

Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content

Paula Kankaala · Sami Taipale · Lu Li ·
Roger I. Jones

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Abstract We used stable carbon and nitrogen isotope analyses to estimate the relative proportions of three putative food sources (1) algae, (2) allochthonous organic matter (but including also heterotrophic bacteria and green-sulphur bacteria having similar isotopic values) and (3) methane-oxidizing bacteria (MOB) in the diets of crustacean zooplankton in five small boreal lakes representing a gradient of dissolved organic carbon (DOC) concentration from ca. 5 to 40 mg C l⁻¹. The lakes were sampled in May, after establishment of stratification, and again in October during autumnal mixing of the water column. IsoSource mixing model outputs indicated that the proportion of algae in the diets of zooplankton was generally higher in May than in October, and that bacteria contributed to the diets of both cladocerans

and copepods. Our results indicate that bacteria, especially MOB, can make an appreciable contribution to zooplankton diets in these small lakes, even in those with relatively low DOC concentrations.

Keywords Boreal lakes · Zooplankton · Stable isotope analyses · Algal food · Bacterial food · Allochthonous organic matter · IsoSource model

Introduction

Although lake food webs have traditionally been perceived to be based on algal primary producers, there is growing evidence that pelagic food webs in lakes are subsidized to varying degrees by allochthonous inputs of dissolved organic matter (DOM) from their catchment area (e.g. Jones 1992; Carpenter et al. 2005; Jansson et al. 2007). This phenomenon seems to be greatest in lakes with high loads of allochthonous DOM (Salonen et al. 1983; 2005; Jansson et al. 2000). Salonen and Hammar (1986) found allochthonous organic matter to be an important food resource for zooplankton, particularly in highly humic lakes, with heterotrophic flagellates likely playing an important role as a food of zooplankton in humic waters. Direct use of colloidal DOM by heterotrophic and mixotrophic flagellates and even by metazoan zooplankton has been postulated (Salonen et al. 1992; Cole et al. 2006), while

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P. Kankaala
Lammi Biological Station, University of Helsinki,
16900 Lammi, Finland

P. Kankaala (✉)
Ecological Research Institute, University of Eastern
Finland, Joensuu Campus, Box 111, 80101 Joensuu,
Finland
e-mail: paula.kankaala@uef.fi

S. Taipale · L. Li · R. I. Jones
Department of Biological and Environmental Science,
University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä,
Finland

flocculation of DOM (see von Wachenfeldt et al. 2009) could also convert it to a form usable directly by zooplankton. However, both these possible pathways remain largely speculative, and bacterial production is probably the most important link converting allochthonous DOM to biomass available for higher trophic levels. Bacteria can either be grazed directly by macrozooplankton or passed through a bacteria–flagellate–macrozooplankton food chain (Jones 1992; Jansson et al. 2007). Particularly when the availability of algae is low, bacteria can be an important, direct food source for zooplankton (Kankaala 1988).

In small, sheltered, boreal lakes with a high concentration of allochthonous DOM, hypolimnetic anoxia is a typical phenomenon during both summer and winter stratification. Anaerobic decomposition of organic matter in the sediment may result in a high concentration of methane (CH₄) in the anoxic hypolimnion of these lakes (e.g. Rudd and Taylor 1980; Riera et al. 1999; Kortelainen et al. 2000). Most of the methane produced (50–100%) is oxidized to CO₂ in the water column by methane-oxidizing bacteria (MOB) in a metalimnetic oxic–anoxic interface zone and is thus partly incorporated into microbial mass (Bastviken et al. 2003; Kankaala et al. 2006a; 2007). In addition, dense communities of phototrophic and/or dark-carbon-fixing bacteria are typically observed in the anoxic hypolimnetic layers of lakes (Kuoppo-Leinikki and Salonen 1992; Camacho et al. 2001; Hadas et al. 2001). Observations of bacterial chlorophyll *d* (Bchl *d*) in the guts of zooplankton have indicated a contribution of these bacteria to their diets (Salonen and Lehtovaara 1992). Thus, the potential bacterial food sources for higher trophic levels may consist of several heterotrophic, chemosynthetic and/or photoautotrophic types.

Stable isotope analysis (SIA) has proved a useful tool in food web studies (e.g. Peterson and Fry 1987; Vander Zanden and Rasmussen 1999), because consumer tissues integrate dietary components over time and also indicate assimilation rather than ingestion (Rounick and Winterbourn 1986). Therefore, SIA may offer advantages over more conventional methods such as gut content analysis. In food web studies, carbon isotopes generally fractionate little (<1‰) between diet and consumer and can therefore act as an indicator of food sources, whereas nitrogen isotopes fractionate more (typically assumed

to be by approximately 3.4‰) and therefore have been widely used to define trophic position of the organism (Vander Zanden and Rasmussen 1999). Crustacean zooplankton consumes food items (algae, bacteria, heterotrophic protozoa) that can rarely be separated in field samples and hence in SIA are usually analysed as bulk particulate organic matter (POM). In some Finnish humic lakes, phytoplankton ($\delta^{13}\text{C}$ –28 to –37‰) is generally ¹³C-depleted relative to bulk POM ($\delta^{13}\text{C}$ –26 to –30‰) (Jones et al. 1999; Taipale et al. 2007). Lake zooplankton are also often ¹³C-depleted relative to POM and phytoplankton (e.g. del Giorgio and France 1996; Jones et al. 1999; Grey et al. 2000; Bastviken et al. 2003). Jones et al. (1999) hypothesized that the observed low zooplankton $\delta^{13}\text{C}$ values (–35 to –45‰) in small boreal lakes could be due to their feeding on isotopically light methanotrophic bacteria, and doing so to a greater extent in the more humic lakes with a higher loading of allochthonous organic matter and greater development of hypolimnetic anoxia. Biogenic methane is extremely isotopically light, with $\delta^{13}\text{C}$ values typically between –45 and –110‰, and the MOB utilizing this methane may further fractionate carbon (Whiticar 1999). Laboratory and field experiments using stable carbon isotope analyses of zooplankton and its food have supported the hypothesis that methane-derived carbon is important for zooplankton diet in some lakes (Kankaala et al. 2006b; Taipale et al. 2007; 2008).

Using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Daphnia longispina* and its food sources obtained in whole-lake DI¹³C addition experiments in a small humic lake, Mekkojärvi, Taipale et al. (2008) estimated the proportional contribution of four putative food sources in the diet of *Daphnia*. The likely contribution of phytoplankton was greatest in spring, while the contribution of MOB clearly increased from spring through summer to become a dominant proportion in autumn. Notable contributions from heterotrophic and green-sulphur bacteria to the diet of *Daphnia* were also observed, especially during spring and summer (Taipale et al. 2008). The aim of our study was to evaluate the wider applicability of these earlier results from polyhumic Mekkojärvi, and especially the contribution of MOB to zooplankton diets in different lakes. Therefore, five small Finnish forest lakes with contrasting water colour and DOC content were studied during spring and autumn using

SIA ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). We applied the IsoSource mixing model (Phillips and Gregg 2003) to estimate minimum and maximum ranges of algae, of allochthonous organic matter and of three bacterial groups in the diets of zooplankton from the lakes. We hypothesized that the contribution of MOB to the diets of zooplankton in small boreal lakes increases with the humic content of lakes, and that the importance of MOB is higher during the autumnal mixing period than during the spring. Because cladocerans are known to be more efficient grazers of bacteria compared with copepods (e.g. Hessen 1985; Nagata and Okamoto 1988), we also expected bacterial carbon to be more important in cladoceran diets than in copepod diets.

