Master's thesis

Variation in zooplankton diets in contrasting small lakes, inferred from stable isotope analyses

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02.09.2007

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Li, Lu: Variation in zooplankton diets in contrasting small lakes, inferred from stable isotope analyses
Master's thesis: 32 p.
Supervisors: Dr. Roger I. Jones, Dr. Paula Kankaala, M.Sc. Sami Taipale
Reviewers: Dr. Roger I. Jones, Dr. Paula Kankaala
September 2007

Keywords: Allochthonous carbon, Cladocera, Copepoda, $\delta^{13}C$, $\delta^{15}N$, Dissolved organic carbon, Humic lakes, Stable isotope analysis, Zooplankton diets

ABSTRACT

Five small Finnish forest lakes with contrasting water colour were studied during May and October 2006, using stable isotope analysis (SIA) to evaluate the variation in zooplankton diets. $\delta^{13}C$ and $\delta^{15}N$ analyses were made for zooplankton, particulate organic matter (POM) and dissolved organic matter (DOM); $\delta^{13}C$ analysis was also made for dissolved inorganic carbon (DIC). At the same time, several lake variables were measured to provide background information. The study suggested that dissolved organic carbon (DOC) concentration in the lakes influenced the zooplankton diets, and that an important carbon flow pathway occurred from allochthonous DOM via methanotrophic bacteria (MOB) to crustacean zooplankton. MOB was a major supplement to zooplankton diet besides phytoplankton, especially in the more humic lakes, and in October when there was generally less phytoplankton available than in May. Zooplankton differed in their use of carbon from MOB, with Cladocera probably more efficient MOB users than Copepoda. Other food sources supplementing zooplankton diet may include photosynthetic bacteria, chemosynthetic bacteria and heterotrophic bacteria, which contributed to a greater extent in less humic lakes and might decrease in October with increasing of MOB consumption.

JYVÄSKYLÄN YLIOPISTO, Matemaattis-luonnontieteellinen tiedekunta

Bio- ja ympäristötieteiden laitos
Limnologia ja hydrobiologia

Li, Lu:	Pienten järvien eläinplanktonin ruokavalion monipuolisuus vakaiden isotooppien perusteella
Pro gradu:	32 s.
Työn ohjaajat:	Dr. Roger I. Jones, Dr. Paula Kankaala, M.Sc. Sami Taipale
Tarkastajat:	Dr. Roger I. Jones, Dr. Paula Kankaala
Syyskuu 2007	

Hakusanat: Alloktoninen hiili, Cladocera, Copepoda, $\delta^{13}C$, $\delta^{15}N$, eläinplanktonin ruokavalio, humusjärvi, liuennut orgaaninen aines, vakaat isotooppisovellutukset

TIIVISTELMÄ

Viiden erilaisen metsäjärven eläinplanktonin ravintokohteet tutkittiin hiilen sekä typen isotooppien avulla touko- sekä lokakuussa 2006. Eläinplanktonin ravintokohteiden analysoimiseksi hiilen ja typen isotoopit määritettiin myös partikulaarisesta orgaanisesta aineksesta (POM), liuenneesta orgaanisesta aineksesta (DOM) sekä liuenneesta epäorgaanisesta hiilestä (DIC). Tämän lisäksi mitattiin useita fysikaalis-kemiallisia sekä biologisia parametreja. Tutkimustulosten mukaan metaania hapettavien bakteereiden välittämällä alloktonisella hiilellä on merkittävä rooli Cladocerans-vesikirppujen ravinnossa kasviplanktonin ohella. Metaania hapettavat bakteerien merkitys eläinplanktonin ravintokohteena oli suurinta järvissä, joissa oli korkeampi liuenneen orgaanisen aineksen pitoisuus suhteessa muihin järviin. Metaania hapettavien bakteerien alhainen hiilen isotooppi arvo oli nähtävissä kaikissa eri järvien Cladocerans-vesikirpuissa syyskierron jälkeen otetuissa näytteissä. Näin ollen kasviplanktonilla oli merkittävämpi rooli eläinplanktonin ruokavaliossa keväällä kuin syksyllä. Koska Copepod-hankajalkaisten hiilen isootooppiarvo oli korkeampi kuin Cladocerans-vesikirpuista mitatuista, metaania hapettavilla bakteereilla on luultavasti vähäisempi merkitys niiden ruokavaliossa. Kasviplanktonin sekä metaania hapettavien bakteereiden lisäksi eläinplanktonien ravinto koostui heterotrofisista bakteereista, fotoautotrofisista bakteereista (viherrikkibakteerit) sekä kemoautotrofisista bakteereista.

Contents

1. INTRODUCTION

Lake food webs have traditionally been described as based on algal primary producers, but recent investigation have shown that most lakes worldwide are actually net heterotrophic, i.e., community respiration exceeds primary production (Cole *et al.* 1994, del Giorgio *et al.* 1999) There is growing evidence that pelagic food webs in lakes are subsidized to varying degrees by allochthonous inputs of organic carbon from their catchment area (Jones 1992, Hessen 1998).This imbalance is greatest in lakes with high subsidies of allochthonous dissolved organic matter (DOM) originating from the catchments (Salonen *et al.* 1983, 2005, Jansson *et al.* 2000).

Ecosystems are supported by organic carbon from two distinct sources. Autochthonous carbon is produced by photosynthesis within an ecosystem by autotrophic organisms, and much of it is readily exploited by consumers. Allochthonous carbon is produced elsewhere and transported into ecosystems; allochthonous fluxes of organic carbon to ecosystems are often large, and much of this material is recalcitrant and difficult to assimilate (Pace *et al.* 2004). Food webs in brown-water lakes must be strongly driven by allochthonous organic carbon, since autochthonous production alone cannot sustain the production at higher trophic levels in these lakes (e.g. Jones 1992, Hessen 1998, Järvinen 2002). Carbon metabolism based largely on allochthonous carbon and a high importance of the microbial loop is typical of the food webs of brown-water lakes (Hessen 1998).

Many studies have shown a significant subsidy of lake ecosystems (humic and less humic) by organic carbon produced outside their boundary (e.g. Pace *et al.* 2004, Salonen *et al.* 2005). Salonen & Hammar (1986) found that allochthonous DOM seems to be an important food resource for zooplankton, particularly in highly humic lakes, and that heterotrophic flagellates appeared likely to play an important role as a food of zooplankton in humic waters. Grey *et al*. (2001) found a seasonal switch in zooplankton dependence between allochthonous and autochthonous sources of organic matter in oligotrophic Loch Ness; the zooplankton diet switched from reliance on allochthonous carbon derived from particulate organic matter (POM) during winter and early spring to heavy dependence on algal production during summer.

In humic lakes, DOM is mainly allochthonous (Salonen *et al.* 1992b). The direct use of colloidal DOM by heterotrophic and mixotrophic flagellates may also be possible, but is poorly understood (Salonen *et al.* 1992b, Pace *et al.* 2004). Bacterial biomass and production is probably an important link converting allochthonous DOM to biomass available for higher trophic levels; it can either be grazed directly by macrozooplankton or passed through a bacteria-flagellate-macrozooplankton food chain (Jones 1992, Hessen 1998, Jones *et al*. 1999, Järvinen 2002). Particularly when the availability of algae is low, bacteria can be an important food source for zooplankton (Kankaala 1988). In small humic lakes, bacterial food sources for higher trophic levels generally include heterotrophic, photoautotrophic and chemoautotrophic types.

Kankaala *et al.* (2006b) pointed out that methane-derived carbon is a more important contribution to carbon flux through lake pelagic food webs than that has previously been suspected. In small sheltered boreal lakes with a high concentration of allochthonous humic DOM, hypolimnetic anoxia is a typical phenomenon during both summer and winter stratification. Organic matter in the anoxic sediment or hypolimnion undergoes anaerobic decomposition, and may produce a high concentration of methane (CH_4) via the activity of methanogenic bacteria in the hypolimnion (Rudd & Taylor 1980, Riera *et al*. 1999, Kortelainen *et al.* 2000). Most of the methane produced (50–100%) is oxidized to CO_2 in the water column in a metalimnetic oxic-anoxic interface zone and is partly

incorporated into microbial mass (Bastviken *et al.* 2003, Kankaala *et al.* 2006a). Thus, methanotrophic bacteria could be an important carbon source for zooplankton in some lakes.

Stable isotopes of carbon have been used to study the role of methanotrophs in the diet of zooplankton in several previous studies (e.g. Kankaala *et al.* 2006b, Taipale *et al.* 2007). Stable isotopes in an animal tissue integrate dietary components over time and also indicate assimilation rather than ingestion (Rounick & Winterbourn 1986). Therefore stable isotope analysis (SIA) may offer advantages over more conventional methods such as gut content analysis. In food web studies, carbon isotopes fractionate little (< 1 ‰) between diet and consumer and therefore act as an indicator of food sources, whereas nitrogen isotopes fractionate more (approximately 3.4 ‰) and therefore have generally been used to define trophic position of the organism.

Crustacean zooplankton consume food items (algae, bacteria, heterotrophic protozoa) that can rarely be separated in field samples and hence are usually analyzed as bulk POM in SIA. In some Finnish humic lakes, phytoplankton (δ^{13}C -28 to -37 ‰) are generally ^{13}C-depleted relative to bulk POM (δ^{13}C -26 to -30 ‰) (Jones *et al.* 1999, Taipale *et al.* 2007). Lake zooplankton are also often ^{13}C-depleted relative to POM and phytoplankton (e.g. del Giorgio & France 1996, Jones *et al*. 1999, Grey *et al*. 2000). Feeding on green sulphur bacteria (δ^{13}C -19 to -33 ‰) would not explain this (Jones *et al.* 1999, Taipale *et al.* 2007). Jones *et al*. (1999) hypothesized that the observed low zooplankton δ^{13}C values (-35 ‰ to -45 ‰) could be due to their feeding on isotopically light methanotrophic bacteria, and doing so to a greater extent in the more humic lakes, with a higher loading of allochthonous organic matter and greater development of hypolimnetic anoxia. Biogenic methane is extremely isotopically light, with δ^{13}C values typically between -45 ‰ and -72 ‰, and the methanotrophic bacteria utilize this methane at the oxic-anoxic interface within the water column fractionate carbon further and have δ^{13}C values between -52 ‰ and -101 ‰ (Rudd & Taylor 1980, Jones *et al*. 1999, Taipale *et al.* 2007).

Bastviken *et al*. (2003) studied three south-central Sweden lakes during summer and winter. They estimated methanotrophic bacterial production (MBP), methanotrophic bacterial growth efficiency (MBGE), heterotrophic bacterial production (HBP), primary production (PP), and the relative contribution of methanotrophic bacteria to overall bacterial biomass; in addition, they measured stable carbon isotope ratios in POM, surface sediments, zooplankton, and methane (Bastviken *et al*. 2003). MBP corresponded to 0.3–7% of the organic C production by primary producers, and 0.5–17% of HBP during summer (Bastviken *et al*. 2003). During winter, MBP was 3–120% of HBP (Bastviken *et al*. 2003). MBP generally dominated the heterotrophic bacterial production at greater depths (Bastviken *et al*. 2003). Methanotrophic biomass was 3–11% of total bacterial biomass on a depth-integrated basis (Bastviken *et al*. 2003). Zooplankton were generally more depleted in ^{13}C than POM (Bastviken *et al*. 2003). If phytoplankton δ^{13}C signatures were -30 to -35 ‰, such as the POM signals, observed zooplankton signatures could be explained by a fraction of 5–15% methanotrophic bacteria in their diet (Bastviken *et al*. 2003).

Kankaala *et al*. (2006b) studied methane-derived carbon in lakes pelagic food webs by replicate laboratory experiments and in field enclosures in a Finnish lake Mekkojärvi. In replicate laboratory cultures, *Daphnia longispina*, a common crustacean zooplankton in humic lakes, were fed microbial suspensions with or without enrichment by biogenic methane (Kankaala *et al*. 2006b). The δ^{13}C values of *Daphnia* indicated consumption of ^{13}C-depleted methanotrophic bacteria, while growth rates, survival, and reproduction of

Daphnia in cultures enriched with methane were equal to or greater than those in non-enriched cultures (Kankaala *et al*. 2006b). Results from lake enclosures during the autumn overturn period revealed a decrease in $\delta^{13}C$ of adult *Daphnia* from -40.5 ‰ to -50.3 ‰, reflecting extensive consumption of ^{13}C-depleted methanotrophic bacteria (Kankaala *et al*. 2006b).

Taipale *et al.* (2007) studied the relative contributions of different carbon sources to zooplankton in Mekkojärvi, by adding/not adding ^{13}C-enriched bicarbonate into the epilimnion of replicate treatment/control surface-to-sediment enclosures during summer and autumn 2004. Carbon stable isotope ratios of *Daphnia*, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and particulate organic carbon (POC) were monitored throughout each experimental period, along with a range of physical, chemical and biological variables (Taipale *et al.* 2007). The data were analyzed with a model modified from Pace *et al.* (2004) and by carbon mass balance calculations (Taipale *et al.* 2007). The results suggested that phytoplankton contributed 64-84% (model results) or 30-40% (pelagic mass balance calculation results) of *Daphnia* diet during the summer experiment, whereas methanotrophic bacteria contributed 64-87% (model results) or 37-112% (pelagic mass balance calculation results) during autumn (Taipale *et al.* 2007). Thus methanotrophic bacteria could supply virtually all the carbon requirement of *Daphnia* during the autumn in Mekkojärvi (Taipale *et al.* 2007).

