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Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake ^{13}C addition experiments

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Abstract

Organic substrates for pelagic bacteria are derived from dissolved organic carbon (DOC) in the water column. DOC is a heterogeneous mixture of molecules, some of which are imported from the watershed (allochthonous DOC) and others that are produced by autotrophs within the system (autochthonous DOC). We examined the importance of autochthonous versus allochthonous DOC in supporting the growth of pelagic bacteria by manipulating the ^{13}C content of autochthonous sources in a whole-lake experiment. $NaH^{13}CO_3$ was added daily to two small forested lakes for a period of 42 d, thereby strongly labeling autochthonous primary production. To obtain bacterial carbon isotopes, bacteria were regrown in vitro in particle-free lake water and in situ in dialysis tubes; little difference was found between the two methods. The contribution of autochthonous versus allochthonous carbon to the bacterial biomass was estimated by applying a two-member mixing model using a ^{13}C of −28‰ as the allochthonous end member. The autochthonous end member, which varied over time, was estimated indirectly by several approaches. The bacterial biomass consisted of 35–70% allochthonous carbon. This result confirms the often-stated hypothesis that autochthonous carbon alone does not support bacterial production. On the other hand, autochthonous DOC was preferentially utilized relative to terrestrial DOC. On the basis of ^{13}C measurements, only 13% of the DOC standing stock was of recent autochthonous origin, but it supported 30–65% of bacterial production.

In recent years, the ecological importance of terrestrially derived carbon to lake food webs has received increased attention (Wetzel 2001). The traditional concept of a lake food web, where heterotrophic production is predominantly supported by autochthonous primary production, has been challenged by evidence that suggests that aquatic systems may be net heterotrophic (Cole et al. 1994; del Giorgio and Peters 1994; Jansson et al. 1999). In net heterotrophic systems, respiration exceeds gross primary production (GPP), which implies that heterotrophic organisms must receive external subsidies of organic C.

Often considered recalcitrant to microbial attack, there is accumulating evidence showing that terrestrial C may support aquatic bacterial production under experimental conditions (Tranvik 1988; Moran and Hodson 1994). Indeed, net heterotrophy results in large part from the bacterial metabolism of terrestrial dissolved organic carbon (DOC; Cole et al. 2000). Still, in many lotic systems, bacterial production is correlated with phytoplankton primary production, which suggests that algal-derived C is important for bacterial growth (Cole et al. 1988). Correspondingly, the results of laboratory studies have shown that bacteria can very rapidly utilize organic C of algal origin (Chen and Wangersky 1996). Because bacteria are clearly capable of using both autochthonous and terrestrial organic C, the question remains: which source is more important under in situ conditions? And if allochthonous sources are significantly utilized by bacteria, how important are bacteria in transforming terrestrial DOC into biomass that subsequently supports consumption by phagotrophic organisms?

Often the most direct way to clarify the origin of C sourc-

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es to heterotrophic organisms is by analyzing stable C isotopes (Fry and Sherr 1984; Lajtha and Michener 1994). There are two challenges in using this approach. First, there must be a distinct isotopic difference between aquatic primary production and terrestrial inputs. In lakes where these differences are large enough, isotopic approaches have shown direct contributions of allochthonous C to zooplankton (Meili et al. 1996; Jones et al. 1998). However, in many lakes, these two sources are not isotopically distinct (Cole et al. 2002). Second, for bacteria, it is necessary to analyze their isotopic content by physical separation from nonbacterial particles, by isolating compounds that serve as bacterial biomarkers (Coffin et al. 1990; Pelz et al. 1998; Boschker et al. 1999) or by regrowing bacteria in ambient, but particle-free, sterile water (Coffin et al. 1989).

To make aquatic primary production isotopically distinct from terrestrial inputs, we enriched the entire epilimnion of two, small, soft-water lakes with inorganic ^{13}C over the course of 6 weeks during summer. To obtain the isotopic signature of bacteria in these lakes, we used two approaches—in vitro bacterial regrowth cultures in particle-free lake water (e.g., Coffin et al. 1989) and in situ bacterial regrowth experiments in dialysis tubes (Herndl et al. 1993). The dialysis membrane is semipermeable and allows DOC to move across the membrane. Whereas the batch culture may result in the uncoupling of bacteria and the natural DOC supply, the dialysis tube method has the advantage of making newly produced C available to bacteria during the course of incubation. A potential problem with the dialysis culture approach is the exclusion of DOC of large molecular size.

Materials and methods

Lake description—The experiments were conducted in Paul and Peter lakes at the University of Notre Dame Environmental Research Center near Land O' Lakes, Wisconsin (89°32′W, 46°13′N). Paul and Peter are moderately unproductive, slightly acidic small lakes with surface areas of 16,289 and 24,782 m^2, respectively. The lakes are similar in many aspects. For example, means from May through August of 2001 for Paul and Peter lakes, respectively, were: DOC, 3.7 and 4.6 g C m^{-3}; color, 1.5 and 1.3 m^{-1} at 440 nm; epilimnetic phosphorous concentration, 11 and 10 mg P m^{-3}; and chlorophyll *a*, 4.4 and 4.1 mg m^{-3}. The lakes differ in pelagic food webs, however. Paul Lake has piscivorous fish (largemouth bass, *Micropterus salmoides*), few minnows, and zooplankton dominated by large-bodied cladocerans (*Daphnia* spp. and *Holopedium gibberum*). Peter Lake has extensive minnow populations (e.g., fathead minnows, *Pimephales promelas;* finescale dace, *Phoxinus neogaussay;* and redbelly dace, *Phoxinus eos*), as well as centrarchids (pumpkin seeds, *Lepomis gibbosus*), and zooplankton are smaller. Wetlands dominated by *Sphagnum* spp., ericacious shrubs and sedges, and forest dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula lutea*) and balsam fir (*Abies balsamea*) surround the lakes. Steep-sided lake basins preclude a significant contribution of organic C from littoral macrophytes, which are sparse in these lakes (Carpenter and Kitchell 1993). Both lakes, in the absence of nutrient enrichment, are strongly net heterotrophic, supersaturated in CO_2 (mean pCO_2 for the 2001 field season, Paul Lake 1,039 μatm and Peter Lake 673 μatm), and undersaturated in O_2 (mean saturation, Paul Lake 88% and Peter Lake 94%).

