Limnol. Oceanogr., 48(6), 2003, 2200–2207

Using the hidden isotopic heterogeneity in phyto- and zooplankton to unmask disparity in trophic carbon transfer

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Abstract

In this study, we show that natural phototrophic populations can be probed individually for their in situ $\delta^{13}C$ signature by linking fluorescence-activated cell sorting and isotope-ratio mass spectrometry (IRMS) using in-line pyrolytic methylation. This novel methodology greatly improved the resolution in discriminating and tracing the differential carbon (C) pathways at the base of the pelagic food web in the cyanobacteria-dominated Lake Loosdrecht (The Netherlands). Our analysis revealed the co-occurrence of phytoplankton taxa differing by 6–10‰ in $\delta^{13}C$. Predominant micro- and mesozooplankton species reflected this difference as the result of preferential grazing and/or selective digestion. Flow cytometric (FCM) retrieval of phytoplankton $\delta^{13}C$ signatures, applied in conjunction with ^{13}C-carbonate labeling, also enabled an assessment of in situ population-specific growth rates. Diatoms and green algae exhibited up to ninefold higher growth rates than those for cyanobacterial species. The coexistence of phytoplankton populations widely differing in $\delta^{13}C$, standing stock, and turnover time has important implications for the interpretation of C transfer in pelagic food webs. Our approach disclosed a disproportional impact on trophic cascades by numerically minor phototrophs that otherwise would have gone unnoticed. Despite the abundance of cyanobacterial-derived C, the zooplankton largely rely on eukaryotic algae for growth. Rotifers take a central position in passing on this algal C to the cyclopoid copepod populations in the lake. The bosminid-dominated cladoceran population uses both the cyanobacterial- and algal-derived C in approximately equal shares.

Stable isotope analysis is increasingly being used for unraveling the structure of food webs and has proven to be indispensable in documenting and understanding trophic shifts in response to stress (Stapp et al. 1999; Vander Zanden et al. 1999). In aquatic systems, however, this tool often lacks the resolution to pinpoint trophic interrelationships at the population level since only crude proxies for the ^{13}C signal of phyto- and zooplankton are established by the available purification protocols (e.g., density-gradient centrifugation) (Hamilton and Lewis 1992), differential filtration (Fry and Wainright 1991; Del Giorgio and France 1996; Bouillon et al. 2000), chlorophyll *a* (Chl *a*)/phytol, and lipid extraction (Laws et al. 1995; Volkman et al. 1998; Bidigare et al. 1999; Pancost et al. 1999; Tolosa et al. 1999; O'Leary et al. 2001). Thus, population-specific isotopic information is lost by the indiscriminate lumping of species. Moreover, the proxies are prone to an unknown degree of isotopic dilution by co-collected but nonrelated materials. As a consequence, information on C transfer in food webs could very well be obscured or biased. This prompted us to examine the feasibility of $\delta^{13}C$-signature analysis by compound-specific isotope-ratio mass spectrometry (GC-combustion-IRMS: Hayes et al. 1989; Pel et al. 1997) of algal fractions selected from natural phytoplankton employing FCM cell sorting. Pigment fluorescence normally provides sufficient resolution in FCM to distinguish predominant populations (Hofstraat et al. 1991; Becker et al. 2002). However, only minute amounts of cell material can be collected by flow sorting within a reasonable time frame (100–200 ng total cells C). Therefore, a procedure was developed to achieve a direct transfer into an IRMS-linked gas chromatograph (GC) of the cellular fatty acids (FAs: C_{12}-C_{22}) volatilized from small amounts of sorted phytoplankton fractions by means of in-line pyrolytic methylation (Py-GC-IRMS; Pel et al. unpubl. data). In this study, defined, mixed algal cultures were used to evaluate the accuracy of the methodology in retrieving population-specific FA profiles and $\delta^{13}C$ signatures, showing that the isotopic signals of the species constituting such mixtures could be reproduced with a precision $\leq$0.4‰.

The tracking of C transfer in food webs using the naturally existing differences in ^{13}C abundance among primary producers relies on a detailed knowledge of the isotopic signatures of these sources and their sinks (Peterson and Fry 1987). Phytoplankton are one of the major sources of organic matter in aquatic systems and will usually comprise the isotopic signals of a variety of different phototrophic species. Environmental conditions and species-related physiology are known to affect the stable C isotope composition of phytoplankton (Gu and Schelske 1996; Burkhardt et al. 1999; Eek et al. 1999). Moreover, the existence of an inverse relationship between the growth rate of algae and the extent of discrimination against $^{13}CO_2$ in photosynthesis has been implied in a number of studies (Fry and Wainright 1991; Kopczyñska et al. 1995; Laws et al. 1995; Popp et al. 1998). Thus, the heterogeneity in $\delta^{13}C$ that may exist within the phytoplankton C pool itself because of a multispecies composition will not easily be shown by the present sampling and cleanup procedures. Similar arguments apply to the zooplankton isotopic signatures derived in the past. In the majority of studies, zooplankton specimens have been lumped on filters for bulk ^{13}C analysis (e.g., Del Giorgio and France 1996; Bouillon et al. 2000), hence obscuring the potentially

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Acknowledgments

We thank R. D. Gulati for his advice in the zooplankton experiments and comments on earlier versions of the manuscript. We also wish to thank two anonymous reviewers for their helpful comments regarding this manuscript. This is publication 3179 of the Netherlands Institute of Ecology, Center for Limnology, KNAW.

present isotopic diversity. In this contribution, we will show how the existing natural differences in isotopic signatures of functional algal groups can be used directly in studies of C transfer to (secondary) consumers and events of apparent preferential grazing. The monitoring of phytoplankton and zooplankton $\delta^{13}C$ signatures in Lake Loosdrecht showed that despite the overwhelming abundance of cyanobacterial species in this eutrophic lake, higher trophic levels depended heavily on the local production by numerically minor eukaryotic algae.

