karen Francl at the University of Notre Dame Environmental Research Center, and Dr. Tim Kratz at the University of Wisconsin Trout Lake Station provided facilities and logistical support. Norma Haubenstock, Robert Drimmie and Marshall Otter provided expertise in stable isotope analysis. Financial support for this research came from the National Science Foundation, the Andrew W. Mellon Foundation and the Anna Grant Birge Fellowship.

References

Bade, D. L., 2004. Ecosystem carbon cycles: whole-lake fluxes estimated with multiple isotopes. Ph.D. University of Wisconsin-Madison, 137 pp.

Bade, D. L. and J. J. Cole, 2006. Impact of chemically enhanced diffusion on dissolved inorganic carbon stable isotopes in a fertilized lake. Journal of Geophysical Research, 111, C01014, doi:10.1029/2004JC002684.

Bade, D. L., S. R. Carpenter, J. J. Cole, P. C. Hanson and R. H. Hesslein, 2004. Controls of δ^{13}C-DIC in lakes: Geochemistry, lake metabolism, and morphometry. Limnol. Oceanogr. **49**: 1160–1172.

Bidigare, R. R., M. C. Kennicutt, W. L. Keeneykennicutt and S. A. Macko, 1991. Isolation and purification of chlorophylls *a* and *b* for the determination of stable carbon and nitrogen isotope compositions. Anal. Chem. **63**: 130–133.

Brenner, M., T. J. Whitmore, J. H. Curtis, D. A. Hodell and C. L. Schelske, 1999. Stable isotope (δ^{13}C and δ^{15}N) signatures of sedimented organic matter as indicators of historic lake trophic state. J. Paleolimnol. **22**: 205–221.

Burkhardt, S., U. Riebesell and I. Zondervan, 1999a. Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. Geochim. Cosmochim. Acta **63**: 3729–3741.

Burkhardt, S., U. Riebesell and I. Zondervan, 1999b. Stable carbon isotope fractionation by marine phytoplankton in response to daylength, growth rate, and CO_2 availability. Mar. Ecol.-Prog. Ser. **184**: 31–41.

Carpenter, S. R. and J. F. Kitchell, 1993. The Trophic Cascade in Lakes. Cambridge University Press, Cambridge, 385 pp.

Cole, J. J., N. F. Caraco, G. W. Kling and T. K. Kratz, 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science **265**: 1568–1570.

Cole, J. J., S. R. Carpenter, J. F. Kitchell and M. L. Pace, 2002. Pathways of organic carbon utilization in small lakes: Results from a whole-lake C-13 addition and coupled model. Limnol. Oceanogr. **47**: 1664–1675.

del Giorgio, P. A. and R. L. France, 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton δ^{13}C. Limnol. Oceanogr. **41**: 359–365.

Falkowski, P. G., 1991. Species variability in the fractionation of ^{13}C and ^{12}C by marine phytoplankton. J. Plankton Res. **13**: S21–S28, Suppl.

Finlay, J. C., 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnol. Oceanogr. **49**: 850–861.

Finlay, J. C., M. E. Power and G. Cabana, 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. Limnol. Oceanogr. **44**: 1198–1203.

Francois, R., M. A. Altabet, R. Goericke, D. C. McCorkle, C. Brunet and A. Poisson, 1993. Changes in the δ^{13}C of surface water particulate organic matter across the subtropical convergence in the SW Indian Ocean. Glob. Biogeochem. Cycle **7**: 627–644.

Goericke, R. and B. Fry, 1994. Variations of marine plankton δ^{13}C with latitude, temperature, and dissolved CO2 in the world ocean. Glob. Biogeochem. Cycle **8**: 85–90.

Goericke, R., J. P. Montoya and B. Fry, 1994. Physiology of isotopic fractionation in algae and cyanobacteria. In: K. Lajtha and R. H. Michener (eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, Cambridge, pp. 187–221.

Graham, L. E. and L. W. Wilcox, 2000. Algae. Prentice-Hall, Upper Saddle River, 700 pp.

Hamilton, S. K., S. J. Sippel and S. E. Bunn, 2005. Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica. Limnology and Oceanography-Methods **3**: 149–157.

Hanson, P. C., D. L. Bade, S. R. Carpenter and T. K. Kratz, 2003. Lake metabolism: Relationships with dissolved organic carbon and phosphorus. Limnol. Oceanogr. **48**: 1112–1119.

