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Contributions of Different Organic Carbon Sources to *Daphnia* in the Pelagic Foodweb of a Small Polyhumic Lake: Results from Mesocosm DI^{13}C-Additions

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Abstract

Freshwater ecosystems derive organic carbon from both allochthonous and autochthonous sources. We studied the relative contributions of different carbon sources to zooplankton in a small, polyhumic, steeply stratified lake, using six replicate surface-to-sediment enclosures established during summer and autumn 2004. We added ^{13}C-enriched bicarbonate to the epilimnion of half the enclosures for three weeks during each season and monitored carbon stable isotope ratios of DIC, DOC, POC and *Daphnia*, along with physical, chemical and biological variables. During summer, ^{13}C-enriched DIC (δ^{13}C up to 44 ± 7.2‰) was soon taken up by phytoplankton (δ^{13}C up to −5.1 ± 13.6‰) and was transmitted to *Daphnia* (δ^{13}C up to −1.7 ± 7.2‰), demonstrating consumption of phytoplankton. In contrast, during autumn, ^{13}C-enriched DIC (δ^{13}C up to 56.3 ± 9.8‰) was not transmitted to *Daphnia*, whose δ^{13}C became progressively lower (δ^{13}C down to −45.6 ± 3.3‰) concomitant with decreasing methane concentration. Outputs from a model suggested phytoplankton contributed 64–84% of *Daphnia* diet during summer, whereas a calculated pelagic carbon mass balance indicated only 30–40% could have come from phytoplankton. Although autumn primary production was negligible, zooplankton biomass persisted at the summer level. The model suggested methanotrophic bacteria contributed 64–87% of *Daphnia* diet during autumn, although the calculated carbon mass balance indicated a contribution of 37–112%. Thus methanotrophic bacteria could supply virtually all the carbon requirement of *Daphnia* during autumn in this lake. The strongly ^{13}C-depleted *Daphnia* values, together with the outputs from the models and the calculated carbon mass balance showed that methanotrophic bacteria can be a greater carbon source for *Daphnia* in lakes than previously suspected.

Key words: lake enclosures, allochthonous organic carbon, 13C-addition, *Daphnia longispina*, food-web models, stable isotopes, methanotrophic bacteria.

Introduction

All ecosystems depend on organic matter to support and fuel their food webs. Freshwater ecosystems receive organic matter from two distinct sources.

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Autochthonous primary production occurs within the system, but freshwaters also receive a variable allochthonous loading of terrestrial organic matter from their catchments. Bacterial utilization of allochthonous dissolved organic matter and bacterial consumption by protozoan and metazoan grazers represents an alternative carbon flow pathway to higher trophic levels. The magnitude and proportion of these two distinct sources vary widely between lakes (Wetzel 2001). In highly eutrophic lakes autochthonous production plays a major role whereas in more oligotrophic lakes allochthonous organic matter may have a key role in whole-lake metabolism (del Giorgio and others 1999). However, the quantitative importance of this alternative pathway is still uncertain, even if its existence is firmly established (Salonen and Hammar 1986; Carpenter and others 2005).

Analysis of stable isotopes of carbon potentially offers an effective way to differentiate between autochthonous and allochthonous organic carbon source in lakes (Jones and others 1998; Grey and others 2001). In temperate lakes, allochthonous organic carbon of terrestrial origin has a $\delta^{13}C$ value around $-27‰$, whereas autochthonous carbon produced by phytoplankton photosynthesis tends to be more ^{13}C-depleted (Vuorio and others 2006). In some cases this difference has permitted natural abundance carbon stable isotope analysis to be used to distinguish carbon sources in zooplankton biomass (for example, Grey and others 2001). However, often the isotopic separation between autochthonous and allochthonous organic carbon is too slight to allow effective distinction.

An alternative approach is then to manipulate the autochthonous (phytoplankton) carbon isotope value by addition of ^{13}C-enriched dissolved inorganic carbon (DIC). When this enriched DIC is utilized by photosynthetic phytoplankton, their own carbon isotope value will become correspondingly enriched, whereas the value for allochthonous, detrital organic carbon will remain unaffected. If autochthonous carbon (phytoplankton) is the main carbon source for zooplankton, they will become labeled with ^{13}C, with the speed and magnitude of labeling depending on the zooplankton feeding and growth rates. However, if allochthonous carbon largely supports zooplankton carbon demands, the transmission of ^{13}C label will be slower or will not occur at all. Because zooplankton is a key link to higher trophic levels, the major freshwater pelagic system carbon source can be estimated if the carbon source of zooplankton is known.

Cole and others (2002) used this approach to study the role of allochthonous organic carbon in East Long Lake in Wisconsin, where they added a single dose of $NaH^{13}CO_3$ to the lake in June. During that experiment, the isotope signature induced in DIC was transmitted rapidly to POC and then to zooplankton, indicating that zooplankton obtained most of their carbon from phytoplankton and that bacteria passed little allochthonous carbon to higher trophic levels. However, Pace and others (2004), using daily additions of $NaH^{13}CO_3$ over 42 days, subsequently suggested that 40–55% of particulate organic carbon and 22–50% of zooplankton carbon was derived from terrestrial sources. In a synthesis of their results, Carpenter and others (2005) noted that allochthony was more important in a dystrophic lake than in an unproductive lake or one with nutrient enrichment. However, these generalizations derive from studies of only very few lakes, and further studies are certainly needed to evaluate both the robustness of the generalizations and the quantitative range of allochthony in lakes.

In particular, because phytoplankton production and composition vary seasonally results from summer when primary production is high are not necessarily representative of carbon sources for grazers over on annul cycle. Grey and others (2001) used natural abundance carbon isotope analysis to show marked seasonal changes in the carbon source used by zooplankton in Loch Ness, Scotland. Moreover, Jones and others (1999) reported that zooplankton $\delta^{13}C$ was substantially lighter than phytoplankton $\delta^{13}C$ in several small boreal lakes in late summer and they proposed that zooplankton may have been partly deriving their carbon from ^{13}C-depleted methanotrophic bacteria. Hence zooplankton in lakes may have access at different seasons to several different food sources with distinct carbon isotope signatures. Here we report results from experimental additions of ^{13}C-enriched DIC to replicate enclosures in a polyhumic lake in southern Finland during two contrasting seasons. We use the results to estimate the proportions of zooplankton carbon derived from various potential organic carbon dietary sources.

Methods

Site Description

Mekkojärvi (61°13′ N, 25°8′ E) is a small, shallow lake (area 0.35 ha, mean depth 3 m) located in the Evo forest area of southern Finland. It is a naturally

acidic (pH 4.6–6.2), highly humic lake surrounded by coniferous forest. The small size and brown water (color 300–700 mg Pt l^{-1}) result in steep thermal and oxygen stratification. The lake is ice-free usually from the beginning of May to mid-November and the whole water column turns over in autumn, but only partly in spring. The concentration of dissolved organic carbon (DOC) varies from 20 to 40 mg C l^{-1} and the concentration of dissolved inorganic carbon is 3–12 mg C l^{-1}. Further characteristics of Mekkojärvi can be found elsewhere (Arvola and others 1992; Kuuppo-Leinikki and Salonen 1992; Münster 1999).