Whole-lake ^{13}C experiment—The present experiment followed many of the protocols and methods in a prior whole-lake ^{13}C addition study (Cole et al. 2002) but differed in several key aspects. In the previous experiment, a single pulse of ^{13}C–dissolved inorganic carbon (DIC) was added to East Long Lake, and the lake was enriched with inorganic N and P. In our experiments, neither lake was fertilized, and a high level of ^{13}C labeling was maintained over time by sustained, daily additions of ^{13}C DIC. After the onset of summer stratification, we increased the ^{13}C of epilimnetic DIC by daily additions of $NaH^{13}CO_3$ (>99 ^{13}C atom %; Isotec) from 11 June through 22 July 2001. At early morning (0600–0800 h), $NaH^{13}CO_3$ dissolved in lake water was discharged at 0.5 m depth by continuous pumping into the epilimnion of each lake from a moving boat. Paul Lake received 15 μmol $m^{-2}d^{-1}$, and Peter Lake received 14 μmol $m^{-2}d^{-1}$, which increased the ^{13}C greatly but increased the total DIC by <1% and did not measurably change pH. Sampling for the isotopic signature of the relevant C pools was made from 28 May through 5 September. During the period for isotope additions, sampling for $DI^{13}C$, $DO^{13}C$, and $PO^{13}C$ was made before the daily $NaH^{13}CO_3$ addition.

Analytical methods—Bacterial abundance was measured using the acridine orange direct count method with 1% formalin preservation (Hobbie et al. 1977). Ten grids with a minimum of 40 cells were counted from duplicate slides. DOC concentration (Pt-catalyzed high-temperature combustion method) was analyzed using a Schimadzu TOC-5000 total C analyzer equipped with an ASI-5000 auto sampler. Samples were GF/F filtered and stored frozen until analyzed. Chlorophyll samples were filtered onto GF/F filters which were frozen and extracted in methanol, and chlorophyll was determined flourometrically (Carpenter et al. 1996).

Carbon isotopes were measured using elemental analysis–isotope ratio mass spectrometry (IRMS; Fry et al. 1992). $DI^{13}C$ was analyzed using a Micromass Isochrome GC-C–IRMS at the University of Waterloo. Analysis of $\delta^{13}C$ for bacteria was carried out using an ANCA-NT system and a 20–20 stable isotope analyzer (PDZ Europa) at the Ecology Department at the University of Lund, Sweden. DOC (after acidification and drying; *see below*), particulate organic carbon (POC), and periphyton were analyzed for isotope content at the University of Alaska Fairbanks stable isotope facility, using a Carlo Erba Elemental Analyzer (NC2500), a Finnigan MAT Conflo II/III interface with a Delta+ mass spectrometer. A series of samples was run in both the Lund and Alaska instruments for comparison of $\delta^{13}C$. The machines had a consistent difference of 0.6‰, which was corrected for. To reduce potential sources of organic C contamination, all glass-fiber filters were precombusted at 450°C, and all membrane filters were prerinsed with deionized water.

Dialysis cultures—To separate bacteria for isotope analyses, bacteria were cultured in dialysis tubes in situ on five occasions for each lake. The initial incubations were done 2 weeks after the onset of isotope additions, and the final incubations were done 2 weeks after the ending of isotope additions. Dialysis tubes (Spectra Por 2; cutoff 12,000–14,000 D, 45-mm flat width) were cut to a length of 2 m to hold 1.2 L. Lake water was collected at noon using a 2-m-long integrated sampler. This water was used for both the dilution cultures and bacterial inocula. Water for the dilution cultures was filtered through 0.2-μm membrane filters (Gelman Supor-200) after prefiltration through glass fiber filters (A/E; Gelman). A grazer-free inoculum was made by filtering whole lake water through a GF/D filter (Whatman). Three replicate dialysis tubes were filled with 1,080 ml of 0.2-μm filtered and 120 ml of GF/D-filtered lake-water (9 : 1 vol : vol). The sealed tubes were incubated in situ, suspended at 0.5-m depth. To confirm the growth of bacteria, the dialysis tubes were sampled for bacterial abundance at the beginning and end of the incubation.

After 48 h of in situ incubation, the contents of the tubes were transferred to dark, acid-washed Nalgene bottles and transported to the lab where the bacteria were harvested by filtering the water through 25-mm GF/F filters (Whatman). The filters were dried at 60°C for 24 h and stored in desiccators for subsequent analysis of ^{13}C.

Batch cultures—For comparison with the dialysis incubations, synchronous batch cultures were run on three occasions for each lake. The batch cultures were done according to a modified version of the method described by Coffin et al. (1989), using water from the same volume as that collected for dialysis cultures: 0.2-μm and GF/D-filtered lake water (9 : 1 vol : vol) was incubated in triplicate glass bottles (1.2 L) in darkness, at in situ temperature, for 48 h. The bacteria were then harvested by filtering the contents of each bottle through a 25-mm GF/F filter, which was later analyzed for stable C isotope ratios.

