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Sami Taipale

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This thesis is dedicated to my mom and dad

ABSTRACT

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Bacterial-mediated terrestrial carbon in the foodweb of humic lakes
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Yhteenveto: Bakteerivälitteisen terrestrisen hiilen merkitys humusjärvien ravintoketjussa
Diss.

The aim of this thesis was to quantity the relative role of allochthonous dissolved organic carbon (DOC) and autochthonous primary production in the food webs of humic lakes during different seasons. Terrestrial detrital organic matter received from the surrounding catchment area is further decomposed by bacteria in lakes, and in stratified lakes much can be decomposed under anoxic conditions, resulting in high production of methane. However, it has been unclear how much of this decomposed carbon is assimilated by bacteria and then transmitted via zooplankton to higher trophic levels. Carbon stable isotope tracer measurements and ^{13}C-manipulations in a small humic lake, Mekkojärvi, combined with different models, were the basic experimental approaches in this study. Phospholipids fatty acids (PLFA) analyses provided additional insights. Laboratory experiments confirmed that low $\delta^{13}C$ values of *Daphnia* originated from consumption of methane-oxidizing bacteria (MOB) and *in situ* decrease in $\delta^{13}C$ of zooplankton was detected during autumn 2004. Results from enclosures indicated that *Daphnia* in the lake used multiple food sources, while carbon mass balance calculations suggested that MOB could supply all *Daphnia* carbon demand during autumn. IsoSource modelling of data from the whole-lake manipulation, revealed that *Daphnia* consumed heterotrophic bacteria (HB), photoautotrophic green sulphur bacteria (GSB), and MOB in addition to phytoplankton, but the relative contribution of these putative food sources varied seasonally. Overall, the study indicated that *Daphnia* diet in Mekkojärvi comprised 29-59%, 65-79% and 76-86% bacterial-mediated carbon of allochthonous origin during spring, summer and autumn, respectively. Results from four lakes with different DOC content sampled in spring and autumn 2006 indicated that heterotrophic bacteria have a proportionally higher role as terrestrial carbon mediators in the lower DOC content lakes, whereas chemoautotrophic bacteria and MOB have a particularly important role in very dystrophic lakes.

Keywords: Allochthonous carbon, Daphnia, humic lakes, methanotrophs, microbial loop, stable isotopes

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by Roman numerals (I-IV). Paper I was first planned and written by Dr. Paula Kankaala. I collected and analyzed the lake data for $\delta^{13}C$ of zooplankton, which supported the laboratory results, and commented on the manuscript. The experiments for papers II and III were planned together with Prof. Roger Jones and Dr. Paula Kankaala. I was largely responsible for field sampling and the majority of analyses, except that Dr. Paula Kankaala measured methane concentration and methanotrophic activity in each experiment and calculated the carbon mass balance budgets. I originally wrote papers II and III, which were revised together with Dr. Paula Kankaala and Prof. Roger Jones. I planned, collected samples, analyzed PLFA-samples and originally wrote paper IV. Dr. Heikki Hämäläinen provided stastical support for paper IV. The paper was revised with Prof. Roger Jones and Dr. Paula Kankaala.

I Kankaala, P., Taipale, S., Grey, J., Sonninen, E., Arvola, L. & Jones, R. I. 2006. Experimental $\delta^{13}C$ evidence for a contribution of methane to pelagic food webs in lakes. Limnology and Oceanography 51: 2821-2827.

II Taipale, S., Kankaala, P. & Jones, R. I. 2007. Contributions of different organic carbon sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: results from mesocosm $DI^{13}C$-additions. Ecosystems 10: 757-772.

III Taipale, S. Kankaala, P., Tiirola, M. & Jones, R. I. Whole-lake $DI^{13}C$ additions reveal seasonal shifts between multiple food source contributions to zooplankton diet. Ecology. In press.

IV Taipale, S., Hämäläinen, H., Kankaala, P. & Jones, R. I. Seasonal shifts in diet of lake zooplankton revealed by PLFA analysis. Manuscript.

ABBREVIATIONS

DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
DOM	dissolved organic matter
GSB	green sulphur bacteria
HB	heterotrophic bacteria
MOB	methane-oxidizing bacteria
MUFA	monounsaturated fatty acids
NLFA	neutral lipid fatty acids
PLFA	phospholipid fatty acids
POM	particulate organic matter
PP	phytoplankton
PUFA	polyunsaturated fatty acids
SAFA	saturated fatty acids
TOC	terrestrial organic carbon
TOM	terrestrial organic matter

1 INTRODUCTION

Carbon is central to all life, and is the most actively cycled element in the biosphere (Canfield et al. 2005). The main basis of lacustrine carbon flow is *in situ* phytoplankton primary production, whereby inorganic carbon is bound into organic forms usable by consumers. This autochthonous production has been traditionally regarded as the ultimate foundation for all carbon flow within pelagic systems (Fig. 1). However, it is now realised that in most lakes world wide, community respiration exceeds primary production, and thus most lakes are actually net heterotrophic (Cole et al. 1994, del Giorgio et al. 1999).

Lakes receive a variable loading of allochthonous terrestrial organic matter from their surrounding catchment areas. This terrestrial detrital organic matter is further decomposed by bacteria. It was long thought that only a small proportion of allochthonous carbon in lakes is assimilated and transmitted to higher trophic levels, but results mainly from boreal lakes have indicated the important role of terrestrial carbon in aquatic ecosystems (e.g. Salonen & Hammar 1986, Hessen et al. 1990, Tranvik 1992, Jansson et al. 2000, 2007, Karlsson et al. 2003). In this pathway terrestrial dissolved organic carbon (DOC) is utilized by bacteria, which are then consumed by phagotrophic micro-organisms (e. g. flagellates and ciliates, Fenchel 1982), or directly by metazoan zooplankton, such as cladocerans and copepods. Since cladocerans filter feed non-selectively on bacteria and small phytoplankton, whereas copepods feed selectively on larger phytoplankton, flagellates and ciliates (Sommer & Sommer 2006), there is approximately one trophic step less in cladoceran-based food chains. However, the direct consumption of bacteria and phytoplankton by cladocerans and copepods is affected by the presence or absence of piscivorous fish. High numbers of piscivorous fish decrease the number of planktivorous fish, and thus the predator pressure on metazoan zooplankton is lessened, which allows increased zooplankton consumption more directly on bacteria and phytoplankton (Jansson et al. 2007).

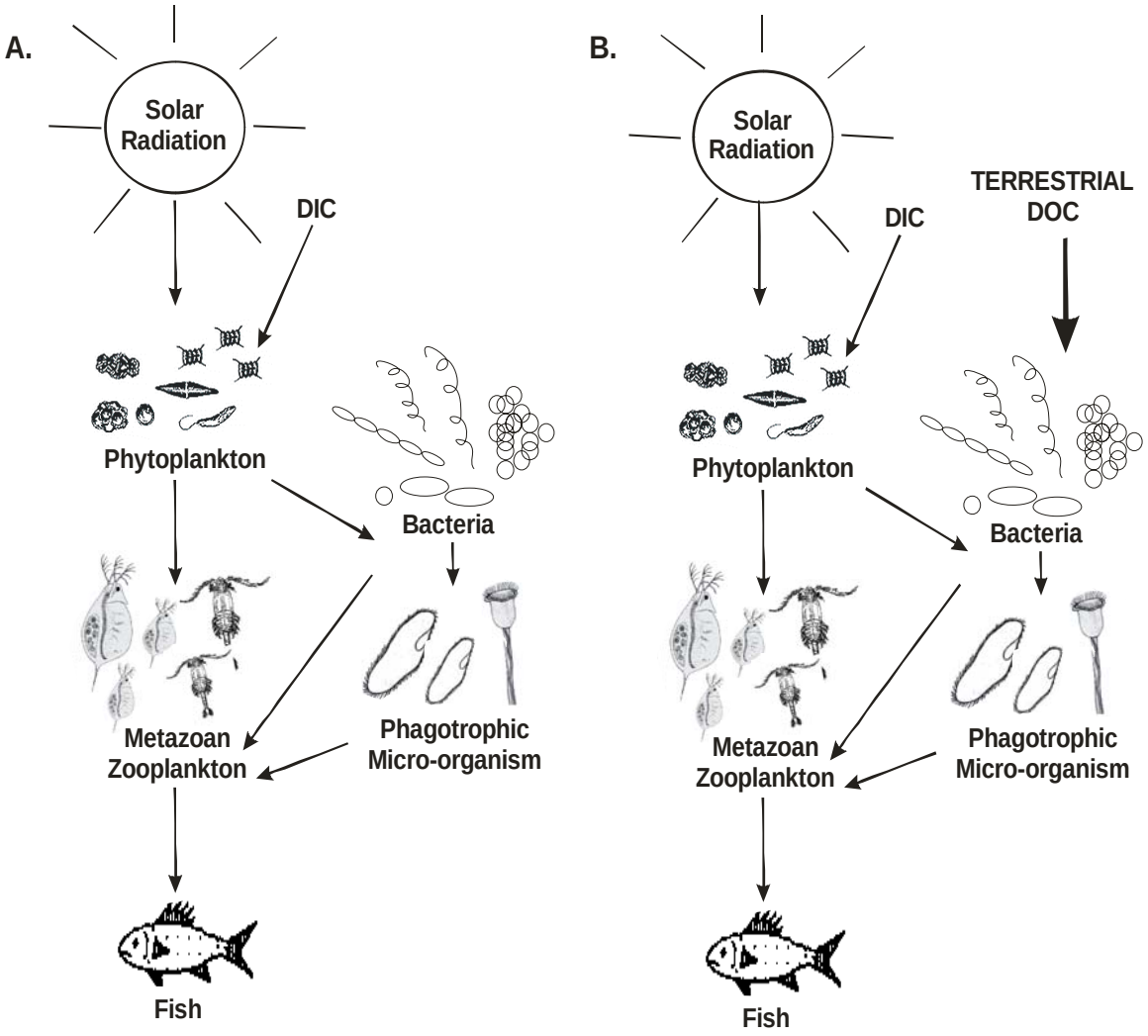

FIGURE 1 **A.** Primary producers require solar radiation and dissolved inorganic carbon (DIC) for photosynthesis whereby inorganic carbon is turned to organic forms usable by consumers. In this traditional autochthonous-based carbon flow model, bacteria only decompose organic carbon from phytoplankton and channel it back to consumers. **B.** Terrestrial dissolved organic carbon (DOC) serves as an alternative carbon source for to higher trophic levels. In this model bacteria decompose and also assimilate this terrestrial organic carbon which is further consumed by metazoan zooplankton and phagotropic micro-organisms. Arrows shows carbon flow in the foodweb. The figure is modified from Jansson et al. (2007).

This allochthonous organic matter source and bacterial-mediated pathway represents an alternative organic carbon pathway alongside the 'traditional' production and transfer of autochthonous carbon and has usually been termed a microbial loop (Azam et al. 1983, Sherr & Sherr 1988). The microbial loop, or microbial food web, was first reported from marine ecosystems (Azam et al. 1983) in which the microbial loop is largely based on release of dissolved organic matter (DOM) from phytoplankton or from other higher trophic level organisms. Thus it differs from the allochthonous DOM-bacteria-flagellate food chain in humic lakes, which should rather be called a microbial link than a microbial loop (Salonen et al. 1992). The magnitude and proportion of autochthonous and allochthonous sources vary widely between lakes according to biological and geological parameters. 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 et al. 1999, Wetzel 2001).

According to lake population statistics from 1987 (Kortelainen 1993), approximately 93% of Finnish lake are humic with total organic carbon (TOC) > 5 mg C l^{-1}, and in 63% of Finnish lakes TOC concentration is > 10 mg C l^{-1}. Humic lakes are often nutrient limited and also exhibit restricted light penetration, conditions which favour bacteria over phytoplankton (e.g. Jansson et al. 2007); hence high bacterial production (Salonen 1981) and low phytoplankton biomass (Johansson 1983) are typical in humic lakes. Additionally, higher bacterial biomass has been measured from humic lakes than from clearwater oligotrophic lakes (Johansson 1983). Hence, allochthonous carbon provides a substantial subsidy alongside phytoplankton production (Fig. 1). Previous studies in Mekkojärvi and other humic lakes have revealed that summer primary production of phytoplankton could account for only a small fraction of the carbon in zooplankton, and that bacterial- mediated terrestrial carbon must therefore be an important carbon supply to zooplankton in humic lakes (Salonen & Hammar 1986, Salonen et al. 2005, Ojala & Salonen 2001, Meili et al. 1996). Additionally, the role of bacteria has been reported to be most important during autumn (Kankaala 1988). Previous studies have also indicated that *Daphnia* feeds on different type of bacteria, such as photosynthetic green sulphur bacteria (GSB, Kuuppo-Leinikki & Salonen 1992) and methane-oxidizing bacteria (MOB, Jones et al. 1999). However, quantifying the role of bacterial-mediated transfer of terrestrial carbon to higher trophic levels in these lakes has proved elusive and remains an important challenge in aquatic sciences.

Small, boreal, humic lakes are usually steeply stratified with respect to temperature and oxygen (Eloranta 2001). The anoxic hypolimnion can be many times thicker than the oxic epilimnion, and thus anoxic decomposition of DOC can have an important role in the carbon cycle. These conditions allow methanogenesis to take place and may result in high methane concentrations (>100 mmol m^{-3}, Kortelainen et al. 2000). Methanogenesis by Archaea usually dominates anaerobic carbon mineralization in anoxic freshwaters (Canfield et al. 2005) which serves as a carbon and energy source for methane-oxidising

bacteria (MOB, methanotrophs) in the water column. It has been estimated that a major part (50-100%, Kankaala et al. 2006, Bastviken et al. 2003, 2004) of methane in the water column is consumed by methanotrophs. This suggests that a large proportion of methane might become incorporated into microbial cells and thus that methane carbon might sustain a high microbial biomass in the water column. From a global perspective it is important that only a minor part of the total methane pool in lakes escapes to the atmosphere, because methane (CH_4) is about 21 times more powerful at warming the atmosphere than carbon dioxide (CO_2) by weight (Anon. 2001).

Since MOB are dependent upon two main substrates, CH_4 and O_2, MOB are expected to be located preferentially at oxic-anoxic interfaces. All MOB first oxidize methane to methanol with the enzyme methane monooxygenase (MMO) and then convert methanol to formaldehyde. After this there are two different carbon assimilation pathways, the ribulose monophosphate pathway (RuMP) and the serine pathway (Canfield et al. 2005).

There are three main classes of MOB which are usually referred to as type I, type II and type X organisms (Hanson & Hanson 1996). Type I and type X are γ-proteobacteria, and they assimilate carbon via the RuMP. Both type I and type X belong to the family of Methylococcaceae. The main type I genera are *Methylomonas*, *Methylobacter*, *Methylomicrobium*, and *Methylosphaera*, whereas type X contains two genera, *Methylocaldum* and *Methylococcus*. Type X can also bind CO_2 using ribulose bisphosphate carboxylase (Taylor et al. 1981). All type II methanotrophs belong to the family of Methylocystaceae and are member of the α-proteobacteria subdivision. Type II utilise the serine pathway for formaldehyde assimilation. The main genera are *Methylosinus* and *Methylocystis* (Hanson & Hanson 1996). Type II can also utilize CO_2, resulting in different $\delta^{13}C$ values for MOB cells.

Biogenic methane in lakes is known to be strongly ^{13}C-depleted (e.g. Whiticar 1999), while microbial oxidation of methane results in further isotope discrimination (Summons et al. 1994). Hence the carbon stable isotope ratio, determined by stable isotope analysis (SIA), is proving a sensitive tracer of incorporation of biogenic methane into lake food webs. There have been reports of very low carbon isotope ratios in both plankton (Jones et al. 1999) and benthic organisms, especially profundal chironomid larvae (Bunn & Boon 1993; Kiyashko et al. 2001, Jones & Grey 2004), indicative of a strong contribution of methane-derived carbon to some lake food webs. The results have confirmed that methane-derived carbon can contribute up to 80% of chironomid body carbon, although there is considerable variation between species, between lakes and with season (Jones & Grey 2004; Grey et al. 2004; Kelly et al. 2004).

In addition to methane, hydrogen sulphide (H_2S) and reduced iron (Fe^{2+}) are formed under anoxic conditions in these dystrophic lakes. These reduced elements are oxidized by different micro-organisms, like chemolithoautorophic bacteria (e.g. *Gallionella* sp.) and photolithoautrotrophic green sulphur bacteria (GSB, *Chlorobium* sp.). Salonen & Lehtovaara (1992) observed that zooplankton migrate to the oxic-anoxic layer to feed on these bacteria and also to avoid

predation pressure from invertebrate predators (*Chaoborus* larvae and *Notonecta*).

