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ECOSYSTEM SUBSIDIES: TERRESTRIAL SUPPORT OF AQUATIC FOOD WEBS FROM ^{13}C ADDITION TO CONTRASTING LAKES

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Abstract. Whole-lake additions of dissolved inorganic ^{13}C were used to measure allochthony (the terrestrial contribution of organic carbon to aquatic consumers) in two unproductive lakes (Paul and Peter Lakes in 2001), a nutrient-enriched lake (Peter Lake in 2002), and a dystrophic lake (Tuesday Lake in 2002). Three kinds of dynamic models were used to estimate allochthony: a process-rich, dual-isotope flow model based on mass balances of two carbon isotopes in 12 carbon pools; simple univariate time-series models driven by observed time courses of $\delta^{13}CO_2$; and multivariate autoregression models that combined information from time series of $\delta^{13}C$ in several interacting carbon pools. All three models gave similar estimates of allochthony. In the three experiments without nutrient enrichment, flows of terrestrial carbon to dissolved and particulate organic carbon, zooplankton, *Chaoborus*, and fishes were substantial. For example, terrestrial sources accounted for more than half the carbon flow to juvenile and adult largemouth bass, pumpkinseed sunfish, golden shiners, brook sticklebacks, and fathead minnows in the unenriched experiments. Allochthony was highest in the dystrophic lake and lowest in the nutrient-enriched lake. Nutrient enrichment of Peter Lake decreased allochthony of zooplankton from 0.34–0.48 to 0–0.12, and of fishes from 0.51–0.80 to 0.25–0.55. These experiments show that lake ecosystem carbon cycles, including carbon flows to consumers, are heavily subsidized by organic carbon from the surrounding landscape.

Key words: allochthonous; allochthony; consumer; dissolved inorganic carbon; food web; lake; models; organic carbon; stable isotope; subsidy; whole-lake experiment.

INTRODUCTION

Microbial and animal consumers frequently use resources transported to their habitats from elsewhere. These allochthonous resources or subsidies influence population dynamics, community interactions, and ecosystem processes (Polis et al. 1997, 2004). There is growing evidence for the significance of cross-boundary inputs and subsidies of populations in a wide range of habitats, including streams, rivers, lakes, islands and riparian terrestrial environments (Kitchell et al. 1999, Fausch et al. 2002, Power and Dietrich 2002, Polis et al. 2004). Allochthonous inputs are a major component of organic carbon (C) budgets for streams and rivers (Fisher and Likens 1972). More recent studies have documented the varying contributions of allochthonous and autochthonous organic carbon sources to consumers in a wide range of flowing-water ecosystems (Webster and Meyer 1997, Fausch et al. 2002, Power and Dietrich 2002, Bunn et al. 2003).

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The importance of subsidies to consumers is also implied by measurements of ecosystem metabolism. Respiration exceeds primary production in many ecosystems, indicating significant input and degradation of allochthonous material. For example, many lakes receive high loadings of dissolved and particulate organic matter from adjacent wetlands and uplands (Wetzel 1995). As a consequence, in these lakes ecosystem respiration commonly exceeds gross primary production (Cole et al. 2000). Thus terrestrial material subsidizes lake metabolism. However, the significance of these subsidies to the support of food webs is less certain.

The relative importance of allochthonous vs. autochthonous resources cannot be discerned from organic carbon budgets alone. Hence there are few examples where direct estimates have been made of the autochthonous and allochthonous support of food web constituents. An obvious way to overcome this problem is to trace the flow of allochthonous and autochthonous matter into food webs using stable isotopes (Kling et al. 1992, France et al. 1997). Where there is a contrast between the stable isotope content of sources, it is pos-

TABLE 1. Means of limnological variables from late May to early September for each lake ^{13}C addition.

Variable	Paul 2001	Peter 2001	Peter 2002	Tuesday 2002
Temperature (°C at 1 m)	21.1	21.4	22.1	22.0
Thermocline (m)	3.5	3.6	3.1	2.6
pH	6.4	6.9	8.5	6.1
Color (m^{-1})	1.5	1.3	1.7	3.5
Secchi (m)	4.6	4.9	1.9	2.3
pCO_2 (μatm)†	1039	673	152	977
DIC (μmol)	93	141	67	70
DOC (μmol)	304	376	483	700
POC (μmol)	35.5	34.1	152.3	76.5
Chlorophyll *a* (μg/L)	4.21	3.55	42.1	6.8
TP (μmol)	0.314	0.261	0.846	0.385
TN (μmol)	26.9	30.3	46.7	28.5
GPP (mmol $O_2 \cdot m^{-2} \cdot d^{-1}$)	43.4	31.3	104.5	42.9
R (mmol $O_2 \cdot m^{-2} \cdot d^{-1}$)	51.8	31	79.7	44.7

Notes: Variables are as follows: pCO_2, partial pressure of CO_2; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; POC, particulate organic carbon; TP, total phosphorus; TN, total nitrogen; GPP, gross primary production; R, respiration. Chemical measurements are means for the epilimnion. Most means were calculated from weekly samples except GPP and R (daily), and POC in 2001, where more frequent samples were taken.

† We report partial pressure as μatm. Using the standard atmosphere conversion, a pCO_2 value of 1000 μatm = 101.325×10^{-3} kPa.

sible to estimate the fraction of consumer carbon flow supported by each using end-member mixing models. For terrestrial and aquatic primary production, some studies have compared components of the food web to these two extremes (Meili et al. 1996, France et al. 1997, Jones et al. 1999, Grey et al. 2001). A common limitation with these natural abundance studies, however, is the small contrast between terrestrial and aquatic primary producers. When these end-member values are close, carbon sources to the food web cannot be resolved (Schiff et al. 1990, Cole et al. 2002).

Whole-lake additions of radioactive ^{14}C demonstrate that it is possible to unambiguously label carbon that is autotrophically fixed within the ecosystem (Hesselein et al. 1980, Bower et al. 1987). We have extended this approach using the stable isotope ^{13}C. We measured the contribution of internal primary production (autochthony) to food webs by altering the ^{13}C of dissolved inorganic carbon (DIC), thereby enriching the ^{13}C of in-lake primary production relative to organic matter from terrestrial inputs (Cole et al. 2002). In many lakes the isotopic composition of the CO_2 moiety of dissolved inorganic carbon (the proximate substrate for photosynthesis), and fractionation of that CO_2 during photosynthesis, causes carbon fixed by aquatic primary producers (especially phytoplankton) to be nearly identical in ^{13}C to organic carbon of terrestrial origin (Karlsson et al. 2003). ^{13}C additions overcome this problem by providing a distinct ^{13}C signature to internal primary production and the consumer carbon derived therefrom. Our previous research used a pulse experiment (Cole et al. 2002) in which a single addition of ^{13}C was made. Press experiments with continuous daily additions of ^{13}C allow greater and sustained labeling of the food web, reducing immediate losses of ^{13}C to the atmosphere and increasing carbon flows to consumers (Pace et al. 2004).

Although research has begun to quantify the contribution of allochthonous carbon to lake food webs, it is not clear how the importance of terrigenous organic carbon varies among lake consumers and among lake trophic types. In this paper, we use press additions of $DI^{13}C$ to estimate the terrestrial subsidy to lake ecosystems and specific consumers. This paper adds to results presented by Pace et al. (2004) by (1) testing whether terrestrial subsidies are more important in a lake with high concentrations of terrestrially derived dissolved organic matter (DOC) than in a lake with low concentrations of terrestrially derived DOC, (2) using a whole-lake manipulation to test whether the importance of terrestrial subsidies is diminished by nutrient enrichment, (3) comparing allochthony among several different groups of consumers, and (4) using three different modeling approaches to evaluate the consistency of estimates of allochthony.

METHODS

Inorganic ^{13}C was added to Paul, Peter, and Tuesday Lakes located at the University of Notre Dame Environmental Research Center near Land O'Lakes, Wisconsin, USA (89°32′ W, 46°13′ N). These lakes have been described in detail (Carpenter and Kitchell 1993), and we focus here mainly on pertinent ecological conditions during the ^{13}C additions of 2001 and 2002. All three basins are small (0.9–2.5 ha) and steep sided. Lakes are fringed by wetlands and forests typical of the upper Great Lakes region. The lakes are all soft water with moderate to high dissolved organic C (DOC) and dissolved inorganic C (DIC), from 80 to 140 μmol among the three systems (Table 1).

DOC in the lakes is rich in chromophoric compounds; hence lakes in this region with high DOC typically have dark water. Water color measured as the absorbance of light at 440 nm (Cuthbert and del Giorgio 1992) is much higher in Tuesday Lake (2002 average = 3.5 m^{-1}) than in Paul (1.5 m^{-1}) or Peter (1.3 m^{-1}) Lakes. During summer the lakes are strongly stratified with relatively shallow thermocline depths near 3 m (Table 1). Periphyton and phytoplankton are the main primary producers, but rates are limited by low nutrients (phytoplankton) and low light (periphyton; Carpenter et al. 2001, Vadeboncoeur et al. 2001). Macrophytes, while present, are sparse, and do not contribute significantly to primary production (Carpenter and Kitchell 1993). The zooplankton community of Paul Lake is dominated in terms of biomass by large cladocerans (*Daphnia* spp. and *Holopedium gibberum*). Peter Lake has a mixture of *Daphnia* spp., *Diaphanosoma* spp., and copepods as biomass dominants. The zooplankton of Tuesday Lake is an assemblage of small-bodied cladocerans and copepods (Carpenter and Kitchell 1993). The planktivorous dipteran, *Chaoborus* spp., is abundant in Paul and Tuesday Lakes but rare in Peter Lake during 2001 and 2002. The lakes also differ in their fish communities. Paul Lake has only largemouth bass (*Micropterus salmoides*). Peter and Tuesday Lakes have mixtures of small-bodied fishes. The dominant species of Peter Lake are pumpkinseeds (*Lepomis gibbosus*), sticklebacks (*Gasterosteus aculeatus*), and fathead minnows (*Pimephales promelas*). The dominant species of Tuesday Lake are golden shiners (*Notemigonus crysoleucas*), sticklebacks, and fathead minnows.

In 2001 we added we added ^{13}C in the form of $NaHCO_3$ (''NaH $^{13}CO_3$'') to Paul and Peter Lakes for 42 days beginning 11 June and ending 27 July. In 2002 we added NaH $^{13}CO_3$ to Tuesday and Peter Lakes for 35 days beginning 17 June and ending 25 July. Each morning shortly after dawn, preweighed NaH $^{13}CO_3$ (99% pure; Isotech, Champaign, Illinois, USA) was dissolved in lake water within gas-tight carboys. The resulting solution was pumped into the upper mixed layer while underway in a boat to promote dispersion of the tracer throughout the mixed layer of the lake. Experiments using rhodamine dye, LiBr, and SF_6 in these lakes indicate that solutes disperse uniformly through the mixed layer in $<$24 hours (Cole and Pace 1998; J. Cole et al., *unpublished data*). Daily loadings of NaH $^{13}CO_3$ were 0.24, 0.35, 0.25, and 0.61 mol ^{13}C/d to Paul (2001), Peter (2001), Tuesday (2002), and Peter (2002) Lakes, respectively. These additions were designed to substantially enrich the ^{13}C of DIC while not significantly altering total DIC (i.e., $^{12}C + {}^{13}C$) concentration. More ^{13}C was added to Peter Lake to compensate for its higher concentration of DIC and the prospect of substantial inputs of atmospheric $^{12}CO_2$ in 2002 due to nutrient enrichment and chemically enhanced diffusion (Bade 2004).

In 2002, Peter Lake was also amended with nutrients to stimulate primary production. Liquid fertilizer was made from NH_4NO_3 and H_3PO_4. The fertilizer had an atomic nitrogen : phosphorus (N:P) ratio of 25. An initial addition of 0.69 mmol P/m^2 and 18.9 mmol N/m^2 was made on 3 June 2002 to stimulate primary producer growth prior to the beginning of the ^{13}C addition. Beginning on 10 June and continuing until 25 August, daily additions were made that corresponded to a P-loading rate of 0.11 mmol $P \cdot m^{-2} \cdot d^{-1}$ (and 2.7 mmol $N \cdot m^{-2} \cdot d^{-1}$). This level of nutrient addition was chosen because prior enrichments at this level generated substantial phytoplankton blooms in Peter Lake (Carpenter et al. 2001).

