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Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake?

Michael L. Pace[1]
Institute of Ecosystem Studies, P.O. Box AB, Millbrook, New York 12545

Stephen R. Carpenter
Center for Limnology, University of Wisconsin, Madison, Wisconsin 53706

Jonathan J. Cole and James J. Coloso
Institute of Ecosystem Studies, P.O. Box AB, Millbrook, New York 12545

James F. Kitchell
Center for Limnology, University of Wisconsin, Madison, Wisconsin 53706

James R. Hodgson
Department of Biology, St. Norbert College, De Pere, Wisconsin 54115

Jack J. Middelburg
Netherlands Institute of Ecology, Korringaweg 7, 4401 NT Yerseke, The Netherlands

Nicholas D. Preston, Christopher T. Solomon, and Brian C. Weidel
Center for Limnology, University of Wisconsin, Madison, Wisconsin 53706

Abstract

Allochthonous organic carbon can subsidize consumers in aquatic systems, but this subsidy may only be significant in relatively small systems with high organic matter loading. We tested the importance of allochthonous carbon to consumers in a relatively large (258,000 m^2) clear-water lake by adding $H^{13}CO_3$ daily for 56 d. Dissolved inorganic carbon (DIC) was substantially enriched in ^{13}C by the addition, but it was also variable over diel cycles because of exchange with the atmosphere and photosynthesis. By measuring the $\delta^{13}C$ value of a physically separated phytoplankton concentrate as well as the $\delta^{13}C$ of phospholipid fatty acids, we were able to follow ^{13}C-labeling dynamics of specific groups of phytoplankton and bacteria. The $\delta^{13}C$ values of particulate organic carbon (POC), dissolved organic carbon (DOC), phytoplankton, bacteria, zooplankton, and the invertebrate predator, *Chaoborus* spp. all increased to a maximum during the addition and declined once the addition ceased. Autochthony (% C derived from internal primary production) of carbon pools (POC, DOC) and consumers was assessed by fitting dynamic models to time series of $\delta^{13}C$. Autochthonous carbon was the dominant source (88–100%) for POC, gram-positive bacteria, a copepod, zooplankton biomass, and *Chaoborus* spp. Autochthonous carbon provided a lower fraction (<70%) of carbon to DOC, gram-negative bacteria, and cladoceran zooplankton. In comparison to smaller and more humic lakes, terrestrially derived allochthonous C was less significant to the pelagic food web in this larger, clear-water lake. Among lakes, the relative importance of autochthonous versus allochthonous carbon to planktonic consumers is positively correlated to the ratio of color (absorbance of light at 440 nm, an indicator of terrestrially derived organic carbon) to chlorophyll.

[1] Corresponding author (pacem@ecostudies.org).

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Aquatic ecosystem carbon budgets are often dominated by large inputs of dissolved and particulate organic carbon from land. This terrestrially derived organic matter partially fuels both system metabolism and secondary production in many streams, rivers, lakes, and estuaries (Webster and Myer 1997; Wetzel 2001; Chanton and Lewis 2002). The relative support of consumers by autochthonous and allochthonous resources, however, is difficult to determine except in rare cases (e.g., Peterson and Howarth 1987). One method for differentiating autochthonous and allochthonous sources is to experimentally manipulate the stable isotope values of one of the pools. We have used this approach in previous work by adding $H^{13}CO_3$ to entire lakes to enrich the $\delta^{13}C$ of dissolved inorganic carbon used by photosynthetic organisms (Cole et al. 2002; Carpenter et

al. 2005). These additions increased the $\delta^{13}C$ of autotrophs and allowed us to measure the utilization of recently fixed autotrophic carbon by consumers (Pace et al. 2004). Carbon-flow simulations as well as time-series statistical models were applied to assess the dynamics of ^{13}C as it moved through the food web in order to estimate utilization of autochthonous and allochthonous carbon by consumers such as bacteria, zooplankton, benthos, and fish (Carpenter et al. 2005). We conducted ^{13}C additions to lakes that contrasted in colored (i.e., chromophoric) dissolved organic carbon (DOC), nutrients, and food webs. Terrestrial support of consumers was greater than 40% in most cases, except when nutrients were added, which increased internal primary production and decreased relative support by allochthonous sources (Carpenter et al. 2005). Consumer utilization of terrestrial subsidies varied depending on the characteristics of the imported material and route of entry into the food web (Cole et al. 2006). For the three lakes studied, the ratio of color (absorbance of light at 440 nm) to chlorophyll was a good indicator of relative use of allochthonous carbon by consumers (Carpenter et al. 2005).

These prior studies were conducted in small lakes (surface areas of 9,100 to 26,500 m^2) that had moderate to high concentrations of DOC and gross primary production (GPP) to respiration (R) ratios <1, indicating considerable heterotrophic metabolism of allochthonous carbon. Food webs of larger lakes may depend less on allochthonous resources than small lakes because areal loading of terrestrial organic carbon should decline as the perimeter-to-area ratio declines with increased lake size. It is also possible, however, that consumers such as fishes orient to littoral zones and, therefore, depend on terrestrial resources to a similar extent in large and small lakes (Schindler and Scheuerell 2002; Vander Zanden and Vadeboncoeur 2002). In addition, terrestrial organic carbon may be processed more completely in larger lakes because of longer water residence times (Molot and Dillon 1997; Curtis 1998), and this mechanism could promote significant utilization of this material by consumers. If these latter two perspectives (i.e., littoral orientation and/or greater processing) are correct, then the extent of the terrestrial subsidy to lake food webs might be independent of lake size.

Evidence for the importance of allochthonous resources across lake-size gradients is mixed and indirect. In larger lakes, the $\delta^{13}C$ value of dissolved inorganic carbon (DIC) is close to atmospheric equilibrium, whereas in smaller lakes, the $\delta^{13}C$ value of DIC is more similar to terrestrial organic matter (Bade et al. 2004). This difference partly reflects the effects of fetch and wind mixing, which promote equilibration of lake CO_2 with the atmosphere (Bade et al. 2004). Nevertheless, surface-water partial pressure of CO_2 (pCO_2) may be an indicator of net heterotrophy and has been found to be negatively correlated with lake area across a range of lake sizes (2.4×10^4 to 8.6×10^{10} m^2) (Kelly et al. 2001). If the $\delta^{13}C$ value of DIC relative to atmospheric equilibrium or the relative saturation of CO_2 predicts the importance of allochthonous carbon to food webs, then the patterns of near-isotopic equilibrium with the atmosphere and lower pCO_2 in larger lakes suggest that allochthony (the relative support of consumers by allochthonous carbon) declines with lake area. However, no correlation between CO_2 flux to the atmosphere and lake size was found for 16 lakes over the size range of 10,000–270,000 m^2 (Jonsson et al. 2003), and even very large lakes can be net heterotrophic (Cole et al. 1994; Urban et al. 2005). Thus, it is uncertain if net heterotrophy as indicated by the balance of metabolic gases (i.e., CO_2 and O_2) is related to the importance of allochthonous resources to consumers or mainly reflects bacterial respiration of terrestrial organic matter. Studies of ambient ^{13}C in zooplankton have indicated significant allochthonous support of zooplankton in both large and small lakes (Meili et al. 1996; Grey et al. 2001). Karlsson et al. (2003) found that allochthonous support of zooplankton varied among lakes as a function of relative pelagic heterotrophic bacterial production and concluded that zooplankton use of allochthonous carbon was not simply a function of relative DOC concentration in lakes. These results suggest that high allochthonous support of zooplankton is not necessarily unique to small lakes or lakes with high DOC concentrations and that allochthonous support may not decline with lake area.