A variety of methods has been used to study diet sources of consumers and the structure of foodwebs in both marine and freshwater ecosystems, and considerable knowledge of zooplankton diets and feeding behaviour has been acquired. Traditionally much knowledge of the diet and the feeding behaviour of different zooplankton species has been obtained from laboratory growth experiments and further algal or bacterial counts by microscopy (reviewed by DeMott 1994). Gut content analysis coupled with modern high-performance liquid chromatography (HPLC) has been used to detect the algal diet of *Daphnia* (Thys et al. 2003, Pandolfini et al. 2000). Identification of bacterial pigments, such as bacteriochlorophyll-*d,* in their guts revealed that *Daphnia* ingested photoautotrophic green sulphur bacteria (GSB) in a humic forest lake (Salonen & Lehtovaara 1992). HPLC has confirmed these findings (Villaneuve et al. 1994) and has made it possible to identify different bacterial pigments in zooplankton guts, for example bacteriochlorphyll-*a* and carotenoids in the okenone series have been found in marine copepods, confirming that they consume purple sulphur bacteria (Proctor 1997). Radiotracers have been used to measure grazing rates of zooplankton on different food in the laboratory and within lakes; for example Kankaala (1988) used ^{14}C- labelled phytoplankton and bacteria to investigate seasonal changes in diet of *Daphnia* from the same lake studied in this thesis.

Carbon stable isotope analysis (SIA) combined with mixing models (Phillips et al. 2001, Fry 2006) has been demonstrated to be an effective tool to investigate food sources of different organism, and has been widely used also in aquatic foodweb studies (Jones et al. 1998; Grey et al. 2001). However, autochthonous and allochthonous carbon sources often have too similar $\delta^{13}C$ values, and thus the contribution of phytoplankton and different bacteria to zooplankton diets cannot be accurately calculated from natural isotope signatures. Therefore ^{13}C-enrichment experiments are crucial to create distinct source $\delta^{13}C$ values and make it possible to estimate the contributions of putative food sources. The autochthonous production can be labelled by adding ^{13}C-enriched bicarbonate to a water column, after which its subsequent transmission to zooplankton can be monitored. The proportion of different carbon sources can then be calculated using various models. However, to date there have been only very few whole-lake ^{13}C addition experiments (Pace et al. 2004, Carpenter et al. 2005, Cole et al. 2006), and thus knowledge of carbon flow in lakes of varying dissolved organic carbon concentration lakes is needed.

In the simplest case, when a predator is consuming only two food sources, their magnitude can be easily calculated using one isotope signature and a simple two-source mixing model. However, this is an unusual situation in natural food webs where consumer diets usually consist of at least of three or four sources, and thus two or three additional isotopic signatures are needed to calculate the precise contribution of food sources. However, in many cases it is impossible to measure more than two or three isotopic signals reflective of the source material. Thus, no unique solution can be calculated, although the range

of possible contributions of each food source can be obtained with the IsoSource mixing model (Phillips & Gregg 2003).

However, it is difficult or even impossible to measure separate bacterial and algal species isotopic ratios from wild assemblages, which contain dozens of species (see Vuorio et al. 2007). Therefore, lipids have been used to get more insight into consumer diets. Stott et al. (1997) demonstrated that it is energetically more efficient to incorporate dietary fatty acids (FAs) directly into body tissue without any modification, and thus these FAs can be used as dietary biomarkers. Lipids were first used to study marine food chains (Lee et al. 1971) and thereafter in terrestrial (Ruess et al. 2005) and freshwater food web investigations (Kainz et al. 2004, Perga et al. 2006, Brett et al. 2006). Lipids have also been used to detect food quality using essential fatty acids and omega-3-polyunsaturated fatty acids PUFAs in freshwater studies (Ahlgren et al. 1990, Müller-Navarra 1995, Park et al. 2002). However, most previous zooplankton diet and aquatic food web studies have focused on total lipids fractions, which have been divided to groups of bacterial, algal or terrestrial origin.

Lipids include various classes such as non-polar storage (neutral, NLFA including hydrocarbons, pigments, sterols, triglycerides, waxes, fatty acids) lipids, and membrane lipids (phospholipids fatty acids, PLFAs), and free fatty acids (including cerebrosides, sulfatides, mono- and digalactosyl diglycerides, sterol glycosides), whose relative composition varies among organism. For example, neutral lipid, phospholipid, and free fatty acid relative composition of total lipids in *Daphnia cuculata* was 59.1%, 39.5% and 1.3%, but was 83.4%, 5.0%, 8.6% respectively in *Eudiaptomus gracilis* (Farkas 1970).

Traditionally NLFA (especially triglycerides) and PLFA classes has been kept as dietary lipids (Goulden & Place 1990, Bychek et al. 2005). Rinchard et al. (2007) has newly experimentally showed that both NLFA (especially triglycerides) as well as PLFA composition of young salmon strongly reflected their diet. However, it has also been reported that fatty acid composition may change according to the external (e.g. water temperature, pH, UV light) and internal (e.g. reproductive state) conditions (Bychek et al. 2005, Farkas 1970).

PLFA-profiles can provide precise information about major microbial groups in the water column, and thus can offer information about zooplankton diet. Moreover, since cell membrane PLFAs have been reported to be decomposed rapidly after cell death, PLFA analysis is suitable for detecting rapid changes in living microbial populations (White et al. 1979; Albers 1994). The PLFA-profile has been widely used to study microbial community structure in terrestrial and aquatic systems (White et al. 1979, Bott & Kaplan 1985, Fritze et al. 2000, Smooth & Findlay et al. 2001, Canuel et al. 1995, Boschker et al. 2005) and has been applied like fingerprints to different microbes (Zelles 1999) and phytoplankton at the group level, (Dikjman & Kromkamp 2005 and 2006). Because *Daphnia* feeds rather non-selectively (DeMott 1995) and all ingested material is not assimilated, the PLFA profile offers an effective possibility to detect assimilated diet.

Phytoplankton can be separated from microbes because each phytoplankton taxonomic group contains some characteristic PLFAs that are rare in bacteria (Dijkman & Kromkamp 2005, 2006). These polyunsaturated fatty acids (PUFAs) can be divided to ω-3 and ω-6 families, which are often termed essential fatty acids (EFAs), because only a minor part of PUFAs can be synthesized *de novo* and have to be acquired from the diet. For example linoleic (18:2w6) and linolenic (18:3w3) acids are only produce by plants and have to be obtained from the plants (phytoplankton) in the diet. Futhermore, all EPA (20:5w3) and DHA (22:6w3) has to be acquired directly from plants (phytoplankton) or by conversion from linolenic acid (18:3w3) (Brett & Müller-Navarra 1997). However, ω-3 and ω-6 family PUFAs can be converted only within families, but ω-3 PUFAs cannot be modified to ω-6 families PUFAs. Thus, the magnitude of PUFAs in zooplankton can reflect the contribution of phytoplankton in their diet during different season. If the PLFA composition of *Daphnia* reflects their diet, and if they are feeding on bacteria, PLFA composition should also reflect bacterial sources. For example, branched-chain fatty acids are common in Gram-positive bacteria which are typical heterotrophic bacteria in lakes (Zwart et al. 2002). In contrast, many of the chemoautotrophic bacteria found in lakes, such as iron- and manganese-oxidizing bacteria, and photoautotrophic green sulphur bacteria (GSB), are Gram-negative bacteria, for which 16:0, 16:1 and 18:1 fatty acids are characteristic PLFAs (Kenyon and Gray 1974, Imhoff 2003). Additionally, methylotrophic bacteria and especially methane-oxidizing bacteria (MOB) contain 16:1ω8c and 16:1ω 5t PLFAs, which are specific to them and have been already reported to be transferred from MOB to the cell membranes of *Chironomus* larvae (Deines et al. 2007).

1.1 Aim of study

The ultimate aim of my studies and this thesis was to evaluate and quantify allochthonous organic carbon pathways via Archaea bacteria to higher trophic levels in a lake that receives a heavy loading of terrestrial organic matter. Three main hypotheses were formulated at an early stage of study: 1) bacteria in lakes transmit little dissolved organic carbon to higher tropic levels; 2) bacterial transmission of DOC to higher trophic levels is seasonally variable; 3) bacterial transmission of DOC to higher trophic levels becomes less important when phytoplankton production increases. These hypotheses were investigated by studying the pelagic food web of polyhumic Lake Mekkojärvi and examining the diet of the main zooplankter, *Daphnia longispina* (O. F. Müller), in enclosure and whole-lake ^{13}C-addition experiments with analysis of carbon and nitrogen isotopes signatures and isotope modelling (II and III). I also studied whether the PLFA-profile of *Daphnia* reflect their diet, and what kind of seasonal variation can be seen in PLFA-profiles of *Daphnia* (IV). In addition to these single lake experiments, four lakes with different DOC content were sampled in

spring and autumn 2006, to study the varying importance of allochthonous carbon among lakes.

Because anoxic methanogenesis and subsequent oxidation of methane by MOB is one important possible pathway to transmit terrestrial detrital carbon to higher trophic levels in lakes, my theses also aimed to quantify the role of MOB in the pelagic foodweb. This was conducted in two steps: first to find out if depleted $\delta^{13}C$ values observed for zooplankton in boreal humic lakes are really due to their feeding on isotopically light MOB (I), and secondly to quantify the role of MOB and methane as a carbon and food source for the pelagic foodweb of boreal polyhumic Lake Mekkojärvi (I, II-IV). These results were compared to $\delta^{13}C$ values of zooplankton in four other lakes, to examine variation in zooplankton diet among lakes with different DOC content.

2 MATERIAL AND METHODS

2.1 Description of Lake Mekkojärvi

Lake Mekkojärvi (61°13′ N, 25°8′ E) is located in the Evo forest area in southern Finland (Fig. 2). It is a naturally acidic (pH from 4 to 6) headwater lake in a lake chain, and is surrounded by forest with spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Mekkojärvi is a small (area 0.35 ha) and shallow (mean depth 3 m) lake.

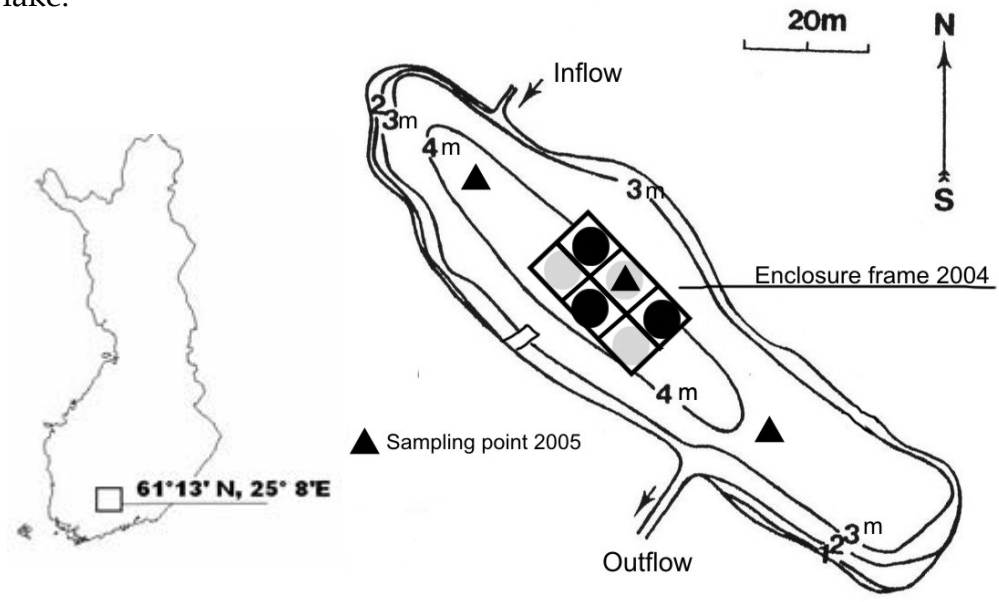

FIGURE 2 Enclosure experiments were carried out during summer and autumn of 2004 at Lake Mekkojärvi. The frame of the enclosures was anchored at the deepest area of the lake. During each experiment three treatment (black circles) and three control (grey circles) enclosures were used randomly. Then during spring, summer and autumn 2005 whole lake ^{13}C-addition experiments were carried out with sampling conducted at three points (black triangles).

Mekkojärvi receives a high loading of terrestrial carbon from the surrounding catchment, and because of its small size the lake water is dark brown (colour

300 – 800 mg Pt l^{-1}) and has a high total DOC concentration (20 to 45 mg C l^{-1}). The concentration of dissolved inorganic carbon (DIC) varies between 3 and 12 mg C l^{-1}.

After ice break-up the surface water warms rapidly and results in steep nutrient, oxygen and temperature gradients. However, spring mixing is sometimes incomplete (Salonen et al. 1984). Because of the thin euphotic zone (0.5-1 m), nutrients such PO_4^{3-} and NO_3^- in the epilimnion are rapidly consumed during the spring phytoplankton bloom, when the highest primary production also occurs. Total P content is between 10 and 15 P µg l^{-1} in the epilimnion and between 25 and 35 µg P l^{-1} in the hypolimnion, whereas total N is 500-1000 µg N l^{-1} and 800-1100 µg N l^{-1} respectively in the epi- and hypolimnion. High precipitation in 2004 led to higher loading of terrestrial organic matter during that year, as seen from the darker colour and greater nutrient concentrations than in the drier year of 2005 (Tables 1, 2, 3).

Bacterial density is greater in the oxic-anoxic boundary zone of the metalimnion (20-45 x 10^6 cells ml^{-1}) than in the oxic epilimnion (2-7 x 10^6 cells ml^{-1}, Arvola et al. 1992). Bacterial biomass increased vertically from spring to autumn and was highest in the hypolimnion during autumnal mixing when bacterial biomass was measured using PLFA as an index (IV). The high bacteriochlorophyll-*d* in the hypolimnion reflects a high density of photosynthetic bacteria, especially during spring and summer at 1.8-3.0 m when the highest bacteriochlorophyll-*d* concentration (> 200 µg l^{-1}) was measured. Further, 16S rRNA gene sequencing (S. Taipale and M. Tiirola, unpublished) revealed that these belonged to *Chlorobium* sp. and group 3 (Imhoff 2003), in which group *Chlorobium ferrooxidans* and *Chlorobium phaeobacterium* were equally abundant among *Chlorobium* species. The former species oxidizes iron and the latter sulphide. In the metalimnion layer (0.6-1.8 m) *Rhodoferax* sp., a photosynthetic purple bacteria, was also found.

Methane concentration is highest at 1.8-3.0 m, where the methane concentration is between 100 and 150 µmol l^{-1} throughout open water season. However, methane oxidation rate is highest at the depth of 0.6-1.2 m throughout all seasons, but especially during autumnal mixing when the methane oxidation rate is high throughout the water column. Methane-oxidising bacteria in Mekkojärvi belong to type I and within the most abundant *Methylobacter* genus, *M. psychrophilus, M. tundripaludum* and *M. luteus* are the most common species according to 16S rRNA gene sequencing (S. Taipale and M. Tiirola, unpublished).

The annual primary production of phytoplankton is below 10 g C m^{-2} (Salonen et al. 2005). The epilimnetic phytoplankton is dominated by flagellate chlorophytes (*Chlamydomonas* spp.) during spring, after which chrysophytes (*Mallomonas* spp.) and cryptophytes (*Cryptomonas* spp.) are abundant in the epilimnion during summer. Cyanophyaceae (*Microcystis sp*. type cell) are the dominant phytoplankton in the hypolimnion throughout the open water season, but are especially abundant during autumn (III). Also, the prasinophyte *Scourfieldia cordiformis*, is rather abundant in the upper hypolimnion (Arvola et al. 1992).

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. The aquatic moss, *Warnstorfia procera,* grows around the shoreline of Mekkojärvi and provides good habitat for many macroinvertebrates.

2.2 Experimental design

The possible role of MOB in the diet of zooplankton was first studied in laboratory experiments by feeding *Daphnia* from Mekkojärvi with microbial suspensions with and without CH_4 addition. DI^{13}C-enrichments were first carried out in enclosures in Mekkojärvi in summer and autumn during 2004 to quantify the relative roles of terrestrial carbon, MOB and autochthonous carbon in a stratified water column at different seasons. To quantify seasonal changes in the contribution to *Daphnia* diet of phytoplankton, heterotrophic bacteria, green sulphur bacteria and MOB, at the most realistic scale possible, DI^{13}C-enrichments were carried out at the whole lake scale in spring, summer and autumn 2005 in Mekkojärvi. The PLFA-profiles of seston and *Daphnia* were analyzed to obtain further insight into differences in bacterial consumption by *Daphnia*. The $\delta^{13}C$ of zooplankton from four nearby lakes was also analyzed to estimate the role of MOB in zooplankton diet in lakes of different DOC content during spring and autumn in 2006.

