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# Seasonal Feeding Strategies of Subarctic Zooplankton



JYVÄSKYLÄN YLIOPISTO

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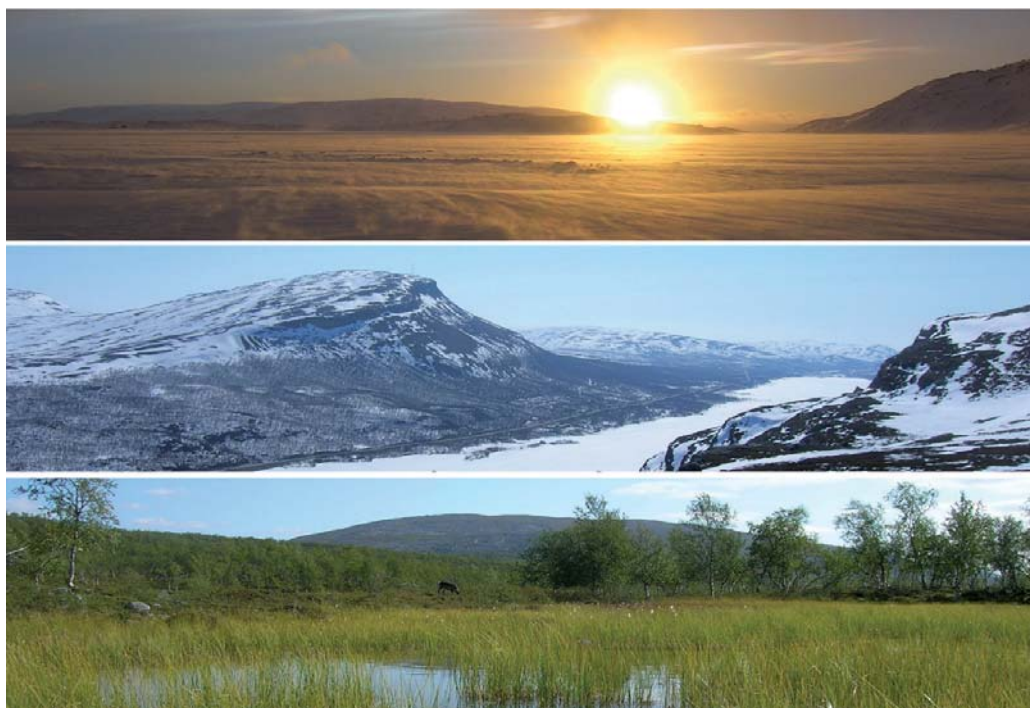
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# ABSTRACT

High-latitude lakes exhibit some of the largest seasonal variation in environmental conditions of aquatic systems globally. The acute difference between the extended dark winters and short cold summers at high-latitudes impose distinct constraints on food availability and consumer survival. This thesis focuses on the extent to which different sources of carbon, from the pelagic zone, the benthic zone, or the terrestrial catchment, contribute to zooplankton diets in the subarctic. This includes a comparison of the different strategies zooplankton use to adapt to arctic winters when carbon sources other than those derived from photosynthetic production are available. Both stable isotopes and fatty acids analysis were used to quantify the contribution of the potential carbon sources and link the physiology of storage lipids to explain how zooplankton can mitigate seasonal food shortages.

The seasonal variation of food sources can be directly related to ice cover and spring melt. Spring melt brings an influx of terrestrial dissolved organic matter into the lake, then in summer phytoplankton and other autotrophs are most abundant in the water column. These seasonal variations in putative food sources are mirrored in the zooplankton carbon stable isotope signatures. The stable isotope results show how zooplankton reliance on terrestrial-derived carbon varies seasonally and between species. Similarly, the fatty acid composition of zooplankton had distinct seasonal and species-specific differences. Zooplankton depend on these storage lipids to buffer winter food shortages. During summer, benthic algae provides all essential fatty acids, and can be an important nutritional subsidy to zooplankton in tundra ponds.

Understanding the strategies and mechanisms that aquatic organisms use to cope with a changing environment on an annual scale may provide indications as to how they will manage in a changing climate to a broader extent. This thesis therefore provides valuable insight into the potential resilience of high-latitude freshwater habitats in a changing climate.

Keywords: Arctic; benthic-pelagic coupling; *Daphnia;* fatty acids; food web; freshwater; stable isotopes.

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# CONTENTS

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## LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-IV.

I made significant contributions to the planning and direction of all the studies. I collected and analysised the data. I wrote all papers where I am first author; as a second author, I made significant contributions towards collecting samples, data analysis, writing and revisions. All papers were finished together with the co-authors.

I    Mariash H., Cazzanelli M., Kainz M., Rautio M. 2011. Food sources and lipid retention of zooplankton in subarctic ponds. *Freshwater Biology* 56: 1851–1862.

II   Rautio M., Mariash H., Forsström L. 2011. Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. *Limnology & Oceanography.* 56:1513–1524

III  Mariash H., Devlin S., Forsström L., Jones RI., Rautio M. 2012. Benthic mats subsidize pelagic consumers in tundra pond food webs. Submitted manuscript.

IV   Mariash H., Kainz M., & Rautio M. 2012. Storage lipids are key to winter life history strategies of *Daphnia* sp. Manuscript.

## ABBREVIATIONS

| | |
|---|---|
| DIC | dissolved inorganic carbon |
| DOC | dissolved organic carbon |
| p-DOC | phytoplankton-derived dissolved organic carbon |
| EPA | eicosapentaenoic acid, 20:5$\omega$3 |
| FA | fatty acids |
| FAME | fatty acid methyl esters |
| HNF | heterotrophic nanoflagellates |
| POM | particulate organic matter |
| PUFA | polyunsaturated fatty acids |
| MUFA | monounsaturated fatty acids |
| SAFA | saturated fatty acids |
| SDA | stearidonic acid, 18:4$\omega$3 |
| SIA | stable isotope analysis |
| SUVA | specific ultraviolet radiation absorption |
| t-DOC | terrestrial-derived dissolved organic carbon |

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# 1 INTRODUCTION

## 1.1 Functioning of aquatic ecosystems

An ecosystem includes all physical and living components within a system (Lindeman 1942). Lakes provide useful ecosystem models because of their relatively discrete basin boundaries. The living components within lakes are governed by a variety of physical components, from temperature to topography. The prevalence of physical factors such as light, altitude, area geology, water mixing regime, temperature, evaporation etc. can influence biological processes, like rate of photosynthesis, species composition, respiration rates, and the organic-inorganic nutritive cycle. Collectively these biological processes are used to describe how aquatic ecosystems function.