Sampling and measurement of ^{13}C

Detailed methods for most of the measurements made in this study are summarized elsewhere (Carpenter et al. 2001, Kritzberg et al. 2004, Pace et al. 2004; also *available online*).[7] For this paper we focus on methods to sample and process lake constituents for ^{13}C measurements and briefly summarize other measurements of physical properties, chemical composition, standing stocks, and rate estimates that supported model analyses. ^{13}C samples for most lake constituents were taken before, during, and after the tracer addition, at either daily, weekly, or biweekly intervals for faster and slower C pools.

Particulate organic carbon (POC) and DIC, which have fast turnover times, were sampled daily. For DI^{13}C, water was pumped into gas-tight 60-mL serum vials and acidified to pH 2 with H_2SO_4. Samples were sent to the University of Waterloo stable isotope facility and analyzed using a Micromass Isochrome GC-C-IRMS (Waters, Milford, Massachusetts, USA). POC was concentrated by filtration through precombusted glass fiber filters (GF/F), dried at 40°C for 48 h, and acid-fumed to remove excess inorganic ^{13}C. POC and all other particulate samples were analyzed for ^{13}C at the University of Alaska Isotope Facility using a Carlo Erba Elemental Analyzer (NC2500; Thermo Electron, Milan, Italy) and a Finnigan MAT Conflo II/III interface with a Delta+ Mass Spectrometer (Thermo Electron, Advanced Mass Spectrometry, Bremen, Germany).

Periphyton ^{13}C was sampled weekly by scraping accumulated algae from colonization tiles. Zooplankton and *Chaoborus* for isotopic analyses were sampled weekly with oblique net hauls through the upper mixed layer at night. Individual animals were separated by taxa under a dissecting microscope, dried, and pulverized. Water in filtrates of the POC samples was acidified to drive off excess DI^{13}C and concentrated by evaporation for isotope analysis of DOC.

Fish were sampled by electrofishing, netting, and angling to obtain animals for isotopic analysis and diet

[7] ⟨http://216.110.136.172/methods.htm⟩

analysis (Hodgson and Kitchell 1987, Carpenter and Kitchell 1993). Gastric lavage was used to obtain gut items for estimating the isotope content of benthic invertebrates (Hodgson and Kitchell 1987). Gut contents were pooled into diet categories (zooplankton, *Chaoborus*, largemouth bass young-of-year, and macroinvertebrates, mainly odonate naiads) for isotope analysis. Invertebrates were also sampled with D-nets, sorted by major taxa, dried, ground, and analyzed for ^{13}C. For larger fish, dorsal muscle samples were taken from three to five individuals for ^{13}C analysis. For smaller fish, a number of individuals were pooled, dried, pulverized, and subsampled for ^{13}C analysis.

To obtain samples of bacteria, cultures were grown in situ in dialysis bags using particle-free lake water and an inoculum of bacteria from the lake (Kritzberg et al. 2004). Cells were concentrated on precombusted GF/F filters, dried, and analyzed for ^{13}C using an ANCA-NT system and a 20–20 Stable Isotope Analyzer (PDZ Europa, Crewe, Cheshire, UK) at the Ecology Department, University of Lund, Sweden. Bacterial isotope estimates were made four times during each experiment.

Isotope data are presented in conventional δ notation in per mil units (‰) following the equation $\delta^{13}C = 1000 \times [(R/0.011237) - 1]$ where R is the ratio of ^{13}C to ^{12}C in the sample and 0.011237 is the ratio in a standard.

Other measurements

A variety of additional measurements were made to aid interpretation of the isotope dynamics, provide flux estimates and parameters for modeling analysis, and provide standing stock estimates for models. DIC, pCO_2 (partial pressure of CO_2), pH, and temperature were measured to calculate the chemical species of inorganic C and their isotopic content (Mook et al. 1974, Zhang et al. 1995). DIC and pCO_2 were determined by gas chromatography following established methods (Cole et al. 2000), while pH was measured with an electrode (Pace and Cole 2002). Gross primary production and total system respiration were estimated from continuous deployment of YSI sondes (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA) that recorded oxygen concentration and temperature at 5-min intervals following methods in Cole et al. (2000, 2002) and Hanson et al. (2003). Gas exchange was estimated from direct measurements of the gas piston velocity (k_{600}) using whole-lake SF_6 additions and wind-based estimates from continuous lakeside wind measurements (Wanninkhof et al. 1985, Cole and Caraco 1998). Bacterial production was estimated from leucine incorporation using the microcentrifuge tube method (Smith and Azam 1993). Planktonic respiration was estimated from the decline of oxygen in dark bottles (Pace and Cole 2000). Weekly vertical profiles of temperature, O_2, irradiance (photosynthetically active radiation, PAR), and chlorophyll *a* were made in each lake to estimate mixed-layer depth and to calculate phytoplankton biomass.

Standing stocks of POC and DOC were derived from mixed-layer water samples using a Carlo-Erba C/N analyzer and a Shimadzu 5050 TOC analyzer (Shimadzu, Kyoto, Japan) for POC and DOC, respectively. Weekly measurements of phytoplankton and zooplankton biomass were derived from vertical profiles of chlorophyll *a* and calibrated net hauls, respectively, using methods described in Carpenter and Kitchell (1993) and Carpenter et al. (2001). *Chaoborus* were sampled with vertical net hauls every week and biomass determined from estimates of abundance and measurements of length and diameter (Carpenter and Kitchell 1993).

Fish abundance, size distribution, and diets were measured using methods described in Hodgson and Kitchell (1987) and Carpenter and Kitchell (1993). Estimates of largemouth bass populations were calculated by mark–recapture methods using data from electroshocking and angling (Seber 1982). Fishes were sampled weekly using minnow traps in Peter and Tuesday Lakes (Carpenter and Kitchell 1993).

Model methods

Changes in $\delta^{13}C$ over time for the major carbon pools were used to estimate allochthony, the proportion of carbon flow into a pool from terrestrial sources. In these tracer experiments, information about flows is obtained from transient changes in $\delta^{13}C$. Therefore, the steady-state mixing models used in studies of natural isotope abundance are not appropriate. At present there is no single standard method for assessment of carbon fluxes through the entire food web in whole-ecosystem tracer experiments. Many modeling approaches are potentially applicable, and we do not know if they will lead to similar or different conclusions. Therefore, we used three different modeling approaches. To the extent that these give similar results, we can have confidence that conclusions are robust. The differences among model results provide information about the uncertainties that derive from model selection.

Initially we developed dual isotope flow (DIF) models for each experiment (Appendix A; Cole et al. 2002). The DIF employs mass-balance of total carbon and ^{13}C for 12 carbon pools. Many pool sizes and flows were directly measured to calibrate the DIF. The DIF provides a detailed analysis that is grounded in the current understanding of the major processes that govern carbon flows in lake ecosystems. While this is an advantage, the DIF depends on a large number and diversity of measurements and could potentially propagate errors in complicated ways. A complete statistical analysis of the DIF is not possible, but we did fit some parameters by least squares, perform numerous sensitivity experiments, and evaluate goodness of fit statistics.

To provide a contrast in complexity, we developed univariate time-series models (Appendix B; Pace et al. 2004). These predicted $\delta^{13}C$ of a response pool (DOC,

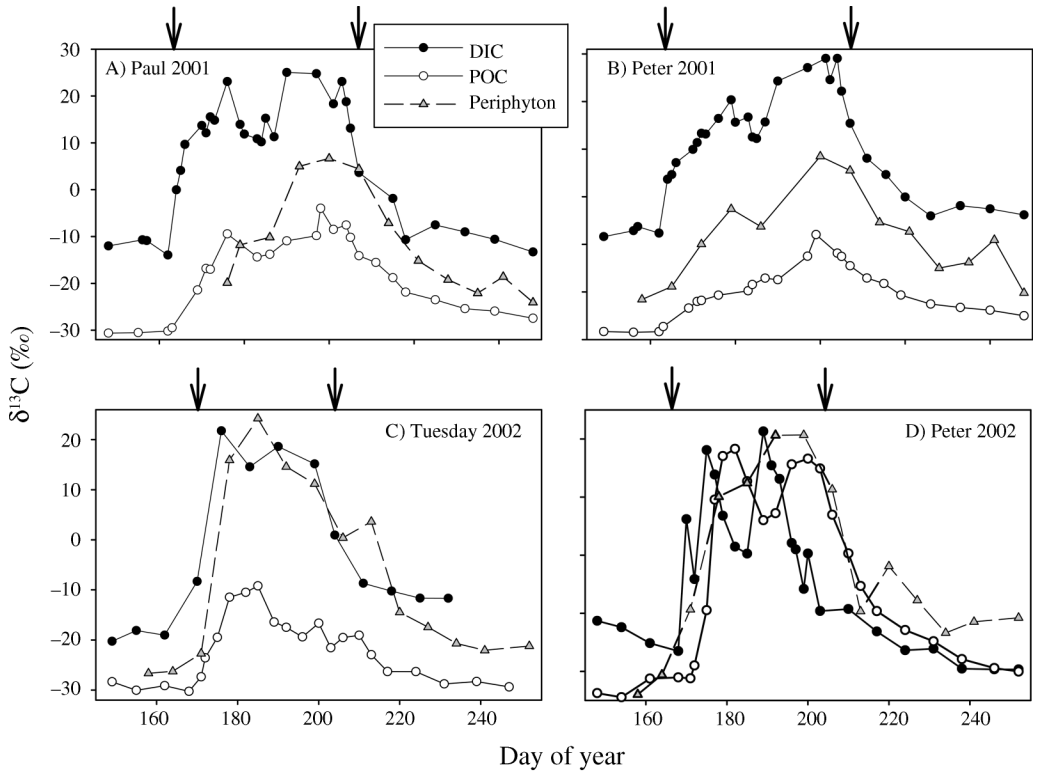

FIG. 1. $\delta^{13}C$ (‰) of dissolved inorganic carbon (DIC), particulate inorganic carbon (POC), and periphyton vs. day of year (day 1 is 1 January) in four whole-lake labeling experiments at Paul, Peter, and Tuesday Lakes in 2001 and 2002.

POC, zooplankton, or *Chaoborus*) from $\delta^{13}C$ of DIC. The univariate models can be fitted by standard statistical methods, and errors can be analyzed by bootstrapping. However, they neglect information in the dynamics of closely related time series and do not attempt to represent the specific ecological processes that govern carbon flows.

To provide a third perspective with an intermediate level of complexity, we fit multivariate autoregression (MAR) models (Appendix C; Ives et al. 2003). These predicted $\delta^{13}C$ of a set of closely interacting carbon pools (e.g., DOC, POC, zooplankton, and *Chaoborus*). Dynamics of $\delta^{13}C$ for the response variables are fitted to the time course of the experimentally manipulated variable, $\delta^{13}C$ of DIC. In addition, more slowly changing carbon pools (such as *Chaoborus* or benthos) are linked to $\delta^{13}C$ of their diets. These models allowed us to evaluate the carbon flows among a few key pools, using relatively simple models that could be analyzed statistically. In addition, we used MAR models to account for possible effects of observation variance on our conclusions about carbon flow.