2.2.1 *Daphnia* feeding experiment with methanotrophic bacterial suspension and methane addition (paper I)

Two laboratory experiments were carried out with *D. longispina* feeding on the natural microbial community (< 10 μm) of Mekkojärvi. The microbial suspension was taken in autumn (M1 experiment) and winter (M2 experiment) to ensure presence of MOB, but to minimize abundance of phytoplankton. The water for M1 experiment was collected from 0.5 m depth on 18 November 2002 after autumnal turnover in the lake, and for M2 experiment on 16 March from under ice. The water was kept dark at 4 °C until the experiment started. Before experiments *D. longispina* was cultured for several generations by feeding on *Scenedesmus* sp. For both M1 and M2 experiments, 40-45 juvenile (<0.5 mm) *Daphnia* were put in to eight replicate chambers. All chambers received the natural microbial suspension from Mekkojärvi, but the microbial suspension of four chambers contained biogenic CH_4 which was added to the suspension 24 h before feeding was started. This methane was obtained from anaerobic fermentation of aquatic plants in 500 mL bottles with gas-tight septa for ca. 3 months, during which the CH_4 concentration in the headspace of the bottles rose to 10-27 x 10^3 ppmv. Experiments were carried out in continuous-flow culture systems (Lampert 1976) in dark and constant temperature (+20°C) for 10 days. To compare the *Daphnia* growth rate with microbial and algal suspension,

Daphnia cultures were also fed on algae in an experiment conducted in addition to the M1 and M2 experiments. The $\delta^{13}C$ was measured from the added food suspension as POM and from *Daphnia* at the beginning and the end of the experiment.

To support this laboratory experiment, juvenile and adult *D. longispina* were collected from Mekkojärvi during autumnal mixing (from 16 September to 11 October 2004) from the control enclosures of the DI^{13}C-addition experiment (see paper II) for $\delta^{13}C$ analysis. Consumption of CH_4 by MOB in the water column was estimated from the linear decrease of CH_4 concentration.

2.2.2 Enclosure DI^{13}C-addition experiment and isotope modelling (paper II)

Two separate mesocosm experiments were made during the open water season 2004, first in mid-summer (6 to 30 July), and again in autumn (14 September to 12 October). The experiments were made in cylindrical enclosures (diameter 2 m, height 4 m, open from the bottom) 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. $NaH^{13}CO_3$ (99 atom %, CK Gas Products Ltd.) was added into the epilimnion of each treatment enclosure, and the water mixed gently to a depth of 0.5 m. Additions of 0.29 mmol were made five times per week, with a double amount added when there was no addition on the next day. Additions were made from 8 to 25 July 2004 during the summer experiment and from 16 September to 5 October 2004 during the autumn experiment. Any scheduled sampling of enclosures was done before isotope additions were made. Changes in carbon stable isotope ratios were monitored for DIC, DOC, POC and zooplankton (*D. longispina*) throughout each experimental period, along with a range of physical, chemical and biological variables (Tables 1 and 2).

Because there were many possible carbon sources for *Daphnia*, in this experiment we estimated the likely contribution of different carbon sources to *Daphnia* using two different modelling approaches. The first model (based on Pace et al. 2004) estimated the relative contributions of allochthonous and autochthonous carbon and took into account the fractionation of ^{12}C and ^{13}C, the proportion of carbon of 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 used this model to estimate the proportional contribution (w) of terrestrial organic matter (TOM) which is probably mainly channelled via heterotrophic bacteria, relative to that of phytoplankton (PP), to *Daphnia* biomass (TOM-PP model). Then in a variation of this model (MOB-PP model), terrestrial organic matter was replaced by methane-oxidizing bacteria (MOB). The assumed $\delta^{13}C$ value for MOB was based on $\delta^{13}CH_4$ values measured from Mekkojärvi (Kankaala et al. 2007a), with values taken from the literature (Templeton et al. 2006) for further carbon isotopic fractionation (-7.8 to -28.5‰) during methane oxidation.

To get an alternative view of *Daphnia* carbon sources and carbon flow in the pelagic food web of Mekkojärvi, we made independent mass balance calculations (mg C m^{-2} d^{-1}) for the summer and autumn periods. The carbon flows through algae, protozoa, heterotrophic bacteria and MOB were estimated for a range of moderate and high growth efficiencies (GE) of MOB and low and high assimilation efficiencies (AE) of *Daphnia*. 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 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 (Salonen et al. 1976). Methanotrophic (MOB) activity in the water column was not measured directly during the enclosure experiments. We therefore made mass balance calculations based on MOB activity measured in 2005 assuming a range of moderate and high growth efficiencies for MOB. An estimate of hypolimnetic green-sulphur bacterial production was obtained from *in situ* anaerobic dark inorganic ^{14}C-uptake results for Mekkojärvi (Kuuppo-Leinikki & Salonen 1992). Net production of protozoan flagellates was estimated from the growth rate results of Salonen et al. (1992).

TABLE 1 Physical and chemical properties of water in the enclosures and in the lake in summer 2004.

Summer 2004	Lake		Enclosures	
	0-0.6 m	0.6-3.0 m	0-0.6 m	0.6-3.0 m
Water Colour (mg Pt l^{-1})	598.0 ± 48.0	518.0 ± 12.0	515.0 ± 42.0	453.0 ± 25.0
pH	4.7 ± 0.0	5.7 ± 0.1	5.9 ± 0.4	6.1 ± 0.1
Alkalinity (mmol l^{-1})	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Conductivity (µS cm^{-1})	47.3 ± 4.4	64.0 ± 2.5	54.7 ± 3.8	70.5 ± 2.5
tot P (µg P l^{-1})	14.5 ± 4.9	24.0 ± 0.0	10.1 ± 2.2	25.8 ± 2.7
tot N (µg N $^{l-1}$)	1024.0 ± 201.0	907.0 ± 9.0	833.0 ± 62.0	900.0 ± 203.0
Chl-a (µg Chl l^{-1})	2.5 ± 0.7	129.2 ± 3.6	7.9 ± 2.9	108.2 ± 15.2
DIC (mg C l^{-1})	4.6 ± 1.0	11.5 ± 2.2	6.9 ± 3.9	9.5 ± 4.0
DOC (mg C l^{-1})	41.7 ± 3.8	31.4 ± 2.8	30.7 ± 1.7	25.2 ± 1.4
POC (mg C l^{-1})	0.4 ± 0.1	1.4 ± 0.0	0.4 ± 0.2	1.6 ± 0.3
CH_4 (µmol l^{-1})	1.4 ± 1.0	59.8 ± 9.3	5.1 ± 3.1	61.2 ± 9.4

TABLE 2 Physical and chemical properties of water in the enclosures and in the lake in autumn 2004.

Autumn 2004	Lake		Enclosures	
	0-0.6 m	0.6-3.0 m	0-0.6 m	0.6-3.0 m
Water Colour (mg Pt l^{-1})	760.0 ± 48.0	652.0 ± 19.0	654.0 ± 76.0	558.0 ± 41.0
pH	5.2 ± 0.3	5.8 ± 0.1	5.9 ± 0.3	5.8 ± 1.1
Alkalinity (mmol l^{-1})	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0
Conductivity (µS cm^{-1})	44.5 ± 1.1	55.1 ± 1.1	53.2 ± 5.1	63.0 ± 3.1
tot P (µg P l^{-1})	13.0 ± 1.4	22.5 ± 0.7	16.6 ± 3.8	31.8 ± 6.4
tot N (µg N $^{l-1}$)	1014.0 ± 28.0	1019.0 ± 22.0	1010.0 ± 50.0	1057.0 ± 85.0
Chl-a (µg Chl l^{-1})	3.3 ± 2.2	52.0 ± 25.2	11.2 ± 3.1	44.1 ± 7.5
DIC (mg C l^{-1})	6.4 ± 1.8	11.8 ± 1.1	8.7 ± 2.3	10.0 ± 2.4
DOC (mg C l^{-1})	42.3 ± 3.7	35.2 ± 1.4	34.2 ± 3.2	28.3 ± 2.4
POC (mg C l^{-1})	0.3 ± 0.0	1.0 ± 0.3	0.6 ± 0.2	1.5 ± 0.3
CH_4 (µmol l^{-1})	9.8 ± 25.6	56.1 ± 26.0	6.5 ± 8.3	45.9 ± 22.4

2.2.3 Whole lake DI^{13}C addition and IsoSource modelling (paper III)

Three separate whole lake DI^{13}C-additions were made during the spring, summer and autumn of 2005. At the beginning of each experiment 0.24 mmol of $NaH^{13}CO_3$ (99 atom %, CK Gas Products Ltd.) and after that 0.18 mmol of $NaH^{13}CO_3$ was spread into the epilimnion by watering can while rowing around the lake. Additions were made three times per week. The first set of additions was made from 16 May to 3 June, the second from 11 July to 1 August 2005, and the final one from 19 September to 3 October 2005. These are referred to simply as spring, summer and autumn experiments, respectively.

Water samples were taken from the epilimnion (0 – 0.6 m), metalimnion1 (0.6-1.2 m), metalimnion2 (1.2-1.8 m) and from pooled anoxic hypolimnion samples (1.8 – 3.0 m) using a Limnos tube sampler (height 60 cm, vol. 4.25 l), after which water was passed through a 100 µm mesh size net. The $\delta^{13}C$ of DIC, and the $\delta^{13}C$ and $\delta^{15}N$ values of POM and zooplankton were monitored twice per week, and the $\delta^{13}C$ and $\delta^{15}N$ of DOM were measured at the beginning, mid and end of each experiment. During each experiment primary production, bacterial production and zooplankton biomass were measured twice per week and methanotrophic activity was measured once per week. Oxygen and temperature were measured five times per week. Various other chemical parameters were measured during each experiment and results are summarised in Table 3. All samples were taken before DI^{13}C additions were made.

Because the diet of the dominant zooplankton species, *D. longispina*, presumably consisted of many sources, including phytoplankton (PP), heterotrophic bacteria (HB), methanotrophic bacteria (MOB) and green sulphur

bacteria (GSB), their contribution could not be estimated from carbon isotopes alone. Therefore, we first measured or estimated likely carbon and nitrogen isotopic signals of food sources. We then used a four sources mixing model and the IsoSource software (Phillips & Greg 2003, www.epa.gov/wed/pages/models.htm) to estimate the likely magnitude of each food source during spring, summer and autumn periods and compared the model outputs to other biological parameters such as primary production, bacterial production and methanotrophic activity.

TABLE 3 Physical and chemical properties of lake water during spring, summer and autumn periods in 2005.

	Spring		Summer		Autumn	
	0-0.6 m	0.6-3.0 m	0-0.6 m	0.6-3.0 m	0-0.6 m	0.6-3.0 m
Water Colour (mg Pt l^{-1})	412.0 ± 38.0	464.0 ± 31.0	411.0 ± 50.0	442.0 ± 24.0	488.0 ± 3.0	461.0 ± 12.0
pH	5.5 ± 0.1	6.0 ± 0.1	5.8 ± 0.2	6.1 ± 0.1	6.0 ± 0.3	6.2 ± 0.1
P/PO_4 (µg P l^{-1})	1.5 ± 0.7	4.0 ± 2.8	1.5 ± 0.7	5.5 ± 2.1	2.5 ± 0.7	9.5 ± 0.7
tot P (µg P l^{-1})	14.5 ± 3.5	29.0 ± 1.4	10.0 ± 2.8	34.0 ± 1.4	10.0 ± 2.8	32.0 ± 1.2
$N/NO_2 + NO_3$ (µg N l^{-1})	27.0 ± 9.9	18.5 ± 13.4	22.0 ± 12.7	13.5 ± 9.2	13.5 ± 0.7	8.5 ± 0.7
N/NH_4 (µg N l^{-1})	12.0 ± 1.4	186.5 ± 40.3	22.3 ± 8.2	258.8 ± 42.2	25.0 ± 4.2	296.5 ± 4.9
tot N (µg N $^{l-1}$)	611.0 ± 7.1	880.0 ± 31.1	574.0 ± 70.7	914.5 ± 34.6	607.0 ± ##	911.0 ± 20.0
Chl-a (µg Chl l^{-1})	3.3 ± 2.2	52.0 ± 25.2	11.2 ± 3.1	44.1 ± 7.5	11.2 ± 3.1	44.1 ± 7.5
Chl-d (µg Chl l^{-1})	4.7 ± 2.3	136.3 ± 10.5	3.5 ± 0.6	220.0 ± 15.2	7.6 ± 6.8	92.4 ± 45.9
DIC (mg C l^{-1})	3.1 ± 0.9	12.2 ± 2.5	3.5 ± 0.8	14.0 ± 1.6	5.0 ± 1.6	11.1 ± 2.9
DOC (mg C l^{-1})	26.8 ± 1.1	26.2 ± 0.7	22.3 ± 1.7	21.9 ± 0.9	24.9 ± 1.6	20.9 ± 0.9
POC (mg C l^{-1})	0.4 ± 0.1	1.5 ± 0.3	0.3 ± 0.1	1.5 ± 0.5	0.3 ± 0.1	0.6 ± 0.1
CH_4 (µmol l^{-1})	1.5 ± 1.1	121.2 ± 5.8	11.6 ± 7.3	133.0 ± 9.0	5.9 ± 7.6	101.6 ± 60.6

2.2.4 The PLFA-profile of seston and *Daphnia* as a tool for foodweb studies (paper IV)

To acquire more understanding of whether the PLFA-profile of *Daphnia* reflects their diet or not, and what kind of seasonal dietary changes can be seen in PLFA-profiles of *Daphnia*, seston and zooplankton samples were collected for PLFA analysis once per week during each ^{13}C-whole lake experiment in 2005.

Zooplankton samples as well as filtered water column (seston) samples were put immediately into extraction tubes containing 7.5 mL of chloroform, 15 mL methanol, and 50 mM phosphate buffer (pH 7.4). Lipids were extracted, fractionated, saponified and methylated according to the method of Bligh & Dyer (1959) modified by White et al. (1979) and Keinänen et al. (2002). Lipids were fractionated to neutral, glyco-, and phospholipids using 10, 20 and 10 mL of chloroform, acetone and methanol, respectively. For quantification, dipentadecanoylphostatidylcholine (c15:0) was added as an internal standard. In mild alkaline methanolysis, tridecanoic and nonadecanoic acid methyl esters were used as fatty acids internal standards. To separate monounsaturated fatty acids (MUFA) from each other the modified timer program of Virtue et al. (1996) was used. The position of the double bond in MUFAs was determined by

capillary GC-MS of their dimethyl disulphide (DMDS) adducts (Nichols et al. 1986).

The phytoplankton species composition and biomass were determined by inverted microscopy using a settling chamber technique (Utermöhl 1958). Bacteriochlorophyll-*d* (Takayashi & Ichimuyara 1979) and methane oxidation rate (Kankaala et al. 2007a) were also measured. Pearson's correlation was computed between phytoplankton biomass and concentrations of individual PLFAs of the upper water column (epi- and metalimnion combined) at significance levels of 0.01 and 0.05 to detect PLFA-biomarkers for the most common phytoplankton groups and species. These PLFA-biomarkers were compared to respective values found in the literature (e.g. Dikjman & Kramkamp 2006). The correlations between individual PLFA concentrations and methane oxidation rates and bacteriochlorophyll-*d* were also calculated to determine whether MOB in our study lake mainly belonged to type I or II, to identify biomarkers for MOB and to detect possible biomarkers for photoautotrophic bacteria and especially for *Chlorobium* sp. After identifying possible biomarkers for phytoplankton, MOB and *Chlorobium* sp., Pearson's correlation was computed between the proportions of PLFAs in seston (mean values of the two first water column layers) and the proportions of PLFAs in adult and juvenile *Daphnia.* In addition to calculating correlations for the concurrent values, correlations were calculated between the *Daphnia* PLFA profile and that of the water column 7 days earlier and for mean values of each studied season (spring, summer and autumn). These correlations were calculated to established whether the PLFA-profile of the feeding environment is mirrored by that of *Daphnia* and hence to clarify the utility of PLFAs in aquatic foodweb studies.

2.2.5 Carbon mass balance calculations for the whole lake in 2005

To combine the dietary estimates derived from carbon and nitrogen isotope-based mixing models with the PLFA-profile of adult *Daphnia*, I here make further, original, carbon mass balance calculations for putative *Daphnia* carbon sources and carbon flow in the pelagic food web of Mekkojärvi (mg C m^{-2} d^{-1}) for the spring, summer and autumn seasons of 2005 based on data from this thesis and from earlier studies on Mekkojärvi. For daily production estimates the mean dry weight biomass of *D. longispina* was converted to carbon by a factor 0.5 (Salonen et al. 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 (Ojala & Salonen 2001). The carbon flows through phytoplankton, protozoa, heterotrophic bacteria, and methanotrophic bacteria were estimated for both 40% (low) and 60% (high) assimilation efficiencies (AE) of *Daphnia.* This range was adapted from He & Wang (2006) who quantified AE of *Daphnia magna* feeding on *Chlamydomonas reinhardtii.* For the mass balance calculations, MOB activity was 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 each experimental period. Here I have used 30% for moderate and 70%

for high growth efficiency of MOB to encompass a proper range of growth estimation. These values are within the range of reported growth efficiency for MOB of 6-77% by Bastviken et al. (2003), and 15-80% by King (1992), and of 55% for M*ethylomonas methanica* by Templeton et al. (2006).