A particular aspect of ecosystem function describes how energy is transferred through the food web, from autotrophs at the base of the food web that mobilize their own energy, through to top consumers who gain their energy through other heterotrophs. The different pathways by which energy moves within the system is the basis of trophic dynamics (Lindeman 1942, Karlsson 2005). Carbon, the basic element from which all organic matter is made, is commonly used to track the flow of energy through the ecosystem. Using carbon enables a quantifiable unit to compare energy transfer among ecosystem components, along both temporal and spatial scales.

According to the carbon budget of a typical shallow arctic lake, the main sources of carbon are from the benthic community (50 %), terrestrial carbon (30 %), and phytoplankton (20 %)(Ramlal 1994). These three sources represent the primary basal carbon pool available to secondary production, such as heterotrophic bacteria, protists and larger zooplankton (Hessen & Tranvik 1988, Hessen 1992, Pace et al. 2007, Solomon et al. 2011)(Fig. 1).

Phytoplankton carbon is mobilised via photosynthesis and includes both living cells and degraded planktonic organisms. Phytoplankton-derived dissolved organic carbon (p-DOC) is regarded as the foundation for pelagic secondary production (Wetzel 2001). Zooplankton may gain p-DOC by feeding

directly on phytoplankton or by feeding on bacteria that have utilised phytoplankton production (Fig. 1). Alternatively, zooplankton production may be derived from terrestrial carbon (t-DOC) either by direct feeding on detrital particles or via bacterial-mediated uptake of t-DOC (Karlsson et al. 2003). Terrestrial carbon is residual carbon that is mostly recalcitrant and thus less efficiently used by bacteria and zooplankton than the much more labile carbon derived from phytoplankton (Wetzel 1995). Further, t-DOC lacks the essential fatty acids required by zooplankton (Brett et al. 2009a), and is poor in phosphorus and nitrogen (Hessen 1992). Therefore the material recycled through microbial pathways, such as bacteria, is an important link converting t-DOC into a particulate form available to zooplankton (Wetzel 1995, Jansson et al. 2007, Taipale 2007).

The third source of carbon can be either heterotrophic or autotrophic mobilized carbon derived from the benthic production (Fig. 1). The same bacterial-mediated pathway of t-DOC and p-DOC as described above, is also relevant for the communities at the sediment surface boundary layer. As the benthic communities represent a spatially and functionally different carbon source for pelagic consumers, the extent and relative importance of benthic-derived carbon promoting pelagic secondary production is important for our understanding of lake trophic dynamics.

A complete understanding of the carbon inputs into a lake is highly dependent on all seasons. These annual physical characteristics intimately connect lakes and their landscapes. Seasonal conditions such as ice cover can affect within-lake processes as well as the external surroundings. Lake ice cover has a significant role in energy and water-balance and directs local climate factors, for example surface air temperatures, precipitation, evaporation, and low-level cloud cover (Prowse et al. 2012). The duration of ice cover determines the length of the open-water growing season, while the changes in ice composition can alter absorption characteristics limiting the rate of primary production (Walsh et al. 2005). The hydrology associated with spring melt transports much of the t-DOC into lakes. As such the annual ice cover period is a critical denominator underlying lake metabolism; ice cover sets the light environment, the timing of carbon inputs, and the subsequent phytoplankton and zooplankton emergence in the lake. It is these seasonal changes in physical and biological process that control the functioning of aquatic ecosystems.

This study focuses on seasonal variation that encompasses many coalescent environmental factors on the annual scale. The annual scale is the only ecologically meaningful scale to investigate food web dynamics and has the advantage of being globally consistent (Wetzel 1995). By taking a broad geographical range and monitoring several "typical" water bodies across latitude, altitude, catchment areas, lake size and depth, and among all seasons, this thesis provides a broader knowledge of northern freshwater ecosystem functioning.
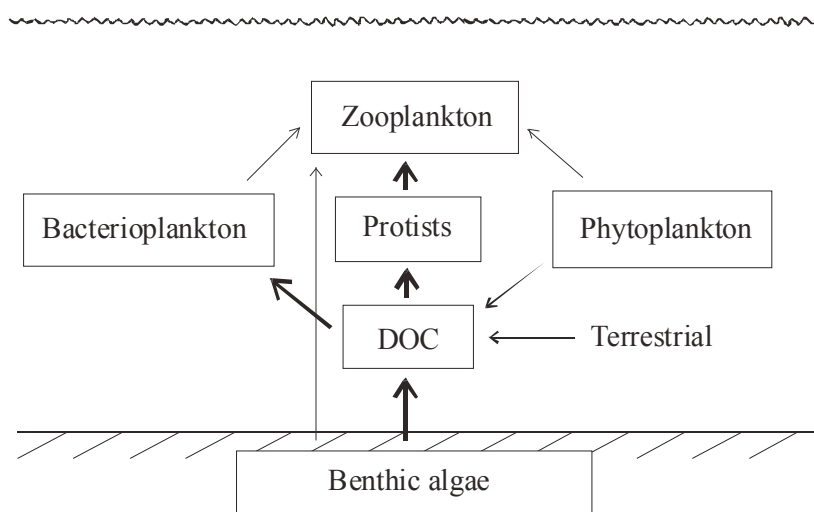
FIGURE 1   Schematic representation of available sources of carbon and potential trophic pathways in aquatic ecosystems (M. Rautio, unpublished).

## 1.2 High-latitude aquatic ecosystems

Whether it is strictly above the Arctic Circle (66° 3′ N), or above the permafrost zone, or tree line, there are various ways to delineate the arctic. Depending on the definition, the arctic may encompasses a gradient of characteristics that include the tundra, subarctic, and high arctic biomes. These biomes provide a more relevant classification based on the biotic characteristics that govern the ecosystems within their boundaries. These boundaries are a gradient of tapering vegetation northward, replaced by increased snow and ice cover. Throughout the northern range, inland waters are seemingly boundless. From dilute surface melt ponds, thermokarst wetlands, to large deep lakes, or epishelf ice lakes, freshwater habitats remain a ubiquitous part of the northern landscape. Arctic and subarctic lakes are typically oligotrophic or ultraoligtrophic clearwater lakes with low conductivity, alkalinity, nutrient and dissolved organic carbon (DOC) concentrations and circumneutral pH (Rautio et al. 2011a).

High-latitude lakes exhibit some of the largest seasonal variation in environmental conditions of aquatic systems globally. Large fluctuations in temperature and light mainly regulate the ecosystem. Small water bodies can have broad range of temperatures during the summer season (5–15 °C), while larger waters have consistently cool summer and winter temperatures, average ± s.d. 6 ± 4 °C and 0.8 ± 0.3 °C respectively. The annual light regime is contrasted between the 24 h of daylight during summer, to the darkness of the polar winter. The light constraints during winter are further compounded by

the months of ice and snow cover. Freeze-up dates can vary by as much as 2 months between small and large lakes in the same region; however, size has no similar effect on ice break up dates, which may only vary by about 2 weeks (Prowse et al. 2012). Many ponds freeze solid during winter and may dry out during the short summer period.