There are several problems in analyzing the importance of carbon sources in lakes using natural abundances of stable isotopes. In many lakes, the $\delta^{13}C$ value of particulate organic carbon (POC) falls within the range of carbon derived from terrestrial photosynthesis (France et al. 1997; Bade et al. 2006). In addition, phytoplankton comprise a variable fraction of the POC in lakes and are not easily separated from nonphytoplankton POC. Further, the nonphytoplankton POC is a mixture of material of aquatic and terrestrial origin. Thus, it is difficult to measure the ^{13}C content of phytoplankton biomass as well as the ^{13}C content of the dead autochthonous carbon that derives from phytoplankton. There is also uncertainty about the expected ^{13}C content of phytoplankton. Photosynthetic carbon fixation discriminates against $^{13}CO_2$. Consequently, phytoplankton organic matter is substantially more depleted than DIC, but photosynthetic fractionation is variable in lakes, and estimates from conventional models based mainly on cultures of marine phytoplankton may not apply in many cases (Bade et al. 2006).

Here, we present results from a $H^{13}CO_3$ addition to an oligotrophic, clear-water lake with low color, low chlorophyll, and GPP : R ratio close to unity. The surface area of the study lake was much larger than the lakes used in previous studies. We tested if autochthonous carbon was sufficient to support pelagic consumers.

Methods

^{13}C addition and site description—$NaH^{13}CO_3$ was added daily to the upper mixed layer of Crampton Lake for 56 d from 13 June to 07 August 2005. These additions were designed to enrich the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) and create a large contrast between the $\delta^{13}C$ of organic matter produced by (or derived from) primary producers within the lake (phytoplankton, periphyton, and macrophytes) and the organic matter that was terrestrially derived. Each morning, 1.9 mol of $NaH^{13}CO_3$ (Isotech Inc,

^{13}C content >99%) was dissolved in carboys containing lake water. The resulting solution was pumped into the upper mixed layer from a moving boat to promote dispersion of the tracer throughout the lake. The addition was designed to enrich the ^{13}C of DIC in the upper mixed layer (average depth = 4 m) while not significantly altering total DIC (i.e., $^{12}C + {}^{13}C$) concentration.

Located at the University of Notre Dame Environmental Research Center near Land O'Lakes, Wisconsin (89°32′W, 46°13′N), Crampton Lake (258,000 m^2) is considerably larger than the small lakes (9,100–26,500 m^2) used for previous ^{13}C addition studies (Carpenter et al. 2005). The lake is an oligotrophic, clear-water system with no visible inlets. The littoral zone of the lake supports several species of macrophytes, including *Eriocaulon aquaticum* and *Sparganium* spp. The crustacean zooplankton community is dominated in terms of biomass by a calanoid copepod (*Leptodiaptomus minutus*) and a cladoceran (*Holopedium gibberum*). The invertebrate predator *Chaoborus* spp. (primarily *C. punctipennis*) is abundant in the lake. The fish community consists mainly of bluegills (*Lepomis macrochirus*), pumpkinseeds (*Lepomis gibbosus*), yellow perch (*Percha flavescens*), and largemouth bass (*Micropterus salmoides*). Bluegill and largemouth bass dominate the fish community in terms of biomass.

Measurement of ^{13}C—The ^{13}C content of the major pelagic carbon pools was measured before, during, and after the tracer addition. Samples were collected daily for DIC and POC and weekly for DOC, algae, bacteria, zooplankton, and *Chaoborus*. For $DI^{13}C$ samples, evacuated 100-mL serum bottles were filled underwater by piercing rubber septa with needles. H_2SO_4 had previously been added to the evacuated bottles to acidify the final sample to pH 2. Samples were sent to the University of Waterloo Environmental Isotope Laboratory and analyzed by gas chromatography–mass spectrometry using a Micromass Isochrome gas chomatograph combustion (GC-C) isotope ratio–mass spectrometer (IRMS). POC was concentrated by filtration through precombusted glass fiber filters (GF/F), dried (60°C for 48 h), and acid-fumed to remove inorganic carbon. Every week, a DOC sample was saved from one of the POC filtrates. This sample was acidified to pH 2 and dried on a petri dish in a dehydrator. Material remaining on the petri dish after drying was scraped off into a vial for isotopic analysis. Zooplankton and *Chaoborus* were sampled weekly using oblique net hauls conducted in the upper mixed layer at night. Individual animals were separated by taxa under a dissecting microscope, dried, and pulverized. POC, DOC, zooplankton, and *Chaoborus* were analyzed for ^{13}C at the University of Alaska Stable Isotope Facility using a Thermo Finnigan Delta-Plus XP isotope ratio–mass spectrometer with a Costech ESC 4010 elemental analyzer. All ^{13}C data are presented as δ values in per mil units following the equation:

$$\delta^{13}C = 1000 \times [(R/0.011237) - 1] \quad (1)$$

where R is the ratio of ^{13}C to ^{12}C in a sample, and 0.011237 is the ratio in a standard.

We measured the $\delta^{13}C$ value of phytoplankton using two methods: first by physically separating phytoplankton from other seston, and second by using phospholipid fatty acids (PLFAs) that represent specific biomarkers for phytoplankton groups (e.g., chlorophytes) as well as gram-negative and gram-positive bacteria.

Physical separation: We separated seston using the density-gradient centrifugation method described by Hamilton et al. (2005). Particles from 40-liter samples were concentrated by continuous centrifugation in a plankton centrifuge (Kahl Scientific Model 026WH106). The retained particles were resuspended and centrifuged at 150 g in silica gel (Ludox TM-50), which was diluted with deionized water to a density of 1.27 g cm^{-3} (Hamilton et al. 2005). Microscopic examination was used to determine where in the gel algae were most concentrated and least contaminated by nonalgal particles. This fraction was collected, and samples were saved for isotopic analysis.

Separation and isotopic composition of PLFA biomarkers: PLFAs in suspended particles collected on GF/F filters were extracted and analyzed as in Boschker et al. (1999, 2005). Briefly, lipids were extracted in chloroform–methanol–water using a modified Bligh and Dyer method and fractionated on silicic acid into different polarity classes. The PLFA fraction was derivatized to fatty acid methyl esters (FAME), and concentrations were determined by gas chromatograph–flame ionization detection (GC-FID). The derivatized PLFAs were identified from retention times compared with those of reference materials and by gas chromatography–mass spectrometry (GC-MS, Thermo Finnigan Voyager). The carbon isotopic composition of individual FAMEs was determined with a gas chromatograph–combustion interface–isotope ratio–mass spectrometer (GC-C-IRMS; an HP 68900 GC [Hewlett Packard] connected to a Delta-Plus IRMS via a type-III combustion interface from Thermo Finnigan [Bremen]). Internal and external FAME standards were used to verify the accuracy, and stable isotope ratios for individual PLFAs were corrected for the one carbon atom added during derivatization.

PLFAs as biomarkers: PLFAs are derived from biological membranes, and individual PLFAs provide the potential to trace specific groups of bacteria and eukaryotic algae. Moreover, PLFAs are readily degraded upon death, and so PLFA patterns and isotope values are directly linked to living biomass (Boschker and Middelburg 2002). Polyunsaturated PLFAs are biomarkers for algae because some compounds dominate the spectra in one group and are not present or are only very minor constituents in other algae. On the basis of PLFA spectra, we attributed PLFA 20:5ω3, 18:3ω3, and 18:4ω3 in our samples from Crampton Lake to diatoms, green algae, and dinoflagellates, respectively. These attributions were supported by measurements of chlorophylls *a*, *b*, and *c* as an independent check. Unfortunately, accessory pigments like fucoxanthin and peridinin, which are diagnostic of diatoms and dinoflagellates, were below detection limits because of limited sample

size. The isotopic composition of the total algal pool was estimated by weighting the $\delta^{13}C$ values of the three algal biomarkers with their contribution to the common PLFA using the polyunsaturated 16:0 concentration ratios reported by Dijkman and Kromkamp (2006). By this means, we derived an algal $\delta^{13}C$ time series and did not further consider the contribution of individual algal groups. The methyl-branched PLFAs (i14:0, i15:0, and ai15:0) were attributed to Cytophaga-Flavobacteria and gram-positive bacteria, while the mono-unsaturated bacterial marker PLFA 18:1ω7c was probably mostly derived from Proteobacteria and gram-negative bacteria (but it also occurs in some algae). For simplicity, we refer hereafter to these two bacterial groups distinguished based on their PLFA biomarkers as gram-positive and gram-negative bacteria, although we recognize that we are actually tracing a mixture of species in these categories (Boschker and Middelburg 2002; Evershed et al. 2006). The $\delta^{13}C$ value of gram-positive bacteria was derived by weighting the $\delta^{13}C$ of methyl-branched PLFAs with their concentration.