The estimates of primary production were obtained from the daily mean values of the lake measured in 2005. The minimum value for heterotrophic bacterial production was taken from daily mean values of ^{14}C-leucine uptake and the maximum value is the mean value of bacterial growth rate (26-65 mg C m^{-3} d^{-1}) reported by Salonen et al. (1992) in the epilimnion of Lake Mekkojärvi. An estimate of hypolimnetic green sulphur bacterial production was obtained from *in situ* anaerobic dark inorganic ^{14}C-uptake results in Lake Mekkojärvi (Kuuppo-Leinikki & Salonen 1992). Net production of protozoan flagellates was estimated from the growth rate results of Salonen et al. (1992).

2.2.6 The δ^{13}C of zooplankton in five lakes with different DOC

During May and September-October 2006, five small forest lakes including Mekkojärvi (Table 4) with various DOC content were sampled. Spring samples represent the period when primary production is expected to be highest and samples after autumnal water column mixing represent the period of expected highest MOB activity. Thus these spring and autumn samplings probably represent two extremes within changing carbon flow patterns during a whole open water season. During sampling, various biological and chemical parameters were measured (Table 4) as well as carbon and nitrogen isotope ratios from zooplankton, especially cladocerans. Among the sampled lakes, colour (~30 mg Pt l^{-1} and DOC content (~5-6 mg C l^{-1}) was the lowest in the epilimnion of Valkea-Mustajärvi, which is an oligotrophic clear water lake, and there the water column was well oxidised down to 7 m in both spring and autumn. The DOC content and colour are around 15 mg C l^{-1} and 150-160 mg Pt l^{-1} in lakes Valkea-Kotinen and Alinen-Mustajärvi, which both exhibited oxygen and temperature stratification in spring 2006. Valkea-Kotinen is partly a spring meromictic lake, and thus the water column is not mixed after every ice-melt, whereas Alinen-Mustajärvi is a meromictic lake, and still had an anoxic hypolimnion after autumnal partial mixing in 2006. During spring 2006, DOC content was highest (26 mg C l^{-1}) in Mekkojärvi, whereas colour was the highest (400 mg Pt l^{-1}) in Nimetön, which is a relatively deep (11 m) meromictic lake, and was anoxic below 3 m after autumnal mixing in 2006.

TABLE 4 Physical and chemical properties of the five lakes sampled during spring, and autumn 2006. Epilimnion represents the oxic surface layer of the water column whereas hypolimnion is the anoxic part of the water column.

Lake	Season	Layer	Colour (mg Pt L^{-1})	pH	DIC (µM L^{-1})	DOC (mg C L^{-1})	POC (mg C L^{-1})	CH_4 (µM L^{-1})	BB (µg L^{-1})	PB (mg L^{-1})	BChl d (µg L^{-1})	Chl a (µg L^{-1})
Valkea-Mustajärvi	Spring	Epi (0-5 m)	30	6.3	119±43	nd	0.43±0.02	0.08±0.02	12	0.6	1.4	3
		Hypo (9 m)	57	6.0	476	nd	1.09±0.01	0.07	45	4.2	1.8	2.7
	Autumn	Epi (0-6 m)	27	6.5	130±4	5.8±0.1	0.31	0.13±0.02	25	2.6	2.2	4.8
		Hypo (9 m)	nd	nd	712	5.4±0.1	1.68±0.08	66.8	64	3.6	30.8	22.8
Alinen-Mustajärvi	Spring	Epi (0-2 m)	149	5.4	151±196	12	0.63	0.34±0.30	59	2.2	3	6.2
		Hypo (5-6 m)	145	5.3	938±19	15.4±0.1	1.48±0.01	285.7±7.5	108	3.7	30.4	24.1
	Autumn	Epi (0-3 m)	125	5.4	199±3	11.1	0.46	2.7±0.1	39	2.4	8	6.8
		Hypo (5 m)	nd	nd	365	14.9±0.4	0.98±0.01	59.8	97	2.1	56.1	39.3
Valkea-Kotinen	Spring	Epi (0-3 m)	163	5.4	166±159	14.5±0.6	1.78±0.06	0.10±0.10	49	20.6	6.3	9.7
		Hypo (5.5-5.8 m)	243	5.5	652	18.2±0.4	1.86±0.02	47.6	39	2	14.4	15.3
	Autumn	Epi (0-3 m)	157	5.6	141±5	13.4±0.1	0.78±0.03	0.70±0.19	31	4.7	9.9	18.1
		Hypo (6-6.3 m)	263	5.9	856	16.5±0.1	1.23±0.05	137.6	77	2	14.2	13.3
Mekkojärvi	Spring	Epi (0-0.6 m)	199	6.0	270	26.3±1.1	0.87	2.3	165	1.2	7.3	11.3
		Hypo (1.8-3.0 m)	425	6.0	1	21.9±1.1	2.27±0.11	77.7±11.0	155	0.7	162.3	101.9
	Autumn	Epi (0-0.6 m)	327	6.0	270	17.6±0.1	1.14±0.02	2.3	81	0.8	13.5	12.9
		Hypo (1.8-3.0 m)	nd	nd	974±170	17.5±0.1	1.07±0.09	22.3±37.9	77	0.7	14.7	11.9
Nimetön	Spring	Epi (0-2 m)	392	5.6	374±291	22.3±0.3	0.68	1.4±1.6	20	0.4	4.2	8.5
		Hypo (6-10m)	618	5.9	0	43.8±0.4	0.72±0.02	631±480	27	0.1	8.1	7.2
	Autumn	Epi (0-1 m)	361	5.4	nd	22.3±0.2	0.47	nd	32	1.1	6.8	7.5
		Hypo (4-10m)	nd	nd	nd	32.0±0.3	0.68	nd	64	0.4	15.9	11.7

nd = not determined, BB = bacterial biomass, PB = phytoplankton biomass

3 RESULTS

3.1 Daphnia feeding experiment with methanotrophic bacterial suspension and methane addition (paper I)

The initial CH_4 concentration in Mekkojärvi was low (0.03±0.0004 mmol m^{-3}) before the M1 experiment started. There was an average 260-fold increase in CH_4 concentration in the chambers with added CH_4, while CH_4 concentration remained negligible in chambers with no added CH_4. The $\delta^{13}C$ of CH_4 was -32.4±1.5‰ during experiment M1. The initial $\delta^{13}C$ signature of *Daphnia* was -19.7±0.1‰, but become more depleted during the experiment in all chambers: -26.6±0.5‰ in chambers with no added CH_4 and -29.6±0.6‰ in chambers with added CH_4; thus *Daphnia* from CH_4 chambers were 3‰ more depleted than *Daphnia* in no CH_4 added chambers.

The initial CH_4 concentration in Mekkojärvi under ice cover and before the start of the M2 experiment (3.9±0.1 mmol m^{-3}) was much higher than before the M1 experiment, but was only 0.07 mmol m^{-3} by the end of the M2 experiment indicating that almost all CH_4 was consumed by methantrophic bacteria. Hence, the CH_4 concentration in chambers with added CH_4 was high (7.1±1.7 mmol m^{-3}). During experiment M2, the $\delta^{13}C$ of CH_4 was -63.4±1.3‰. Initially *Daphnia* were quite ^{13}C enriched ($\delta^{13}C$ -18.5±0.1‰), but became more than 20‰ depleted by the end of the experiment. *Daphnia* from chambers with added CH_4 (-41.0±0.2‰) were slightly more depleted than *Daphnia* from chambers with no CH_4 addition (-40.5±0.3‰).

In the algal food experiment, the $\delta^{13}C$ of *Daphnia* was initially -18.6±0.1‰ and declined only slightly to -23.0±0.1‰ at the end. Hence these laboratory experiments confirmed that the light $\delta^{13}C$ values of *Daphnia* are related to their feeding on methanotrophic bacteria. The calculated growth rate of *Daphna* was highest (0.28 d^{-1}) during the algal food experiment, but was also quite high (0.19 d^{-1}) during the M2 experiment in relation to the M1 experiment (0.06 d^{-1}).

During the field experiment period in 2004 (see paper II), the mean CH_4 concentration in the water column of the enclosures decreased from 75 to 23

mmol m^{-3}. The $\delta^{13}C$ of adult and juvenile *Daphnia* became concurrently more depleted along with autumnal mixing, and $\delta^{13}C$ of adult and juvenile *Daphnia* from control enclosures dropped from -40.5‰ to -50.3‰, reflecting *Daphnia* feeding extensively on methanotrophic bacteria also at the field scale.

3.2 Enclosure $DI^{13}C$-addition experiment and isotope modelling (paper II)

During both summer and autumn enclosure 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 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 (cf. Kankaala et al. 2006). 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.

For the summer experiment the TOM-PP and MOB-PP models fitted the *Daphnia* $\delta^{13}C$ results best 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. During the summer experiment the TOM-PP model suggested that 37% of the diet originated from terrestrial sources. For the summer the MOB-PP model suggested that the proportion of methanotrophic bacteria in the diet of *Daphnia* was 11-20%, when the $\delta^{13}C$ of CH_4 varied between -58.7 to -101.4‰. According to the standard deviation of residual means, the TOM-PP model fitted the data a little better than the MOB-PP model. However, the TOM-PP model did not provide a good fit to the data from the autumn experiment and actually gave its best solution when the proportion of memory carbon (m) was 0.15 and TOM (w) was greater than 1.00. This implies that during the autumn experiment *Daphnia* acquired negligible autotrophic carbon. The MOB-PP model estimated the contribution of methanotrophic 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.

The calculated carbon mass balances at moderate-high GE of MOB and low –high AE of *Daphnia* for summer suggested that phytoplankton could have supplied 21-32% of *Daphnia* carbon demand. Correspondingly, heterotrophic bacteria (HB), methanotrophic bacteria (MOB) and green sulphur bacteria (GSB) could have supplied 5-7%, 7-21%, and 15-30% of *Daphnia* carbon demand, respectively. The protozoa would have supplied only 2-3% of all *Daphnia* carbon demand during summer. However, according to this model unknown food sources could have made up 18-53% of *Daphnia* carbon demand. For the autumn period this unknown food sources component shrunk to 0-25%, and MOB could have supplied 37-113% of all of *Daphnia* carbon demand. The magnitude of primary production was negligible (< 1%) and the magnitude of protozoa unknown. The heterotrophic bacteria and green sulphur bacteria could have supplied 11-12.5% and 15-23% of all *Daphnia* carbon demand, respectively. Additionally, bacterial production measured as ^{14}C-leucine incorporation likely underestimates total bacterial production and true net bacterial growth rate may have been approximately 10-fold higher (26-65 mg C m^{-3} d^{-1}), as measured by Salonen et al. (1992) in the epilimnetic water of Mekkojärvi. If those are more realistic estimates of heterotrophic bacterial production in the epilimnion of Mekkojärvi, then a higher proportion (10-36%) of *Daphnia* carbon demand may actually have been supported by heterotrophic bacteria, close to the TOM-PP model estimate.

3.3 Whole lake DI^{13}C addition and IsoSource modelling (paper III)

After ice-melt at the beginning of May 2005, Mekkojärvi was soon sharply stratified with respect to both temperature and oxygen. Stratification persisted until mid-October when the whole water column mixed. During spring and summer, oxygen was at the limit of detection (0.3 mg l^{-1}) at 1.5 m and during autumn at 2.0 m. The total *Daphnia* biomass was highest during the autumn period (243±85 mg C DW m^{-2}) but was at similar lower levels in spring and summer (142±140 mg C DW m^{-2} and 153±22 mg C DW m^{-2}, respectively). Bacterial production and methane oxidation were also highest during the autumn period, and at similarly lower levels in spring and summer. The warm autumn gave unusually high mean primary production (15.5±6.6 mg C m^{-2} d^{-1}) that was only slightly less than during the summer (25.6±13.3 mg C m^{-2} d^{-1}). However, mean primary production in spring (58.0±42.0 mg C m^{-2} d^{-1}) was twice that in summer or autumn (see Fig. 3).

During the spring, summer and autumn periods, added $NaH^{13}CO_3$ quickly increased the epilimnetic DIC δ^{13}C from an initial δ^{13}C of -20 to -22‰ up to between 18 and 21‰ during ^{13}C-enrichments, but had little influence on DIC in the meta- and hypolimnion. The ^{13}C-enrichment of DIC was transmitted to POM and on to adult and juvenile *Daphnia,* which became about 10‰

heavier than initial $\delta^{13}C$ values. This indicates that during spring and summer *Daphnia* were feeding on autochthonous carbon and primary producers at an adequate level to transmit the ^{13}C-label.

According to the position of *Daphnia* in the isotope diet polygons (see Fig. 5 in paper III) *Daphnia* consumed each of the four putative food sources during all seasons, except for the last weeks of autumn when *Daphnia* was positioned between phytoplankton and methanotrophic bacteria and hence *Daphnia* diet would then have consisted half of phytoplankton and half of methanotrophic bacteria (MOB). The IsoSource mixing model gave a narrow range for proportions of MOB and also for phytoplankton (see Fig. 3). The proportion of phytoplankton was greatest (37-71%) during the spring period, and its magnitude decreased first to 25-61% in summer, then to 31-56% in the first autumn period, and finally to 42-49% during the second autumn period. In contrast to phytoplankton, the proportion of MOB in *Daphnia* diet rose during the open water season 2005, and ranged from 9% to 50%, achieving the smallest proportion during spring and the largest during the second autumn period.

Estimated deviations in the contribution of each food source were widest for heterotrophic bacteria (HB), mainly because their estimated nitrogen and carbon isotope values were similar to those of *Daphnia*. This could be caused either because *Daphnia* very strongly favoured HB in their diet or because HB carbon and nitrogen isotope values were intermediate between other food sources. According to the IsoSource model outputs, the magnitude of HB in *Daphnia* diet would have been greatest during the summer (0-58%) and spring (0-54%) periods, and was lowest during the last weeks of the autumn period (0-10%). The IsoSource estimations for *Chlorobium* were similar for spring (0-20%), summer (0-16%) and first autumn (0-19%) periods, but in the second autumn period its role in *Daphnia* diet was clearly negligible (<1%).

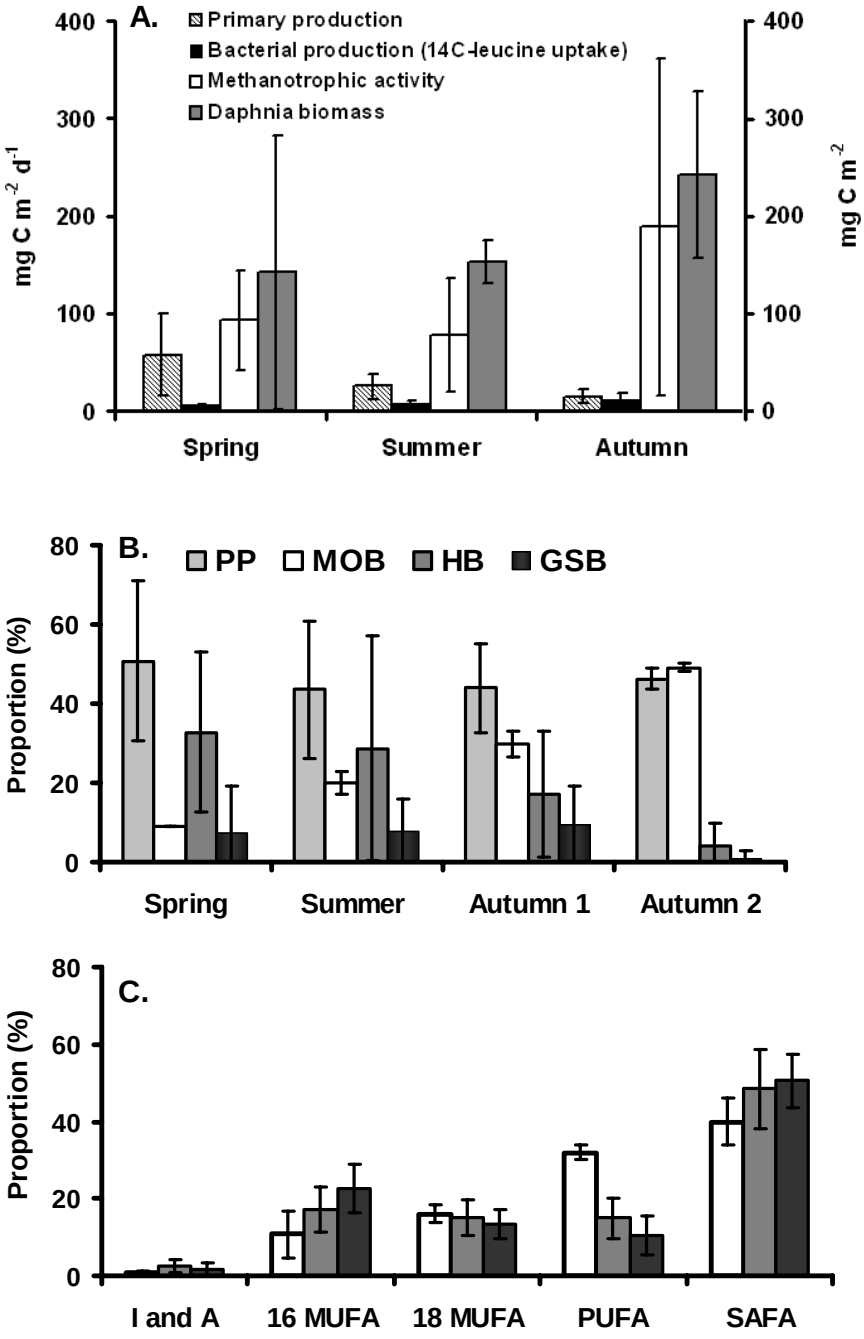

FIGURE 3 **A.** Primary production, bacterial production and methanotrophic activity (all mg C m^{-2} d^{-1}) and the biomass of Daphnia (mg C m^{-2}) during 2005 in Mekkojärvi. **B.** Mean values with 99% confidence intervals of IsoSource estimated proportions of different food sources in adult *Daphnia* diet during the spring, summer, autumn1 and autumn2 periods in Mekkojärvi. **C.** The contribution of *iso*- and *anteiso* –branching, 16 MUFA, 18 MUFA, PUFA and SAFA PLFAs in adult *Daphnia* during spring, summer and autumn periods in 2005.