The technique we present is, to our knowledge, the most specific and sensitive method presently available for determining the stable C isotopic composition of the pelagic microflora and microfauna.

Materials and methods

Study site—Lake Loosdrecht is a shallow eutrophic seepage lake, the water column of which is generally completely mixed by the prevailing western winds ($A = 9.8$ km^2; $\bar{z} = 1.85$ m; $P_{total} = 40$–60 μg L^{-1}; and $N_{total} = 1.4$–1.9 mg L^{-1}). Littoral communities are poorly developed in the lake, and benthic producers are absent. The phytoplankton show a perennial predomination by filamentous prokaryotic species (trichome lengths up to 350 μm; mean lengths, 90–120 μm; diameters, approximately 2–3 μm), mainly oscillatorioid cyanobacteria belonging to the genus *Limnothrix* and a lower share of *Prochlorothrix* spp., reaching total concentrations of 1.75–2.25×10^5 trichomes ml^{-1} in summer (Pel et al. unpubl. data). *Oscillatoria* cf. *limnetica* (strain MR1, Centre for Limnology) isolated in the past as one of the prominent filamentous cyanobacterial species present in Lake Loosdrecht (Rijkeboer et al. 1992), has recently been reclassified in a taxonomic study by Suda et al. (2002) and is now considered a representative of the genus *Limnothrix.* The second most abundant filamentous population closely resembles *Prochlorothrix hollandica* in its FA profile (Pel et al. unpubl. data). *P. hollandica,* originally isolated from the lake in 1986 (Burger-Wiersma et al. 1986), does not contain phycobilins and has little Chl *b* (Matthijs et al. 1994). The population density of eukaryotic algae, mainly comprising diatom and green algal species, is relatively low and mostly well below 15×10^3 ml^{-1}. The seston (20–25 mg L^{-1} dry wt) is characterized by a low phytoplankton : detritus ratio of ~0.3, and the euphotic depth is 0.7–1.0 m (Gons et al. 1992*b*).

The zooplankton composition is typical of eutrophic lakes: within the Crustacea, small-bodied cladocerans dominate, mainly *Bosmina* spp. and cyclopoid copepods, and an abundance of rotifers, predominantly *Keratella, Anuraeopsis, Filinia,* and *Polyarthra* spp. (Gulati 1990; Ooms-Wilms et al. 1999). The annual means of crustacean densities may vary more than twofold in the lake, between approximately 400 and 940 individuals (ind.) L^{-1} (*see* Gulati et al. 1992 for the densities recorded in 1981–1991). From about one half to two thirds (45–69%) of the crustaceans in the lake on the average are cyclopoid copepods (Gulati et al. 1992). Apart from a short period in spring with low densities of *Daphnia cucullata* (<3 ind. L^{-1}), large-bodied daphnids are absent in this lake (DeMott et al. 2001*a*). Littoral cladocerans such as *Chydorus sphaericus* have also rarely been encountered in the last decade (Gulati pers. comm.).

Flow cytometry and cell sorting—FCM analyses and cell sorting of phytoplankton groups were conducted with a Epics Elite (Coulter) cell sorter equipped with an ion argon laser (excitation, 488 nm; 30 mW of power). The instrument optics were set to collect the red fluorescence of Chl *a* (bandpass, 675 ± 20 nm) and the orange fluorescence of phycoerythrin (bandpass, 637 ± 10 nm). Sort criteria were defined by drawing amorphous gates in the bivariate dot plots of red and orange fluorescence. Typically, three major cell clusters were resolved using red and orange fluorescence emission in FCM analysis of Lake Loosdrecht phytoplankton. Microscopic examination after sorting showed that two clusters contained morphologically similar, filamentous cyanobacteria (species belonging to *Limnothrix* and *Prochlorothrix,* respectively) and that the third one contained a mixture of diatoms and green algae (Pel et al. unpubl. data). For pyrolytic methylation of phototrophs (*see below*), 5×10^4 to 2×10^5 cells trichome^{-1} were collected by FCM cell sorting, depending on the FA content of the target and the size (trichome lengths).

In-line pyrolytic methylation of cellular FAs and IRMS—Pyrolysis was conducted using a microvolume Curie-point reactor (Pel et al. unpubl. data). In short, the cell suspensions delivered by FCM were concentrated further by centrifugation in a final volume of approximately 4 μl and quantitatively deposited on the tips of ferromagnetic wires (Curie-point temperature, 480°C), together with a 1-μl drop of 0.25 mol L^{-1} trimethylphenylammonium hydroxide (TMPAH) in methanol as the derivatizing agent. Loaded wires were allowed to dry at room temperature under reduced pressure and continuous rotation. A release of the cellular FA fraction from the sample by in situ methylation was achieved applying a 3-s pyrolysis time. The volatilized FA methyl esters (FAMEs) were then swept, splitless, into a capillary GC coupled to a Finnigan Delta-S IRMS via a Finnigan type II combustion interface (Py-GC-IRMS). Zooplankton specimens were hand picked using a syringe under a stereomicroscope, and, depending on size, 1–30 individuals were used in pyrolytic methylation.