The annual primary production of phytoplankton is below 10 g C m^{-2} (Salonen and others 2005). The epilimnetic phytoplankton is dominated by chrysophytes (*Mallomonas* spp.) and cryptophytes (*Cryptomonas* spp.), whereas flagellated chlorophytes (*Chlamydomonas* spp.) and the prasinophyte *Scourfieldia cordiformis*, are rather abundant in the upper hypolimnion (Arvola and others 1992). Bacterial density is greater in the oxic-anoxic boundary zone of the metalimnion (20–45 $\times 10^6$ cells ml^{-1}) than in the oxic epilimnion (2–7 $\times 10^6$ cells ml^{-1}). There are abundant photosynthetic green sulfur bacteria (*Chlorobium*) in the oxic-anoxic boundary zone. Ciliates are also more abundant in the hypolimnion (5–30 cells ml^{-1}) than in the oxic epilimnion. *Daphnia longispina* is the most abundant crustacean zooplankton in the lake, where planktivorous fish are absent, and the main invertebrate predators are *Chaoborus* larvae and *Notonecta* spp. This simple food web facilitates the detection of the contribution of different sources of organic matter to the crustacean zooplankton.

Experimental Setup

Two separate mesocosm experiments were performed during the open water season, first in mid-summer (6th to 30th July), and again in autumn (14th September to 12th October). The experiments were made in cylindrical enclosures (diameter 2 m, height 4 m) constructed of 2 mm flexible polyethylene and extending from the surface to the sediment. Three replicate control enclosures and three replicate treatment enclosures were used in each experiment. Additionally, in the summer experiment, the abundance of the *Daphnia longispina* population was first estimated in each enclosure by sampling with a zooplankton net (100 µm mesh) and then approximately equalized between the enclosures by adding *Daphnia* to those enclosures with lower numbers.

Sample Collection and Analyses

Oxygen and temperature were measured five times per week using a YSI 55 probe (Yellow Springs Instruments, Ohio, USA, accuracy 0.3°C and 0.3 mg O_2 l^{-1}). Water samples were taken from the oxic (0–0.6 m) and anoxic (0.6–3.0 m) layers using a Limnos tube sampler (height 60 cm, vol. 4.25 l). Two replicate samples from the epilimnion were pooled. A pooled hypolimnetic sample was derived from four samples collected from discrete depths (0.6–1.2, 1.2–1.8, 1.8–2.4 and 2.4–3.0 m). Samples were passed through a 100 µm mesh zooplankton net and zooplankton retained in the net were used for biomass calculation and stable isotope analysis (SIA). Bacterial production was measured by ^{14}C leucine incorporation (Kirchman and others 1985) as modified by Tulonen (1993), using 60 nm and 30 nmol of ^{14}C leucine (Amersham Biosciences) during the summer and the autumn experiments, respectively. Primary production was determined from 24 h incorporation of $NaH^{14}CO_3$ according to Keskitalo and Salonen (1994). Bacterial and primary production was measured twice per week. Total nitrogen, total phosphorus, alkalinity, pH, conductivity, DOC, POC and chlorophyll-*a* were analyzed twice per week using the validated routine methods of the Finnish Standard Association (http://www.sfs.fi/en/). Total DIC and methane samples (30 ml) were taken into 60 ml polypropylene syringes, which were kept in crushed ice for less than 4 h before analyses with a headspace equilibrium technique (McAuliffe 1971). Before the addition of 30 ml N_2 headspace into syringes, the samples were acidified to approximately pH 2 with HNO_3. Well-mixed headspace gas from the syringes was injected into pre-evacuated LABCO exetainers (12 ml), from which the samples were delivered by a GILSON 222 XL autosampler through a 1 ml VALCO 10-port valve into an AGILENT 6890 N Gas Chromatograph equipped with TCD and FID detectors (temperatures FID 210°C, TCD 120°C, oven 40°C, PlotQ capillary column, flow rate 12 ml min^{-1}, He as a carrier gas). The total amount of DIC and the methane concentration were determined in comparison to 103 and 999 ppm CO_2 reference samples and 10 and 493 ppm CH_4 reference samples.

Stable Isotope Addition

A stock solution containing 0.29 mmol of $NaH^{13}CO_3$ (99 atom %, CK Gas Products Ltd) was first diluted to one liter with lake water and then poured into the epilimnion of each treatment

enclosure, and the water mixed gently to a depth of 0.5 m. Although this addition increased total DIC by only less than 0.1%, to control for the effect of added inorganic carbon, 0.30 mmol of $NaHCO_3$ (MERCK, $\delta^{13}C$-5.0‰) was added into control enclosures. Additions were made five times per week, with a double amount added when there was no addition on the next day. Additions were made from 8th to 25th June 2004 during the summer experiment and from 16th September to 5th October 2004 during the autumn experiment. Any scheduled sampling of enclosures was done before isotope additions were made.

Stable Isotope Samples and Analyses

Samples for analysis of DIC were taken into 20 ml glass bottles and 200 µL of 25.6 g $CuSO_4 \times 5H_2O$ 100 ml^{-1} were added to prevent microbial activity in samples (Winslow and others 2001). Sample bottles were sealed with an aluminum cap containing a PTFE/silicon septum (VWR). DIC samples for SIA were stored at 4°C prior to analysis. For analysis, 500 or 1000 µL of H_3PO_4 (o-H_3PO_4, 85%, MERCK) were first added into 12 ml exetainers (Labco), which were then flushed with helium to expel all CO_2. Then 2 or 4 ml of sample (according to total DIC concentration) were injected into the exetainer and left more than 24 h for CO_2 to equilibrate in the helium headspace. Prior to analysis the exetainers were mixed using a vortex shaker. Samples were analyzed using a Gas Bench II (Thermo Finnigan) connected to Delta Plus Advantage IRMS (Thermo Finnigan). Sample $\delta^{13}C$ of DIC was determined against IAEA standards NBS-19 and limestone was used as a working standard. Results were linearly corrected using NBS-19 values at different intensity. Standard deviation between repeated measurements was less than 0.5‰.

Daphnia samples were rinsed into deionized water and after gut evacuation approximately 20 h later, the animals (adults and juveniles separately) were picked into pre-weighed tin cups (5–80 individuals in each) and then dried at 60°C yielding 0.1–1.2 mg dry weight (DW) for SIA analyses. The rest of the samples were preserved with formaldehyde (final concentration 4%) for later microscopical counts. For $\delta^{13}C$ analyses of POC, 500 ml of water were filtered through preignited Whatman GF/C glass-fiber filters (pore size ca. 1 µm) which were then dried and 0.2–1.0 mg of the retained material was scraped into tin cups. For $\delta^{13}C$ analyses of DOC, 100 ml samples of the filtrates passed through GF/C filters were acidified and freeze-dried (Christ alpha 1–4, B. Braun biotech International), and 0.2–0.4 mg of the dry material were weighed into tin cups. The solid samples were analyzed with a Carlo-Erba Flash 1112 series Element Analyzer connected to a Thermo Finnigan Delta Plus Advantage IRMS and run against NBS-22 standard using dried and homogenized fish muscle as an internal laboratory working standard. The precision of the $\delta^{13}C$ analysis was 0.2‰ for *Daphnia* and 0.3‰ for POC and DOC.

Modeling

The large diversity of potential food sources for *Daphnia* in Mekkojärvi (Table 1) means it is not possible to calculate the precise contribution of each source at any time. Therefore, to estimate the likely contribution of different carbon sources to *Daphnia* we used two different modeling approaches. We used several variations of the third model of Pace and others (2004), which gave the best fit to their data. This model estimates the relative contributions of allochthonous and autochthonous carbon and takes into account the fractionation of ^{12}C and ^{13}C, the proportion of carbon from terrestrial origin, the lag time (u) between carbon production from recent photosynthesis and its assimilation by *Daphnia*, and the proportion of memory carbon (m) in *Daphnia* from u lags before the present day (t). We first used this model to estimate the proportional contribution (w) of terrestrial organic matter (TOM), which is probably mainly channeled via heterotrophic bacteria, relative to phytoplankton (PP) to *Daphnia* biomass (TOM-PP model). The measured mean $\delta^{13}C$ value (−27.8‰) of DOC in Mekkojärvi was used in the model to represent organic matter from terrestrial C_3 plant detritus. In fact, according to the model presented by Kritzberg and others (2006) and given the very high allochthonous DOC concentration in Mekkojärvi it is probable that the heterotrophic bacteria largely rely on allochthonous carbon and hence would have a $\delta^{13}C$ value close to that of terrestrial detritus.

