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Experimental $\delta^{13}C$ evidence for a contribution of methane to pelagic food webs in lakes

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Abstract

We tested the hypothesis that low stable carbon isotope ($\delta^{13}C$) values commonly observed for zooplankton in humic lakes are due to their feeding on isotopically light methane-oxidizing microbes, and thus that methane-derived carbon is important in the food webs of these lakes. In replicate laboratory cultures, *Daphnia longispina*, a common crustacean zooplankter in humic lakes, were fed microbial suspensions with or without enrichment by biogenic methane. The $\delta^{13}C$ values of *Daphnia* indicated consumption of ^{13}C-depleted methanotrophic bacteria, while growth rates, survival, and reproduction of *Daphnia* in cultures enriched with methane were equal to or greater than those in nonenriched cultures. Results from lake enclosures during the autumn overturn period revealed a decrease in $\delta^{13}C$ of adult *Daphnia* from −40.5‰ to −50.3‰, reflecting extensive consumption of ^{13}C-depleted methanotrophic bacteria. Methane-derived carbon is a more important contribution to carbon flux through lake pelagic food webs than has previously been suspected.

Although lake food webs have been traditionally described as based on algal primary producers, recent investigations have shown that most lakes worldwide are actually net heterotrophic, i.e., community respiration exceeds primary production (Cole et al. 1994; del Giorgio et al. 1999). This imbalance is greatest in lakes with high concentrations of dissolved organic matter (DOM) originating from the catchment (Salonen et al. 1983; Jansson et al. 2000). In small sheltered boreal lakes with a high concentration of allochthonous humic DOM, hypolimnetic anoxia is a typical phenomenon during summer and winter stratification. As a consequence of the anaerobic decomposition of organic matter in the sediment, the concentration of methane (CH_4) may be high (>100 mmol m^{-3}) in the hypolimnion (Riera et al. 1999; Kortelainen et al. 2000). Most of the CH_4 produced (50–100%) is oxidized to CO_2 in the water column in a metalimnetic oxic-anoxic interface zone and partly incorporated into microbial mass (Bastviken et al. 2003; Kankaala et al. 2006). Thus, methanotrophic bacteria could be an important, and hitherto largely ignored, carbon source for zooplankton in some lakes.

Stable carbon isotope analyses ($\delta^{13}C$) are now widely applied in studies of the sources and fluxes of organic matter in lake pelagic food webs (Grey et al. 2001; Pace et al. 2004). Crustacean zooplankton consume food items (algae, bacteria, heterotrophic protozoa) that can rarely be separated in field samples and hence are usually analyzed as bulk particulate organic matter (POM, >0.5 μm). Lake zooplankton are often ^{13}C-depleted (lower $\delta^{13}C$) relative to POM (del Giorgio and France 1996; Grey et al. 2000). This might be accounted for by selective feeding on isotopically light photosynthetic algae, which have utilized dissolved inorganic carbon (DIC) originating from microbial respi-

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ration. However, Jones et al. (1999) reported that in small boreal forest lakes, the zooplankton $\delta^{13}C$ actually *increased* with phytoplankton biomass but decreased with increasing concentration of humic substances. They hypothesized that the observed low zooplankton $\delta^{13}C$ values (−35‰ to −45‰) could be due to feeding on isotopically light methanotrophic bacteria. Biogenic methane (CH_4) is highly ^{13}C-depleted (Whiticar 1999), while CH_4-oxidizing bacteria can further fractionate the carbon isotopes (Summons et al. 1994).

We experimentally tested the hypothesis that methanotrophic bacteria could serve as food for lake zooplankton. We compared $\delta^{13}C$ values and growth and reproduction of *Daphnia longispina* (Cladocera, Crustacea), which is known to be an efficient bacterial feeder (Kankaala 1988), in two continuous-flow culture experiments: one where *Daphnia* were fed with natural lake microbial food with ($+CH_4$) or without ($-CH_4$) added biogenic methane, and one additional culture experiment where *Daphnia* were fed with predominantly algal food. Further evidence was obtained from enclosures in a polyhumic lake, in which $\delta^{13}C$ signature was followed in relation to CH_4 concentration in the water column during the autumn turnover period.