The C isotopic composition of FAME is reported in δ-notation. $\delta^{13}C$ in parts per thousand (‰) = $[(^{13}C/^{12}C_{sample} - {}^{13}C/{}^{12}C_{reference}) - 1] \times 10^3$ (Pel et al. 1997). The reproducibility in isotopic measurements of the individual FAME in FCM-retrieved profiles was ≤0.4‰ (Pel et al. unpubl. data). Since the isotopic value of the methyl in the TMPAH was not known, the cellular FA $\delta^{13}C$-values in this study will be reported without the usual correction for the methyl group originating from the derivatizing reagents. If the $\delta^{13}C$-value of tetramethylammonium-hydroxide, where all C originates from the four methyl groups (approximately −42‰), is used as a proxy in an isotopic mass balance correction of the cellular FAs, the FAMEs of diatoms and green algal species reported in this study ($\delta^{13}C$, approximately −34‰) will become 0.4–0.6‰ enriched (depending on FA chain length), whereas this correction is negligible at isotope ratios of approximately −42‰ (such as in cyanobacterial FAs).

Using one-laser excitation of pigments at 488 nm, diatoms and green algae cannot be distinguished and separated in fluorescence-activated cell sorting and thus are collected in the same sort fraction. However, in the composite FA profile that results from pyrolytic methylation, (poly)unsaturated C_{18} ($C_{18:n}$) can be attributed mainly to the green algae since freshwater diatoms are almost devoid of this type of FA (Napolitano 1999). Vice versa, $C_{16:1}$ is largely absent from green algae but ubiquitous in diatoms (Napolitano 1999). Thus, the distributions of common and otherwise not very discriminative FAs can complement the cell sorting (Pel et al. unpubl. data). Unfortunately, the specific diatom FA marker $C_{20:5}$ proved to be unstable under pyrolysis conditions using TMPAH, showing signs of (multiple) isomerization and severe degradation (Blokker et al. 2002).

^{13}C-carbonate labeling—In situ–specific growth rates of phototrophs were estimated from the uptake of inorganic C using enclosures of lake water (1.4 liters), of which total dissolved inorganic C (DIC) had been enriched ~1,400‰ in δ^{13}C, by supplementing an appropriate amount of ^{13}C sodium bicarbonate (99 atom% ^{13}C). Because DIC in Lake Loosdrecht is relatively high throughout the year (24–28 ppm C), this pool will normally be far in excess of the CO_2 demand of the phytoplankton at the ambient lake water pH of 8.0–8.5. Diel-averaged population-specific growth rates (μ_C d^{-1}) were determined from the rate of ^{13}C-CO_2 incorporation into cellular FAs of flow-sorted phototrophs during 24-h periods, analogous to the calculation of C-specific growth rates as reported by Welschmeyer and Lorenzen (1984).

$$\mu_C = -1/t \ln[1 - (\Delta\delta^{13}C_{FA}/\Delta\delta^{13}C_{DIC})] \quad (1)$$

where $\Delta\delta^{13}C_{FA}$ is the enrichment in ^{13}C of population-specific FAs after the incubation (t in days), and $\Delta\delta^{13}C_{DIC}$ is the enrichment of the DIC at time zero. Provided the light regime applied to such enclosures is consistent with the light history of the phytoplankton prior to their confinement, the use of diel incubations is considered appropriate for a reliable assessment of in situ growth rates (Goericke and Welschmeyer 1993). The C stable isotope ratio of total DIC was determined and monitored during these incubations essentially as described by Miyajima et al. (1995), using a Carlo Erba 1106 Elemental Analyzer coupled to a Finnigan Delta-S IRMS for the $\delta^{13}CO_2$ headspace analysis.

The enclosures were temperature controlled and stirred at low speeds (~20 rpm) to keep the cells and other sestonic material in suspension. Light was provided by a halogene 7 IPR lamp (230 V, 1,500 W, Mazda) in a light : dark cycle that depended on the time of the year. The strong light fluctuations that cells experienced in the lake because of their circulation throughout the entire water column (water depth, ~1.9 m; euphotic depth, 0.7–1.0 m) were simulated in the enclosures by switching the lamp on and off at 15-min intervals during the light period. Using a model QSL-100 sensor from Biospherical Instruments, the irradiance of the enclosures was adjusted to a level approximating the average light dose that individual cells had received in the field prior to their confinement (for further details, *see ''Results''*).