In a second variation of this model, terrestrial organic matter was replaced by methane oxidizing bacteria (MOB; MOB-PP model). The assumed $\delta^{13}C$ value for methanotrophic bacteria (Table 2) was based on $\delta^{13}CH_4$ values measured from Lake Mekkojärvi in September (Kankaala and others 2007) and assuming further isotopic fractionation during methane oxidation from −7.8 to −28.5‰ (Templeton and others 2006). The summer $\delta^{13}CH_4$ value was from the measured $\delta^{13}CH_4$ value of the lake water at three different depths (Table 2) on 15th

Table 1. The Major Functional Groups of Phytoplankton and Bacteria in Mekkojärvi representing Potential Food Sources for *Daphnia* in the Lake

Metabolic type	Species	References	Carbon source	δ^{13} of carbon source (‰)	Fractionation (‰)	References	Final $\delta^{13}C$ value (‰)
Phytoplankton							
Autotrophs	*Mallomonas* sp., *Cryptomonas* sp.	Arvola and others (1992)	Epilimnetic CO_2	−18.3 to −21.5	−10 to −15	Estimated here	−28 to −37
Photosynthetic bacteria							
Green sulfur bacteria (GSB)	*Chlorobium* sp.	Kuuppo-Leinikki and Salonen (1992); Taipale and Tiirola (Unpublished data)	Hypolimnetic CO_2	−17.1 to −20.9	−12 to −13.7	Sirevåg and others (1977); Hob and Sirevåg (1986)	−29 to −35
Chemosynthetic autotrophs							
Methane oxidizing bacteria (Mainly type 1)	*Methylobacter* spp. *Methylophilus* sp.	Taipale and Tiirola (Unpublished data)	CH_4 and other one carbon compounds	−45.1 to −72.9	−7.8 to −28.4	Templeton and others (2006)	−52 to −101
Iron oxidizing bacteria	*Gallionella* sp.	Taipale and Tiirola (Unpublished data)	Hypolimnetic CO_2	−17.1 to −20.9	−20 to −25	Ruby and others (1987); Hadas and others (2001)	−38 to −43
Heterotrophs							
Terrestrial organic carbon utilizers	*Polynucleobacter* sp., *Actinobacteridae*	Taipale and Tiirola (Unpublished data)	Organic substances	−27.4 to −28.1	up to 1		−27 to −29

Most of the groups and species were reported from earlier investigations (as denoted in column 3), whereas the presence of others is based on current unpublished studies. The $\delta^{13}C$ values for different groups were either derived from measured carbon sources with carbon fractionation values for different groups estimated from the experiments, or else were taken from the literature (as denoted in column 8).

Table 2. Estimated $\delta^{13}C$ Values for Cells of Methane Oxidizing Bacteria derived by Adding, as Alternative Values for Fractionation during Methane Oxidation, either −7.8, −14.3 or −28.5‰ to the Measured $\delta^{13}CH_4$ Values

	15. 9. 2005			22. 9. 2005		
	Depth (m)			Depth (m)		
	0–0.6	0.6–1.2	1.2–1.8	0–0.6	0.6–1.2	1.2–1.8
$\delta^{13}CH_4$ (‰)	−50.9	−55.5	−72.9	−45.1	−47.1	−48.7
Fractionation						
−7.8	−58.7	−63.3	−80.7	−52.9	−54.9	−56.5
−14.25	−65.1	−69.8	−87.2	−59.4	−61.4	−62.9
−28.5	−79.4	−84.0	−101.4	−73.6	−75.6	−77.2

The $\delta^{13}CH_4$ values are values measured from different depths in Mekkojärvi during late stratification (15.9.2005) or during autumnal turnover (22. 9. 2005).

September 2005, when the lake was still stratified. The autumn $\delta^{13}CH_4$ value was from 22nd September 2005 when the water column had started mixing. The MOB-PP model was run using minimum, maximum and intermediate values for fractionation during methane oxidation added to the $\delta^{13}CH_4$ values for each season.

The algal $\delta^{13}C$ values were estimated from a two source mixing model assuming that POC consisted only of algae and terrestrial detritus. The proportion of algae in POC was estimated by multiplying the amount of chlorophyll-*a* by 25, a mean value for carbon:chlorophyll ratio in algae according to Gosselin and others (2000). The carbon:chlorophyll ratio for phytoplankton is certainly variable, but the higher values such as 40 used by Bade and others (2006) yielded a proportion of algae in POC in excess of 100% for Mekkojärvi, which is not realistic. Because detrital algae with degraded or no chlorophyll would further bias the carbon:chlorophyll ratio towards higher values, the inappropriateness of higher values for our study suggest that detrital algae were unimportant in the carbon pool of Mekkojärvi. The $\delta^{13}C$ value of POC was linearly related with the proportion of chlorophyll-*a* in POC ($y = -27.2 - 4.233x$, $n = 22$, $p < 0.01$, $r^2 = 0.33$). At zero chlorophyll the equation indicated a $\delta^{13}C$ value of terrestrial detritus (−27.2‰) which agreed well with the measured $\delta^{13}C$ of DOC in Mekkojärvi (−27.8 ± 0.3‰). The algal $\delta^{13}C$ was then calculated from the following equation:

$$\delta^{13}C_{algae} = [\delta^{13}C_{POC} - (-27.2‰ \times \text{proportion of detritus})]/\text{estimated proportion of algae}$$

Because the pH in Mekkojärvi was generally only slightly above 5.0, we assumed that the $\delta^{13}C$ of dissolved CO_2 ($\delta^{13}CO_{2(aq)}$) could be approximated by the measured $\delta^{13}C$ of DIC.

A photosynthetic fractionation value between DIC and algae (ε) of 13.1 ± 2.8 was then obtained by comparing the calculated algal $\delta^{13}C$ value with the measured DIC $\delta^{13}C$ in the control enclosures during the summer experiment. This fractionation value (13.1‰) is similar to the estimate by Bade and others (2006) of phytoplankton fractionation between 12 and 16‰ in their unenriched lakes. The fractionation (ε_{dph}) value for *Daphnia* relative to DIC was then calculated by adding the widely used 1‰ ^{13}C enrichment between consumer and food source. In the next step, the proportion (w) of terrestrial organic matter as heterotrophic bacteria or of methanotrophic bacteria and the proportion of memory carbon (m) were calculated for combined results using nonlinear regression as an estimation method in SPSS with the following equation:

$$\delta^{13}C_{Dt} = (1 - w)[(1 - m)(\delta^{13}CO_{2(aq)} - \varepsilon_p)_t + m(\delta^{13}CO_{2(aq)} - \varepsilon_p)_{t-u}] + w(x)$$

where $\delta^{13}C_{Dt}$ is the measured $\delta^{13}C$ of *Dahpnia* at time t, w is the proportion of carbon in *Daphnia* from heterotrophic or methanotrophic bacteria, m is the proportion of memory carbon in *Daphnia* from u lags before day t, background values for $\delta^{13}CO_{2(aq)}$ (measured as $\delta^{13}C$ of DIC) during the summer and autumn experiments were −18.8 and −21.3‰, respectively), x in the TOM-PP model is $\delta^{13}C$ of DOC and in the MOB-PP model the $\delta^{13}C$ of methanotrophs (Table 3).The model best fit our data when the lag time u was 6 days. This

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Table 3. Parameter Values for Models of *Daphnia* $\delta^{13}C$ Dynamics in Treatment Enclosures in Lake Mekkojärvi during the Summer and Autumn Experiments