Methods

Laboratory experiments—For laboratory experiments, we used *Daphnia longispina* isolated from polyhumic Lake Mekkojärvi, southern Finland (Arvola et al. 1992). A stock of *Daphnia* was maintained in batch cultures in water from Lake Pääjärvi, southern Finland, to which green algae, *Scenedesmus* sp., grown in an appropriate algal medium (Lynch et al. 1986), was added (about 20–30 × 10^3 cells mL^{-1} d^{-1}) to provide high-quality food. In two growth experiments (referred to as M1 and M2), natural microbial suspension from polyhumic Lake Mekkojärvi was provided as food, while in one further experiment (ALG), *Daphnia* was fed with predominantly algal food. The experiments were carried out in darkness in flow-through chambers (Lampert 1976; volume: 240 mL, flow rate: ~1.8 L d^{-1}) at constant temperature (20 ± 0.3°C). For both M1 and M2 experiments, juvenile *Daphnia* <0.5 mm were transferred to eight replicate chambers (40–45 individuals in each). Four chambers ($+CH_4$) received the natural microbial food suspension from Lake Mekkojärvi to which biogenic CH_4 was added 24 h before feeding was started, and four chambers ($-CH_4$) received the same microbial suspension without added CH_4. The rationale for this experimental setup was to keep the original microbial community as similar as possible in both treatments, but provide the $+CH_4$ treatments with greater availability of CH_4.

The natural microbial food for the M1 and M2 experiments was collected from polyhumic Lake Mekkojärvi in autumn and winter to ensure the presence of methanotrophic bacteria but negligible density of photosynthetic algae. About 500 L of near-surface water was pumped into a 1000-L polyethylene tank and stored at +4°C in darkness to prevent algal growth. Water for the M1 experiment was taken on 18 November 2002 after autumnal turnover in the lake, and for the M2 experiment, on 16 March 2003 from under ice. The food suspension was prepared daily from this water by filtration through a 10-μm net and was kept for 24 h at 20°C in darkness before use. Biogenic CH_4 was obtained from anaerobic fermentation of aquatic plants in 500-mL bottles with gas-tight septa. For the M1 experiment, *Spirodela polyrhiza* and *Lemna minor* (wet weight 10 ± 0.8 g $bottle^{-1}$) from Lake Pääjärvi were fermented for 82–89 d, and for the M2 experiment, submerged moss (*Warnstorfia procera*) from Lake Mekkojärvi (wet weight 32 ± 3 g $bottle^{-1}$) was fermented for 89–98 d according to the procedure described in Kankaala et al. (2003). During fermentation, the headspace CH_4 concentration of the bottles rose to 10–27 × 10^3 ppmv. Microbial food suspension was prepared in glass-stoppered, 11-L bottles. Gas overpressure from the headspace of a fermentation bottle was released via an aquarium air stone as fine bubbles, raising the CH_4 concentration for the $+CH_4$ food suspension. Methanotrophic consumption of CH_4 was measured in 50-mL glass syringes filled after the CH_4 addition. CH_4 concentration was measured immediately in three syringes and in another three after 24-h dark incubation at +20°C. Methane concentration was determined using a headspace equilibrium technique (McAuliffe 1971) with an Agilent 6890 N (Agilent Technologies, Wilmington, DE, USA) gas chromatograph (flame ionization detector [FID] 205°C, oven 40°C, CarbonPlot capillary column, He as carrier gas).

Biomass of food was obtained daily from samples of food suspensions taken before feeding to *Daphnia* and 24 h later before replacement with new food bottles. Samples were preserved in Lugol's iodine solution, and numbers and volumes of bacteria in the food suspensions were determined from acriflavine-stained (Bergström et al. 1986) samples (first decolorized with thiosulfate) using epifluorescence microscopy (Olympus BX60, Olympus Optical Co., Tokyo, Japan) and analySIS 3.1 Soft Imaging System (www.soft-imaging.net). The total bacterial cell volume was then converted to carbon using a factor of 0.36 (Tulonen 1993) to provide a measure of carbon food concentration available to *Daphnia*. In addition, in the M2 experiment, the concentration of total particulate organic carbon (POC) was measured from 1-L samples filtered on pre-ignited Whatman glass-fiber GF/F filters (Whatman International Ltd., Maidstone, England) according to Salonen (1979).

In the ALG experiment, *D. longispina* was cultured in six flow-through chambers with predominantly algal food <10 μm from mesohumic Lake Pääjärvi (Kankaala et al. 1996) that had been further enriched with cultured *Scenedesmus* sp. (12–13 × 10^3 cells mL^{-1}). The food biomass was estimated by analyzing POC of 50-mL suspension filtered on pre-ignited Whatman GF/C filters as described above.

