Master's Thesis

Responses of zooplankton to cane sugar additions to a small humic lake, Alinen Mustajärvi

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ABSTRACT

The response to projected increase in dissolved organic carbon (DOC) loading to boreal lakes is being studied in lake Alinen Mustajärvi, a small humic lake in southern Finland, where labile DOC concentration has been artificially increased during 2008 and 2009 by monthly additions of cane sugar. As part of this wider project, in this thesis the responses of the zooplankton community to cane sugar addition were studied. Zooplankton samples were collected monthly in the ice-free periods of 2007, 2008 and 2009 and the mean densities and mean carbon biomasses of rotifers and crustacean zooplankton (cladocerans and copepods) were calculated. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were made for the crustacean zooplankton samples. Zooplankton respond to additions of DOC by an appreciable increase in the mean density of rotifers and carbon biomass of copepods, while both the mean density and carbon biomass of cladocerans showed a decrease between years. Values for $\delta^{13}C$ increased progressively across the years, especially for cladocerans, but less so for copepods. This suggests strongly that appreciable DOC from sugar additions was being transferred to zooplankton by heterotrophic bacteria and zooplankton dependence on heterotrophic bacteria appeared to increase with the increased loading of DOC in lake Alinen Mustajärvi.

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TIIVISTELMÄ

Lisääntyneen liuenneen orgaanisen hiilen (DOC) kuormituksen vaikutuksia boreaalisen vyöhykkeen järviin tutkittiin eteläsuomalaisessa pienessä humusjärvessä, Alinen Mustajärvessä, missä labiilin DOC:in pitoisuutta keinotekoisesti nostettiin lisäämällä järveen vuosina 2008 ja 2009 kuukausittain ruokosokeria. Osana tätä laajempaa projektia, tämä Pro gradu –työ tutkii sokerin lisäysten vaikutuksia eläinplanktonyhteisöön. Eläinplanktonnäytteitä kerättiin kuukausittain sulanveden aikana vuosina 2007–2009, ja niistä määritettiin rataseläinten (Rotifera) ja äyriäisplanktonin, eli vesikirppujen ja hankajalkaisten (Cladocera ja Copepoda) keskitiheydet ja keskimääräiset hiilibiomassat. Äyriäisplanktonille tehtiin lisäksi hiilen ja typen stabiili-isotooppianalyysit ($\delta^{13}C$ ja $\delta^{15}N$). DOC-lisäysten seurauksena rataseläinten keskitiheys ja hankajalkaisten hiilibiomassa kasvoivat, kun taas vesikirppujen keskitiheys ja hiilibiomassa laskivat. DOC-lisäysten myötä $\delta^{13}C$-arvot kasvoivat, erityisesti vesikirpuilla, mutta vähemmän hankajalkaisilla. $\delta^{13}C$-arvojen kasvu viittaa vahvasti siihen, että huomattava määrä lisätystä DOC: sta siirtyi heterotrofisten bakteerien kautta eläinplanktoniin ja eläinplanktonin riippuvuus heterotrofisista bakteereista näytti kasvavan DOC-lisäysten seurauksena Alinen Mustajärvessä.

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1. INTRODUCTION

Lake food webs have traditionally been described as based on algal primary producers, but recent investigations have shown that most lakes worldwide are actually net heterotrophic, that is, community respiration exceeds primary production (Cole et al. 1994, del Giorgio et al. 1999). An estimated 30-70% of the organic carbon content of organisms at all trophic levels in lakes can be of terrestrial origin and bacterioplankton using dissolve organic carbon (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 (Jansson et al. 2007). 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).

Accordingly, the food web in which zooplankton occurs is now viewed as rather complex, with some carbon and energy flowing directly from algae to zooplankton, some from bacteria to zooplankton, and some indirectly from bacteria to zooplankton by way of a 'microbial loop' involving intermediate protozoan consumers, including ciliates and heterotrophic flagellates (Fig. 1). Thus, freshwater zooplankton plays a critical role in transferring carbon and energy from phytoplankton, bacteria and protozoa to higher trophic levels in lakes (Thorp & Covich, 1991).

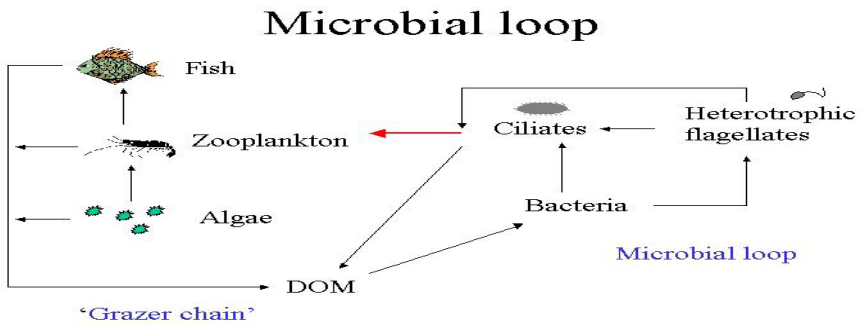

Figure 1. An illustration of the concept of microbial loop in small boreal lakes (from www.esf.edu/efb/schulz/Limnology/microbialloop.jpg).

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 can be 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).

1.1. Background and relevance of the study

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 & Hammer (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 DOM or 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. 1992). The direct use of colloidal DOM by heterotrophic and mixotrophic flagellates may also be possible, but is poorly understood (Salonen et al. 1992, Pace et al. 2004). Bacterial 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. In small humic lakes, bacterial food sources for higher trophic levels can include heterotrophic, methanotrophic (MOB), photoautotrophic and chemoautotrophic types (Kankaala 1988).

Kankaala et al. (2006b) argued that methane-derived carbon is a more important contribution to carbon flux through lake pelagic food webs than 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. Kankaala et al. (2010) further indicated that when methane that is accumulated in the hypolimnion becomes mixed with oxygen from the upper layers, it provides favourable conditions for MOB in the whole water column. This ensures a particularly high availability of MOB as bacterial food for zooplankton during the autumnal turnover period.

Stable isotopes of carbon have been used to study the diets 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 (Post 2002, Fry 2006).

A combination of carbon stable isotope analysis with mixing models (Phillips and Gregg 2001, Fry 2006) has been demonstrated to be an effective tool to investigate food sources of different organisms, and has been widely used also in aquatic food web studies (Jones et al. 1998; Grey et al. 2001). However, autochthonous and allochthonous carbon sources often have too similar $\delta^{13}C$ values, and then the contribution of phytoplankton and different bacteria to zooplankton diets cannot be accurately calculated from natural isotope signatures (Taipale et al. 2007). Thus, the relative support of consumers by autochthonous and allochthonous sources is difficult to determine except in rare cases. ^{13}C-enrichment experiments offer one means to create more distinct source $\delta^{13}C$ values and make it possible to estimate the contributions of putative food sources (Taipale 2007).

Crustacean zooplankton consumes food items (algae, bacteria, heterotrophic protozoa) that can rarely be separated in field samples and hence in SIA are usually analyzed as bulk POM. In some Finnish humic lakes, phytoplanktons ($\delta^{13}C$ -28 to -37 ‰) are generally ^{13}C-depleted relative to bulk POM ($\delta^{13}C$ -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 ($\delta^{13}C$ -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 $\delta^{13}C$ 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 $\delta^{13}C$ values typically between -45 ‰ and -72 ‰, and the methanotrophic bacteria that utilize this methane at the oxic-anoxic interface within the water column fractionate carbon further and have $\delta^{13}C$ values between -52 ‰ and -101 ‰ (Rudd & Taylor 1980, Jones et al. 1999, Taipale et al. 2007).

Taipale et al. (2007) studied the relative contributions of different carbon sources to zooplankton in a small Finnish lake, Mekkojärvi, by adding/not adding 13C-enriched bicarbonate into the epilimnion of replicate treatment/control surface-to-sediment enclosures during summer and autumn 2004. Carbon stable isotope ratios of *Daphnia*, 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).

Notably, current climate change scenarios suggest increased loading of DOC to northern lakes in the future associated with increased rainfall and runoff from catchments. One possible effect of this might be to enable increased bacterial production at the expense of phytoplankton, and hence to shift the overall lake energy balance to a more heterotrophic state. This study was part of a larger project to test these effects being carried out at Alinen Mustajärvi, a small, humic, forest lake in southern Finland where cane sugar was added at monthly intervals during the ice-free period in 2008 and 2009. Cane sugar acts as source of readily utilisable DOC but does not alter the light conditions for autotrophic phytoplankton, unlike brown organic matter from peatlands. Moreover, cane sugar (from a tropical C_4 plant) also has a higher carbon stable isotope signature ($\delta^{13}C$ ~12 ‰) than local DOC from C_3 plants ($\delta^{13}C$ ~28 ‰) and can therefore act as a stable isotope tracer in the food web. Zooplankton (especially crustacean zooplankton, but also rotifers) are a key link in lake food webs between lower trophic levels (phytoplankton and bacteria) and higher consumers (fish).