RESULTS

Additions of NaH $^{13}CO_3$ increased $\delta^{13}C$ of DIC from pretreatment values of -8 to -20‰ (depending on the lake) to highly enriched values exceeding $+20$‰ (Fig. 1). When isotope additions ended, $\delta^{13}C$ of DIC returned within a few weeks to values near those observed before treatment. The added $DI^{13}C$ had two immediate fates: loss to the atmosphere and uptake by primary producers. Daily additions helped reduce losses because there was a lower $DI^{13}C$ gradient from lake to atmosphere relative to a single large pulse. Primary producers were effectively labeled, as indicated by the increase in $\delta^{13}C$ of POC and periphyton during each addition (Fig. 1). In Paul Lake 2001, Peter Lake 2001, and Tuesday Lake 2002, periphyton was labeled more than POC, because POC included nonalgal material, such as bacteria and terrigenous POC. In these three experiments, $\delta^{13}C$ of DIC exceeded that of primary producers because of photosynthetic fractionation. In contrast to the large changes seen in the labeled lakes, variation over time of $\delta^{13}C$ in unlabeled lakes was negligible (Pace et al. 2004).

In Peter Lake 2002, $\delta^{13}C$ of DIC was comparable to that of primary producers (Fig. 1D). In this experiment, nutrient enrichment stimulated primary production (Table 1) resulting in the near complete depletion of aqueous CO_2. Since the entire CO_2 pool was utilized, photosynthetic fractionation was near 0. Further, the CO_2 depletion also greatly increased the pH. Consequently HCO_3 rather than CO_2 may have been the substrate for photosynthesis (Rau et al. 2001, Bade 2004).

In all experiments, additions of $DI^{13}C$ were transferred throughout the food web. Labeled carbon ap-

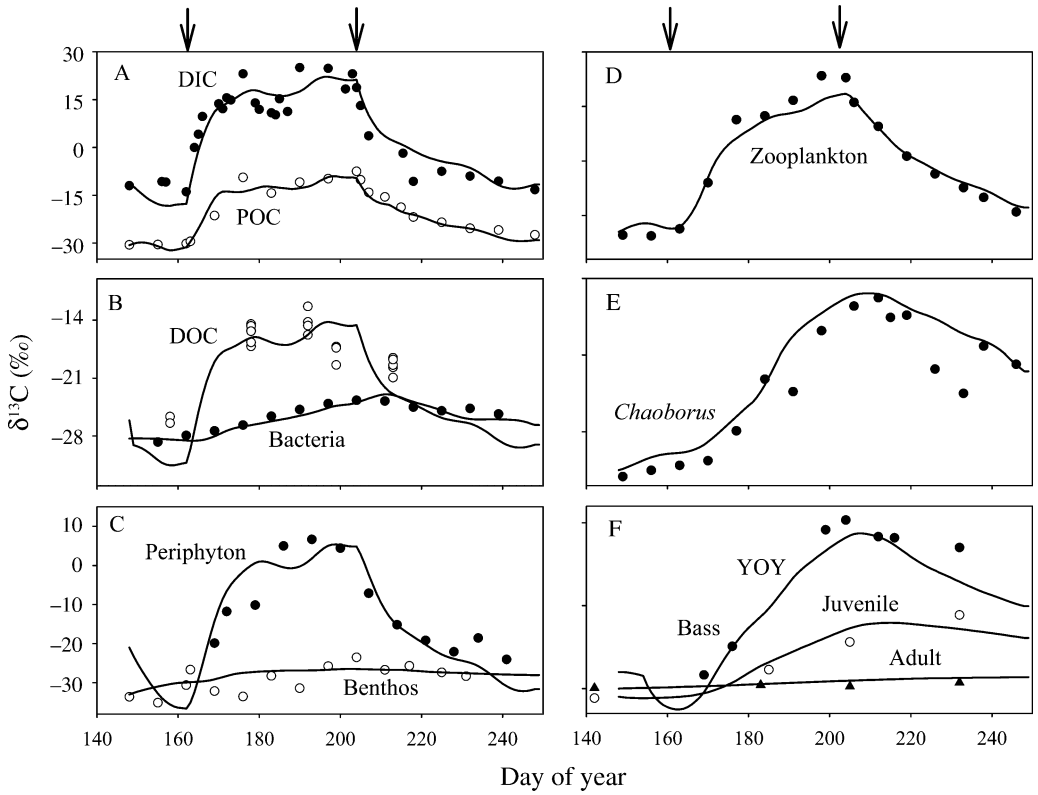

FIG. 2. $\delta^{13}C$ (‰) predicted by the dual isotope flow (DIF) model (lines) and observed (points) vs. day of year (day 1 is 1 January) for Paul Lake in 2001. (A) DIC and POC, (B) DOC and bacteria, (C) periphyton and benthos, (D) zooplankton, (E) *Chaoborus*, and (F) three size classes of largemouth bass: young-of-year (YOY; solid circles), juveniles (open circles), and adults (solid triangles). Arrows indicate the start and end of the isotope addition.

peared in bacteria shortly after initiation of the ^{13}C addition (Fig. 2B). DOC was also labeled, though to a lesser extent because of the large size of this carbon pool. Although periphyton rapidly accumulated ^{13}C, labeled carbon accumulated slowly in benthic invertebrates in this experiment (Fig. 2C).

Zooplankton accumulated ^{13}C shortly after ^{13}C appeared in the POC (Fig. 2D), and labeled carbon in zooplankton was transferred to *Chaoborus* (Fig. 2E). Among fishes of Paul Lake, young-of-year largemouth bass accumulated ^{13}C to the greatest extent, consistent with their more rapid carbon turnover rate and zooplanktivorous habit (Fig. 2F). Juvenile largemouth bass accumulated some ^{13}C as a consequence of eating zooplankton, *Chaoborus*, benthos, and young-of-year bass. Adult largemouth bass were labeled only slightly. This result was predicted by the bass bioenergetics model and is consistent with the slow carbon turnover rate of these large but slow-growing fishes. Both juvenile and adult largemouth bass consume terrestrial prey items which are not enriched in ^{13}C.

The DIF model appeared to fit the observed $\delta^{13}C$ (Fig. 2 and Appendix D). This model includes a comprehensive analysis of the carbon cycle by employing a substantial amount of field data on carbon pool sizes and flux rates (Appendix A). That richness of process-level detail is an advantage. Discrepancies between model predictions and observations are small relative to the overall changes in the data. But because so many observations must be accommodated simultaneously, there can be systematic departures between predicted and observed $\delta^{13}C$. For example, in Paul Lake 2001, the model underestimates labeling of zooplankton and overestimates labeling of *Chaoborus* (Fig. 2E, F).

Similar results occurred in the other experiments. Residual standard deviations for most compartments were <1‰, and these deviations were typically small relative to the large range of ^{13}C created by the manipulation (Appendix D). Overall, correspondence between observed $\delta^{13}C$ and predictions of the DIF model was similar in all four experiments (Appendix D).

The univariate models focus on one carbon pool at a time predicting dynamics from the $DI^{13}C$ time series and a fixed pool of carbon with a terrestrial signature of -28‰ (Pace et al. 2004). These parsimonious models fit the data closely in most cases, as illustrated for Peter Lake in Fig. 3. The model simulates the increase and decline of ^{13}C, with the exception of underpredicting POC observations in the 2002 experiment at maximum labeling (Fig. 3). Fits of similar quality were found for other experiments (Appendix D). The univariate models are limited in that fits for a given carbon

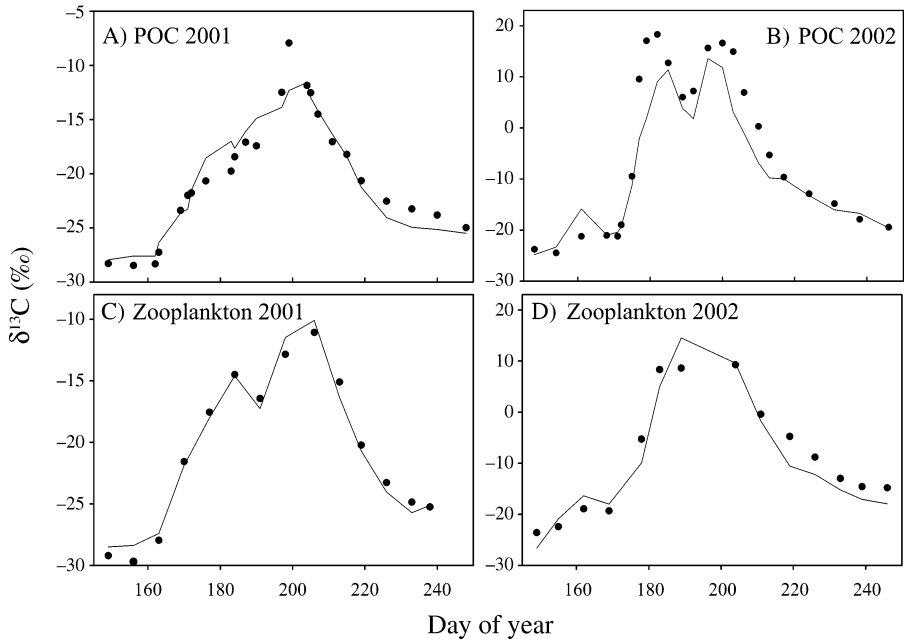

FIG. 3. $\delta^{13}C$ (‰) predicted by univariate models (lines) and observed (points) vs. day of year (day 1 is 1 January) for Peter Lake.

pool do not take advantage of information in closely related carbon pools. Also, dynamics of $\delta^{13}C$ in slowly changing pools, such as benthos or fishes, are not easily predicted from the relatively rapid changes of $\delta^{13}C$ in DIC and the many transformations that occur as carbon moves through the food web to these consumers. Therefore we did not attempt to fit univariate models for these slowly changing pools.

MAR models incorporate additional information by including the dynamics of closely related variables. Predictions of the MAR models closely match observed $\delta^{13}C$ in most cases, as shown in Fig. 4 for Tuesday Lake in 2002. The MAR approach considers three subsystems of the food web. The modest response of the benthos to the substantial enrichment of periphyton is captured (Fig. 4A). Bacterial ^{13}C falls between POC and DOC but more closely reflects the dynamics of $PO^{13}C$, reflecting preferential utilization of the autotrophic component of POC (Fig. 4B; Kritzberg et al. 2004). Predicted zooplankton and *Chaoborus* ^{13}C dynamics fit the data well, and the MAR model represents the expected lag in labeling of *Chaborus* relative to their prey (Fig. 4C). Fits of similar quality were found for other experiments (Appendix D). We did not attempt to fit MAR models for fishes. Instead we combined MAR estimates of allochthony of diet items with data on composition of fish diets to calculate allochthony of fishes.

Allochthony, the proportion of carbon flow from terrestrial sources, was calculated for all organic carbon pools in the DIF model, and for as many carbon pools as could be fitted for the univariate and MAR models (Table 2). All models indicate that the major carbon pools POC and DOC had significant allochthonous components. For example, in the experiments without nutrient enrichment (Paul Lake 2001, Peter Lake 2001, Tuesday Lake 2002) POC allochthony ranged from 0.29 to 0.59, depending on the model. In the nutrient enrichment experiment (Peter Lake 2002), allochthony of POC ranged from 0 to 0.07, depending on the model. Thus nutrient enrichment increased the contribution of phytoplankton to POC.

DOC was more allochthonous than POC (Table 2). In the unenriched experiments, allochthony of DOC ranged from 0.53 to 0.96, depending on the lake and the model. In dystrophic Tuesday Lake, model estimates of DOC allochthony were consistently high (0.92–0.96). For each model, the lowest estimate of DOC allochthony occurred in the enrichment experiment (Peter Lake 2002).

Carbon flow through bacteria was dominated by allochthonous sources in the unenriched experiments (allochthony range, 0.60–0.76 depending on the model and experiment). In the enriched experiment, the DIF model estimated bacterial allochthony as 0.39, but data were insufficient for analysis using the other models.

Allochthony of zooplankton was similar in Paul Lake and Peter Lake in 2001 (0.22–0.48). In Tuesday Lake, zooplankton were more allochthonous (0.49–0.75). In Peter Lake during enrichment in 2002, zooplankton were supported almost entirely by within-lake primary production, and allochthony estimates ranged from 0 to 0.12. The same general pattern—more allochthony

TABLE 2. Allochthony (proportion of carbon flow from terrestrial sources) for major carbon pools, estimated using three different models.