Methodological issues—We checked the possibility that DOC might leak from the cellulose of the dialysis tubes and thereby affect bacterial growth. Before filling, the dialysis tubes were thoroughly rinsed and soaked in deionized water for 1 h. This procedure was repeated five times, and, after, rinsing, no measurable DOC leaked from the tubes within 48 h (one-way ANOVA, $P = 0.88$, $n = 5$), compared with DOC concentration in water with no dialysis membrane. To affirm that no other substance that affected bacterial growth leaked from the tubes, the bacterial abundance was measured in water with and without dialysis membranes over 72 h, and no differences were observed (repeated-measurements ANOVA, $P = 0.30$, $n = 6$). The relation of water volume to the amount of dialysis membrane used in the tests was the same as that in the in situ bacterial incubations. Inspection with epifluorescent microscopy revealed that the GF/D filtrate used as inoculum was essentially free of bacterial grazers but contained most (93% on average) of the indigenous bacteria.

One additional 0.5-m dialysis tube, with water filtered as for the dialysis and batch cultures, was incubated to evaluate the possibility that phytoplankton growth occurred in the tubes. Chl *a* was measured on samples taken at the start and termination of incubation. Another dialysis tube, which initially contained deionized water, was incubated parallel to the tubes used to measure bacterial growth, to determine the diffusion of lake water DOC into tubes over 48 h. Samples for the measurement of DOC concentrations were taken from the tubes at the beginning and end of incubation, as well as from the lake.

To determine the rate of diffusion across the dialysis membrane, triplicate dialysis tubes containing deionized water were incubated in either a NaCl or glucose solution or in a solution with DOC (<0.2 μm) from a phytoplankton culture. Conductivity or DOC concentration ([DOC]) on the inside and outside of the tubes was measured over time. The exchange should conform with an exponential rise to a maximum according to the model

$$C_{in} = C_{out} \times (1 - e^{-kt}) \quad (1)$$

where C_{in} is conductivity/[DOC] inside the dialysis tube, C_{out} is conductivity/[DOC] outside, k is the diffusion coefficient, and t is time. This equation is an expression of diffusion and follows Fick's law.

Sampling for stable isotope pools—Although a wide variety of stable isotope pools were monitored over the course of the study, only the pools relevant to bacterial production are discussed in the present article. These include samples for DIC, DOC, POC, bacteria and peripyton. Samples for $DI^{13}C$, $DO^{13}C$, and $PO^{13}C$ were collected from 0.5 m depth at daily intervals. DIC samples were collected directly in 60-ml serum bottles, acidified with 5 mol L^{-1} H_2SO_4 to pH $<$ 2, and stored in darkness for later ^{13}C analysis. POC was collected by filtration through Whatman GF/F filters, after prefiltration at 153 μm to remove zooplankton, and dried at 60°C. DOC was collected as the filtrate of the GF/F filtration, and samples of 900 ml were acidified with 1 mol L^{-1} HCl to pH <2 and dried to a residue at 60°C (Cole et al. 2002). Periphyton was collected by suspending acid-cleaned tiles at 0.5 m. Periphyton that grew on the tiles were scraped off weekly and dried at 60°C.

Results

Dialysis and batch cultures—Filtration of the water used for dialysis incubations reduced the Chl *a* concentration to ~1% of the concentration in the unfiltered lake water. During the course of incubation, Chl *a* decreased further, by ~50%, to an average of 0.03 μg L^{-1} (SD 0.02, $n = 6$). Thus, the growth of phytoplankton in the tubes was insignificant, and phytoplankton C did not significantly contaminate the bacterial regrowth cultures. Microscopic inspection revealed no presence of cyanobacteria in either the inocula or the incubated cultures.

On the basis of direct counts, ~90% of the cultured bacteria were retained on the GF/F filters used to harvest cells from the incubations. The dialysis tubes contained 450% more organic C at the end of the incubation than at the start. This increase corresponds fairly well with the simultaneous increase in bacterial abundance of 390%. The cells were con-