Therefore, the aim of this study in 2006 was to evaluate how those previous results from Mekkojärvi (Jones *et al.*1999, Kankaala *et al.* 2006, Taipale *et al.* 2007) may apply more generally. Five small Finnish forest lakes with contrasting water colour and DOC content were studied during spring and autumn using SIA ($\delta^{13}C$ and $\delta^{15}N$): (1) to estimate the role of methanotrophic bacteria in the diet of different zooplankton species in different seasons in lakes with varied humic content, and its importance related to phytoplankton; (2) to identify possible other food sources (e.g. heterotrophic bacteria, green sulphur bacteria) used by different zooplankton species; and (3) to explore patterns between lakes related to different DOC concentrations, and any seasonal changes in such patterns.

2. MATERIAL AND METHODS

2.1. Study area and sampling

The study lakes are situated in the Evo state forest area, Lammi, Finland. The lakes were selected to provide a wide range of DOC concentration. Valkea Mustajärvi is a clear water lake; the other lakes, Alinen Mustajärvi, Valkea-Kotinen, Mekkojärvi and Nimetön, have higher DOC concentrations, with the last two having the highest DOC.

Each lake was sampled at the deepest point in May and October, 2006, and from epilimnion (epi), metalimnion (meta), and hypolimnion (hypo). The layers were gauged on site by measuring oxygen content and temperature at 0.5 m intervals with a YSI 55 probe (Yellow Springs Instruments, Ohio, USA, accuracy ±0.3°C, ±0.3 mg O_2 L^{-1}). Water samples were taken with a 60-cm-long Limnos tube sampler (volume 4.25 L) and were passed through a net with a mesh size of 100 µm, except that at Valkea-Kotinen for the hypolimnion a 40-cm-long Limnos tube sampler (volume 2 L) was used. The zooplankton retained on the net were used for counting and species determination. The lake water passed through the 100-µm net was saved for laboratory analysis of POC, DOC, chlorophyll *a* (Chl *a*), bacteriochlorophyll *d* (Bchl *d*), bacterial biomass, phytoplankton biomass, $\delta^{13}C$ and $\delta^{15}N$ of POM, $\delta^{13}C$ and $\delta^{15}N$ of DOM and $\delta^{13}C$ of DIC. Generally 40 L of filtered lake water was collected for these purposes. Zooplankton samples for stable

isotope analysis were collected by hauling a net of mesh size 100 μm through the whole water column at several sites around the lake, to avoid possible effects of zooplankton migration and patchiness. Methane and DIC samples were taken with a 60-cm-long Limnos tube sampler at every 1 m interval (except at Mekkojärvi every 0.6 m interval), then injected into 60 ml polypropylene syringes, which were kept in crushed ice for <4h before analysis.

2.2. SIA analysis

Zooplankton samples were rinsed into deionised water and, after gut evacuation ca. 20 h later, the animals were generally sorted manually into genus or into species when the amount permitted, or mixed together when there were insufficient animals. The sorted animals were put into pre-weighed tin cups. Generally three replicates were prepared for one sample, each with dry weight around 0.5 mg. The samples were then dried at 60°C overnight to constant dry weight and wrapped into tight balls excluding air, ready for $\delta^{13}C$ and $\delta^{15}N$ analysis.

For spring stable isotope analyses of POM, around 200 mL of water was filtered through Whatman Anodisc 47 filters, dried at 60°C overnight to constant weight and ground into powder, transferring all the powder into one tin cup. Another spring POM sample and the autumn samples (2 replicates) were prepared by filtering 500 ml of water though pre-ignited Whatman GF/C glass fibre filters, drying at 60°C to constant weight, and then scraping the retained material into one tin cup.

For DOM samples, 100 mL sample of the filtrate passed through pre-ignited Whatman GF/C filters was acidified and freeze dried (Christ alpha 1-4, B. Braun biotech International), two replicates (5 mg of the dry material each) were prepared from each layer. If a sample size was insufficient, samples from different layers of same lake were mixed.

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

Samples for $\delta^{13}C_{DIC}$ were taken into 20 mL glass bottles and 200 μL of 25.6 g $CuSO_4$* $5H_2O$ $100mL^{-1}$ was added to prevent microbial activity in the sample. Each sample bottle was sealed with an aluminium cap containing a PTFE/silicon septum. Three replicates were prepared for each layer and were stored at 4ºC prior to analysis. The analysis was done by Sami Taipale, Department of Biological and Environmental Sciences, University of Jyväskylä, using a Gas Bench II (Thermo Finnigan) connected to DELTA^{plus} Advantage IRMS (Thermo Finnigan). $\delta^{13}C_{DIC}$ was determined against IAEA standards NBS-19 and limestone was used as a working standard. Results were linearly corrected using NBS-19 values at different intensity. Standard deviation between repeated measurements was normally <0.5 ‰ (Taipale *et al.* 2007).

2.3. Other laboratory analyses

For bacterial and phytoplankton counting, 200 mL samples were immediately fixed with 1 mL Lugol's solution. For analysis, bacterial samples were first decolorized with thiosulfate, and then stained with acriflavine on polycarbonate black 0.22 micron filter. Ten random fields per filter were counted with an epifluorescence microscope (Olympus BX60, Olympus Optical Co., Tokyo, Japan) at 1000 × magnification connected with

analySIS 3.2. Soft Imaging System (www.soft-imaging.net). The number and volume of bacteria were calculated according to the geometric form of bacteria based on the software results. The total bacterial cell volume was then converted to carbon using a factor of 0.36 to provide a measure of carbon food concentration available to zooplankton (Kankaala *et al*. 2006b).

Species composition and biomass of phytoplankton samples were determined with an inverted microscope at 600 × magnification using a settling chamber technique. A 50 mL sample was settled for 24 hours, random points were counted and every species observed was recorded with the cell number and size. Random points were counted until the recorded number of most species reached 200. The phytoplankton abundance and biomass were calculated based on the counted number, size and geometric form of phytoplankton.

Zooplankton samples were immediately fixed with 10% formalin for later microscopical counting. The composition and abundance of zooplankton were determined using a Leica L2 microscope for Cladocera and Copepoda, and an inverted microscope at 100 × magnification for protozoa and rotifers.

For Chl *a* and BChl *d* analysis, one water sample from each layer was filtered in the dark through pre-ignited GF/C Whatman glass microfibre filters until there was noticeable colour on the filter (enough chlorophyll on the filter). The pigments were extracted in ERAX A ethanol, and measured with a SHIMADZU UV-2100 Visible Recording Spectrophotometer from 320 nm to 772 nm, 665 nm and 750 nm for Chl *a* and 654 nm for BChl *d*. The concentration of Chl *a* was calculated using the equation of Lorenzen, and BChl *d* using the equation of Takahashi & Ichimura (Salonen *et al.* 1992a).

POC was detected by high temperature combustion to CO_2 (Salonen 1979). One water sample from each layer was filtered through a pre-ignited Whatman GF/C glass fibre filter. The filter of known area and filtered water volume was burned in a Heraeus oven at 800°C and the CO_2 concentration was detected by a H&B Uras3G Infrared gas analyzer.

DOC samples were analysed by Riitta Ilola of Lammi Biological Station, Finland, using a SHIMADZU TOC-5000A Total Organic Carbon Analyzer. DIC and methane concentrations were analysis by Paula Kankaala of Lammi Biological Station, Finland, by using an AGILENT 6890 N (Agilent Technologies) gas chromatograph equipped with FID and TCD detectors.

2.4. Data analysis

For phytoplankton and zooplankton, only the most abundant genera/species were compared. Methane and DIC results were measured from every 1m/0.6m interval; they were averaged into the results of layer to fit into the analysis with other lake variables.

For statistical analysis, ANOVA test was used to analyse the lake variables. Correlation analysis was done for every individual lake during May and October for 7 pairs: DOC and bacteria biomass, CH_4 and bacteria biomass, Bchl *d* and bacteria biomass, Chl *a* and phytoplankton biomass, DIC and phytoplankton biomass, POC and phytoplankton biomass, and DOC and CH_4. Principle components analysis (PCA) was used for analysis $\delta^{13}C$ values of zooplankton, and the correlations between principle components (PC) and lake variables were then analysed. More correlation analyses were done for $\delta^{13}C$ of DIC, DOC, POC, DIC_{epi}, DOC_{epi} and POC_{epi} separately with DOC; for $\delta^{13}C_{zpl}$ ($\delta^{13}C$ of zooplankton) and $\delta^{13}C_{zpl}$-$\delta^{13}C_{POC}$ separately with DOC; and for $\delta^{13}C_{zpl}$ with phytoplankton biomass.

The proportional contribution of methanotrophic bacteria to the food of zooplankton (% MOB) was estimated from a two source mixing model, assuming that zooplankton use only algae and methanotrophic bacteria (MOB) as food (equation [1]):

$$\% \text{ MOB C} = 100 * (\delta^{13}C_{Zooplankton} - F - \delta^{13}C_{algae}) / (\delta^{13}C_{MOB} - \delta^{13}C_{algae}) \quad [1]$$

where F is the fractionation factor for which the widely used value of 0.4 ‰ was adopted. $\delta^{13}C_{MOB}$ was based on measurements from Mekkojärvi on 15 and 22 September 2005, which ranged from -52.9 ‰ to -101.4 ‰ (Taipale *et al.* 2007). Therefore an averaged $\delta^{13}C_{MOB}$ value of -70 ± 13 ‰ was used for the calculation.

Such a two source mixing model was also applied for other food sources relative to algae: POM, DOM, heterotrophic bacteria ($\delta^{13}C$ -27 to -29 ‰ in Mekkojärvi, in other lakes calculated as $\delta^{13}C_{DOM}$ subtract by fractionation 1 ‰), green sulphur bacteria ($\delta^{13}C$ -19 to -33 ‰ in Mekkojärvi, in other lake calculated as hypolimnetic $\delta^{13}C_{DIC}$ subtract by fractionation 2.5 ‰ to 12.2 ‰) and iron oxidizing bacteria ($\delta^{13}C$ -19 to -33 ‰ in Mekkojärvi, in other lake calculated as hypolimnetic $\delta^{13}C_{DIC}$ subtract by fractionation 20 ‰ to 25 ‰) (see Taipale *et al.* 2007).

The algal $\delta^{13}C$ values were estimated from a two source mixing model assuming that POM consisted only of algae and terrestrial detritus. The proportion of algal carbon in POM was estimated by multiplying the amount of Chl *a* by 25, a mean value for carbon: Chl *a* ratio in algae (Gosselain *et al.* 2000, Taipale *et al.* 2007), and then divided by the POC concentration. The $\delta^{13}C_{POM}$ was then linearly related with the proportion of algal carbon in POM ($\delta^{13}C_{POM}$ = b + a * % of algae C, where a = slope, b = intercept). At 0% of algal C the equation indicate a $\delta^{13}C$ value of terrestrial detritus. Then the algal $\delta^{13}C$ was calculated from equation [2] (Taipale *et al.* 2007).

$$\delta^{13}C_{algae} = (\delta^{13}C_{POM} - (b * \% \text{ of detritus C})) / \text{estimated \% of algae C} \quad [2]$$

3. RESULTS

3.1. Lake variables

All the lakes were first sampled during May when the lakes had stratified and again during October when they began to mix (Figure 1). In May, all the lakes were steeply stratified; mixed layer (epilimnion) generally shallower in lakes with higher DOC; all lakes showed hypolimnetic anoxia, but least acute in clear water lake Valkea Mustajärvi (Figure 1). In October, the water columns were less mixed in humic lakes Mekkojärvi and Nimetön (Figure 1).

ANOVA analysis showed that bacterial biomass, Bchl *d*, methane, Chl *a*, DOC and phytoplankton biomass varied significantly ($P<0.05$) among lakes during May and October (Table 1). Significant correlations between lake variables for individual lakes are presented in Figure 2.