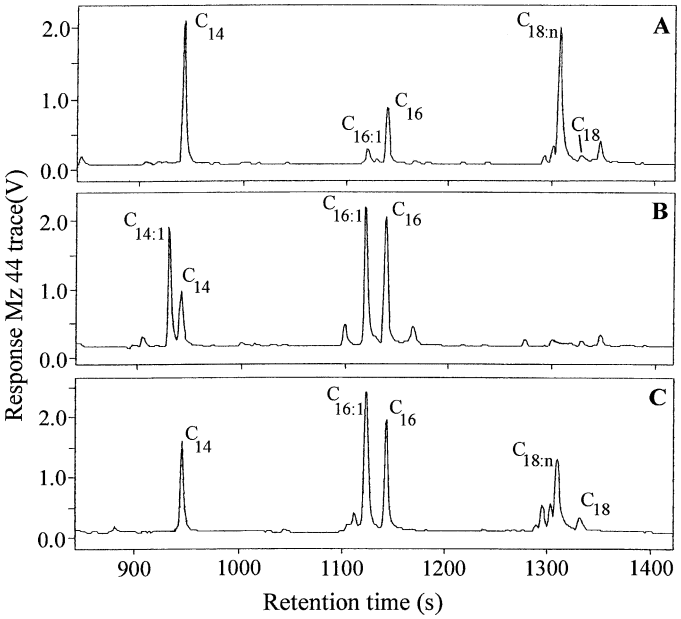

Fig. 1. Typical population-specific FAME profiles retrieved from Lake Loosdrecht phytoplankton using flow sorting and Py-GC-IRMS of collected cell fractions (12 April 1999): A) Oscillatorioids (*Limnothrix* sp. strain MR-1–like, mean trichome length = ~110 μm, ~7 × 10^4 filaments applied in pyrolysis), B) Prochlorophytes (*P. hollandica*–like, mean trichome length = ~120 μm, ~7 × 10^4 filaments applied), and C) diatoms plus green algae (~5 × 10^4 cells applied).

Results

FCM/Py-GC-IRMS analysis of Lake Loosdrecht phytoplankton—Using fluorescence-activated cell sorting and in-line Py-GC-IRMS, the standing stocks, FA profiles, and isotopic signatures of the predominant phytoplankton populations of Lake Loosdrecht were monitored weekly from spring to early summer 1999. Apart from a shift in the $C_{16:1}/C_{18:n}$ ratio from $\gg 1$ to ≤ 1 in the algal FA profile (*see Fig. 1C and ''Materials and methods''*), indicating the partial replacement of diatom populations by green algal species with time, the profiles shown in Fig. 1 are typical of the phytoplankton fractions retrieved from the lake by cell sorting. The wax and wane of trichomes and unicellular cells belonging to the three major clusters resolved by FCM are depicted in Fig. 2A. Within the retrieved FA profiles, the isotopic values of the individual FAs fell in narrow and characteristic ranges (Table 1). FAs constituting the profile of the green algae/diatoms cluster were strikingly more enriched in ^{13}C than in oscillatorioid and prochlorophyte profiles, with the strongest depletions occurring in the latter (Table 1). By taking the weighted average of the δ^{13}C-values of major FAs in a profile, an overall population-specific isotopic signal was determined. Monitoring these δ^{13}C signals at weekly intervals showed that the eukaryotic algae and the two cyanobacterial groups conspicuously differed in their isotope composition by 6–10‰ (Fig. 2B).

Zooplankton FA profiles and C isotopic signatures—In addition to the isotopic monitoring of the phytoplankton, the