Lake	Year	Method	DOC	Bacteria	POC
Paul	2001	DIF	0.53	0.60	0.29
Paul	2001	MAR	0.83 ± 0.01	0.71 ± 0.18	0.38 ± 0.03
Paul	2001	univar.	0.85 ± 0.02		0.40 ± 0.03
Peter	2001	DIF	0.69	0.73	0.50
Peter	2001	MAR	0.87 ± 0.01		0.47 ± 0.04
Peter	2001	univar.	0.87 ± 0.01		0.55 ± 0.03
Peter	2002	DIF	0.43	0.39	0.06
Peter	2002	MAR	0.55 ± 0.10		0.07 ± 0.00
Peter	2002	univar.	0.70 ± 0.02		0.00 ± 0.01
Tues	2002	DIF	0.92	0.76	0.48
Tues	2002	MAR	0.95 ± 0.02	0.67 ± 0.04	0.57 ± 0.05
Tues	2002	univar.	0.96 ± 0.01		0.59 ± 0.05

Notes: For univariate and multivariate autoregression (MAR) models, bootstrapped standard deviations are presented. In Paul Lake, Fish 1 is young-of-year largemouth bass, Fish 2 is juvenile largemouth bass, and Fish 3 is adult largemouth bass. In Peter Lake, Fish 1 is pumpkinseed, Fish 2 is stickleback, and Fish 3 is fathead minnow. In Tuesday Lake, Fish 1 is golden shiner, Fish 2 is stickleback, and Fish 3 is fathead minnow. DIF refers to the dual isotope flow model (Appendix A). Other abbreviations are as in Table 1.

in Tuesday Lake, less allochthony in Peter Lake with enrichment—was evident for *Chaoborus*.

Benthic invertebrates had similar allochthony in the unenriched experiments (0.60–0.85). Benthos tended to be more allochthonous than zooplankton. Under enrichment, allochthony of benthos appeared to decrease, although the gap between DIF estimates and MAR estimates was large.

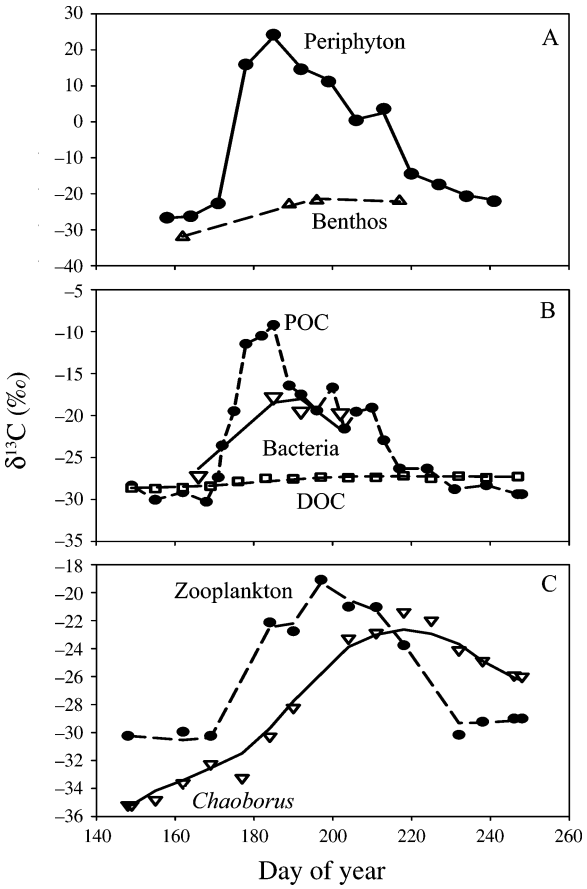

FIG. 4. $\delta^{13}C$ (‰) predicted by multivariate autoregression (MAR) models (lines) and observed (points) vs. day of year (day 1 is 1 January) for Tuesday Lake in 2002.

In Paul Lake, flow of carbon to juvenile and adult largemouth bass (Fishes 2 and 3 in Table 2) was more than half allochthonous. Diets of these fishes include substantial numbers of terrestrial prey (Hodgson and Kitchell 1987). Young-of-year largemouth bass were less allochthonous, partly because these fish feed primarily on zooplankton during the first few weeks of life (Post et al. 1997). In Tuesday Lake, we estimated high allochthony for a different set of fishes: golden shiner, stickleback, and fathead minnow.

In Peter Lake, allochthony of pumpkinseed sunfish, stickleback, and fathead minnow (Fishes 1, 2, and 3, respectively, in Table 2) declined with nutrient enrichment. Prior to enrichment, fish allochthony was comparable to that of the other lakes (0.51–0.80). After enrichment, fish allochthony declined to 0.25–0.55. These results indicate that nutrient enrichment of Peter Lake caused a decrease in the contribution of terrigenous carbon, relative to carbon fixed in the lake, to fishes during the course of the experiment.

DISCUSSION

Evaluation of allochthony estimates

Our experiments label new, autochthonous primary production of phytoplankton and periphyton in the mixed layer of the lakes for 35–42 d. The experiments show clearly that some portion of secondary production is directly supported by this contemporaneous, surface-layer, labeled primary production and some is not. Some portion of secondary production may, therefore, be supported by terrestrially derived organic C (allochthony) but there are additional possibilities. Consumers may utilize contemporaneous primary production from waters or sediments deeper than the mixed

TABLE 2. Extended.

Zooplankton	*Chaoborus*	Benthos	Fish 1	Fish 2	Fish 3
0.37	0.37	0.60	0.38	0.59	0.73
0.24 ± 0.04	0.36 ± 0.06	0.84 ± 0.06	0.67	0.72	0.76
0.22 ± 0.05	0.53 ± 0.09				
0.34	0.34	0.85	0.69	0.71	0.51
0.41 ± 0.01	0.41	0.78	0.80	0.65	0.54
0.48 ± 0.03					
0.12	0.12	0.07	0.40	0.45	0.33
0.08 ± 0.00	0.20 ± 0.04	0.41 ± 0.11	0.55	0.30	0.25
0.00 ± 0.02	0.12 ± 0.08				
0.75	0.75	0.83	0.93	0.93	0.84
0.49 ± 0.04	0.49 ± 0.04	0.72 ± 0.23	0.56	0.65	0.58
0.74 ± 0.04	0.65 ± 0.56				

layer that is not labeled with added ^{13}C. Alternatively, consumers may consume detritus from primary production that occurred prior to the time ^{13}C was added. Several lines of evidence suggest that these processes are not important in these experiments. We evaluate this evidence for POC and DOC inputs to the epilimnion, for vertical migration and feeding of planktonic organisms, and for sources of C consumed by epilimnetic benthos.

POC inputs.—The three study lakes are strongly stratified during summer. Solutes added to the upper mixed layer do not move across the thermocline (see Cole and Pace 1998, Houser 2001). There is no mechanism, except thermocline deepening, that can add DOC or POC from below the thermocline to the mixed layer. For POC, the DIF and MAR models calculate that losses of epilimnetic POC from sedimentation and consumption are rapid, and hence the epilimnetic POC pool turns over in a few days. In the case of POC, the standing stock is replaced many times over the course of the experiment, and its ^{13}C content represents the introduction of new inputs. In addition to autochthonous primary production, the possible inputs of new POC include flocculation of DOC (which the DIF model accounts for), terrestrial inputs (accounted for), and resuspension of previously deposited material on epilimnetic sediments. To the extent that a portion of this resuspended material could be both autochthonous in origin and older than the experiment, our estimate of allochthony for POC might be compromised. A simple calculation suggests that the total amount of resuspension from these sediments could be only a trivial portion of the POC input to the epilimnion. Using the MAR model, POC inputs can be estimated as daily turnover × allochthony × mean areal density of POC (Appendix C). Estimated POC inputs during the experiments were 62 $mg \cdot m^{-2} \cdot d^{-1}$ in Paul Lake, 47 $mg \cdot m^{-2} \cdot d^{-1}$ in Peter Lake 2001, 35 $mg \cdot m^{-2} \cdot d^{-1}$ in Peter Lake 2002, and 104 $mg \cdot m^{-2} \cdot d^{-1}$ in Tuesday Lake. Epilimnetic sediments comprise ~10% of the surface area of these lakes. If 10% of annual primary production were deposited on these sediments, only 50% of this decomposed in place, and all of the rest was resuspended into the lake during the ice-free season, resuspension of autochthonous material would supply <5 mg $C \cdot m^{-2} \cdot d^{-1}$ (to the whole lake), which is much smaller than the required POC input to the water column. This calculation of resuspended POC is certainly an overestimate in these small lakes, which experience little wave action and low resuspension of sediments. We conclude that epilimnetic POC was primarily derived from autochthonous primary production and new terrestrial inputs during the course of the manipulations.

Origins of DOC.—DOC is a mixture of both autochthonous and allochthonous sources, and the average pool turns over slowly, ~3%/d (Bade 2004). Could DOC produced autochthonously prior to the experiment compromise our interpretation of allochthony? Kritzberg et al. (2004) demonstrated that bacteria preferentially utilize DOC of fresh algal origin in the study lakes, and this preference is accounted for in the DIF model. This preference rapidly depletes much of the fresh DOC of algal origin from the DOC standing stock. Bade (2004), using a kinetic modeling approach, estimates that, except for the nutrient-enriched lake (Peter 2002), terrestrial DOC comprises from 80 to 90% of the DOC standing stock, in agreement with the results presented here (Table 1). Thus while there is both allochthonous and autochthonous input to the DOC pool, these findings imply that most of the bulk standing stock DOC at any point in time is terrestrial in origin (as is the case for many lakes, Hessen and Tranvik 1998). Thus any effects of older DOC derived from phytoplankton are minor. In the case of the nutrient-enriched lake (Peter 2002) as much as 40% of the DOC pool is of algal origin (Bade 2004). In this case, however, the nutrients and the ^{13}C were added in the same season, precluding a large role for algal DOC produced prior to the experiment. We conclude that DOC was primarily allochthonous and not transiently enriched in autochthonous carbon prior to the ^{13}C additions. Using the MAR model, DOC input rates can be estimated as daily turnover × allochthony × mean areal density of DOC (Appendix C). Estimated input rates during the

TABLE 3. Allochthony for benthos and fishes from the multivariate autoregression (MAR) model and fish diet composition, under contrasting assumptions about the sources of unlabeled detrital carbon for benthos.

Lake	Year	Source of unlabeled detritus	Benthos	Fish 1	Fish 2	Fish 3
Paul	2001	terrestrial	0.84	0.67	0.72	0.76
Paul	2001	terrestrial and aquatic	0.51	0.48	0.53	0.59
Peter	2001	terrestrial	0.78	0.80	0.65	0.54
Peter	2001	terrestrial and aquatic	0.76	0.79	0.64	0.53
Peter	2002	terrestrial	0.41	0.55	0.30	0.25
Peter	2002	terrestrial and aquatic	0.04	0.33	0.08	0.17
Tuesday	2002	terrestrial	0.72	0.56	0.65	0.58
Tuesday	2002	terrestrial and aquatic	0.69	0.55	0.63	0.57

Notes: ''Terrestrial'' means that all unlabeled detritus was assumed to be terrestrial. ''Terrestrial and aquatic'' means that the proportion of autochthonous material in littoral sediments was estimated using the dual isotope flow (DIF) model. In this case, the unlabeled detritus includes an autochthonous component. In Paul Lake, Fish 1 is young-of-year largemouth bass, Fish 2 is juvenile largemouth bass, and Fish 3 is adult largemouth bass. In Peter Lake, Fish 1 is pumpkinseed, Fish 2 is stickleback, and Fish 3 is fathead minnow. In Tuesday Lake, Fish 1 is golden shiner, Fish 2 is stickleback, and Fish 3 is fathead minnow.

experiments were 204 $mg \cdot m^{-2} \cdot d^{-1}$ in Paul Lake, 254 $mg \cdot m^{-2} \cdot d^{-1}$ in Peter Lake 2001, 189 $mg \cdot m^{-2} \cdot d^{-1}$ in Peter Lake 2002, and 194 $mg \cdot m^{-2} \cdot d^{-1}$ in Tuesday Lake.