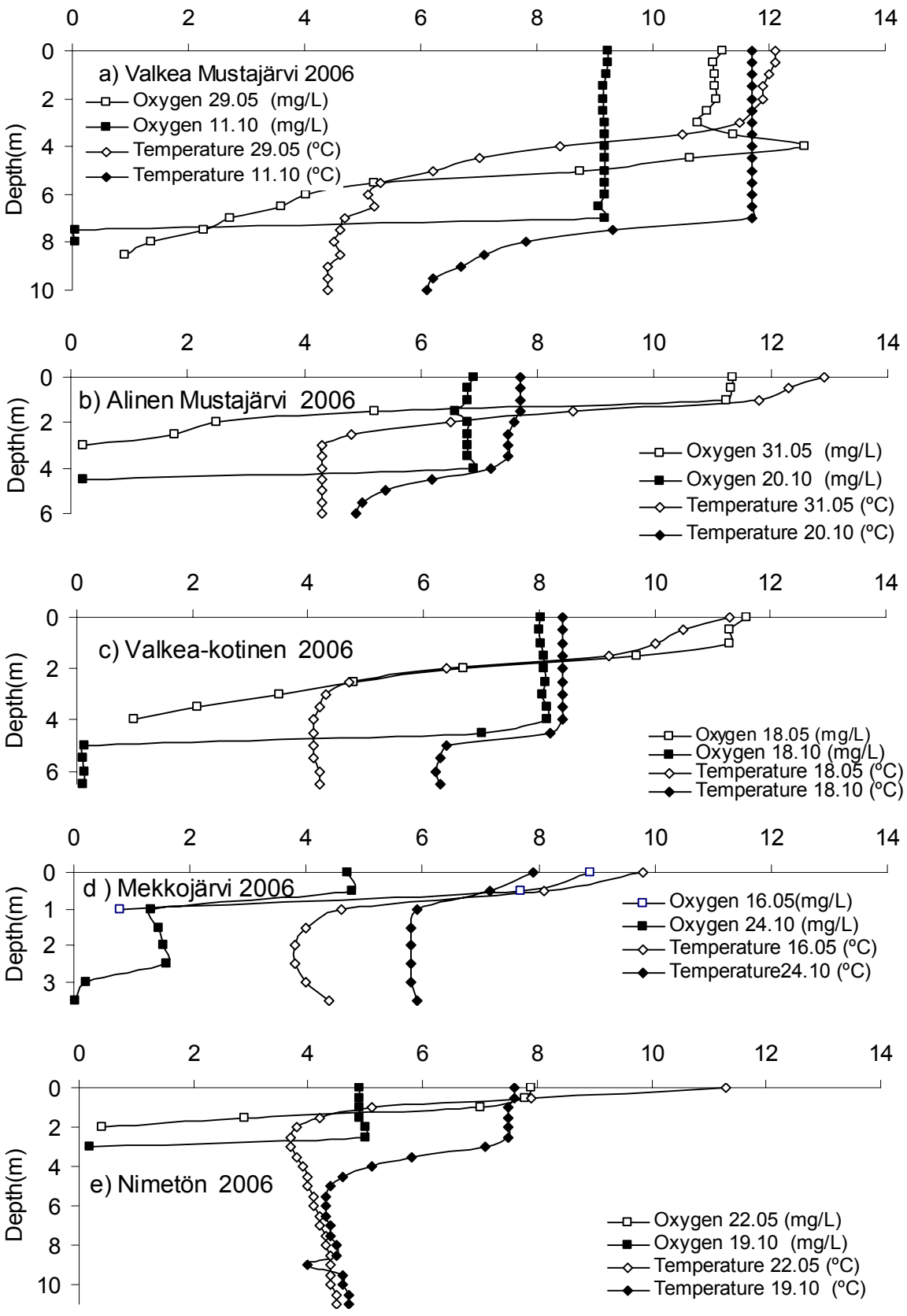


Figure 1. Oxygen and temperature profiles of 5 contrasting forest lakes in May and October 2006. Figures a) to e) are arranged in order of increasing lake DOC concentration.

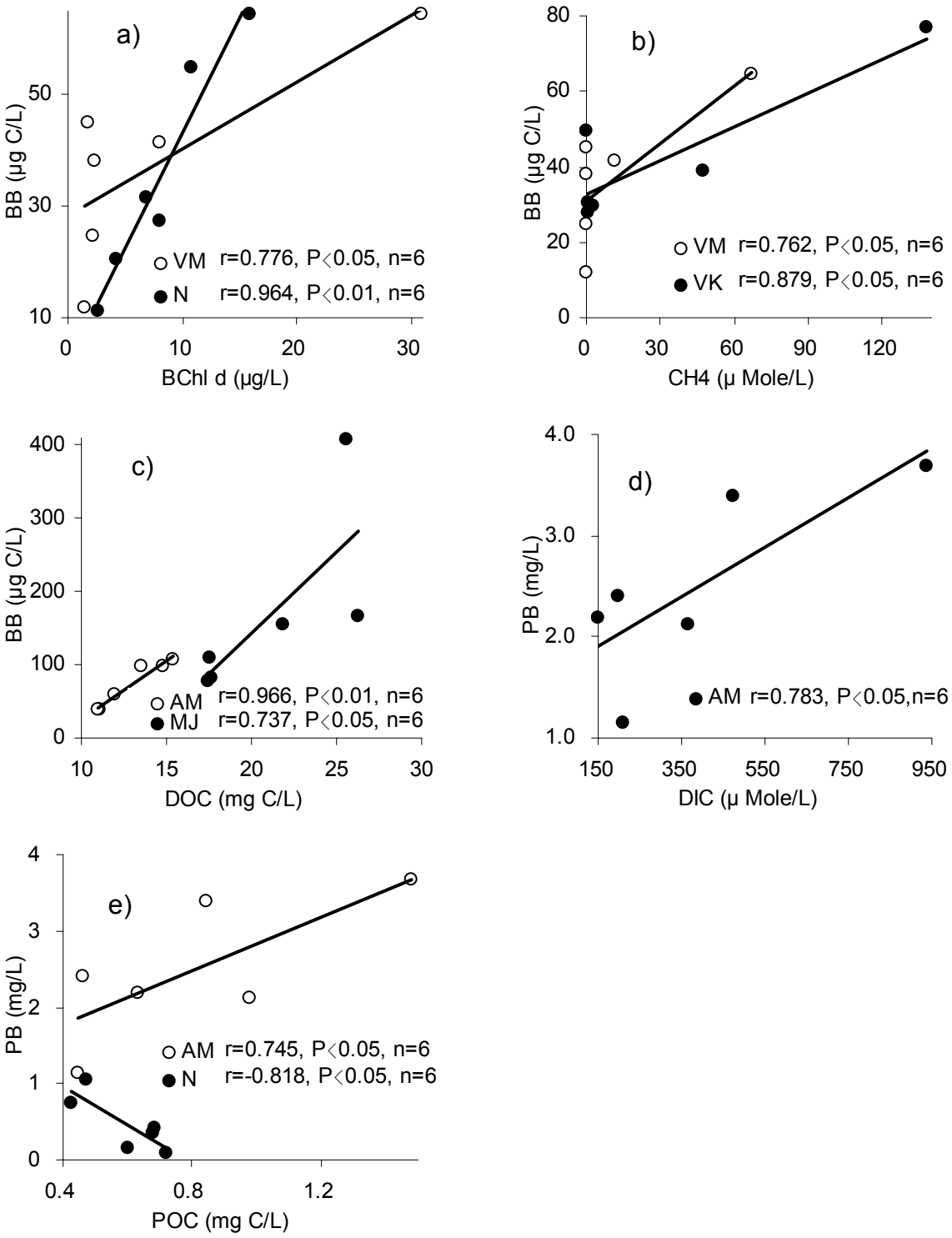


Figure 2. a) Bacteria biomass (BB) as a function of Bchl *d* for Valkea Mustajärvi (VM) and Nimetön (N). b) BB as a function of CH_4 for VM and Valkea-Kotinen (VK). c) BB as a function of DOC for Alinen Mustajärvi (AM) and Mekkojärvi (MJ). d) Phytoplankton biomass (PB) as a function of DIC for AM. e) PB as a function of POC for AM and N.

Table 1. ANOVA results for lake variables of 5 contrasting forest lakes during May and October 2006.

Variables	F	P
Bacteria biomass	3.111	0.033
Bchl *d*	6.028	0.002
CH_4	13.369	0.000
Chl *a*	6.548	0.001
DIC	2.042	0.123
DOC	4.594	0.008
Phytoplankton biomass	4.670	0.006
POC	1.932	0.136

Table 2. Bacterial biomass (BB) (µg C/L), Bchl *d* (µg/L), CH_4 (uM/L), Chl *a* (µg/L), DIC (uM/L), DOC (mg C/L), phytoplankton biomass (PB) (mg/L), POC (mg C/L) and phytoplankton volume (PV) (μm^3/cell) of 5 contrasting forest lakes during May and October 2006. Values are means ± standard deviations (SD), except for BB, Bchl *d*, Chl *a*, PB and PV for which there was only one sample for each layer. There are no data for CH_4 and DIC from Nimetön in October and for DOC from Valkea Mustajärvi in May.

Lake	Month	layer	BB	BChl *d*	CH_4	Chl *a*	DIC	DOC	PB	POC	PV
VM	May	Epi	12	1.4	0.08±0.02	3.0	119±43	-	0.6	0.43±0.02	16
		Meta	38	2.4	0.02±0.003	4.4	429±44	-	1.5	0.69±0.02	1
		Hypo	45	1.8	0.07	2.7	476	-	4.2	1.09±0.01	1
	Oct.	Epi	25	2.2	0.13±0.02	4.8	130±4	5.8±0.1	2.6	0.31	8
		Meta	41	8.0	11.5±16.0	7.4	377±307	5.4±0.1	1.2	0.94±0.01	1
		Hypo	64	30.8	66.8	22.8	712	5.4±0.1	3.6	1.68±0.08	1
AM	May	Epi	59	3.0	0.34±0.30	6.2	151±196	12.0	2.2	0.63	20
		Meta	98	7.0	2.2±3.2	7.6	473±35	13.5±0.1	3.4	0.85±0.05	2
		Hypo	108	30.4	286±8	24.1	938±19	15.4±0.1	3.7	1.48±0.01	3
	Oct.	Epi	39	8.0	2.7±0.1	6.8	199±3	11.1	2.4	0.46	3
		Meta	40	11.8	5.6	9.2	212	11.0±0.1	1.2	0.45	4
		Hypo	97	56.1	59.8	39.3	365	14.9±0.4	2.1	0.98±0.01	10
VK	May	Epi	49	6.3	0.10±0.10	9.7	166±159	14.5±0.6	20.6	1.78±0.06	52
		Meta	29	7.7	2.5±3.5	9.6	391±210	20.5±0.4	2.9	1.49±0.06	19
		Hypo	39	14.4	47.6	15.3	652	18.2±0.4	2.0	1.86±0.02	9
	Oct.	Epi	31	9.9	0.70±0.19	18.1	141±5	13.4±0.1	4.7	0.78±0.03	11
		Meta	28	7.8	0.55	11.5	142	14.1±0.1	3.1	0.65±0.01	10
		Hypo	77	14.2	138	13.3	856	16.5±0.1	2.0	1.23±0.05	4
MJ	May	Epi	165	7.3	2.3	11.3	270	26.3±1.1	1.2	0.87	60
		Meta	409	62.4	21.4±21.7	45.4	684±343	25.6±0.4	1.4	1.64	2
		Hypo	155	162.3	77.7±11.0	101.9	1299±131	21.9±1.1	0.7	2.27±0.11	1
	Oct.	Epi	81	13.5	2.3	12.9	270	17.6±0.1	0.8	1.14±0.02	1
		Meta	109	15.0	0.58±0.15	13.1	870±34	17.5	0.8	1.22±0.01	2
		Hypo	77	14.7	22.3±37.9	11.9	974±170	17.5±0.1	0.7	1.07±0.09	1
N	May	Epi	20	4.2	1.4±1.6	8.5	374±291	22.3±0.3	0.4	0.68	226
		Meta	11	2.7	16.5±7.6	3.6	723±31	30.2±0.2	0.2	0.60	43
		Hypo	27	8.1	631±480	7.2	1688±730	43.8±0.4	0.1	0.72±0.02	60
	Oct.	Epi	32	6.8	-	7.5	-	22.3±0.2	1.1	0.47	5
		Meta	55	10.8	-	9.8	-	23.0±0.1	0.7	0.43	4
		Hypo	64	15.9	-	11.7	-	32.0±0.3	0.4	0.68	1

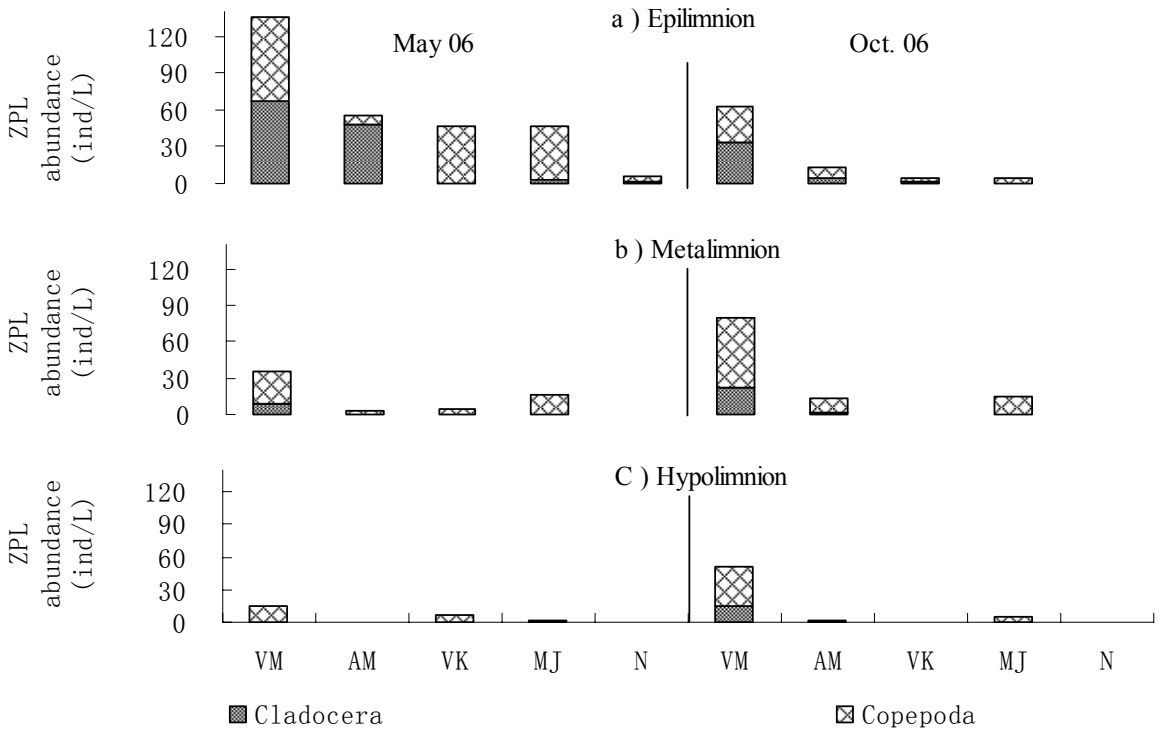


Figure 3. Cladocera and Copepoda abundance in 5 contrasting forest lakes during May and October 2006. The lakes are arranged in the order of increasing mean DOC concentration in both seasons.