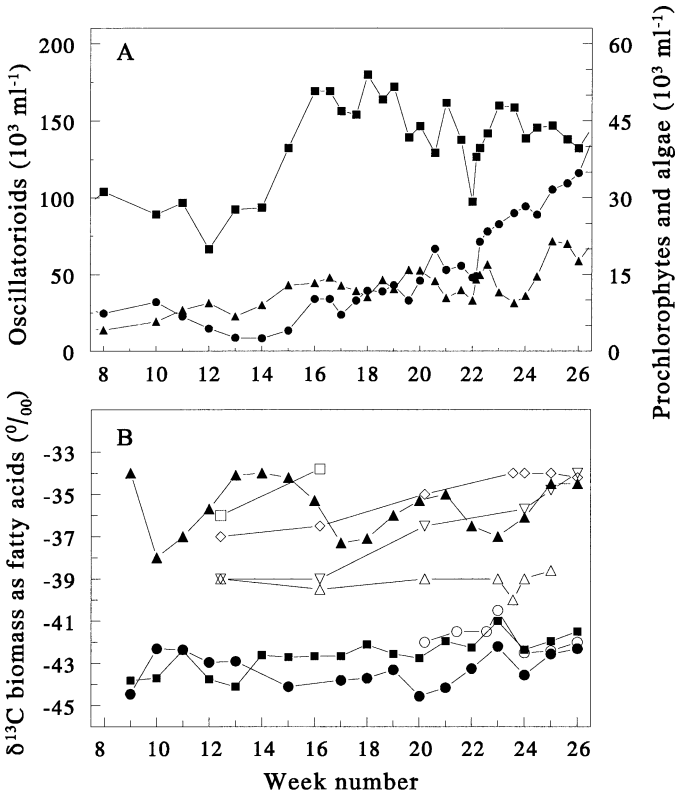

Fig. 2. Results of weekly monitoring (Lake Loosdrecht, spring 1999) of the numerical abundances of phytoplankton groups as resolved by flow cytometry and the δ^{13}C signatures for major phyto- and zooplankton groups. A) Cell or trichome densities of phytoplankton: oscillatorioids (squares), prochlorophytes (circles), diatoms plus green algae (triangles). B) Phytoplankton δ^{13}C signatures (filled symbols): oscillatorioids (squares), prochlorophytes (circles), diatoms plus green algae (triangles). Zooplankton δ^{13}C signatures (open symbols): *Brachionus* sp. (squares), *A. priodonta* (diamonds), Copepods (inverted triangles), *Bosmina* sp. (triangles), *E. dilatata* (circles). Isotopic compositions are expressed as the weighted average of the δ^{13}C-values of individual FAs present in a group-specific FA profile ($C_{20:5}$ and $C_{22:6}$ were not included in this average because of their substantial degradation in pyrolysis, *see Table 2 and ''Materials and methods''*). Each data point is the mean of a replicate measurement of the weighted average, with standard deviations <0.4‰ in FCM-sorted phytoplankton and ≤0.3‰ in hand-picked zooplankton.

δ^{13}C signatures of predominant zooplankton taxa were also established at a regular temporal basis using Py-GC-IRMS. Typical zooplankton FA profiles are given in Table 2, together with the δ^{13}C-values of the individual FAs ($\delta^{13}C_{FA}$) within these profiles as retrieved in late spring. Similar to the phytoplankton, zooplankton population-specific isotopic signals were determined as the weighted-average δ^{13}C-value of major FAs present in a profile ($C_{20:5}$ and $C_{22:6}$ were not included in this average because of their degradation in pyrolysis; *see Table 2 and ''Materials and methods''*). Apart from the depleted ^{13}C signal of the rotifer *Euchlanis dilatata*, which was always close to the δ^{13}C signatures of the cyanobacterial populations, all other examined zooplankton taxa were isotopically significantly heavier and much more often resembled the algal isotopic signals (Fig. 2B).

Table 1. Typical δ^{13}C-values of major individual FAs present in the FA profiles obtained from Lake Loosdrecht by Py-GC-IRMS analysis of whole lake water and of population-specific sort fractions from the phytoplankton in spring (12 Apr 99).

	δ^{13}C of signature FAs (‰)*				
	$C_{14:1}$	$C_{14:0}$	$C_{16:1}$	$C_{16:0}$	$C_{18:n}$†
Whole lake water	−42.1	−41.7	−39.4	−40.5	−43.2
Oscillatorioids	ND‡	−42.8	ND	−41.1	−43.3
Prochlorophytes	−43.7	−41.9	−45.0	−43.7	ND
Eukaryotic algae	ND	−33.7	−34.3	−34.0	−35.8

* Mean values are given. Standard deviations in δ-values of all FAMEs were ≤0.4‰ ($n = 2$).

† Mainly composed of $C_{18:2}$ with contributions (<20% of total) of $C_{18:1}$ and $C_{18:3}$ FAs. Because sufficient chromatographic separation in GC-combustion-IRMS analysis was not achieved, the overall δ^{13}C-value of the entire FA complex is given.

‡ ND, not determined because of insufficient peak intensity or total absence.

^{13}C-carbonate labeling of lake phytoplankton and label transfer to zooplankton—In situ, diel-averaged growth rates of the FCM-retrieved populations were estimated by means of ^{13}C-carbonate labeling on four occasions in the period of isotopic monitoring using microcosm confinement of lake water. ^{13}C-carbonate labeling of chemostat-grown representatives of the predominant functional phototrophic groups in Lake Loosdrecht showed the general validity of the use of unsaturated, primarily membrane-derived FAs in the growth rate assessments (Pel et al. unpubl. data). In the present study, $C_{18:n}$-values were taken for oscillatorioids and green algae, $C_{14:1}$ for prochlorophytes, and $C_{16:1}$ for diatoms (Fig. 3A; Table 3). The growth rates of the diatom and green algae populations appeared to be substantially higher (five- to ninefold) than those of the oscillatorioids and prochlorophytes.

By taking advantage of the presence of differentially ^{13}C-labeled phytoplankton groups in the ^{13}C-carbonate–spiked enclosures (Fig. 3A), we were also able to examine the grazing behavior of the zooplankton. To this end, the illumination in the microcosm confinement was turned off permanently at $t = 2$ d, and the isotopic signals of the phyto- and zooplankton were monitored for another 7 d (Fig. 3A,B). The zooplankton populations examined responded differently in the presence of the various ^{13}C-enriched C sources (compare panel B with A in Fig. 3), strongly suggesting the occurrence of preferential grazing for some of them (e.g., predominant consumption of algae—at least initially—by *Asplanchna* and the small-bodied rotifers).

Discussion

The monitoring of Lake Loosdrecht phytoplankton for their in situ δ^{13}C signatures showed that the FCM-retrieved algal and cynanobacterial clusters differed significantly in isotopic composition (Fig. 2B). Ecophysiological factors can affect the C isotope composition of phytoplankton (Gu and Schelske 1996; Burkhardt et al. 1999; Eek et al. 1999), and the phytoplankton growth rate and degree of discrimination against $^{13}CO_2$ in photosynthesis are known to be inversely related (Fry and Wainright 1991; Kopczyñska et al. 1995;

Table 2. Typical FA profiles of Lake Loosdrecht main zooplankton taxa and the δ^{13}C-values of the individual FAMEs as determined on 22 Jun 99 (*Brachionus* sp. sample from 20 Apr 99).