For growth rate and stable carbon isotope analyses of *Daphnia*, three subsamples (100–150 individuals in each) of the juveniles (<0.5 mm) used to initiate growth experiments were dried at 60°C in aluminium foil cups, and their dry weight (DW) was determined on a microbalance (precision 0.01 mg). At the end of the experiment,

individuals from each growth chamber (juveniles and adults separately, 15–150 individuals per batch) were treated similarly. The growth rate (d^{-1}) was calculated as

$$\frac{\ln W_t - \ln W_{t0}}{t}$$

where W_{t0} = initial weight, W_t = final weight, and t = duration of the experiment (d).

Field enclosures—A field experiment was carried out in polyhumic Lake Mekkojärvi (61°13′N, 25°08′E, area 0.0035 km^2, max. depth 3.5 m). Due to its sheltered position and high concentration of dissolved organic carbon (20–40 g C m^{-3}), the lake is steeply stratified in summer, and the concentration of CH_4 can be >150 mmol m^{-3} in the anoxic hypolimnion below 1 m depth. For the period of the autumnal overturn of water masses (16 September to 11 October 2004), three flexible polyethylene cylinders (wall thickness 2 mm, diameter 1.9 m, volume 11.3 m^3) were installed in the middle of the lake to enclose the whole water column from the sediment surface to around 15 cm above the water surface. The enclosures contained a natural population of *D. longispina*. Temperature and oxygen concentration in the water column were measured at 0.5-m intervals with a combined probe (YSI 55, Yellow Springs Instruments, Ohio, USA, accuracy ±0.3°C, ±0.3 mg L^{-1} O_2). Samples for carbon stable isotope analysis, density, and biomass of *D. longispina* were taken every 3–4 d with a 60-cm-long Limnos tube sampler (volume 4.25 L) and were retained on a net with a mesh size of 100 μm. Samples were taken separately from the euphotic zone (two pooled samples from 0 to 0.6 m) and from the water column beneath (four pooled samples from 0.6 to 3 m). The samples for the other variables were taken from a 40 L bucket that collected the water passed through the 100-μm-mesh net. Photosynthetic production was measured in the euphotic zone (0–60 cm) as uptake of inorganic ^{14}C according to Keskitalo and Salonen (1994). Methane samples were taken into 60-mL polypropene syringes, kept in crushed ice until analyses within 4 h with gas chromatography as described above. The sampling procedure used probably underestimated CH_4 concentrations in the water column. However, in autumn 2005 in the same temperature and CH_4 range as in 2004, the CH_4 concentrations, measured in water samples taken with the same procedure as described above, were linearly related with those of samples taken in parallel from the same depths directly from the Limnos sampler into glass-stoppered bottles ($r^2 = 0.824$, $p = 0.002$, $n = 8$, 2–4 replicate measurements in each).

Consumption of CH_4 by methanotrophs in the water column was estimated as a linear decrease of CH_4 (weighted average in the water column) during the experiment. The proportion released to the atmosphere was calculated with boundary-layer diffusion equations (Phelps et al. 1998) from the concentration of CH_4 in the surface layer (0–0.6 m) assuming the range of wind speed from 0 at the enclosure surfaces to mean daily wind speed during the study period (0.3–2.1 m s^{-1}) at 1 m above the surface of a nearby lake (Valkea-Kotinen, distance ~4 km).

$\delta^{13}C$ analyses—Stable carbon isotope ratios ($\delta^{13}C$) of CH_4 from anaerobic fermentation of aquatic plants were determined from aliquots of headspace samples mixed in He gas offline. CH_4 was oxidized to CO_2 at 1000°C and introduced online to a mass spectrometer (PreCon Gas-Bench II Delta XL, Thermofinnigan Bremen, Germany). Carbon isotope ratios of POM were analyzed from around 1 L of food suspension collected on pre-ignited Whatman glass-fiber filters (GF/F, nominal pore size ~0.7 μm in M1 and M2 experiments, and GF/C, nominal pore size ~1 μm in ALG and field experiments). *Daphnia* carbon isotope ratios were determined from 0.2–0.5 mg of dried animals (i.e., 10–150 individuals). Isotope analyses were made using a Micromass Isoprime continuous-flow isotope ratio mass spectrometer interfaced with a Carlo-Erba NA1500 elemental analyzer (Carlo-Erba, Milan, Italy). Values are expressed as conventional $\delta^{13}C$ ‰ notation (relative to international standard Pee-Dee Belemnite; Peterson and Fry 1987).