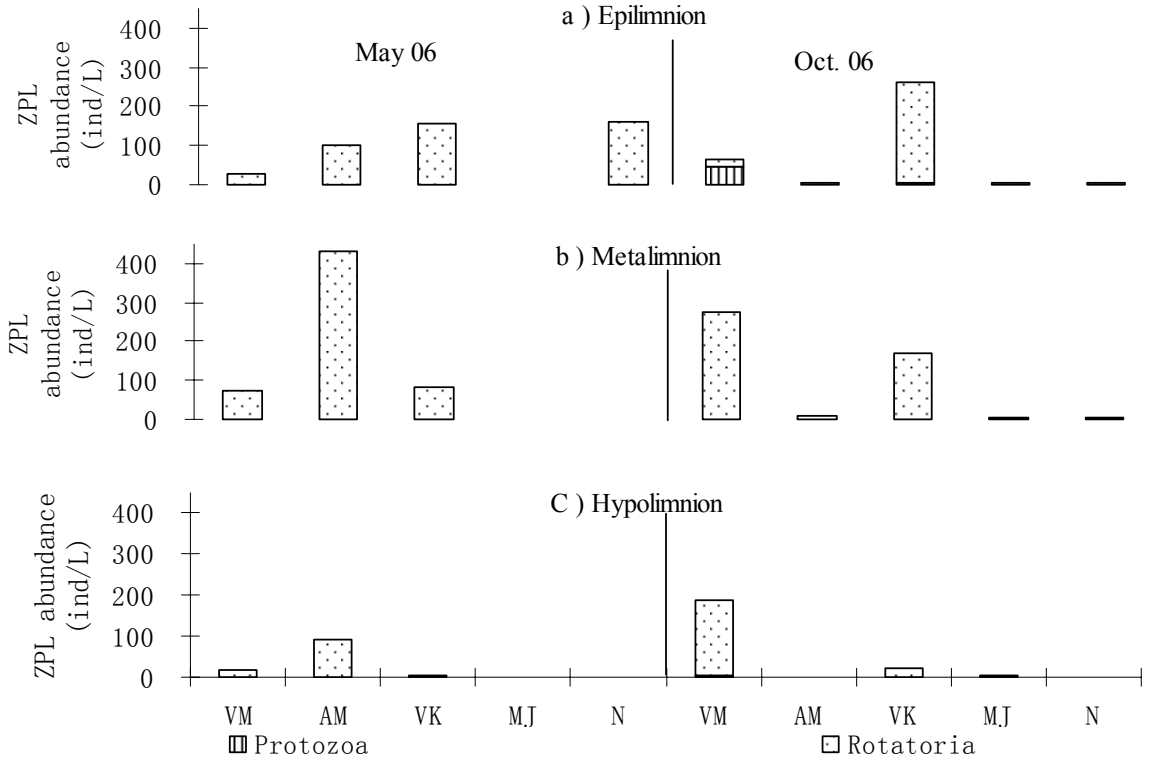


Figure 4. Protozoa and Rotatoria in 5 contrasting forest lakes during May and October 2006. The lakes are arranged in the order of increasing mean DOC concentration in both seasons.

Table 3. Mean phytoplankton biomass (PB) and the percentage of species biomass of 5 contrasting forest lakes during May and October 2006. Chl.=Chlorophyceae, Chr.=Chrysophyceae, Con.=Conjugatophyceae, Cra.=Craspedomonadina, Cry.=Cryptophyceae, Cya.=Cyanophyceae, Dia.=Diatomae, Din.=Dinophyceae, Eug.=Euglenophyceae, Rap.=Raphidophyceae, and Tri.=Tribophyceae.

Lake	Month	Mean PB	Percentage of biomass (%)										
		(mg/L)	Chl.	Chr.	Con.	Cra.	Cry.	Cya.	Dia.	Din.	Eug.	Rap.	Tri.
VM	May	2.1	4	17	0.3		8	57	1	7			1
	Oct.	2.5	29	9	2	0.1	5	53	1				
AM	May	3.1	0.3	6	1		0.3	14	1	47		29	
	Oct.	1.9	12	49	0.1	0.4	0.5	13	2	1		20	
VK	May	8.5	1	1			1	4	0.1	92			
	Oct.	3.3	6	1		0.2	1	9	16	66	1		
MJ	May	1.1	24	5	0.1		2	41	4	5			
	Oct.	0.8	5	13		2	34	44					0.3
N	May	0.2	25	12			17	16	14	6			
	Oct.	0.7	1	13	14	0.4	32	34	5	0.3			

Mean DOC concentrations increased in the order of Valkea Mustajärvi, Alinen Mustajärvi, Valkea-Kotinen, Mekkojärvi and Nimetön both in May and October (Table 2). Methane occurred in every lake but at different concentrations and was mainly highest in the hypolimnion (Table 2). Methane and DOC correlated positively with bacterial biomass in some lakes (Figure 2). Bchl *d* was present in every lake, with highest concentration in the hypolimnion, indicating the presence of green sulphur bacteria (Table 2). Mekkojärvi had the highest Bchl *d* in May among all the lakes, but the concentration decreased dramatically in October; however, in the other lakes, Bchl *d* concentration was general higher or similar in October compared with May (Table 2). Chl *a* had a similar pattern as Bchl *d* (Table 2). Mekkojärvi showed the highest mean bacterial biomass, Bchl *d* and Chl *a* concentration among the 5 lakes in May (Table 2). Mean DIC concentrations were higher in May than in October, as were POC concentrations, except that Valkea Mustajärvi had higher concentration in October (Table 2).

Zooplankton species composition and abundance varied among lakes and between seasons. Generally Valkea Mustajärvi had the highest abundance of macrozooplankton both in May and October; while in more humic lakes, there were lower abundances (Figure 3). The apparent scarcity of Cladocerans in Mekkojärvi might reflect use of number of individuals rather than biomass, since a relatively low density of rather large *Daphnia* in Mekkojärvi actually gives a relatively large zooplankton biomass in this lake (Arvola *et al.* 1992). However, zooplankton biomass was not measured in this project. Rotatoria were more widely detected in the lakes than Protozoa, although their abundance was very low in the most humic lakes, Mekkojärvi and Nimetön, especially in October (Figure 4).

The lowest phytoplankton biomass occurred in the most humic lakes Mekkojärvi and Nimetön, and the highest concentration occurred in Valkea-Kotinen both in May and October; while Valkea Mustajärvi and Alinen Mustajärvi had similar mean phytoplankton biomass (Table 3). Mean phytoplankton biomass was lower in October in Alinen Mustajärvi, Valkea-Kotinen and Mekkojärvi; and slightly higher in the other two lakes (Table 3). More phytoplankton species were recorded in lakes of lower DOC concentration: 53 species in the clear water lake Valkea Mustajärvi, 51 species in Alinen Mustajärvi, 42 species in Valkea-Kotinen, 36 species in Mekkojärvi and 41 species in Nimetön. In Valkea Mustajärvi, Cyanophyceae gave the highest biomass percentage both in May (57%) and

October (53%) (Table 3). In Alinen Mustajärvi, the highest biomass percentage was for Dinophyceae (47%) in May and Chrysophyceae (49%) in October (Table 3). In Valkea-Kotinen, the phytoplankton biomass was very high in the epilimnion in May (Table 2), with *Peridinium* sp. making 97% of biomass; in autumn, the biomass of this species decreased but still represented an average percentage of 66% (Table 3). In Mekkojärvi, the highest biomass percentage was for Cyanophyceae (41% in May and 44% in October); Cryptophyceae made only 2% in May but 34% on October (Table 3). In Nimetön, biomass was more evenly distributed than in other lakes in May, in which the highest percentage occurred for Chlorophyceae (25%); however, Cryptophyceae (32%) and Cyanophyceae (34%) gave more biomass in October (Table 3). *Mallomonas* sp. was widely detected in all the lakes in both seasons. In all the lakes, the mean phytoplankton cell volume was generally higher in the epilimnion than in the other water layers, and the mean cell volume was bigger in May than in October (Table 2).

The microscope examinations of phytoplankton also detected iron oxidizing bacteria (*Ferribacterium* sp.) from Mekkojärvi in May and from Valkea Mustajärvi in October, but its biomass only contributed 1% compared to phytoplankton biomass in Mekkojärvi and 7% in Valkea Mustajärvi.

3.2. SIA results

Zooplankton $\delta^{13}C$ values were generally depleted in October relative to May in all the lakes, with the highest depletion in Alinen Mustajärvi (6.5 ‰) and Mekkojärvi (5.6 ‰), although in Nimetön a slight enrichment of 0.2 ‰ occurred (Figure 5). Zooplankton generally had lower $\delta^{13}C$ values than DIC, DOC and POC; the only exceptions were *Ceriodaphnia* sp. and *Holopedium* sp. from Alinen Mustajärvi in May (Figure 6).

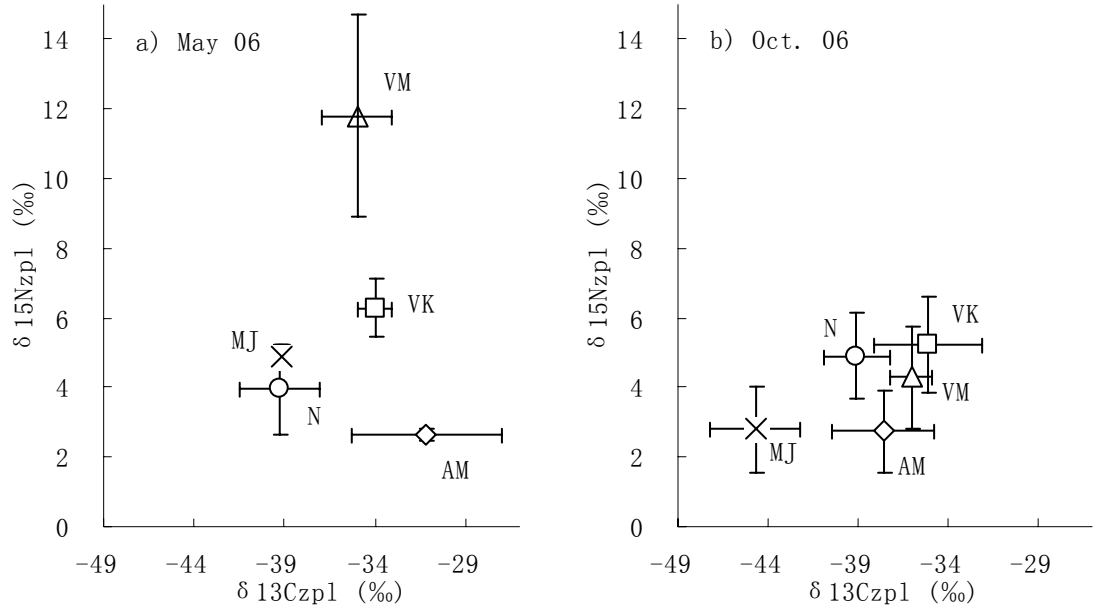


Figure 5. Mean zooplankton $\delta^{15}N$ ($\delta^{15}Nzpl$) plotted against mean zooplankton $\delta^{13}C$ ($\delta^{13}Czpl$) in 5 contrasting forest lakes during May and October 2006. Error bars indicate SD.