Measurement of other variables—To characterize lake conditions and to provide additional data to support model calculations, we measured several other variables. DIC, pCO_2, and temperature were measured to calculate pH and the relative concentration and isotopic composition of aqueous CO_2 and bicarbonate in the DIC pool (Mook et al. 1974; Zhang et al. 1995). DIC and pCO_2 were measured by gas chromatography (Cole et al. 2000). Gross primary production and system respiration of the upper mixed layer were estimated with YSI-Endeco sondes that recorded dissolved oxygen concentration and temperature at 5-min intervals (Cole et al. 2000, 2002; Hanson et al. 2003). Gas exchange between the lake and atmosphere was estimated from wind measurements as described below (Wanninkhof et al. 1985; Cole and Caraco 1998).

Methods used for measuring additional variables are only briefly presented here as these are detailed elsewhere (Carpenter and Kitchell 1993; Carpenter et al. 2001; online manual: http://216.110.136.172/Pages/methods.htm). POC was collected by filtering water through GF/F filters and measured using a Carlo-Erba C:N analyzer. Filtrates from POC analysis were used to measure DOC and color. DOC was determined using a Shimadzu high-temperature carbon analyzer, where acidification and bubbling prior to sample analysis provided estimates of the nonpurgeable organic carbon fraction. Color was measured with a spectrophotometer as absorbance at 440 nm in a 10-cm cell. Weekly measurements of phytoplankton biomass were derived from vertical profiles of chlorophyll *a* (Chl *a*). Weekly measures of zooplankton abundance and biomass were derived from calibrated net hauls. *Chaoborus* were sampled weekly with vertical net hauls taken at night, and biomass was determined from estimates of abundance and measurements of length and diameter.

Model of the isotopic composition of DIC

DIC concentrations were very low in Crampton Lake, and the $DI^{13}C$ isotopic composition was highly dynamic even over the course of a single day. This dynamic was caused by loss of the $DI^{13}C$ to the atmosphere and by photosynthesis and respiration. Changes in wind speed (which controls k, the gas-exchange piston velocity), the mixing depth (Z_{mix}, which affects the mass of DIC in the upper mixed layer, and interacts with k to influence the gas residence time), and the daily addition of $DI^{13}C$ (which occurred near 07:00 h each day) all caused $\delta^{13}C$ values of DIC to vary rapidly on timescales shorter than it was possible to measure. In addition, $DI^{13}C$ measurements were always near the limit of detection because of low DIC concentration.

In order to reconstruct the rapid dynamics of $DI^{13}C$ and to compare these dynamics with our limited measurements of this pool, we calculated the hourly values of $DI^{13}C$ using a simplified version of the dual isotope flow model (DIF) described in Carpenter et al. (2005) and Cole et al. (2006). The DIF model is a mass-balance calculation with two differential equations, one for total DIC ($^{12}C + {}^{13}C$) and one for $DI^{13}C$. In the model, significant inputs of DIC come from incoming groundwater and the excess of respiration over primary production. For $DI^{13}C$, there was also an input caused by our addition of 1.9 mol d^{-1}. For both carbon isotopes (i.e., ^{12}C and ^{13}C), the major loss term was evasion to the atmosphere. The gas piston velocity was calculated from wind speeds measured at 5-min intervals on a floating raft in the middle of the lake at a height of 1 m. We estimated the wind at 10-m height using the equations of Smith (1985) and then calculated k_{600} according Cole and Caraco (1998). We calculated k for CO_2 at the ambient measured temperature from the Schmidt number (Jahne et al. 1987). Temperature was measured at 5-min intervals using YSI-Endeco sondes deployed at 1-m depth continuously at a central station. The mixing depth (Z_{mix}) was measured weekly from detailed temperature profiles. We assumed that Z_{mix} changed linearly between measurements. The model calculated total DIC and $DI^{13}C$ each hour.

Models for estimating autochthony and allochthony

Autochthony, the proportion of carbon flow from within-lake primary production, was estimated by fitting dynamic models to time series of selected ecosystem components. Allochthony was considered to be one minus autochthony. Ecosystem labeling experiments use transient dynamics of the added isotope (^{13}C) to estimate quantities such as autochthony. Therefore, the steady-state mixing models used in studies of natural isotope abundances do not apply. In previous papers, we used three different kinds of models to estimate carbon flows. We found that all three models gave similar results (Carpenter et al. 2005). Simple compartmental models allow us to estimate standard deviations of autochthony by bootstrapping. This information is useful for assessing precision and comparing measurements of autochthony across ecosystem components or lakes. Therefore, we employed simple models with bootstrapped estimates of standard deviations in the analysis of Crampton Lake. Because we measured phytoplankton $\delta^{13}C$ (by two methods) directly in this study, the

models used in previous studies were modified to incorporate this new kind of data.

Autochthony of POC was assessed by fitting the following model to time series of $\delta^{13}C$ for algae (A) and POC (P):

$$A_{t+1} = A_t + [-\lambda A_t + \alpha(C_t - \varepsilon)]\Delta t$$
and
$$P_{t+1} = P_t + [-\lambda P_t + \alpha(C_t - \varepsilon) - 28\tau]\Delta t \quad (2)$$

The input time series C was $\delta^{13}C$ (per mil) of aqueous CO_2 derived from the $DI^{13}C$ values calculated by the model described already, and the time step (Δt) was measured in d. The parameters α, λ, and τ have units of d^{-1}, and ε has units of per mil. Four parameters (α, ε, λ, and τ) and the initial values of A and P were estimated by minimizing the sum of squared errors in predictions of A and P. Note that the model addresses the carbon isotope ratio of a pool measured in per mil composition, not the quantity of carbon in that pool. Thus, the model predicts the movement of the ^{13}C between the end members, not increases or decreases in the carbon pool. The coefficient α is the daily uptake of inorganic carbon as determined by the $\delta^{13}C$ of the aqueous CO_2 adjusted by a fractionation term ε. The proportion of material lost at each time step (due to grazing, sinking, or other loss processes) is λ. This parameter is constrained between 0 and 1. The daily incorporation of terrestrial material with a $\delta^{13}C$ of $-28‰$ (*see* Pace et al. 2004) into POC is τ. Note, we assumed that losses (λ) for A and P and gains (α) for A and P were the same. This assumption simplified the model and reduced the number of parameters that had to be estimated. Autochthony was calculated as $\alpha/(\alpha + \tau)$. Allochthony, the proportion of carbon flow from terrestrial sources, was calculated as $\tau/(\alpha + \tau)$. Equation 2 was also used to fit the $\delta^{13}C$ DOC data, replacing P above with the DOC time series.