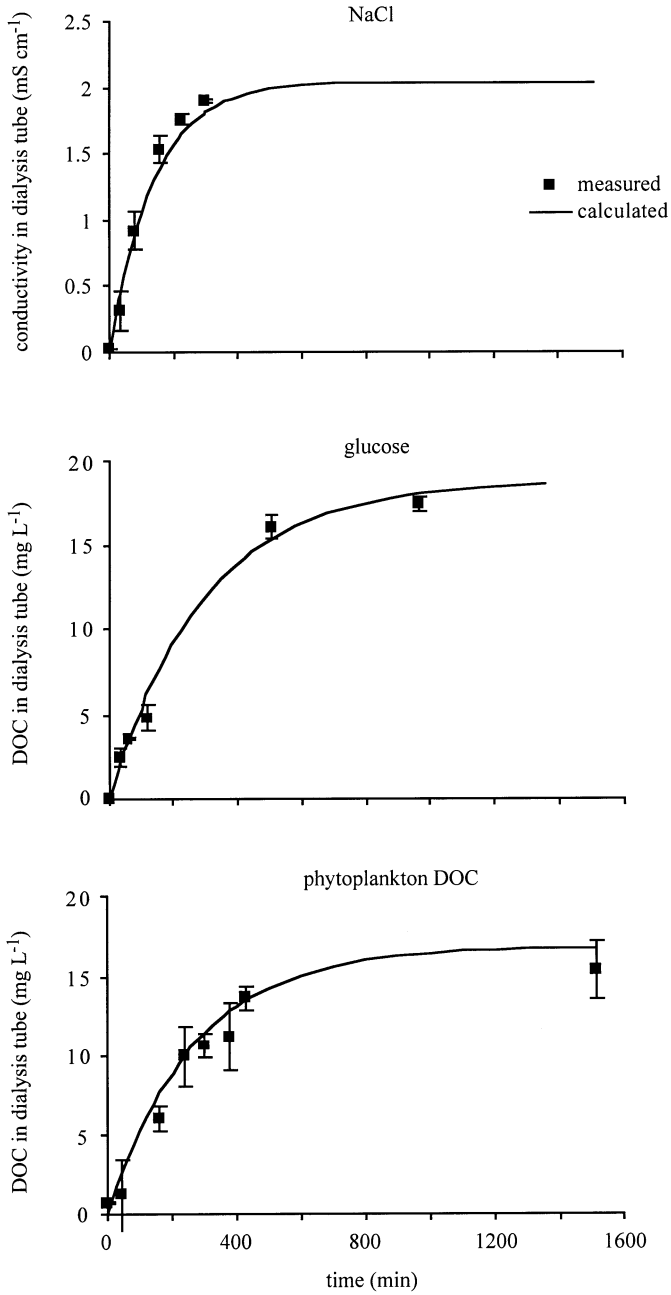

Fig. 1. Measured and calculated conductivity or DOC concentration in the dialysis tubes over time from exchange experiments with NaCl, glucose and phytoplankton derived C. Error bars show ±1 SD.

siderably larger (visual microscope inspection; no data) at the end of the incubations; therefore, the increase in bacterial C was larger than was suggested by bacterial numbers only. The C : N ratio of the bacterial samples averaged 5.8 (SD 1.3, $n = 9$), which is consistent with the expectation that the organic matter was primarily composed of bacterial cells. The limited time for bacterial regrowth (48 h) was possibly too short for bacterial ^{13}C to reach equilibrium. Because bacterial C in the inoculum equaled only 22% of the harvested bacteria and the added bacteria at least partly turned over

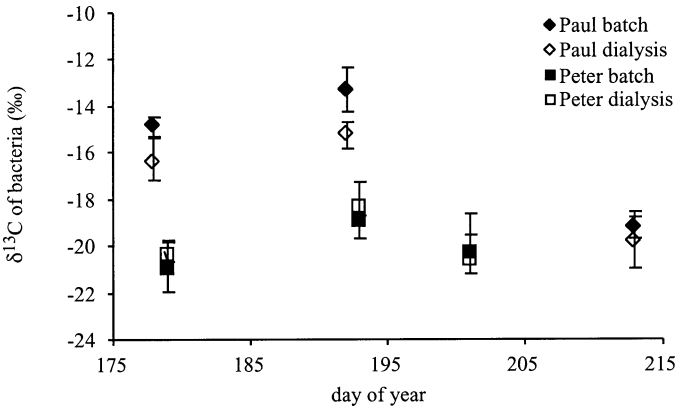

Fig. 2. C isotope signatures of bacteria from dialysis (open symbols) and batch incubations (filled symbols) on three dates in Paul (diamonds) and Peter (squares) lakes. Sample size, $n = 3$. Error bars show the range of isotope values.

during incubation, this did not significantly influence the ^{13}C level of the harvested bacteria.

The DOC concentration in the control tubes that initially contained deionized water was 59% and 57% of that in the lake after 48 h of incubation in Paul and Peter lakes, respectively, whereas 92% of the phytoplankton-derived C used in the exchange experiment was able to diffuse through the membrane. Hence, a greater fraction of phytoplankton DOC than of ambient DOC can enter the tubes.

In the experiments to assess the exchange over the dialysis membrane, half the volume of the dialysis tube was replaced in 1.6, 3.0, and 3.2 h for NaCl, glucose, and phytoplankton DOC, respectively. The measured values of conductivity and DOC over time were described well by Eq. 1 ($r^2 = 0.99$, $P < 0.05$ for NaCl; $r^2 = 0.99$, $P < 0.05$ for glucose; and $r^2 = 0.98$, $P < 0.05$ for phytoplankton DOC; Fig. 1).

When comparing parallel dialysis incubations and batch cultures (Fig. 2), the bacterial signatures were similar and overlapping (one-way ANOVA, $P = 0.49$). This suggests that the DOC cutoff of the dialysis tubes (12,000–14,000 D) did not result in enhanced bacterial dependence of phytoplankton-derived DOC.

^{13}C dynamics of major C pools—DI^{13}C values before the addition of $NaH^{13}CO_3$ were around −12 and −8‰ in Paul and Peter lakes, respectively (Fig. 3). DI^{13}C responded quickly to $NaH^{13}CO_3$ additions and leveled off at ~25‰ after 2 weeks of daily additions in Paul Lake ($\Delta DI^{13}C$ = 37‰). After another 3 weeks, when additions were terminated, the signature decreased, and levels returned to approximately prespike values after 2 weeks. In Peter Lake, DI^{13}C dynamics were similar (Fig. 3), but the response of the DIC pool to the added label was somewhat slower than that in Paul Lake. DI^{13}C peaked at +29‰ ($\Delta DI^{13}C$ = 37‰).