The acute seasonality between the extended dark winters and short cold summers governs the temporal variability of aquatic productivity (Karlsson & Säwaström 2009). Primary productivity is enhanced in ponds compared to lakes, since ponds can heat congruently with air temperature and are constantly circulated with the benthic nutrient flux and catchment carbon inputs (Rautio et al. 2011a). For lakes, the deeper water column provides proportionally higher dominance from planktonic processes, and the greater light attenuation with depth further inhibits benthic production in lakes. Benthic primary production is especially important in shallow, clear ponds where a combination of restricted depth, high light, and low concentrations of DOC allows for high production of benthic material (Vadeboncoeur et al. 2003, 2008a, Rautio & Vincent 2006, Karlsson & Säwaström 2009). The relative importance of benthic productivity compared to pelagic is a distinctive characteristic of high-latitude pond functioning (Rautio et al. 2011a).

Similar seasonal stressors as outlined for primary production also constrain secondary production. Short summers and cold temperatures limit the reproduction, dispersal, and species diversity of consumers in northern waters, many of which are fishless, making metazoan zooplankton the top consumer within the food web (Rautio et al. 2011b). Specific adaptations used by zooplankton to survive in the ephemeral waters or months under the ice are largely dependent on the dormant stages of their life cycles. Classically, cladocerans in these water bodies were thought to be restricted to diapausing species whose adults make ephippia, resting eggs that sit in the sediments during winter (Larsson & Wathne 2006). However, a growing body of evidence shows high densities of zooplankton, including both cladocerans and copepods, remaining active under the ice (Rautio 2000, Karlsson & Säwström 2009). During the active life stages, zooplankton must acquire their energy for growth and reproduction within the seasonal limitations. However there is an imbalance between primary production to summer zooplankton production (Sierszen et al. 2003, Cazzanelli et al. 2011) which forces zooplankton to supplement their diet with alternative forms of carbon, such as from the biomass-rich benthos (Rautio & Vincent 2007) or from t-DOC (Karlsson et al. 2002).

## 1.3 Sources of carbon

### 1.3.1 Phytoplankton and primary productivity

Phytoplankton relies on light, nutrients, temperature, and carbon for its production (Wetzel 2001, Forsström et al. 2005a). These physical and chemical factors and their associated seasonal changes are linked to the considerable annual fluctuations in phytoplankton production, biomass, and composition (Forsström 2006). Typical of polar ecosystems, low temperatures together with low nutrient availability and a short growing season mean that primary productivity is usually low and food webs consist of relatively small numbers of species arranged along few trophic levels (Rautio et al. 2011a). Flagellates, including Chrysophyta, Cryptophyta, and Dinophyta are typical phytoplankton (Laybourn-Parry & Marshall 2003, Forsström et al. 2005b, Bonilla et al. 2009).

The classic paradigm of freshwater ecosystems is centered on phytoplankton being the primary base of the food web (Wetzel 2001). This is mainly attributed to phytoplankton being the best quality and most easily accessed food source for the pelagic filter-feeding consumers (DeMott & Tessier 2002). Another index of quality is the presence of polyunsaturated fatty acids (PUFA) in all phytoplankton. The PUFA that cannot be synthesized within zooplankton are termed essential FA (Brett 1997). The essential phytoplankton-derived lipids not only enhance growth and reproduction, but are also selectively metabolised by zooplankton (Brett et al. 2009).

The seasonal differences in irradiance, ice cover, and DOC inputs govern primary production rates at high latitudes. In spring, with increased light intensity, long periods of daylight and excess nutrients after winter mineralization, phytoplankton can develop already under ice, a common feature in both arctic and alpine lakes (Pugnetti et al. 1999, Forsström et al. 2007). These pulses of primary production are essential to replenishing the higher trophic levels with energy and nutrition after winter. The timing of phytoplankton, ephippia emergence, and parthenogenetic young from the overwintering population is critical for population growth and survival. The coupling of phytoplankton-zooplankton dynamics has implications to community structure and life history strategies (de Senerpont Domis et al. 2007).

### 1.3.2 Alternative carbon sources

When phytoplankton productivity is low, there is a need for consumers to use alternative food sources. Alternative carbon sources for zooplankton may include benthic algae and associated heterotrophic organisms, and/or terrestrial organic matter made available via microbial-mediated pathways (Matthews & Mazumder 2006, Taipale et al. 2008). Benthic- derived carbon is a product of benthic primary production, decomposition of detritus, and sedimentation of pelagic organic matter accumulating at the sediment-surface

boundary. The resulting microbial biofilm includes algae, bacteria, protists, and associated nutrients that are constantly in flux between the sediment and over-laying water. This circulation of nutrients and slow decomposition rates from cold temperatures are the main factors determining benthic biomass (Hansson 1992). Even in temperate ponds with higher light attenuation, benthic productivity and biomass can be important for higher trophic levels (Vander Zanden & Vadeboncoeur 2002). While light is the limiting factor for benthic production in temperate ponds, oligotrophic waters are nutrient limited (Hansson 1992). The transfer of benthic-derived primary production into lake food web models is especially important in shallow, clear lakes where a combination of restricted depth, higher nutrient loads, low concentrations of DOC, and high light allows for high production of benthic material (Vadeboncoeur et al. 2003, Rautio & Vincent 2006, Ask et al. 2009). Catchment characteristics like vegetation type and coverage, soil stability, and drainage of spring thaw have important consequences for the timing, type, and form of t-DOC entering the water (Schindler & Scheuerell 2002, Rautio et al. 2011a). Early winter rains and late winter snowmelt are the two seasonal peaks transporting t-DOC into the lakes. These pulses of carbon help to stimulate production, especially as t-DOC is a main source of carbon for bacteria (Hessen 1992, Cole et al. 2006). Depending on lake size and water residence times, t-DOC can also have counter effect supressing primary production due its absorption qualities that inhibit light. The strong seasonal variations in the quantity and quality of the carbon sources (Hessen 1992) and that lake trophic status and food chain structure can affect the relative contributions of p-DOC and t-DOC sustaining secondary production (Karlsson et al. 2003).