	% total FA and δ^{13}C of signature fatty acids (‰)									
	Copepods		*Bosmina*		*Asplanchna*		*Euchlanis*		*Brachionus*	
	%	δ^{13}C	%	δ^{13}C	%	δ^{13}C	%	δ^{13}C	%	δ^{13}C
$C_{14:1}$	ND*	—	tr*	—	ND	—	1.8	−40.5	ND	—
$C_{14:0}$	5.3	−34.1	13.4	−38.6	8.4	−34.7	17.1	−41.1	21.3	−34.1
$C_{16:1}$	3.5	−34.3	7.8	−37.9	3.2	−34.7	5.0	−41.6	7.5	−32.2
$C_{16:0}$	20.1	−34.8	24.5	−37.9	13.3	−30.9	12.5	−42.7	26.1	−33.1
$C_{18:n}$†	14.1	−34.4	30.0	−39.3	19.1	−33.0	26.1	−43.1	13.5	−35.6
$C_{18:0}$	7.6	−35.2	6.3	−38.6	3.7	−33.6	4.2	−41.8	4.5	−34.4
$C_{20:5}$‡	12.9	−34.2	4.2	−33.7	15.8	−34.1	tr	—	5.5	−34.0
$C_{20:2}$	tr	—	tr	—	ND	—	29.2	−42.4	ND	—
$C_{20:0}$	tr	—	tr	—	tr	—	0.4	−41.7	ND	—
$C_{22:6}$‡	22.7	−33.2	tr	—	22.1	−28.6	tr	—	17.8	−32.7

* ND, not detectable; tr, trace.

† Mainly composed of $C_{18:2}$ with contributions (<30% of total) of $C_{18:1}$ and $C_{18:3}$ FAs. Because sufficient chromatographic separation in GC-combustion-IRMS analysis was not achieved, the overall δ^{13}C-value of the entire FA complex is given.

‡ Because of the degradation and isomerization of these FAs in pyrolytic methylation, the percentages of total FA and δ^{13}C-values are given as tentative figures.

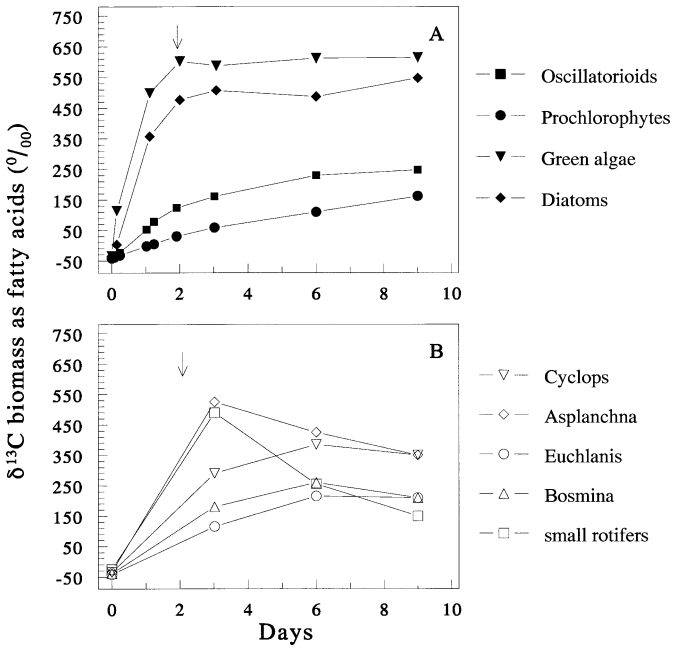

Fig. 3. A) Results of ^{13}C-carbonate labeling of phytoplankton and B) label transfer to zooplankton populations in an enclosure of ^{13}C-DIC–enriched lake water (~1,400‰) (Lake Loosdrecht; June 1999). For the incubation conditions of the enclosure, *see Table 3* (22 June). At $t = 2$ d, the illumination in the enclosure was permanently turned off (arrows). ^{13}C-CO_2 incorporation in FCM-retrieved phytoplankton is presented as an accumulation of labeled C in a group-specific major FA: oscillatorioids and green algae, $C_{18:n}$; prochlorophytes, $C_{14:1}$; diatoms $C_{16:1}$. Mean δ^{13}C-values are given. Standard deviations for all FAMEs were <15‰ ($n = 2$). ^{13}C label uptake by the zooplankton examined is given as an accumulation of the label in major FAs (as the weighted average) present in the population-specific FAME profiles (*see Table 2*; $C_{20:5}$ and $C_{22:6}$ not included).

Laws et al. 1995; Popp et al. 1998). This relationship results in a relative enrichment in ^{13}C of photosynthetic biomass with higher growth rates. The laboratory confinements with ^{13}C-carbonate–spiked lake water indeed confirmed that the growth rates of diatoms and green algae were substantially higher than those of oscillatorioids and prochlorophytes (Fig. 3A; Table 3). The oscillations observed in the algal δ^{13}C signal (Fig. 2B) may, in fact, reflect accelerating and decelerating growth. Growth rates of oscillatorioids and prochlorophytes determined in June (*see Table 3*) compare well with the summer growth rate estimates of Lake Loosdrecht phytoplankton measured in the past ($\mu \sim 0.1$ d^{-1}) using chlorophyll-specific column-integrated oxygen production and in situ grazing-free protein production in dialysis bags (Gons et al. 1992*a*). Despite their higher growth rates, the algae do not replace the cyanobacteria: algal cell numbers remain at 10–20 $\times$ 10^3 ml^{-1} (Fig. 2A). Apparently, at these population densities, the growth is balanced, on average, by equally high losses.