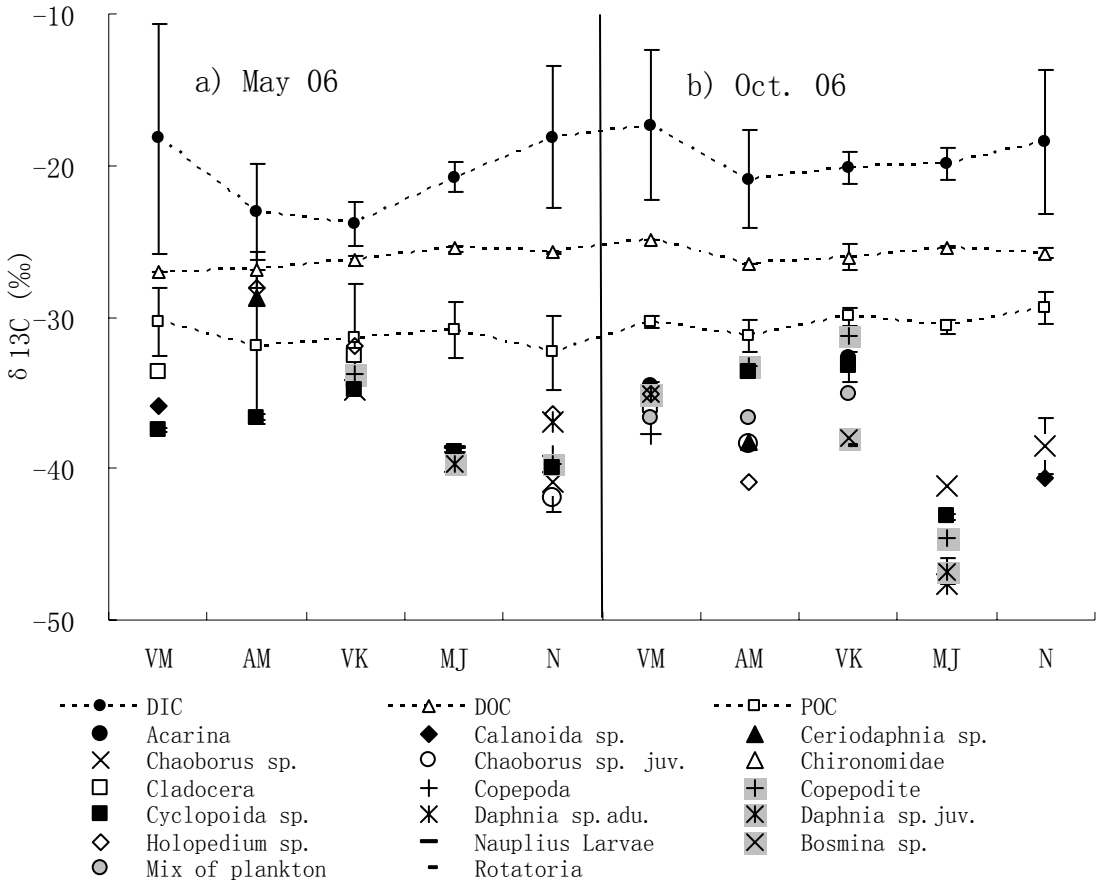


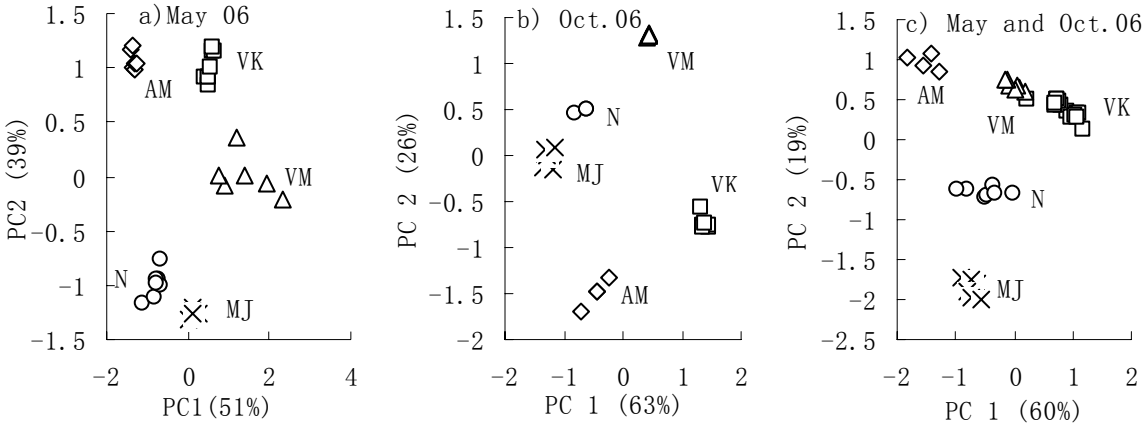
Figure 6. Mean SIA results ($\delta^{13}C$ of zooplankton, DIC, DOC and POC) of 5 contrasting forest lakes during May and October 2006. The lakes are arranged in the order of increasing mean DOC concentration in both seasons. Error bars indicate SD.

Table 4. Mean $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of DIC (only $\delta^{13}C$ values), DOM, POM and zooplankton for 5 contrasting forest lakes during May and October 2006. Values are means ± SD. Some DOM samples do not have SD because only one sample was analysed.

Lake	Month	$\delta^{13}C_{DIC}$	$\delta^{13}C_{DOM}$	$\delta^{13}C_{POM}$	$\delta^{13}C_{ZPL}$	$\delta^{15}N_{DOM}$	$\delta^{15}N_{POM}$	$\delta^{15}N_{ZPL}$
VM	May	-18.2±7.6	-27.0	-30.3±2.3	-35.0±1.9	0.8	8.8±10.1	11.8±2.9
	Oct.	-17.4±5.0	-24.9	-30.3±0.4	-36.0±1.2	2.8	2.1±0.4	4.3±1.5
AM	May	-23.1±3.1	-26.9±1.2	-31.9±4.9	-31.2±4.2	-0.3±4.0	-2.9±9.8	2.6±0.2
	Oct.	-20.9±3.3	-26.5	-31.3±1.1	-37.6±2.8	0.9	-2.1±0.6	2.8±1.2
VK	May	-23. 9±1.5	-26.3±0.3	-31.4±3.6	-34.0±1.0	1.6±4.8	0.4±2.7	6.3±0.8
	Oct.	-20.2±1.1	-26.1±0.9	-30.0±0.6	-35.1±3.0	2.8±1.3	0.7±0.8	5.2±1.4
MJ	May	-20.8±1.0	-25.5±0.2	-30.9±1.8	-39.2±0.5	-0.4±2.4	-2.5±4.4	4.9±0.4
	Oct.	-19.9±1.1	-25.4±0.1	-30.7±0.4	-44.7±2.5	2.3±0.6	-1.2±1.0	2.8±1.2
N	May	-18.2±4.7	-25.8±0.1	-32.3±2.5	-39.3±2.2	2.5±4.0	-2.5±3.2	4.0±1.3
	Oct.	-18.4±4.8	-25.8±0.3	-29.4±1.1	-39.1±1.8	2.2±2.0	-0.1±1.1	4.9±1.3

$\delta^{15}N_{zpl}$ values (Figure 5) were slightly enriched in October in Alinen Mustajärvi (0.1 ‰) and Nimetön (1.0 ‰), but depleted in Valkea-Kotinen (1.1 ‰), Mekkojärvi (2.1 ‰) and Valkea Mustajärvi (7.5 ‰). $\delta^{15}N$ of zooplankton was 2.2 - 7.4 ‰ higher than that of POM, while $\delta^{15}N_{zpl}$ and $\delta^{15}N_{POM}$ of Valkea Mustajärvi in May were much higher than in

the other lakes (Table 4). In addition, $\delta^{15}N$ of zooplankton was 0.5 – 11.0 ‰ higher than that of DOM (Table 4).



Correlation:	r	p	n
PC1&CH4hypo:	−0.72	<0.01	33
PC1&CH4:	−0.619	<0.01	33
PC1&DIC:	−0.533	<0.01	33
PC2&PB:	0.778	<0.01	33
PC2&DOC:	−0.87	<0.01	27
PC2&BB:	−0.427	<0.01	33

Correlation:	r	P	n
PC1&CH4hypo:	0.946	<0.01	23
PC1&PB:	0.815	<0.01	26
PC1&CH4:	0.651	<0.01	23
PC2&POC:	0.388	<0.05	26
PC2&Chla:	−0.842	<0.01	26
PC2&Bchld:	−0.487	<0.01	26

Correlation:	r	p	n
PC1&PB:	0.604	<0.01	38
PC1&POC:	0.544	<0.01	38
PC2&PB:	0.393	<0.01	38
PC2&Chla:	0.378	<0.01	38
PC2&Bchld:	0.276	<0.05	38
PC2&DIC:	−0.843	<0.01	38
PC2&DOC:	−0.524	<0.01	38
PC2&BB:	−0.468	<0.01	38
PC2&CH4:	−0.311	<0.05	38

Figure 7. PCA results for zooplankton SIA values from the 5 contrasting forest lakes during May and October 2006; and the correlations of lake variables with PC. a) For May, test $\delta^{13}C_{cladocera}$, $ln\delta^{15}N_{cladocera}$, $\delta^{13}C_{copepoda}$ and $ln\delta^{15}N_{copepoda}$; b) For October, test $\delta^{13}C_{copepoda}$, $\delta^{13}C_{predator}$, $ln\delta^{15}N_{copepoda}$, $ln\delta^{15}N_{predator}$ and $\delta^{13}C_{DOC}$. c) For combining 2 months, test $\delta^{13}C_{zpl}$ values tested in a) and b).

According to Principle Component Analysis (PCA) of $\delta^{13}C_{zpl}$ (Figure 7), $\delta^{13}C_{zpl}$ values were affected by the variables which had statistically correlation with PC1 (principle component 1) and PC2; a higher correlation indicates stronger effects of the variables in the respective lakes, and PC1 with higher variance suggests more important characters.

In May, Nimetön and Alinen Mustajärvi were characterized by highest CH_4 and DIC, and especially by $CH_{4\ hypo}$; Mekkojärvi was moderately affected by these variables. Phytoplankton biomass, DOC and bacterial biomass (correlated with PC2) had less effect than DIC, CH_4 and $CH_{4\ hypo}$ (Figure 7). Alinen Mustajärvi and Valkea-Kotinen were mostly affected by phytoplankton biomass, Mekkojärvi and Nimetön less so (Figure 7). Compared with phytoplankton biomass, bacterial biomass and DOC had a stronger effect in Mekkojärvi and Nimetön and less in Alinen Mustajärvi and Valkea Kotinen (Figure 7).

In October, the position of each lake changed compare with May, which indicates a change of variable effects on $\delta^{13}C_{zpl}$ (Figure 7). Mekkojärvi, Nimetön, and Alinen Mustajärvi had lowest CH_{4hypo} and CH_4 concentration, which might indicate more CH_4 consumption in these lakes, and high methanotrophic bacteria activities (Figure 7). Phytoplankton biomass was also low in the three lakes (Figure 7). POC, Chl *a* and Bchl *d* (correlated with PC2) had less effects on the lakes than CH_4, CH_{4hypo} and phytoplankton biomass (Figure 7). POC affected the lakes differently; from highest to lowest were Valkea Mustajärvi, Nimetön, Mekkojärvi, Valkea-Kotinen and Alinen Mustajärvi (Figure 7). Chl *a* and Bchl *d* affected the lakes in inverse order compared with POC (Figure 7).

Overall, the PCA results indicated that Mekkojärvi and Nimetön showed the same pattern, being mostly affected by CH_{4hypo} and CH_4, and also by DOC. Alinen Mustajärvi and Valkea-Kotinen showed another pattern, being affected mainly by phytoplankton biomass, CH_{4hypo}, CH_4, POC and Chl *a*, and Valkea Mustajärvi showed a third pattern, affected mainly by phytoplankton biomass in May, and CH_{4hypo}, POC and phytoplankton biomass in October.

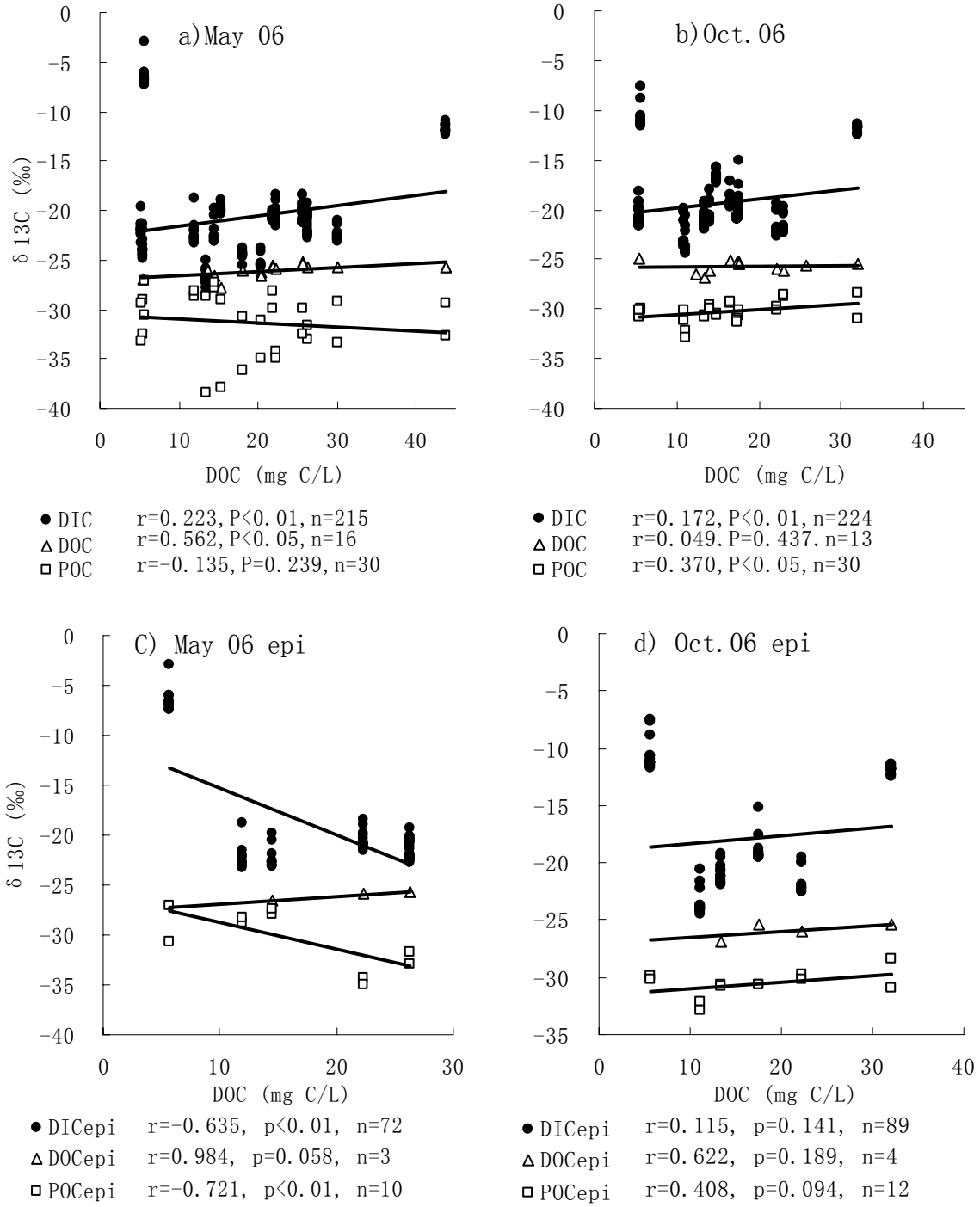


Figure 8. $\delta^{13}C$ of DIC, DOC and POC as a function of DOC concentration of 5 contrasting forest lakes during May and October 2006. DOC concentrations of October for Valkea Mustajärvi were used for both May and October of this lake. a) and b) include the results from the whole water column. c) and d) analysis only the epilimnion data.