Results

Laboratory experiments—In the M1 experiment, the initial CH_4 concentration in lake water was low (0.03 ± 0.004 mmol m^{-3}), and addition of biogenic CH_4 caused an average 260-fold increase in CH_4 concentration in the $+CH_4$ treatments (Fig. 1). The methanotrophic activity in the $+CH_4$ food suspension was 3.3 ± 2.1 mmol m^{-3} d^{-1} during the first 4 d, but during the last 6 d, methanotrophic activity was lower. This was reflected in a larger difference between the POM $\delta^{13}C$ values in the $+CH_4$ and $-CH_4$ food suspensions during the first half of the experiment (Fig. 2). The bacterial food concentration was slightly higher in the $+CH_4$ than in $-CH_4$ treatments (Table 1). However, *Daphnia* growth and survival, and particularly the proportion of *Daphnia* individuals reaching maturity by the end of experiment, were all higher in the cultures receiving $+CH_4$ microbial food (Table 1). Moreover, the $\delta^{13}C$ values of *Daphnia* at the end of experiment were significantly lower (3‰) in $+CH_4$ than in $-CH_4$ cultures.

The original CH_4 concentration of water taken below the ice cover of Mekkojärvi for the M2 experiment was ~100-fold (3.9 ± 0.1 mmol m^{-3}) that in the water taken in autumn for the M1 experiment (Fig. 1). During 4 d storage, the concentration decreased to ~1 mmol m^{-3}, and by the end of the experiment, it was only ~0.07 mmol m^{-3}. In the $+CH_4$ food suspension, the CH_4 concentration increased to 7.1 ± 1.7 mmol m^{-3}, and most of this methane had been consumed by methanotrophic bacteria before the suspension was fed daily to *Daphnia*; methanotrophic activity during the whole experiment consumed methane at an average rate of 6.94 ± 1.67 mmol m^{-3} d^{-1}. The difference in the $\delta^{13}C$ of POM was significant, although the isotopic ratio decreased in both $+CH_4$ and $-CH_4$ treatments (from −31.2‰ to −32.6‰ and from −30.0‰ to −32.4‰, respectively; Fig. 2). The bacterial food concentration was slightly

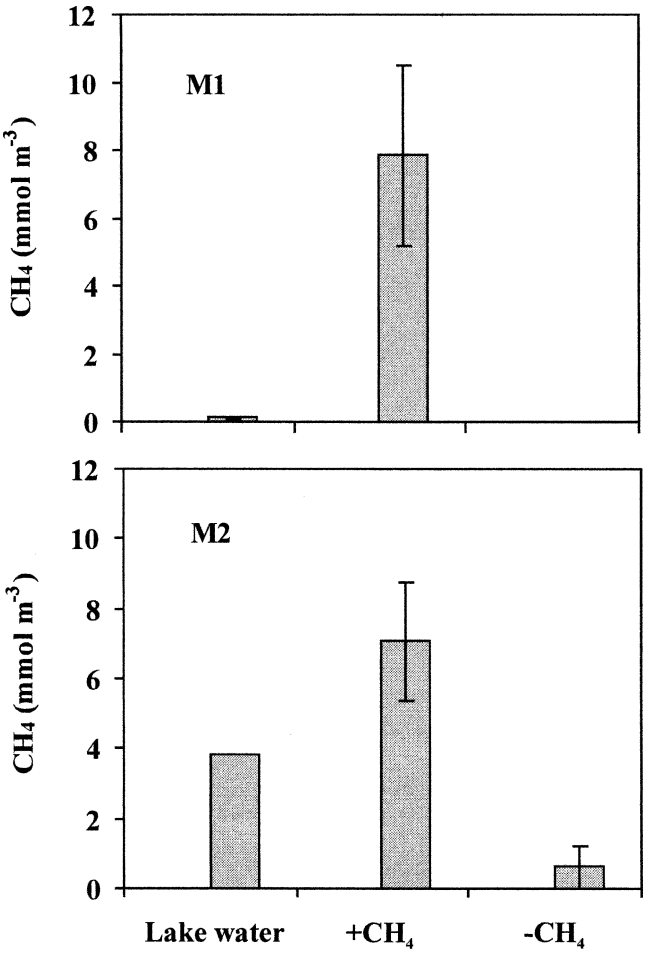

Fig. 1. Initial concentration of methane (CH_4 mmol m^{-3}) in lake water sampled on 18 November 2002 for M1 and on 16 March for M2 experiments, as well as mean CH_4 concentration in microbial food suspension one day prior to feeding to *Daphnia* in $+CH_4$ and $-CH_4$ treatments.