Over the past two decades, aquatic food web research has had active discourse on alternative carbon sources infiltrating the phytoplankton-based trophic structure. Advances in methodology have fuelled the autocthonous versus allochthonous carbon debate. From natural abundance $\delta^{13}C$ (Jones et al. 1992), to isotope enrichment experiments (Carpenter et al. 2005, Pace et al. 2007, Taipale et al. 2008), to the inclusion of fatty acid analysis (Brett et al. 2009) and hydrogen stable isotope ($\delta D$) (Doucett et al. 2007, Solomon et al. 2011), every new approach has furthered our understanding of the complexity and variability of aquatic ecosystem functioning. The pathways by which benthic and terrestrial carbon contribute to consumer growth and reproduction have important implications for food web theory regarding cross-habitat linkages stabilizing or destabilizing trophic dynamics (Polis et al. 1997, Schindler & Scheuerell 2002, Vadeboncoeur et al. 2002). Considering the large fluctuation and rapid oscillation of primary productivity, the large pool of slowing degrading carbon in the benthos and of terrestrial origin can provide ecosystem stability (Polis et al. 1997, Schindler & Scheuerell 2002, Vadeboncoeur et al. 2002). Moreover, the trophic interactions between habitat compartments provide a mechanism for how habitat coupling and resource subsidies can stabilize food webs (Marcarelli et al. 2011).

## 1.4   Tracking carbon

Being able to track the sources of carbon through the ecosystem is an essential component of food web ecology. Food web ecology is based on identifying particular basal food sources, then tracing how the sources move through the food chain. A classical method to track trophic transfer, the sequential movement of matter through the food web, is by using stable isotopes. Isotopes are the different forms of an element based on atomic weight. For example, carbon is an ecologically relevant isotope; the ratio between heavy $^{13}$C and light $^{12}$C isotope gives a particular signature that can be traced through the food web. The consumer isotope signature is the outcome of a mixture of the diet source signatures and its own metabolic discrimination between the heavy and light isotopes, with the latter referred to as fractionation.

Fatty acid analysis has become a contemporary alternative to discern trophic transfer. It is not a replacement, but rather a complement to stable isotopes analysis. Both analyses use carbon as the basic element to measure food web interactions, but are methodologically distinct. Because fatty acids are an integrated part of an organism's biochemistry, fatty acid analysis can quantify the nutritional value of diet sources to consumers.

Fatty acids (FA) have important physiological roles within an organism, from enzyme activity, to basic cell structure, to energy storage and reproduction. Fatty acids are named by their structure, in terms of the number of carbons within the fatty acid chain and the number and position of carbon double bonds. Certain fatty acids are termed essential, because the organism lacks the biosynthetic pathways to make them internally. These FA must be obtained from external sources. For zooplankton, the essential FA, mainly the polyunsaturated fatty acids (PUFA) must be gained from the diet (Marcarelli et al. 2011). PUFA are physiologically important to the cell fluidity, reproduction, and commonly found in all algal groups (Desvilettes et al. 1997, Brett et al. 2009a, Taipale et al. 2011). The analysis of fatty acids considers both the composition and concentrations of consumer fatty acids to track diet sources.

## 1.5   Thesis objectives

This thesis consists of four papers that focus on the seasonal changes in carbon sources and the consequences these changes have for zooplankton in high latitude freshwater ecosystems.

The main objectives of this thesis can be characterized as follows:
1. To evaluate the carbon sources and the subsequent seasonality of food availability. (II, IV)
2. To identify seasonal feeding strategies of zooplankton and, using stable isotopes and fatty acids, to evaluate the trophic transfer of available carbon to zooplankton . (I, II, III)
3. To identify the physiological adaptations used by zooplankton to cope in extreme environmental conditions. (III, IV)

The seasonal fluctuations in the p-DOC and t-DOC resource pools and their relative contributions to zooplankton diets throughout the year were investigated in Saanajärvi, Finland, using stable isotope analysis (SIA) (II). The ecological importance of benthic-derived material to pond food webs was studied for a series of ponds in Kilpisjärvi, Finland and in Kangerlussuaq, Greenland. A summer field study was conducted to evaluate the dietary role of pelagic and benthic food sources for zooplankton in a set of subarctic ponds using SIA and FA (I). Next, an experiment approach was used to address the questions of accessibility and the nutritional quality of benthic mats for zooplankton (III). The nutritional contribution of benthic mats was quantified to the whole-lake fatty acid resource pool (III). By investigating zooplankton during winter, we compared the different life history strategies that zooplankton have evolved to cope with food limitation, months of ice cover, and non-phytoplankton based food sources (IV). Comparing the composition of storage lipids between the two overwintering strategies gives insight into the physiological requirements of zooplankton and may indicate how the selective retention of specific FA can regulate feeding and life history strategies.

Northern freshwater ecosystems provide a model for themes fundamental to limnology. Specifically, the structurally simple food web in arctic lakes provides an opportunity to tease apart the complex interactions between ecosystem components.   This thesis addresses three fundamental themes: landscape-lake interactions, food web dynamics, and adaptations in extreme environments; thereby this thesis is relevant not only to polar science but provides fresh insight into basic limnological questions.

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## 2   METHODS

### 2.1   Study sites

This thesis is largely based on samples collected from a series of lakes and ponds in the Kilpisjärvi region in northwest Finland, but additional secondary sites are included (Fig. 2).



FIGURE 2     Geographical locations of study sites by region, from left to right: Lac Simoncouche, Québec; Kangerlussuaq, Greenland; Kilpisjärvi, Finland; Lunz am See, Austria.

The Kilpisjärvi region is situated at 69° N within the Scandinavian mountain chain. This area is classified as subarctic, as it is above at the tundra-forest boundary but just shy of the 10 °C isotherm, with an average July temperature of 11 °C. The Kilpisjärvi ponds presented in I and III represent the spectrum of pond characteristics in the area (Fig. 3a). Four of the ten ponds are below the treeline (*Betula pubescens* (Ehrh)), and have a characteristic brown colour due to higher levels of dissolved organic carbon (DOC). The ponds above the tree line have catchment vegetation dominated by low dwarf shrubs, mosses, grasses, and sedges; in consequence these ponds are clear with very low concentrations

of DOC. All ponds were sampled in the summers of 2008 and 2010. Malla South was the only pond to be sampled in the winter, as the other ponds freeze solid or were inaccessible. Additional samples for III were obtained during a study of nine ponds near Kangerlussuaq, Greenland, during summer 2010 (Fig. 2).