The PO^{13}C dynamics followed that of DI^{13}C (Fig. 3). The good correlation between DI^{13}C and PO^{13}C ($r^2 = 0.89$, $P < 0.05$ in Paul Lake and $r^2 = 0.83$, $P < 0.05$ in Peter Lake) indicates that phytoplankton became enriched in response to the ^{13}C manipulations. Because POC is likely to contain some C of terrestrial origin that would move the POC sig-

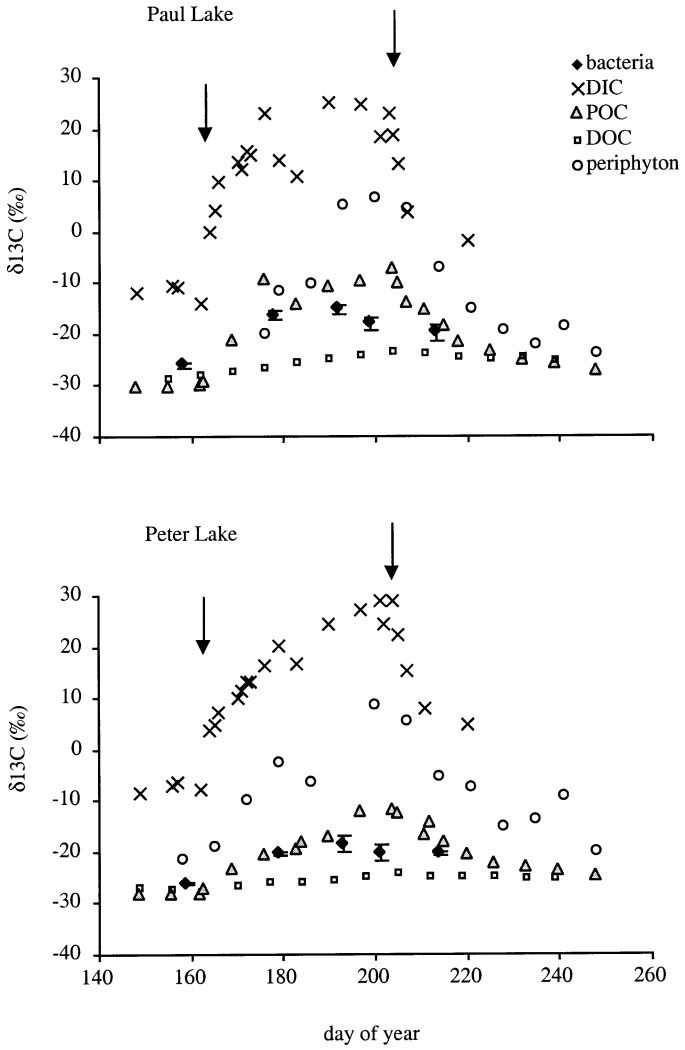

Fig. 3. $\delta^{13}C$ values of bacterial biomass in dialysis tubes, DIC, POC, DOC, and periphyton in Paul and Peter lakes. Error bars show ±1 SD, and arrows denote the first and last day of isotope additions.

nature toward terrestrial values (i.e., lowering it during the period of ^{13}C manipulations), the true phytoplankton signature was equal to or greater than that of POC during the period of isotope manipulation. $PO^{13}C$ increased less than $DI^{13}C$ ($\Delta PO^{13}C$ = 23‰ and 16‰ in Paul and Peter, respectively), which demonstrates that not all POC was the result of recent photosynthesis by phytoplankton.

The preaddition $\delta^{13}C$ values of the DOC were −28.7‰ and −27.2‰ respectively, similar to reported values for terrestrially produced organic C (Lajtha and Michener 1994). The slow increase in the DOC signature (Fig. 3) probably resulted from losses of labeled DOC by phytoplankton and heterotrophs. Periphyton was also labeled as a result of the $NaH^{13}CO_3$ additions and was enriched, compared with POC, by, on average, 9‰ and 12‰ in Paul and Peter, respectively (Fig. 3).

C sources for bacteria—The pattern of C isotope signatures in the two lakes was roughly similar (Fig. 3). The dynamics of bacterial ^{13}C resembled those of $PO^{13}C$, which indicates that bacteria were utilizing some C of phytoplankton origin. However, it is apparent that bacteria were not entirely dependent on newly produced phytoplankton C, given that the bacteria were consistently lighter than POC. Bacteria were enriched in ^{13}C compared with DOC, both before (by 1.4‰ and 1.2‰) and during ^{13}C additions.

The relative contribution of allochthonous C to bacterial biomass was calculated using a two–end member mixing model

$$\%\ \text{allochthonous C} = \frac{(\delta^{13}C_{\text{bacteria}} - \delta^{13}C_{\text{autochthonous}})}{(\delta^{13}C_{\text{allochthonous}} - \delta^{13}C_{\text{autochthonous}})} \times 100$$

The allochthonous end member was estimated at −28‰, after Lajtha and Michener (1994), and was similar to preexperiment DOC levels and direct measurements of terrestrial vegetation in the area (mean −29‰, SD 1.7, n = 10). Because we could not physically separate all phytoplankton from POC of other origin, we used multiple estimates of the autochthonous end member, which changed dramatically during the course of the experiments.

The first approach assumed that POC was 100% autochthonous carbon and that the $PO^{13}C$ value was the same as that of phytoplankton. To the extent that POC was of terrestrial origin, this approach underestimates the ^{13}C content of phytoplankton. This approach yielded the lowest contribution of allochthonous C to bacterial biomass (Fig. 4). Means (and ranges) for four dates in each lake were 35% (26–51%) and 37% (22–54%) in Paul and Peter lakes, respectively. The assumption that bacterial C is ~2‰ depleted with respect to their substrate (see ''Discussion'') increases the mean estimates to 48% and 43% allochthonous C.

The second approach assumed that regrown periphyton had the ^{13}C of phytoplankton. Because periphyton tend to deplete local CO_2 concentrations, isotopic fractionation tends to be lower than that of phytoplankton, and this approach may overestimate the ^{13}C of phytoplankton. This approach gave estimated allochthonous contributions to bacterial C of 54% (21–71%) and 70% (66–79%) (Fig. 4).

