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Carpenter, S R; Cole, J J; Pace, M L; Van de Bogert, M; Bade, D L; Bastviken, D; Gille, C M; Hodgson, J R; Kitchell, J F; Kritzberg, Emma

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

STEPHEN R. CARPENTER,1,6 JONATHAN J. COLE,2 MICHAEL L. PACE,2 MATTHEW VAN DE BOGERT1,2 DARREN L. BADE,1,2 DAVID BASTVIKEN,3 CAITLIN M. GILLE,1 JAMES R. HODGSON,4 JAMES F. KITCHELL,1 AND EMMA S. KRITZBERG5

1Center for Limnology, University of Wisconsin, Madison, Wisconsin 53706 USA
2Institute of Ecosystem Studies, Millbrook, New York 12545 USA
3Department of Water and Environmental Studies, Linköping University, SE 581 83, Linköping, Sweden
4Department of Biology, St. Norbert College, De Pere, Wisconsin 54115 USA
5Department of Ecology/Limnology, Lund University, S-223 62, Lund, Sweden

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, $\textit{Chaoborus}$, 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).

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-
son et al. 2003). 13 C additions overcome this problem synthesis), and fractionation of that CO2 during pho-

tochthony) to food webs by altering the13 C of dissolved 

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 CO2 moiety of dissolved inorganic carbon (the proximate substrate for photosynthesis), and fractionation of that CO2 during photosynthesis, causes carbon fixed by aquatic primary producers (especially phytoplankton) to be nearly identical in 13 C to organic carbon of terrestrial origin (Karls-son 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 atmo-
sphere and increasing carbon flows to consumers (Pace et al. 2004).

Although research has begun to quantify the contribu-
tion 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 D13 C to estimate the terrestrial subsidy to lake eco-
systems 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 terrestrial DOC than in a lake with low concentrations of terrigenous 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 differ-
ent 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).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Paul 2001</th>
<th>Peter 2001</th>
<th>Peter 2002</th>
<th>Tuesday 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C at 1 m)</td>
<td>21.1</td>
<td>21.4</td>
<td>22.1</td>
<td>22.0</td>
</tr>
<tr>
<td>Thermocline (m)</td>
<td>3.5</td>
<td>3.6</td>
<td>3.1</td>
<td>2.6</td>
</tr>
<tr>
<td>pH</td>
<td>6.4</td>
<td>6.9</td>
<td>8.5</td>
<td>6.1</td>
</tr>
<tr>
<td>Color (m⁻¹)</td>
<td>1.5</td>
<td>1.3</td>
<td>1.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Secchi (m)</td>
<td>4.6</td>
<td>4.9</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>pCO₂ (μatm)†</td>
<td>1039</td>
<td>673</td>
<td>152</td>
<td>977</td>
</tr>
<tr>
<td>DIC (μmol)</td>
<td>93</td>
<td>141</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>DOC (μmol)</td>
<td>304</td>
<td>376</td>
<td>483</td>
<td>700</td>
</tr>
<tr>
<td>POC (μmol)</td>
<td>35.5</td>
<td>34.1</td>
<td>152.3</td>
<td>76.5</td>
</tr>
<tr>
<td>Chlorophyll a (μg/L)</td>
<td>4.21</td>
<td>3.55</td>
<td>42.1</td>
<td>6.8</td>
</tr>
<tr>
<td>TP (μmol)</td>
<td>0.314</td>
<td>0.261</td>
<td>0.846</td>
<td>0.385</td>
</tr>
<tr>
<td>TN (μmol)</td>
<td>26.9</td>
<td>30.3</td>
<td>46.7</td>
<td>28.5</td>
</tr>
<tr>
<td>GPP (mmol O₂·m⁻²·d⁻¹)</td>
<td>43.4</td>
<td>31.3</td>
<td>104.5</td>
<td>42.9</td>
</tr>
<tr>
<td>R (mmol O₂·m⁻²·d⁻¹)</td>
<td>51.8</td>
<td>31</td>
<td>79.7</td>
<td>44.7</td>
</tr>
</tbody>
</table>

*Notes:* Variables are as follows: pCO₂, partial pressure of CO₂; 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 pCO₂ value of 1000 μatm = 101.325 × 10⁻³ kPa.
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 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 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 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 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.

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In 2002, Peter Lake was also amended with nutrients to stimulate primary production. Liquid fertilizer was made from NH$_4$NO$_3$ and H$_3$PO$_4$. 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·m$^{-2}$·d$^{-1}$ (and 2.7 mmol N·m$^{-2}$·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). 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$_2$SO$_4$. Samples were sent to the University of Waterloo stable isotope facility and analyzed using a Micromass Isochrom GC-C-IRMS (Waters, Milford, Massachusetts, USA). POC was concentrated by filtration through precombusted glass fiber filters (GF/F), dried, and acid-fumed to remove excess DI$^{13}$C and concentrated by evaporation. Water in filtrates of the POC samples was acidified. Water in filtrates of the POC samples was acidified. 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...
analysis (Hodgson and Kitchell 1987, Carpenter and Kitchell 1993). Gastric lavage was used to obtain gut items for estimating the isotopic content of invertebrates (Hodgson and Kitchell 1987). Gut contents were pooled into diet categories (zooplankton, Chaoborus, largemouth bass young-of-year, and macroinvertebrates, mainly odonate naïads) 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 ($\%\delta$) 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 isotopic dynamics, provide flux estimates and parameters for modeling analysis, and provide standing stock estimates for models. DIC, $p$CO$_2$, (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 $p$CO$_2$ 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 ($k_{gas}$) 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.
POC, zooplankton, or Chaoborus) from δ13C 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 δ13C of a set of closely interacting carbon pools (e.g., DOC, POC, zooplankton, and Chaoborus). Dynamics of δ13C for the response variables are fitted to the time course of the experimentally manipulated variable, δ13C of DIC. In addition, more slowly changing carbon pools (such as Chaoborus or benthos) are linked to δ13C 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 NaH13CO3 increased δ13C 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, δ13C of DIC returned within a few weeks to values near those observed before treatment. The added DI13C had two immediate fates: loss to the atmosphere and uptake by primary producers. Daily additions helped reduce losses because there was a lower DI13C gradient from lake to atmosphere relative to a single large pulse. Primary producers were effectively labeled, as indicated by the increase in δ13C 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, δ13C 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 δ13C in unlabeled lakes was negligible (Pace et al. 2004).