Materials and methods

Study area

The study lakes (Table 1) are situated in the Evo state forest area, Lammi, Finland. The five lakes were selected to provide a wide range of DOC concentration (ca. 5–40 mg C l⁻¹) from oligohumic to polyhumic conditions, but form a parallel gradient of size with areas from 0.145 to 0.003 km². The lakes also differ in their mixing patterns. The largest lake, Valkea Mustajärvi, is clearly dimictic, whereas in Valkea-Kotinen the spring turnover is often incomplete, although complete mixing of the water column

takes place in autumn. In the three smallest lakes (Alinen Mustajärvi, Nimetön and Mekkojärvi), the spring turnover is always incomplete and, although the shallowest lake, Mekkojärvi, is usually mixed to the bottom during autumn, in Alinen Mustajärvi and Nimetön the autumnal mixing probably never reaches the deepest areas (>6 m depth). The duration of the ice cover in winter varies from 4.5 to 5.5 months. In all the lakes, anoxic conditions form in the deepest area during summer stratification, thus promoting sediment methane production and its accumulation in the hypolimnion. During the summer stratification, the proportion of the oxic layer volume in the whole lake volume also varies in these lakes according to lake area (Table 1). The water in all the lakes is acidic (pH 5–6.5), being least so in the largest lake (Valkea Mustajärvi). In the smallest lakes, the DOC concentration and colour of the water may vary annually in strong correlation with precipitation; the values presented in Table 1 are observed ranges from dry and wet years. No planktivorous fish are present in the smallest lake (Mekkojärvi), whereas the other lakes support populations of perch and pike, and the largest lake (Valkea Mustajärvi) also contains a stocked population of whitefish.

Sampling

All the lakes were sampled at the deepest point between 16 and 29 May 2006, when the lakes had stratified, and again between 11 and 24 October 2006,

Table 1 Main characteristics of the study lakes

Lake	Coordinates	Area (km ²)	Max. depth (m)	Mean depth (m)	Volume (10 ³ m ³)	Oxic layer volume as % of total volume	pH	Colour in epilimnion (mg Pt l ⁻¹)	DOC (mg l ⁻¹)	Max. CH ₄ concentration measured in hypolimnion (μmol l ⁻¹)
Valkea-Mustajärvi (VM)	61°13'N; 25°07'E	0.145	11	4	558	96–100	6.0–6.5	27–30	5–6	67
Valkea-Kotinen (VK)	61°14'N; 25°04'E	0.040	6.5	3	103	62–80	5.4–5.9	100–180	13–18	230
Alinen-Mustajärvi (AM)	61°12'N; 25°06'E	0.008	6.5	4	31	50–63	5.3–5.4	120–150	11–15	900
Nimetön (N)	61°13'N; 25°10'E	0.005	11	8	36	26–40	5.4–5.9	360–400	22–44	>1,000
Mekkojärvi (MJ)	61°13'N; 25°08'E	0.003	4	3	11	30–45	5.9–6.0	200–600	18–40	160

Data for pH are from the measurements made during this study, and other values are from unpublished databases of Lammi Biological Station, University of Helsinki. Oxic layer volume is calculated as mean percentage of total lake volume through the summer stratification period

when the autumnal mixing of the water column was underway. Methane (CH₄) and dissolved inorganic carbon (DIC) samples were taken into 60-ml polypropylene syringes directly from the water in a 60-cm-long Limnos sampler (vol 4.25 l) collected at 1-m intervals (except in Mekkojärvi at 0.6-m intervals). The syringes were kept in crushed ice for <4 h before analysis. The other samples for chemical analyses, plankton biomass and density were pooled to give a single integrated sample for epilimnion (Epi), metalimnion (Meta) and hypolimnion (Hypo) (see Table 3 for the depth zones). The layers were gauged on site by measuring oxygen content and temperature at 0.5 m intervals with a YSI 55 probe (Yellow Springs Instruments, accuracy $\pm 0.3^\circ\text{C}$, $\pm 0.3 \text{ mg O}_2 \text{ l}^{-1}$). Transition zones from hypoxia to anoxia were detected from measurements of redox potential with a Schott redox-combination electrode (BlueLine 31 Rx) connected to a WTW MultiLine P3 pH/conductivity metre. From each sampling depth, measurements were made by inserting the tip of the electrode directly into the Limnos sampler filled with water, and the readings (mV) were noted after a stabilization period of ca. 1–3 min.

Water from the Limnos sampler was passed through a net with a mesh size of 100 μm . Zooplankton retained on the net was used for quantitative counting and species determination. The lake water passed through the net was saved for laboratory analysis of particulate organic carbon (POC), dissolved organic carbon (DOC), chlorophyll, bacterial biomass, phytoplankton biomass, and SIA of POM and DOM. Generally, 40 l of pre-filtered lake water was collected for these purposes. Zooplankton samples for SIA were collected by hauling a net of mesh size 100 μm through the whole water column at

several sites around the lake to avoid possible effects of zooplankton migration and patchiness.

SIA

Samples for $\delta^{13}\text{C}_{\text{DIC}}$ were taken directly from the Limnos sampler into 12 ml LABCO exetainers, which had previously been flushed with helium to expel all CO₂ and then injected with 0.5 ml H₃PO₄ (o- H₃PO₄, MERCK). Three 4-ml replicates were taken at 1-m intervals and were stored at 4°C prior to analysis. The analysis was done with a Gas Bench II (Thermo Finnigan) connected to DELTA^{plus} Advantage IRMS (see Taipale and Sonninen 2009, for details).

For stable isotope analyses of POM, 500 ml of water was filtered on pre-ignited Whatman GF/F glass fibre filters (1–2 subsamples per layer). The filters were dried at 60°C to constant weight, and the material retained on them was then scraped into tin cups.

For DOM samples, 100 ml of the filtrate passed through pre-ignited Whatman GF/F filters was acidified and freeze-dried (Christ alpha 1–4, B. Braun biotech International), and two replicates (ca. 0.5 mg of the dry material each) were prepared from each layer. If a sample size was insufficient, samples from different layers (Epi, Meta, Hypo) of the same lake were amalgamated to a whole water column sample.

Zooplankton samples were rinsed into deionised water and, after gut evacuation for ca. 20 h, crustaceans were sorted manually using watch-maker forceps into sub-classes (Cladocera, Copepoda). The animals were sorted into pre-weighed tin cups containing a drop (30 μl) of deionized water. Generally, three replicates were prepared for one sample,

Table 2 Range of stable carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}\text{‰}$) values used for the food source types: algae, allochthonous organic matter (AlloOM) including heterotrophic

Lake	$\delta^{13}\text{C}_{\text{algae}}$	$\delta^{15}\text{N}_{\text{algae}}$	$\delta^{13}\text{C}_{\text{AlloOM+HB+GSB}}$	$\delta^{15}\text{N}_{\text{AlloOM+HB+GSB}}$	$\delta^{13}\text{C}_{\text{MOB}}$	$\delta^{15}\text{N}_{\text{MOB}}$
VM	–33 to –37	2.1 to 5.0	–26 to –30	1.6 to 2.7	–62 to –64	–2.8
VK	–26 to –30	1.1 to 5.0	–26 to –30	1.4 to 1.6	–62 to –64	–2.8
AM	–26 to –30	–1.9 to 5.0	–26 to –30	–2.8 to 0.9	–62 to –64	–2.8
N	–37 to –41	0.6 to 5.0	–26 to –30	0.4 to 2.0	–62 to –64	–2.8
MJ	–37 to –41	0.0 to 5.0	–26 to –30	–2.3 to 2.4	–62 to –64	–2.8

See “Materials and methods” for details

bacteria (HB) and green-sulphur bacteria (GSB) and methanotrophic bacteria (MOB) used in the IsoSource model for estimating the diet of zooplankton in the five study lakes

each with dry weight around 0.5 mg. The samples were dried at 60°C overnight to constant dry weight and the cups were then compacted into tight balls excluding air, ready for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis.