From correlation analyses of $\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$, $\delta^{13}C_{POC}$, $\delta^{13}C_{DICepi}$, $\delta^{13}C_{DOCepi}$ and $\delta^{13}C_{POCepi}$ separately with DOC, only $\delta^{13}C_{DICepi}$ and $\delta^{13}C_{POCepi}$ in May had a very strong inverse relationship with DOC. However, when considering the whole water column, such inverse relationship was very weak for $\delta^{13}C_{POC}$ and DOC, and was actually positive for $\delta^{13}C_{DIC}$ and DOC (Figure 8). The correlations for other pairs were positive (Figure 8).

Figures 9, 10 and 11 show that the zooplankton had consumed some isotopically light (^{13}C-depleted) food sources (e.g. methanotrophic bacteria) other than phytoplankton and/or POM both in May and October, and that the $\delta^{13}C_{zpl}$ values generally decreased with increasing DOC concentration, and increased with increasing phytoplankton biomass. The correlation analyses suggest that in October Cladocera may make particular use of methanotrophic bacteria (Figures 9 & 10).

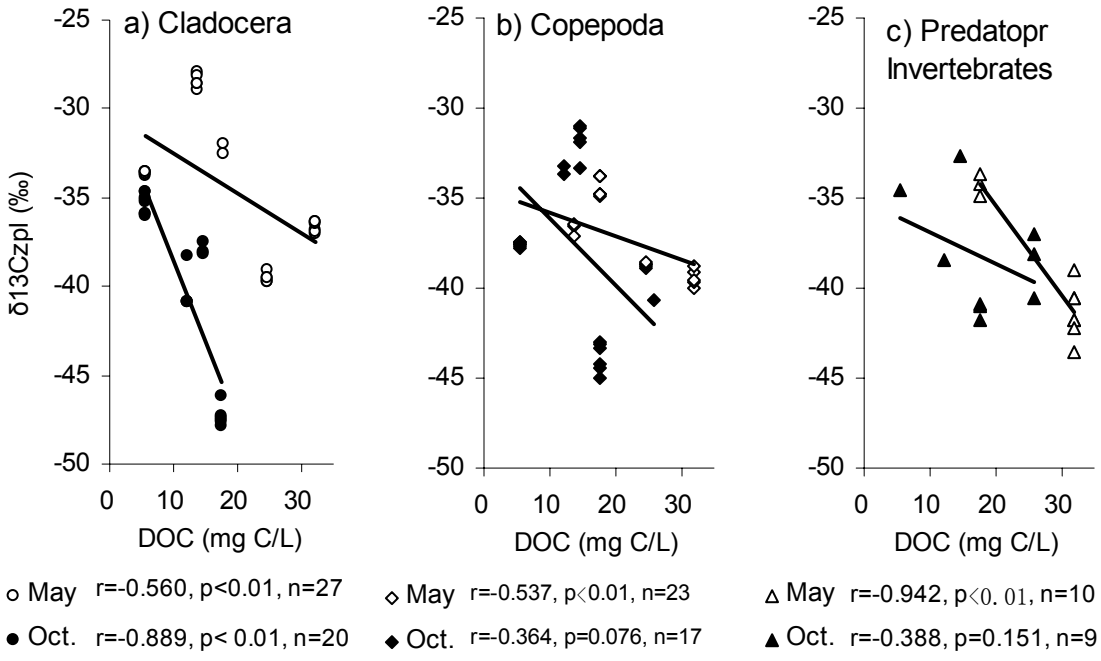


Figure 9. δ^{13}Czpl (Cladocera, Copepoda and predator invertebrates) as a function of DOC concentration in 5 contrasting forest lakes during May and October 2006. (Note that October DOC concentrations in Valkea Mustajärvi were used for both May and October.)

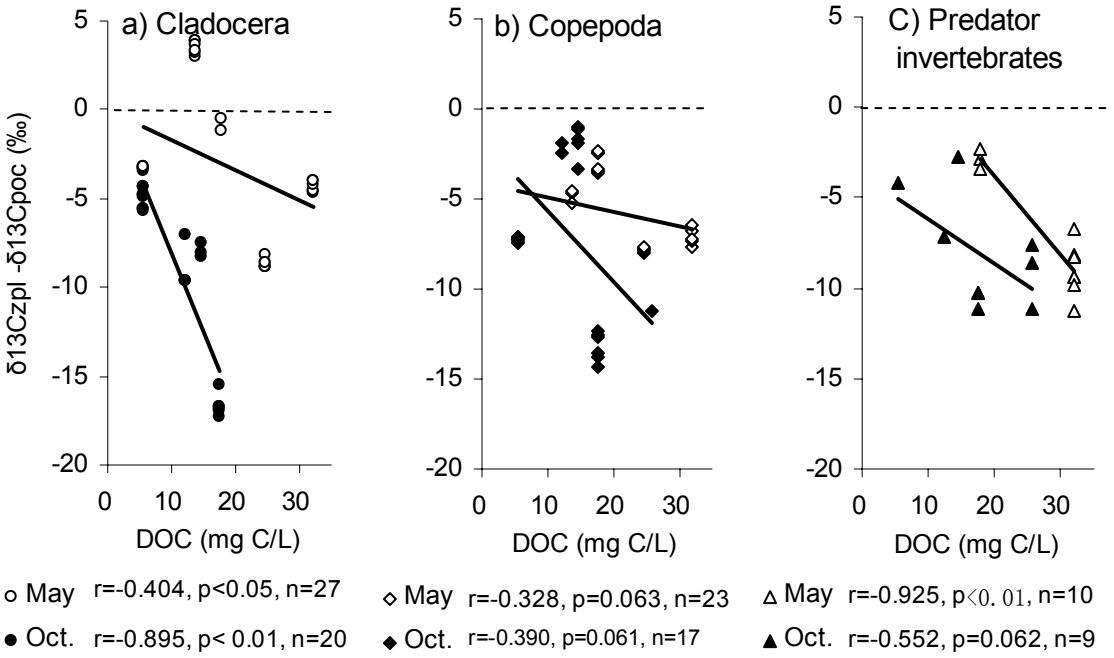


Figure 10. The difference between zooplankton (Cladocera, Copepoda and predator invertebrates) $\delta^{13}C$ and that of $\delta^{13}C_{POC}$, as a function of DOC concentration for 5 contrasting forest lakes during May and October 2006. (Note that October DOC concentrations in Valkea Mustajärvi were used for both May and October.)

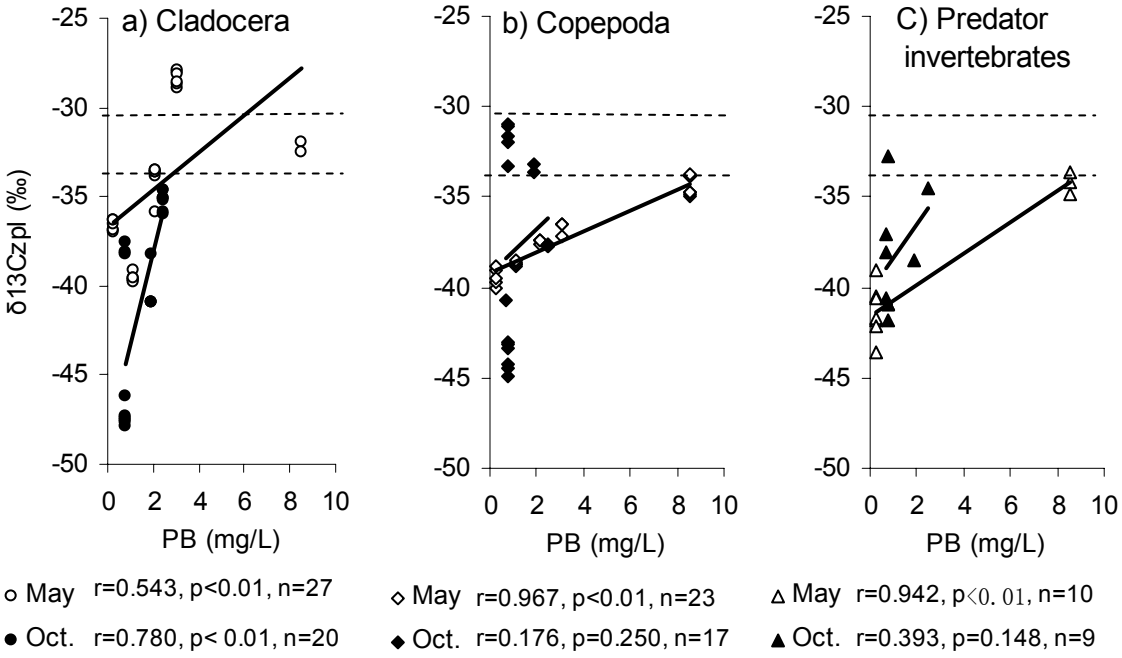


Figure11. Zooplankton $\delta^{13}C$ (Cladocera, Copepoda and predator invertebrates) as a function of mean phytoplankton biomass (PB) for 5 contrasting forest lakes in May and October 2006. The dashed lines indicate the range of estimated values of $\delta^{13}C$ for phytoplankton (-30.4‰ to -33.8‰).

3.3. Approximate proportion of zooplankton carbon from methanotrophic bacteria

The estimates of the proportion of methanotrophic bacteria C contribution to zooplankton (Figure 12) indicated that the more ^{13}C-depleted species got a higher proportion of their carbon from methanotrophic bacteria, and the proportion generally increased with the DOC concentration (Figure 13). For Cladocera, there was a weak, non-significant positive correlation between proportion of C from methanotrophic bacteria and DOC in May, but the correlation became strongly positive and statistically significant in October. For Copepoda, there were only very weak, non-significant positive correlations both in May and October. For predatory invertebrates, the correlation changed from strongly positive and significant in May to non-significant in October (Figure 13).

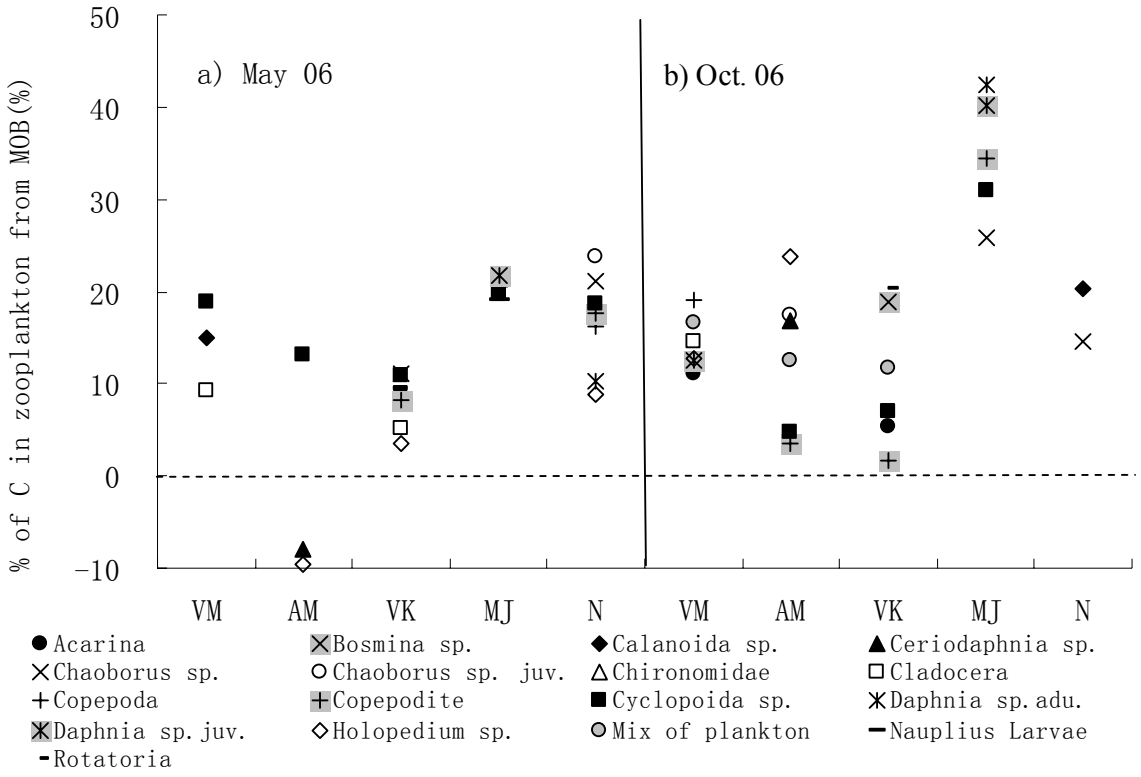


Figure12. Estimated proportion of carbon from methanotrophic bacteria in zooplankton species in 5 contrasting forest lakes in May and October 2006. The results were calculated with a two source mixing model using methanotrophic bacteria and phytoplankton as end members. The lakes are arranged in the order of increasing mean DOC concentration in both seasons.