higher in the $+CH_4$ than in the $-CH_4$ treatments (Table 1), but overall, the biomass of bacterial food was ~10-fold that in both treatments of the M1 experiment. The total POC of the food suspensions was 834 ± 133 and 584 ± 215 mg C m^{-3} in the $+CH_4$ and $-CH_4$ treatments, respectively, and thus was four to five times higher than the bacterial biomass (Table 1). This total POC contained loose, brown, colloidal humic matter, which settled at the bottom of the food suspension bottles. This humic matter must have greatly influenced the mean $\delta^{13}C$ of POM. In the M2 experiment, the $\delta^{13}C$ value of *Daphnia* in both treatments decreased to a level typical for *Daphnia* in Lake Mekkojärvi (−40‰; cf. Jones et al. 1999), and the decrease was significantly greater (0.5‰) in the $+CH_4$ treatment (Table 1). Reflecting the greater food availability, the growth rate of *Daphnia* was higher in this experiment compared with M1, but there were no between-treatment differences. However, the proportion surviving and the number of juveniles produced were both higher in the $+CH_4$ treatment.

At the end of the ALG experiment, the $\delta^{13}C$ value of *Daphnia* (−23‰) was the same as that of the POM

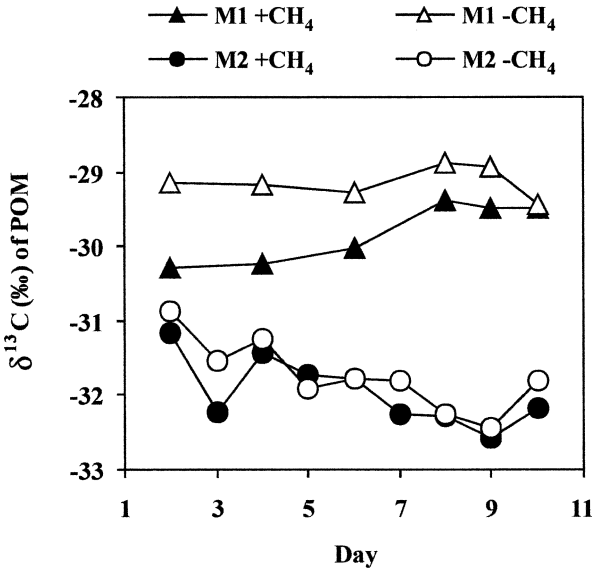

Fig. 2. $\delta^{13}C$ (‰) of particulate organic matter (POM) during the laboratory growth experiments in which *Daphnia longispina* was provided with natural microbial food suspension (M1 and M2) with ($+CH_4$) or without ($-CH_4$) added biogenic methane.

consisting predominantly of algal food. The much greater food availability in this experiment was reflected in a higher growth rate of *Daphnia* compared with that in the M1 and M2 experiments (Table 1). Irrespective of food type, the growth rate of *Daphnia* in the experiments was linearly related to the natural logarithm of food concentration (Fig. 3).

Field enclosures—During the field experiment period, when the autumnal turnover of the lake was proceeding, the mean CH_4 concentration in the water column of the enclosures decreased from 75 to 23 mmol m^{-3} (Fig. 4) simultaneously with disappearing anoxia and following the same pattern as observed in the lake (data not shown). Assuming wind speeds ranging from 0 to 2.1 m s^{-1}, 33–48% of this reduction in CH_4 concentration was estimated to have been due to release to the atmosphere; this proportion is very similar to that observed during the autumn overturn in nearby Lake Valkea-Kotinen (Kankaala et al. 2006). Thus the other 52–67% of the reduction in the CH_4 concentration was presumably due to consumption by methanotrophic bacteria at a rate between 3.3 and 4.2 mmol C m^{-2} d^{-1}. This was at the same level as algal primary production measured in the lake in July (4.8 ± 4.4 mmol C m^{-2} d^{-1}; Taipale, Kankaala, and Jones, unpubl. data). However, during the autumn period, algal primary production in this brown-water lake was undetectable in the enclosures, while dark uptake of inorganic ^{14}C, reflecting chemoautotrophic production, was only 0.6 ± 0.3 mmol C m^{-2} d^{-1}. However, the biomass of *Daphnia* at this time (890 ± 270 mg dry-weight m^{-2}) was similar to that observed in July (910 ± 510 mg dry weight m^{-2}). At the start of the experiment, $\delta^{13}C$ of *Daphnia* was −37.9‰ in juveniles and −40.5‰ in