The third approach estimated the ^{13}C of phytoplankton from the ^{13}C content of the CO_2 moiety of the DIC and an assumed or computed fractionation factor (ε_p) from CO_2 to phytoplankton. In this last approach, we estimated ε_p in two ways. First, we used the approach of Laws et al. (1995), modeling the fractionation factor (ε_p) by assuming a linear dependence on the ratio of μ (the specific growth rate of the phytoplankton) and CO_2 concentration ([CO_2]). The concentration of $^{13}CO_2$ was calculated according to the method of Mook et al. (1974) from measured temperature, $DI^{13}C$, DIC, and pCO_2. μ was calculated from pelagic GPP, as estimated by continuous O_2 measurements (Cole et al. 2000) and phytoplankton biomass, assessed by multiplying Chl *a* concentrations by a C : Chl *a* factor of 40. This approach gave average ε_p values for Paul of −20.1 (SD 1.0, n = 5) and for Peter of −18.6 (SD 1.7, n = 5), which are in agreement with physiological data for marine phytoplankton. However, these estimates of ε_p yielded values of the autochthonous end member that, on several dates, were more ^{13}C depleted than the measured bacterial values. This implies that there

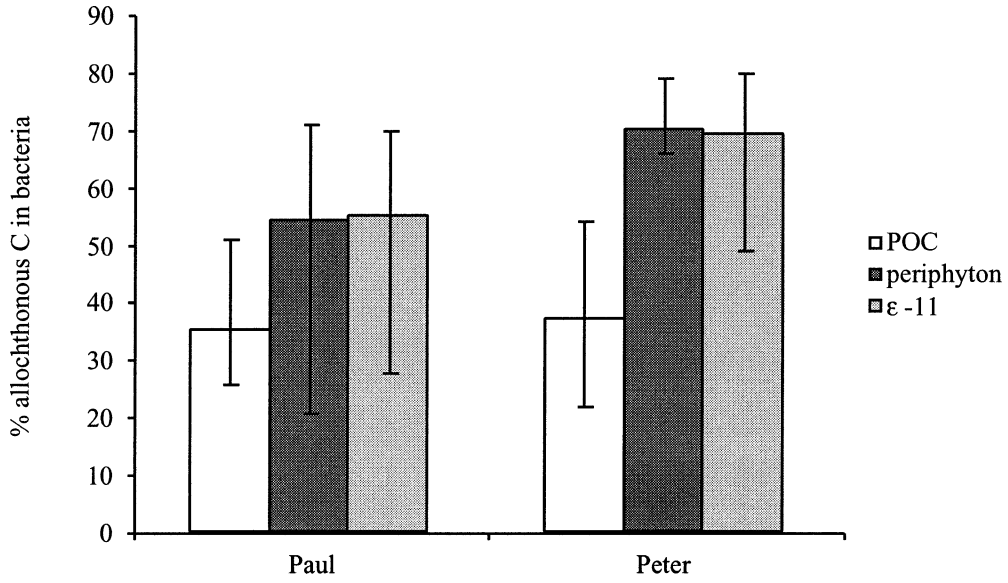

Fig. 4. Calculated values of the relative contribution of allochthonous C to bacterial biomass. $PO^{13}C$, $\delta^{13}C$ of periphyton, and $^{13}CO_2$ −11‰ were used as the isotope signature of the autochthonous end member (see text). The bars show mean values, and error bars confine the range.

was either a more ^{13}C-enriched pelagic substrate that we did not measure or, more likely, that this estimate of ε_p is too large. Using values only from the dates when the allochthonous and autochthonous end members bracket the bacterial signature, the allochthonous contribution to bacterial C would be 51% (42–58%) and 69% (65–76%).

We also set ε_p at a single value of −11.5‰ in Paul Lake and −11.4‰ in Peter Lake based on time-dynamic models for this experiment (Pace et al. 2004). In brief, these models used the aqueous $^{13}CO_2$ time series to predict $PO^{13}C$ by fitting parameters for photosynthetic fractionation (ε_p) and the proportion of POC that is allochthonous. Means and ranges using these values of ε_p were 55% (28–70%) and 69% (49–80%) allochthonous C (Fig. 4). These values agree well with the approach in which the ^{13}C of periphyton was used as the autochthonous isotope signature.

Bacteria were enriched in ^{13}C compared with bulk DOC, which suggests that the more labeled, autochthonous DOC was utilized preferentially relative to its representation in the DOC pool (Fig. 3). The relative contribution of allochthonous C to the DOC pool was calculated using the same two–end member mixing model as for bacterial biomass, exchanging $\delta^{13}C_{bacteria}$ for $\delta^{13}C_{DOC}$ and applying the −11‰ fractionation factor to assess $\delta^{13}C_{autochthonus}$. Comparing the origin of the DOC pool and bacterial biomass over the season (Fig. 5), it appears that, although DOC of autochthonous origin was a small part of the total DOC pool, it was preferentially utilized by bacteria over DOC of terrestrial origin. The bacterial preference for autochthonous C can be expressed as

$$P = \frac{(\text{autofrac}_{\text{bacteria}}/\text{allofrac}_{\text{bacteria}})}{(\text{autofrac}_{\text{DOC}}/\text{allofrac}_{\text{DOC}})}$$

where autofrac is the fraction of bacterial C and standing stock DOC that is autochthonous and allofrac is the fraction that is allochthonous. The average P for Paul and Peter lakes, respectively was 7.0 (SD 5.2) and 4.2 (SD 1.0). $P = 1$ would correspond to no preference, and $P < 1$ would imply a preference for allochthonous C.