Vertical migration and feeding.—Vertically migrating organisms, such as some zooplankton and fishes, may feed below the mixed layer of the lake that we labeled with ^{13}C. If we captured these deeper-feeding organisms in the epilimnion, we could erroneously attribute their lack of labeling to allochthony. In two of the lakes, Tuesday and Peter (in both the 2001 and 2002 experiments), the zooplankton, which are small cladocerans and copepods, have negligible migrations (Dini et al. 1987). In Paul Lake, both *Chaoborus* and larger-bodied cladocerans migrate. Although *Chaoborus* migrates from the hypolimnion, it feeds above the thermocline at night (Elser et al. 1987). Migrating *Daphnia* may indeed feed in both shallow and deep waters, but the similarity in labeling pattern to *Chaoborus* argues against this. Zooplankton collected from the epilimnion during the day and night had nearly identical ^{13}C labeling patterns. Further, the ^{13}C in the gut contents of planktivorus fish reflected the labeling patterns of zooplankton. We conclude that zooplankton and *Chaoborus* were receiving the bulk of their carbon from feeding in the portion of the lake that was labeled with ^{13}C.

Benthos and fish.—C flow to benthos appeared to be more allochthonous than that to zooplankton. Unlabeled organic carbon consumed by benthos could be terrestrial in origin, or it could be autochthonous carbon accumulated in sediments prior to our labeling experiments. To assess this possibility, allochthony was estimated using the MAR model under two contrasting assumptions about the origin of unlabeled detritus consumed by benthos (Table 3). First, we assumed that unlabeled detritus was allochthonous in origin (''terrestrial'' rows in Table 3). As an alternative, we assumed that unlabeled detritus included an autochthonous component (''terrestrial and aquatic'' rows in Table 3). In this case, the allochthony of unlabeled detritus was set equal to the allochthony of sedimenting organic matter calculated by the DIF model.

In Paul Lake 2001, the autochthonous contribution to detritus could reduce benthic allochthony from 0.84 to 0.51, with corresponding decreases in allochthony of largemouth bass. However, the terrestrial contribution to largemouth bass carbon is still substantial, ranging from 0.48 for young-of-year to 0.59 for adults. In Peter Lake 2001 and Tuesday Lake 2002, the autochthonous contribution to detritus has less effect on allochthony of benthos or fishes. In Peter Lake 2002, the autochthonous contribution to detritus could substantially decrease the allochthony of benthos and fishes. However, in this estimate the autochthony of detritus was substantially increased by nutrient enrichment, and this may be a transient effect. We conclude that benthic trophic pathways are derived from a mixture of sources but that a substantial component of the benthic carbon has an allochthonous origin in unenriched lakes. We believe that the ''terrestrial and aquatic'' estimates in Table 3 are the most plausible estimates of allochthony using MAR models.

Implications of allochthony estimates

There is no established way of estimating source contributions for nonequilibrium whole-ecosystem isotope studies. The three models used in this study represent three different and apparently reasonable approaches to the problem. The univariate model is the simplest method with the fewest assumptions. It focuses on one compartment at a time. MAR models consider several interacting compartments simultaneously. Unlike the univariate approach, MAR provides an estimate of daily biomass turnover for each compartment. Both univariate and MAR models employ isotope time series from the source and consumer

compartments, and no other rate measurements. Error estimates for univariate and MAR models are easily computed by bootstrapping. The DIF model, in contrast, uses many field measurements of ecosystem rates, all available isotope time series, and many assumptions about ecosystem structure and feedbacks. This added complexity allows the DIF model to estimate more fluxes among ecosystem compartments than the other models, thereby providing a more detailed breakdown of ecosystem carbon flows. It is not possible to compute a statistically rigorous estimate of errors for the DIF model. However, errors in predicting $\delta^{13}C$ were similar for the three models (Appendix D).

In general there was good agreement among the three models, with the univariate and MAR models producing the most similar estimates. The correspondence of these two approaches results partly from the importance of the time-series data of the focal compartment common to both estimates. DIF model estimates differed in some cases from the univariate and MAR models, but these differences were usually not consistent when results were compared among lakes. For example, DIF model estimates of zooplankton allochthony were 15% higher than the univariate model for Paul Lake in 2001, but this difference was reversed for Peter Lake 2001 where the DIF model estimate was 14% lower than the univariate model. Hence we conclude that the differences among allochthony estimates for zooplankton largely reflect model uncertainty. The DIF model consistently produced lower estimates of the autochthonous contribution to DOC than the univariate and MAR estimates. Except in Tuesday Lake, the DIF model indicates DOC has a significant autochthonous component in contrast with the other two models. This discrepancy suggests that autochthonous fluxes by a number of mechanisms (phytoplankton release, phytoplankton mortality, consumer release) are important and not well captured by the indirect, empirical approaches of the MAR and univariate models. If the DIF model estimates are more realistic, additional study of these mechanisms is warranted, especially in terms of how these sources produce autochthonous DOC that accumulates.

All three models indicate that allochthony was substantial. Carbon flow to ''herbivorous'' zooplankton was 22–75% allochthonous in unenriched lakes, due to consumption of terrigenous POC and bacterial carbon derived from terrigenous DOC. Carbon flow to fishes was more allochthonous than that to zooplankton (for a given model). Fish allochthony is higher, because of greater reliance on allochthonous benthic resources and direct consumption of terrestrial prey (Hodgson and Kitchell 1987). Allochthonous organic carbon represents a substantial subsidy to food webs of these lakes.

Many ecosystems receive substantial inputs of organic carbon from outside their boundaries. Ecologists have only recently begun to evaluate the contribution of these carbon inputs to food webs. In a number of cases, species populations or consumer guilds are subsidized by exogenous food sources (Polis et al. 1997, 2004). Our experiments demonstrate substantial organic carbon subsidies to entire food webs of ecosystems. This finding is not consistent with the simplification often made for lakes where the food web is viewed as largely supported by endogenous primary production. Instead, lake ecosystems, such as stream ecosystems (Wallace et al. 1997, Nakano and Murakami 2001), are open, and consumers derive significant amounts of carbon from exogenous sources.

Allochthony is reduced if nutrients are added. The relative importance of allochthonous carbon flow to all consumers decreased as a result of nutrient enrichment of Peter Lake. This result is consistent with an earlier pulse labeling experiment of an entire lake, in which nutrients were added and zooplankton were found to be supported largely by autochthonous carbon (Cole et al. 2002). Eutrophication results from increased flow of nutrients from land to lakes, but the increase in autochthonous primary production reduces the dependency of aquatic consumers on terrigenous organic carbon. Thus changes in landscapes that increase nutrient flow to lakes, such as land conversion for agriculture or urbanization (Carpenter et al. 1998), may reduce the terrestrial subsidy of organic matter to aquatic consumers and thereby decouple the aquatic food web from its watershed.

Terrigenous subsidies were more important in dystrophic Tuesday Lake than in the other lakes. Changes in landscapes that increase the flux or concentration of terrigenous organic matter in lakes (Canham et al. 2004) may increase the terrestrial subsidy to aquatic food webs. The relative importance of terrestrial subsidies may wax or wane over decades to millennia as changes in hydrology, soils, and watershed vegetation alter nutrient and organic matter inputs to lakes.

Allochthony is related to color : chlorophyll *a* ratio, which is an easily measured index of terrigenous organic carbon relative to endogenous producer biomass (Fig. 5). Means of the three models represent our best estimate of allochthony for four consumer compartments, and ranges represent the variability among models (Table 2 for zooplankton and *Chaoborus* from all models, Table 2 for benthos and fish from DIF model, Table 3 for benthos and fish from MAR model). All increase with the color : chlorophyll *a* ratio except benthos, where allochthony is high for three of four cases. A similar positive relationship between percent allochthony and the ratio of color : chlorophyll *a* also occurs for the two major pelagic C pools, DOC and POC (*data not shown*). Color (light absorbance at 440 nm) is a measure of chromophoric dissolved organic matter (CDOM), which is largely of terrestrial origin (Hessen and Tranvik 1998). CDOM is probably proportional to the amount of terrestrially derived organic C potentially available to consumers in a given lake. Chlorophyll *a* is proportional to phytoplankton biomass, an index of

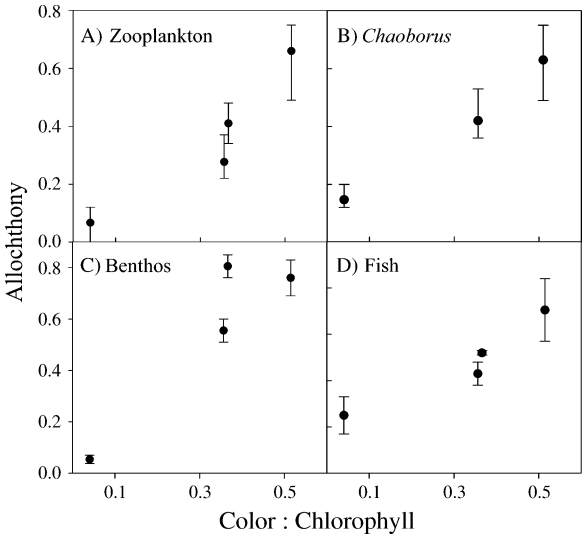

FIG. 5. Allochthony (the proportion of carbon flow from terrestrial sources) vs. ratio of color to chlorophyll *a* for (A) zooplankton, (B) *Chaoborus* (not available in Peter Lake 2001), (C) benthos, (D) fish (YOY bass in Paul Lake 2001, fathead minnows in the other three experiments). Symbols show the means, and error bars show the maximum and minimum values observed. Experiments in order of color : chlorophyll are: Peter Lake 2002, Paul Lake, Peter Lake 2001, Tuesday Lake.

the amount of autochthonous C potentially available to consumers. Allochthony is inversely related to primary producer biomass and positively related to terrestrially derived CDOM. While allochthony in our experiments also tracks other measures of autochthonous primary production and terrestrial C-loading (e.g., measured gross primary production and estimated terrestrial inputs of DOC), color and chlorophyll *a* data are widely available for a large number of lakes and may ultimately prove to be a useful predictor of allochthony. Because few measurements of whole-ecosystem allochthony are available, other variables such as lake size, morphometry, or water residence time may also be important.

There is long history of research on material fluxes from land to water in ecosystem ecology (Likens and Bormann 1974). More recently, ecologists have addressed the role of cross-boundary subsidies for population and community ecology (Polis et al. 2004). In order to be important for the receiving ecosystem, cross-boundary fluxes must be used by consumers in that ecosystem. Our experiments show that consumer production in small, relatively unproductive lakes is heavily subsidized by organic carbon from the surrounding landscape. The importance of this subsidy is reduced by nutrient enrichment, and is greater in a dystrophic lake with high concentrations of terrigenous DOC.

Our experimental lakes are near the average size for the Northern Highland Lake District (median area, 0.33 ha; range, 0.008 to 1625 ha; $n = 6928$; North Temperate Lakes Long-Term Ecological Research site; S. Carpenter et al., *unpublished data*). However, a substantial proportion of the landscape's lake area and fresh-water volume is found in larger lakes. The importance of terrigenous organic carbon in larger lakes is uncertain. Inputs at the perimeter may be simply diluted in larger lakes, leading to the expectation that autochthony drives the lake food web. Alternatively, consumers may orient toward the littoral zone, a highly productive ecotone (Schindler and Scheuerell 2002, Vander Zanden and Vadeboncoeur 2002), and thereby remain highly dependent on terrigenous carbon even in larger lakes.

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APPENDIX A

Dual isotope flow models are available in ESA's Electronic Data Archive: *Ecological Archives* E086-146-A1.

APPENDIX B

Univariate models are available in ESA's Electronic Data Archive: *Ecological Archives* E086-146-A2.

APPENDIX C

Multivariate autoregression models are available in ESA's Electronic Data Archive: *Ecological Archives* E086-146-A3.