Experiment	Model	$\delta^{13}C_{PP}$ (‰)	ϵ_{PP}	ϵ_{MOB}	$\delta^{13}C_{w}$(‰)	u	m	w	r^2	RSD	AIC
Summer	TOM-PP	DIC + ϵ	14.1		−27.8	6	0.60	0.37	0.54	10.2	28.1
	MOB-PP	DIC + ϵ	14.1	−7.8	−58.7	6	0.46	0.20	0.47	10.9	28.5
	MOB-PP	DIC + ϵ	14.1	−14.3	−69.8	6	0.47	0.17	0.45	11.1	28.6
	MOB-PP	DIC + ϵ	14.1	−28.5	−101.4	6	0.48	0.11	0.41	11.5	28.8
Autumn	TOM-PP	DIC + ϵ	14.1		−27.8	6	0.15	1.31	–	7.8	26.5
	MOB-PP	DIC + ϵ	14.1	−7.8	−52.9	6	0.06	0.87	–	4.1	22.6
	MOB-PP	DIC + ϵ	14.1	−14.3	−61.4	6	0.16	0.77	–	6.3	25.2
	MOB-PP	DIC + ϵ	14.1	−28.5	−77.2	6	0.22	0.64	–	9.4	27.6

Because the residual sums of squares were higher than the corrected sums of squares, r^2 for the autumn experiment is not shown in the table. See text for explanation of parameters and variables.
$\delta^{13}C_w$ is the $\delta^{13}C$ value of either TOM or MOB.
r^2 = 1- (residual sum of squares/corrected sum of squares), - indicates residual sum of squares higher than corrected sum of squares.
RSD = mean of residuals; during the summer experiment n was 7 and during the autumn experiment n was 8.
AIC = Akaike Information Criterion for low sample sizes.

was presumably related to the instar duration of *Daphnia*, which is from 3–8 days at a temperature range of 10–18°C (Bottrell and others 1976).

Pelagic Carbon Mass Balance

In addition to model results derived from the $NaH^{13}CO_3$ enrichments, we made mass balance calculations (mg C m^{-2} d^{-1}) for summer and autumn periods to obtain another, independent assessment of carbon flow in the pelagic food web of Mekkojärvi.

The estimates of primary production and heterotrophic bacterial production (^{14}C-leucine uptake) were obtained from the daily mean values of the enclosure experiments. For daily production estimates the mean dry weight biomass of *D. longispina* was converted to carbon by a factor 0.5 (Salonen and others 1976) and biomass was converted to net production (mg C m^{-2} d^{-1}) assuming conservative daily growth rates of 0.2 in summer and 0.1 in autumn. These values were obtained from field and laboratory growth experiments with *D. longispina* (Ojala and others 1995; Ojala and Salonen 2001), in which the daily growth rate of *Daphnia* varied between 0.1 and 0.4 depending on the quantity and quality of food. Methanotrophic (MOB) activity in the water column was not measured during the enclosure experiments. Thus, for the mass balance calculations, MOB activity measured in 2005 as 24 h consumption of CH_4 in glass syringes at in situ temperatures for each respective 0.6 m sampling interval during the periods 11 July–1 August and 12 September–18 October (Kankaala unpublished; methods in Kankaala and others 2006a) was used for the summer and autumn periods, respectively. An estimate of hypolimnetic green-sulfur bacterial production was obtained from in situ anaerobic dark inorganic ^{14}C-uptake results for Mekkojärvi (Kuuppo-Leinikki and Salonen 1992). Net production of protozoan flagellates was estimated from the growth rate results of Salonen and others (1992).

We made mass balance calculations assuming a range of moderate and high growth efficiencies of MOB and low and high assimilation efficiencies of *Daphnia*. Growth efficiency of MOB has been reported to range between 6 and 77% (Bastviken and others 2003), but we used a narrower range between 25%, which is typical for pelagic bacteria in general (del Giorgio and Cole 1998), and 50% reported by Rudd and Hamilton (1978) for methanotrophs in a Canadian shield lake (see also Templeton and others 2006). The assimilation efficiency of *Daphnia* has been reported to range from 17 to 96%, but is commonly between 30–70% (Urabe and Watanabe 1991; He and Wang 2006). The efficiency has been found to be lowest at algal food concentrations above 2 mg C l^{-1}, a level which is higher than the total POC concentration in the epi- and hypolimnion of Mekkojärvi. Thus, we assumed values of 33 and 50% respectively, representing low and high assimilation efficiencies of *D. longispina*.

Results

Physical and Chemical Conditions

During the open-water season of 2004 there was unusually frequent rain and consequent discharge into the lake, which increased water color in the

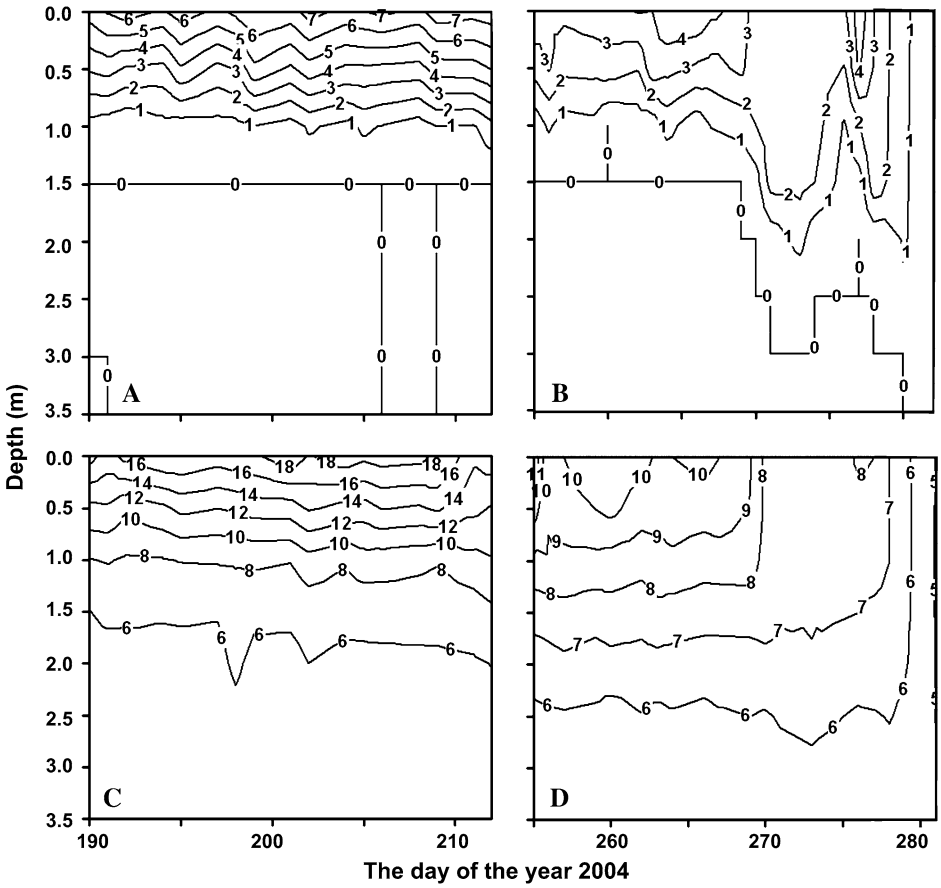

Figure 1. (**A**, **B**) Mean oxygen (mg l^{-1}) and (**C**, **D**) temperature (°C) from six replicate enclosures during the summer and autumn experiments. Maximum deviations between enclosures were 1.6 mg O_2 l^{-1} and 1.1°C.

lake itself from 280 mg Pt l^{-1} in June through 550 mg Pt l^{-1} in July up to 700 mg Pt l^{-1} in September. Total phosphorus and nitrogen in enclosures decreased slightly during the summer experiment, but were constant during the autumn experiment. Mean values down the whole water column were 18.1 ± 0.8 µg P l^{-1} and 876 ± 152 µg N l^{-1} during the summer experiment and 23.7 ± 8.1 µg P l^{-1} and 1038 ± 62 µg N l^{-1} during the autumn. In both experiments the water columns in the enclosures were sharply stratified, but by the end of the autumn experiment the whole water column had mixed (Figure 1). For most of the time oxygen was at the limit of detection (0.3 mg l^{-1}) by 1.5 m depth. Because of this sharp stratification no added $DI^{13}C$ reached the hypolimnion until the water column started to mix towards the end of the autumn experiment.