1.2. Research questions

The aim of this thesis was to examine the responses of zooplankton in lake Alinen Mustajärvi to the cane sugar additions. Specific questions were as follows:
1) Does the abundance and species composition of the zooplankton in the lake change in response to the increased loading of DOC in the form of cane sugar?
2) How much do the different zooplankton types in the lake depend on autochthonous or allochthonous carbon sources for their food?
3) Does the relative dependence of zooplankton on the heterotrophic food web (based on bacterial production) increase with increased loading of DOC?

2. MATERIAL AND METHODS

2.1. Study area

The study lake was Alinen Mustajärvi in the Evo state forest area, Lammi, Southern Finland ($61^0$12'N, $25^0$7'E), about 20 km north from Lammi Biological Station (Fig. 2). Alinen Mustajärvi is an autumn monomictic, oligo-mesohumic lake with a surface area of 0.008 km^2 and a volume of 31x 10^3 m^3. The lake has a maximum depth of 6.5 m and mean depth of 4 m. It is a headwater lake with no inlets and only a small outlet that does not allow any immigration or emigration of fishes. The lake has a high concentration (11-15 mg l^{-1}) of DOC and thus provides a good site for experimental work with the addition of cane sugar. About 66 kg of cane sugar was added to the lake every month during the ice-free periods of 2008 and 2009. This amount was calculated to produce approximately a 2 mg l^{-1} rise in the DOC concentration in the epilimnion, and this was intended to raise labile DOC concentration from around the first quartile level for boreal lakes to that in lakes around the third quartile level (Henriksen et al. 1998). The study lake has a pH of about 5.3-5.4, the colour in the epilimnion ranges between 120-150 mg Pt l^{-1}, and the maximum CH_4 concentration in the lake stands at 900 µmol l^{-1} measured in the hypolimnion.

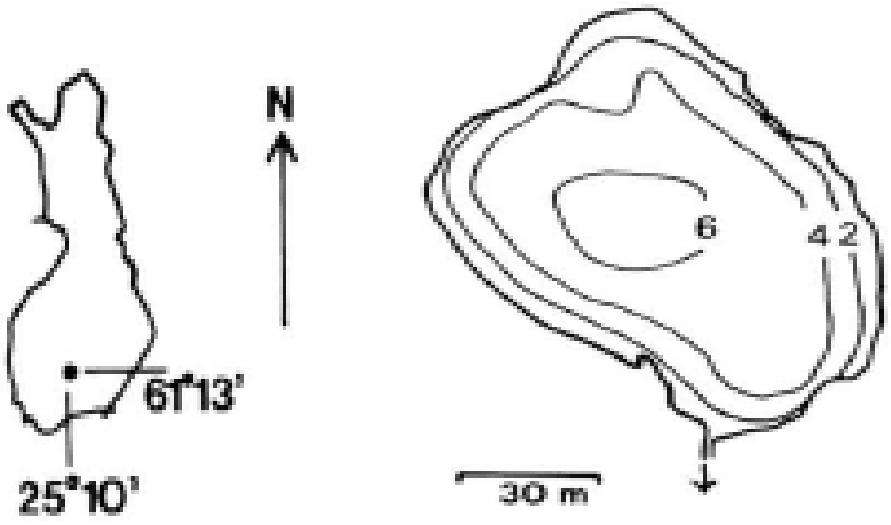

Figure 2. The location and bathmetry of lake Alinen Mustajärvi (from Rask & Arvola, 1985).

The oxic layer volume as a percentage of total volume ranges between 50-63 % (Kankaala et al. 2010). The bacterial biomass of the study lake ranges between 0.1-0.2 mg C l^{-1} and the phytoplankton biomass ranges between 0.4-1.0 mg C l^{-1}. Dinophyceae represents the most dominant algal taxonomic group in the phytoplankton biomass of the epilimnion in the study lake in May, while Chrysophyceae and Raphidophyceae co-dominate in October (Kankaala et al. 2010).

The amount of macrophytes in the lake is low, consisting of some *Carex* vegetation inshore and *Nuphar lutea* L. (Smith) slightly offshore. One third of the shoreline is surrounded by a floating *Sphagnum* mat. The original fish species in the lake were perch, *Perca fluviatilis* L. and pike, *Esox lucius* L. In the autumn of 1975 a small population of whitefish, *Coregonus muksun pallas* was introduced into the lake (Rask & Arvola, 1985). The total fish biomass of the lake was estimated at 73 kg ha^{-1} and fish production was 30 kg ha^{-1} (Rask & Arvola, 1985).

2.2. Data collection for zooplankton community structure

The lake was sampled at its deepest point during the ice-free period of May to early November (2007-2009). Zooplankton samples were collected two to three times monthly for the epilimnion (epi), metalimnion (meta) and hypolimnion (hypo), respectively. In 2007 and 2008, separate samples were collected from the epilimnion (6x 2 L Limnos tube samples, total volume 12 l) and from the metalimnion and hypolimnion (4x 2 L Limnos tube samples, total volume 8 l). In 2009, zooplankton samples were collected jointly from the epilimnion and metalimnion (6x 2 L Limnos tube samples, total volume 12 l) and separately from the hypolimnion (4x 2 L Limnos tube samples, total volume 8 l). The zooplankton retained on a 50 mm mesh size net from the 12 and 8 litres of zooplankton samples collected separately for the different water depths were then concentrated into 100 ml samples and preserved with formalin for later counting and species determination.

During the laboratory identification and counting process, the zooplankton samples were first rinsed on a 50 mm mesh size net with water left at room temperature. This was done to reduce the concentration of formalin before placing the zooplankton in a 50 ml settling chamber for counting. A 25 ml zooplankton sample size was used for the 2008 zooplankton samples. The sample was left to settle for about 25 minutes, and then placed on the microscope and every individual observed was identified, counted and recorded. The species composition and abundance of zooplankton was determined using an inverted microscope at x100 magnification. The zooplankton abundance and species composition was calculated based on the counted individual species and their total numbers per 25 ml sample size.

The counting and species determination for the zooplankton samples collected in 2007 had been made previously by another student (Adewale Ariwo-Olo) in a slightly different manner. The 100 ml cup samples were split into two or three different sample sizes before counting, but counts were combined to treat the 100 ml samples as one main sample. Therefore, the total numbers of individuals counted in 2007 were divided by 12, 8 and 8 (i.e. by the exact number of litres originally collected) for the epilimnion, metalimnion and hypolimnion, respectively, in order to get the total number of zooplankton individuals counted per litre for 2007. For 2008, 25 ml zooplankton samples were counted from the concentrated samples of 100 ml. To obtain the total number of individual zooplankton per litre for 2008, the total number of zooplankton individuals counted in 25 ml samples were therefore divided by 3, 2 and 2 (i.e. by a quarter of the original 12, 8 and 8 litres of zooplankton samples collected) for the epilimnion, metalimnion and hypolimnion, respectively. This approach gave estimated values for the total number of zooplankton individuals counted per litre for the different water depths, months and sampling years. Mean carbon biomasses for rotifer species were obtained using the standard values of carbon content of some freshwater rotifers reported by Telesh et al. (1998). The numbers of individual rotifer species counted per litre were converted to carbon biomass (µgC l^{-1}) by multiplying the mean dry weight of individual rotifer species by the number of individuals counted per litre for each species. Where mean dry weight for a particular species was not available, the mean dry weight of a closely related species was used. For example, the mean dry weights for *Kellicottia longispina* and *Kellicottia bostoniensis* were not available, and thus the mean dry weight for *Keratella ticiniensis* was used.

To convert crustacean zooplankton (copepods, cladocerans, *Holopedium*, *Chaoborus* and *Bosmina*) mean density (inds l^{-1}) to carbon biomass (µgC l^{-1}), the number of individual zooplankters counted per litre was multiplied by half the mean individual dry weight (µg) for each taxon (assuming carbon to be half the dry weight biomass). Individual dry weights for each crustacean zooplankton taxon were obtained when weighing the zooplankton samples for stable isotope analysis (SIA). To obtain the mean individual dry weight per taxon, the total measured dry weight for each taxon was divided by the number of individuals counted for each taxon to be used for SIA. To obtain the total population of zooplankton per m^2 of lake surface area for each water layer, the total number of individuals counted per litre was multiplied by 1000 (volume expressed as m^3) = number inds m^{-3}. Then, we further multiplied the obtained values for number of inds m^{-3} for each water depth by 3, 2 or 2 (i.e. the depth metres of the layer from which samples were collected) for the epilimnion, metalimnion and hypolimnion, respectively, to obtain values for the total population of zooplankton per m^2 of lake water column for each water layer. The sum of the values obtained for the different layers

then gave the total water column population of zooplankton under m^2 of lake surface area (i.e. zooplankton density/biomass in the water column).

2.3. Data collection for Stable isotope analyses

Qualitative samples of crustacean zooplankton for SIA were collected by hauling a net of mesh size 100 µm through the whole water column at several sites around the lake, to avoid possible effects of zooplankton migration and patchiness. The zooplankton collected were subsequently grouped to those >500 µm and those 100-500 µm. *Chaoborus* samples were collected separately with a 100 µm mesh size net at the deepest point (6.5 m) in the lake. Samples were placed in 100 ml jars and then frozen to await sorting. Watch-maker forceps, tin cups, ELISA-plates, a Finn-pipette for water volumes of 30 µl and deionized water were used as materials during the sorting of the samples to be used for SIA. Because of their small individual sizes, the rotifer component of the zooplankton community could not be sorted and analysed for stable isotopes.