The zooplankton, POM and DOM samples were then analysed with a Carlo-Erba Flash 1112 series Elemental Analyzer connected to a DELTA^{plus} Advantage IRMS (Thermo Finnigan) and run against IAEA standard NBS-22 using dried and homogenized fish muscle as an internal laboratory working standards. The standard deviation between replicates was normally within 0.2‰ for both carbon and nitrogen.

Other laboratory analyses

For bacterial and phytoplankton counting, 200-ml samples were immediately fixed with 1-ml Lugol's solution. For analysis, bacterial samples were first decolorized with thiosulfate, and then stained with acriflavine (Bergström et al. 1986) on black polycarbonate filters (pore size 0.22 μm). Ten random fields per filter were counted with an epifluorescence microscope (Olympus BX60, Olympus Optical Co., Tokyo, Japan) at 1,000 \times magnification connected to analySIS 3.2. Soft Imaging System (www.soft-imaging.net). The numbers and volumes of bacteria were calculated according to the geometric forms of bacteria based on the software results. The total bacterial cell volume was then converted to carbon using a factor of 0.36 $\text{pg } \mu\text{m}^{-3}$ (Tulonon 1993).

Species composition and biomass of phytoplankton samples were determined with an inverted microscope at 600 \times magnification using a settling chamber technique. A 50-ml sample was settled for 24 h, and random points were counted until the recorded number of most species reached 200. The phytoplankton abundance and biomass were calculated based on the counted number, size and geometric form of phytoplankton. Biovolume was converted to carbon with a factor 0.2 $\text{pg } \mu\text{m}^{-3}$ (Reynolds 2006). Zooplankton samples were immediately fixed in formaldehyde (final concentration 4%) for later microscopical counting. The composition and abundance of zooplankton were determined using a Leica L2 microscope at 25 \times magnification for Cladocera and Copepoda, and a Nikon Optiphot inverted microscope at 100 \times magnification for rotifers.

For chlorophyll measurements, one pooled water sample (0.5–1 l) from each layer was filtered in the

dark on pre-ignited Whatman GF/C filters. The pigments were extracted in ethanol and measured with a SHIMADZU UV-2100 Visible Recording Spectrophotometer from 320 to 772; 665 and 750 nm for Chl *a* and 654 nm for BChl *d*. The concentration of Chl *a* was analysed and calculated with standard methods (SFS 5772, www.SFS.fi), and of BChl *d* calculated using the equation of Takahashi and Ichimura (1970). Because the area of spectral peaks of Chl *a* and BChl *d* overlap each other, the results are presented as total chlorophyll dominated by either *a* or *d* type depending on the position of the peak (665 or 654 nm) in the spectrum.

Particulate organic carbon (POC) was measured from water samples filtered on a pre-ignited Whatman GF/F glass fibre filter with a high temperature combustion method according to Salonen (1979). DOC concentration (fraction <0.22 μm) was analysed with a SHIMADZU TOC-5000A Total Organic Carbon Analyzer. DIC and methane concentrations were analysed from acidified samples with a headspace equilibrium technique using an AGILENT 6890 N (Agilent Technologies) gas chromatograph equipped with FID and TCD 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).

Data analysis

We used the IsoSource model (Phillips and Gregg 2003) to estimate min–max ranges of different food source types: algae, allochthonous organic matter and bacteria [heterotrophic (HB), green-sulphur (GSB) and methanotrophic bacteria, MOB] in the diets of zooplankton (Cladocera and Copepoda). Estimates of values for the $\delta^{13}\text{C}$ of algae and bacteria were obtained from compound-specific analyses (see Boschker and Middelburg 2002 for a review) of algal and bacterial fatty acids (FA) in the studied lakes. Extraction of FAs is described in Taipale et al. (2009), and $\delta^{13}\text{C}$ analyses of FAs were determined with a GC-C TA III connected to an Isotope Ratio Mass Spectrometer (IRMS, DELTAPLUS[®], Thermo Co, Taipale, unpublished). Algal $\delta^{13}\text{C}$ values for the biomarker ω -3 FAs (sum of stearidonic, eicosapentaenoic and docosahexaenoic acids) differed between oligohumic (Valkea Mustajärvi), mesohumic (Valkea-Kotinen, Alinen-Mustajärvi) and polyhumic (Nimetön, Mekkojärvi) lakes (Table 2). Thus, the

ranges of specific $\delta^{13}\text{C}$ -FA values, corrected with 6–10‰ fractionation (Van Dongen et al. 2002) were used for each lake. The $\delta^{13}\text{C}$ values of 15:0-*iso*- and *anteiso*-branched FAs from the epilimnion of oligohumic and mesohumic lakes were used for HB, $\delta^{13}\text{C}$ values of 14:0, 16:0, *anteiso*-15:0 and 16:1 ω 7c from the hypolimnion of Mekkojärvi were used for GSB. FAs typical of MOB were found from all lakes, and the $\delta^{13}\text{C}$ values of 16:0 ω 5c, a biomarker of type I MOB (Bowman et al. 1991; Taipale et al. 2009), were used for MOB (the range of the values was similar in all lakes). All values obtained for different bacterial groups were corrected for 2–5‰ fractionation (Hayes 2001). Because the ranges of $\delta^{13}\text{C}$ values for HB (–26 to –29‰), for GSB (–28 to 30‰) and for allochthonous organic matter (AlloOM) originating from terrestrial C3 plants (commonly measured to range from –27 to –28‰) overlapped, these potential food sources were treated together in the IsoSource model (Table 2).

The $\delta^{15}\text{N}$ values for algae could not be estimated by any direct method. Thus, we assumed (see Table 2) that these values could vary from those measured for epilimnetic POM in each lake to the $\delta^{15}\text{N}$ value ($5 \pm 2\%$) obtained by Taipale et al. (2007) for algae in Mekkojärvi during the seasonal phytoplankton maximum. The possible range of $\delta^{15}\text{N}$ values for AlloOM + HB + GSB in each lake was obtained from $\delta^{15}\text{N}$ values of DOM in the whole water column (assumed representative of AlloOM + HB) and from those of hypolimnetic POM (assumed representative of GSB present so abundantly in anoxic conditions that they dominated total POM in the hypolimnion, cf. Tables 3 and 4). The $\delta^{15}\text{N}$ value of MOB was assumed to be –2.8‰ as estimated by Taipale et al. (2008) in Mekkojärvi. We further assumed that zooplankton did not enrich $\delta^{13}\text{C}$ relative to its food sources. Cladocerans were assumed to enrich $\delta^{15}\text{N}$ by 2.5‰ (Matthews and Mazumder 2008). Because $\delta^{15}\text{N}$ values of copepods were higher and correlated with those of cladocerans sampled simultaneously in the study lakes (see “Results”), the copepods were assumed to enrich $\delta^{15}\text{N}$ by 4.8‰ relative to algae, bacteria and allochthonous OM.