Table 1. Summary of results from the continuous-flow culture experiments with *Daphnia longispina*. M1 and M2 refer to cultures supplied with natural microbial food with ($+CH_4$) and without ($-CH_4$) added biogenic methane; ALG refers to the culture supplied with algal food. Food concentrations supplied in the different treatments, $\delta^{13}C$ values of added CH_4 gas, POM during the experiments, and *Daphnia* at the beginning and at the end of the experiments, as well as population parameters for *Daphnia* are presented. Values are means ± standard deviations, with number of replicate analyses shown in parentheses. Statistical significance between means from $+CH_4$ and $-CH_4$ treatments for food concentration and POM $\delta^{13}C$ was tested with paired-sample *t*-test and for the other variables with *t*-test assuming equal variances; n.s. = not significant.

Experiment	M1+CH_4	M1–CH_4	M2+CH_4	M2–CH_4	ALG
Duration (d)	10	10	10.5	10.5	7
Food concentration (mg C m^{-3})	21±5 (10)	18±3 (10)	185±50 (11)	160±40 (11)	530±60 (9)
	(t=1.498, p=0.084)		(t=1.514, p=0.08)		
CH_4 $\delta^{13}C$ (‰)	−32.4±1.5 (3)		−63.4±1.3 (3)		
POM $\delta^{13}C$ (‰)	−29.8±0.4 (6)	−29.1±0.2 (6)	−32.0±0.5 (9)	−31.7±0.5 (9)	−23.0±0.4 (2)
	(t=4.060, p=0.005)		(t=2.433, p=0.02)		
Daphnia initial $\delta^{13}C$ (‰)	−19.7±0.1 (3)	−19.7±0.1 (3)	−18.5±0.1 (3)	−18.5±0.1 (3)	−18.6±0.1 (3)
Daphnia final $\delta^{13}C$ (‰)	−29.6±0.6 (4)	−26.6±0.5 (4)	−41.0±0.2 (6)	−40.5±0.3 (6)	−23.0±0.1 (6)
	(t=8.079, p<0.001)		(t=3.452, p=0.003)		
Growth rate (d^{-1})	0.06±0.01 (4)	0.04±0.01 (4)	0.19±0.01 (4)	0.19±0.02 (4)	0.28±0.02 (6)
	(t=2.067, p=0.042)		(t=0.047, n.s.)		
% survival	87.3	83.1	95.0	91.1	95.3
% individuals reaching maturity by end of experiment	44.0±8.2	24.6±6.8	100	100	100
	(t=3.644, p=0.005)				
Young per female at end of experiment	0	0	2.5±0.2 (4)	2.2±0.2 (4)	2.2±0.2 (6)
			(t=2.506, p=0.02)		

adults. However, during the experiment, *Daphnia* $\delta^{13}C$ decreased to −47.2‰ in juveniles and to −50.3‰ in adults; these decreases were strongly correlated (r^2 = 0.96 and 0.94) with the decrease in CH_4 concentration (Fig. 4). The $\delta^{13}C$ of DIC and POM (size fraction ~1–100 μm) both remained stable during the experiment (−20.9 ± 0.5‰ and −32.0 ± 1.0‰, respectively).

Discussion

Our results from the laboratory culture experiments indicate that $\delta^{13}C$ of both POM and *D. longispina* decreased significantly with increased availability of CH_4 to the microbial community in the food suspension. Moreover, the different plant material used for anaerobic