Zooplankton samples were thawed and then rinsed several times with tap water at room temperature and the animals were sorted manually, using forceps, from a 100 µm moist net cloth suspended to a Petri-dish under a microscope, into groups or into genus when the amount permitted. Using a Leica L2 microscope, the samples were sorted into cladocerans, copepods, *Holopedium* and *Chaoborus*. The sorted animals were placed into pre-weighed tin cups containing 30 µl of deionised water in Elisa plates kept in a desiccator. The name, size and number of individuals of the sorted species were recorded on a paper sheet having the corresponding locations and weights of empty tin-cups. After the tin cups were filled, they were stored in a separate Elisa-plate for a few hours at room temperature, and then transferred to tin cups in an Elisa-plate in a freezer. The same codes were used in the record sheet, in the "transfer" Elisa plate and in the Elisa plate used for storage in the freezer in order to avoid mixing the tin cups.

During the sorting, three or more replicates were prepared for one sample (when numbers permitted), and the dry weight target for samples in each tin cup was around 0.3 to 0.5 mg. The samples were then stored in a freezer and later dried at 60°C overnight in their Elisa plates. After drying and cooling in a desiccator, tin cups were weighed and wrapped into tight balls excluding air and were analyzed using the isotope ratio mass spectrometer. The zooplankton samples were 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.

Stable isotope ratios are reported using delta notation. This notation compares the ratio of the heavier isotope to the lighter isotope in a sample to that of a standard. Delta (δ) values measure how 'heavy' or 'light' a sample is relative to the standard and are generated from R values for a sample and its standard (where R is the isotopic ratio of interest). Values are positive (heavier or enriched) when the ratio of heavy to light forms of the isotope is greater in the sample than in the standard. Values are negative (lighter or depleted) when the ratio is less than that for the standard. A zero value denotes identical isotope abundance to the standard.

Delta values are defined as follows:

$$\delta^{H}X = \left[\frac{(R_{spl} - R_{std})}{R_{std}}\right] * 1000 = \quad \delta^{H}X = \left[\frac{R_{spl}}{R_{std}} - 1\right] * 1000$$

where, R is the isotopic ratio of interest. The above equation represents the delta (δ) notation for a sample as a function of the isotope ratio (R) for the standard (std) and sample (spl). Isotopic data are therefore reported relative to a standard and because the variations are small, values are multiplied by 1000 and the resulting delta value is therefore in per mill (‰). The accepted international standard for carbon is PeeDee belemnite and for nitrogen is atmospheric N_2.

2.4. Data analysis

Comparisons were made for the zooplankton community structure in terms of its mean monthly, seasonal and annual variations, and the corresponding variations in the zooplankton carbon biomass for each species and taxa were quantified in percentages. Further comparisons were made for: zooplankton and heterotrophic bacteria consumption, zooplankton and phytoplankton consumption, DOC additions and zooplankton mean density, DOC additions and zooplankton carbon biomass, DOC additions and zooplankton volume (inds m^2) and zooplankton biomass and phytoplankton biomass. Variations of zooplankton abundance and species composition were compared with lake variables such as depth, and as monthly, seasonal and annual variations for the different sampling years. The seasons were defined based on the available sample months for each year. For 2007, April, May and June were grouped as spring, July and August were grouped as summer and September and October were grouped as autumn. For 2008, while the summer grouping remained the same, May and June were grouped as spring and September, October and November were grouped as autumn. SIA values for $\delta^{13}C$ and $\delta^{15}N$ of zooplankton were used to compare the variations of these isotopic signatures across the sampled years for the different taxa (cladocerans including *Holopedium*, and copepods). Thus any changes in the zooplankton $\delta^{13}C$ for the different taxa following cane sugar additions could be explored from the SIA results.

3. RESULTS

3.1. Abundance and species composition of the zooplankton community

Zooplankton abundance and species composition varied greatly among the years (2007, 2008 and March of 2009) and between the months, seasons and in the different water depths. A total of 17 zooplankton species were detected from Alinen Mustajärvi for 2007, 2008 and March of 2009, but here only the 12 most abundant species are presented and compared. Generally, the zooplankton community was numerically dominated by rotifers (9 species), with *Keratella ticiniensis* and *Kellicottia bostoniensis* being the most abundant. The highest zooplankton mean densities (inds l^{-1}) were mostly recorded in the metalimnion in 2007, 2008 and in March 2009 (Table 1a and b). Zooplankton species composition indicated no major variations across the years. In 2007, the highest zooplankton mean densities were recorded

Table 1. Zooplankton abundance and species composition for a) 2007 and b) 2008 and March 2009 in lake Alinen Mustajärvi for the different water layers for each sample month. Values are presented as mean numbers of individual species for each sample counted per litre for each water layer and for each month. In 2009 data were available only for March.

Year	Mon.	Layer	K.C	K.Q	K.T	Kk.L	Kk.B	Py	Na	Cy	Ce	Ho	Ds	Ch
a) 2007	April	Epi	0	57	0	5	0	0	0	13	0	0	0	0
		Meta	2	10	60	1	1	0	0	1	0	0	0	0
		Hypo	3	12	16	2	1	0	1	1	0	0	0	0
	May	Epi	1	105	1	45	37	17	1	8	0	0	0	0
		Meta	3	312	854	32	1	0	0	2	0	0	0	0
		Hypo	2	24	65	11	1	0	0	0	0	0	0	1
	June	Epi	0	14	3	118	37	15	0	1	5	5	1	0
		Meta	9	165	1495	54	70	40	2	1	0	2	0	1
		Hypo	1	28	177	15	3	0	0	0	0	0	0	1
	July	Epi	1	1	1	219	2	3	2	1	3	10	8	0
		Meta	2	1	306	107	30	0	18	0	0	2	0	1
		Hypo	3	1	53	42	3	0		0	0	0	0	1
	August	Epi	0	0	0	67	0	0	8	2	7	27	0	1
		Meta	0	0	14	25	8	0	3	0	1	2	0	1
		Hypo	0	0	0	12	0	0	0	0	0	0	0	1
	Sept.	Epi	0	0	0	12	0	0	16	0	5	16	0	0
		Meta	1	0	0	7	8	0	12	0	3	9	0	1
		Hypo	0	0	0	2	1	0	0	0	0	0	0	1
	Oct.	Epi	0	0	1	2	6	0	10	0	1	5	0	0
		Meta	1	1	1	3	13	0	14	0	0	2	0	1
		Hypo	0	1	0	2	9	0	7	0	0	1	0	1

Year	Mon.	Layer	K.C	K.Q	K.T	Kk.L	Kk.B	Py	Na	Cy	Ce	Ho	Ds	Ch
b) 2008	May	Epi	2	85	37	139	13	0	17	51	1	1	1	0
		Meta	1	35	39	125	7	9	11	5	0	0	1	2
		Hypo	1	12	9	34	1	0	3	3	0	0	0	2
	June	Epi	0	4	1	160	147	18	6	4	6	56	3	1
		Meta	2	6	312	54	218	1	9	8	0	1	0	1
		Hypo	2	2	18	22	10	13	1	1	1	2	0	3
	July	Epi	3	1	2	18	3	89	1	3	23	36	10	1
		Meta	4	1	453	7	1407	6	41	5	5	8	5	0
		Hypo	3	1	29	5	41	48	1	1	1	12	1	3
	August	Epi	19	1	43	2	416	378	81	16	30	3	4	1
		Meta	5	0	136	1	668	8	43	2	6	0	1	2
		Hypo	3	0	12	1	44	6	8	1	0	0	0	2
	Sept.	Epi	7	0	84	0	72	5	5	4	6	0	0	1
		Meta	10	0	172	0	95	1	3	3	2	0	0	1
		Hypo	3	0	9	0	18	0	0	1	0	0	0	0
	Oct.	Epi	10	0	256	0	648	0	7	1	0	0	0	0
		Meta	24	1	314	0	707	0	7	1	1	0	0	0
		Hypo	6	0	82	2	156	0	1	1	0	0	0	1
	Nov.	Epi	1	0	214	0	2413	0	0	0	0	0	0	0
		Meta	8	0	211	0	1884	0	1	2	0	0	0	0
		Hypo	12	2	643	0	815	0	8	1	0	0	0	1
2009	March	Epi	7	1	976	0	1477	0	4	10	0	0	0	0
		Meta	9	1	6781	0	619	0	0	2	0	0	0	0
		Hypo	1	0	241	0	56	0	0	1	0	0	0	0

K.C= *Keratella cochlearis*, K.Q= *Keratella quadrata*, K.T= *Keratella ticiniensis*, Kk.L= *Kellicottia longispina*, Kk.B= *Kellicottia bostoniensis*, Py.= *Polyarthra*, Na.= Nauplii, Cy.= Cyclopoids, Ce.= *Ceriodaphnia*, Hol.= *Holopedium*, Ds.= *Diaphanosoma* and Ch.=*Chaoborus*.

in the metalimnion in June and in March 2009, with *Keratella ticiniensis* numerically dominating the zooplankton population; while in 2008, the highest zooplankton mean densities were recorded in the epilimnion in November, with *Kellicottia bostoniensis* dominant (Table 1b). In both 2007 and 2008, zooplankton mean density was also very high in the metalimnion in May and July, with *Keratella ticiniensis* and *Keratella bostoniensis* co-dominating during both months. However, the epilimnion in October 2007 and July 2008 yielded the lowest zooplankton mean densities. Similarly, the metalimnion in October 2007 and May 2008 yielded the lowest zooplankton mean densities (Table 1a and b).