APPENDIX D

Information about goodness of fit of the models is available in ESA's Electronic Data Archive: *Ecological Archives* E086-146-A4.

APPENDICES FOR THE PAPER

ECOSYSTEM SUBSIDIES:
TERRESTRIAL SUPPORT OF AQUATIC FOOD WEBS FROM ^{13}C ADDITION TO CONTRASTING LAKES

A paper submitted to *Ecology* by

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APPENDIX 1 DUAL ISOTOPE FLOW MODELS

The dual-isotope flow (DIF) model calculates C flow for both ^{12}C and ^{13}C among 12 compartments within the lake and across the external boundaries of the ecosystem (Figure A1.1). The model is similar to a 6-compartment model presented in a prior paper (Cole et al. 2002), so we explain it only briefly here, highlighting the differences. The boundaries of the ecosystem are the bottom of the mixed layer, the atmosphere and the sediments. Two differential equations (one for mass balance dynamics of each C isotope) describe each of the 12 components of the model (DIC, DOC, pelagic bacteria, phytoplankton, detrital POC, zooplankton, *Chaoborus*, periphyton, benthic invertebrates, and three fish compartments representing different functional groups). Thus, there are 24 differential equations, one for the mass balance dynamics of ^{13}C and one for the mass balance of total C in each of 12 compartments. The model was parameterized separately for each of the four experiments.

Each of the 24 differential equations is a mass-balance equation (Cole et al. 2002). Many of the fluxes in each equation were directly measured. One flux for each carbon pool was estimated by difference. Eighteen parameters were estimated by constrained least squares using the Matlab Optimization Toolbox. The constraints were provided by literature values, our own measurements, or sensitivity analyses. These 18 parameters were input rate of POC; photosynthetic fractionation parameters for periphyton and phytoplankton; assimilation efficiencies of zooplankton and *Chaoborus*; respiration coefficients of benthos, zooplankton and *Chaoborus*; sedimentation coefficients of phytoplankton and zooplankton feces; flocculation and photodegradation coefficients of DOC; DOC release coefficient of phytoplankton; proportion of periphyton in benthos diets; selectivity coefficient of bacteria for DOC derived from phytoplankton; selectivity coefficient of zooplankton for phytoplankton; proportion of periphyton in benthos diets; relative contributions of zooplankton and *Chaoborus* to fish diets. The least squares estimates are the values of these parameters that minimize the sum of squared deviations between simulated and observed $\delta^{13}C$ during the experiment. As a measure of goodness of fit, we present the residual standard deviation (standard deviation of the difference between simulated and observed $\delta^{13}C$ values). This represents the average error of the model projections in the same units as $\delta^{13}C$ (Appendix 4).

The DIF model includes fish carbon fluxes computed by a fish bioenergetics model (Hanson et al. 1997). Bioenergetic parameters were taken from standard parameter tables for the fishes represented in the model (Hanson et al. 1997). For each fish compartment, the bioenergetics model was used to compute daily consumption of each prey item, respiration, and egestion. Growth and biomass dynamics were measured directly and interpolated to daily values input to the bioenergetics model. Fish functional groups were chosen to represent differences in body size and diet, and to include the most abundant fishes found in each experiment. In Paul Lake, Fish 1 is young-of-year largemouth bass, Fish 2 is juvenile largemouth bass, and Fish 3 is adult largemouth bass. Ontogenetic changes in diets of largemouth bass are reported in Carpenter and Kitchell (1993). In Peter Lake, Fish 1 is pumpkinseed (a sunfish that is primarily benthivorous), Fish 2 is stickleback (a small-bodied benthivore and planktivore), and Fish 3 is fathead minnow (a benthivorous cyprinid). In Tuesday Lake, Fish 1 is golden shiner (a primarily planktivorous cyprinid), Fish 2 is stickleback, and Fish 3 is fathead minnow.

In Tuesday Lake 2002, we did not simulate $DI^{13}C$ dynamics because good fits to observed $\delta^{13}C$ of DIC could not be obtained. For that experiment, daily $DI^{13}C$ values were interpolated from measurements and used as inputs to the DIF model to solve the other 23 differential equations.

DIC is a pH-dependent mixure of inorganic C species (CO_{2aq}, HCO_3 and CO_3) and the reactions among these species fractionate ^{13}C in different ways. Only the $\delta^{13}C$ of the total DIC pool can be measured directly. The $\delta^{13}C$ of the C species was calculated according to the equations in Zhang et al. (1995) and Mook et al. (1974).

In nutrient-enriched Peter Lake in 2002, the very high pH and low CO_{2aq} created conditions in which chemically-enhanced diffusion with the atomosphere occurred (Wanninkhof and Knox 1996). The uncertainty with isotope fractionation during chemically-enchanced diffusion made it difficult for us to accurately model all aspects of the DIC pool. Thus, the DIF for Peter Lake in 2002 uses actual measured DIC and its C-species isotopes as input data and does not model this compartment. A more complete treatment of the isotopic aspects of chemically enchanced diffusion in this experiment is presented by Bade (2004).

The DIF model was solved in Matlab using a numerical method that accounted for the extremely rapid dynamics of $DI^{13}C$ in comparison with dynamics of ^{13}C in other carbon pools. The 23 differential equations for compartments other than $DI^{13}C$ were integrated using the fourth-order fixed-interval Runge-Kutta method (Press et al. 1989). At each time step, $DI^{13}C$ was calculated using the analytic solution of the differential equation for $DI^{13}C$. The analytical solution was derived by assuming that the other, more slowly-changing variables were constant over the short time step.

Programs for the model analysis and parameter bootstrapping were written by the authors using Matlab (versions 5.3 and 6.2).

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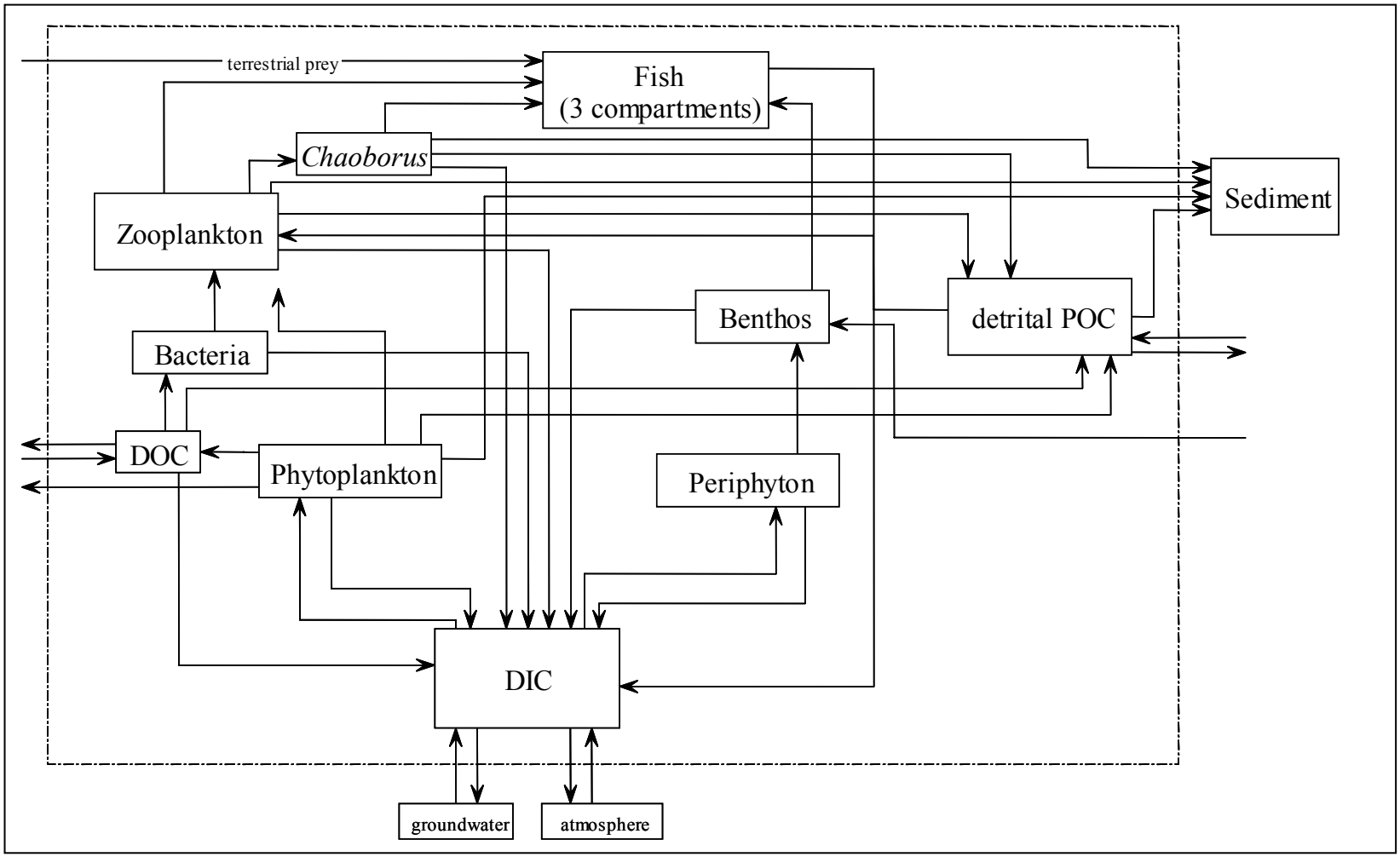
Figure A1.1. Flow diagram of the dual isotope flow model. Both carbon isotopes were tracked for all flows and pools.



APPENDIX 2. UNIVARIATE MODELS

We used univariate statistical models to estimate sources supporting carbon pools and food web constituents. The models are univariate because they only attempt to explain the response of a single variable (e.g. zooplankton) in contrast to other model approaches which are multivariate (Appendices 1 and 3). The models are statistical because they are based on fitting data from the C-13 manipulations using the equation:

$$\delta^{13}Cx_t = (1\text{-}w)[(1\text{-}m)(\delta^{13}CO_{2\,(aq)} - \varepsilon_p)_t + m(\delta^{13}CO_{2\,(aq)} - \varepsilon_p)_{t\text{-}u}] + w(\text{-}28).$$

A schematic diagram of this model is presented in Figure A2.1. The left hand side of the equation represents time series of $\delta^{13}C$ of POC, DOC, zooplankton, or *Chaoborus* (x_t). The $\delta^{13}C$ of these variables is modeled as a function of two sources - the $\delta^{13}C$ of aqueous CO_2 that varies during the manipulation and the $\delta^{13}C$ of terrestrial C-3 plants that did not vary during the manipulation. The parameter w ($0 \le w \le 1$) estimates the relative contribution from these two sources. In addition, the contribution from aqueous CO_2 is further divided into current production on day t and past production on day t - u where u is a time delay in days. The parameter m ($0 \le m \le 1$) estimates the relative contribution from current and past production. The distinction between current and past production accounts for the turnover of labeled carbon in the system. Comparisons showed that this distinction significantly improved the fit of the models to data (Pace et al. 2004).

Aqueous carbon dioxide is the primary form of $DI^{13}C$ taken up by most phytoplankton and hence represents the carbon derived from primary production in the lake. The fractionation of ^{13}C between $CO_{2(aq)}$ and HCO_3 was calculated from direct measurements of DIC, $DI^{13}C$, pH, and temperature using the equations of Mook et al. (1978) and Zhang et al. (1995). A daily time series of $\delta^{13}CO_{2(aq)}$ was established from measured values interpolated to daily values with a cubic spline. The model was evaluated for all the dates when $\delta^{13}C$ of the response variable (POC, DOC zooplankton, or *Chaoborus*) was measured.