The wet conditions influenced enclosure DIC, DOC, POC and chlorophyll-*a* values (Figure 2). The overall concentration of DIC was higher during the autumn experiment (5.7–9.3 mg l^{-1}) than in the summer experiment (3.5–5.7 mg l^{-1}) due to higher inflow and to mixing of the water column towards the end of the autumn experiment. DIC in the hypolimnion was about twice that in the epilimnion. DOC was initially higher in the epilimnion (summer 35.9 ± 1.0 mg l^{-1} and autumn 40.5 ± 3.2 mg l^{-1}) than in the hypolimnion (summer 25.6 ± 1.1 mg l^{-1} and autumn 28.3 ± 3.32 mg l^{-1}), but the concentration of DOC in the epilimnion decreased to the hypolimnetic level during both experiments.

The POC concentration was similar during summer and autumn experiments. POC concentration in the hypolimnion was substantially higher than in the epilimnion. POC and chlorophyll-*a* values did not vary significantly between enclosures (ANOVA, $F = 1.108$, $P = 0.363$ for POC and $F = 0.775$, $P = 0.571$ for Chl-*a*). Based on the estimated C:Chl-*a* ratios, POC in the epilimnion comprised 20–70% algae during the summer experiment. In the hypolimnion during the summer experiment the concentrations of chlorophyll, much of which was probably bacterial chlorophyll-*d* (Kuuppo-Leinikki and Salonen 1992), and POC showed similar patterns, suggesting that during the summer experiment much of the POC in the hypolimnion consisted of green-sulfur bacteria. During the autumn experiment total POC was

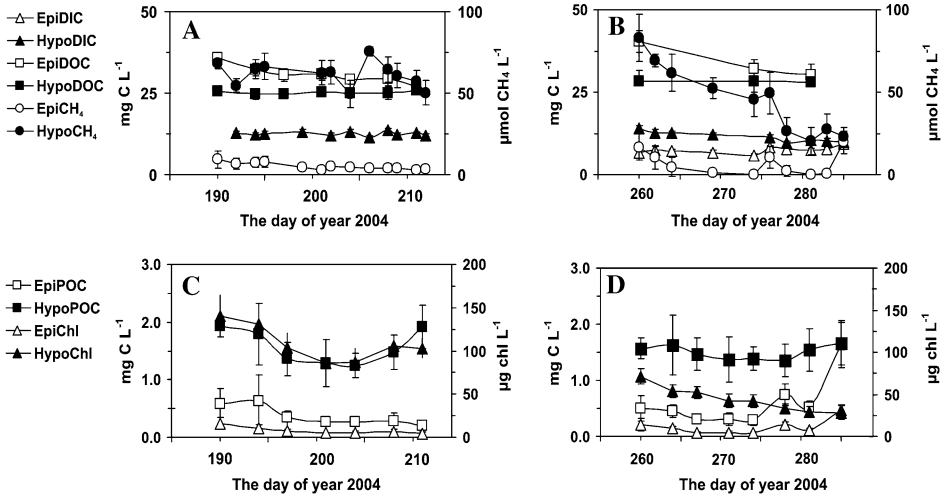

Figure 2. The concentrations of DIC, DOC (mg C l^{-1}) and CH_4 (µmol CH_4 l^{-1}) in the epilimnion (epi) and the hypolimnion (hypo) through the summer (**A**) and the autumn (**B**) experiments. Also shown are POC (mg C l^{-1}), chlorophyll (µg C l^{-1}) in the epilimnion for the summer (**C**) and autumn (**D**) experiments.

steady when hypolimnetic chlorophyll values were lower than in summer and further decreased through the experiment.

In the enclosures, the 0–1 m integrated primary production during the summer experiment (Figure 3A) was 58 ± 6 mg C m^{-2} d^{-1} (mean ± SE), but varied greatly, from 5 to 275 mg C m^{-2} d^{-1}, both between enclosures and within enclosures between days. At the beginning of the autumn experiment, the 0–1 m integrated primary production was only 5 mg C m^{-2} d^{-1} and subsequently dark fixation exceeded light fixation. Bacterial volumetric production ranged from 1 to 15 mg C m^{-3} d^{-1} between enclosures. The depth-integrated (epilimnion + hypolimnion) bacterial production (Figure 3B) from all enclosures was 13.0 ± 0.4 and 10.9 ± 0.4 mg C m^{-2} d^{-1} in the summer and autumn experiments, respectively. The density and biomass of *Daphnia* also varied greatly between enclosures, but the average was similar during the summer and autumn experiments (Figure 3C). The density of adult *Daphnia* was highest in the oxic epilimnion of the enclosures, being 10,000–110,000 ind. m^{-3} during the summer experiment and 10,000–70, 000 ind. m^{-3} during the autumn experiment. *Daphnia* density in the hypolimnion was similar during both experiments, varying between 0 and 10,000 ind. m^{-3}. The mean (±SE) integrated biomass of *Daphnia* was 914 ± 41 mg DW m^{-2} in the summer experiment and 885 ± 23 mg DW m^{-2} in the autumn experiment.

Stable Isotope Values

In control enclosures, carbon stable isotope values of DIC, POC and DOC were similar in both experiments (Figure 4), although DIC was slightly more ^{13}C-enriched during the summer (−18.8 ± 0.88‰) than during the autumn experiment (−21.32 ± 0.62‰). The mean δ^{13}C of DOC was −27.9 ± 0.62‰ during both experiments and there was only a minor difference in δ^{13}C of POC between the summer (−29.6 ± 1.5‰) and the autumn (−30.9 ± 0.6‰) experiments.

In the enriched enclosures there was striking variation in the δ^{13}C values of DIC, POC and *Daphnia*. During the summer experiment (Figure 4A, b), DIC in the epilimnion became enriched with ^{13}C soon after addition of ^{13}C bicarbonate was started on 8th July, with δ^{13}C reaching 44 ± 7.2‰ after 16 days. The hypolimnetic DIC of enriched enclosures was slightly enriched (−12.1 ± 4.2‰) in comparison to control enclosures (−20.6 ± 0.8‰) during the summer experiment (data not shown). The δ^{13}C values of POC in the epilimnion responded to DIC enrichment and were highest (−5.0 ± 13.6‰) after 18 days. This ^{13}C labeling transmitted to *Daphnia*, which had highest δ^{13}C (8.8 ± 16.4‰) 21 days after starting ^{13}C-bicarbonate addition, whereas the δ^{13}C of *Daphnia* was stable in control enclosures (−41.5 ± 0.9‰). Enrichment did not influence the δ^{13}C of DOC, which was very stable (−27.6 ± 0.3‰).