Generally, the lowest zooplankton mean density was recorded in the hypolimnion. In 2007, the hypolimnion in September yielded the lowest zooplankton mean density (Table 1a). For example, in September 2007, *Keratella cochlearis*, *Keratella quadrata*, *Keratella ticiniensis*, *Ceriodaphnia* and *Diaphanosoma* were hardly detected in this water layer (Table 1a). In September 2008, the hypolimnion yielded the lowest zooplankton mean density (Table 1b). Similarly, the hypolimnion in August 2007 and June 2008 also showed very low zooplankton mean densities. Only in July 2008 was the zooplankton population more uniformly distributed through all the water layers, in which the highest abundance occurred for

Table 2. Monthly mean density (inds l^{-1}) for the most abundant zooplankton individual species for 2007, 2008 and March 2009 for lake Alinen Mustajärvi. In 2009 data were available only for March.

Year	Month	K.C	K.Q	K.T	Kk.L	Kk.B	Py	Na	Cy	Ce	Ho	Ds	Ch	Da
2007	April	4	79	77	8	3	0	1	15	0	0	0	0	ND
	May	6	441	920	88	38	17	1	9	0	1	0	1	ND
	June	11	207	1676	186	109	55	2	2	5	7	1	2	ND
	July	5	2	361	369	36	3	20	1	3	12	8	2	ND
	Aug.	0	0	14	105	8	0	11	2	8	29	1	3	ND
	Sept.	0	0	0	22	8	0	27	1	13	25	0	2	ND
	Oct.	1	2	1	7	28	1	31	1	1	8	0	2	ND
2008	May	4	132	85	298	21	3	31	59	1	1	1	1	4
	June	3	12	330	235	374	7	16	12	7	58	3	12	4
	July	10	3	484	30	1450	19	43	9	28	56	16	19	4
	Aug.	27	1	191	4	1127	67	131	18	36	3	5	19	4
	Sept.	19	0	264	0	184	2	8	7	7	0	0	1	1
	Oct.	40	1	651	2	1512	0	14	2	1	0	0	1	1
	Nov.	21	2	1068	0	5112	0	9	3	0	0	0	0	1
2009	March	16	1	7998	0	2152	0	4	13	0	0	0	1	0

ND= Not detected, K.C= *Keratella cochlearis*, K.Q= *Keratella quadrata*, K.T= *Kera*tella *ticiniensis*, Kk.L= *Kellicottia longispina*, Kk.B= *Kellicottia bostoniensis*, Py.= *Polyarthra*, Na.= Nauplii, Cy.= Cyclopoids, Ce.= *Ceriodaphnia*, Hol.= *Holopedium*, Ch.=*Chaoborus*, Ds.= *Diaphanosoma* and Da.= *Daphnia*.

Keratella bostoniensis than in the other sampled months of 2008 and 2007. In the other months of the sample years, one or more species were either poorly detected or in low numerical abundance (Table 1a and b). The most abundant cladocerans in the study lake were *Ceriodaphnia, Diaphanosoma, Holopedium* and *Daphnia* in 2008, while among the copepods, young nauplii and cyclopoids were more abundant than calanoids (Table 2).

Variation in crustacean zooplankton mean densities among months was very high and cladoceran zooplankton were considerably more abundant in 2007 samples than in 2008. Generally, cladocerans were very poorly represented in the samples in both 2007 and 2008, but were widely detected in 2007 than in 2008, although more were recorded in some months 2008 than in 2007 (Table 2). For example, in April, May and to a greater extent June of 2007, very few cladocerans were observed, unlike in June, July and August of 2008. Nevertheless, in September and October of 2007, more cladocerans were recorded compared to September, October and November of 2008, and also March of 2009 (Table 2). However, young *Daphnia* were far more abundant than adults and were detected in 2008 (e.g. in June, July and August), but not detected in 2007 (Table 2). *Bosmina* were also very poorly represented in the 2007 samples, but were more prevalent in 2008 although still at very low mean densities during July and August (data for *Bosmina* not included in Table 2). Copepods and their nauplii were widely recorded in all the samples from both 2007 and 2008, but more were recorded in 2008 (Table 2). *Chaoborus* was widely detected (at very low mean density but representing high carbon biomass) in most of the samples from 2007 and 2008, except for April 2007 and March 2009. Overall, *Chaoborus* mean density increased in 2008 (Table 2).

Generally, the zooplankton species population showed great variations among the months and seasons in each year, but increased progressively across the years. The highest monthly zooplankton mean density (inds l^{-1}) occurred for *Keratella ticiniensis* and *Kellicottia bostoniensis sps*. and increased in November 2008 and in March 2009, respectively, after cane sugar additions (Table 2). Comparatively, rotifer species dominated the zooplankton mean density and species composition in the sample months of both years (Table 2). Although some rotifer species (e.g *Kellicottia longispina, Keratella quadrata and Polyphemus sps.*) decreased in mean density across 2007 and 2008, overall the zooplankton mean densities for August, September, October and November 2008 were substantially higher than those of the same months in 2007 (Table 2). Thus in both years, the zooplankton population (inds l^{-1}) was dominated by the more rapidly reproducing and smaller bodied rotifer species.

The zooplankton populations counted as inds l^{-1} were converted to carbon biomass as µg l^{-1} for each taxon based on the individual mean dry weight and the results are presented below (Table 3). Generally, the mean carbon biomass of zooplankton varied greatly within and among species in different sampled months and years. Cyclopoid copepods and their nauplii jointly dominated the zooplankton mean carbon biomass in both 2007 and 2008, but with an appreciable increase and highest values recorded in most of the sample months of 2008, especially in May and August, after additions of DOC (Table 3). *Keratella ticiniensis* and *Kellicottia bostoniensis*, unlike the other rotifer species detected (e.g. *Keratella cochlearis, Kellicottia longispina, Polyarthra* etc), also showed increased carbon biomass in 2008, with highest values recorded in November 2008 and March 2009 (Table 3), corresponding to the months of their highest mean densities (Table 2).

Ceriodaphnia, *Holopedium*, *Diaphanosoma* and *Daphnia* showed great variations in their carbon biomasses in 2007, 2008 and in March 2009, with, surprisingly, lower mean carbon biomass values in September, October and November 2008 and in March 2009 after additions of DOC (Table 3), corresponding to the months of their lowest mean densities (Table 2). Nevertheless, *Ceriodaphnia* and *Holopedium* showed comparatively higher mean carbon biomass values than all rotifer species in August and July 2008, but with values for these two species higher in most months in 2007 than in 2008 (Table 3).

Table 3: Mean monthly carbon biomass (µgC l^{-1}) for the most abundant zooplankton species for 2007, 2008 and March 2009.

Year	Month	K.C	K.Q	K.T	Kk.L	Kk.B	Py	Na	Cy	Ce	Ho	Ds	Da
2007	April	0.06	4.6	1.3	0.13	0.05	0	5.81	50.3	0	0	0	ND
	May	0.08	25.6	15.6	1.5	0.65	0.63	2.91	37.8	0	0.33	0	ND
	June	0.15	12	28.5	3.17	1.86	1.99	13	8.68	8.07	10.7	1.67	ND
	July	0.08	0.12	6.13	6.27	0.61	0.11	115.5	7.34	5.24	18.7	13.5	ND
	Aug.	0	0	0.24	1.78	0.14	0	64.7	12.3	16.1	59.3	0.43	ND
	Sept.	0	0	0	0.37	0.14	0	94	1.21	13.1	40.5	0	ND
	Oct.	0.01	0.08	0.02	0.12	0.47	0.02	106,1	1.21	1.77	17.4	0	ND
2008	May	0.05	7.7	1.44	5.1	0.4	0.09	192.3	337	0.3	0.15	0.32	0.17
	June	0.04	0.7	5.6	4	6.4	0.25	104.5	69	2.6	20.2	0.81	4.66
	July	0.15	0.08	8.23	0.5	24.7	0.7	238.3	54	21	35.5	9.6	12.8
	Aug.	0.38	0.03	3.24	0.1	19.2	2.4	731.5	126	25.4	2.2	0.36	13.3
	Sept.	0.27	0	4.49	0	3.1	0.05	44.7	55	5.1	0	0	0.7
	Oct.	0.58	0.03	11.07	0.03	25.7	0	77.3	11	0.4	0	0	0.07
	Nov.	0.3	0.15	18.2	0	86.9	0	44	11	0	0	0	0
2009	March	0.23	0.06	136	0	36.6	0	19.6	44	0	0	0	0.07

ND= Not detected, K.C= *Keratella cochlearis*, K.Q= *Keratella quadrata*, K.T= *Keratella ticiniensis*, Kk.L= *Kellicottia longispina*, Kk.B= *Kellicottia bostoniensis*, Py.= *Polyarthra*, Na.= Nauplii, Cy.= Cyclopoids, Ce.= *Ceriodaphnia*, Hol.= *Holopedium*, Ch.=*Chaoborus*, Ds.= *Diaphanosoma* and Da.= *Daphnia*.