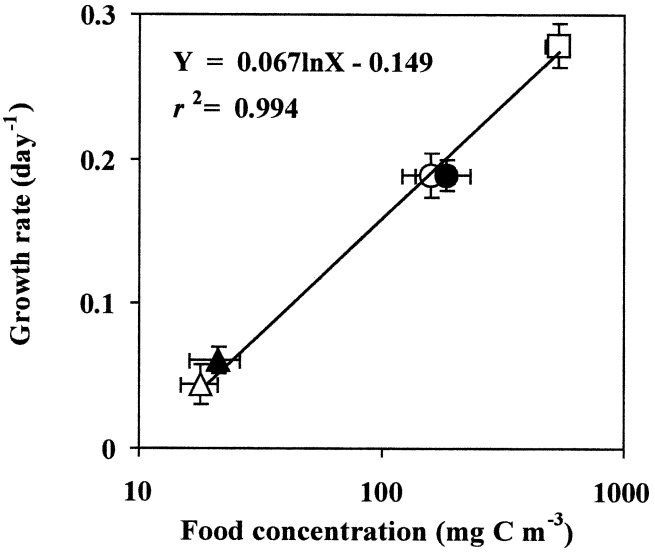

Fig. 3. Growth rates of *Daphnia longispina* in the laboratory culture experiments in the dark as a function of food concentration with food supplied as cultured algae (square) or as natural microbial suspension (M1 and M2, triangles and circles, respectively) with (solid symbols) or without (open symbols) added biogenic methane. Values are means ±SD (standard deviation).

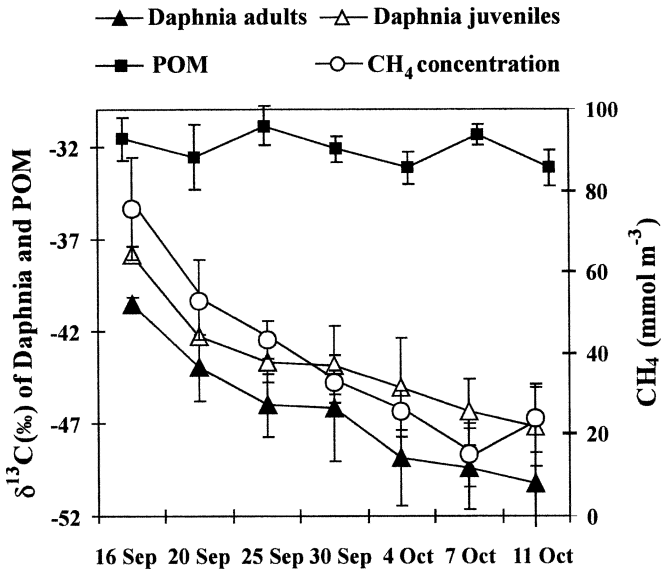

Fig. 4. $\delta^{13}C$ (‰) of particulate organic matter (POM), adult and juvenile *D. longispina*, and the concentration of CH_4 in the water column in enclosures in Lake Mekkojärvi during the autumn turnover 16 September to 11 October 2004. Values are means ±SD from three replicate enclosures.

fermentation in the M1 and M2 experiments yielded CH_4 with different $\delta^{13}C$ values (Table 1), and this strongly influenced the $\delta^{13}C$ values of POM and *Daphnia* observed in the two experiments. The discrepancy between the less-depleted $\delta^{13}C$ of POM relative to more-depleted $\delta^{13}C$ of *Daphnia* in the M2 experiment indicates that a high amount of allochthonous detrital carbon of terrestrial origin ($\delta^{13}C$ of C-3 terrestrial plants is typically −28‰; Peterson and Fry 1987) was retained on glass-fiber filters and contributed greatly to the overall $\delta^{13}C$ of POM, but that this particulate detritus was less important as a direct food source for *Daphnia*. Dominance of terrestrial detritus in total POM also accounted for the rather stable $\delta^{13}C$ of POM during the field experiment (Fig. 4).

In the M1 experiment, the bacterial food concentration, based on microscope counts, corresponded to that observed in the epilimnion (0–1 m) of Lake Mekkojärvi during summer stratification, whereas in experiment M2, it was ~10-fold higher, which is the level of bacterial biomass at the metalimnetic oxic-anoxic interface in the lake (Arvola et al. 1992). Quantitative analyses of phospholipid fatty acid (PLFA) composition (Taipale, unpubl. data) revealed that during winter stratification below ice (March), the proportion of type I methanotrophs (MOB I) was high. PFLAs specific for MOB I (16:1ω8, 16:1ω6, and 16:1ω5; see Guckert et al. 1991) constituted 12% of the total microbial PFLAs at the depth of 0–0.6 m and 24% at 0.6–3 m, while during the other seasons, the proportion of MOB I PLFAs was also significant in the microbial community in this lake. Thus, the presence of a high proportion of MOB I in the winter microbial community that was used as culture media in our M2 experiment was very probable, although not specifically quantified. In this experiment, methanotrophic bacteria consumed methane and produced biomass potentially available to *Daphnia* in both $+CH_4$ and $-CH_4$ treatments. Assuming 25% growth efficiency of pelagic bacteria (del Giorgio and Cole 1998), the methanotrophic activity in the M2 experiment would have caused a daily biomass increase of ~10 mg C m^{-3} during the first 4 d in the water stored in the tank, and ~21 mg C m^{-3} in the $+CH_4$ treatment. These potential mass increments are within the fluctuations of the total bacterial biomass during the experiment, but indicate the order of magnitude of the total bacterial biomass present in the M1 experiment (cf. Table 1).