All statistical analyses (Pearson correlation, regression analysis) were done with SPSS 16.0 programme package.

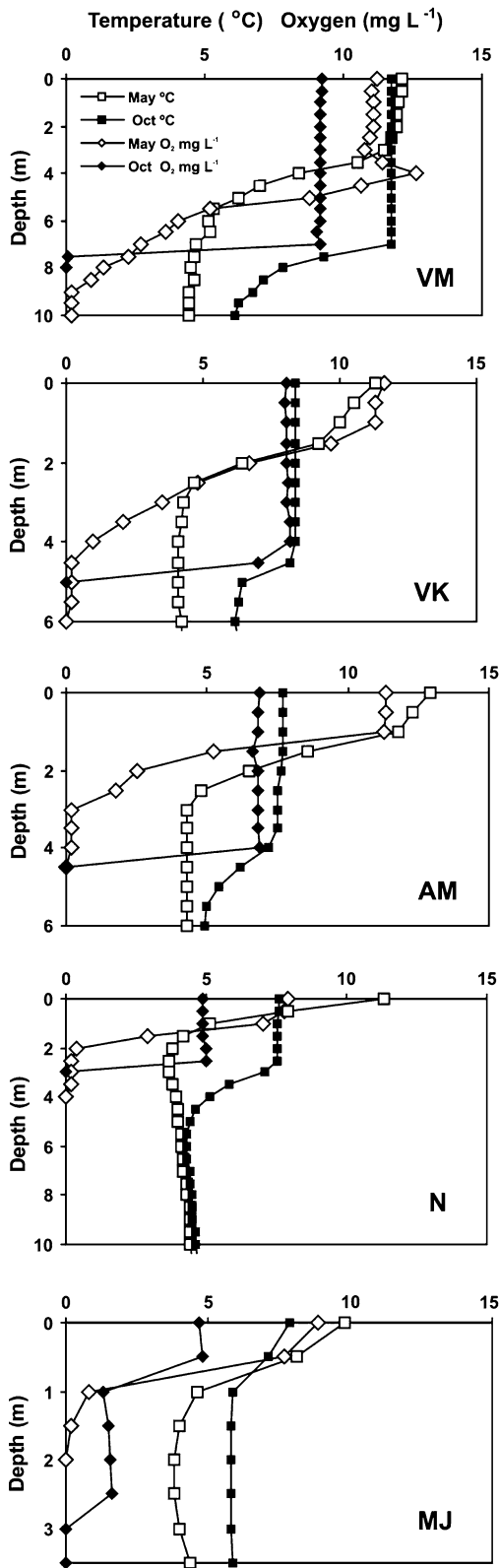
Results

Lake variables

All the lakes were steeply stratified in May (Fig. 1). The mixed layer (epilimnion) was generally shallower in lakes with higher DOC. All lakes showed hypolimnetic anoxia with the exception of Valkea Mustajärvi, where residual oxygen concentration (ca. 0.3 mg l^{-1}) could still be detected at 9–10 m depth, and the redox potential did not indicate complete anoxia. In October, the thermocline in the lakes was ca. 2–4 m deeper than in May, with the exception of Mekkojärvi where no distinct thermocline was observed in October. Hypolimnetic anoxia then prevailed in all lakes. In Valkea Mustajärvi a steep oxycline was found at about 7.5 m depth, and in the other lakes the oxycline in October was 1–2 m deeper than in May.

The concentration of DOC in the study lakes varied between 5 and 44 mg C l^{-1} (Table 3), being lowest in the largest lake (Valkea Mustajärvi) and highest in the two smallest lakes (Mekkojärvi and Nimetön) especially in the hypolimnion. In all the lakes, concentrations of DIC and CH_4 were higher in the hypolimnion than in the epilimnion, the difference being especially pronounced in May in Alinen Mustajärvi and Nimetön. In the whole dataset, including epi- meta- and hypolimnion, the concentrations of DIC, CH_4 and DOC were significantly correlated (Table 4).

The lowest phytoplankton biomass ($0.1\text{--}0.2 \text{ mg C l}^{-1}$) occurred in the most humic lakes, Mekkojärvi and Nimetön, and the highest biomass occurred in Valkea-Kotinen in both May and October (3.4 and 0.9 mg C l^{-1} , respectively; Fig. 2a). In Valkea Mustajärvi, chrysophytes and cryptophytes were the dominant algal groups in May forming ca. 60% of the biomass, whereas in October ca. two-thirds % of the biomass consisted of chlorophytes (Table 5). In Valkea-Kotinen, 97% of phytoplankton biomass in May consisted of a dinophycean, *Peridinium* sp., and in October the proportion of this genus was almost as high. In Alinen Mustajärvi, the highest biomass percentage was for dinophytes (74%) in May but in October chrysophytes and a rapidophycean, *Gonyostomum semen*, formed ca. 70% of the biomass. In Nimetön, the highest biomasses were formed



◀ **Fig. 1** Stratification of temperature and oxygen in the study lakes in May and October

by Cryptophyceae and also by Chrysophyceae in October. In Mekkojärvi, unidentified flagellates (size 5–15 μm) formed ca. half of the biomass in May, but in October cryptophytes and small *Microcystis*-like cyanophyte colonies dominated.

Mean bacterial biomass in the whole water column of the three clearest lakes was <30% of that of phytoplankton (0.04–0.16 mg C l⁻¹), but in the two most humic lakes the bacterial biomass was about the same as or higher than the phytoplankton biomass (Fig. 2b). The mean cell volumes and biomasses were generally higher in the anoxic hypolimnion than in the epilimnion (data not shown). With the exception of Valkea Mustajärvi in May, the bulk of chlorophyll in the hypolimnion and in most lakes also in the metalimnion consisted of bacteriochlorophyll *d* (Bchl *d*) (Table 3), indicating presence of green-sulphur bacteria in the anoxic conditions in all lakes. For the whole dataset bacterial biomass, total particulate organic carbon (POC) and chlorophyll concentrations were significantly correlated (Table 4).

The zooplankton community in Valkea-Kotinen and Alinen Mustajärvi was numerically dominated by rotifers (Fig 2c), with *Synchaeta* sp., *Keratella* spp., *Kellicottia longispina* and *K. bostoniensis* the most abundant. In Valkea Mustajärvi, the most abundant cladocerans were *Holopedium gibberum*, *Daphnia cristata*, *D. longiremis* and *Bosmina* sp. Among the copepods, cyclopoids were more abundant than a calanoid *Eudiaptomus gracilis*. In Valkea-Kotinen, the cyclopoids were the most abundant crustaceans, but cladocerans (*Bosmina longirostris*, *H. gibberum* and *Daphnia* sp.) also appeared. In Alinen Mustajärvi, cladocerans (*H. gibberum*, *Ceriodaphnia quadrangula*, *Daphnia* sp., *Bosmina* sp.) dominated over cyclopoid copepods. In Nimetön, cyclopoid copepods and cladocerans *H. gibberum* and *D. longispina* were found in May, but in October no cladocerans were observed. In the fishless lake Mekkojärvi, cladocerans were dominated by *D. longispina*, which is far bigger than the cladocerans in the other lakes, reaching an adult length of >2 mm. Small cyclopoid copepods were also found in Mekkojärvi especially in October. Although the bulk of zooplankton (>50%) appeared in the