Equation 2 was fit simultaneously to time series of $\delta^{13}C$ for POC (or DOC), the average $\delta^{13}C$ of PLFAs from three groups of phytoplankton (greens, dinoflagellates, and diatoms), and phytoplankton separated physically from POC using the Hamilton et al. (2005) method. A value of 3‰ was added to the measured phytoplankton PLFA $\delta^{13}C$ to correct for fractionation that occurs during lipid synthesis. The isotopic values measured by the two methods (physical separation versus biomarker) generally agreed well. The initial physical separation value, however, was more enriched than expected based on the biomarker and POC samples. We assumed that on this date, the centrifugation did not result in a good separation between algae and other particles and excluded this value from the analysis.

The time series for algae, obtained by fitting Eq. 2, was used to estimate autochthony of consumers. Each consumer time-series Z was fit to the model:

$$Z_{t+1} = Z_t + [-\lambda Z_t + \alpha A_t - 28\tau]\Delta t \quad (3)$$

Three parameters (α, λ, and τ) and the initial value of Z were estimated by minimizing the sum of squared errors in predictions of Z. The parameters α, λ, and τ, have the same interpretations as in Eq. 2, and autochthony and allochthony were calculated as explained above.

Equation 3 was fit independently to $\delta^{13}C$ data for two bacterial and three zooplankton time series. Bacterial time series were $\delta^{13}C$ of PLFA biomarkers for gram-positive and gram-negative bacteria. Zooplankton time series were $\delta^{13}C$ of animals handpicked from oblique tows, specifically: *Leptodiaptomus minutus*, cladocerans (*Holopedium gibberum* and *Diaphanosoma birgei*), and a biomass composite of all dominant zooplankters combined (*L. minutus*, the two cladocerans, and *Mesocyclops edax*). For *Chaoborus*, A_t in Eq. 3 was replaced by the model predictions for $\delta^{13}C$ of the fitted zooplankton biomass composite.

Least-squares model fits and bootstrap analyses to estimate parameter uncertainty were computed in Matlab using programs written by the authors. From an initial guess of the parameter values and the initial value of the time series, the entire time series was simulated, the sum of squared errors was computed, and estimates of parameters and initial conditions were iteratively improved until they converged on values that minimized the sum of squared errors (Hilborn and Mangel 1997). Initial values were estimated for each time series on day 152 to standardize the starting point of the analysis because we used multiple series and data for individual time series were not always available for the same starting date. We compared least-squares with maximum likelihood fits, and differences were negligible. Least-squares analysis was preferred because it was faster to compute and did not require additional parameters for the error of each observed time series. Equations 2 and 3 were compared to several more complicated models, but these did not improve the fits, and we therefore preferred the simpler models presented here.

Results

The general conditions of Crampton Lake were characterized before, during, and after the ^{13}C addition (late May to early September 2005), and we present means and standard deviations for some variables derived from weekly or daily (as noted) measurements. The mixed layer of Crampton was slightly acidic (pH = 6.4 ± 0.1). The lake was relatively clear, when compared to other lakes in the area (Pace and Cole 2002), with a Secchi depth of 5.2 ± 0.5 m and water color of 0.57 ± 0.05 m^{-1}. Lake Crampton was unproductive, with a mixed-layer total phosphorus concentration of 7.6 ± 2.5 $\mu g\ L^{-1}$. Gross primary production and system respiration based on average daily mixed-layer values were 47.9 ± 33.8 and 50.8 ± 41.9 mmol $O_2\ m^{-2}\ d^{-1}$, respectively. Hence, the average P : R ratio for the sampling period was 0.94. Average crustacean biomass for the water column was 1.8 ± 0.8 g dry wt m^{-2}. *Chaoborus* were present throughout the season, and biomass averaged 0.38 ± 0.28 g dry wt m^{-2}.

Temporal variability was relatively low. For example, daily average temperature at 1 m increased until day 198 and then declined, but the range in surface-water temperature over the period was $<10^{\circ}C$ (Fig. 1a). Daily average

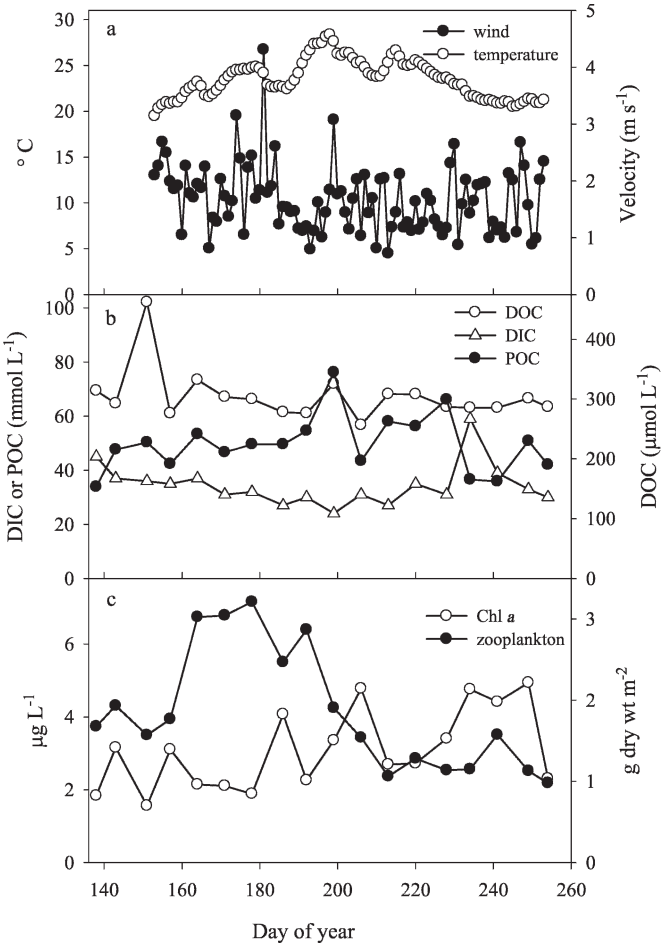

Fig. 1. Seasonal variability of (a) 1-m temperature (open circles) and wind speed (closed circles), (b) DIC (open triangles), POC (closed circles), and DOC (open circles), and (c) Chl *a* (open circles) and crustacean zooplankton biomass (closed circles).

wind speeds were low and typically in the range of 1–3 m s^{-1}, with no seasonal trend (Fig. 1a). There were also no strong seasonal trends in DIC, DOC, POC (Fig. 1b), or Chl *a* concentrations (Fig. 1c). Crustacean zooplankton biomass was dominated by *Leptodiaptomus minutus*, and *Holopedium gibberum* was typically the second most important species. There was a seasonal trend where zooplankton biomass was greatest in early summer and declined to a lower stable value in late summer, reflecting the dynamics of *L. minutus* (Fig. 1c). In general, the temporal variability of the major carbon pools (i.e., DIC, POC, DOC) and consumer biomass was not strongly related to dynamics of ^{13}C. Physical variables, such as temperature and wind, as well as fluxes, such as gross primary production, were important to $DI^{13}C$ dynamics as described next.

$DI^{13}C$ measurements and model

The pre-addition $\delta^{13}C$ value of DIC was in the range of −11 to −15‰. The $\delta^{13}C$ value of DIC rapidly increased after the addition began on day 164 and reached a maximum value of +16‰ on day 192 (Fig. 2). DIC

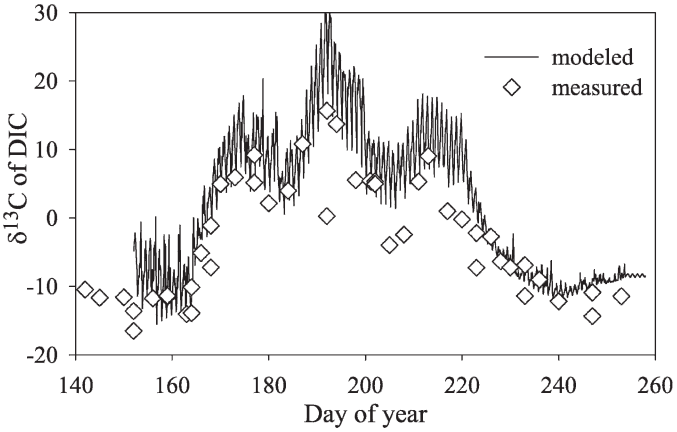

Fig. 2. Measured (open diamonds) and modeled (solid line) values of $DI^{13}C$ for Crampton Lake. Measurements were made about 07:00 h prior to the daily ^{13}C addition and should be close to daily minimum values.