Zooplankton abundance and species composition for various taxa (cladocerans, copepods and rotifers) varied greatly within the months, seasons and among the years (2007- year before cane sugar additions, 2008 and March of 2009- years of cane sugar additions). The trends of monthly zooplankton mean density and mean carbon biomass for both 2007 (control year) and 2008 (experimental year) further showed great variations and fluctuations within the sample months and years (Fig. 3a and b). Monthly zooplankton mean density increased appreciably in the later sample months (October and November) in 2008 than in 2007 (Fig. 3a) and the mean carbon biomass was also higher in most of the sample months (May and August) in 2008 than 2007 (Fig. 3b). The monthly proportion (%) of mean density and mean carbon biomass for the various zooplankton taxa targeted also showed great variations within the sample months and years and from one taxon to another and these is illustrated below (Figs. 4 and 5).

Generally, large-bodied copepods that were never numerically abundant (Fig. 4) dominated the zooplankton mean carbon biomass for most of the sampled months in both years (Fig. 5). However, the relative carbon biomasses for copepod zooplankton were higher in most of the sample months in 2008, with highest proportions in May (97 %), August (93 %) and September (88 %) than in the same months in 2007, except for October (Fig. 5).

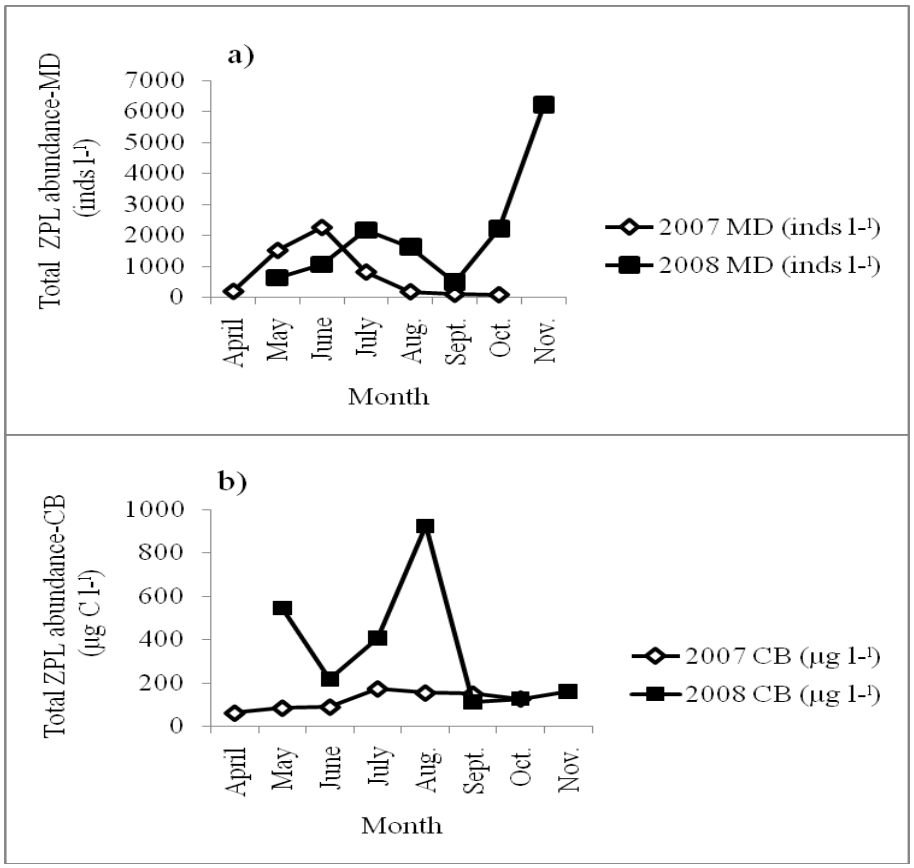

Figure 3. Monthly total zooplankton a) mean density (MD) and b) mean carbon biomass (CB) for 2007 and 2008.

Cladocerans were neither numerically abundant, except for September 2007 (Fig. 4), nor dominant in mean carbon biomass in both 2007 and 2008, but they did constitute an appreciable proportion of the total zooplankton mean carbon biomass in the August (49 %) and September (36 %) of 2007, with comparatively lower proportions throughout 2008 (Fig. 5). Rotifers that were numerically most abundant throughout the sample months in both years, except in September 2007 (Fig. 4), constituted comparatively lower proportions of the zooplankton mean carbon biomass in most of the sample months, except in May (52 %) and June (53 %) 2007, November (66 %) 2008 and 73 % in March (data for March 2009 not included in the figure) 2009 (Fig. 5). In accordance therefore, both the proportion (%) of mean density and that of mean carbon biomass for cladocerans fluctuated considerably in 2007 and 2008, with a decrease detected in 2008 (Figs. 4 and 5).

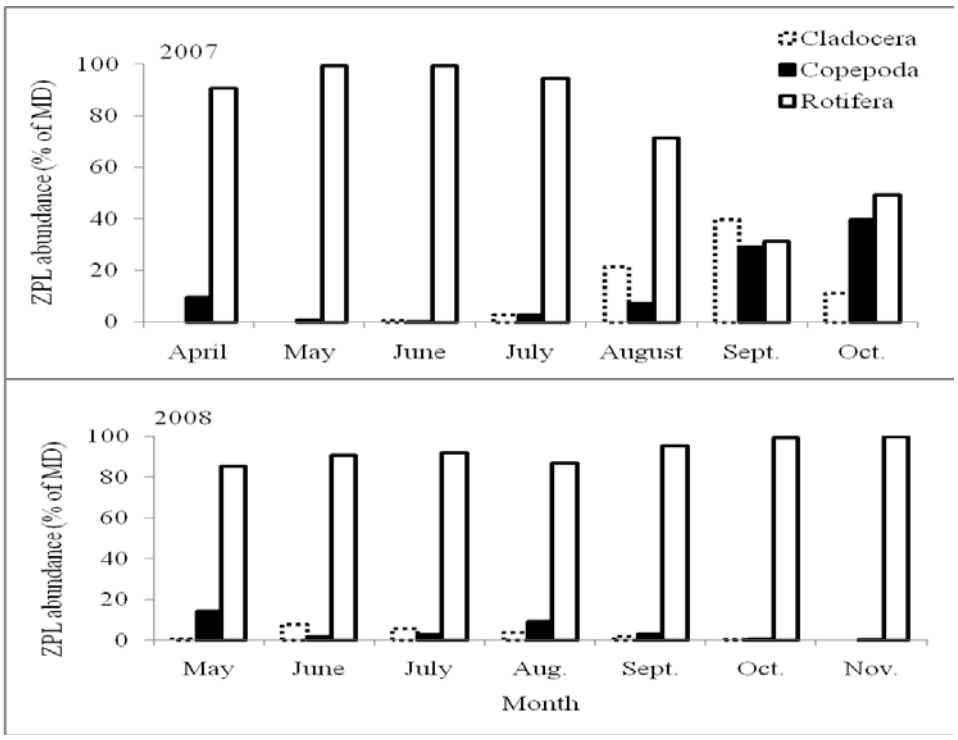

Figure 4. Monthly proportions (%) of mean density of zooplankton for 2007 and 2008 for cladoceran, copepod and rotifer zooplankton.

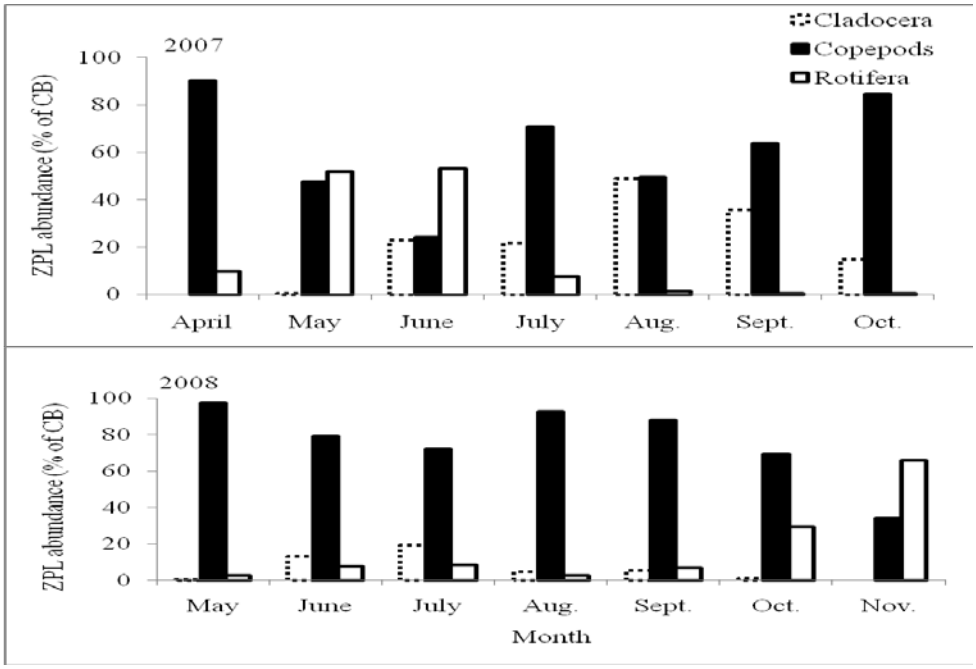

Figure 5. Monthly proportions (%) of mean carbon biomass of zooplankton for 2007 and 2008 for cladoceran, copepod and rotifer zooplankton.