Table 3 Mean (\pm SD) concentrations of different forms of carbon and of chlorophyll in different depth layers in May and October in the study lakes

Lake	Layer	DIC ($\mu\text{M L}^{-1}$)	CH ₄ ($\mu\text{M L}^{-1}$)	DOC (mg C L ⁻¹)	POC (mg C L ⁻¹)	Chl ($\mu\text{g L}^{-1}$)	Dominance of Chl <i>a</i> or <i>d</i>	
May	VM	Epi (0–5 m)	119 \pm 43	0.08 \pm 0.02	ND	0.43 \pm 0.02	3	a
		Meta (6–8 m)	429 \pm 44	0.02 \pm 0.003	ND	0.69 \pm 0.02	4.4	a
		Hypo (9 m)	476 \pm 57	0.07 \pm .04	ND	1.09 \pm 0.01	2.7	a
	VK	Epi (0–3 m)	166 \pm 159	0.10 \pm 0.10	14.5 \pm 0.6	1.78 \pm 0.06	9.7	a
		Meta (4–5 m)	391 \pm 210	2.5 \pm 3.5	20.5 \pm 0.4	1.49 \pm 0.06	7.7	d
		Hypo (6 m)	652 \pm 2	47.6 \pm 0.9	18.2 \pm 0.4	1.86 \pm 0.02	14.4	d
	AM	Epi (0–2 m)	151 \pm 196	0.34 \pm 0.30	12.0	0.63	6.2	a
		Meta (3–4 m)	473 \pm 35	2.2 \pm 3.2	13.5 \pm 0.1	0.85 \pm 0.05	7.0	d
		Hypo (5–6 m)	938 \pm 19	285.7 \pm 7.5	15.4 \pm 0.1	1.48 \pm 0.01	30.4	d
	N	Epi (0–2 m)	374 \pm 291	1.4 \pm 1.6	22.3 \pm 0.3	0.68	8.5	a
		Meta (3–5 m)	723 \pm 31	16.5 \pm 7.6	30.2 \pm 0.2	0.6	2.7	d
		Hypo (6–10 m)	1,688 \pm 730	631 \pm 480	43.8 \pm 0.4	0.72 \pm 0.02	8.1	d
	MJ	Epi (0–0.6 m)	270 \pm 12	2.3 \pm 0.1	26.3 \pm 1.1	0.87	11.3	a
		Meta(0.6–1.8 m)	684 \pm 343	21.4 \pm 21.7	25.6 \pm 0.4	1.64	62.4	d
		Hypo (1.8–3 m)	1,299 \pm 131	77.7 \pm 11.0	21.9 \pm 1.1	2.27 \pm 0.11	162.3	d
Oct	VM	Epi (0–6 m)	130 \pm 4	0.13 \pm 0.02	5.8 \pm 0.1	0.31	4.8	a
		Meta (7–8 m)	377 \pm 307	11.5 \pm 16.0	5.4 \pm 0.1	0.94 \pm 0.01	8.0	d
		Hypo (9 m)	712 \pm 13	66.8 \pm 3.2	5.4 \pm 0.1	1.68 \pm 0.08	30.8	d
	VK	Epi (0–3 m)	141 \pm 5	0.70 \pm 0.19	13.4 \pm 0.1	0.78 \pm 0.03	18.1	a
		Meta (4–5 m)	142 \pm 2	0.55 \pm 0.08	14.1 \pm 0.1	0.65 \pm 0.01	11.5	a
		Hypo (6 m)	856 \pm 19	137.6 \pm 0.7	16.5 \pm 0.1	1.23 \pm 0.05	14.2	d
	AM	Epi (0–3 m)	199 \pm 3	2.7 \pm 0.1	11.1	0.46	8.0	d
		Meta (4 m)	212 \pm 2	5.6 \pm 0.3	11.0 \pm 0.1	0.45	11.8	d
		Hypo (5 m)	365 \pm 31	59.8 \pm 9.8	14.9 \pm 0.4	0.98 \pm 0.01	56.1	d
	N	Epi (0–1 m)	ND	ND	22.3 \pm 0.2	0.47	7.5	a
		Meta (2–3 m)	ND	ND	23.0 \pm 0.1	0.43	10.8	d
		Hypo (4–10 m)	ND	ND	32.0 \pm 0.3	0.68	15.9	d
	MJ	Epi (0–0.6 m)	693 \pm 42	0.21 \pm 0.05	17.6 \pm 0.1	1.14 \pm 0.02	13.5	d
		Meta(0.6–1.8 m)	870 \pm 34	0.58 \pm 0.15	17.5	1.22 \pm 0.01	15.0	d
		Hypo (1.8–3 m)	974 \pm 170	22.3 \pm 37.9	17.5 \pm 0.1	1.07 \pm 0.09	14.7	d

ND no data

epilimnion, some individuals were always found in meta- and hypolimnion of the lakes.

Stable isotope ratios of food web compartments

The overall mean water column $\delta^{13}\text{C}$ values of DIC in the five lakes varied between -17.4 and -23.9% (Table 6). The most ^{13}C -enriched individual values (-6.7 to -10.8%) were observed for the epilimnion of Valkea Mustajärvi, and the most ^{13}C -depleted (-25.1 to -26.9%) in spring for the metalimnion of

Valkea-Kotinen and Alinen Mustajärvi. Across the whole data set, $\delta^{13}\text{C}$ of DIC was not related to concentrations of DIC or DOC (Table 4) or to any other measured variables. However, in the hypolimnion $\delta^{13}\text{C}$ of DIC was positively correlated with DOC concentration ($r = 0.795$, $P < 0.01$, $n = 10$). In all lakes and at all depths, particulate organic matter (POM) was consistently ^{13}C -depleted relative to DIC (Table 6), but no significant relationship was found between them (Table 4). In May, with the exception of Nimetön, $\delta^{13}\text{C}$ of POC was more enriched in the

Table 4 Pearson correlation coefficients (*r*) between concentrations of dissolved inorganic and organic carbon (DIC, DOC), particulate organic carbon (POC), chlorophyll, phytoplanktonand bacterial biomass (PhytoBio, BacBio) as well as $\delta^{13}\text{C}$ -DIC and $\delta^{13}\text{C}$ -POC in the whole dataset of five lakes sampled for epilimnetic and hypolimnetic in May and in October

	DIC	$\delta^{13}\text{C}$ -DIC	CH_4	DOC	POC	$\delta^{13}\text{C}$ -POC	Chl	PhytoBio	BacBio
DIC	1.000	.090	.738**	.607**	.440*	-.219	.417*	-.277	.230
$\delta^{13}\text{C}$ -DIC		1.000	.363	.134	-.301	.336	-.024	-.206	-.073
CH_4			1.000	.588**	.075	-.203	.074	-.126	-.025
DOC				1.000	.041	-.081	.147	-.234	.209
POC					1.000	-.168	.642**	.334	.444*
$\delta^{13}\text{C}$ -POC						1.000	.068	.162	-.050
Chl							1.000	-.071	.543**
PhytoBio								1.000	-.072
BacBio									1.000

Correlation significant at the 0.05 level*, at the 0.01 level**, $n = 27\text{--}30$

epilimnion than in the meta- and hypolimnion, but in October the differences in $\delta^{13}\text{C}$ -POC values were minor between the layers (Table 6).