Hessen, D. O., T. Andersen, P. Brettum and B. A. Faafeng, 2003. Phytoplankton contribution to sestonic mass and elemental ratios in lakes: Implications for zooplankton nutrition. Limnol. Oceanogr. **48**: 1289–1296.

Hollander, D. J. and J. A. McKenzie, 1991. CO_2 control on carbon-isotope fractionation during aqueous photosynthesis: A paleo-pCO_2 barometer. Geology **19**: 929–932.

Karlsson, J., A. Jonsson, M. Meili and M. Jansson, 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. Limnol. Oceanogr. **48**: 269–276.

Keller, K. and F. M. M. Morel, 1999. A model of carbon isotopic fractionation and active carbon uptake in phytoplankton. Mar. Ecol.-Prog. Ser. **182**: 295–298.

Lajtha, K. and R. H. Michener, 1994. Sources and variations in the stable isotopic composition of plants. In: K. Lajtha and R. H. Michener (eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell, Boston, pp. 1–21.

Laws, E. A., R. R. Bidigare and B. N. Popp, 1997. Effect of growth rate and CO_2 concentration on carbon isotopic fractionation by the marine diatom *Phaeodactylum tricornutum*. Limnol. Oceanogr. **42**: 1552–1560.

Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt and S. A. Macko, 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and [CO2]aq: Theoretical considerations and experimental results. Geochim. Cosmochim. Acta **59**: 1131–1138.

Leavitt, P. R. and S. R. Carpenter, 1990. Regulation of pigment sedimentation by photo-oxidation and herbivore grazing. Can. J. Fish. Aquat. Sci. **47**: 1166–1176.

Marker, A. F. H., C. A. Crowther and R. J. M. Gunn, 1980. Methanol and acetone as solvents for estimating chlorophyll and pheopigments by spectrophotometry. Ergeb. Limnol. **14**: 52–69.

Meyers, P. A. and E. Lallier-Verges, 1999. Lacustrine sedimentary organic matter records of Late Quaternary paleoclimates. J. Paleolimnol. **21**: 345–372.

Mook, W. G., J. C. Bommerson and W. H. Staverman, 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet. Sci. Lett. **22**: 169–176.

Oana, S. and E. S. Deevey, 1960. Carbon 13 in lake waters and its possible bearing on paleolimnology. Am. J. Sci. **258**: 253–272.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. Van de Bogert, D. L. Bade, E. S. Kritzberg and D. Bastviken, 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**: 240–243.

Pel, R., H. Hoogveld and V. Floris, 2003. Using the hidden isotopic hetergeneity in phyto- and zooplankton to unmask disparity in trophic carbon transfer. Limnol. Oceanogr. **48**: 2200–2207.

Popp, B. N., E. A. Laws, R. R. Bidigare, J. E. Dore, K. L. Hanson and S. G. Wakeham, 1998. Effect of phytoplankton cell geometry on carbon isotopic fractionation. Geochim. Cosmochim. Acta **62**: 69–77.

Rau, G. H., F. P. Chavez and G. E. Friederich, 2001. Plankton $^{13}C/^{12}C$ variations in Monterey Bay, California: evidence of non-diffusive inorganic carbon uptake by phytoplankton in an upwelling environment. Deep Sea Res. (I Oceanogr. Res. Pap.) **48**: 79–94.

Rau, G. H., T. Takahashi and D. J. D. Marais, 1989. Latitudinal variations in plankton $\delta^{13}C$: implications for CO_2 and productivity in past oceans. Nature **341**: 516–518.

Raven, J. A., 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biol.Rev. **45**: 167–221.

Schelske, C. L. and D. A. Hodell, 1995. Using carbon isotopes of bulk sedimentary organic matter to reconstruct the history of nutrient loading and eutrophication in Lake Erie. Limnol. Oceanogr. **40**: 918–929.

Sharkey, T. D. and J. A. Berry, 1985. Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. In: W. J. Lucas and J. A. Berry (eds.), Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. American Society of Plant Physiologists, Rockville, pp. 389–401.

Stumm, W. and J. J. Morgan, 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, 3 edition. John Wiley and Sons, New York, 1022 pp.

Yoshioka, T., 1997. Phytoplanktonic carbon isotope fractionation: equations accounting for CO_2-concentrating mechanisms. J. Plankton Res. **19**: 1455–1476.

Zohary, T., J. Erez, M. Gophen, I. Berman-Frank and M. Stiller, 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnol. Oceanogr. **39**: 1030–1043.