3.4 The PLFA-profile of seston and *Daphnia* as a tool for foodweb studies (paper IV)

Correlations between phytoplankton biomasses and the quantitative amounts of PLFAs revealed that there were typical PLFAs for major phytoplankton groups in a wild assemblage. Correlations between PLFAs and phytoplankton groups matched earlier findings (Wood 1979, Dijkman & Kromkamp 2006) with some exceptions. *Chlorophyceae*, and especially *Chlamydomonas* sp. (987 µg fresh weight l^{-1}) were the most abundant phytoplankton and correlated strongly with linolenic acid 18:3ω3 PLFA, which is a characteristics PLFA of *Chlamydomonas*.

Methane oxidation rate in the epi- and metalimnion correlated significantly with all 16 MUFAs of the water column, but best with 16:1ω8c ($r=0.81$, $p=0.01$), 16:1ω6c ($r=0.78$, $p=0.01$) and 16.1ω5t ($r=0.77$, $p=0.01$), which are all considered specific for MOB type I. Thus, MOB in our study lake evidently belonged to type I, which was confirmed by DNA analysis (Taipale & Tiirola, unpublished). Bacteriochlorophyll-*d* correlated significantly ($P < 0.01$) with many PLFAs, but best with 14:0 ($r=0.71$, $p=0.01$) and a-15:0 ($r=0.70$, $p=0.01$).

During the spring, summer and autumn, 35 PLFAs were identified in seston, of which 29 were also detected from *Daphnia* (Table 5). There were seasonal changes (Fig. 3) in abundances of SAFA, MUFA, PUFA and *iso* and *anteiso* -branching PLFAs and also between adult female, and juvenile and male *Daphnia.*

Daily primary production correlated very strongly ($r=0.73$, $p<0.01$) with the proportion of PUFAs in adult *Daphnia.* Also, the primary production of each season correlated very strongly ($r=0.9994$, $p=0.02$) with the mean values calculated for each season (spring, summer, autumn) of PUFA percentages in adult *Daphnia*. The percentages of MOB-specific PLFAs in adult *Daphnia* correlated ($r=0.9938$, $p<0.07$) with the measured methane oxidation rate of each season. Additionally, the contribution of *iso*- and *anteiso* –branching PLFAs in adult *Daphnia* correlated ($r=0.576$, $p<0.05$) with daily bacterial production in epilimnion (0-1.2 m).

Furthermore the PLFA composition of adult *Daphnia* correlated significantly with the PLFA composition of seston (0-1.2 m) one week earlier, indicating how the PLFA-profile reflects zooplankton diet also during growth and development. This one week transition interval was most clearly seen from similar shifts in abundances in seston and in adult *Daphnia* of 18:3ω3 (Fig. 4.). However, this one-week interval correlation was not so strong as that between spring, summer and autumn seasons, perhaps indicating that membrane FAs are not changing completely according to concurrent diet, and especially PUFAs can remain longer in membrane according to the environment conditions. Additionally, total PUFAs correlated even more strongly than individual PUFAs, which can reflect lipid modification within ω-3 and ω-6 series PLFAs in *Daphnia*. Especially, arachidonic acid (20:4ω6) was negligible (<1%) in seston, but contributed more than 1.4% in *Daphnia*. This was noted

previously in the total FA fraction (Kainz et al 2004, Brett et al. 2006), and was proposed by them that arachidonic acid is converted from linoleic (18:2ω6) and γ-linolenic acid (18:3ω6). Because individual 16 MUFAs correlated even stronger than total 16 MUFAs, probably modification within 16 MUFAs is minor, and proportional changes in 16 MUFAs of zooplankton reflect changes in bacterial sources. Because PUFAs and 18 MUFAs in juvenile *Daphnia* correlated significantly and positively only with the concurrent water column PLFA-profile, it is evident that metabolism is quicker in juveniles than in adults.

Altogether, PLFA-profiles of *Daphnia* revealed significant changes in their diet (see Fig. 3 and Table 5). During the spring period, algal PUFAs dominated *Daphnia* PLFAs, after which their contribution was first reduced by half in summer and then in autumn was only a third of the spring magnitude. In contrast the contribution of 16 MUFAs typical of Gram-negative bacteria and MOB increased in *Daphnia* towards autumn. During autumn, 16 MUFAs dominated among *Daphnia* PLFAs, and thus indicated a dominance of Gram-negative bacteria and MOB in *Daphnia* diet. During the summer period, algal PUFAs, 18 MUFAs and 16 MUFAs were contributing rather equally to *Daphnia* PLFAs, indicating multiplicity of *Daphnia* diet. The PLFA composition of *Daphnia* adults correlated significantly with the PLFA composition of seston (0-1.2 m) from the preceding week and seasonally, indicating that PLFA-profiles reflected zooplankton diet.

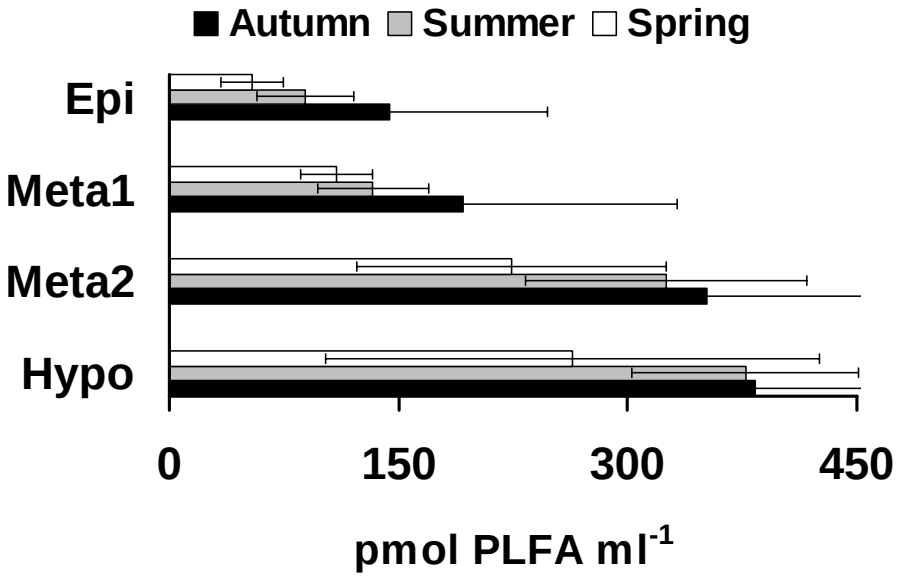

FIGURE 4 The proportional contribution of PLFA 18:3ω3, which is typical for Chlorophyceae, to total seston PLFA (mean value for 0.0-1.2 m) and to Daphnia PLFA (Dph, a), together with the abundance of Chlorophyceae (µg l-1).

TABLE 5 The PLFA-profiles of Daphnia adults, juveniles and males and combined seston (0-1.2 m). The proportions (Mean ± SD%) were calculated for each season.

	Daphnia adult			Daphnia juveniles and males			Combined seston (0-1.2 m)		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
I and A Branching									
i-14:0	0.1 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.4
i-15:0	0.1 ± 0.0	0.5 ± 0.5	0.6 ± 0.8	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.3	1.9 ± 0.7
a-15:0	0.0 ± 0.1	0.8 ± 1.1	0.5 ± 0.8	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.1	1.3 ± 0.3	1.6 ± 0.5
i-16:0	0.1 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2
i-17:0	0.3 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	1.5 ± 2.1	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
a-17:0	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.0 ± 0.0	1.9 ± 2.9	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
Σ I and A	0.9 ± 0.6	2.5 ± 1.7	1.8 ± 1.6	0.4 ± 0.5	4.2 ± 4.8	1.4 ± 0.5	0.8 ± 0.2	3.9 ± 0.8	4.9 ± 1.7
Satutated									
14:0	3.2 ± 2.1	3.3 ± 1.9	1.7 ± 0.9	3.5 ± 1.5	2.8 ± 1.0	2.3 ± 0.7	9.4 ± 0.2	8.2 ± 3.1	3.0 ± 0.5
16:0	28.0 ± 7.2	27.7 ± 7.6	28.2 ± 5.1	45.3 ± 3.5	32.4 ± 0.9	30.7 ± 10.0	13.7 ± 1.0	14.2 ± 1.9	16.3 ± 8.1
17:0	0.5 ± 0.2	1.2 ± 0.4	1.1 ± 0.3	0.3 ± 0.4	0.9 ± 0.6	1.1 ± 0.5	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
18:0	6.8 ± 3.8	16.8 ± 3.9	20.1 ± 6.4	34.0 ± 13.0	18.7 ± 4.3	25.5 ± 7.4	0.6 ± 0.3	2.5 ± 0.8	6.2 ± 4.5
20:0	1.9 ± 2.7	0.8 ± 0.2	0.6 ± 0.6	1.7 ± 1.5	0.8 ± 0.4	0.5 ± 0.4	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.1
Σ SAFA	40.0 ± 6.2	48.5 ± 10.3	50.6 ± 6.9	84.4 ± 13.4	54.7 ± 4.8	58.9 ± 10.7	23.8 ± 1.1	25.2 ± 4.5	25.7 ± 12.3
16 Monounsaturated									
16:1ω8	1.0 ± 0.6	1.1 ± 0.3	2.9 ± 1.9	0.0 ± 0.0	1.0 ± 0.8	1.5 ± 1.0	2.3 ± 0.4	10.0 ± 5.4	9.8 ± 6.9
16:1ω7c	6.4 ± 2.9	12.7 ± 4.7	16.4 ± 5.3	2.4 ± 3.1	9.4 ± 3.4	11.9 ± 7.4	36.9 ± 21.3	25.5 ± 4.5	31.5 ± 7.5
16:1ω6	0.7 ± 0.6	0.7 ± 0.4	0.7 ± 0.5	0.2 ± 0.2	0.5 ± 0.6	2.1 ± 3.3	1.2 ± 0.5	1.5 ± 0.8	2.8 ± 1.9
16:1ω5c	1.0 ± 0.9	1.0 ± 0.5	1.1 ± 0.5	0.0 ± 0.0	0.7 ± 0.8	0.8 ± 0.7	1.9 ± 0.7	2.6 ± 0.6	3.0 ± 1.7
16:1ω5t	1.0 ± 1.0	1.1 ± 0.5	1.3 ± 0.6	0.0 ± 0.0	0.7 ± 0.8	1.0 ± 0.9	2.5 ± 0.9	3.9 ± 1.0	5.9 ± 3.5
16:1ω13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
Σ 16 MUFA	10.8 ± 6.1	17.1 ± 5.9	22.5 ± 6.3	2.5 ± 2.9	12.3 ± 5.3	17.4 ± 7.8	45.4 ± 23.2	43.7 ± 2.9	53.1 ± 20.3
18 Monounsaturated									
18:1ω9	9.4 ± 1.5	6.9 ± 1.3	6.5 ± 2.7	7.3 ± 9.2	7.0 ± 2.6	6.7 ± 2.3	2.8 ± 2.9	1.1 ± 0.6	0.5 ± 0.6
18:1ω7c	6.5 ± 1.0	8.1 ± 3.4	6.4 ± 2.3	2.7 ± 2.7	7.0 ± 1.5	7.4 ± 2.5	7.1 ± 4.9	6.0 ± 1.0	6.4 ± 2.1
18:1ω7t	0.1 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.3	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1ω6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
18:1ω5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Σ 18 MUFA	16.0 ± 2.3	15.1 ± 4.5	13.4 ± 3.7	10.0 ± 11.9	14.1 ± 3.9	14.3 ± 3.5	9.9 ± 7.8	7.3 ± 1.2	7.1 ± 2.5
Polyunsaturated									
16:3ω3	0.8 ± 0.1	0.4 ± 0.3	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	1.3 ± 2.5	0.1 ± 0.1
18:3ω3	16.9 ± 7.1	5.7 ± 2.0	2.9 ± 1.8	1.2 ± 1.8	5.3 ± 3.6	1.2 ± 0.8	14.6 ± 11.6	5.3 ± 0.7	1.5 ± 0.9
18:4ω3	2.7 ± 1.4	0.3 ± 0.4	0.9 ± 0.4	0.0 ± 0.0	0.1 ± 0.2	0.4 ± 0.5	1.0 ± 0.5	2.1 ± 0.9	1.0 ± 0.6
20:5ω3	6.5 ± 3.8	2.0 ± 1.0	3.4 ± 2.6	0.4 ± 0.5	2.0 ± 2.2	2.0 ± 1.5	0.7 ± 0.2	1.3 ± 0.8	1.3 ± 0.8
Σ w-3	26.9 ± 3.1	8.4 ± 0.9	7.2 ± 1.2	1.6 ± 0.6	7.4 ± 1.5	3.7 ± 0.7	16.7 ± 3.2	10.0 ± 1.2	4.0 ± 0.6
18:2ω6	4.4 ± 2.2	4.5 ± 1.5	2.0 ± 0.4	0.1 ± 0.1	4.0 ± 3.1	1.9 ± 0.5	1.8 ± 0.3	2.8 ± 1.1	0.8 ± 0.5
18:3ω6	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0
20:4ω6	1.6 ± 1.5	2.1 ± 0.8	1.4 ± 1.2	0.1 ± 0.1	2.1 ± 0.7	1.4 ± 0.8	0.1 ± 0.0	0.9 ± 0.7	0.0 ± 0.0
22:5ω6	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.4 ± 0.3	0.0 ± 0.0
Σ w-6	6.0 ± 0.9	6.9 ± 0.7	3.5 ± 0.4	0.2 ± 0.1	6.4 ± 1.1	3.3 ± 0.3	2.3 ± 0.1	4.4 ± 0.5	0.9 ± 0.1
Σ PUFA	32.0 ± 1.9	15.0 ± 5.2	10.6 ± 5.1	1.8 ± 1.2	13.8 ± 6.4	7.0 ± 2.7	19.1 ± 13.7	14.8 ± 3.6	5.0 ± 2.4
Σ w-3/w-6 ratio	0.8	0.6	0.7	0.9	0.5	0.5	0.9	0.7	0.8
MOB	2.6 ± 2.1	1.9 ± 0.6	5.0 ± 1.8	0.2 ± 0.2	2.2 ± 2.0	4.6 ± 2.3	5.9 ± 1.0	15.4 ± 6.3	18.5 ± 11.8
Algae	30.5 ± 0.4	12.9 ± 4.5	9.2 ± 4.7	1.7 ± 1.3	11.7 ± 6.8	5.6 ± 2.1	18.0 ± 12.7	11.8 ± 3.4	3.8 ± 1.8

3.5 Carbon mass balance for Mekkojärvi in 2005

During the spring, summer and autumn periods, the calculated *Daphnia* net production was 85, 92 and 73 mg C m^{-2} d^{-1}, and thus *Daphnia* carbon demand

would have been for each season 122-213, 184-276 and 104-182 mg C m^{-2} d^{-1}, respectively. The possible carbon flows from putative food sources to *Daphnia* are represented in Figure 5; here I concentrate more on possible contributions of each food source to *Daphnia* diet. When assuming low (40%) assimilation efficiency, the carbon demand of *Daphnia* would be satisfied by the putative food sources only in autumn, when unknown food sources would have contributed 0-25% of all carbon demand. For spring and summer the contribution of unknown food sources could have been 3-20% and 29-42%, correspondingly. However, when assuming high (70%) assimilation efficiency, all carbon demand of *Daphnia* in each season could have been satisfied by the putative food sources.