Lake Saanajärvi, as presented in Papers II and IV, is an oligotrophic clear-water lake (0.7 km², max depth of 26 m), having a small catchment (4.6 km²) located all above the tree line. The lake is ice covered for eight months of the year, from October through till June. Samples were collected between November 2007 and February 2011 from the deepest part of the North basin (Fig. 3b).
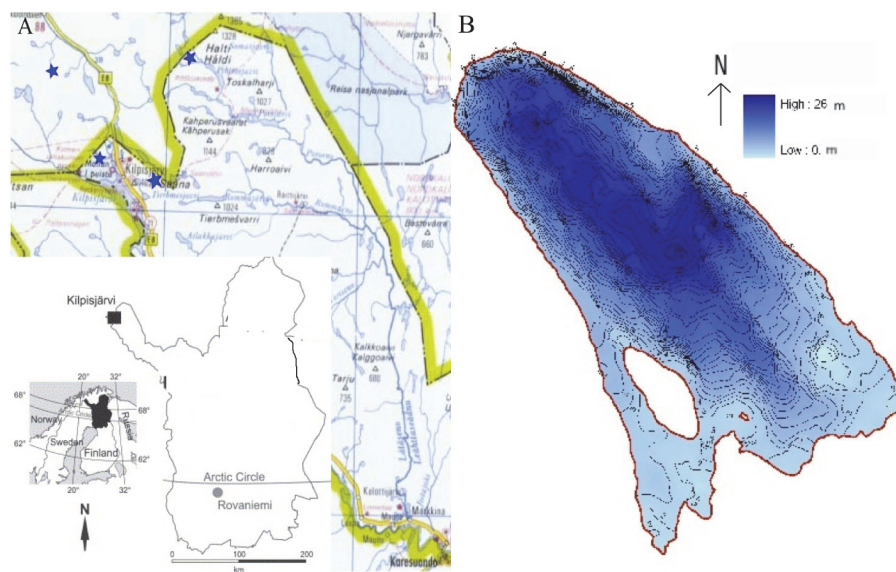


FIGURE 3    Map of Kilpisjärvi study sites (A) and a bathymetric map of Saanajärvi, Finland (B). Sannajärvi map is based on echo sounded depth measurements (m), which were interpolated by kriging method (Annina Kiilunen & Tarmo Virtanen, unpublished data). Stars represent several ponds in the area, Saanajärvi is under the lowermost star.

A variety of environmental variables were determined, which were used to describe the physical and chemical characteristics of the water bodies. The basic limnological data for the Kilpisjärvi ponds, Kangerlussuaq ponds, and Saanajärvi lake during the open water and ice cover periods are summarized in Table 1.

TABLE 1    Basic limnological characteristics of the study ponds in the Kilpisjärvi and Kangerlussuaq regions, and the seasonal variation for lake Saanajärvi. Header legend: max.z= max depth, Chl.pel= pelagic chlorphyll-a, chl.ben= benthic chlorohyll-a, DIC= dissolved inorganic carbon, DOC= dissolved organic carbon, $k_{dPAR}$= vertical light attenuation)

| Pond location & months | n | Paper # | Season | max.z m | Temp °C | chl.pel µg l$^{-1}$ | chl.ben mg m$^{-2}$ | DIC mg l$^{-1}$ | DOC mg l$^{-1}$ | $k_{dPAR}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **Kilpisjärvi, Finland** | | | | | | | | | | |
| Below treeline | 4 | I, III | summer | 1.1 ±0.4 | 12.0 ±2.3 | 1.3 ±0.6 | 11.5 ±13 | 3.9 ±1.4 | 5.7 ±1.9 | 1.0 ±0.5 |
| Above treeline | 5 | I, III | summer | 2.7 ±2.5 | 9.8 ±2.6 | 0.6 ± 0.3 | 5.6 ±3.8 | 2.7 ±1.9 | 2.5 ±0.9 | 0.4 ±0.2 |
| **Kangerlussuaq, Greenland** | | | | | | | | | | |
| Above treeline | 9 | III | summer | 1.5 ±0.5 | 14.9 ±1.7 | 0.5 ±0.1 | 47 ± 58 | 9.4 ±0.7 | 23.5 ±18 | 3.7 ±.03 |
| **Saanajärvi, Finland** | | | | | | | | | | |
| June- Oct | 4 | II, IV | summer | 26.2 | 5.8 ±4.0 | 0.6 ±0.2 | 4.6 ±3.1 | 2.7 ±0.4 | 2.1 ±0.1 | 0.3 ±0.02 |
| Nov- May | 4 | II, IV | winter | 26.2 | 0.9 ±0.3 | 0.1 ±0.1 | - | - | 1.7 ±0.3 | - |

## 2.2 Sample collection and analysis

### 2.2.1 Field sampling

The on-site sampling procedure remained consistent for each sampling date throughout the projects. In brief, water was collected at the deepest point in the lake, integrating all depths of the water column, and was passed through a 50 µm mesh sieve to remove zooplankton. The bulk water samples were either retained, preserved, filtered, or fractioned depending on the analysis. Zooplankton were collected by net hauls (50 µm mesh) through the water column where depth would permit, and then hand sorted to species under a dissecting microscope. Horizontal sweeps using a 210 µm hand net was used in the shallow ponds to collect zooplankton. Physical and chemical characteristic like depth, water temperature, pH, nutrients, snow and ice thickness were recorded.

### 2.2.2 Analysis of water components

To analyse dissolved organic matter (DOM) water samples were filtered through 0.2 µm pore size, pre-rinsed, cellulose acetate filters. The filtrate was used for determination of dissolved organic carbon (DOC) concentration and for the optical properties. The optical properties of the DOM used a spectrophotometer to determine the specific UV absorbance (SUVA) at 254 nm wavelength in relation to DOC concentrations. Increasing SUVA can indicate a greater contribution of terrestrial-derived DOM (Hood et al. 2003, 2005).

Chlorophyll *a* (Chl-a) was determined fluorometrically after filtering a 500 ml water sample on a fine glass fibre filter (GF/F). Phytoplankton

identification and counts were from a 500 ml bulk water sample preserved with Lugol's iodine, allowed to settle in chambers before viewing with an inverted microscope (II, III). Bacteria samples were prepared from water samples preserved with formaldehyde, filtered onto black 0.2 µm Nucleopore membranes and stained with DAPI, a nucleic acid fluorocrome stain that allows visible fluorescence of cells under a fluorescence microscope. Examination by fluorescence microscopy allowed discrimination between bacteria and autofluorescing picophytoplankton, and between larger heterotrophic nanoflagelates (HNF) with DAPI fluorescence and autofluorescing phytoplankton. The enumeration of these latter samples was from prepared 0.6 µm pore size black polycarbonate membrane filters stained with DAPI and mounted on slides with immersion oil. Phytoplankton, bacteria, picoplankton, and HNF were components used to determine the autotrophic and heterotrophic proportions of the plankton community in Saanajärvi (II).

Besides phytoplankton counts and Chlorophyll-a, another method to determine the autotrophic contribution is to measure the photosynthetic rate of autotrophs. Primary production represents the total basal autochthonous carbon resource within the pond food webs. Both pelagic and benthic primary production was determined from in situ incubations using the $^{14}$C-bicarbonate protocol (Rautio & Vincent 2006) (I). The radioactivity of samples was determined with a liquid scintillation counter.

### 2.2.3   Stable Isotope Analysis (I, II)

Freeze dried seston filters, benthic pellets and lipid-extracted zooplankton samples were analysed for $\delta^{13}$C and $\delta^{15}$N using a FlashEA 1112 elemental analyser coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer. Samples were weighed (seston filters 0.3–1.0 mg, benthic 0.5–1.0 mg , zooplanknton 0.7 mg) measured in triplicates, with replicate standards of pike white muscle tissue (seston and zooplankton) and potato leaves (benthic samples) used as internal working standards set repeatedly through every analysis correcting for linearity and drift to ensure accuracy. Internal precision for standard was usually < 0.3 ‰ for both C and N in each run.