During the autumn experiment (Figure 4B, D), epilimnetic DIC was also soon enriched, with highest δ^{13}C (45.9 ± 21.5‰) 21 days after addition started. This enrichment also affected the δ^{13}C of DIC in the hypolimnion (data not shown), especially when the lake began to mix, with δ^{13}C increasing to 18.6 ± 19.4‰, whereas the δ^{13}C of DIC was stable in the hypolimnion of control enclosures (−20.6 ± 0.8‰). In the autumn experiment the enrichment only slightly affected the δ^{13}C of POC (−29.6 ± 2.6‰) which was just 1.6‰

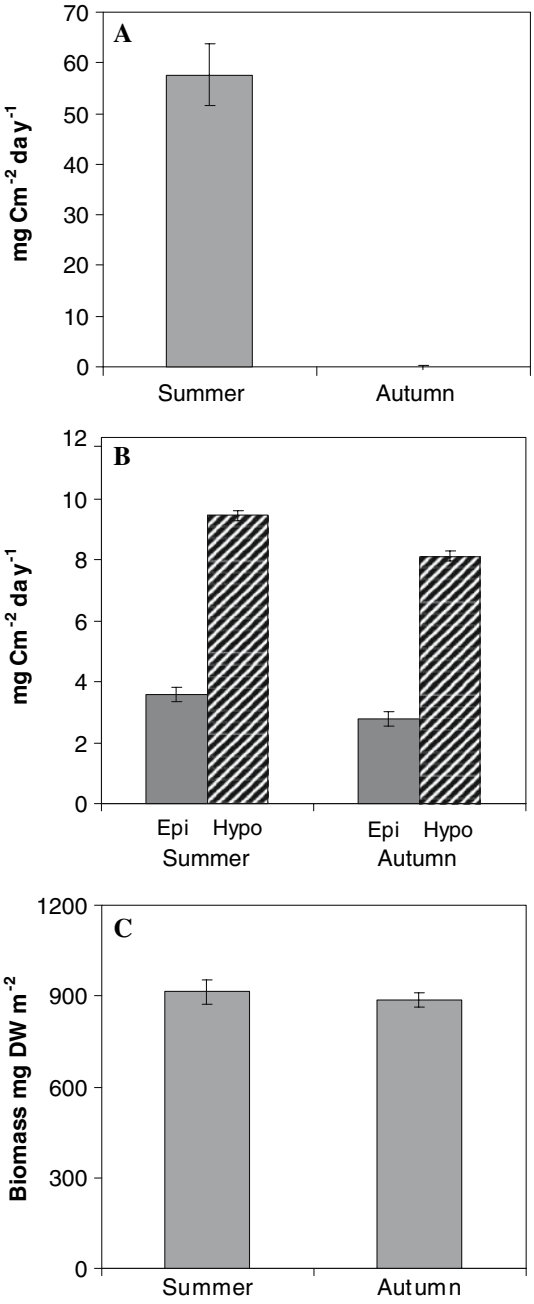

Figure 3. The mean values (±SE) of **A** primary production, **B** bacterial production and **C** zooplankton biomass in the enclosures during the summer and autumn experiments. Primary production and zooplankton biomass values are the whole water column, but bacterial production is separated onto the epilimnion (0–0.6 m) and hypolimnion (0.6–3 m).

higher than the $\delta^{13}C$ of epilimnetic POC in control enclosures. Nevertheless, this slight ^{13}C-enrichment was evidently transmitted to *Daphnia* because, although they actually became progressively more ^{13}C-depleted through the experiment, this ^{13}C-depletion of *Daphnia* was slightly less in the experimental enclosures (from −40.6 ± 0.4 to −45.6 ± 3.3‰) than in the control enclosures (from −40.4 ± 0.4‰ to −50.4 ± 2.8‰). The $\delta^{13}C$ of DOC was again stable (−27.9 ± 0.6‰) and similar to the summer experiment.

Origin of *Daphnia* Carbon, Model Results

For the summer experiment the TOM-PP and MOB-PP models best fit the *Daphnia* $\delta^{13}C$ when lag time (u) was 6 days and the proportion of memory carbon was 0.60 and 0.46–0.48 in the two models, respectively (Table 3). The TOM-PP model suggested that 37% of the diet originated from terrestrial sources. When the model was run with the lowest ($\varepsilon = 10$) and the highest ($\varepsilon = 15$) fractionation values for phytoplankton, the proportion of TOM (w) in *Daphnia* diet only varied between 0.36 and 0.41. The MOB-PP model suggested that the proportion of methanotrophic bacteria in the diet of *Daphnia* was 11–20%. The TOM-PP model fit the data a little better than the MOB-PP model, according to the standard deviation of residual means (Table 3). The TOM-PP model did not provide a satisfactory fit to data from the initial point and neither model fit the last point of the summer experiments, which explains the rather high residuals. This is probably because these points represent phases of transition between unenriched and enriched conditions in the enclosures.

The TOM-PP model did not provide a good fit to the data from the autumn experiment and actually gave its best solution (Figure 5) when the proportion of memory carbon (m) was 0.15 and TOM (w) was greater than 1.00 (Table 3). Because this implies that during the autumn experiment *Daphnia* acquired negligible autotrophic carbon, we used the MOB-PP model for this experiment. However, because there was actually little variation in *Daphnia* δ^{13} carbon values during the autumn period, the predicted values from the MOB-PP model did not significantly correlated with the observed values. Nevertheless, the predicted values matched observed data well and produced low RSD values (Table 3). The MOB-PP model estimated the contribution of methanotropic bacteria (w) to *Daphnia* carbon to be 64–87%, when the lag time (u) was 6 days and the proportion of memory carbon (m) was 0.06–0.22.

Carbon Mass Balance

Algal primary production was sufficient to support 21–32% of the carbon demand of *Daphnia* during summer, but was less than 1% in autumn

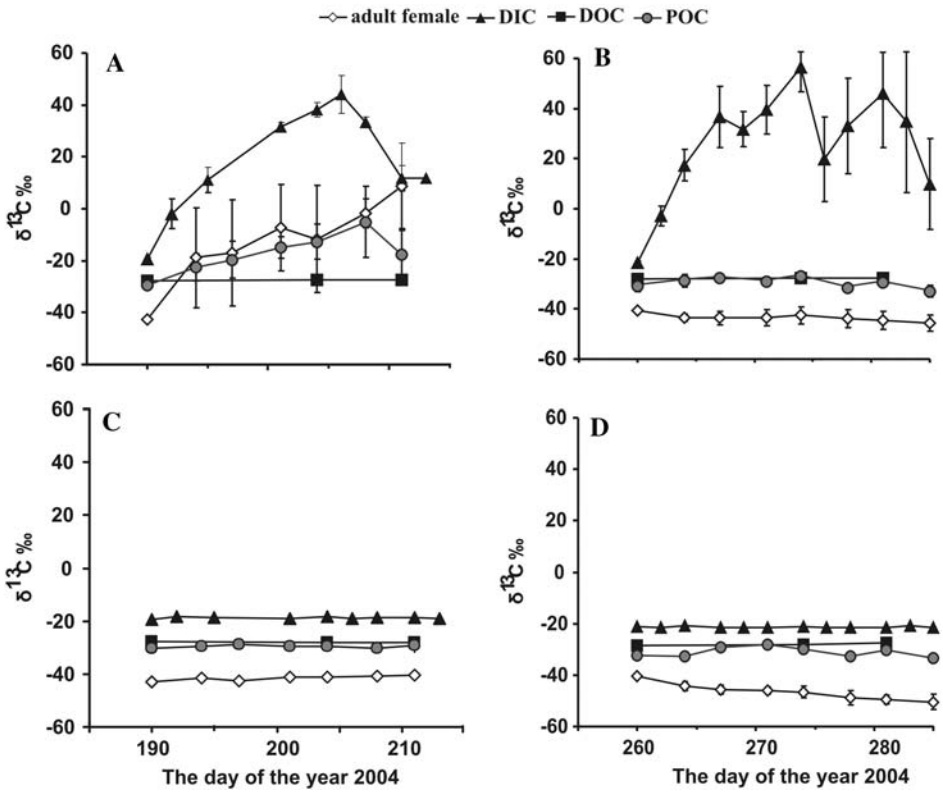

Figure 4. The mean values (±SD) for $\delta^{13}C$ of DIC, DOC, POC, and adult female *Daphnia* in the epilimnion of treatment (**A**, **B**) and control (**C**, **D**) enclosures during the summer and autumn experiments.