The proportion of mean carbon biomass for copepods, although relatively higher, fluctuated in 2007, but increased appreciably and remained high in 2008, though finally decreasing in the later months (Fig. 5).

The proportion of mean density for rotifers for most of the sample months in 2007 was high and showed minor fluctuations, but increased and remained steadily high in the later months in 2008 and March 2009 (Fig. 4). The proportion of mean carbon biomass for rotifers indicated a progressive increase in 2008 and March 2009 compared to the later sample months of 2007 (Fig. 4).

Generally, copepods and their nauplii dominated the zooplankton biomass in all the sample seasons of both years, and particularly in the spring (May and June) and summer (July and August) of 2008 than in same seasons in 2007 (Fig. 6a and b). Rotifer species dominated the zooplankton community in all the sample seasons in terms of mean density (Fig. 6a) but, not in terms of mean carbon biomass (Fig. 6b), with very little variations. While cladocerans and copepods showed variations within the sample seasons, copepods consistently registered the highest zooplankton carbon biomass in all the sample seasons, with only slight variations (Fig. 6b).

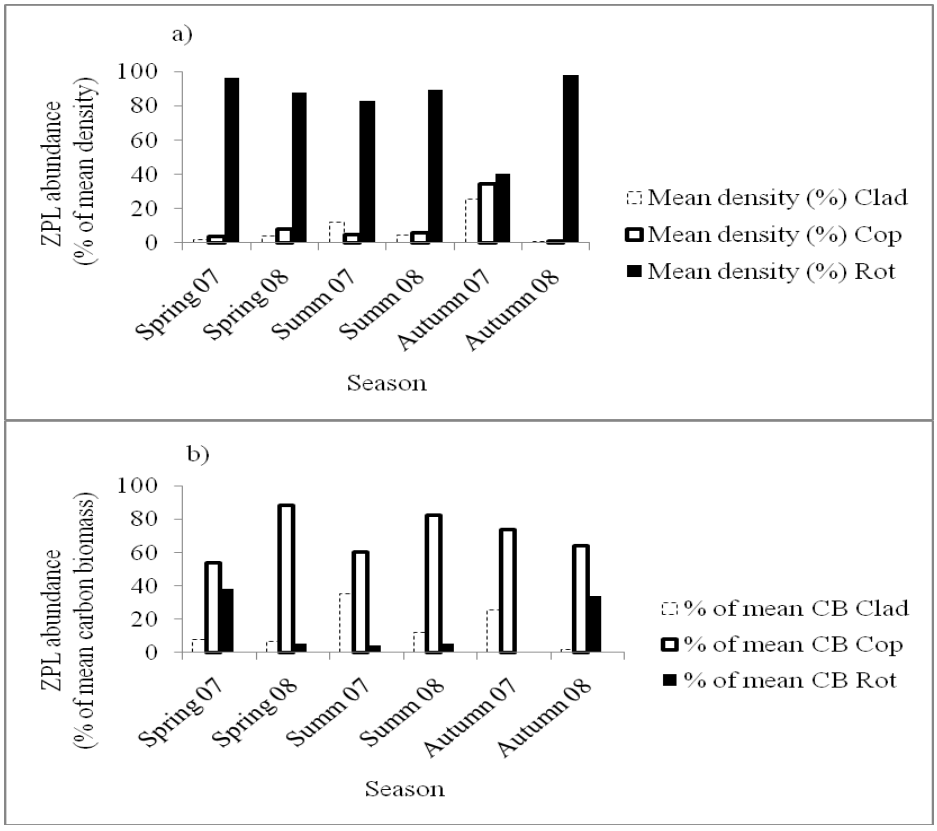

Figure 6. Seasonal proportions (%) of zooplankton a) mean density (MD) and b) mean carbon biomass (CB) for 2007 and 2008 for cladoceran, copepod and rotifer zooplankton.

Cladoceran carbon biomass was considerable higher in summer 2007 (35 %) than in summer 2008 (12 %) and also higher (26 %) in autumn (September and October) 2007 than in autumn 2008 (2 %) (Fig. 6b). The carbon biomass for rotifer zooplankton was higher in spring 2007 (38 %) than in spring 2008 (5 %), but higher in autumn 2008 (34 %) than in autumn (1 %) 2007, while almost level in the summer of both years (Fig. 6b).

3.2. Stable isotope analyses

Crustacean zooplankton $\delta^{13}C$ varied within and among the months and years, and generally increased progressively across the years from 2007 to 2009, especially for cladocerans and *Holopedium* (Fig. 7). Zooplankton generally had lowest $\delta^{13}C$ values in 2007 (control year), especially in April (-44 ‰) and May (-38 ‰) for copepods and in October for cladocerans (-38 ‰) and *Holopedium* (-39 ‰) (Fig. 7).

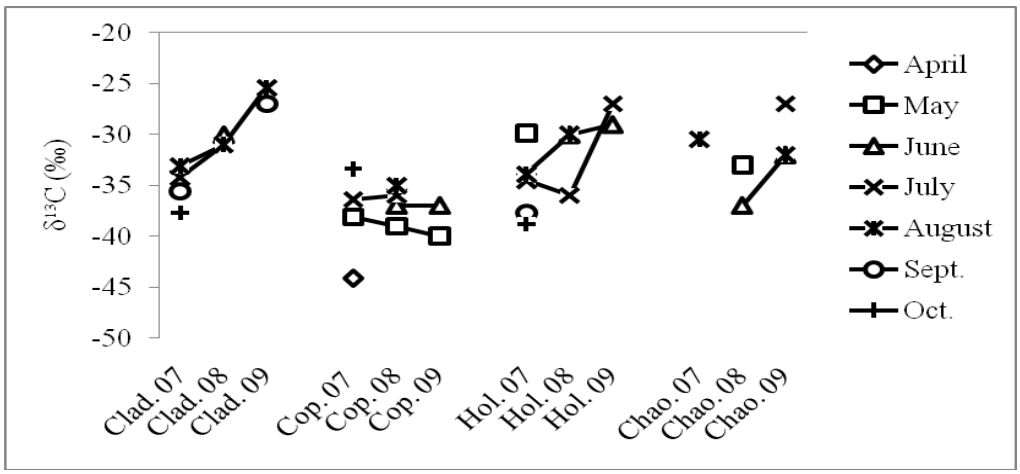

Figure 7. SIA results for mean zooplankton $\delta^{13}C$ for cladoceran, copepod, *Holopedium* and predatory *Chaoborus* for 2007, 2008 and 2009. Data were not available for all the sample months of every year.

The highest $\delta^{13}C$ value in 2007 was recorded in May for *Holopedium* (-30 ‰). Generally, with increased loading of DOC in 2008 and 2009, values for $\delta^{13}C$ showed more enrichment and cladocerans (including *Holopedium*) recorded the highest $\delta^{13}C$ values, strongly indicating an increase from 2007 to 2009, but less so for copepods, whose $\delta^{13}C$ values appear rather constant between years (Fig. 7). Nevertheless, in 2009, copepods recorded an appreciably higher $\delta^{13}C$ values in May (-40 ‰) and June (-37 ‰), compared to -45 ‰ in April 2007. Overall, zooplankton $\delta^{13}C$ values were generally highest in 2009 (second year of cane sugar additions), and cladocerans (including *Holopedium*) showed a progressive increase (highest enrichment), from 2007 to 2009, particularly in August (-33 to -25 ‰) and September (-36 to -27 ‰), respectively (Fig. 7).

Values for $\delta^{15}N$ from SIA results although showing great seasonal fluctuations, did not show any clear pattern of change between the years (Fig. 8a, b and c). However, since additions of cane sugar do not directly alter nitrogen, the between year consistency in $\delta^{15}N$ values is not surprising. Values were generally lowest in the months of spring and autumn and

highest in the months of summer. The few available $\delta^{15}N$ values for the predatory *Chaoborus* were several ‰ higher than other taxa, reflecting its higher trophic level (Fig. 8a and c).