Unknown parameters for the univariate models are ε_p, w, m and u, where w, m, and u are as explained above and ε_p is photosynthetic fractionation. . The value of u that minimized variance was identified using a profile likelihood analysis (Burnham and Anderson 1998). Model parameters ε_p, w, and m were estimated using a nonlinear optimization routine in Matlab. Overall model fit was determined by least squares. Maximum likelihood analysis gave results equivalent to least squares for these models (Hilborn and Mangel 1997), but required estimating an additional parameter (variance). We only report the least squares results here. Goodness of fit was evaluated using mean residual standard deviation and the relationship between predicted and observed values. Parameter uncertainty was estimated by bootstrapping randomized data (Efron and Tibshirani 1993) and estimating parameters for 1000 iterations. The thousand estimates of each parameter were then used to calculate bootstrapped standard deviations. Bootstrapped parameter distributions for the models were approximately normally distributed, and parameter bias was only a few percent of the standard deviation (Efron and Tibshirani 1993).

The general model was modified for analysis of the ^{13}C addition to Peter Lake in 2002. In this case photosynthetic fractionation clearly varied and declined to low values with aqueous

CO_2 drawdown promoted by nutrient addition. For this manipulation photosynthetic fractionation (ε_p) was modeled as a function of the concentration of CO_2.

$$\varepsilon_p = \varphi\ [CO_2]$$

In this equation, the fitted parameter φ is negative, so ε_p becomes more negative as the concentration of CO_2 increases. CO_2 concentrations were measured or interpolated to match the date of sampling for modeled variables. φ was estimated using the same approach as described above for other parameters. Substituting equation 2 into equation 1 resulted in a model that provided a much better fit to the data for Peter Lake in 2002.

For the analysis of DOC ε_p was fixed at the value derived for POC models excepting the Peter Lake 2002 manipulation. We assumed the autotrophic contribution to DOC is derived from the algae in the POC suggesting that photosynthetic fractionation from aqueous CO_2 for these pools (POC and DOC) should be the same. In solutions that fit ε_p for the DOC data, fractionation was estimated near zero for the Paul and Peter 2001 additions. As an alternative, ε_p was fixed at the values derived for POC and parameters estimated using equation 1. Regardless of whether ε_p was fit or fixed the percent of allochthony for DOC was > 85% in the three manipulations that did not include nutrient addition. Here, we report results of models that used fixed values of ε_p.

Programs for the model analysis and parameter bootstrapping were written by the authors using Matlab (version 6.2).

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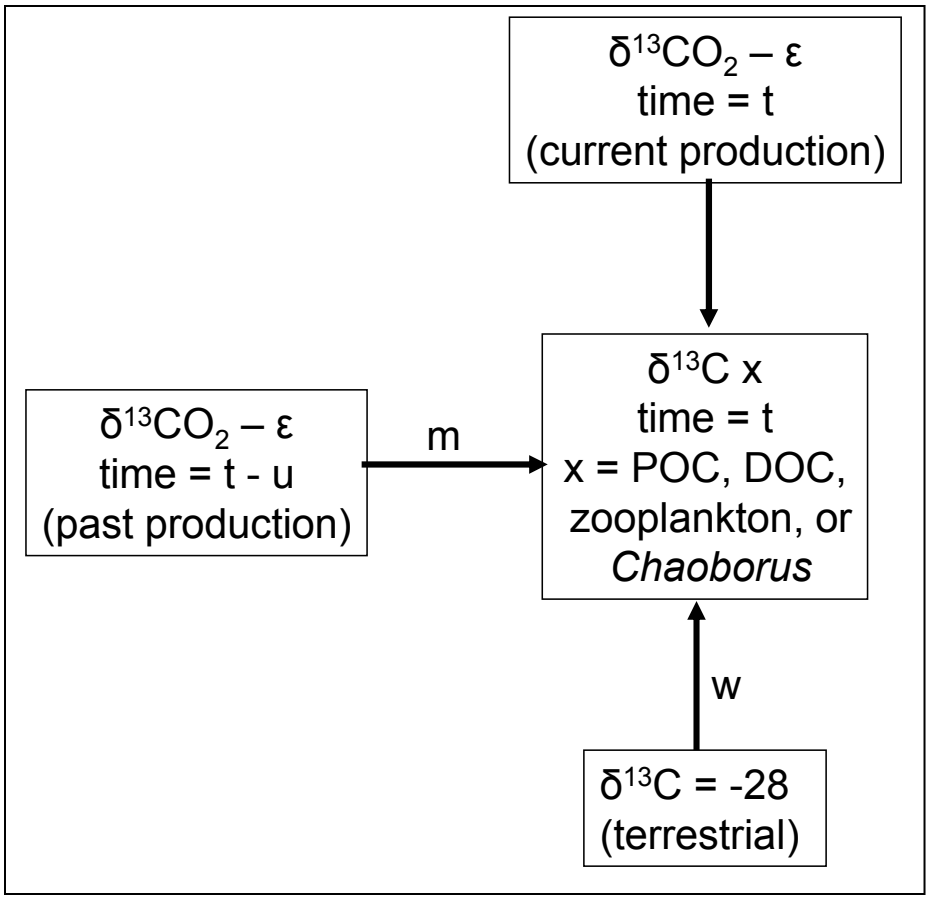
Figure A2.1. Schematic diagram of the univariate models. Symbols are defined in the text.



APPENDIX 3 MULTIVARIATE AUTOREGRESSION MODELS

Multivariate autoregressions (Ives et al. 2003) were used to estimate autochthony and allochthony. The general form of the transition equation was

$$\boldsymbol{\alpha}_t = \mathbf{B}\,\boldsymbol{\alpha}_{t-1} + \mathbf{C}\,\mathbf{U}_t + \boldsymbol{\omega}_t$$

In the transition equation, $\boldsymbol{\alpha}$ is a vector of $\delta^{13}C$ in components of the food web at a specified time. Elements of the transition matrix **B** describe the interactions among food web components. **U** is a matrix of covariates, and **C** is a matrix of parameters for the effects of the covariates. The errors $\boldsymbol{\omega}$ are assumed to follow a multivariate normal distribution with mean 0 and covariance matrix **Q**.

We accounted for sampling errors in observing $\delta^{13}C$. Although analytical errors in measuring $\delta^{13}C$ are small, it may be important to account for variation among replicate samples of consumers. We accounted for observation errors using an equation

$$\mathbf{y}_t = \boldsymbol{\alpha}_t + \boldsymbol{\upsilon}_t$$

Here y is vector of observed $\delta^{13}C$ at a specified time and the observation errors $\boldsymbol{\upsilon}$ were assumed to follow a multivariate normal distribution with mean 0 and covariance matrix **H**.

Given data for **y** and **U** and estimates of the observation covariance matrix **H**, a Kalman Filter was used to compute maximum likelihood estimates of **B**, **C** and **Q** (Harvey 1989). Using the elements of **B** and **C**, we estimated autochthony and allochthony (see below). We computed standard deviations of the elements of **B** and **C**, and of autochthony and allochthony, by parametric bootstrapping as recommended by Ives et al. (2003).

To understand the calculation of autochthony and allochthony, it is necessary to examine the specific transition equations that were analyzed. For each lake-year, we analyzed two transition equations, one for the littoral data and one for the pelagic data.

In the benthic transition equation (Figure A3.1),

$$\alpha = \begin{bmatrix} p \\ b \end{bmatrix}$$

$$B = \begin{bmatrix} 1-m_p & 0 \\ 0 & 1-m_b \end{bmatrix}$$

$$C = \begin{bmatrix} g_p & -g_p\varepsilon & w_p \\ g_b & -g_b\varepsilon & w_b \end{bmatrix}$$

$$U = \begin{bmatrix} \delta^{13}CO_2 \\ 1 \\ -28 \end{bmatrix}$$

Periphyton $\delta^{13}C$ is represented by p and benthic $\delta^{13}C$ is represented by b. The parameters estimated from the data are the autoregressive parameters m_p and m_b, parameters for autochthonous carbon assimilation g_p and g_b, fractionation by periphyton photosynthesis ε, and parameters for allochthonous carbon assimilation w_p and w_b. Autochthony of benthos is defined as the proportion of new benthos carbon derived from periphyton, $g_b / (g_b + w_b)$. Allochthony of benthos is defined as the proportion of new benthos carbon derived from terrestrial sources, $w_b / (g_b + w_b)$.

In Peter Lake 2002, photosynthetic fractionation clearly varied and declined to low values with aqueous CO_2 drawdown promoted by nutrient addition. To account for this effect, we modified U to fit the apparent change in ε when $[CO_2]$ declined below 1 μmol/L:

$$U = \begin{bmatrix} \delta^{13}CO_2 \\ \arctan([CO_2]-1) \\ -28 \end{bmatrix}$$

where arctan is the arc tangent function. This transformation has no ecological interpretation, but did produce a good fit to the data.

In the pelagic transition equation (Figure A3.2)

$$\alpha = \begin{bmatrix} P \\ D \\ Z \\ C \end{bmatrix}$$

$$B = \begin{bmatrix} 1-m_P & 0 & 0 & 0 \\ 0 & 1-m_D & 0 & 0 \\ 0 & 0 & 1-m_Z & 0 \\ 0 & 0 & g_C & 1-m_C \end{bmatrix}$$

$$C = \begin{bmatrix} g_P & -g_P\varepsilon & w_P \\ g_D & -g_D\varepsilon & w_D \\ g_z & -g_z\varepsilon & w_Z \\ 0 & 0 & w_C \end{bmatrix}$$

and **U** is the same as in the benthic equation. Compartment $\delta^{13}C$ values are POC (P), DOC (D), zooplankton (Z) and *Chaoborus* (C). For each compartment, there are three parameters, an autoregressive parameter (m), a parameter for incorporation of phytoplankton carbon (g), and a parameter for incorporation of terrestrial carbon (w). Photosynthetic fractionation by phytoplankton is ε. For POC, DOC and zooplankton, autochthony is g / (g + w) and allochthony is w / (g + w). For *Chaoborus*, allochthony is $w_C / \{g_C [w_Z / (g_Z + w_Z)] + w_C\}$ in order to account for the allochthonous component of zooplankton.

We considered an alternative model structure in which **α** and **U** were the same, but B and C were defined as

$$B = \begin{bmatrix} 1-m_P & 0 & 0 & 0 \\ 0 & 1-m_D & 0 & 0 \\ g_Z & 0 & 1-m_Z & 0 \\ 0 & 0 & g_C & 1-m_C \end{bmatrix}$$

$$C = \begin{bmatrix} g_P & -g_P\varepsilon & w_P \\ g_D & -g_D\varepsilon & w_D \\ 0 & 0 & w_Z \\ 0 & 0 & w_C \end{bmatrix}$$

This alternative model derives the labeling of zooplankton from POC $\delta^{13}C$, whereas the previous model derives the labeling of zooplankton from phytoplankton $\delta^{13}C$, a component of the POC. The alternative model did not fit the data as well, based on Akaike's information criterion (Harvey 1989). Therefore the alternative model was not used to estimate autochthony and allochthony.

In Peter Lake during 2001, *Chaoborus* were found only infrequently and were therefore omitted from the multivariate autoregression. We assumed that autochthony and allochthony of *Chaoborus* were identical to the values for zooplankton.

In Paul Lake for 2001 and Tuesday Lake for 2002, data were available for $\delta^{13}C$ of bacteria. To estimate allochthony of bacteria, we used a multivariate autoregression similar to the pelagic model described above. We defined **α**, **B** and **C** as

$$\alpha = \begin{bmatrix} P \\ D \\ M \end{bmatrix}$$

$$B = \begin{bmatrix} 1-m_P & 0 & 0 \\ 0 & 1-m_D & 0 \\ 0 & 0 & 1-m_M \end{bmatrix}$$

$$C = \begin{bmatrix} g_P & g_P\varepsilon & w_P \\ g_D & g_D\varepsilon & w_D \\ g_M & g_M\varepsilon & w_M \end{bmatrix}$$

Symbols are identical to those for the pelagic model, except that M represents bacterial $\delta^{13}C$. Autochthony of bacteria is $g_M / (g_M + w_M)$ and allochthony is $w_M / (g_M + w_M)$.