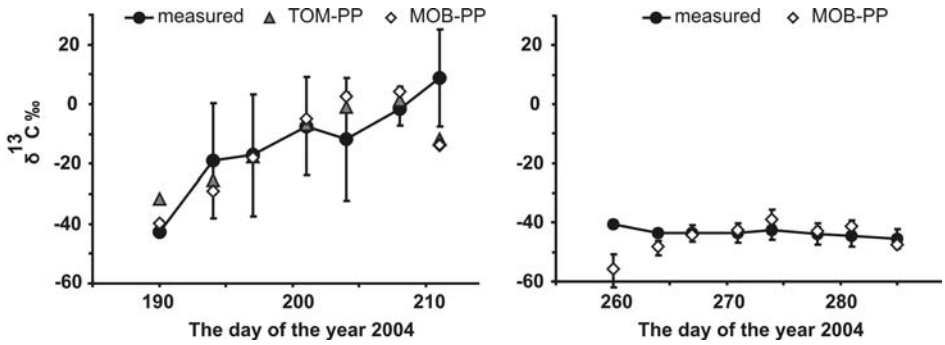

Figure 5. The measured and modeled $\delta^{13}C$ of adult female *Daphnia* in the epilimnion of enclosures during the summer and autumn experiments (means with standard deviation). For the MOB-PP model represented values are mean values, when ε_{cells} is −14.3‰ and the deviation represent a range of ε from −7.8 to −28.5.

(Figure 6). Assuming moderate growth efficiency of MOB and low assimilation efficiency of *Daphnia*, the estimated contribution of methanotrophs to the food carbon demand of *Daphnia* was 7% in summer and 37% in autumn (Figure 6). When high growth and assimilation efficiencies were assumed the estimated proportion of MOB rose to 21% in summer, whereas in autumn MOB was sufficient to support the entire food carbon demand of *Daphnia*. In summer the productivity by green-sulfur bacteria could support 15–22% of the carbon demand of *Daphnia*, but the mass balances indicate rather high proportions of unknown food sources (18–52%) necessary to sustain the productivity of *Daphnia*.

Discussion

During both summer and autumn experiments, the added $NaH^{13}CO_3$ quickly increased the epilimnetic DIC $\delta^{13}C$ from −20 to 50‰, but had little influence on DIC in the hypolimnion until the water column started to turn over towards the end of the autumn experiment. This added $NaH^{13}CO_3$ was rapidly

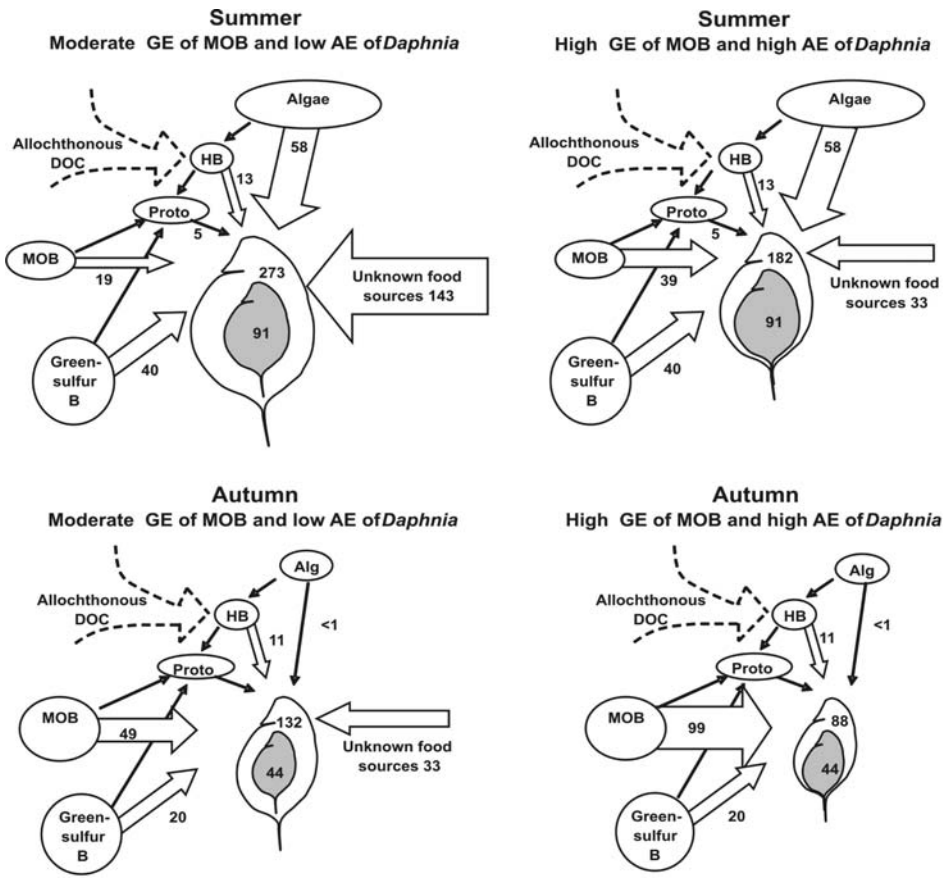

Fig. 6. Mean carbon mass balance (mg C m^{-2} d^{-1}) in the food web of Lake Mekkojärvi during summer and autumn calculated by assuming moderate growth efficiency (GE) of methane oxidizing bacteria (MOB) and low assimilation efficiency (AE) of *D. longispina* and assuming high GE of MOB and high AE of *D. longispina*, respectively. Other *symbols HB*, heterotrophic bacteria; *Proto*, Protozoa; *grey* area of *Daphnia* denotes net production and *grey* + *white* area of *Daphnia* denote total carbon demand by *Daphnia*.

incorporated by photosynthetic phytoplankton during the summer experiment, but apparently not during the autumn experiment when net primary production was very low. However, *Daphnia* biomass was similar during the summer and autumn periods, which indicates that there must have been alternative food sources utilized by *Daphnia* besides autotrophs (phytoplankton). These other food sources not only keep the *Daphnia* population alive, but sustain a *Daphnia* growth rate similar to that with phytoplankton as food (compare Kankaala and others 2006b). These other food sources include heterotrophic, chemoautotrophic and photoautotrophic bacteria and, given the low phytoplankton production and the dominance of terrestrial organic matter in Mekkojärvi, they presumably all depend primarily on allochthonous sources of carbon. With so many alternative food sources available in this lake, quantifying their exact contribution to zooplankton diets is a rather intractable challenge.

The residuals of our models were higher than those from models for individual lakes (Pace and others 2004) because we used mean values from three replicate enclosures with rather high deviations due to somewhat different initial conditions in these enclosure systems. *Daphnia* actually became most rapidly ^{13}C-enriched in the enclosure with the highest primary production (81.6 mg C m^{-2} d^{-1}) indicating an increase in the proportional contribution of algae to *Daphnia* diet when phytoplankton biomass increases. All the carbon model approaches include some parameters, such as the proportion of memory carbon in *Daphnia*, which ideally should be studied separately. This would decrease the number of unknown parameters, and increase the reliability of model results, because if the model is just allowed to select the best combination of too many unknown parameters, it can produce unrealistic outputs and even ignore the possible contribution of other food sources such as methanotrophic bacteria (Pace and others 2004). However, models used together with calculated carbon mass balances appear for now to offer the best approach to estimate the contributions of different carbon sources to the pelagic foodweb.