### 2.2.4   Fatty Acid Analysis (I, III, IV)

All samples for fatty acid analysis were freeze dried and stored in –80 °C, and weighed (seston filters 0.5–2.0 mg, benthic pellets 10–25 mg, zooplankton 0.8–8 mg) before analysis. The analysis is a three-step process. First lipids were extracted using a chloroform-methanol wash and gravimetrically determined, which permits quantitation of final fatty acid methyl esters (FAME). In the second step, FAME were produced by methylating the extracted lipids using toluene and sulphuric acid-methanol solution. Finally, these samples were evaporated under nitrogen and solubilized in hexane. FAMEs (C14–C24) were separated using a highly polar capillary column in a gas chromatograph with a flame ionisation detector (TRACE GC_FID THERMO$^{tm}$). Fatty acid

concentrations were calculated using calibration curves of known standard concentrations. Quantifying the FAME concentrations using the actual carbon weight rather than dry weight allowed for more accurate comparisons of food sources, circumventing differences between lipid content and sample weight.

## 2.3 Daphnia feeding experiment (III)

In order to address benthic resource use and accessibility of zooplankton, a series of feeding experiments was conducted. In all experiments *Daphnia longispina* were fed low concentrations (0.25, 0.5, 1.0 mg C l$^{-1}$) of phytoplankton from a culture of the green alga *Scenedesmus oliquus*. We specifically used low concentrations of high quality *Scenedesmus*, to test for the threshold at which the low quality food source, in this case benthic mats, would be used to subsidize the diets. One treatment was fed only *Scenedesmus*, while the second treatment had both *Scenedesmus* and benthic algae added. The experiments were done in two parts, once using filtered lake water, intact benthic mats directly from the pond and pond *Daphnia*. The second set of experiments used laboratory cultured *Daphnia* in medium (AdaM), and benthic mats were added in a slurry form, to represent the particle size effectively filtered by *Daphnia*. The first set of experiments addressed the accessibility of benthic mats to *Daphnia*, while the second experimental run focused on whether benthic mats could provide a nutritional subsidy and hence improve survival if the mats were accessible.

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# 3    RESULTS AND DISCUSSION

## 3.1   Seasonal variation in carbon sources (I, II)

There were distinct seasonal patterns in the availability of carbon sources in Saanajärvi (Fig. 4). During winter, bacteria and HNF dominated the particulate organic carbon (Fig. 4c). With the first signs of spring the initial catchment snowmelt brought the highest influx of terrestrial carbon into the lake, according to the optical index SUVA (Fig. 4a). Due to light limitation under the ice, Chl-*a* and autotrophic algal abundance were very low during winter, but the increased irradiance during spring stimulated the photosynthetic autotrophies (Fig. 4b). Shortly after ice off, this group made up a larger proportion of POM than heterotrophs (Fig 4c). A similar trend was shown with stable isotopes (Fig. 4e) in which $\delta^{13}$ C values became more negative when the phytoplankton contribution to POM was highest (II).

The major summer contribution of p-DOC can come from either phytoplankton or benthic algae. Despite the differences in specific algae communities, both habitats provide the FA essential for zooplankton, albeit in different quantities and forms. Based on primary production, phytoplankton does not have sufficient production to sustain pelagic zooplankton in tundra ponds (III). Pelagic productivity is roughly 8 mgC $m^{-2}$ $h^{-1}$, and comparative benthic productivity is 50 mgC $m^{-2}$ $h^{-1}$ (Marcarelli et al. 2011). Benthic-derived FAs contribute 75–97 % of the whole-lake FA standing stock. The results showed that the benthic mats have the production potential both in quality and quantity to support pond zooplankton in the summer.
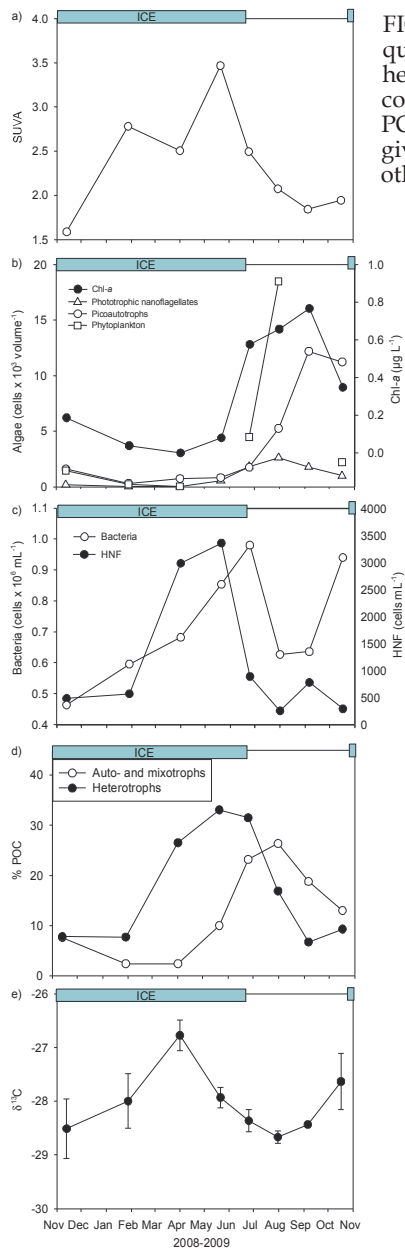
FIGURE 4 Seasonal variation in Saanajärvi of a) DOM quality as determined by SUVA b) autotrophic and c) heterotrophic organisms in the POM, d) carbon composition of the POM and e) δ13 C values of the POM from Saanajärvi (mean ± SE). Algal abundance is given in cells ml$^{-1}$ for phytoplankton and cell l$^{-1}$ for other autotrophs.

## 3.2   Trophic transfer of available carbon (I, II)

Bacteria, flagellates, and ciliates were likely supported by the allochthonous DOM in winter and are intermediates in the transfer of terrestrial carbon to larger zooplankton. *Daphnia* had higher $\delta^{13}$C values in winter and outputs from mixing models indicated > 60 % of *Daphnia* diets is derived from allochthonous carbon in winter. In contrast, neither copepod species showed any apparent feeding during winter, with the carbon isotope signatures of both species remaining constant during the 8 months of ice cover. Active grazing during the long period of food scarcity would have increased the metabolic rate and perhaps resulted in an unfavorable cost–benefit situation in grazing effort versus gained energy. In light of our results, the cladocerans in Saanajärvi could not have survived the 8 winter months under the ice if they had not been able to utilize food sources driven by allochthonous carbon. Likely, when autochthonous carbon is very low or absent, allochthonous carbon-fuelled secondary food sources become critical for the survival of aquatic organisms. Such microbial loop– mediated trophic transfer from allochthonous carbon to higher consumers has been suggested to be the main channel that couples terrestrial and aquatic carbon (Jones et al. 1992, Cole et al. 2000).