With the exception of cladocerans in Alinen Mustajärvi in May, crustacean zooplankton carbon was more ^{13}C -depleted than POM in both May and October (Table 6). Cladocerans were always more ^{13}C -depleted in October than in May (but no data from Nimetön), while copepods in Valkea Mustajärvi, Nimetön and Mekkojärvi were also more ^{13}C -depleted in October than in May. In May, the $\delta^{13}\text{C}$ values of cladocerans (mean of the whole water column) were negatively correlated with metalimnetic and hypolimnetic POC $\delta^{13}\text{C}$ values ($r = -0.900$ and -0.944 , $P < 0.05$, $n = 5$), presumably indicating low dependence of cladocerans on these resources in spring. In October, the $\delta^{13}\text{C}$ values of cladocerans were correlated only with bacterial biomass (volume-weighted for the whole water column, $r = -0.981$, $p < 0.05$, $n = 4$); thus, the most negative $\delta^{13}\text{C}$ values of cladocerans was found in lakes with the highest bacterial biomass. In May, the $\delta^{13}\text{C}$ values of copepods were positively correlated with epilimnetic POC $\delta^{13}\text{C}$ values ($r = 0.931$, $P < 0.05$, $n = 5$), indicating the importance of the epilimnetic food sources, but in October copepod $\delta^{13}\text{C}$ values were not correlated with any measured variable (cf. list of variables in Table 4). The $\delta^{15}\text{N}$ values of zooplankton were generally quite variable and higher in May than in October (Table 6). For the whole dataset, $\delta^{15}\text{N}$ values of cladocerans and copepods were correlated for samples taken simultaneously

($r = 0.917$, $n = 9$). The intercept of the linear regression equation ($y = 2.339 + 0.624x$) reveals that $\delta^{15}\text{N}$ values of copepods were 2.3‰ more enriched than those of cladocerans.

Proportions of algae, allochthonous organic matter and bacteria in the diets of crustacean zooplankton—IsoSource mixing model results

By combining two isotopes and three food sources (Table 2), the IsoSource mixing model gave solutions for zooplankton diet in our datasets of five lakes (Table 7). However, for the May data from the clearest lake, Valkea Mustajärvi, solutions could only be found using a one-isotope ($\delta^{13}\text{C}$) model, due to the rather high and variable $\delta^{15}\text{N}$ values of both cladocerans and copepods. Due to the much lower $\delta^{13}\text{C}$ values of MOB, their proportion in the diet of zooplankton was generally within a relatively narrow min–max range, whereas for algae and AlloOM + HB + GSB the ranges were often wider and overlapping. In general, the model results suggest a higher proportion of algae in the diets of cladocerans in May than in October. The proportion of MOB in the diets of cladocerans was higher in October than in May, with the highest proportions in Alinen Mustajärvi (34–36%) and in Mekkojärvi (35–50%).

The IsoSource model also suggests that bacteria and/or terrestrial detritus played significant roles in the diets of copepods; only in Mekkojärvi in May and in Alinen Mustajärvi in October did >50% of the

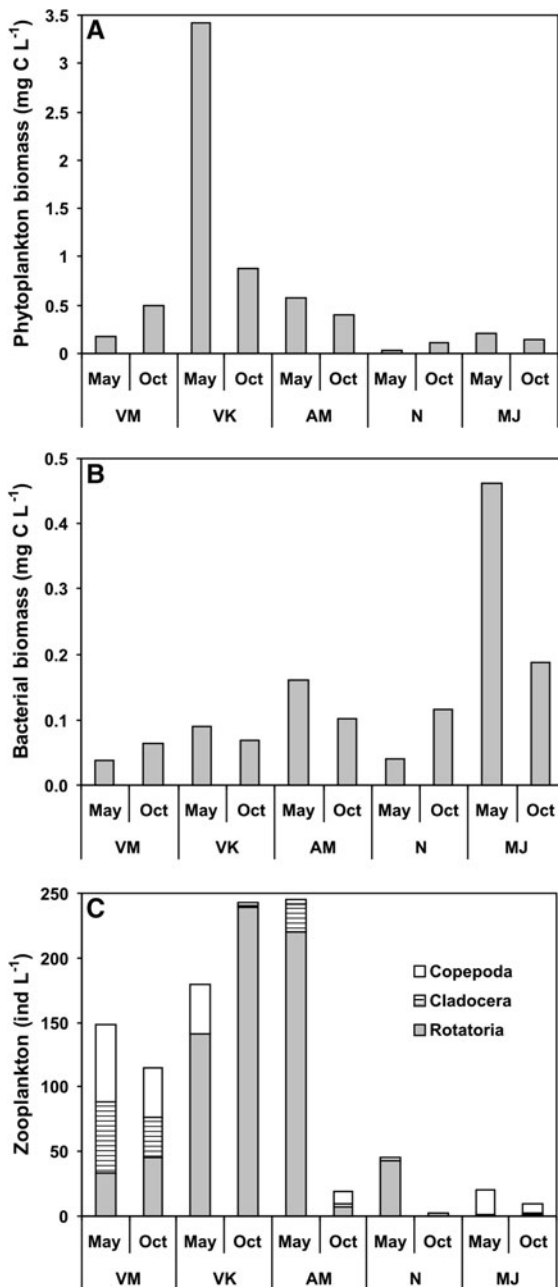


Fig. 2 Biomass of phytoplankton (a) and bacteria (b), and density of zooplankton (c) in the study lakes. All values are volume-weighted averages for the whole water column

copepod diets originate from algae. The proportion of MOB was highest in October in the polyhumic lakes Nimetön (35–40%) and Mekkojärvi (37–50%), when the proportion of algae was also estimated to be low (0–13 and 0–29%, respectively) in the diets of copepods. The $\delta^{13}\text{C}$ values of copepods, which were

close to those obtained for algae in these lakes (cf. Table 2), suggest that the proportion of algae in their diets could have been higher in the autumn. By relying on $\delta^{13}\text{C}$ values alone for the three food sources and for copepods, the one-isotope ($\delta^{13}\text{C}$) IsoSource model generates very wide probable ranges of algae in copepod diets, from 0–97% in Nimetön and 1–83% in Mekkojärvi, in October.

Discussion

In our study of five boreal lakes with a gradient of DOC from 5 to ca. 40 mg C l⁻¹, we found no relation between $\delta^{13}\text{C}$ of DIC and concentrations of either DIC or DOC. This result contrasts with that reported by Lennon et al. (2006) from a survey of north-eastern US lakes along a gradient of terrestrial-derived DOC (ca. 2.5–12 mg C l⁻¹) and sampled during late summer (July–early September). They used naturally occurring $\delta^{13}\text{C}$ values of CO₂, POM and crustacean zooplankton, as well as gas measurements and culture-independent assessments of microbial community composition to make inferences about the flow of terrestrial carbon in lake food webs. Stable isotope ratios of POM and zooplankton decreased with DOC and were often depleted in ¹³C relative to terrestrial carbon, suggesting the importance of an isotopically light carbon source (Lennon et al. 2006). They concluded that the observed low $\delta^{13}\text{C}_{\text{Zooplankton}}$ values could be explained by consumption of ¹³C-depleted phytoplankton, which increased their use of heterotrophically respired and ¹³C-depleted CO₂ with increasing concentrations of terrestrial-derived DOC (Lennon et al. 2006). Mohamed and Taylor (2009) also found significant correlations between CO₂ concentration and epilimnetic $\delta^{13}\text{C}$ -DIC, $\delta^{13}\text{C}$ -POC and $\delta^{13}\text{C}$ -zooplankton in Canadian lakes with DOC concentration ranging from 2 to 13 mg C l⁻¹. In our study, lakes we found only copepod $\delta^{13}\text{C}$ values in May to be significantly correlated with the $\delta^{13}\text{C}$ values of epilimnetic POC.