We also considered an alternative model in which labeling of bacteria was linked to bulk DOC, instead of to carbon released by phytoplankton. For this alternative model, **B** and **C** are defined as

$$B = \begin{bmatrix} 1-m_P & 0 & 0 \\ 0 & 1-m_D & 0 \\ 0 & g_M & 1-m_M \end{bmatrix}$$

$$C = \begin{bmatrix} g_P & g_P\varepsilon & w_P \\ g_D & g_D\varepsilon & w_D \\ 0 & 0 & w_M \end{bmatrix}$$

This alternative model did not fit as well, according to Akaike's information criterion (Harvey 1989). Therefore it was not used to compute autochthony and allochthony.

Programs for the model analysis and parameter bootstrapping were written by the authors using Matlab (version 5.3).

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Figure A3.1. Schematic diagram of the MAR model used to estimate allochthonous and autochthonous carbon flow to benthos. Benthos are linked to periphyton through consumption of current production. Measurements of periphyton $\delta^{13}C$ could have included small amounts of terrestrial detritus as well as in-lake primary production, represented by the arrow from terrestrial carbon to periphyton. Symbols are defined in the text.

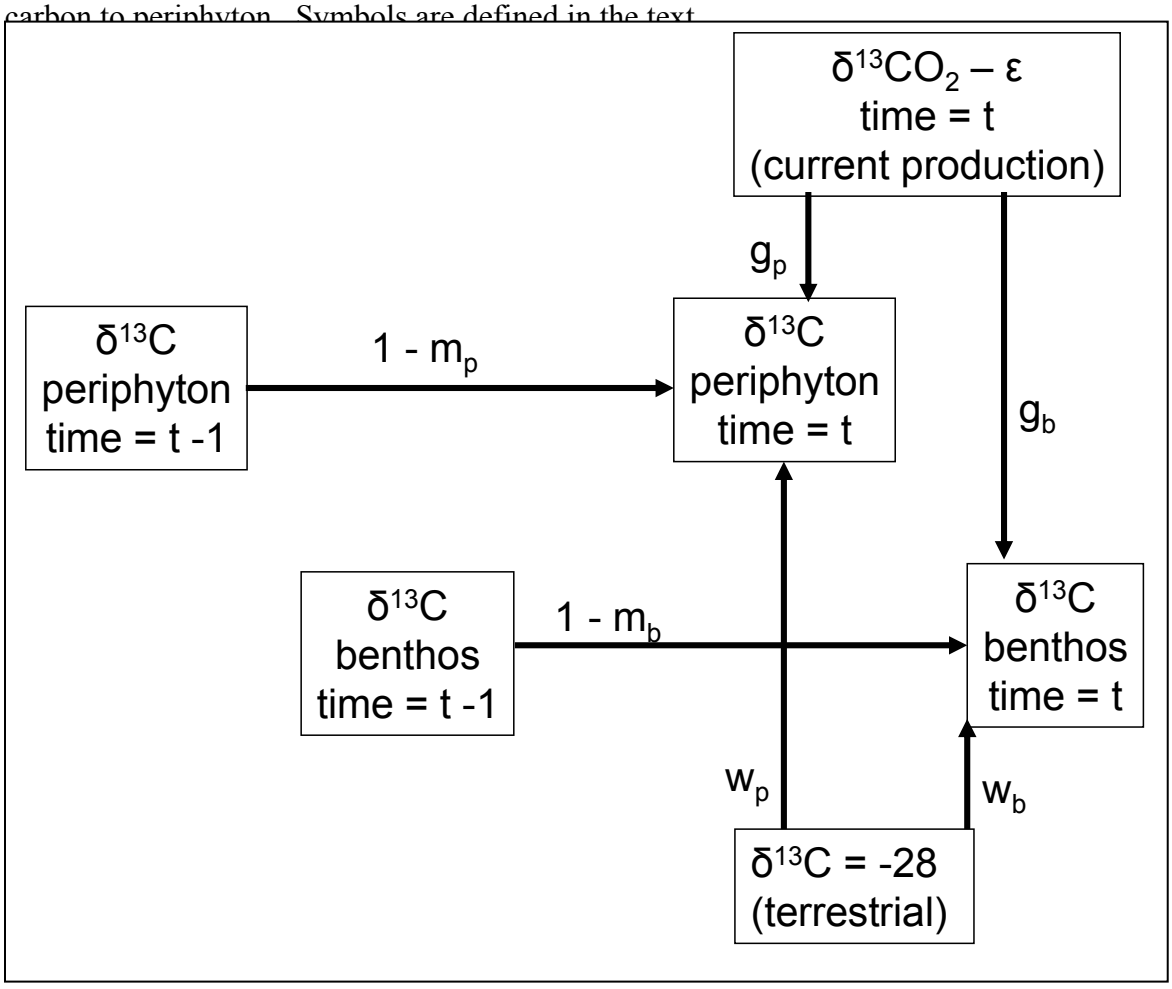
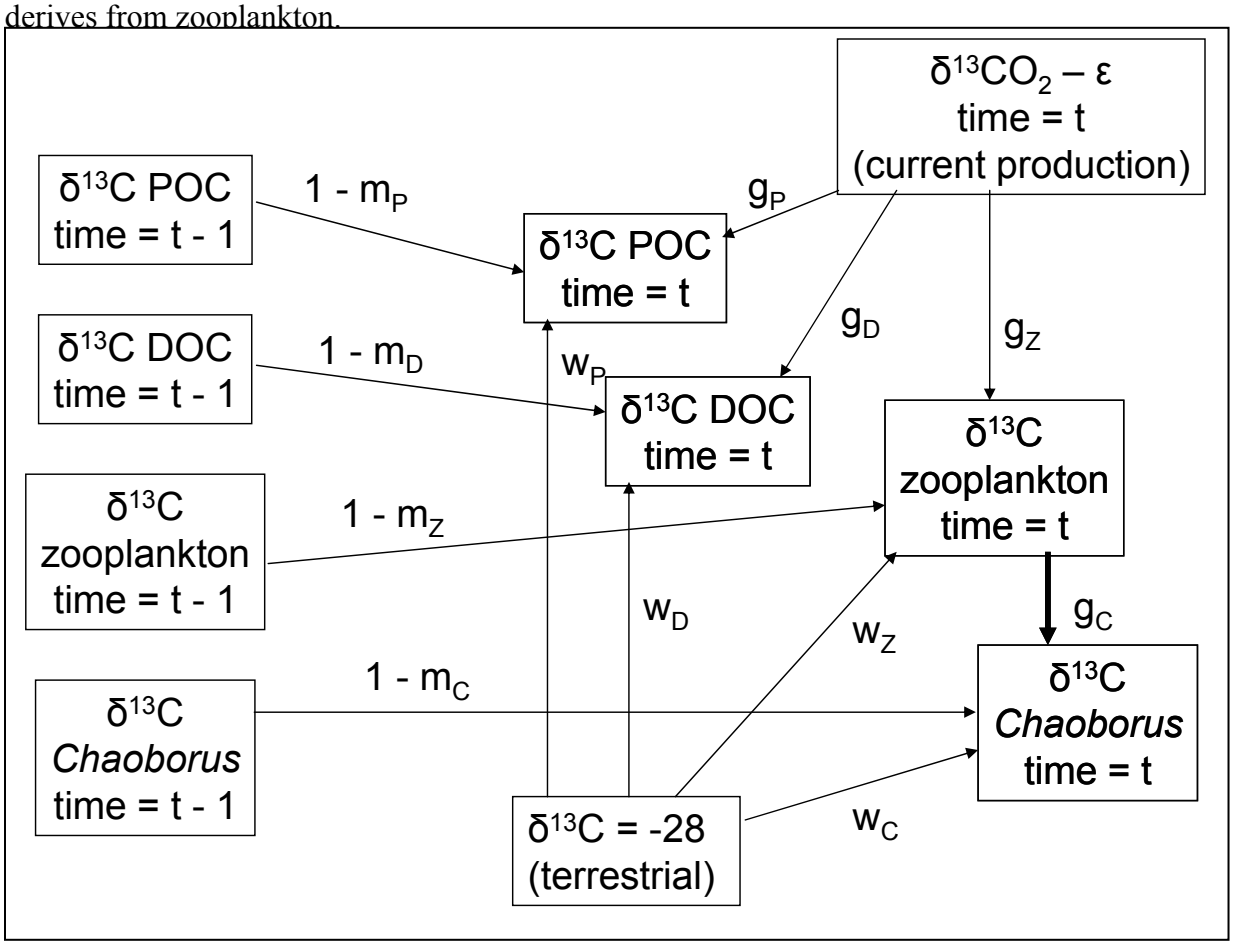


Figure A3.2. Schematic diagram of the MAR model used to estimate allochthonous and autochthonous carbon flow to POC, DOC, zooplankton and *Chaoborus*. POC, DOC and zooplankton are linked to phytoplankton through current production, whereas *Chaoborus* carbon derives from zooplankton.



APPENDIX 4 GOODNESS-OF-FIT OF THE MODELS

Standard deviations of residuals for each carbon pool were similar for the three models (Table A4.1). Standard deviations of residuals were substantially smaller than the ranges of $\delta^{13}C$ caused by the tracer additions.

Table A4.1. Ranges (per mil) and standard deviations of residuals (per mil) for $\delta^{13}C$ of each carbon pool. For each experiment, we present the range of observed $\delta^{13}C$ and the residual standard deviation from predictions of univariate (Uni), multivariate autoregressive (MAR), and dual-isotope flow (DIF) models. Residual standard deviations were not available (n/a) for carbon pools that were not predicted by a model. In Paul Lake, Fish 1 is young-of-year largemouth bass, Fish 2 is juvenile largemouth bass, and Fish 3 is adult largemouth bass. In Peter Lake, Fish 1 is pumpkinseed, Fish 2 is stickleback, and Fish 3 is fathead minnow. In Tuesday Lake, Fish 1 is golden shiner, Fish 2 is stickleback, and Fish 3 is fathead minnow.

Compartment	Paul Lake, 2001				Peter Lake, 2001				Peter Lake, 2002				Tuesday Lake, 2002			
	Range	**Uni**	**MAR**	**DIF**	**Range**	**Uni**	**MAR**	**DIF**	**Range**	**Uni**	**MAR**	**DIF**	**Range**	**Uni**	**MAR**	**DIF**
DIC	39.0	n/a	n/a	0.9	37.4	n/a	n/a	0.6	41.0	n/a	n/a	n/a	42.0	n/a	n/a	3.4
DOC	5.0	1.0	0.2	0.2	3.0	0.6	0.2	0.2	9.5	1.9	0.05	0.4	1.9	0.4	0.2	0.1
POC	23.1	1.6	0.5	0.7	16.6	1.5	0.01	0.4	42.8	5.8	0.03	0.7	21.1	2.8	0.2	1.2
Bacteria	14.1	n/a	1.9	0.5	7.7	n/a	n/a	0.7	22.3	n/a	n/a	1.6	10.2	n/a	1.3	0.4
Zooplankton	29.4	2.2	0.4	0.5	18.6	0.8	1.0	0.7	37.9	3.3	0.5	1.8	11.2	1.6	0.4	1.7
Chaoborus	23.3	4.5	0.2	0.9	8.5	n/a	n/a	4.2	28.4	5.3	0.3	1.9	13.8	2.0	0.7	0.8
Periphyton	30.7	n/a	2.3	2.1	30.0	n/a	2.7	1.5	44.7	n/a	1.6	2.8	50.9	n/a	0.4	3.0
Benthos	11.6	n/a	2.2	0.8	n/a	n/a	n/a	n/a	23.4	n/a	1.4	1.0	18.7	n/a	0.3	0.7
Fish 1	18.7	n/a	n/a	0.8	6.8	n/a	n/a	0.4	16.1	n/a	n/a	1.0	5.5	n/a	n/a	0.3
Fish 2	10.0	n/a	n/a	0.9	11.0	n/a	n/a	0.6	16.4	n/a	n/a	0.9	12.2	n/a	n/a	0.8
Fish 3	0.7	n/a	n/a	0.5	11.4	n/a	n/a	0.6	16.4	n/a	n/a	0.9	9.9	n/a	n/a	0.3