The linear relationships of *Daphnia* growth rate to the log food concentration, of both microbial and algal origin (Fig. 3), as well as the "threshold" food concentration (at which growth rate drops to zero) of around 0.01 mg C m^{-3}, are both consistent with expectations for *Daphnia* of this size (Gliwicz 1990). Hence our data demonstrate that *Daphnia longispina* can grow and reproduce equally well on a diet rich in methanotrophic bacteria as on an algal diet; growth is principally a function of food quantity, but food quality evidently is not reduced by inclusion of a high proportion of methanotrophic bacteria relative to algae.

Evidence for the potential quantitative importance of methanotrophic bacteria in lake food webs came from field enclosures in Lake Mekkojärvi during the autumn turnover of water masses. In accordance with decreasing CH_4 concentration in the water column, $\delta^{13}C$ of juvenile *Daphnia* fell to −47.2‰ and that of adults fell to −50.3‰. These $\delta^{13}C$ values appear to be the lowest ones ever reported for freshwater zooplankton. The 3‰ lower $\delta^{13}C$ values in adults with respect to juveniles were likely due to the higher lipid content in eggs and embryos, since lipids are known to be ^{13}C-depleted (Matthews and Mazumder 2005). Since the $\delta^{13}C$ signature in DIC and POM remained stable and the algal primary production and chemoautotrophic fixation of DIC was negligible, the striking decrease in *Daphnia* $\delta^{13}C$ could not have been due to selective ingestion of isotopically light algae or chemoautotrophic bacteria. Due to the sampling procedure, the value for methanotrophic activity during the autumn turnover period in the enclosures (3.3–4.2 mmol C m^{-2} d^{-1}) was more likely underestimated than overestimated. In fact, during the following autumn in 2005, the methanotrophic activity in Mekkojärvi ranged from 4 to 41 mmol C m^{-2} d^{-1}, measured as CH_4 consumption in glass syringes for 24 h at respective in situ temperatures (Kankaala, unpubl. data). Hence our enclosure data clearly show that the high biomass of *Daphnia* in the lake in autumn was to a large extent sustained by feeding on methanotrophic bacteria. Thus, both laboratory and field results support the hypothesis that a pathway from detrital carbon via methane to methanotrophs can play a significant role in the food webs of humic lakes.

Ecosystems based on chemosynthesis are now well described from the deep oceans (Rau 1981). Chemosynthetic processes have been described from lakes (Wetzel 2001) but have not generally been considered quantitatively important in lake food webs. Anoxic, methane-producing zones are found in most lakes, especially in the sediments. In lakes with an oxic water column throughout the year, methane produced in sediments is mainly consumed by methanotrophic bacteria at the sediment surface and can help support benthic fauna (Kiyashko et al. 2001; Grey et al. 2004). However, in lakes with temporary or permanent anoxia in the hypolimnion, methanotrophic bacteria consume methane at the oxic-anoxic interface in the water column where they can be accessible to pelagic consumers (Rudd and Hamilton 1978; Bastviken et al. 2003). In a humic, boreal lake (Valkea-Kotinen) in southern Finland, net production of methanotrophs corresponded to 23–81% of total heterotrophic bacterial production and to 5–10% of algal primary production during the summer stratification and autumnal turnover periods (Kankaala et al. 2006). Even in the world's largest lakes, such as Tanganyika, annual methane oxidation is at least 10% of primary productivity (Rudd 1980). Our results have shown that methanotrophic bacteria provide suitable food for the growth and reproduction of a typical pelagic consumer, the crustacean zooplankter *Daphnia longispina*. We have also provided an in situ example of how methanotrophic bacteria can be quantitatively important in the diet of *D. longispina*. Since crustacean zooplankton like *Daphnia* are key links between lower and higher trophic levels in lake food webs, we suggest that a carbon flux from biogenic methane via methanotrophic bacteria may be more important in fuelling lake food webs than has previously been considered.

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