$\delta^{13}C$ values declined rapidly after the addition ended on day 219 and returned to pre-addition values by about day 240. These dynamics indicate that the manipulation substantially enriched $DI^{13}C$.

Measurements of $DI^{13}C$ were made early in the morning prior to daily additions. The model of DIC $\delta^{13}C$ indicates substantial daily variation (Fig. 2) resulting from the spike of added $DI^{13}C$ and subsequent losses both to the atmosphere and to uptake by primary producers. The strong daily dynamic of addition and loss is clear in the simulation of hourly values (Fig. 2). Additional variation in the DIC $\delta^{13}C$ time series was caused primarily by changes in wind and the depth of the mixed layer. For example, the decline in DIC $\delta^{13}C$ from day 193 to 206 occurred during a period when loading of $DI^{13}C$ was constant, the mixed layer deepened, and surface-water temperature declined (Fig. 1a). The model also reveals that the DIC $\delta^{13}C$ sampled prior to addition each day during the ^{13}C manipulation underestimated daily average values by as much as 10‰ to 20‰. Hence, we used the daily means from the model in analyses, that incorporate the DIC $\delta^{13}C$ time series below. Measured values reflected daily minima in $\delta^{13}C$ (Fig. 2), and there was good agreement between the modeled (daily minimum at 6:00 h) and measured values taken in the early morning (regression of predicted vs. observed: slope = 0.86 ± 0.09, intercept = 1.28 ± 0.81, $r^2 = 0.86$, $n = 40$, $p < 0.0001$).

Autochthony of POC and DOC

The addition caused an increase in $\delta^{13}C$ of PLFA biomarkers for phytoplankton and physically separated phytoplankton. Peak $\delta^{13}C$ values ranged from −3‰ to −5‰. Peak values of POC $\delta^{13}C$ were near −12‰ (Fig. 3). The $\delta^{13}C$ values of algal biomarkers and physically separated algae were similar (Fig. 3), indicating that these measures were good indicators of the actual phytoplankton biomass $\delta^{13}C$. A common model (Eq. 2) fit the data for the three time series—PLFA phytoplankton $\delta^{13}C$, physically separated phytoplankton $\delta^{13}C$, and POC $\delta^{13}C$ (Fig. 3).

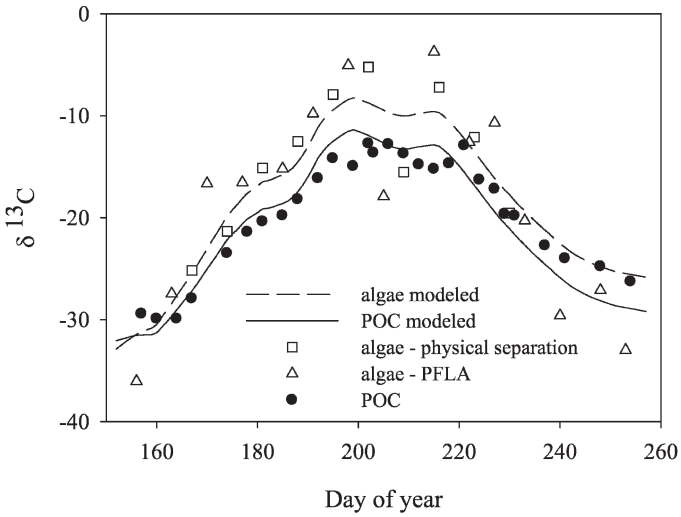

Fig. 3. Measured (symbols) and modeled δ^{13}C (solid and dashed lines) of phytoplankton and POC from day 152 through 257. The phytoplankton are represented by physically separated (open square) and PLFA (open triangle) data. The dashed line is the model fit to phytoplankton δ^{13}C (*see* Table 1). Closed circles are measured POC δ^{13}C values, and the solid line is the model fit to POC δ^{13}C.

Model error standard deviations for the three series were in the range of 1.8‰ to 4.6‰, which were considerably smaller than the total data range of more than 30‰ (Table 1). Correlations of predictions and observations were 0.89 (PLFA phytoplankton), 0.92 (physically separated phytoplankton), and 0.97 (POC). The model estimate for photosynthetic fractionation (ε) of 20.3 is within the commonly reported range of values (20–28‰). Autochthony of POC was 0.88 ± 0.037 (Table 1). Thus, the model suggests that about 12% of the POC on average was of terrestrial origin.

The pre-addition values δ^{13}C of dissolved organic carbon (DOC) averaged 28.3‰, consistent with a terrestrial origin for this material. The δ^{13}C value of DOC increased 4‰ to a maximum of −24.4‰ over the course of the addition (Fig. 4a), demonstrating some contribution of autochthonous carbon to DOC. A model based on the phytoplankton δ^{13}C series and a nominal terrestrial δ^{13}C of −28‰ (Eq. 3) fit the data well (r = 0.91) with a model error of 0.5‰ (Table 1). Based on the model, autochthony of DOC was 0.15, which was much lower than the other components of the system. Unlike some other carbon pools (e.g., POC or zooplankton), the DOC pool was very large and turned over slowly. The model may underestimate autochthony of DOC because the labeling period was probably short relative to the turnover time of the DOC. However, the pre-addition δ^{13}C value of DOC was an exact match for terrestrially derived carbon and about 3‰-enriched compared to our estimates of pre-addition phytoplankton carbon. Taken together, the data suggest that DOC was largely derived from terrestrial inputs.

Autochthony of planktonic consumers

The PLFA biomarkers used to characterize gram-positive and gram-negative bacteria exhibited dynamics similar to phytoplankton, POC, and DOC (Fig. 4b). The PLFA δ^{13}C values of gram-positive bacteria were consistently higher than the algal indicators (cf. Fig. 3 with Fig. 4b). Thus, the model estimate of autochthony of gram-positive bacteria was 100% (Table 1), which is consistent with their carbon always being more enriched than the primary producers. Model error standard deviations were 3.5‰, and the correlation of predictions and observations was 0.86. The model did not fit gram-positive bacteria as well as it fit other consumer groups.

Gram-negative bacteria followed labeling patterns observed for bulk bacterial populations in prior addition experiments (Kritzberg et al. 2004). Gram-negative bacteria were more enriched than DOC (Fig. 4b) but less enriched than phytoplankton (Fig. 3). The PLFA biomarker of gram-negative bacteria attained peak δ^{13}C values similar to those of POC (cf. Fig. 3 with Fig. 4b). The model provided a better fit for these data than for the gram positives, with a model standard deviation of 2.1‰. The correlation of predictions with observations was 0.89. Gram-negative bacteria had much faster turnover rates than POC (λ of 0.31 for bacteria versus 0.06 for POC, Table 1), based on comparisons of the models for each. Autochthony of the

Table 1. Statistics for models used to estimate autochthony. For each time series, the equation analyzed, mean error (‰), correlation coefficient (r) of predictions and observations, the parameters α (daily carbon uptake), ε (photosynthetic fractionation), λ (daily loss rate), and τ (daily terrestrial carbon incorporation), autochthony, and the standard deviation of autochthony are presented. Standard deviations were computed with 1,000 bootstrap iterations.