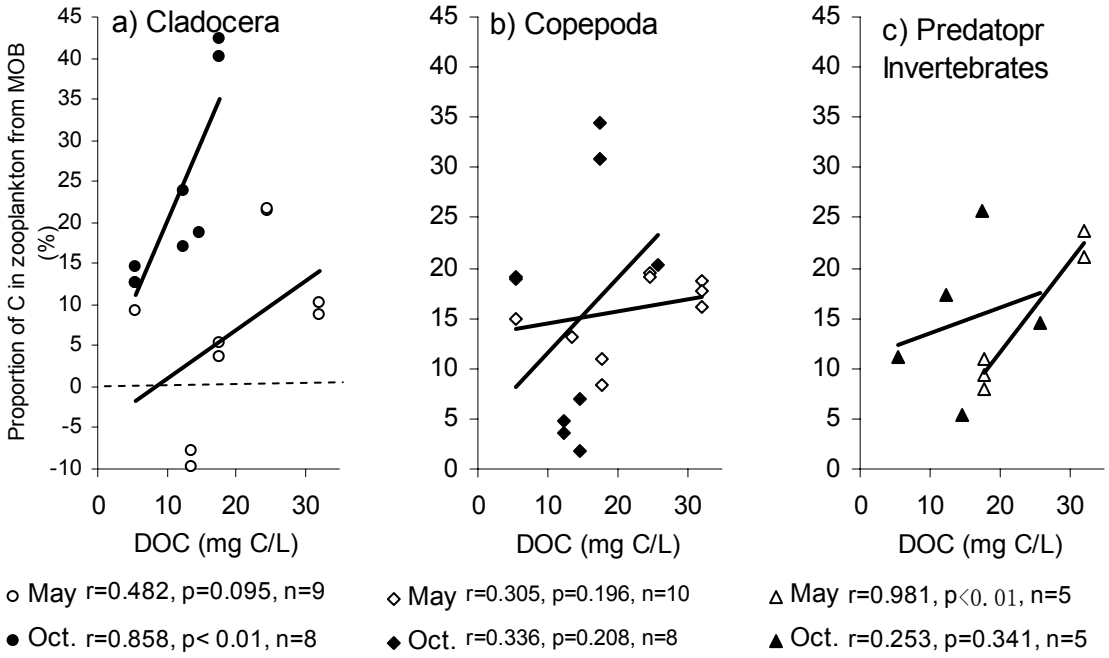


Figure 13. Estimated proportion of carbon from methanotrophic bacteria in zooplankton as a function of DOC concentration in 5 contrasting forest lakes in May and October 2006. (Note that October DOC concentrations in Valkea Mustajärvi were used for both May and October.)

Negative Results of MOB% were obtained for *Holopedium* sp. (-10%) and *Ceriodaphnia* sp. (-8%) from Alinen Mustajärvi in May (Figures 12 & 13), indicating these species had not consumed methanotrophic bacteria at all. Applying other two source mixing models suggested that DOM, heterotrophic bacteria and green sulphur bacteria may contribute to the food of these two species (Table 5).

Table 5. Estimated proportion of DOM, heterotrophic bacteria (HB) and green sulphur bacteria (GSB) contribution to the carbon of two Cladocera species in Alinen Mustajärvi in May 2006; the results are from a two source mixing model using each source separately with algae as the two end members.

Lake & month	Species	DOM (%)	HB (%)	GSB (%)
AM, May	*Holopedium* sp.	71	87	38
AM, May	*Ceriodaphnia* sp.	58	71	31

For other species this model either gave negative or >100% results; except iron oxidizing bacteria got reasonable results (10 - 80%) for most species in the lakes exclude Nimetön. More sophisticated multiple source models would be required to estimate the proportional contributions of these different putative zooplankton diet components, but this was beyond the scope of this project.

4. DISCUSSION

4.1. Phytoplankton as food for zooplankton

Lennon *et al.* (2006) conducted a comparative lake survey in north-eastern U.S. along a gradient of terrestrial-derived DOC; they used naturally occurring carbon stable isotopes of CO_2, POM, and crustacean zooplankton, as well as gas measurements and

culture-independent assessments of microbial community composition to make inferences about the flow of terrestrial carbon in lake food webs. Stable isotope ratios of POM and zooplankton decreased with DOC and were often depleted in ^{13}C relative to terrestrial carbon, suggesting the importance of an isotopically light carbon source (Lennon *et al.* 2006). However, there was weak evidence for the incorporation of biogenic methane into plankton food webs on the basis of relationships of methane, methanogenic archaebacteria, and the methanotrophic bacteria in the lakes; instead, they found that low $\delta^{13}C_{zpl}$ values could be explained by consumption of ^{13}C-depleted phytoplankton, which increase their use of heterotrophically respired CO_2 (^{13}C-depleted) with increasing concentrations of terrestrial derived DOC (Lennon *et al.* 2006).

However in the Finnish lakes studied here, $\delta^{13}C$ of DIC showed no trend to decrease with increasing lake DOC, suggesting no between-lake differences in the availability of respired and ^{13}C-depleted CO_2 (Figures 6 & 8). Moreover, the calculated $\delta^{13}C$ of phytoplankton suggested values between -30.4 ‰ and -33.8 ‰, which is 3.0 ‰ to 13.1 ‰ more ^{13}C-enriched than $\delta^{13}C_{zpl}$ values, except for a depletion of 0.9 ‰ to 1.0 ‰ in Alinen Mustajärvi in May (Figures 6 & 11). Thus the consumption of ^{13}C-depleted phytoplankton would not explain the lower $\delta^{13}C_{zpl}$ with increasing DOC concentration both in May and October, and consumption of strongly ^{13}C-depleted methanotrophic bacteria appears a more plausible explanation (Figures 8, 9 & 10). Nevertheless, phytoplankton is an important food for zooplankton. Phytoplankton biomass and composition may affect the contribution of other food sources to zooplankton. Therefore careful analysis for phytoplankton was needed for better understanding of the variation of zooplankton diets among different lakes at different seasons.

There are two basic factors affecting phytoplankton production in humic lakes (Tulonen 2004). Firstly the penetration of solar radiation is depressed due to humic substances, and secondly the large DOM pool affects the chemical environment by altering the bioavailability of inorganic nutrients and potentially toxic chemicals (Tulonen 2004). Therefore reduced species richness of the phytoplankton community could occur in humic lakes and phytoplankton production is evidently lower in humic lakes compared with clear-water lakes (Tulonen 2004). This was the case in the studied lakes, in which the lowest phytoplankton biomass and species richness occurred in the most humic lakes Mekkojärvi and Nimetön (Table 3). Nevertheless, the highest phytoplankton biomass occurred not in clear water Valkea Mustajärvi, but in moderately humic Valkea-Kotinen, both in May and October (Tables 2 & 3). This might due to the high proportion of mixotrophic algae and migratory ability of flagellated algae, especially in small humic lakes, which may compensate for the poor light environment (Tulonen 2004).

Cyanophyceae made the highest percentage both in May (57%) and October (53%) in Valkea Mustajärvi, and their concentration was also high in Mekkojärvi and Nimetön (Table 3). Cyanobacteria have been considered less important and even harmful food source for zooplankton (Ojala *et al.* 1995). Therefore, zooplankton in these lakes might need compensation from other food sources both in May and October. Meanwhile, phytoplankton biomass was lowest in the most humic lakes Mekkojärvi and Nimetön for both seasons, and was lower in October than in May in other less humic lakes, except that in Valkea Mustajärvi a slight increase occurred; such biomass may not be enough to support the zooplankton community especially in October. Furthermore, *Mallomonas* sp. was widely detected in all the lakes in both seasons, but it is not such favoured food as small cryptomonads and soft bodied flagellates because its silicate bristles and scales make *Mallomonas* sp. difficult for *Daphnia* to handle (Arvola *et al.* 1992). Other phytoplankton may also have adaptive behaviour against predation by zooplankton. Arvola *et al.* (1992)

found that the flagellated chlorophytes, *Chlamydomonas* sp. and *Scourfieldia cordiformis*, stayed mainly in the upper hypolimnion in Mekkojärvi close to the oxic-anoxic boundary zone where only a small proportion of *Daphnia longispina* was continuously present. Therefore it can be concluded that in all the lakes zooplankton needed other food sources (e.g. methanotrophic bacteria) especially in October when the phytoplankton biomass was generally lower related to May.

Meanwhile, phytoplankton biomass could affect the stable isotope signature of zooplankton and the $\delta^{13}C_{zpl}$ values generally increased with increasing phytoplankton biomass (Figure 11). Mean phytoplankton cell volume in October was smaller than in May (Table 2); therefore Cladocera should be more efficient phytoplankton grazers than Copepoda in October, and Copepoda more efficient in May; because Cladocera mainly feed on the smallest members of the plankton, and Copepoda feed selectively on larger ones (Jansson *et al*. 2007). This is consistent with the results (Figure 11), which show that $\delta^{13}C_{Copepoda}$ (r=0.967) was influenced more by phytoplankton biomass than $\delta^{13}C_{Cladocera}$ (r=0.543) in May, and $\delta^{13}C_{Cladocera}$ was influenced more (Cladocera r=0.780, Copepoda r=0.176) in October.

4.2. Bacteria as food for zooplankton

Jansson *et al*. (2007) reviewed recent studies and showed that 30-70% of the organic carbon content of organisms at all trophic levels in clear water, humic and mesotrophic lakes can be of terrestrial origin; bacterioplankton using DOC of terrestrial origin as a source of carbon and energy is one important way in which such carbon is introduced to lake food webs. In the five studied lakes, bacterial biomass was mainly higher in the meta- and hypolimnion than in the epilimnion (Table 2), where the oxygen was lower than in the epilimnion (Figure 1). However, zooplankton can migrate in the water column to maximize their grazing potential across epi-, meta- and hypolimnion; this activity can also minimize predation pressure (Salonen & Lehtovaara 1992). For instance, *Daphnia* has the ability to synthesize haemoglobin, which is an adaptation to unpredictable environments where the oxygen concentration may suddenly drop to lethally low levels (Salonen & Lehtovaara 1992). In this way, the zooplankton in May and October could supplement their diets with more bacterial products, and to a different degree in different lakes. *In situ* grazing experiments have revealed that bacterial food is important for *Daphnia* in humic lakes during periods of algae shortage, and the bacterial diet does not seem to be deficient in any essential nutrients or fatty-acids (Kankaala 1988, Ojala *et al* 1995).

Bacteria are efficiently grazed by phagotrophic microorganisms (e.g. flagellates and ciliates) and by filter feeding zooplankton such as large Cladocera. Cladocera are mainly non-selective filter-feeders, feeding to a large extent on the smallest members of the plankton, including bacteria. By contrast, Copepoda feed selectively on larger phytoplankton, flagellates and ciliates (Jansson *et al*. 2007). The direct link from bacteria to Cladocera implies a higher transfer efficiency than the longer pathway from bacteria via phagotrophic microorganisms to Copepoda (Jansson *et al*. 2007). In the five study lakes Cladocera were apparently more efficient users of methanotrophic bacteria than copepods Figures 9 & 10). This may also have been true for other bacteria in this study, but necessary data to show this were not available. Meanwhile, even the same zooplankton species might differ in their ability to consume bacteria. For example, small and large *D. longispina* may differ in their ability to eat bacteria, and the availability of food may be highly size specific (Kankaala 1988). Juvenile *Daphnia* harvest bacteria and algae with almost equal efficiency, but adults (>1.5mm) clear algal size particles at 2-10 times higher rate than bacteria, thus, juveniles and adults have different food niches along the size

spectrum of food particles (Salonen *et al.* 1992b). This might be the reason that adults and juveniles have different stable isotope values even in the same season within one lake (Figure 6). The differences between adults and juvenile zooplankton stable isotope values might also because that isotopic variation exists among different tissues and metabolites within individual animals. For example, lipids in fat reserves are 2 to 8 ‰ depleted in ^{13}C (Peterson & Fry 1987). $\delta^{13}C$ of adult *Daphnia* might be a bit lower than juvenile *Daphnia*; due to the higher lipid content in eggs and embryos (Matthews & Mazumder 2005). Kling *et al.* (1992) also suggested that accumulation of ^{13}C depleted lipids may shift the isotopic composition of the zooplankton significantly. However, this has been disputed by Zohary *et al*. (1994), and when Grey & Jones (1999) removed lipid from *Daphnia hyalina* in the laboratory, they found no significant isotopic shift.