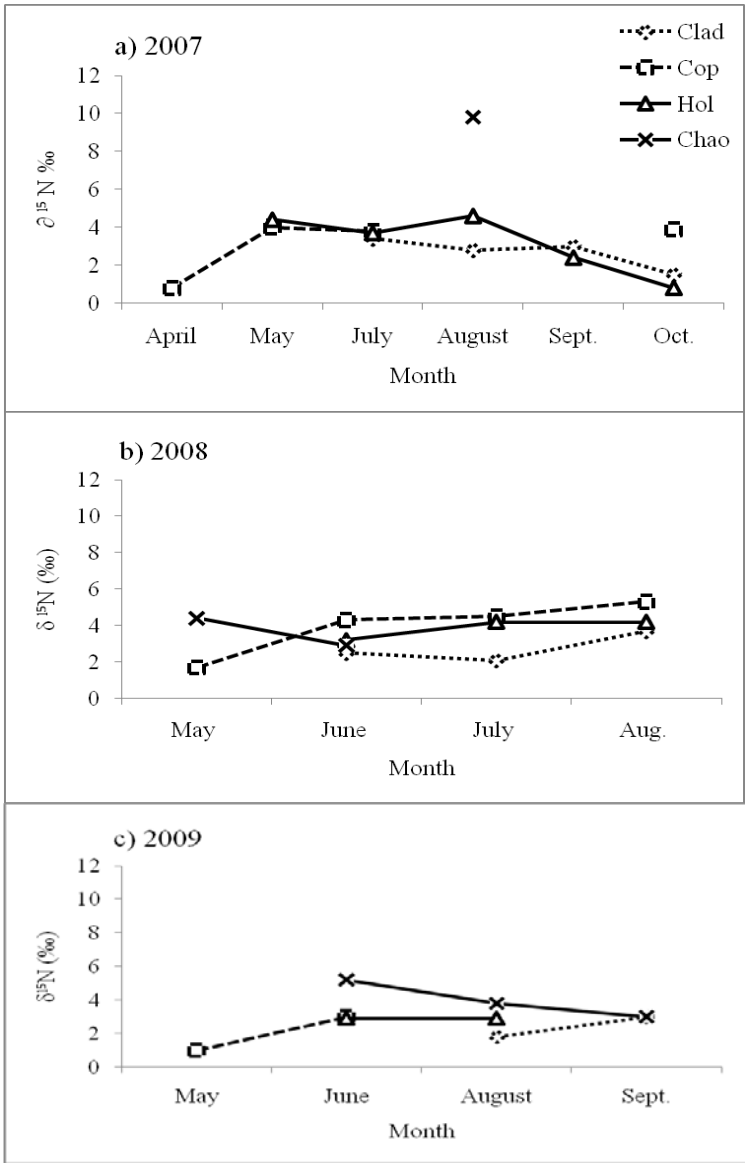

Figure 8. SIA results for mean zooplankton $\delta^{15}N$ for Cladoceran, Copepod, *Holopedium* and predatory *Chaoborus* for a) 2007, b) 2008 and c) 2009. Data were not available for all sample months of every year.

4. DISCUSSION

4.1. Previous estimates of the proportions of zooplankton diet components in small boreal lakes

Kankaala et al. (2010) used stable carbon and nitrogen isotope analyses to estimate the relative proportions of three putative food sources in the diets of crustacean zooplankton in five small boreal lakes in southern Finland, including Alinen Mustajärvi, representing a gradient of DOC concentration from ca. 5 to 40 mg C l^{-1}. The three putative food sources were: 1) algae; 2) allochthonous organic matter (AlloOM) (but including also heterotrophic bacteria (HB) and green sulphur bacteria (GSB), having similar isotopic values), and 3) methane-oxidising bacteria (MOB). The five lakes were sampled in May 2006, after ice-melt and establishment of stratification, and again in October 2006 during autumnal mixing of the water column. They used the IsoSource mixing model (Phillips & Gregg 2003) to estimate minimum-maximum ranges of the different food types. The results obtained by Kankaala et al. (2010) from Alinen Mustajärvi are summarised in Table 4. Their results provide a useful basis for discussion of the results from the present study both in terms of Alinen Mustajärvi and also in relation to similar nearby lakes with different DOC concentrations. This may help to provide insights into the possible effects of changing the DOC concentration in Alinen Mustajärvi by the additions of cane sugar in 2008 and 2009, particularly since the changes observed in Alinen Mustajärvi when sugar was added may include some year-to-year variation that was unrelated to the sugar addition.

Table 4. IsoSource model results showing the proportions (min-max range in %) of three putative food sources: algae, AlloOM together with HB and GSB, and MOB in the diets of cladoceran and copepod zooplankton in Alinen Mustajärvi in May and October 2006.

		Iso-Source model (%)		
Taxa	Month	Algae	AlloOM+HB+GSB	MOB
Cladocera	May	14-39	60-80	1-6
	October	17-26	40-47	34-36
Copepoda	May	45-66	9-31	24-25
	October	61-69	16-23	15-16

Data from Kankaala et al. (2010)

4.2. Response of zooplankton community to sugar addition

Further studies on the impact of DOC in pelagic food webs has also been studied in a whole lake experiment in a clear water lake in Northern Sweden by adding white sugar (Sucrose). The study showed a significant increase in bacterial biomass with a decrease in autotrophic phytoplankton biomass due to additions of DOC, suggesting that it is the carbon component of humic material and its utilization by bacterioplankton that determines the structure and function of the pelagic food web in humic lakes (Blomqvist et al. 2001). Similarly, 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 and 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.

Lake Alinen Mustajärvi is more heterotrophic than autotrophic, meaning that bacterial biomass including AlloOM dominates the lake energy balance at the expense of phytoplankton. This is so because increased loading of DOC in the study lake provides more substrate for pelagic heterotrophic bacteria and they might be expected to use more available inorganic nutrients at the expense of phytoplankton. However, it might be argued that in general the impact of increased loading of DOC on lake food webs could be more striking in clear water lakes than in humic lakes, since humic lakes already contain very high concentration of DOC. Clear water lakes usually have lower DOC concentration, lower bacterial biomass and also higher phytoplankton biomass than humic lakes, thus additions of sugar can provide more striking impacts and results on the zooplankton community in clear water lakes than in humic lakes. This is consistent with the results of Kankaala et al. (2010). They reported that mean bacterial biomass in the whole water column of the three clearest lakes (with the lowest DOC concentration) was less than 30 % of that of phytoplankton, but about the same or higher in the two most humic lakes with the highest DOC concentration. Nutrient enrichment may also decrease allochthony of zooplankton by stimulating autochthonous production by phytoplankton (Carpenter et al. 2005). It is worth noting that, even if cane sugar was not added in lake Alinen Mustajärvi, changes might have still occurred in the lake between the sample years (since lakes are not constant but dynamic systems), thus it is not fully certain whether the observed changes and/or responses of zooplankton in the study lake could be wholly due to additions of cane sugar. Nevertheless, it was expected that additions of sugar should have provided more substrate for pelagic bacteria and consequently, an alternative food source for zooplankton in the lake, hence providing even more impressive and striking results than if cane sugar was not added in these years.

In this study, additions of cane sugar appeared to have an appreciable impact on the mean densities and carbon biomasses of zooplankton taxa. The mean density of rotifer species increased tremendously with cane sugar additions and was constantly high in most of the sample months in 2008 and March 2009 (but not with a corresponding increase in their carbon biomass). Unlike rotifers, it was the carbon biomass of copepods and their nauplii that increased following cane sugar additions, but also not with a corresponding increase in their mean density. This is true because small bodied rotifers are well known to increase exponentially in number, especially if nutrient addition is persistent, but usually may contain low organic carbon content because of their very small size, compared to larger body and more predatory crustacean zooplanktons such as cladocerans and copepods.

However, in this study, the mean density and mean carbon biomass for cladocerans fluctuated considerably in 2007 and 2008, with a decrease detected in 2008 with cane sugar additions, unlike with copepods. This could be explained by the fact that, cladocerans may be facing higher predation pressure from *Chaoborus* (considering the paucity of fish in small humic lakes) and this may account for their very low recorded mean density and mean carbon biomass despite additions of cane sugar. Cladocerans generally have poor predator escaping or migrating capability since they most often move in a series of hops, compared to jerky-moving

copepods (Pennak 1978), which may be more capable of escaping predation, thus making copepods more abundant than cladocerans, both in terms of mean density and carbon biomass.

Furthermore, in this study, more individual rotifer species than crustacean zooplankton species were recorded between the years, but overall, additions of DOC did not clearly increase the zooplankton species composition in the study lake, except that more young *Daphnia* species could only be detected in the manipulation years.

The results for the carbon biomass of rotifer species in this study are consistent with those reported by Telesh et al. 1998 from a survey of the carbon biomass of some freshwater rotifers from a number of freshwater bodies of different types in Russia and Finland. They established body length/carbon mass and body volume/carbon mass regressions to study the rotifers and to express numerically general relationships between carbon content and length/volume/wet weight of rotifers. They reported that carbon content of rotifers (just as with crustacean zooplanktons) was positively correlated with their size length and body volume. Therefore, zooplankton in the study lake appeared to respond to increased loading of DOC in the form of cane sugar by an appreciable increase in their mean density and mean carbon biomass, especially for rotifer and copepods, respectively, but without a corresponding increase in terms of zooplankton species composition.

In this regard, if one considers that the highest zooplankton carbon biomass was recorded in May (early spring) and August (late summer) following cane sugar additions in 2008, then one could presume that zooplankton in the study lake were not only deriving DOC from available bacteria and AlloOM. Certainly, they also benefited from the higher phytoplankton abundance typical in these months, unlike in the later sample months of September and October when phytoplankton abundance is usually lower. Thus, changing environmental conditions and species-specific seasonal changes (e.g. in body composition, growth and abundance) could also explain the variations in the carbon biomass (dry weight/mean density) within and between species/taxa and between the sample months and seasons.