Time series	Eq.	Mean error	r	α	ε	λ	τ	Autochthony ± SD
Algal PLFA	2	4.8	0.89	0.0525	20.3	0.060	–	–
Algal separation	2	2.6	0.92	0.0525	20.3	0.060	–	–
POC	2	1.8	0.97	0.0525	20.3	0.060	0.0072	0.880 ± 0.037
DOC	3	0.5	0.91	0.0067	–	0.045	0.0378	0.150 ± 0.050
Gram-positive bacteria	3	3.6	0.86	0.0606	–	0.099	0	1 ± 0.092
Gram-negative bacteria	3	2.1	0.90	0.1760	–	0.308	0.1211	0.592 ± 0.075
Leptodiaptomus	3	1.5	0.96	0.0409	–	0.031	0.0007	0.983 ± 0.066
Cladocera	3	1.3	0.97	0.0603	–	0.084	0.0270	0.691 ± 0.052
Combined crustacean zooplankton	3	0.6	0.99	0.0442	–	0.041	0.0037	0.922 ± 0.035
Chaoborus	3	1.3	0.99	0.0681	–	0.0682	0	0.922 ± 0.038

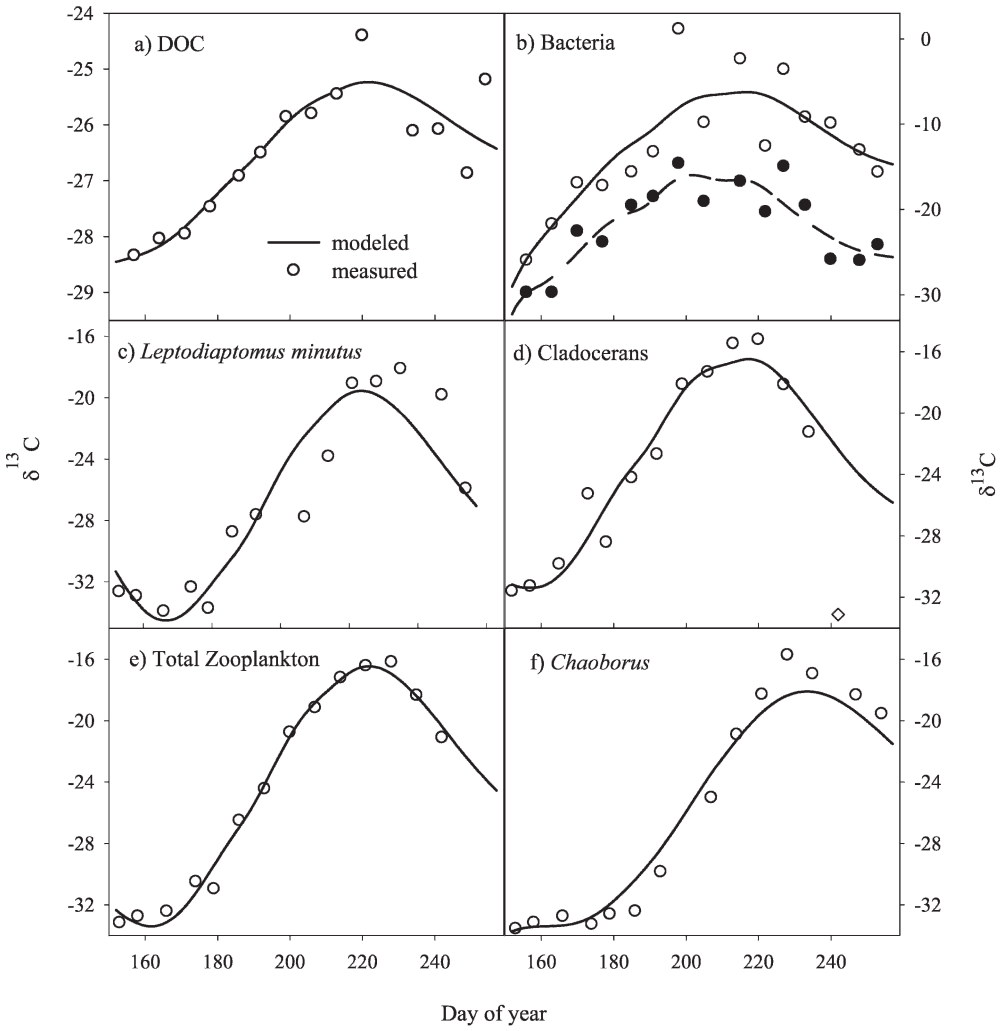

Fig. 4. Measured (open circles) and modeled (solid line) (a) DOC δ^{13}C, (b) gram-positive bacteria and gram-negative bacteria (closed circles, modeled dashed line) based on PLFA profiles, (c) *Leptodiaptomus minutus*, (d) cladocerans (*Holopedium gibberum* and *Diaphanosoma birgei*), (e) total zooplankton, and (f) *Chaoborus* spp. The value represented by an open diamond in panel d was excluded from the model (*see* text).

gram-negative bacteria was 0.59 ± 0.075, which is less than that estimated for POC.

For the three zooplankton groups and *Chaoborus*, the model provided good fits (all $r > 0.9$) with low model errors (all <1.5‰). *Leptodiaptomus minutus*, the dominant zooplankter in Crampton Lake, was almost completely supported by autochthonous carbon (Fig. 4c; Table 1). Cladocerans were less dependent on phytoplankton; they had an autochthonous support of 0.69 ± 0.052. Note, we excluded the final cladoceran observation (Fig. 4d), because it strongly influenced the model fit. However, the estimate of autochthony was very similar (0.70) when this value was included. The total zooplankton δ^{13}C series was a weighted average based on the biomass of the major crustaceans and their measured δ^{13}C. The model fit these data very well (Fig. 4e; $r = 0.99$) and indicated that zooplankton in Crampton Lake were supported primarily by autochthonous carbon (0.92 ± 0.035).

Chaoborus δ^{13}C values reached peak values similar to zooplankton. The time series for *Chaoborus* δ^{13}C values can be explained entirely by the predicted δ^{13}C curve for total crustacean zooplankton (Fig. 4f). Thus, the autochthony of *Chaoborus* was identical to that of total crustacean zooplankton, consistent with diet studies of *Chaoborus* and results of previous whole-lake ^{13}C-labeling experiments (Carpenter et al. 2005).

Discussion

In the low-DIC water of Crampton Lake, DIC and its isotopic composition cycle very rapidly. Our measurements of DI^{13}C taken just prior to the daily additions of ^{13}C represent daily minimum values as indicated by the model-derived, daily averages of ^{13}C-DIC that were substantially more enriched. This difference between the measured ^{13}C-DIC value and the time-dynamic values that phytoplank-

ton experience is more extreme in Crampton Lake than in our prior experiments because DIC is lower and wind-driven gas exchange is faster for this larger lake. The model also suggests that even prior to the start of the ^{13}C addition, $DI^{13}C$ exhibited diel change. For the purposes of analyzing the results of the ^{13}C addition, the model provided average daily values more suitable for estimating photosynthetic fractionation.

The analysis in this study used isotope ratios as expressed in Eqs. 2 and 3. The ratios in a given compartment change because both the ^{13}C changes (in response to the manipulation) and the total carbon ($^{12}C + {}^{13}C$) changes due to many factors. Change in pool size due to manipulation/addition is only one of these factors. Thus, the isotope ratios trace the relative change in ^{13}C and ^{12}C, not change in pool size. The experiment could also be analyzed by constructing a mass balance, but this would require good estimates of the losses of ^{13}C and total C from each pool. The largest losses owe to respiration for the biologic components and sinking for some of the biological components and POC. Since we did not, in this study, measure or estimate either the respiratory or sinking-rate losses, we cannot constrain the mass balances. We have done complete, component-by-component mass balances in other studies (Carpenter et al. 2005; Cole et al. 2006). Estimates of allochthony from the mass-balance model compared favorably with two classes of time-series models (Carpenter et al. 2005). In this study, we adopted the time-series model approach that did not require mass balances. Our results would be enhanced by evaluations of mass balance and by other methods of analyzing allochthony such as natural tracers. Building on the approach we have presented here, future work should explore multiple methods toward measuring allochthony.