SIA was able to differentiate between benthic and pelagic carbon sources. Benthic mats had a heavier carbon isotopic signal than seston (Fig. 5). Zooplankton isotopic signals were consistently more depleted in $^{13}$C than either bulk potential food resource, indicating preferential feeding on the isotopically light, photosynthetic portion of the POM (Brett et al. 2009). Benthic mats could also be fuelled by t-DOC; therefore grazers accessing the benthic mats could be yet another way allochthonous carbon supports aquatic food webs and infiltrates to higher trophic levels.
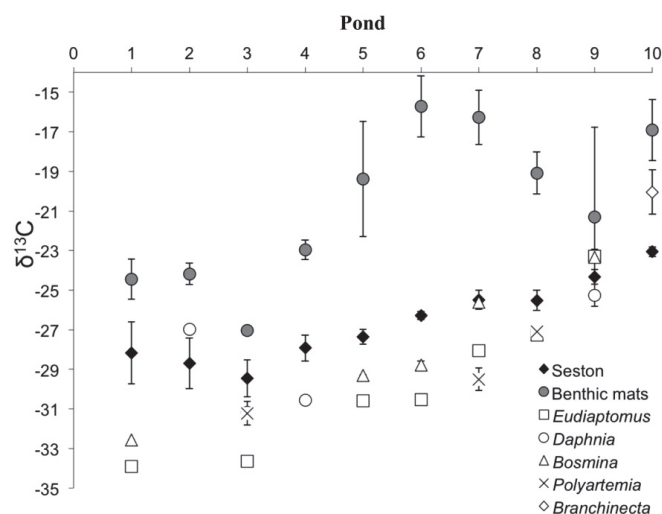
FIGURE 5    Mean (±SE) carbon isotope values for seston, benthic mats, and zooplankton taxa for ponds ordered by increasing altitude. Kilpisjärvi ponds 5-10 are above the tree line.

## 3.3   Implications of benthic-pelagic coupling (I, III)

Along with this thesis on tundra ponds, several studies from temperate lakes have also indicated that lake phytoplankton production is insufficient to support aquatic food webs (Salonen et al. 1992, Pace et al. 2004, Christoffersen et al. 2008). Grazers accessing benthic mats as an alternative to pelagic phytoplankton could act as important habitat integrators, influencing both abiotic and biotic communities and subsequent trophic cascades (Kankaala et al. 2006, Rautio & Vincent 2007). Often it is such movement of nutrient, detritus, and consumers across habitats that controls community dynamics and the local food web structure (Kankaala et al. 2006, Rautio & Vincent 2007). Based on pond morphometry, which includes small surface area to volume ratio, the shallow depth increases the potential for physical and chemical interactions between the benthic and pelagic habitats (Vadeboncoeur et al. 2008b).

The accessibility of benthic habitats to pelagic grazers was experimentally tested. First an experiment keeping the benthic mats intact was used to test whether *Daphnia* can access this form of algae. In the second set of experiments, the benthic mats were added as a suspended slurry, representing the particle size that is accessible to *Daphnia*. Under conditions of food limitation, it was experimentally observed that additions of benthic mats did improve the survival, growth rate, and FA concentrations of *Daphnia*. However, when benthic mats were left intact, *Daphnia* in benthic treatments had poor survival. The different constituents of benthic mats, along with particle size, could be factors contributing to the different results between experiments. In all

experiments, benthic material alone did not prevent *Daphnia* mortality. Bacteria, protists, seasonal changes in algal community, as well as the biofilm boundary layer and associated nutrient-flux may all interact to contribute to the overall trophic transfer of benthos to pelagic grazers.

From a survey of the Kilpisjärvi ponds, the results show that both benthic and pelagic habitats have all essential dietary fatty acids, with no significant changes in FA concentration during the summer (Fig. 6). The quantity of zooplankton PUFA was higher than in either food source (Fig. 6). Gladyshev et al. (2011) found that specifically PUFA had twice higher transfer efficiency between phytoplankton and zooplankton. The consistent source of PUFA in the benthic habitat and the efficient trophic transfer of these compounds could buffer periods of low pelagic phytoplankton availability. This form of benthic-pelagic coupling can promote ecosystem stability by providing alternative energy pathways thereby dampening effects of ecosystem oscillations (Vadeboncoeur et al. 2002, Marcarelli et al. 2011). Further understanding of energy acquisition is needed for more accurate assessment of subarctic food web structure and function. For example, consumer competitive relationships and species composition are affected by resource quality, particularly when the acquisition of nutritional requirements has consequences for somatic growth and reproductive success of consumers (Säwström et al. 2009).
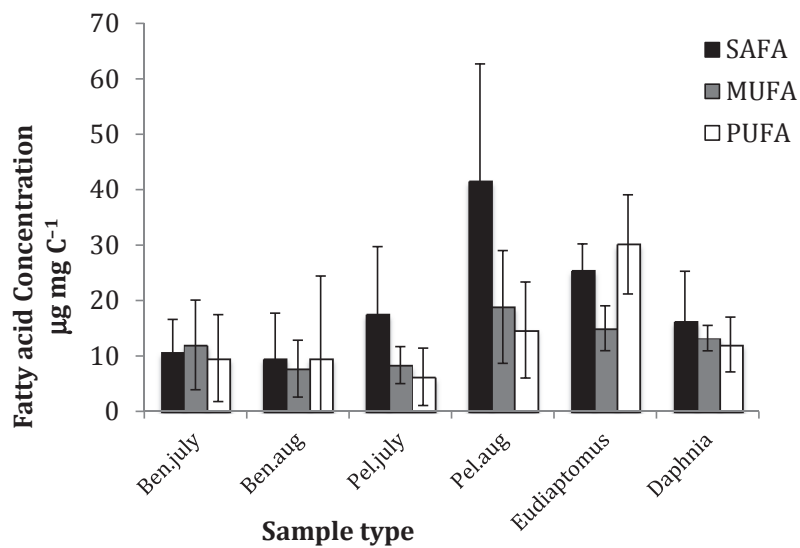


FIGURE 6    Grouping all FAME concentrations into PUFA, MUFA, SAFA, from both sampling periods (July and August, 2010) from benthic (Ben.), pelagic (Pel.) habitats, and zooplankton. These values are an average of nine ponds ± SD.

## 3.4 Mechanisms of zooplankton resilience (I, III, IV)

Organisms must evolve the ability to maintain their metabolic needs in harsh environmental conditions for optimal success (Säwström et al. 2009). Specifically, this could include life history strategies or feeding strategies.