The largest carbon source for *Daphnia* in spring would have been phytoplankton according to all IsoSource outputs. Phytoplankton could then have contributed 21-41% of all *Daphnia* carbon demand. However, it should be noted that there was high temporal deviation in primary production and at the start of the spring period PP could have supplied up to 71% of *Daphnia* carbon demand. Heterotrophic bacterial production could have supplied only a minor proportion (5-8%) of *Daphnia* carbon when using values measured by the ^{14}C-leucine incorporation method, but could have contributed 21-31% of all *Daphnia* carbon demand when using a mean value of 44 mg C m^{-2} d^{-1} from Salonen et al. (1992). The carbon supplied to *Daphnia* from green sulphur bacteria ranged from 19 to 28% during the spring period. However, the widest range was for methanotrophic bacteria, which could have supplied 13-31% of *Daphnia* carbon demand in the low AE case and 20-46% in the high AE case. The possible contribution of protozoan was small (2-4%) in all cases.

During the summer period, the possible contribution of different putative food sources was quite even. Primary production could have supplied 11-17% of all *Daphnia* carbon demand, and the corresponding range for GSB would have been 17-26%. Heterotrophic bacterial production could have supplied 19-29% of *Daphnia* carbon demand when using values of Salonen et al. (1992). MOB could have supplied 10-24% of *Daphnia* carbon demand in the low AE case and 15-35% in the high AE case.

During the autumn period, MOB was a dominant food source for *Daphnia*, and could even have supplied all *Daphnia* carbon demand when assuming 70% GE for MOB and 60% AE for *Daphnia*. The primary producers could have supplied only 8-13% of all *Daphnia* carbon demand, and 11-16% of *Daphnia* carbon could have originated from GSB. Heterotrophic bacterial production could have contributed 24-36% of *Daphnia* carbon demand. The contribution of MOB ranged from 31 to 72% for low AE of *Daphnia* and 47 to 109% for high AE of *Daphnia*.

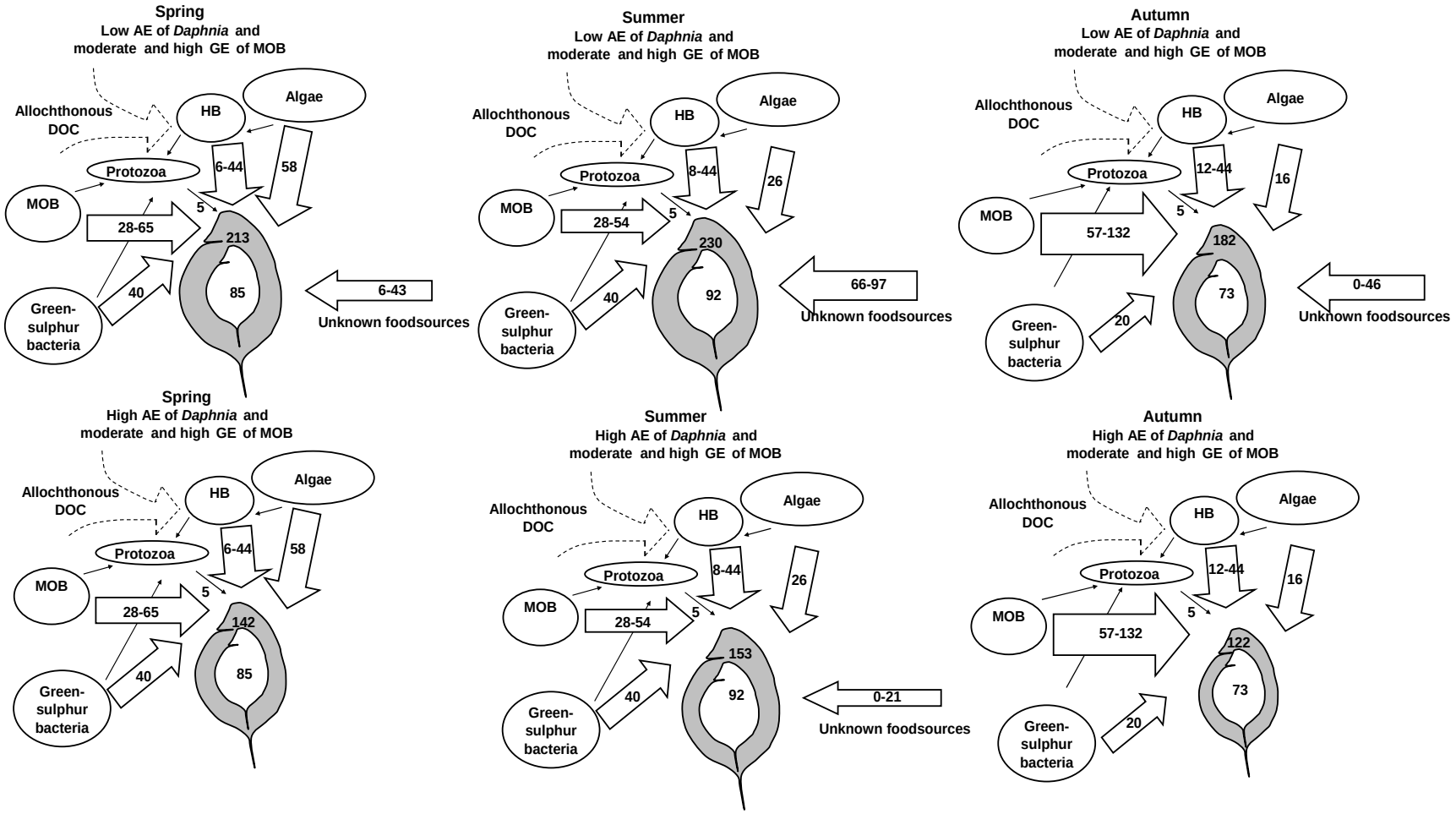

FIGURE 5 Carbon mass balance calculations (mg C m^{-2} d^{-1}) for spring, summer and autumn in 2005. The moderate (30%) and high (70%) growth efficiency (GE) of MOB is applied for each low (40%) and high (60%) assimilation efficiency (AE) of *Daphnia* calculation to give the predicted range. Grey *Daphnia* represents total carbon demand (mg C m^{-2} d^{-1}) and inner white *Daphnia* represents calculated *Daphnia* net production (mg C m^{-2} d^{-1}) for each season.

3.6 The $\delta^{13}C$ of zooplankton in five lakes with different DOC

During spring 2006, phytoplankton biomass was higher in Valkea-Kotinen (20.6 mg C l^{-1}) than in other lakes (0.4 to 2.2 mg C l^{-1}), but dramatically decreased in Valkea-Kotinen (to 4.7 mg C l^{-1}) in autumn. In contrast, bacterial biomass was highest in the epilimnion and in the hypolimnion of Mekkojärvi during spring 2006, but was more even among all lakes during autumn 2006. However, in comparison to other lakes bacterial biomass was also high in the hypolimnion of Alinen-Mustajärvi in spring and autumn 2006.

During spring 2006, methane concentration was extremely high in the hypolimnion of Nimetön, but was also much higher in the hypolimnion of Alinen-Mustajärvi than in the hypolimnion of Mekkojärvi. After autumnal mixing in 2006, methane concentration decreased in Alinen-Mustajärvi and Mekkojärvi, likely reflecting stimulated methanotrophic activity in autumn. Unfortunately no autumn methane concentration value is available from Nimetön. However, methane concentration in the hypolimnion of Valkea-Mustajärvi and Valkea-Kotinen increased from spring to autumn, perhaps indicating higher methanogenic activity in sediments and the overlying water column than methanotrophic activity in the water column.

During spring and autumn 2006, $\delta^{13}C$ of DIC was much higher (6.7‰ and 10.6‰ respectively) in the epilimnion of Valkea-Mustajärvi than in other lakes where $\delta^{13}C$ value of DIC in spring was -20.5 to - 22.3‰ and in autumn -18.9 to -23.5‰ (Tables 6 and 7). However, $\delta^{13}C$ value of DIC in the hypolimnion was higher in Nimetön (~11.7‰) than in other lakes in spring and autumn 2006. The $\delta^{13}C$ of DOC was similar in all lakes and seasons and was between -25.4 and -27.0 ‰.

The $\delta^{13}C$ of cladocerans (Table 8) was lowest (-39.5‰) in Mekkojärvi, even though the $\delta^{15}N$ value of cladocerans was higher (5.2‰) than in Alinen-Mustajärvi and Nimetön. However, $\delta^{13}C$ of cladocerans was highest (-28.4‰) in Alinen-Mustajärvi, but $\delta^{15}N$ value was the highest (12.0‰) in Valkea-Mustajärvi. Altogether, during spring season $\delta^{13}C$ values of cladocerans can be divided to the three following groups: 1) relatively low $\delta^{13}C$ values in Mekkojärvi and Nimetön, 2) medium (32-34‰) $\delta^{13}C$ value, but relatively high $\delta^{15}N$ value in Valkea-Kotinen and Valkea-Mustajärvi, and 3) relatively high $\delta^{13}C$ value but relatively low $\delta^{15}N$ value in Alinen-Mustajärvi.

After autumnal mixing, $\delta^{13}C$ value of cladocerans decreased most (-11.9‰) in Alinen-Mustajärvi, -8.1‰ in Mekkojärvi, 5.7‰ in Valkea-Kotinen, and -1.6‰ in Valkea-Mustajärvi reflecting possible seasonal changes in cladoceran diets in the studied lakes.

TABLE 6 The $\delta^{13}C$ values of DIC, POC and DOC from the five lakes sampled in spring 2006.

	Depht	Alinen Mustajärvi	Mekkojärvi	Nimetön	Valkea Mustajärvi	Valkea-Kotinen
DIC	EPI	-22.2 ± 1.2	-21.4 ± 1.0	-20.5 ± 0.9	-6.7 ± 0.4	-22.2 ± 1.1
	HYPO	-19.9 ± 0.4	-20.6 ± 0.3	-11.7 ± 0.3	-23.7 ± 1.0	-24.5 ± 0.4
POC	EPI	-28.5 ± 0.4	-32.3 ± 0.9	-34.6 ± 0.6	-28.9 ± 2.5	-27.6 ± 0.4
	HYPO	-33.6 ± 6.3	-29.1 ± 1.2	-31.0 ± 2.3	-30.8 ± 2.5	-33.5 ± 3.8
DOC	EPI	-26.1	-25.7	-25.7	-27.0 *	-26.6
	HYPO	-27.8	-25.6	-25.8	-27.0 *	-26.1

* measured from whole water column

TABLE 7 The $\delta^{13}C$ values of DIC, POC and DOC from the five lakes sampled in autumn 2006.

	Depht	Alinen Mustajärvi	Mekkojärvi	Nimetön	Valkea Mustajärvi	Valkea-Kotinen
DIC	EPI	-23.5 ± 1.2	-18.9 ± 1.2	-21.8 ± 1.0	-10.6 ± 1.4	-20.9 ± 0.8
	HYPO	-16.7 ± 0.5	-20.6 ± 0.5	-11.8 ± 0.3	-21.0 ± 0.6	-19.2 ± 0.7
POC	EPI	-32.5 ± 0.6	-30.6 ± 0.0	-30.0 ± 0.3	-30.0 ± 0.2	-30.8 ± 0.1
	HYPO	-30.6 ± 0.1	-31.1 ± 0.2	-29.6 ± 1.9	-30.8 ± 0.1	-29.4 ± 0.1
DOC	EPI	-26.5 *	-25.3		-24.9 *	-26.8
	HYPO	-26.5 *	-25.3	-25.4	-24.9 *	-25.1

* measured from whole water column

TABLE 8 The $\delta^{13}C$ and $\delta^{15}N$ values of cladocerans from the five lakes sampled in spring and autumn 2006.

	Isotope signal (‰)	Alinen Mustajärvi	Mekkojärvi	Nimetön	Valkea Mustajärvi	Valkea-Kotinen
Spring	$\delta^{13}C$	-28.4 ± 0.4	-39.5 ± 0.3	-36.7 ± 0.3	-33.6 ± 0.1	-32.2 ± 0.4
	$\delta^{15}N$	2.7 ± 0.2	5.2 ± 0.1	2.6 ± 0.3	12.9 ± 8.1	5.7 ± 0.2
Autumn	$\delta^{13}C$	-40.2 ± 1.3	-47.6 ± 0.2		-35.2 ± 0.5	-37.9 ± 0.4
	$\delta^{15}N$	1.5 ± 0.3	1.5 ± 0.1		3.0 ± 0.7	4.3 ± 0.6

4 DISCUSSION

During both the enclosure and whole lake ^{13}C-addition experiments, the epilimnetic DIC was rapidly ^{13}C-enriched. This enrichment was transmitted first to POM and then to *Daphnia* relatively soon in spring and summer, but not during the autumn experiments, when methanotrophic activity was at a high level. Thus, our experiments revealed great seasonal variation in *Daphnia* diet, and demonstrated how important it is to study different seasons to get a full view of true annual carbon flow in pelagic food webs.

The importance of autochthonous *in situ* production was highest during spring, when it contributed 37-71% or 27-41% in 2005 according to IsoSource models and calculated carbon budgets, respectively. The importance of allochthonous carbon then increased along with season and, according to the carbon balance budget for 2005, only 11-17% or 8-13% of *Daphnia* carbon demand could have derived from primary production during summer and autumn, respectively. According to the IsoSource model 25-61% and 31-56% of *Daphnia* diet would have originated from phytoplankton, and thus there is a small contradiction between the two modelling approaches in the estimated magnitudes of autochthonous carbon.

Because PUFAs dominate and are specific PLFAs in phytoplankton, and because this decreasing trend was seen also in total primary production, it is very likely that in summer phytoplankton contributed only half of their spring value and only a third in autumn. In this case, if 41% is the maximum contribution from primary production during summer 2005, then primary producers and autochthonous carbon would have supplied 20.5% and 13.7% of *Daphnia* carbon demand during the summer and autumn periods, respectively. These contributions for summer and autumn are similar to the maximum proportions according to the carbon budget calculation (17% and 13%) for 2005. Thus, allochthonous terrestrial carbon would have dominated in each season. Even if daily primary production would underestimate (Carignan et al. 2000, Pace & Prairie 2005) real primary production during each season and it could have supplied 71% of *Daphnia* carbon demand in spring, autochthonous carbon could have supplied only 35.5% and 23.7% of *Daphnia* diet during summer and

autumn, respectively. This confirms that *Daphnia* diet in this humic pelagic foodweb is based mainly on allochthonous carbon for most of the open water season. Only after ice melt, and the associated nutrient pulse which sustains temporary high primary production, does the autochthonous carbon source for zooplankton dominate.

During ^{13}C-enclosure experiments, 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. This model only takes into account two food sources and thus the true contribution can be somewhere between these two estimates. Actually, during the summer enclosure experiment primary production was 58±6 mg C m^{-2} d^{-1} which is similar to the spring level (58±42 mg C m^{-2} d^{-1}) of primary production in Mekkojärvi. Based on their results from whole-lake additions of $NaH^{13}CO_3$ to four contrasting lake types during summer, Carpenter et al. (2005) concluded that the allochthony of zooplankton was highest (49-75%) in dystrophic Tuesday Lake, but contributed 22-48% in Peter and Paul Lakes. Moreover, nutrient enrichment of Peter Lake decreased allochthony of zooplankton from 34-48% to 0-12%. These data reflect the enhanced importance of allochthonous carbon when primary production decreases, as seen also in the lower contribution of terrestrial carbon to *Daphnia* in the Mekkojärvi enclosures with higher primary production during summer 2004.

The transmission of allochthonous carbon to *Daphnia*, was evidently mediated by all putative functional bacterial groups, such as heterotrophic bacteria, methanotrophic bacteria and photosynthetic bacteria. Additionally, it is very likely that the DOC in the lake is overwhelmingly allochthonous carbon, because the $\delta^{13}C$ value of DOC is very close to that of terrestrial detritus (C_3 plants). This argument is reinforced by the fact that $\delta^{13}C$ of DOC in Mekkojärvi did not change at all during our ^{13}C-enrichments, indicating negligible influence on DOC from new autochthonous production. During spring and summer 2005, $\delta^{13}C$ and $\delta^{15}N$ of *Daphnia* corresponded very closely to the estimated values for heterotrophic bacteria, and thus the IsoSource models gave a wide range of estimations of their contribution in *Daphnia* diet. Although most epilimnetic bacteria have been reported to be Gram-positive bacteria (Zwart et al. 2002), the bacteria in Mekkojärvi were predominantly (~ 64%) Gram-negative (S. Taipale and M. Tiirola, unpublished). Moreover, *iso*- and *anteiso* -branching PLFAs typical of Gram-positive bacteria were only a small part of all *Daphnia* PLFAs, indicating their minor role in the *Daphnia* diet. Actually, according to the PLFA analyses, only the Gram-negative heterotrophic bacteria *Polynucleobacter* sp. could have contributed significant carbon to *Daphnia*, so the maximum estimation of the IsoSource model is unrealistic, and the contribution (3-10%) based on the ^{14}C-leucine incorporation values are more likely. In any case, it is most likely that most bacterial-mediated carbon in higher trophic levels derived from chemoautorophic and photoautotrophic bacteria, such as green sulphur bacteria, methylotrophs, iron-oxidizing and methane oxidizing

bacteria, which together contributed > 70% of the bacterial community at 0.6-1.2 m depth (S. Taipale and M. Tiirola, unpublished molecular analyses). The significant role of these bacteria is also supported by the higher PLFA-biomass in the meta- and hypolimnon than in the epilimnion (paper IV).