The specific PLFA biomarkers provided useful proxies to estimate the $\delta^{13}C$ of phytoplankton. While it is difficult to fully separate phytoplankton from detrital POC of other origins, the centrifugation, density-separation method of Hamilton et al. (2005) agreed reasonably well with the estimates generated from the PLFAs. Neither approach, however, is flawless. We had unambiguous specific PLFAs for three algal groups (diatoms, chlorophytes, and dinoflagellates). These groups represented most but not all of the algal biomass in Crampton Lake. There were some differences in the ^{13}C labeling among the algal groups (data not shown), suggesting that isotopic fractionation was not identical in these diverse taxa, a result consistent with other studies (Boschker et al. 2005). In the physical separation approach, the least-dense fraction ideally consists of concentrated phytoplankton in the absence of terrestrial detritus. The least-dense fraction was not obviously contaminated with nonphytoplankton particles based on our microscopic examinations. However, the carbon-to-chlorophyll ratio of this fraction was quite variable from week to week and was often higher than expectations for a pure phytoplankton sample ($<100:1$). Nevertheless, the physical separation method provided estimates consistent with the expected $\delta^{13}C$ values of phytoplankton. These samples were almost always enriched in ^{13}C over bulk POC during the ^{13}C addition, which was consistent with the biomarker data. The pattern of the physically separated samples suggested a significant, but relatively small, contribution of terrestrial material to the bulk POC pool. The model analysis confirmed that POC, on average, was composed primarily of autochthonous (88%) material. POC is less terrestrially influenced in Crampton Lake than in any of the other lakes we studied in the absence of added nutrients (*see* Carpenter et al. 2005).

Our data can also be used to estimate the isotopic discrimination by the phytoplankton community during photosynthesis. This fractionation, denoted by ε, is expressed as the difference in the $\delta^{13}C$ of phytoplankton biomass and the CO_2 moiety of DIC. For this experiment, the best fit for ε was 20.3‰, close to that predicted from physiological models based largely on marine algae (e.g., Bidigare et al. 1997; Laws et al. 1997). Using various approaches, Bade et al. (2006) found ε to be highly variable among lakes in the region considered in our study and generally lower than would be predicted by models based primarily on cultures and marine studies. Similarly, models from prior whole-lake experiments (Cole et al. 2002; Pace et al. 2004) suggested ε values in the range of 5‰ to 15‰. In the Crampton Lake experiment, the close agreement between physically separated samples and phytoplankton biomarkers provides greater confidence in the algal ^{13}C values than in our prior work. The highly dynamic ^{13}C value of DIC, however, makes the daily ^{13}C value for CO_2 uncertain such that any estimate of instantaneous ε is difficult. The estimate of ε derived from fitting Eq. 2 to the data represents a time-averaged value for the analysis period (105 d).

We obtained diverse estimates of photosynthetic fractionation from a variety of relatively low-DIC lakes considered in a comparative study and from lakes where we have made ^{13}C additions, including this study (Cole et al. 2002; Bade et al. 2006). At present, our understanding of this fractionation is limited and not transferable from one system to the next. Future research estimating allochthony with similar isotope methodology will need good empirical estimates of photosynthetic fractionation. Establishing the actual $\delta^{13}C$ value of primary producers is important because large errors might derive from simple assumptions about fractionation. Approaches using biomarkers, such as the PFLA measures used in our current study, can overcome the uncertainties associated with estimating fractionation because these methods provide a more direct estimate of the $\delta^{13}C$ value of primary producer biomass.

The PLFA biomarkers allowed us to estimate the importance of allochthonous carbon separately for gram-positive and gram-negative bacteria. Gram-positive bacteria were slightly more enriched in ^{13}C than phytoplankton and, according to the model, were completely supported by autochthonous carbon. One possible explanation for the high ^{13}C values of gram-positive bacteria is that these bacteria preferentially utilize low-molecular-weight compounds released by phytoplankton that are enriched (above the average composition of phytoplankton) in ^{13}C (e.g., carbohydrates). Whatever the cause of the relatively high $\delta^{13}C$ values of this group, a model that assumed an autochthonous contribution of 100% without assuming

any use of compounds enriched above phytoplankton values provided a reasonable fit to the data. Gram-negative bacterial $\delta^{13}C$ dynamics were similar to those observed by Kritzberg et al. (2004), who used dialysis cultures to assess bacterial resource use in prior ^{13}C additions. The gram-negative bacteria of Crampton Lake were more autochthonous (~60%) than the bacteria in smaller lakes (~20% to 50%) (Kritzberg et al. 2006). Nevertheless, even in a system like Crampton Lake, where the plankton have a strong dependence on autochthony, gram-negative bacteria incorporate significant quantities of carbon from terrestrial sources.

There are two caveats to this interpretation of the carbon sources supporting Crampton Lake bacteria. First, some phytoplankton share the biomarker (18:1ω7c) we used for gram-negative bacteria. If this cross contamination was significant in Crampton Lake, then the allochthony of gram-negative bacteria would be greater than we estimated. The $\delta^{13}C$ dynamics of gram-negative bacteria suggest, however, that the PLFA marker primarily reflected bacterial biomass in Crampton Lake. This result is similar to results from benthic ^{13}C additions, which illustrate that bacterial PLFA 18:1ω7c tracks the ^{13}C labeling of sediment POC (Middelburg et al. 2000). The second caveat is that the labeling pattern of the gram-negative bacteria could be affected, in part, by the slow labeling of the very large DOC pool in the lake. Since the DOC pool turns over slowly, it is conceivable that autochthonous carbon from this source is more significant to bacteria than suggested by our estimate of DOC autochthony of 15%. Our conservative estimate of autochthony for the DOC pool, however, is consistent with DOC $\delta^{13}C$ values of −28‰ observed prior to the manipulation, which were essentially the same as terrestrial material and higher than the $\delta^{13}C$ of phytoplankton before and after the addition (Fig. 3). Thus, it is possible that the gram-negative bacteria are supported to a greater degree by autochthonous carbon than estimated in our analysis, but it is unlikely that gram-negative bacteria have autochthony similar to gram-positive bacteria or zooplankton.

While all zooplankton were primarily autochthonous, the lower dependence of cladocerans relative to copepods on autochthonous sources was an interesting difference. This difference might reflect a greater utilization by cladocerans of gram-negative bacteria, which were over 40% allochthonous. However, we have previously argued that bacterial production is insufficient to support much more than 10% of crustacean zooplankton carbon, and we have inferred that consumers like cladocerans use terrestrial carbon directly through feeding on terrestrially derived POC (Cole et al. 2006). If cladocerans were feeding nonselectively on POC in Crampton Lake, then their support by allochthonous carbon should have been close to the value for POC (12%). Hence, it appears that cladocerans are either obtaining carbon selectively from some allochthonous source (such as bacteria) or that some cladocerans are feeding below the mixed layer on material that is more depleted in ^{13}C than the mixed-layer phytoplankton.