Although we found no overall relation between $\delta^{13}\text{C}$ of DIC and DOC concentration in our study lakes, in the hypolimnion of the lakes $\delta^{13}\text{C}$ of DIC actually became more enriched with increasing concentration of DOC (the opposite of the trend reported by Lennon et al. 2006 and Mohamed and Taylor 2009). This may be related to use of the more

Table 5 Proportion (%) of different algal taxonomic groups in the phytoplankton biomass of the epilimnion of the study lakes in May and in October

		Cya	Crypt	Dinoph	Chryso	Diatom	Raphid	Chloro	Others and unidentified
May	VM	3	20	15	40	3	0	8	11
	VK	2	0	97	1	0	0	0	0
	AM	7	0	74	17	0	0	0	2
	N	0	36	19	15	11	0	15	4
	MJ	2	1	9	4	2	0	37	55
Oct	VM	6	6	0	12	2	0	66	8
	VK	5	1	92	1	1	0	0	0
	AM	18	0	4	38	0	35	3	2
	N	12	35	1	27	3	0	1	21
	MJ	38	51	0	4	0	0	6	1

Cya Cyanophyceae, Crypt Cryptophyceae, Dinoph Dinophyceae, Chryso Chrysophyceae, Diatom Diatomophyceae, Rapid Rapidophyceae, Chloro Chlorophyceae

depleted DIC as a carbon source by green-sulphur bacteria and methanogens (see Van Gernerden and Mas 1995; Whiticar 1999; Gu et al. 2004) in the anoxic zone of our lakes, leading to ^{13}C -enrichment of the remaining DIC. During summer stratification in our lakes, the anoxic water proportion of the total lake volume increased with the DOC concentration (Table 1), which may also have influenced the poor overall relationship between $\delta^{13}\text{C}$ of DIC and DOC concentration.

In stratified lakes, where several food sources are potentially available for zooplankton, estimating the proportion of different food sources in the diet is challenging. We used the IsoSource model to estimate min–max ranges of algae, bacteria and allochthonous OM in the diets of zooplankton. The outputs from the model depend on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values assigned to the possible food sources. Phytoplankton carbon fractionation relative to $\delta^{13}\text{C}$ -DIC can vary greatly, depending on species composition, growth rate and CO_2 availability (e.g. Popp et al. 1998; Bade et al. 2006); thus, algal $\delta^{13}\text{C}$ values derived from $\delta^{13}\text{C}$ -DIC are often uncertain (Marty and Planas 2008). Epilimnetic POC $\delta^{13}\text{C}$ values seldom directly indicate $\delta^{13}\text{C}$ values of phytoplankton (Vuorio et al. 2006; Marty and Planas 2008), but are often results of a mixture of algae, terrestrial detritus and bacteria (Mohamed and Taylor 2009). To avoid these problems, we applied $\delta^{13}\text{C}$ values based on compound-specific $\delta^{13}\text{C}$ values of fatty acids for phytoplankton and bacteria. In our dataset,

epilimnetic $\delta^{13}\text{C}$ -POC of Valkea-Kotinen and Alinen Mustajärvi were in May within the range of $\delta^{13}\text{C}$ values based on algal-specific FAs (see Tables 2 and 6), suggesting that in these lakes, with the highest phytoplankton biomasses dominated by peridiniaceans (cf. Fig 2a, Table 5), the $\delta^{13}\text{C}$ -POC was mainly due to algae. However, this conclusion is uncertain due to the overlapping $\delta^{13}\text{C}$ values for HB and AlloOM (Table 2). In the other lakes, the FA-derived $\delta^{13}\text{C}$ values for phytoplankton were more depleted than those for epilimnetic POC, indicating the common situation of a high proportion of AlloOM and/or bacteria in the bulk POC.

To obtain a better separation between algae, AlloOM and bacteria in the diets of zooplankton, we applied the two-isotope IsoSource model. However, estimation of $\delta^{15}\text{N}$ values for algae, bacteria and allochthonous OM was more problematic than those of $\delta^{13}\text{C}$. For example, in a dataset from four lakes in SW Finland, Vuorio et al. (2006) found wide taxon-specific and between-lake variation of $\delta^{15}\text{N}$ values of phytoplankton (−1.5 to 12.8‰). We applied a range $\delta^{15}\text{N}$ values, from those obtained for the epilimnetic bulk POM of each lake to that for algae previously obtained for the smallest and most humic lake (5.0‰). The applied $\delta^{15}\text{N}$ ranges for AlloOM, HB and GSB (from DOM and hypolimnetic POM) and MOB (experimental values, Taipale et al. 2008) were generally slightly lower than those used for algae (Table 2), but we acknowledge the uncertainty of these $\delta^{15}\text{N}$ estimates. Nevertheless, the $\delta^{15}\text{N}$ ranges

Table 6 Mean (\pm SD) $\delta^{13}\text{C}$ values (‰) for DIC, DOC and POC (epi- meta and hypolimnion), together with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for Cladocera and Copepoda in the whole water column of the study lakes in May and October

Lake	DIC $\delta^{13}\text{C}$	DOC $\delta^{13}\text{C}$	EpiPOC $\delta^{13}\text{C}$	MetaPOC $\delta^{13}\text{C}$	HypoPOC $\delta^{13}\text{C}$	Cladocera $\delta^{13}\text{C}$	Cladocera $\delta^{15}\text{N}$	Copepoda $\delta^{13}\text{C}$	Copepoda $\delta^{15}\text{N}$
May	VM	-18.6 \pm 7.3 (38)	-27.0	-30.6	-33.2	-32.5	12.9 \pm 8.1 (6)	-37.1 \pm 0.8 (4)	10.2 \pm 3.0 (4)
	VK	-23.9 \pm 1.5 (29)	-26.3 \pm 0.3	-27.9	-35.0	-36.2	5.7 \pm 0.2 (2)	-34.4 \pm 0.6 (7)	6.4 \pm 0.3 (7)
	AM	-23.1 \pm 3.1 (42)	-26.9	-28.8	-38.4	-38.0	2.7 \pm 0.2 (6)	-36.7 \pm 0.4 (3)	2.5 \pm 0.0 (3)
	N	-18.2 \pm 4.7 (42)	-25.8 \pm 0.1	-34.2	-33.4	-32.7	2.6 \pm 0.3 (6)	-39.4 \pm 0.5 (5)	3.8 \pm 0.5 (5)
	MJ	-20.8 \pm 1.0 (63)	-25.5 \pm 0.2	-31.7	-32.5	-30.0	5.1 \pm 0.2 (6)	-38.7 \pm 0.1 (5)	4.7 \pm 0.4 (5)
Oct	VM	-17.4 \pm 5.0 (45)	-24.9	-30.0	-30.2	-30.8	3.0 \pm 0.7 (7)	-37.7 \pm 0.1 (3)	6.0 \pm 0.2 (3)
	VK	-20.2 \pm 1.1 (45)	-26.1 \pm 0.9	-30.8	-29.8	-29.4	4.3 \pm 0.6 (3)	-31.8 \pm 0.9 (5)	5.8 \pm 0.3 (5)
	AM	-20.9 \pm 3.3 (44)	-26.5	-32.5	-30.6	-30.6	1.5 \pm 0.3 (4)	-33.4 \pm 0.4 (2)	3.3 \pm 1.3 (2)
	N	-18.4 \pm 4.8 (45)	-25.8 \pm 0.3	-30.0	-30.2	-29.6	ND	-40.7 (1)	3.1 (1)
	MJ	-19.9 \pm 1.1 (45)	-25.4 \pm 0.1	-30.6	-30.2	-31.1	1.5 \pm 0.1 (6)	-44.4 \pm 0.8 (6)	2.9 \pm 0.3 (6)