4.3. Stable isotope evidence

Crustacean zooplankton $\delta^{13}C$ values showed great variation within and among the months and years, and increased progressively across the years from 2007 to 2009, especially for cladocerans (including *Holopedium),* but $\delta^{13}C$ values for copepods appeared to be rather constant between the years, despite additions of cane sugar. Essentially, additions of cane sugar was intended to raise labile DOC concentration from around the first quartile level for boreal lakes to that in lakes around the third quartile level (Henriksen et al. 1998). Thus, in this study, we expected cane sugar additions with its higher $\delta^{13}C$ to increase $\delta^{13}C$ of especially crustacean zooplankton (cladocerans and copepods). However, additions of cane sugar did not clearly increase $\delta^{13}C$ in copepods ($\delta^{13}C$ values for copepods were rather constant between years), but did so most appreciably for cladocerans (including *Holopedium),* whose $\delta^{13}C$ values increased progressively across years. This might be because copepods are known to be less efficient at grazing free-living bacteria. Therefore, in my study lake, cladoceran zooplankton appeared to show increasing dependence on the heterotrophic food web based on

bacterial production with increased loading of DOC in the lake, especially as shown by the progressively more enriched $\delta^{13}C$ values for cladocerans (including *Holopedium*) across the years; though less so for copepods. Also, if it was possible to sort rotifers for SIA, their $\delta^{13}C$ values would have surely showed increased enrichment, considering their increased mean density and carbon biomass following cane sugar additions, since many rotifers also graze on bacteria.

The rather constant $\delta^{13}C$ values for copepods probably reflects that, unlike cladocerans, copepods are well known to derive a greater proportion of their carbon not directly from assimilating bacteria (which are increasingly important diet components for zooplankton in the study lake), but from photosynthetic algae or from protozoan (including mixotrophic phytoplankton) that have consumed bacteria and hence utilised DOC only indirectly. Thus, the amount of carbon derived directly from bacteria (including AlloOM) by copepods is usually lower than that derived indirectly from algae; this could have had a negative impact on the $\delta^{13}C$ enrichment of copepods. Kankaala et al. (2010) reported that cladoceran zooplankton in Alinen Mustajärvi derived 60-80 % of their carbon directing from allochthonous sources (including HB and GSB), while copepods derived just 9-31 % of their carbon directly from the same sources in May. Still in May, however, copepods derived 45-66 % of their carbon indirectly from autochthonous sources and up to 60-70 % in October, for example (Table 4).

Hence, the relatively constant $\delta^{13}C$ values for copepods could be explained by the fact that copepods are generally known to be less efficient than cladocerans at grazing free-living bacteria and cladocerans also ingest particles of bacterial size presumably using a feeding mechanism other than filter feeding (Hessen 1985). This implies that DOC through bacteria could be seen as a less important diet for copepods in the study lake. Thus, additions of cane sugar showed higher impact on the $\delta^{13}C$ values of cladocerans (more enriched $\delta^{13}C$ values) which feed more directly and efficiently on bacteria carbon, than on those in copepods. Therefore, though not directly measured in this study, cladocerans could depend more directly for their diet on AlloOM sources including HB and GSB (60-80 %), while copepods could depend more indirectly on autochthonous sources (60-70 %), as observed in May and October, respectively (Kankaala et al. 2010).

The Iso Source model used by Kankaala et al. (2010) in their study of small boreal lakes gave further insights into the sources of copepod diet component in such lakes. They suggested that bacteria attached to flocculated allochthonous particulate organic matter and/or transfer via mixotrophic algae could be important routes of bacterial carbon and even nitrogen to copepods in their small lakes including Alinen Mustajärvi. Hence, the bulk of the algae biomass could be considered more favoured diet for copepods but, presumably less so for cladocerans zooplankton who might prefer small cryptomonads and soft bodied flagellates.

The results of this study suggest firmly that the increased loading of DOC in the form of cane sugar was efficiently assimilated by heterotrophic bacteria and thus transferred appreciably to lake zooplankton. This is consistent with the findings of Carpenter et al. (2005) in their experimental study of whole-lake additions of DI ^{13}C to measure lake allochthony. They reported that in all their experiments, additions of DI ^{13}C were transferred throughout the food web. Labelled carbon was reported to appear in bacteria, accumulated in zooplankton and

transferred to *Chaoborus* and fish shortly after the initiation of ^{13}C additions. However, this result seems not to be consistent with that reported by Cole et al. (2002) in their whole-lake DI ^{13}C addition to trace the pathway of organic carbon utilisation in small lakes. Instead they found very low bacteria assimilation of DOC and very little transfer of the added DOC by bacteria to higher trophic levels under conditions of their experiment. Zooplankton in their study lake predominantly derived their carbon from the current autochthonous carbon sources.

It must be mentioned here that in Alinen Mustajärvi, the phytoplankton community did not show any clear increase with increased loading of DOC; thus the current phytoplankton biomass may not have been enough to support the increased zooplankton mean density and/or even provide enough energy to higher trophic levels within the manipulation years, suggesting increased dependence of zooplankton on heterotrophic bacteria in the study lake. Hence, it is evident that in the study lake, zooplankton needed other food sources (heterotrophic bacteria) especially in autumn when phytoplankton biomass is known to be generally lower compared to late spring and summer. This is consistent with the results of (Taipale 2008, Kankaala et al. 2010). Although the phytoplankton biomass could affect the stable isotope signature of zooplankton (this could be seen for copepods), the $\delta^{13}C$ values for cladocerans in this study generally increased progressively across the years with increased loading of DOC in the form of cane sugar.

In my study, copepods were seen to have higher $\delta^{15}N$ values than cladocerans in the manipulation years (2008 and 2009), suggesting that copepods were at a higher trophic position than cladocerans. This result is also consistent with that of Kankaala et al. (2010). They reported that the more enriched $\delta^{15}N$ values of copepods compared to those in cladocerans in their study lakes suggest that copepods were at a higher trophic position and that bacterial contribution to their diet probably originated indirectly from protozoa grazing on bacteria. This is also consistent with the experimental results of Karlson et al. (2007) and field results of Mathews and Mazumder (2008). Although there is not yet firm evidence for this, Kankaala et al. (2010) further suggested that the more enriched $\delta^{15}N$ values of copepods compared with those in cladocerans could as well be as a result of a greater systematic isotopic fractionation of $\delta^{15}N$ by copepods.

The traditional concept is that the higher the $\delta^{15}N$ value of a consumer, the higher its trophic position, a widely accepted figure is an increase in $\delta^{15}N$ by 3.4 ‰ per trophic level (Post 2002, Fry 2006). 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 unlikely for the study lake, where baseline $\delta^{15}N$ values could be expected to be rather stable over the period of the study. Nevertheless, in Alinen Mustajärvi values for $\delta^{15}N$ in the control year (2007) suggested a similar trophic position for cladocerans and copepods, while the predatory invertebrate *Chaoborus* was at a higher trophic position with the highest (more enriched) $\delta^{15}N$ value in August 2007 (Fig. 8a).

However, in the manipulation years (2008 and 2009), copepods had somewhat higher values for $\delta^{15}N$ than cladocerans, suggesting a different trophic position, especially in most of

the sample months in 2008 (Fig. 8b). This may reflect copepod dependence on protozoans in the microbial loop, with more trophic steps from DOC to copepods than for cladocerans grazing more directly on bacteria. *Chaoborus* could also have exerted higher predation pressure on cladocerans seen to be at lower trophic position than on copepods, and thus affected the mean density and carbon biomass of cladocerans more than copepods across the sample years. Hence, *Chaoborus* had the highest $\delta^{15}N$ values across the years, especially in August 2007, May 2008 and June 2009 with the highest trophic position (Fig. 8a, b, and c).

5. CONCLUSIONS

In this study, zooplankton appeared to respond to increased loading of DOC by changes in their mean densities, carbon biomasses and $\delta^{13}C$ (of crustacean zooplankton), though with slight variations. Specifically, the zooplankton community in Alinen Mustajärvi responded to additions of DOC in 2008 and 2009 by an appreciable increase in the mean density of rotifer species (but not in terms of their carbon biomasses), with two species (*Keratella ticiniensis* and *Kellicottia bostoniensis*) co-dominating. Copepods and their nauplii dominated the zooplankton carbon biomass in most of the sample months in 2008 (although they were hardly numerically abundant), while both the mean density and carbon biomass of cladocerans showed a decrease. Nevertheless, values for $\delta^{13}C$ increased appreciably and progressively across the years (from 2007-2009), especially for cladocerans (including *Holopedium*), but less so for copepods. This suggests strongly that DOC from the cane sugar additions is being transferred appreciably to zooplankton by heterotrophic bacteria in lake Alinen Mustajärvi (also see Kankaala et al. 2010), and zooplankton dependence on heterotrophic bacteria appeared to increase with increased loading of DOC in the study lake, confirming that humic lakes are net heterotrophic systems and important CO_2 sources. This study provides further evidence and support for the view that a greater proportion of the carbon fixed and/or added to the lake from terrestrial sources is not just emitted to the atmosphere but, also greatly consumed by zooplankton through pelagic bacteria of various types, especially heterotrophic bacteria in this study lake. This confirms that allochthonous DOC forms a particularly important subsidy to the diets of zooplankton in small humic lakes such as Alinen Mustajärvi.

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