Bacterial abundance in lake water seems to correlate positively with humic concentration (Kuuppo-Leinikki & Salonen 1992); and bacterial biomass and production are higher in humic lakes than in clear-water lakes (Hessen 1985, Tranvik 1988). All the studied lakes had higher DOC in May than in October, except that in Valkea Mustajärvi no May data were available，although a similar low DOC level as in October can be assumed and it should be the lake with lowest DOC. Higher DOC in May means the bacteria biomass should also be higher in this month; however, such a situation was found only in Alinen Mustajärvi and Mekkojärvi (Figure 2). This might be because only one sample was measured from each layer, and more replicates might alter the relationship.

Methanotrophic bacteria — Methanotrophic bacteria appear to be an important supplement in the food of zooplankton in the studied lakes (Figures 6 & 12). DOC concentration in the lakes influenced the production of methanotrophic bacteria. Humic lakes with characteristic high DOC concentration provide a steady supply of organic matter and sufficiently low hypolimnetic oxygen levels for a vigorous methanogenesis (Hessen 1998). The methane produced in sediments or anoxic water may subsequently serve as an energy and carbon source for methanotrophic bacteria in the water column. This pathway would represent a link between anoxic and oxic communities in the lake (Bastviken *et al*. 2003). In lakes with an oxic water column throughout the year, methane produced in sediments is mainly consumed by methanotrophic bacteria at the sediment surface and can help support benthic fauna (Kiyashko *et al*. 2001, Grey *et al*. 2004). However, in lakes with temporary or permanent anoxia in the hypolimnion, methanotrophic bacteria consume methane at the oxic-anoxic interface in the water column where they can be accessible to pelagic consumers (Rudd & Hamilton 1978, Bastviken *et al*. 2003). For these reasons, the lakes with higher DOC concentration may produce more CH_4, which means more energy and carbon for methanotrophic bacteria and potentially more of them available for zooplankton. This is consistent with the study results from five lakes, in which zooplankton apparently consumed more carbon from methanotrophic bacteria with increasing of DOC concentration (Figure 9). Jones *et al*. (1999) originally suggested that methane production and its utilization by methanotrophic bacteria could be expected to be greater in the more coloured lakes, hence potentially contributing more to the carbon nutrition of the zooplankton.

A previous study on Valkea-Kotinen (Kankaala *et al*. 2006a) found that net production of methanotrophs corresponded to 23–81% of total heterotrophic bacterial production and to 5–10% of algal primary production during the summer stratification and autumn turnover periods, indicating that methanotrophs offer a potentially significant source of carbon to zooplankton in stratified humic lakes. Kankaala *et al*. (2006b) reported that *D. longispina* can grow and reproduce equally well on a diet rich in methanotrophic bacteria as on an algal diet; therefore, the high carbon fixation by methanotrophs in the

studied humic lakes might contribute significantly to zooplankton nutrition. This study did not determine the biomass of methanotrophic bacteria, only methane concentration which might enable production of methanotrophic bacteria. Methane was mainly produced in hypolimnion (Table 2). Clear water Valkea Mustajärvi had lower methane concentration than Valkea-Kotinen both in May and October, and consequently may have had fewer methanotrophic bacteria. Alinen Mustajärvi had slightly lower DOC concentration than Valkea-Kotinen, so the methanotrophic bacteria available for zooplankton might also be slightly lower; the higher bacteria biomass in this lake than in Valkea-Kotinen both in May and October, this higher biomass might therefore come from other bacteria, especially green sulphur bacteria since Alinen Mustajärvi had higher Bchl *d* concentration than Valkea-Kotinen both in May and October，and these green sulphur bacteria may have contributed to zooplankton diet, especially in May for *Holopedium* sp. and *Ceriodaphnia* sp. (Figures 6 & 12, Table 5). Compared with Valkea-Kotinen, Mekkojärvi and Nimetön are more humic lakes and therefore should have had more methanotrophic bacteria available for zooplankton. Methane concentration was higher in these two lakes than in Valkea-Kotinen in May, while lower in October (but no data available for Nimetön), which might be because of more active usage of methane by methanotrophic bacteria in the two most humic lakes in October when autumnal mixing increased the necessary contact between methane and oxygen.

The estimates of approximate proportion of zooplankton carbon from methanotrophic bacteria indicated that the zooplankton in humic lakes generally used more carbon from methanotrophic bacteria than those in clear water lake (Figure 12), presumably because of the higher methanotrophic bacteria biomass in the more humic lakes. MOB% should be generally higher in October than in May, because less phytoplankton were available in October and the zooplankton had to depend more on methanotrophic bacteria (Figures 6 & 12). This is true for Mekkojärvi, but in October zooplankton from Nimetön, the most humic lake of the study, had slightly higher mean $\delta^{13}C$ values than those from Mekkojärvi (Figure 5), which indicates less consumption of methanotrophic bacteria. This might due to the absence from Nimetön in October of Cladocera, which are probably more efficient methanotrophic bacteria users than Copepoda (Figure 3, 9 & 10). Zooplankton in Alinen Mustajärvi and Valkea-Kotinen generally had lower MOB% than in Mekkojärvi and Nimetön, and also higher MOB% in October. In Valkea Mustajärvi, the ranges of MOB% were lower than most humic lakes, but similar in two seasons (Figure 12). The MOB% of Valkea Mustajärvi was similar to Alinen Mustajärvi and Valkea-Kotinen in October, but the standard deviation was much lower (Figure 12), this might mean the use of less food sources, especially less bacterial sources in this clear water lake.

However, the estimates of MOB% are only rough. Firstly, they derive from a two source mixing model, which assumed that zooplankton only consume phytoplankton and methanotrophic bacteria, whereas zooplankton actually consume more food sources. Secondly, $\delta^{13}C_{MOB}$ used in calculation was not measured directly in this study, but came from Taipale *et al.* (2007), which introduced some uncertainties. Finally, the proportion of "memory carbon" in zooplankton (Pace *et al*. 2004, Taipale *et al.* 2007) could not be calculated in this study because there were not enough data.

Other bacterioplankton — the other bacterioplankton supplementing the food of zooplankton might include photosynthetic bacteria, chemosynthetic bacteria and heterotrophic bacteria.

Small, sheltered, forests lakes exhibit steep stratification which is most pronounced in the more humic lakes (Bowling & Salonen 1990). Where the stratification is sufficiently steep that some light penetrates to the top of the anoxic hypolimnion, a narrow layer can occur of photosynthetic bacteria, mostly green sulphur bacteria (*Chlorobium*), reaching high cell densities and provide food for migrating zooplankton and protozoa (Arvola *et al*. 1992, Järvinen 2002). Estimates of the photosynthetic bacterial contribution to total planktonic primary production in humic and eutrophic lakes range from 0.3 - 6.3%; in meromictic clear-water lakes, bacterial photosynthesis may contribute 50 - 80% of primary production (Kuuppo-Leinikki & Salonen 1992). Although there was no direct measurement for the biomass of photosynthetic bacteria, Bchl *d*, the indicator of *Chlorobium*, was presented at every studied lake with highest concentration generally at hypolimnion (Table 2). In May Mekkojärvi had the highest Bchl *d* concentration, and Alinen Mustajärvi also had some which might explain the enriched $\delta^{13}C$ signatures for *Holopedium* sp. and *Ceriodaphnia* sp.. However, Jones *et al*. (1999) suggested that green sulphur bacteria cannot represent the isotopically light food sources evidently used by the zooplankton in some small forest lakes in southern Finland, because their production and growth rate is rather small, and the isotopic fraction of CO_2 during photosynthesis by *Chlorobium* is less than that of C_3 phytoplankton.

There was also no direct measurement of the biomass of chemosynthetic bacteria. Methanotrophic bacteria are one kind of chemosynthetic bacteria, but other chemosynthetic bacteria (e.g. iron oxidizing bacteria) might also contribute to the food of zooplankton. From the microscopic calculation for phytoplankton, iron oxidizing bacteria *Ferribacterium* sp. was only detected from Mekkojärvi in May and Valkea Mustajärvi in October, and there was no other evidence that iron oxidizing bacteria also exist in other lakes. For these two lakes where iron oxidizing bacteria were present, their biomass only contributed 1% compared to phytoplankton biomass in Mekkojärvi in May and 7% in Valkea Mustajärvi in October. Therefore the approximate calculation for the contribution of zooplankton carbon from iron oxidizing bacteria may show such bacteria could be one food source of zooplankton, but its abundance may not enough to support much of the zooplankton carbon.

Heterotrophic bacterial production represents an important route of external carbon and energy into pelagic food webs in many lakes (Hessen 1998, Tranvik 1998). The heterotrophic bacteria in lakes utilize both allochthonous and autochthonous organic matter as sources of carbon and energy. There was no direct measurement of the biomass of heterotrophic bacteria either, but the zooplankton consumption of heterotrophic bacteria should be lower than of methanotrophic bacteria, because their carbon stable isotope signature is more enriched.

4.3. Trophic level of zooplankton

Nitrogen isotopic values increase by 10 to 15 ‰ in many food webs. These increases may due to the presence of 3 to 5 successive trophic transfers, generally 3 - 4 ‰ (mean = 3.4 ‰) per trophic level (Peterson & Fry 1987). For the studied lakes except Valkea Mustajärvi, the changes of $\delta^{15}N$ was within 01 - 2.1 ‰ for two seasons (Table 4), therefore the changes were within one trophic level, and the trophic position of zooplankton may not changed in these lakes during different seasons. Meanwhile, Table 4 indicates that zooplankton was one or two trophic level higher than phytoplankton; this is possible because predator invertebrates were included in the calculation, which were generally two trophic levels higher than phytoplankton.

In Valkea Mustajärvi, $\delta^{15}N_{zpl}$ was very higher in May (11.8 ± 2.9 ‰) than in October (4.9 ± 1.5 ‰). The traditional concept is that the higher the $\delta^{15}N$ value of a consumer, the higher its trophic position. However Vander Zanden & Rasmussen (1999) pointed out that a consumer with a higher $\delta^{15}N$ signature may simply be feeding in a food chain with a high $\delta^{15}N$ baseline; hence consumers with very different $\delta^{15}N$ values may have similar trophic positions if the baseline $\delta^{15}N$ values vary between different food webs, within or between lakes. This might be a plausible explanation for lake Valkea Mustajärvi, because $\delta^{15}N_{POM}$ was also higher in May (8.8 ± 10.1 ‰) than in October (2.1 ± 0.4 ‰) (Table 4), which in turn indicates that higher $\delta^{15}N$ signature of phytoplankton in May. High $\delta^{15}N_{POM}$ signature in May might result from an enriched ^{15}N inorganic nitrogen pool as the size of the pool was reduced (Syväranta *et al.* 2006), but there was no data for dissolved inorganic nitrogen in this study to test this hypothesis.

The higher standard deviation of $\delta^{15}N_{POM}$ in May related to October in all the studied lakes might arise from the use of two different filter papers (Whatman Anodisc 47 filters and Whatman GF/C glass fibre filters) during analysis.

5. CONCLUSION

In conclusion, in May methanotrophic bacteria in the most humic lakes, Mekkojärvi and Nimetön, may contribute a bigger proportion of zooplankton diets than in the other three less humic lakes. Other food sources supplementing zooplankton diet may include photosynthetic bacteria, chemosynthetic bacteria (e.g. iron oxidizing bacteria) and heterotrophic bacteria, and may play a bigger role in less humic lakes (e.g. *Ceriodaphnia* sp. and *Holopedium* sp. in Alinen Mustajärvi). In October, less phytoplankton was available than in May and the zooplankton depended more on methanotrophic bacteria. Dependence on other food sources might also decrease with increasing of methanotrophic bacteria consumption. Zooplankton in Mekkojärvi consumed the biggest amount of methanotrophic bacteria among all the lakes. Zooplankton in another very humic lake, Nimetön, apparently consumed less than those in Mekkojärvi. This might be due to the absence from Nimetön of Cladocera, which are probably more efficient than Copepoda as users of methanotrophic bacteria in October. The zooplankton in the other three lakes consumed similar amount of methanotrophic bacteria, although in Valkea Mustajärvi, the variance of $\delta^{13}C_{zpl}$ was lower than in the other two lakes, which might indicate use of fewer food sources than in the other two lakes.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Professor Roger I. Jones for accepting me into this project, and for his patience, encouragement, valuable guidance and constructive comments. I would especially like to thank Dr. Paula Kankaala and PhD student Sami Taipale for co-supervising this work, for their thoughtful assistance, excellent advice, and useful discussions. I am also grateful to Dr. Lauri Arvola and PhD student Elina Peltomaa for helping me with the phytoplankton identification, and to Jari Syvaranta and Mikko Kiljunen for sharing their experiences on stable isotopes and for help with stable isotope analysis. My sincere compliments go also to the staff of Lammi Biological Station, University of Helsinki, for their advice, help and pleasant cooperation during the past months.

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