Our *Daphnia* laboratory growth experiment with MOB, confirmed that *Daphnia* can grow well on MOB suspension and that depleted $\delta^{13}C$ values really originate from MOB. This was confirmed by the steep drop (~ -10‰) of $\delta^{13}C$ of *Daphnia* during the autumn 2004 enclosure experiment and then during autumnal mixing in 2005. The model suggested methanotrophic bacteria contributed 64-87% of *Daphnia* diet during autumn, while the calculated carbon mass balance indicated a contribution of 37-112% during the 2004 enclosure experiment. During the warmer 2005 autumn, IsoSource model outputs suggested that MOB could have supplied 26-50% of all *Daphnia* carbon demand, while the calculated carbon mass balance indicated a contribution of 31-109%. Thus methanotrophic bacteria could supply virtually all the carbon requirement of *Daphnia* during both autumns in this lake. However, the role of MOB is not only restricted to the autumn period, being significant also during the spring and summer periods. Actually, during the spring and summer periods the contribution of specific MOB PLFAs was around half that in autumn. According to the IsoSource model ouputs, MOB could have made up 9-10% and 17-23% of *Daphnia* diets, while carbon budget calculations proposed that even 13-46% and 10-45% of *Daphnia* diets could have consisted of MOB during the spring and summer periods, respectively. In fact, if the carbon isotope fractionation between CH_4 and MOB cells is lower (< 10‰) than assumed, these higher magnitudes of the carbon budgets are also possible within the IsoSource outputs. In any case the strongly ^{13}C-depleted *Daphnia* values, together with the outputs from the different models and the calculated carbon mass balance all showed that methanotrophic bacteria are a greater carbon source for *Daphnia* in lakes than previously suspected, and their importance will likely increase with climate change and increasing lake temperatures. Methane-oxidizing bacteria can reduce accumulation of methane in water columns and lower emissions to the atmosphere, although their impact might be constrained by zooplankton grazing (Kankaala et al. 2007b). According to the Finnish Meteorological Institute (Jylhä et al. 2004), the annual temperature is estimated to rise by 1-3 °C and the annual mean precipitation by 0-15% by the 2020s, relative to baseline period 1961-1991. This would mean enhanced terrestrial loadings to lakes, and thus increase anaerobic DOM decomposition and methanogenesis. Hence better knowledge of the diversity of functional methane oxidizers and their role in food webs is important when discussing future scenarios.

The exact quantities of photoautotrophic green sulphur bacteria in *Daphnia* diet are difficult to detect, because their PLFA-profile is typical for all Gram-negative bacteria. Nevertheless, it is evident that *Daphnia* consume these dominant (according to the DNA-analysis, unpublished data) meta- and hypolimnetic bacteria in Mekkojärvi, especially during spring and summer. Therefore, the importance of these bacteria may be somewhat greater than the

IsoSource model outputs suggested (0-20% and 0-16%, for spring and summer periods in 2005). Indeed, the carbon balance budget calculations estimated that 19-28% and 17-26% of *Daphnia* diet could have originated from green sulphur bacteria.

When using low assimilation efficiencies for *Daphnia*, "missing carbon" contributes a significant part, especially during the summer periods in 2004 and 2005. However, He & Wang (2006) showed from laboratory experiments that *Daphnia* AE is higher when there is less food available, and thus in Mekkojärvi it is likely that AE of *Daphnia* is nearer to the high (60%) AE estimate than the low (40%) AE estimate. Therefore, it is likely that *Daphnia* carbon demand is essentially satisfied from a mixture of phytoplankton, heterotrophic bacteria, green sulphur bacteria, methanotrophic bacteria and other chemoautotrophic bacteria.

The contribution of these bacterial sources could be even higher than the IsoSource model outputs reported here if the true $\delta^{15}N$ values of phytoplankton were higher than our estimates. Certainly the IsoSource model estimations for phytoplankton contribution in autumn seem unrealistically high and, because GSB are then almost absent with the increased oxygen after mixing, most *Daphnia* carbon at that time comes from methanotrophic and other chemoautotrophic bacteria. Actually, iron-oxidizing as well as methylotrophic bacteria can use either ammonium or nitrate as their nitrogen sources, and thus exhibit higher $\delta^{15}N$ values. Thus, they can constitute an additional subsidy to *Daphnia* diet and might actually represent part of the calculated proportion from HB due to their similar isotopic signatures.

The role of heterotrophic flagellates (HF) was not estimated here, but can be a possible link between bacterial assimilated carbon and zooplankton, and could have partly explained the relatively enriched $\delta^{15}N$ values of zooplankton throughout the seasons. The low estimates of production of heterotrophic flagellates in the carbon balance mass budget calculations were taken from the epilimnetic tank experiments of Salonen et al. (1992), and because the major bacterial biomass is found from meta- and hypolimnetic, these estimates are probably too low. However, these tank experiments revealed that the high predation pressure of zooplankton on heterotrophic flagellates keep HF production low, and thus their role is probably not very high, although they could be crucial as a source of certain essential fatty acids in this alternative carbon flow pathway.

Many experiments and field studies have now demonstrated conclusively that terrestrial carbon mediated mainly by chemoautotropic bacteria has a dominant role in zooplankton diet in polyhumic Lake Mekkojärvi. Therefore, although this lake may represent an extreme case, it can be taken as a "reference lake" against which to evaluate the contribution of allochthonous carbon in other nearby studied lakes. Some characteristics can be taken into account when estimating zooplankton diet in these other lakes. Firstly, phytoplankton carbon isotope fractionation was between -10 and -15‰ in Mekkojärvi, and similar phytoplankton carbon fractionation in other lakes with similar DIC can be

assumed. If zooplankton is mainly feeding on phytoplankton, their $\delta^{13}C$ value should be in that range. If the $\delta^{13}C$ value of zooplankton differs from the $\delta^{13}C$ value of epilimnetic DIC by more than -10 to -15‰, they can be considered to be consuming mainly chemoautorophic bacteria like in lake Mekkojärvi, but if the difference is less than -10‰, then zooplankton is more likely to be feeding mainly on heterotrophic bacteria alongside phytoplankton. The $\delta^{13}C$ and $\delta^{15}N$ values of cladocerans from all five studied lakes (including "reference lake" Mekkojärvi), suggest some characteristic features. According to the low $\delta^{13}C$ value of cladocerans and a similar difference (~-17‰) between $\delta^{13}C$ of DIC and cladocerans in reference lake Mekkojärvi and in Nimetön, the diet of cladocerans seems to have been similar in both lakes, indicating the importance of methanotrophic bacteria. These lakes both have a very high DOC content and water colour. Additionally they have high methane concentration in the hypolimnion which can sustain high methanotrophic activity in the oxic-anoxic boundary layer. However, the $\delta^{15}N$ value of cladocerans was higher in Mekkojärvi during spring in comparison to Nimetön, indicating phytoplankton play a more important role in Mekkojärvi than in Nimetön. Because the whole water column of Nimetön was not mixed during autumn, such a high increase in methanotropic activity as in Mekkojärvi was not likely in Nimetön and therefore the importance of MOB may be more seasonally steady in Nimetön than in Mekkojärvi.

The $\delta^{13}C$ values of cladocerans were similar in the clearest lakes, Valkea-Mustajärvi and Valkea-Kotinen, and additionally the cladocerans from these two lakes had high $\delta^{15}N$ values. However, there was -29.6‰ and -10.0‰ difference respectively from $\delta^{13}C$ values of DIC. Because phytoplankton biomass was also high in Valkea-Kotinen, cladocerans in that lake were presumably feeding extensively on phytoplankton. However, the relatively low carbon isotope fractionation value might arise if the consumed phytoplankton (*Peridinium* sp.) was mixotrophic or if the cladocerans consumed much heterotrophic bacteria; most likely they consumed phytoplankton together with heterotrophic bacteria. During autumn, $\delta^{13}C$ of cladocerans was much lower in comparison to spring values, indicating an importance of MOB in cladoceran diet also in Valkea-Kotinen. The $\delta^{13}C$ of cladocerans was unusually low in comparison to $\delta^{13}C$ value of DIC in the epilimnion and $\delta^{13}C$ values of POM in Valkea-Mustajärvi. This indicates that cladocerans were feeding more on phytoplankton and bacteria in the meta- or hypolimnion than in the epilimnion. Actually, bacterial and phytoplankton biomass increased with depth and was highest in the hypolimnion. However, because phytoplankton consisted 90% of cyanobacteria that were probably not consumed and because $\delta^{13}C$ value of cladocerans was lower than $\delta^{13}C$ values of POM in the hypolimnion, it is evident that cladocerans were also feeding on heavier $\delta^{13}C$ food, such as chemoautotrophic bacteria and MOB. Indeed, there was a small decrease in the $\delta^{13}C$ value of cladocerans, even though the $\delta^{13}C$ value of DIC (and therefore presumably also of phytoplankton) in the meta- and hypolimnion remained similar to that in spring in Valkea-Mustajärvi.

The spring $\delta^{13}C$ value of cladocerans was highest in Alinen-Mustajärvi, and there was only 8.5‰ difference from DIC. Actually, the $\delta^{13}C$ value of cladocerans (-28.4‰) was near to the $\delta^{13}C$ value of DOC (-27.0‰), suggesting feeding mainly on heterotrophic bacteria. The $\delta^{13}C$ value of cladocerans dropped by -11.8‰ from spring (-28.4‰) to autumn (-40.2‰), indicating an increased and significant role of MOB in cladoceran diet also in Alinen-Mustajärvi during autumnal mixing. Altogether from the studies of these five lakes, it appears that terrestrial carbon mediated by various bacterial types has a particularly significant role in humic lakes but is also an important supplement to phytoplankton production in clearer lakes.

5 CONCLUSIONS

The aim of my thesis was to increase the knowledge of alternative DOM-bacteria pathways in humic lakes. It was found that in Mekkojärvi the largest part of *Daphnia* carbon demand was satisfied through these alternative pathways. Additionally, according to the model presented by Kritzberg et al. (2006) and given the very high allochthonous DOC concentration in Mekkojärvi, it is also clear that a very large (>90%) part of the DOM utilised by these bacteria must ultimately derive from terrestrial carbon. Thus, it can be concluded that bacteria not only decompose terrestrial carbon, but also assimilate a large part and transmit it to higher trophic levels, so that original hypothesis (1) can be rejected.

The contribution of DOM-bacterial pathways to zooplankton diets varies seasonally, and also between lakes mainly according to their DOC concentration and primary production. Our results showed that the importance of this alternative pathway increases seasonally from spring to autumn, in accordance with original hypothesis (2). The highest contribution of phytoplankton in zooplankton diet was thus during spring when primary production was stimulated by increased light availability and nutrient inputs following ice-melt and water mixing. During summer, primary production was lower and zooplankton consumed phytoplankton along with heterotrophic, phototrophic and chemotrophic bacteria in approximately equal proportions. The highest significance of this bacterial-mediated pathway was during autumnal mixing, when the highest zooplankton biomass was also measured. This was mainly based on high methanotrophic activity during autumnal mixing. According to the whole lake ^{13}C-experiment and further IsoSource model outputs, together with carbon mass balance calculations and PLFA-profiles results, 29-59%, 65-79%, 76-86% of *Daphnia* diet consisted of this alternative pathway during spring, summer, and autumn, respectively.

The $\delta^{13}C$ results of zooplankton from three other humic lakes and one clear lake indicated terrestrial carbon mediated by different bacterial types to be important in all lake types. Additionally, this carbon was mediated by chemoautotrophic bacteria and especially MOB in high DOC lakes both in

spring and autumn, but heterotrophic bacteria were a more important carbon mediator in the lakes with lower DOC concentrations. The contribution of phytoplankton in *Daphnia* diet correlated positively with primary production and nutrient concentration in enclosure experiments, in accordance with original hypothesis (3). Nevertheless, terrestrial carbon and bacterial-mediated carbon had an important role in many kinds of lakes. However, in view of the strong seasonality, to specify the overall contribution of bacterial-mediated carbon in zooplankton diet in different DOC content lakes, it is important to evaluate carbon flow through bacteria in all seasons of the year.

Additionally, it seems that much of this bacterial carbon flow goes through photo- and chemoautotrophic bacteria in dystrophic lakes. Our results emphasise the particular role of methanotrophic bacteria and confirmed that very low $\delta^{13}C$ values of zooplankton are related to their feeding on isotopically light methanotrophic bacteria. Our results indicated that MOB could even supply all zooplankton carbon demand during autumnal mixing in Mekkojärvi. Although that may be an extreme case, MOB seemed to be important food source in other studied lakes during autumnal mixing. However, it is very likely that heterotrophic bacteria have a proportionally higher role in lower DOC content lakes (Alinen-Mustajärvi, Valkea-Kotinen) than in very dystrophic lakes (Mekkojärvi, Nimetön). More studies including bacterial groups from lakes of variable DOC content and covering spring, summer and autumn are still required to get a wider overview of the importance of bacterial-mediated carbon flow among different lake types, including large lakes.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Bakteerivälitteisen terrestrisen hiilen merkitys humusjärvien ravintoketjussa

Tämän väitöskirjan tarkoituksen oli selvittää vaihtoehtoisen hiilenreitin eli DOM-bakteeri–reitin merkitystä hiilen lähteenä humusjärvien ravintoketjussa. Tarkoituksena on erityisesti kvantifioida erilaisten bakteerien assimiloiman hiilen siirtymistä ylemmille trofiatasoille. Uudet tekniikat, kuten vakaiden isotooppien käyttö sekä rasvahappoanalytiikka, tarjosivat oivan mahdollisuuden DOM-bakteeri–reitin merkityksen testaamiseen useammasta näkökulmasta.

Järvien hiilivirran on perinteisesti ajateltu perustuvan järvissä itsessään tapahtuvaan kasvinplanktonin fotosynteesiin eli perustuotantoon (autoktoninen tuotanto), jossa epäorgaanisessa muodossa oleva hiili (CO_2) sidotaan orgaaniseen muotoon. Heterotrofiset (toisenvaraiset) eliöt eli kuluttajat saavat energiansa orgaanisen aineen hapettamisesta sekä käyttävät orgaanista ainetta. Autoktonisen tuotannon lisäksi järviin tulee ympäröivältä valuma-alueelta vaihteleva määrä orgaanista hiiltä, jota kutsutaan alloktoniseksi hiileksi. Järvessä elävät mikrobit käyttävät tätä hiiltä energian- sekä hiilenlähteenään. Pitkään ajateltiin, että orgaaninen hiiltä on rakenteeltaan liian monimutkaista assimiloituakseen soluihin sekä vaikuttaakseen solujen kasvuun. Tämän vaihtoehtoisen hiilenväylän seuraavassa portaassa ovat fagotrofiset alkueläimet, kuten ripsieläimet ja flagellaatit, jotka käyttävät ravinnokseen bakteereita. Myös eläinplankton, ja etenkin Cladocera-vesikirput käyttävät ravintonaan suoraan erilaisia bakteereita. Tätä vaihtoehtoista ravintoväylää, joka pohjautuu bakteerien eläinplanktonille välittämään terrestrisiseen hiileen, kutsutaan mikrobiravintoverkoksi.