Discussion

Bacterial incubations—Bacterial ^{13}C values in aquatic environments have been determined through analyses of bacterial cells or CO_2 respired from bacteria grown in bioassay experiments (Coffin et al. 1989; Hullar et al. 1996; Waichman 1996). Alternative methods for estimating bacterial ^{13}C include extractions of bacterial biomarkers such as nucleic acids (Coffin et al. 1990), amino acids from the bacterial peptidoglycan cell wall (Pelz et al. 1998), or polar lipid-derived fatty acids (Boschker et al. 1999). The extraction methods measure the response of in situ bacteria and are not affected by potential artifacts from culture conditions. However, there are problems with the biomarker methods, such as the coextraction of unwanted compounds (Coffin et al. 1990), differences in isotope signatures of the biomarker and whole cells (Pelz et al. 1998), low recovery of bacteria and the biomarker (Coffin et al. 1990), and labor-intensive procedures. The bioassay method avoids these problems but may result in a decoupling of bacteria from their natural substrates.

The objective of incubating bacteria in dialysis tubes in situ was to obtain bacterial biomass that grew on a natural suite of organic C substrates. The results of the various methodological tests we used suggested that this was the case. Tests showed that most organic C originating from phytoplankton (pDOC) and ambient DOC could effectively exchange over the membrane. A greater fraction of pDOC (92%) than ambient DOC (58%) could move across the membrane within 48 h. The similar isotope signatures of bacteria from parallel dialysis and batch incubations (Fig. 1)

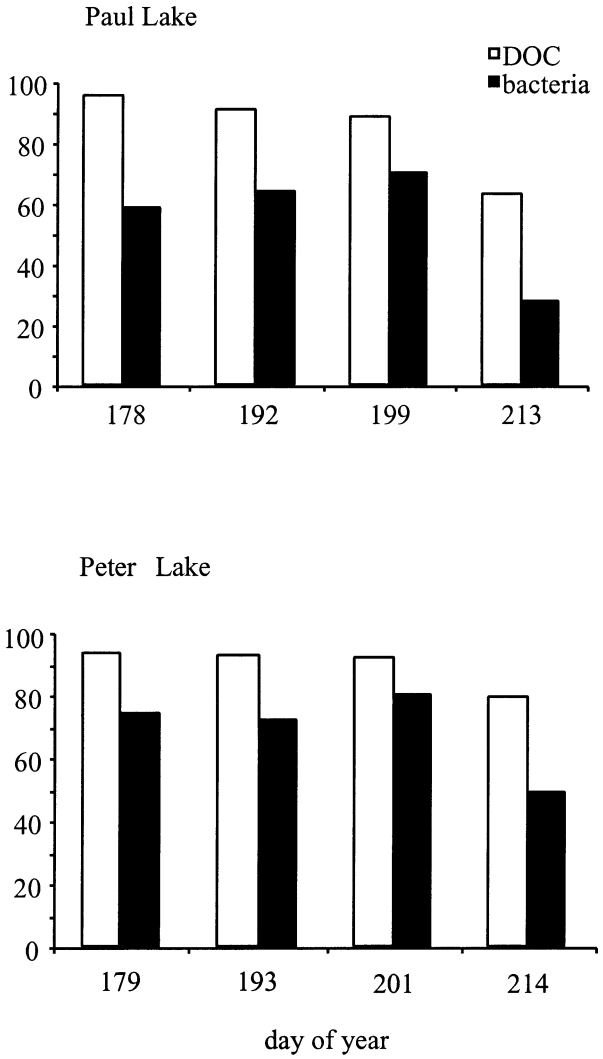

Fig. 5. The relative contribution of allochthonous C to the DOC pool and bacterial biomass at four different dates. The autochthonous end member was estimated under the assumption of a constant fractionation from CO_2 to phytoplankton of −11‰.

suggested that this did not cause an overestimation of the bacterial dependence on pDOC in the dialysis tubes. However, we cannot exclude the possibility that the similarity was a result of counteracting effects of DOC cutoff and batch effects.

The exchange of DOC through the dialysis membrane was rapid, so that the DOC within the tubes resembled that of the lake. The short exchange time for NaCl shows that chemical conditions in the dialysis tubes were similar to those in the lake.

The low and decreasing Chl *a* concentrations and the concurrent increase in bacterial abundance during incubation imply that the measured isotope values of POC in the tubes can be attributed to bacteria. Moreover, C : N ratios of the particulate organic matter in the tubes were within the reported range of C : N ratios for bacteria (Vrede et al. 2002).

Bacterial C sources in a forested lake—The addition of inorganic ^{13}C and its subsequent uptake by phytoplankton in the study lakes made it possible to distinguish between newly produced pDOC and the unlabeled fraction of DOC that may consist of subpools of terrestrially derived and ''older'' DOC made by photosynthesis prior to the addition of ^{13}C. All three approaches for the autochthonous end member suggested that pelagic bacteria utilized both allochthonous and autochthonous sources; however, the calculated relative importance of the two sources differed with the approach. Replacing a measured phytoplankton signature with $PO^{13}C$ yielded the lowest contribution of allochthonus C to bacterial biomass. The ratio of POC to chlorophyll in the lakes, 123 (51–318) and 128 (77–192), respectively, was much higher than C : Chl *a* in pure phytoplankton, which confirms that POC was not entirely composed of phytoplankton. As a consequence, this approach overestimates the contribution of recent phytoplankton production to bacteria. Periphyton $\delta^{13}C$ represents a theoretical maximum value of phytoplankton C, because periphyton fractionate equally or less than phytoplankton (France 1995). Therefore, adopting periphyton $\delta^{13}C$ as the autochthonous end member gave the largest contribution of allochthonous C to bacterial biomass. The periphyton layers established in a week were thin, and isotope fractionation caused by diffusion could be assumed to be similar to that for free-living phytoplankton. Therefore, the isotope values in the periphyton might have reflected those of phytoplankton. The average fractionation from CO_2 to periphyton was −7.4‰ and −9.7‰ in Peter and Paul lakes, respectively.