We can address this latter possibility with data on *Holopedium gibberum*, which was an abundant cladoceran in Crampton Lake throughout most of the period of our observations. This species was abundant below the mixed layer, while the animals we sampled for the analysis presented here were taken in oblique tows through the mixed layer at night. If these samples included some animals that lived primarily at greater depths, the autochthony of *Holopedium* would be underestimated, because deeper animals would be exposed to food resources with lower $\delta^{13}C$ values. The extent of *Holopedium* diel migration was modest (data not presented), and *Holopedium* sampled at 5-m depth on one occasion near peak labeling had an isotopic value similar to but lower than animals collected in the mixed layer (5-m $\delta^{13}C = -19.1$‰ vs. oblique $\delta^{13}C = -18.0$‰). Hence, we conclude that our samples were representative of that portion of the population that fed primarily in the upper mixed layer, but we cannot completely exclude the lower apparent autochthony of *Holopedium* as a partial result of vertical migration.

Methane production and subsequent oxidation by methanotrophs provide a highly depleted source of $\delta^{13}C$ in lake food webs (Jones et al. 1999; Bastviken et al. 2003; Kankaala et al. 2006). Crampton Lake has an aerobic hypolimnion, and methane does not accumulate (Houser et al. 2003). Hence, there is likely very little methane oxidation in the water column and limited potential for this source of carbon to be important in supporting zooplankton. This inference is supported by the PLFA analysis. Substantial concentrations of 16:1ω8c and 18:1ω8c, which might indicate the presence of methanotrophs type I and II (highly characteristic biomarkers), were not detected, implying low biomass of these forms. Moreover, none of the PLFAs measured had the highly depleted $\delta^{13}C$ values that one would expect for carbon derived from methane-oxidizing bacteria.

The $\delta^{13}C$ dynamics of *Chaoborus* closely mirrored the $\delta^{13}C$ of the weighted zooplankton biomass samples. *Chaoborus* do undergo extensive nocturnal vertical migrations in Crampton Lake. The excellent fit of the weighted zooplankton biomass series with *Chaoborus* $\delta^{13}C$ values indicates that these animals must feed primarily on zooplankton residing in the upper mixed layer. As a consequence, *Chaoborus* carbon is primarily derived from autochthonous sources.

A distinguishing feature of lakes in forested temperate and boreal areas is their variable ratio of color to chlorophyll. Color is measured as absorbance at 440 nm and reflects the concentration of dissolved organic matter in lakes. Chlorophyll is an index of primary production. The color-to-chlorophyll ratio varies widely among lakes and is negatively related to lake area (Fig. 5a). Crampton Lake is large and has a low color-to-chlorophyll ratio relative to 23 nearby lakes (Fig. 5a). The allochthony of zooplankton consumers varies positively with the color : chlorophyll ratio for lakes where we have conducted ^{13}C additions (Fig. 5b). The lowest color : chlorophyll ratio in Fig. 5b was for a nutrient-fertilized lake, which had high chlorophyll and hence a low ratio. Allochthony was <10% in this system. Crampton Lake also has a relatively low color : chlorophyll ratio that results primarily from low

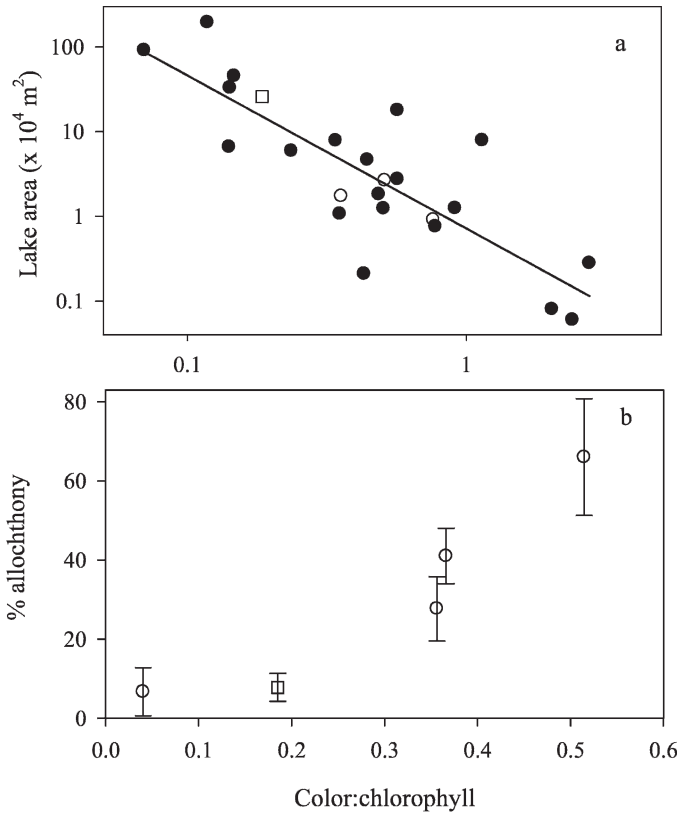

Fig. 5. (a) Average color : chlorophyll (m^{-1} [μg L^{-1}]$^{-1}$) ratio for 24 lakes located at the University of Notre Dame Environmental Research Center versus lake area (note log scale). (b) Percent allochthony of zooplankton versus color : chlorophyll. Errors are standard deviations from three model estimates or the parameter error estimate for allochthony of zooplankton biomass from Crampton Lake. In (a) and (b), ^{13}C addition to lakes is noted by open circles (Paul, Peter, and Tuesday lakes) or by an open square (Crampton Lake). Note that in panel b, the point with the lowest color-to-chlorophyll ratio represents Peter Lake when nutrients were added (color : chlorophyll = 0.04). Peter Lake is also represented when no nutrients were added (color : chlorophyll = 0.37). Solid circles are for lakes studied by Pace and Cole (2002) as well as unpublished data.

color (i.e., clear water), and zooplankton are, on average, <10% allochthonous. We infer that allochthony is low in both eutrophic lakes and oligotrophic, clear-water lakes. When the color : chlorophyll ratio is >0.3, zooplankton allochthony exceeds 20% and increases with the color : chlorophyll ratio (Fig. 5b). The pattern of zooplankton allochthony derived from our ^{13}C additions is consistent with other studies that indicate greater utilization of allochthonous carbon by zooplankton in lakes with greater humic content (i.e., higher color and DOC) (Jones et al. 1999). Note, however, that our analysis does not fully span the distribution of color : chlorophyll observations for the area (Fig. 5a). The relative contribution of allochthonous carbon to consumers for small, highly colored lakes, which are numerous in north temperate and boreal regions, requires additional study. In addition, the relative importance of lake size as an indicator of allochthony versus color-to-chlorophyll ratios cannot be resolved with our current results because these indicators are correlated (Fig. 5a) and because of the limited number of measures of allochthony (Fig. 5b). Comparisons of contrasting systems (e.g., lakes of equal area but different color : chlorophyll) are needed. A simpler approach to measuring allochthony (than ^{13}C additions) would facilitate comparisons and allow a broader assessment of the use of allochthonous resources.

While our analysis focuses on the pelagic zone, Crampton Lake also has significant littoral areas and an oxygenated hypolimnion. Consequently, littoral and sublittoral benthic populations of invertebrates and fishes are important. The high degree of autochthony of planktonic consumers we present here may not be indicative of the ultimate sources of carbon supporting littoral and benthic consumers. In our previous studies, we intentionally focused on small lakes with limited littoral zones and substantial anoxic benthic areas in order to conduct our initial ^{13}C additions in systems that had less spatial complexity and simpler food webs. In Crampton Lake, more extensive macrophyte beds as well as attached and benthic algae provide potential autochthonous resources to consumers, and the system has inherently greater spatial complexity. An evaluation of the sources of organic carbon for littoral and benthic consumers is beyond the scope of this paper. We expect, however, based on our prior studies, greater allochthony of benthic animals and fish than for plankton because sediment organic matter is a large pool that appears to have a significant allochthonous component (Carpenter et al. 2005). Nevertheless, the relative importance of autochthonous carbon in supporting benthic and littoral consumers in clear-water lakes is an open question. This problem, especially in the context of the coupling of benthic-pelagic food webs, remains an important area for research.

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