For the summer experiment the TOM-PP model suggested that 37% of the diet of *Daphnia* originated from terrestrial organic matter (and thus from heterotrophic bacteria) and the rest of the diet would have consisted of phytoplankton. However, according to the calculated pelagic carbon mass

balance, primary producers could have supplied only 21–31% of *Daphnia* carbon demand in summer. This in turn is higher than the estimate by Ojala and Salonen (2001) that carbon fixed by primary producers was only 25% of the carbon demand for somatic production and only 10–15% of the total food requirement of *Daphnia* in Lake of Mekkojärvi. Therefore either the TOM-PP model estimate of greater than 63% of *Daphnia* diet from PP is too high or the 24 h $NaH^{14}CO_3$ incorporation method underestimated true primary production in this polyhumic lake. The MOB-PP models suggested that 11–20% of *Daphnia* diet could have been methanotrophic bacteria, which is consistent with the range obtained from the carbon mass balance calculations (7–21%). Nevertheless, primary producers and MOB together could have supplied only about a half of *Daphnia* carbon demand in summer. The carbon mass balance calculations actually indicated that only 5–7% of *Daphnia* food came from heterotrophic bacteria, which is much lower than TOM-PP model estimate of 37%, but production of heterotrophic bacteria may have been underestimated by the ^{14}C-leucine uptake method in the polyhumic water. This is actually indicated by the approximately tenfold net higher bacterial growth rate (26–65 mg C m^{-3} d^{-1}) found by Salonen and others (1992) in the epilimnetic water of Mekkojärvi. If those are realistic estimates of heterotrophic bacterial production in the epilimnion of Mekkojärvi, a higher proportion (10–36%) of *Daphnia* carbon demand may actually have been supported by heterotrophic bacteria, close to the TOM-PP model estimate.

Our assumption that *Daphnia* also consumed green photosynthetic bacteria is based on the findings of Salonen and Lehtovaara (1992) who detected bacteriochlorophyll-*d* from guts of *Daphnia* in Mekkojärvi. Additional evidence that *Daphnia* in Mekkojärvi do migrate vertically to anoxic water layers comes from our recovery of ^{13}C-enriched *Daphnia* from the hypolimnion even when hypolimnetic DIC was not enriched. Therefore it is likely that *Daphnia* were mainly living in the oxic epilimnion, but undertaking short forays to the anoxic hypolimnion when food quantity or quality was inadequate in the upper oxic layers. Our results suggest that during both the summer and the autumn experiments *Daphnia* migrated to the oxic-anoxic boundary zone to feed on phototrophic, methanotrophic and other chemotrophic bacteria. *Daphnia* likely also fed on ciliates, whose density is highest in that layer (Arvola and others 1992).

The model results of Pace and others (2004) and Carpenter and others (2005) from inorganic ^{13}C-enrichments into three contrasting temperate lakes suggested that a significant proportion of zooplankton carbon (22–74%) ultimately originated from terrestrial sources, with the highest proportion in the dystrophic lake. Cole and others (2006) concluded that the pathway from allochthonous DOC via bacteria was less important compared with a direct use of terrestrial POC by zooplankton. In polyhumic Mekkojärvi, the proportion of detrital carbon in total POC was 20–60% and 20–70% in summer and autumn, respectively, and potentially could have been a food source for *Daphnia*, especially in summer, as indicated by the unknown food sources in the mass balance estimates. However, in laboratory experiments detritus proved to be poor food for *Daphnia* (Ojala and others 1995). Most *Daphnia* are known as efficient bacterial feeders (Brendelberger 1991; Jürgens 1994) and in zooplankton communities dominated by *Daphnia* a significant proportion of bacterial production may be channelled further in the food web (Pace and others 1990; Jürgens 1994). A moderate proportion of bacteria in the diet of *D. longispina* in summer (2–44%) and a higher proportion in autumn (55–73%), was also found in in situ grazing measurements in Mekkojärvi (Kankaala 1988), which supports the conclusions drawn in this study from the TOM-PP and MOB-PP models and the mass balance calculations.

Because primary production by phytoplankton was negligible during the autumn experiment, its contribution to *Daphnia* diet could have been only a few per cent at most. Actually, *Daphnia* from the ^{13}C-enriched enclosures were 4.8‰ heavier than *Daphnia* from the control enclosures, indicating some small carbon contribution from primary producers. According to the MOB-PP models, 64–87% of *Daphnia* diet was methanotrophic bacteria during autumn. In view of the high growth rate of MOB and the consequent reduced fractionation (Templeton and others 2006), it seems likely that more than 77% would have consisted of MOB. Indeed, the carbon mass balance estimate suggested that MOB could supply all the carbon demand of *Daphnia*, if a high growth efficiency of MOB and a high assimilation efficiency of *Daphnia* were assumed. Thus, the results from the MOB-PP model are consistent with the calculated range of 37–100% of MOB from the carbon mass balance. The consistency of results from these two independent approaches gives us confidence that methanotrophic bacteria really do make a highly significant contribution to *Daphnia* diet in this lake, as is directly suggested by the exceptionally low δ^{13}C values of *Daphnia* (compare Jones and others

1999). The carbon mass balance for autumn suggested that 15–23% of *Daphnia* carbon demand could then have come from green sulfur bacteria (*Chlorobium* sp.). However, as the water column mixed the *Chlorobium* sp. biomass decreased rapidly and finally disappeared with the increasing oxygen that is lethal for *Chlorobium* species (Van Gemerden and others 1995). Therefore it is most likely that *Daphnia* were feeding mainly on methanotrophic bacteria during the autumn experiment with some 8–12% of carbon heterotrophic bacteria as the carbon mass balance indicated.

An increase in MOB production during water column mixing has been reported previously (Rudd and Hamilton 1978; Kankaala and others 2006a). MOB have been estimated to provide a substantial carbon source for zooplankton (Bastviken and others 2003) and benthic fauna (Grey and others 2004; Jones and Grey 2004; Eller and others 2005). In laboratory experiments, Kankaala and others (2006b) found the $\delta^{13}C$ signature of *Daphnia* to be significantly more depleted when feeding on CH_4-supplemented food suspension and that a food suspension with a high proportion of MOB was not poorer food than that consisting mainly of phytoplankton. Lennon and others (2006) argued that ^{13}C-depletion of zooplankton during summer and early autumn stratification in lakes with a DOC gradient ranging from approximately 1–14 mg C l^{-1}, which is far less than in Mekkojärvi (20–40 mg C l^{-1}), was due to selective feeding on phytoplankton utilizing ^{13}C-depleted respiratory carbon, rather than to feeding on methanotrophic bacteria. However, we found the greatest importance of MOB in the diet of *Daphnia* during autumnal mixing of water masses, which again emphasizes the importance of considering seasonal changes in the use of different food sources by zooplankton.

The high biomass and production of *Daphnia* in Mekkojärvi indicate that *Daphnia* readily consume all available groups of potential food, such as phytoplankton, ciliates, green sulfur bacteria, heterotrophic and chemoautotrophic bacteria, and methanotrophic bacteria during summer and autumn. Our results indicate that the proportion of contemporaneous autochthonous carbon is relatively high (about 30–40%) in summer, but only 1–5% in autumn; the remainder of the *Daphnia* carbon requirement would come from sources that ultimately depend on non-contemporaneous primary production carbon. In view of the low phytoplankton production and the high content of terrestrial organic matter in this lake, we must conclude that most of this carbon from non-contemporaneous primary production is of allochthonous origin. In particular, our model outputs and carbon balance calculations, together with the very low $\delta^{13}C$ *Daphnia* values in the control enclosures, provide the first demonstration that a major part, or even all, of the carbon demand of *Daphnia* can be supplied by MOB during autumn in certain lake types.

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