The presence of phytoplankton-derived C sources differing in isotope signature enabled a study of the substrate consumption of micro- and mesozooplankton populations. An important feature of FAs as trophic markers is that the FA composition of a zooplankter does provide a time-integrated measure of the animal's dietary intake (Olsen 1999). Because extensive catabolism and anabolism of FAs normally do not seem to occur, the lipid composition of the animal's tissues will be very close to that of the feed (Olsen 1999). We can therefore anticipate that the animals will also be similar in isotopic composition to their diets, except for modest shifts in δ^{13}C between 0‰ and +1.0‰ per trophic level, as have been observed in studies involving bulk C or protein measurements (Peterson and Fry 1987). Indeed, both with respect to cellular FA composition and $\delta^{13}C_{FA}$ signature, the rotifer *Brachionus calyciflorus* closely resembled its diet, which was the green algae *Scenedesmus acutus,* in controlled feeding experiments (data not shown). The δ^{13}C signatures

Table 3. Population-specific growth rates* of predominant phototrophs in Lake Loosdrecht as assessed using ^{13}C-carbohydrate–spiked lake water enclosures simulating field conditions in spring 1999.

	25 Mar 99	20 Apr 99	18 May 99	22 June 99
μ_c, oscillatorioids	0.033	0.031	0.05	0.08
μ_C, prochlorophytes	0.014	0.021	0.023	0.046
μ_C, diatoms	0.14	0.12	0.15	0.33
μ_C, green algae	ND†	0.14	0.2	0.43
Incubation temperature (°C)	8	10	14	18
Light : dark cycle (h)	13 : 11	15 : 9	16 : 8	17 : 7
PAR‡ (joule cm^{-2} d^{-1})	290	600	880	960

* Diel-averaged growth rates (μ_C d^{-1}) estimated from ^{13}C incorporation into FCM-retrieved population-specific FAs: $C_{18:n}$ was used for oscillatorioids and green algae, and $C_{14:1}$ and $C_{16:1}$ were used for prochlorophytes and diatoms, respectively.

† ND, not determined because of insufficient population density in cell sorting.

‡ Outdoor photosynthetically available radiation (PAR) given as the daily average for the 5 days prior to confinement. The irradiance of the enclosures was set at a fivefold lower level to approximate the average light dose individual cells had received in the field.

of the rotifer taxa examined in Lake Loosdrecht strikingly overlapped with those of either the diatoms/green algae or the cyanobacteria (Fig. 2B). Both *Brachionus* sp. and *Asplanchna priodonta* exhibited ^{13}C signals similar to diatoms/green algae, implying that their diets had largely consisted of these phototrophs. In contrast, *Euchlanis* reflected the ^{13}C-depleted signals of the oscillatorioid and prochlorophyte groups. The explicit food preference of this rotifer group confirms the laboratory observations that indicate the efficient ingestion and assimilation of cultured filamentous cyanobacteria by *E. dilatata* (Gulati et al. 1993). Copepods and bosminids, each group accounting for nearly half of the total crustacean biomass in Lake Loosdrecht (Gulati 1990), were intermediate in δ^{13}C with respect to the primary C resources available in early spring. However, from week 16 onward, the isotopic signature of the copepods shifted toward and ultimately overlapped with the algal δ^{13}C signals, whereas the *Bosmina* δ^{13}C remained around −39‰ (Fig. 2B). Bosminids can exploit both the small-sized as well as the coarse particles and were observed to assimilate cyanobacterial trichomes to some extent (Irvine 1986). Their intermediate δ^{13}C signal (approximately −39‰), therefore, most likely results from the assimilation of both cyanobacterial and algal cells or derived detritus. Because of their very low densities and infrequent appearance in the lake, we have no isotopic data on daphnids. In contrast to bosminids that can discriminate particles by taste, daphnids are thought to show only size selectivity (Kerfoot and Kirk 1991). Also, the high density of cyanobacterial filaments in the lake ($>10^5$ trichomes ml^{-1}) may interfere with the feeding mechanism of daphnids (Gilbert 1990; DeMott et al. 2001*b*). Thus, the results for *Bosmina* should not be extrapolated to other cladoceran genera. The observed tendency in the copepods toward algal-derived C is surprising since none of the five dominant species in the lake belongs essentially to the herbivorous calanoids (Gulati 1990). Apparently, the copepods predate selectively on those fractions of the herbivorous zooplankton that, in their turn, feed preferentially on diatoms/green algae. The size of prey suitable for the cyclopoids are the ciliates, their own (herbivorous) nauplii, and particularly small-bodied rotifers (<100 μm) such as *Keratella*, *Anuraeopsis*, *Filinia*, and *Polyarthra* spp., which are very abundant (5,000–8,000 ind. L^{-1}) in the lake (Ooms-Wilms et al. 1999).