In Peter Lake 2002, δ13C 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 CO2. Since the entire CO2 pool was utilized, photosynthetic fractionation was near 0. Further, the CO2 depletion also greatly increased the pH. Consequently HCO3 rather than CO2 may have been the substrate for photosynthesis (Rau et al. 2001, Bade 2004).

In all experiments, additions of DI13C were transferred throughout the food web. Labeled carbon ap-
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 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 $\Delta^{13}$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 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 Chaoborus 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 allocloathy of diet items with data on composition of fish diets to calculate allocloathy of fishes.

Allocloathy, 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 allocloathous components. For example, in the experiments without nutrient enrichment (Paul Lake 2001, Peter Lake 2001, Tuesday Lake 2002) POC allocloathy ranged from 0.29 to 0.59, depending on the model. In the nutrient enrichment experiment (Peter Lake 2002), allocloathy 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 allocloathous than POC (Table 2). In the unenriched experiments, allocloathy of DOC ranged from 0.53 to 0.96, depending on the lake and the model. In dystrophic Tuesday Lake, model estimates of DOC allocloathy were consistently high (0.92–0.96). For each model, the lowest estimate of DOC allocloathy occurred in the enrichment experiment (Peter Lake 2002).

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

Allocloathy of zooplankton was similar in Paul Lake and Peter Lake in 2001 (0.22–0.48). In Tuesday Lake, zooplankton were more allocloathous (0.49–0.75). In Peter Lake during enrichment in 2002, zooplankton were supported almost entirely by within-lake primary production, and allocloathy estimates ranged from 0 to 0.12. The same general pattern—more allocloathy
Table 2. Allochthony (proportion of carbon flow from terrestrial sources) for major carbon pools, estimated using three different models.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Year</th>
<th>Method</th>
<th>DOC</th>
<th>Bacteria</th>
<th>POC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul</td>
<td>2001</td>
<td>DIF</td>
<td>0.53±0.01</td>
<td>0.71±0.18</td>
<td>0.38±0.03</td>
</tr>
<tr>
<td>Paul</td>
<td>2001</td>
<td>MAR</td>
<td>0.83±0.02</td>
<td>0.40±0.03</td>
<td>0.50±0.00</td>
</tr>
<tr>
<td>Peter</td>
<td>2001</td>
<td>univar.</td>
<td>0.73±0.01</td>
<td>0.47±0.04</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>Peter</td>
<td>2001</td>
<td>MAR</td>
<td>0.87±0.01</td>
<td>0.55±0.03</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Peter</td>
<td>2002</td>
<td>univar.</td>
<td>0.43±0.01</td>
<td>0.07±0.01</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td>Peter</td>
<td>2002</td>
<td>MAR</td>
<td>0.55±0.10</td>
<td>0.00±0.01</td>
<td>0.57±0.05</td>
</tr>
<tr>
<td>Tues</td>
<td>2002</td>
<td>univar.</td>
<td>0.92±0.02</td>
<td>0.76±0.04</td>
<td>0.59±0.05</td>
</tr>
</tbody>
</table>

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.

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...
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 $\times$ allochthony $\times$ mean areal density of POC (Appendix C). Estimated POC inputs during the experiments were 62 mg·m$^{-2}$·d$^{-1}$ in Paul Lake, 47 mg·m$^{-2}$·d$^{-1}$ in Peter Lake 2001, 35 mg·m$^{-2}$·d$^{-1}$ in Peter Lake 2002, and 104 mg·m$^{-2}$·d$^{-1}$ in Tuesday Lake. Epilimnetic sediments comprise $\sim$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·m$^{-2}$·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, $\sim$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 $\times$ allochthony $\times$ mean areal density of DOC (Appendix C). Estimated input rates during the

| Table 2. Extended. |  |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| **Zooplankton**   | **Chydorus**      | **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       | 0.85              | 0.69              | 0.71              | 0.51              |
| 0.34              | 0.34              | 0.41              | 0.78              | 0.80              | 0.65              |
| 0.41 ± 0.01       | 0.41              | 0.72 ± 0.23       | 0.56              | 0.65              | 0.58              |
| 0.48 ± 0.03       | 0.49 ± 0.04       | 0.49 ± 0.04       | 0.75              | 0.75              | 0.56              |
| 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       | 0.83              | 0.93              | 0.93              | 0.84              |
| 0.75              | 0.75              | 0.72 ± 0.23       | 0.56              | 0.65              | 0.58              |
| 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       | 0.65 ± 0.56       | 0.65 ± 0.56       | 0.65 ± 0.56       | 0.65 ± 0.56       |
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, such as some zooplankton and fishes, but that a substantial component of the benthic carbon trophic pathways are derived from a mixture of sources. 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 δ¹³C 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 terrestrial derived organic C potentially available to consumers in a given lake. Chlorophyll a is proportional to phytoplankton biomass, an index of
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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**LITERATURE CITED**


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.