Humusjärvien perustuotanto on usein ravinteiden sekä valon rajoittamia ja ovat näin ollen soveliaampia kasvuympäristöjä bakteereille kuin kasviplanktonille. Tämän vuoksi kasviplanktonin tuotanto on näissä järvissä pieni suhteessa bakteerituotantoon. Tämän lisäksi humusjärvet ovat yleensä voimakkaasti sekä termisesti että kemiallisesti kerrostuneita, minkä vuoksi hapettoman vesipatsaan tilavuus suhteessa hapelliseen on suuri. Tämän vuoksi suuri osa orgaanisen hiilen mineralisaatiosta tapahtuu vesipatsaan hapettomassa osassa. Kun sulfaatin pitoisuus laskee, metanogeneesin (metaanin muodostumisen eri subtraateista) merkitys anaerobisena hajotusreittinä kasvaa. Suomalaisista järvistä onkin mitattu korkeita metaanipitoisuuksia (>100 mmol m^{-3}). Eri tutkimusten mukaan suurimman osan (50-100%) tästä arkkibakteerientuottamasta metaanista hapettavat metaania hapettavat bakteerit, jotka voivat toimia energian- ja hiilenlähteenä eläinplanktonille. Tämän biogeenisesti tuotetun metaanin hiilen isotooppiarvo on poikkeuksellisen alhainen (-50 — -80 ‰). Koska ravintokohteen hiilen isotooppiarvo säilyy sellaisenaan myös kuluttajassa, tämä alhainen $\delta^{13}C$-arvo näkyy myös eläinplanktonissa, mikäli eläinplankton käyttää metaania hapettavia bakteereita ravintokohteenaan. Anaerobisissa kerroksissa muodostuu metaanin lisäksi merkittäviä pitoisuuksia rikkivetyä (H_2S) sekä pelkistynyttä rautaa (Fe^{2+}). Erilaiset bakteerit, kuten kemolitoautrofit sekä fotolito-

autotrofit, käyttävät näitä pelkistyneitä alkuaineita energianlähteenään. Merkittävimpiä näistä ovat fotoautotrofiset viherrikkibakteerit sekä kemolitoautotrofiset bakteerit, kuten rautaa hapettava *Gallionella* sp. Näiden kemolitoautrofisten bakteerien määrä on suurin hapettoman ja hapellisen vesipatsaan rajapinnassa. Viherrikkibakteerien määrä on korkein täysin hapettomassa vyöhykkeessä, jossa on kuitenkin hiukan valoa jäljellä. Aikaisempien tutkimusten mukaan eläinplankton (*Daphnia*) vaeltaa hapellisen ja hapettoman rajavyöhykkeeseen ruokailemaan näillä bakteereilla sekä välttämään hapellisessa kerroksessa eläviä saalistajia.

Tutkimusjärvenä oli Mekkojärvi (61°13′ N, 25°8′ E), joka on pieni (pinta-ala 0,35 ha) ja matala (keskisyvyys 3 m) humusjärvi. Järven pH on luonnostaan alhainen (4-6), ja järveen tulee runsaasti terrestrisistä orgaanista aineista, minkä vuoksi vesi on tumman ruskeaa (väriluku 300 – 800 mg Pt l^{-1}) ja DOC-pitoisuus (20–45 mg l^{-1}) on erityisen korkea. Jäiden lähdettyä vesipatsas kerrostuu nopeasti ja hapellinen eufoottinen kerros on hyvin ohut (0,5-1 m). Kasviplanktonin perustuotanto on suurinta keväällä, jolloin ravinteita on runsaasti saatavilla. Bakteeribiomassa kasvaa vertikaalisesti pinnalta pohjaan päin siirryttäessä, mikä viittaa anaerobisten arkkien ja bakteerien merkittävään rooliin suhteessa heterotrofisiin aerobisiin bakteereihin. Alusveden 16S rRNA-geenin sekvensoinnin perusteella järven viherrikkibakteerit ovat *Chlorobium* spp. -taksonia ja kuuluvat erityisesti ryhmään 3, josta runsaimpia ovat rautaa hapettava *Chlorobium ferrooxidans* sekä rikkivetyä käyttävä *Chlorobium phaeobacterium*. Metaanipitoisuus on korkeimmillaan 1,8-3,0 m syvyydessä (100 – 150 µmol l^{-1}), mutta metaanin hapetus on suurimmillaan 0,6-1,2 metrissä. Metanotrofit kuuluvat tyyppiin I ja sukuun *Methylobacter* sp. Keväällä *Chlamydomonas* on runsain ja hallitseva kasviplanktonsuku, minkä jälkeen *Mallomonas* spp. sekä *Cryptomonas* spp. runsastuvat ja hallitsevat pintaveden kasviplanktonia. *Daphnia longispina* on Mekkojärven runsain eläinplanktonlaji, ja *Chaoborus*-toukat sekä *Notonecta* spp. ovat järven tärkeimmät selkärangattomat pedot kalojen puuttuessa.

Ensimmäisen osatutkimuksen laboratoriokokeessa (julkaisu I) tutkittiin hiilen isotooppien avulla metaania hapettavien bakteerien merkitystä eläinplanktonin ravinnonkohteena. Laboratoriokasvatuskokeet *Daphnia*-vesikirpuilla osoittivat, että metaania hapettavien bakteereiden alhaiset $\delta^{13}C$-isotooppiarvot siirtyvän bakteereilta eläinplanktonille. Erityisen alhaisia $\delta^{13}C$-arvoja mitattiin Mekkojärven eläinplanktonista täyskierron aikaan 2004, jolloin vesikirppujen $\delta^{13}C$-arvo oli kokeen alussa kontrollialtaissa -40,5 ‰, mutta kokeen lopussa -50,3 ‰, joka on toistaiseksi kevyin eläinplanktonista koskaan mitattu hiilen isotooppiarvo.

Toisessa osatutkimuksessa (julkaisu II) tutkittiin v. 2004 allaskokeella alloktonisen sekä autoktonisen hiilen merkitystä eläinplanktonin, lähinnä *Daphnia*-vesikirppujen, ravinnossa. Tutkimusta varten Mekkojärveen asennettiin kuusi (2 x 4 m, halk. x korkeus) allasta, joista kolmen altaan pintaveteen tehtiin $NaH^{13}CO_3$ (^{13}C 99 atomi %, CK Gas Products Ltd.) lisäys viisi kertaa viikossa 8.–25. kesäkuuta välisenä aikana sekä 16. syyskuuta – 5. lokakuuta välisenä aikana. Muutoksia liuenneen epäorgaanisen hiilen (DIC), liuenneen orgaanisen hiilen (DOC), partikulaarisen orgaanisen hiilen (POC) sekä eläinplanktonin

$\delta^{13}C$-arvoissa seurattiin läpi koejakson. Alloktonisen ja autoktonisen hiilen osuus laskettiin kahden eri mallin avulla, joista ensimmäinen malli huomio heterotrofiset bakteerit sekä kasviplanktonin (TOM-PP-malli) ja toinen malli metaania hapettavat bakteerit sekä kasviplanktonin (MOB-PP-malli). Lisätty rikastusleima siirtyi kesäkokeen aikana nopeasti kasviplanktoniin, mikä näkyi POM:n hiilen korkeina isotooppiarvoina. Syyskokeen aikana hiilen rikastumista ei ollut nähtävissä POM:ssa alhaisen perustuotannon vuoksi. Tästä huolimatta eläinplanktonin biomassa oli samanlainen kesällä ja syksyllä, mikä todistaa, että erilainen ravintokäyttäytyminen ylläpitää korkeata eläinplanktonbiomassaa. TOM-PP -malli arvioi, että 37% ravinnosta tuli terrestrisestä lähteestä heterotrofisten bakteereiden välittämänä. MOB-PP-malli arvioi, että metaania hapettavat bakteerit muodostivat 11-20% eläinplanktonin ravinnosta. TOM-PP-malli ei pystynyt laskemaan realistista osuutta eläinplanktonin käyttämälle terrestriselle hiilelle syyskokeen aikana, mikä viittaa metaania hapettavien bakteereiden suureen merkitykseen. MOB-PP -mallin mukaan 64-87% eläinplanktonin ravinnosta tulikin metaania hapettavista bakteereista. Lasketun hiilimassabalanssin mukaan metaania hapettavat bakteerit olisivat syksyllä 2004 pystyneet välittämään kaiken eläinplanktonin tarvitseman hiilen.

Kolmannessa osastutkimuksessa (julkaisu III) $NaH^{13}CO$ lisäykset tehtiin suoraan Mekkojärven pintaveteen keväällä, kesällä sekä syksyllä 2005. Mekkojärven runsaimman eläinplanktonlajin, *Daphnia longispina* -vesikirpun, ravinto koostuu kasviplanktonista, heterotrofisista bakteereista, metaania hapettavista bakteereista sekä viherrikkibakteereista. IsoSource–ohjelman laskelmien mukaan kasviplanktonin osuus eläinplanktonin ravinnosta oli kevätkokeen aikana suurin eli 37–71%, kesäkokeen aikana 25–61% ja syyskokeen aikana 31–56%. Päinvastoin kuin kasviplanktonilla, metaania hapettavien bakteereiden osuus kasvoi keväästä syksyä kohden ja oli 9–10%, 17–23% ja 26–50% kevät-, kesä- sekä syyskokeen aikana. IsoSource–ohjelman laskelmien mukaan heterotrofisten bakteereiden osuus vaihteli runsaasti jokaisen kokeen aikana ja oli 0–54%, 0–58% sekä 0–35% kevät-, kesä- sekä syyskokeiden aikana. Virherrikkibakteerien osuus oli IsoSource–laskelmien mukaan 0–20% tasaisesti läpi avovesikauden aina täyskiertoon asti, jolloin viherrikkibakteerien biomassa romahti.

Neljännessä osatutkimuksessa (julkaisu IV) POM- sekä eläinplanktonnäytteistä analysoitiin fosfolipidien rasvahappoprofiilit (PLFA) eläinplanktonin ravintokäyttäytymisen arvioimiseksi. Koska fosfolipidien rasvahapot siirtyvät pääosin sellaisenaan eläinplanktonin solukalvolle, voidaan rasvahappoprofiilia käyttää ravintokohteita arvioitaessa. Koska ympäristöolosuhteiden, kuten lämpötilan, on raportoitu myös vaikuttavan membraanikalvon rasvahappokoostumukseen, tutkin PLFA-profiilin käyttökelpoisuutta eläinplankton ravintokäyttäytymisen arvioimisessa. Kevät-, kesä- ja syyskokeen aikana tunnistettiin eläinplanktonista 35 eri rasvahappoa, joista 29:ssä oli myös mitattavissa oleva pitoisuus. Eri rasvahapporyhmien osuudet vaihtelivat yli avovesikauden, mutta suoraketjuiset rasvahapot muodostivat merkittävän osuuden kaikkina vuodenaikoina. Korrelaatiot kvantitatiivisen PLFA:n sekä eri kasviplanktontaksonien biomassojen välillä paljastivat eri ryhmille tyypilliset rasvahapot. Vuonna 2005 *Chlamydomonas* sp. oli Mekkojärven pintavedessä yleinen kasviplanktonlaji, ja

18:3ω3 oli tyypillisin rasvahappo kyseille kasviplanktonlajille. Tämä rasvahappo runsastui eläinplanktonissa viikon viiveellä. Eläinplanktonin PLFA-profiili paljasti suuria muutoksia *Daphnia*-vesikirppujen ravinnossa eri vuodenaikoina. Kevätkaudella kasviplaktonille tyypilliset monityydyttymättömät rasvahapot olivat runsaimmillaan, minkä jälkeen niiden määrä laski ensi puoleen kesäkaudella ja sitten syysjaksolla kolmannekseen kevätjakson osuudesta. Tämä osoitti kasviplanktonin merkityksen radikaalia pienenemistä vesikirppujen ravintokohteena. Päinvastoin kuin monityydyttymättömät rasvahapot 16 monoeenien määrä kasvoi avovesikauden läpi ja oli runsaimmillaan syysjaksolla. 16 monoeenit ovat tyyppillisiä Gram-negatiivisille bakteereille, kuten metaania hapettaville bakteereille sekä viherrikkibakteereille. Tämän lisäksi Gram-positiivisille bakteereille tyypilliset *iso-* ja *anteiso* -haaroittuneiden rasvahappojen osuus *Daphnia*-vesikirppujen PLFA-profiilissa korreloi pintaveden bakteerituotannon kanssa.

Bakteerivälitteisen terrestrisen hiilen merkitystä DOC-pitoisuudeltaan erilaisissa järvissä tutkittiin keväällä ja syksyllä 2006 keräämällä näytteitä viidestä eri järvestä. Tutkittujen eri humusjärvien eläinplanktonin hiilen isotooppiarvot sekä ravintokäyttäytyminen voidaan jakaa kolmeen eri ryhmään. Ensimmäiseen ryhmään kuuluvat Mekkojärvi sekä Nimetön, joista molemmissa DOC-pitoisuus on hyvin korkea. Kummankin järven eläinplanktonin hiilen isotooppiarvo oli alhainen sekä keväällä että syksyllä ja erosi pintaveden DIC:n hiilen isotooppiarvosta keväällä (-16 — -18‰) ja syksyllä (-28‰), mikä viittaa siihen, että näiden järvien eläinplankton käyttää runsaasti kemoautotrofisia bakteereita sekä etenkin metaania hapettavia bakteereita ravintonaan. Toiseen ryhmään kuuluvat Valkea-Mustajärvi sekä Valkea-Kotinen, joiden eläinplanktonin hiilen isotooppiarvot olivat -32 — -34 ‰ ja typen isotooppiarvot korkeampia kuin muissa tutkimusjärvissä, mikä viittaa runsaaseen kasviplanktonin käyttöön. Valkea-Kotisen eläinplanktonin hiilen isotooppiarvo erosi -10 ‰ pintaveden DIC:n hiilen isotooppiarvosta, mikä osoittaa, että keväällä sekä kasviplankton että heterotrofiset bakteerit olivat merkittäviä vesikirppujen ravinnonkohteita. Valkea-Mustajärven eläinplanktonin hiilen isotooppiarvo erosi -26,9 ‰ pintaveden DIC:n hiilen isotooppiarvosta viitaten siihen, että hiili tuli enemminkin väli- sekä alusvedestä kuin pintavedestä. Syanobakteerit olivat väli- ja alusveden runsain kasviplanktonryhmä Valkea-Mustajärvessä, joten eläinplankton käytti runsaasti joko syanobakteereita tai heterotrofisia bakteereita ja kemoautotrofisia bakteereita. Syksyllä molempien järvien vesikirppujen hiilen isotooppiarvot olivat alhaisempia kuin keväällä, vaikka DIC:n hiilen isotooppiarvot eivät muuttuneet, mikä osoitti metaania hapettavien bakteereiden olleen tärkeä ravinnonkohde syksyllä. Kolmannen ryhmän muodosti Alinen-Mustajärvi, jonka vesikirppujen hiilen isotooppiarvot erosivat DIC:n hiilen isotooppiarvosta vain -6,1 ‰ eli heterotrofisten bakteereiden käyttö oli runsasta Alinen-Mustajärven vesikirppujen hiilen isotooppiarvot alenivat -11,9 ‰ kevätjaksosta syysjaksoon siirryttäessä, mikä kuvannee suurta muutosta syyskauden ravintokäyttätymisessä sekä metaania hapettavien bakteereiden merkityksen kasvua.

Kun kaikki eri tulokset laitetaan yhteen, voidaan todeta kasviplanktonin ja autoktonisen hiilen määrän olleen suurimmallaan kevätjaksolla, minkä jälkeen

sen osuus ensin puoliintui kesäjaksolle tultaessa ja oli lopulta syyskaudella kolmannekseen kevättasosta. Näiden arvioiden pohjalta voidaan todeta allokto-nisen hiilen sekä vaihtoehtoisen mikrobiravintoverkon olleen tärkein Mekko-järven eläinplanktonin hiilen sekä ravinnon alkuperä kaikkina vuodenaikoina. Alloktoninen hiili kulkeutui usean eri bakteeriryhmän, kuten heterotrofisten bakteerien, viherrikkibakteerien ja metaania hapettavien bakteerien, välityksellä eläinplanktonille. Tulosten perusteella suurin yksittäinen ja merkittävin eläin-planktonin hiilenlähde on metaania hapettavat bakteerit, jotka muodostivat merkittävän osuuden kaikkina vuodenaikoina, mutta erityisesti syksyllä, jolloin ne olisivat voineet tarjota kaiken *Daphnia*-vesikirppujen tarvitseman hiilen. Tä-mä näkyi eläinplanktonin erityisen alhaisina hiilen isotooppiarvoina syksyllä 2004 sekä 2005. Näin ollen metaania hapettavilla bakteereilla on merkittävä roo-li humusjärven ravintoketjussa, mutta myös globaalisti, sillä ne estävät suurelta osin voimakkaan kasvihuonekaasun, metaanin, pääsyn ilmakehään. Metaania hapettavien bakteerien merkitys korostunee tulevaisuudessa ilmaston lämme-tessä sekä sadannan kasvaessa, minkä vuoksi järveen tulleen terrestrisen or-gaanisen aineksen määrä mahdollisesti kasvaa tulevaisuudessa, ja näin ollen orgaanisen aineksen anaerobinen hajotus myös kasvaisi.

Yhteenvetona voidaan todeta bakteerivälitteisen terrestrisen hiilen olevan merkittävä hiilen lähde humusjärvien eläinplanktonille sekä terrestrisen hiilen olevan tärkeä hiilen lisä myös kirkasvetisissä järvissä. Tämän lisäksi terrestrinen hiili välittyy humusjärvissä heterotrotrofisten bakteereiden kautta sekä poly-humusjärvissä kemoautotrofisten ja etenkin metaania hapettaviin bakteereiden kautta. Syyskierron aikaan metaania hapettavien bakteereiden alhainen hiilen isotooppiarvo näkyi eri humusjärvien sekä yhden kirkasvetisen järven eläin-planktonin hiiilen isotooppiarvon alenemisena. Näin ollen terrestrisellä hiilellä sekä metaania hapettavilla bakteereilla on merkittävämpi rooli järvien ravinto-ketjussa kuin aikaisemmin on arveltu.

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