Number of analyses 1–4 in DOC and POC, others in brackets, ND = no data

Table 7 IsoSource model results showing the proportions (min–max range in %) of three putative food sources, algae, allochthonous organic matter (AlloOM) together with heterotrophic (HB) and green-sulphur bacteria (GSB), and methanotrophic bacteria (MOB) in the diets of cladocerans in the five study lakes in May and in October

	Lake	Iso-Source model		
		Algae	AlloOM + HB + GSB	MOB
<i>Cladocera</i>				
May	VM	0–76	23–82	0–17
	VK	34–86	0–58	7–14
	AM	14–39	60–80	1–6
	N	8–44	39–68	10–24
	MJ	42–69	19–39	12–19
Oct	VM	0–21	63–83	15–17
	VK	38–47	24–35	27–29
	AM	17–26	40–47	34–36
	N	No data		
	MJ	20–65	0–32	35–50
<i>Copepoda</i>				
May	VM	0–85	15–75	0–28
	VK	23–28	52–57	17–20
	AM	45–66	9–31	24–25
	N	0–17	53–70	29–35
	MJ	92–98	2–6	0–2
Oct	VM	4–67	18–71	15–28
	VK	24–51	41–68	8–12
	AM	61–69	16–23	15–16
	N	0–13	50–64	35–40
	MJ	0–29	33–53	37–50

we used for parameterization of the IsoSource model originate from results derived from the study lakes, lending greater credence to the model outputs.

The model outputs indicate that bacteria and terrestrial OM contributed appreciably to the diets of cladocerans and copepods during both seasons in the study lakes. Despite higher biomass of algae than bacteria in most lakes, the bulk of the algal biomass consisted of taxa (e.g. Dinophyceae and Rapidophyceae) in the lakes Valkea-Kotinen and Alinen Mustajärvi, which probably were not preferred food for zooplankton. One route from bacterial carbon and nitrogen to crustacean zooplankton may pass through mixotrophic algae, such as chrysophytes (cf. Bird and Kalff 1987; Rothhaupt 1996), which graze on bacteria and can thus mask differences between algal and

bacterial diets. The algal communities in the lakes generally contained a high proportion of taxa like chrysophytes, cryptophytes and dinophytes that are known to be capable of mixotrophy. Especially in October, less favourable light conditions than in May could have favoured algal mixotrophy. Taipale et al. (2008) achieved better separation between algae and different bacterial groups than we obtained in this study by labelling lake phytoplankton with DI^{13}C in Mekkojärvi, but still the separation of HB from the other food sources in the cladoceran diet was poor (wide min–max range).

Although there are intraspecific differences between cladoceran taxa in their efficiency of filtering small bacteria, related to intersetular differences of their filter meshes (Hessen 1985; Brendelberger 1991), the differences in species composition between our study lakes seemed not to have any great impact on the estimated importance of bacteria in the diet of cladocerans. For example, *D. longispina*, which dominated in Mekkojärvi and was also found in Nimetön, is known to filter bacteria efficiently (Børshem and Andersen 1987; Kankaala 1988). On the other hand, a ‘macrofiltrator’ *Holopedium gibberum* (see Geller and Müller 1981; Hessen 1985), which was abundant in all lakes except Mekkojärvi, has also been observed to ingest particles of bacterial size probably using a feeding mechanism other than filter feeding (Hessen and Nordby 1988).

We expected that bacteria would be less important for copepod than for cladoceran diets, but the IsoSource model outputs did not clearly support this assumption. Generally, copepods are known to be less efficient than cladocerans at grazing free-living bacteria (Hessen 1985; Nagata and Okamoto 1988), but in our small humic lakes bacteria attached to flocculated allochthonous particulate organic matter (see von Wachenfeldt et al. 2009) and/or transfer via mixotrophic algae may have been important routes of bacterial carbon and nitrogen into copepods. The more enriched $\delta^{15}\text{N}$ values of copepods compared with those in cladocerans suggest that copepods were at a higher trophic position and that bacterial contribution to their diet probably originated also via protozoan grazing on bacteria, which is consistent with the experimental results of Karlsson et al. (2007) and field results of Matthews and Mazumder (2008). An alternative explanation for the more enriched

$\delta^{15}\text{N}$ values of copepods compared with those in cladocerans would be a greater systematic fractionation of $\delta^{15}\text{N}$ by copepods, although we are not aware of any evidence for this.

Although we could not separate GSB from HB and AlloOM, detection of bacterial chlorophyll *d* (Bchl *d*) in the guts of zooplankton (Salonen and Lehtovaara 1992; Camacho et al. 2001) and observations of phospholipid fatty acids (PLFA) typical for GSB (*Chlorobium* sp.) in the tissues of *Daphnia* (Taipale et al. 2009) have independently indicated a contribution of GSB in the food webs of some stratified lakes. The significant correlation between bacterial biomass, POC and total chlorophyll also indicated the potential importance of this food source for zooplankton in the study lakes.

The $\delta^{13}\text{C}$ values of MOB, analysed from their compound-specific PFLAs (–62 to –64‰), were the most depleted values (cf. Boschker and Middelburg 2002) of the potential carbon sources to zooplankton, and thus MOB was distinguished with the smallest min–max range in the diets of zooplankton. The contribution of MOB in the cladoceran diets was greater in October than earlier in the year (cf. Taipale et al. 2008), and this proportion increased in parallel with the DOC concentration of the lakes as we originally hypothesized. In the two most humic lakes, the proportion of MOB in the diets of copepods also seemed to be higher in October (35–40%) than in May. In these small boreal lakes, the seasonally highest MOB activities are typically measured during the autumnal mixing (Kankaala et al. 2006a; 2007; Taipale et al. 2008), when CH_4 accumulated in the hypolimnion becomes mixed with oxygen from the upper layers, providing favourable conditions for MOB in the whole water column. This ensures a particularly high availability of MOB as bacterial food for zooplankton during the autumn turnover period.

Conclusions

Despite the uncertainty in correctly parameterizing the IsoSource mixing model, our results provide further support for the view that in small boreal lakes with a steep stratification of temperature and oxygen in summer pelagic bacteria of various types and allochthonous organic matter form an important

component of the food of zooplankton, even in lakes at the low end of the DOC concentration gradient. Moreover, our results provide fresh evidence that biogenic methane can be a carbon and energy source for some pelagic food webs, as has already been shown to be the case for profundal food webs in many lakes (Jones et al. 2008; Ravinet et al. 2009).

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