Under the assumption that $PO^{13}C$ can be predicted from $^{13}CO_2 - \varepsilon_p$ (the algal signature) and the relative proportion of autochthonous and allochthonous C (−28‰) in POC, Pace et al. (2004) concluded that ε_p values of −11‰ give the best fit to the $PO^{13}C$ data from the present study. Applying these estimates to the mixing model gives a bacterial dependence on allochthonous C that was very similar to that produced by using periphyton as the autochthonous end member. The physical separation of algae in a nearby oligotrophic lake showed ε_p to average −12‰ (Bade pers. comm.), which supports the use of the ε_p achieved from modeling. A low value of ε_p was also seen by Cole et al. (2002) in a nearby lake with similar chemistry.

Before the manipulation of ^{13}C began, bacteria were enriched compared with both DOC (by 1.4‰ and 1.2‰ in Paul and Peter lakes, respectively) and POC (by 4.2‰ and 2.2‰). These values are well within the reported range of values for uptake fractionation between substrate and bacterial cells (Coffin et al. 1989, 1990; Hullar et al. 1996). Fractionations from −0.6‰ to +2.4‰ have been reported, with most values in the positive range, ~2‰ (Blair et al. 1985; Coffin et al. 1989, 1990; Hullar et al. 1996; Pelz et al. 1998). When working in systems with end members of very distinct isotope signatures, such as estuarine and coastal environments and the ^{13}C-enriched lakes examined in the present study, the potential fractionation between bacteria and their substrates is relatively small compared with differences in the source materials and can be ignored.

If bacteria utilized the DOC pool nonselectively, they would have had the same (±2‰, see above) ^{13}C signature as DOC. However, bacteria were consistently enriched compared with DOC (Fig. 3), which suggests that freshly pro-

duced phytoplankton C was selectively utilized (Fig. 5). Moreover, the increasing ^{13}C signature of DOC (Fig. 3) shows that part of phytoplankton C remained in the DOC pool. Thus, bacteria did not deplete the entire supply of DOC of phytoplankton origin. Whether the labeling of the DOC pool is a result of continuous accumulation of phytoplankton C or a steady-state phenomenon is uncertain, so the actual fraction of DOC that is algal is not well known.

The extent to which bacteria utilize autochthonous versus allochthonous C for growth is presumably a function of supply, lability, and quality of the two sources. In clear and large lakes, inputs of organic C from the plankton may be most significant, but, in small and in humic lakes, the dominant source is likely to be allochthonous C (Tranvik 1998). Because terrestrially derived C has already undergone some degradation and transformation before entering the pelagic environment, it is potentially less labile than substrates originating from recent algal production. Allochthonous DOC is commonly considered to be recalcitrant to bacterial utilization because of its relatively high molecular weight and aromaticity (Moran and Hodson 1994) and qualitatively deficient because of its low content of nitrogen and phosphorous (Wetzel 2001). However, ectoenzymes can make polymeric DOC available as a source of energy and nutrition to bacteria (Chrôst 1990). Moreover, UV radiation increases the availability of terrestrial DOC to bacteria (Anesio et al. 2000). pDOC is characterized as being mainly of low molecular weight and highly available for bacterial uptake (Chen and Wangersky 1996). Yet a substantial part of pDOC can also be recalcitrant (Sundh 1992; Chen and Wangersky 1996). UV exposure seems to have little effect in promoting the decomposition of pDOC (Thomas and Lara 1995) and can even decrease biological availability in the presence of humic acids (Tranvik and Kokalj 1998). Correspondingly, in a whole-lake ^{14}C-addition experiment, Schindler et al. (1992) found that, although most pDOC was degraded quickly, a substantial part remained in the water for >1 yr without decreasing in concentration. According to Sundh (1992), the slow turnover of high-molecular-weight pDOC contributes to the accumulation of refractory DOC in the pelagic zone of some lakes.

The bacterial isotope values appeared to level off after day 180, whereas the periphyton signature, and, to a lesser degree the $PO^{13}C$, continued to get heavier until day 200 (Fig. 3). Although the bacterial observations are limited in time, the pattern suggests that recent phytoplankton production was used less as the experiment progressed. This trend cannot be explained by a decreased supply of autochthonous C in relation to allochthonous C. It is possible, however, that the lability of allochthonous C increased as a result of extended periods of solar radiation.

Through the whole-lake manipulation of ^{13}C, along with the isolation of the bacteria in regrowth experiments for isotope analysis, we could study the bacterial utilization of autochthonous versus allochthonous C in situ. Our results provide some important new insights regarding bacterial C sources and the origin and utilization of the DOC pool. First, pelagic bacteria in Paul and Peter lakes consisted of 35–70% allochthonous organic C. Although there was a wide range in our estimates of the percentage of allochthony, the conclusion that allochthonous organic C is heavily utilized by bacteria is robust and in accordance with an emerging consensus that autochthonous C alone does not satisfy bacterial C demand. Second, results from batch regrowth experiments in the laboratory and dialysis incubations in situ were consistent, which implies that the decoupling of bacteria from their natural substrates in batch cultures may not be a major problem when measuring bacterial isotopic signatures. Finally, DOC of recent autochthonous origin is a small part of the total DOC pool (Fig. 5) but is preferentially utilized by bacteria over DOC of terrestrial origin. However, bacteria do not simply utilize all autochthonous C that is produced, since some of the labeled C remains in the DOC pool.

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