^{13}C-CO_2 labeling of phytoplankton and label transfer to zooplankton—Zooplankton grazing was also examined by ^{13}C-carbonate spiking of the lake water (*see Fig. 3A,B*) and, indeed, corroborated most of the trophic interrelationships inferred from the natural stable isotope distributions of various plankton compartments, such as the apparent preferential feeding of the small-bodied rotifers mentioned above, on eukaryotic algae. Both *Asplanchna* and the group of small-bodied rotifers responded to our experimental conditions by showing an initial strong enrichment in ^{13}C close to the tracer levels attained by diatoms and green algae (compare panels A and B in Fig. 3). In contrast, the *Euchlanis* ^{13}C signal approached the enrichment level in the oscillatorioids (Fig. 3A,B). The observed decline in ^{13}C enrichment of *Asplanchna* and small-bodied rotifers after $t = 3$ d probably results from a shift in diets elicited by the exhaustion of their preferred C resources, as the eukaryotic phytoplankton were rapidly grazed to cell densities <1,000 ml^{-1} (data not shown) from the moment the enclosure was maintained under a continuous dark condition ($t = 2$ d in Fig. 3A,B). The diet plasticity observed in *A. priodonta* by Kappes et al. (2000), which varied from zoophagy to exclusive phytophagy, corroborates an opportunistic feeding behavior of this rotifer in our labeling experiment. The ^{13}C signal in copepods peaked with a delay of 2–3 d with respect to the group of small-bodied rotifers, which is in accordance with their suspected preference for this resource. Bosminids, however, did not exhibit the accumulation of labeled C expected from a mixed diet unless the intake of live prochlorophyte trichomes (δ^{13}C, ≤100‰) and/or the cyanobacterial-derived detritus already present at the start of the experiment (δ^{13}C, approximately −40‰) offset the incorporation of algal C highly enriched with tracer. Our observations on the natural ^{13}C abundance differences and similarities among plankton groups and the short-term ^{13}C-tracer experiments clearly point to a central position of rotifers in cropping on the eukaryotic algal C and passing this C on to the cyclopoid copepod population. Quite surprisingly, the funneling of cyanobacterial-derived (detrital) C (δ^{13}C, approximately −40‰) via the microbial loop and then by rotifer grazing (Ooms-Wilms 1998) toward the copepods is not apparent from our isotope data. Probably, the overall efficiency of the microbial loop in processing the cyanobacterial C is low

because of the cascades involved (bacteria, nanoflagellates, and ciliates) before rotifers manage to harvest this C flux.

This study demonstrates the importance of identifying and sampling the different phototrophic C sources available to consumers in pelagic food webs. Even in the well-mixed water column of a shallow lake, which provided relatively uniform physical and chemical conditions, a pronounced distinction in $\delta^{13}C$ signals occurred between the major representatives of the phytoplankton. We also observed this phenomenon in three other Dutch lakes: Tjeukemeer, Volkerak-Zoommeer, and IJsselmeer, dominated by *Planktothrix agardhii*, *Microcystis/Aphanizomenon*, and *Microcystis*, respectively, and we suspect this also to be true for many other lakes. This would imply a biased perception of C pathways in past studies, in which ''bulk'' procedures have been used to establish the ^{13}C proxies of sources and sinks. Our population-specific examination of in situ isotopic signatures indicates that most members of the micro- and mesozooplankton in Lake Loosdrecht depend largely on the algal C, despite the overwhelming abundance of cyanobacterial biomass and derived detritus (Gons et al. 1992*a*). The occurrence of specific energy/C fluxes in pelagic systems where small masses of primary producers apparently support much larger masses of herbivores has repeatedly attracted attention in previous decades. Our data now provide direct proof of this phenomenon that has been suspected from more indirect evidence so far (Sommer 1989; Lampert and Sommer 1997) in a eutrophic habitat dominated by long–life span phytoplankton. In a number of marine-oriented isotope studies, a disproportionate impact on food chains exerted by apparently minor phototrophic populations has been postulated to occur (coastal and mangrove habitats) but has remained speculative because of the insufficient resolution in isotopic analysis (Fry and Wainright 1991; Kopczyñska et al. 1995; Bouillon et al. 2000). An opposite phenomenon is observed for *E. dilatata* in Lake Loosdrecht. Despite the abundance of its preferred food (Gulati et al. 1993; this study), this rotifer species does not actually achieve high densities in the lake, being even periodically absent for weeks altogether (data not shown). The question as to what prevents the population from proliferating remains unanswered.

The adequacy of our method to probe trophic links in depth may provide a sensitive means to detect and come to grips with the impact of anthropogenic activities and climate-mediated stresses on pelagic food webs at their very core, a prerequisite to predicting the possible perturbations of functions at the ecosystem level (Stapp et al. 1999; Vander Zanden et al. 1999; Scheffer et al. 2001). In this respect, it is noteworthy that individual phyto- and zooplankton species, rather than the functional groups, have been hypothesized as the nexus between stress factors and ecosystem changes (Adrian and Deneke 1996; Möllmann et al. 2000; Downing and Leibold 2002). Water management measures to initiate and sustain the restoration of degraded lakes like Loosdrecht may greatly benefit from a detailed monitoring and evaluation of the standing stocks and turnover rates of individual phyto- and zooplankton populations, the related C transfer functions, and the changes therein.

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Received: 1 October 2002
Accepted: 16 May 2003
Amended: 23 July 2003