Feeding strategies include both selective feeding and/or metabolic selection of acquired diet components. Selective feeding, the ability to choose between diet sources, is an example of behavioural plasticity. The results have demonstrated the ability of zooplankton to switch from phytoplankton to benthic algae, or to t-DOC-supported heterotrophs when necessary. Consumers can also metabolically select how the energy acquired is sequestered within the soma (Smyntek et al. 2008, Müller-Navarra 2008). The higher retention of PUFA and $\omega$-3 FA of Calanoids in ponds above the tree line compared to below, provides field evidence of metabolic plasticity. Internal regulation of these physiologically important FA with increasing altitude may indicate the potential metabolic flexibility of zooplankton in times of low food availability.

In winter, the combination of food limitation and low temperature together act to lower metabolism, thus increasing life span. The surviving winter *Daphnia* have a distinct ecological advantage, as at high-latitudes active *Daphnia* are better timed to take advantage of spring phytoplankton growth than those hatching from ephippia. At lower latitudes, this advantage is lost with longer summer open-water periods, as individuals from the diapausing strategy have time to emerge and start reproducing within the season. Storing lipids is another metabolic adaption by which zooplankton can mitigate seasonal food shortages. Actively overwintering zooplankton have their highest lipid concentrations at the beginning of ice cover, with these storage lipids gradually decreasing throughout the winter months (Fig. 7). Our results show preferential retention of unsaturated fatty acids, specifically EPA and SDA, in *Daphnia* preparing to actively overwinter. The specific concentrations were for active *Daphnia,* 399 ± 213 µg DW $l^{-1}$ (average± s.d., n=9), while *Daphnia* that use diapause to overwinter have only 106 ± 18 µg DW $l^{-1}$ (n=8). The diapause strategy stores proportionally equal SAFA, MUFA, PUFA, (25, 32, 35 % respectively). The lower concentrations of total lipids in diapausing *Daphnia* could be due to channeling FA to ephippia production or catabolized for metabolism.

Understanding zooplankton life histories and the associated changes in trophic interactions as a consequence of ice cover can indicate the degree of population plasticity in response to ongoing environmental change. Whether through feeding strategies, behavioural or physiological, or by alternate life histories, the mechanisms zooplankton use to adapt to environmental conditions ultimately determines the diversity and structure of high-latitude aquatic ecosystems (Rother et al. 2010).

(A) Saanajärvi ice cover        (B) *Eudiaptomus graciloides*        (C) *Daphnia umbra*



**October**. Last month of open water, zooplankton have **60% body fat**.



**January**. Third month of ice cover, zooplankton have **40% body fat**



**May**. Seventh month of ice cover, zooplanknton hve **10% body fat**
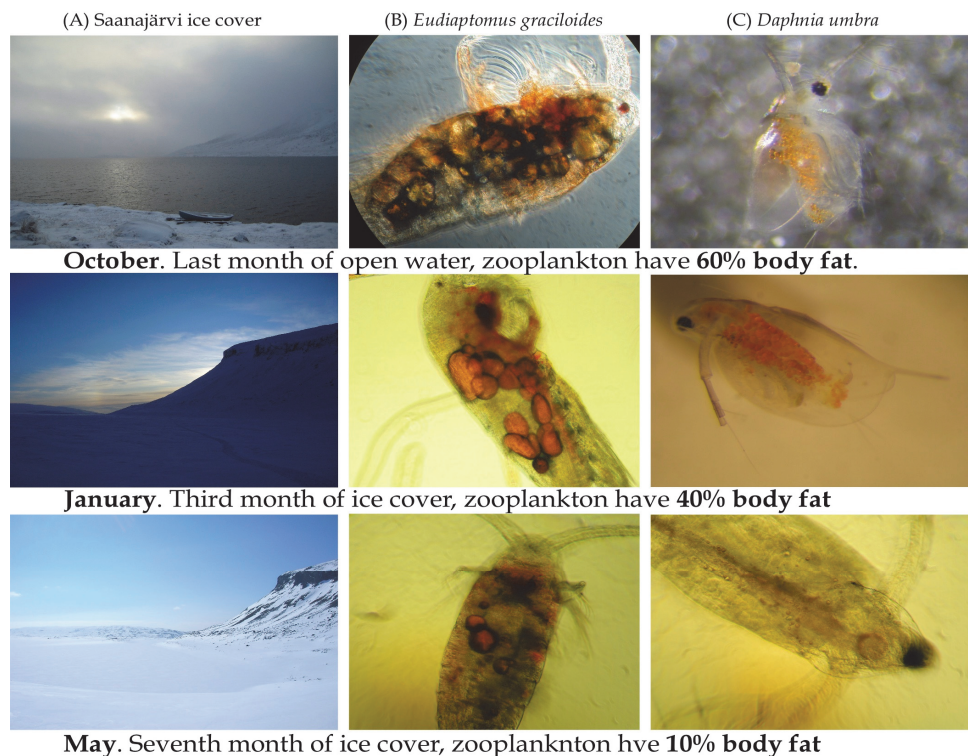
FIGURE 7      Changes in ice cover (A) and percent body fat, calculated from total fatty acids, in *Eudiaptomus graciloides* (B)and *Daphnia umbra* (C) through winter in Saanajärvi, a subarctic lake. Photographs: H. Mariash and M. Rautio.

## 3.5 Clarification

An inherent part of research is discovering a better way to approach a problem. This was the case with the distinct, primary field material collected separately in 2008 and in 2010. Both projects (I, III) address whether benthic mats can provide a complementary food source to pelagic grazers, but the projects came to different conclusions due to the differences in methodology. Paper I used both stable isotopes and fatty acid analysis in tandem to evaluate the dietary contribution of benthic and pelagic food sources. Conclusions from the results suggest that phytoplankton remains the dominant diet source of pelagic zooplankton; although, the caveats of the stable isotopes and fatty acids methods were discussed (I).

Using community composition and distinct FA data collected in 2010, the importance of benthic mats could more broadly be evaluated and compared between habitats. Because of the inherent differences in sample collection and

weight between seston and benthic mats, FA in 2010 were standardized to carbon weight rather than dry weight. This was a more accurate way to approach the fatty acid quantification and thus the importance of benthic habitats was re-evaluated (III).

The change in methodology to analyze the 2008 and 2010 FA data ultimately changed results and hence the conclusions between paper I and III. Despite these changes in conclusions, both studies provide valuable discussions to address the benthic-pelagic coupling and zooplankton adaptations to extreme environmental conditions. Mariash et al. (2011) considers how zooplankton can balance their individual metabolic needs during times of food limitation either by selective feeding (ie. whether zooplankton actively access benthic resources), an example of behavioural plasticity, or by metabolic retention of specific fatty acids, and example of metabolic selection of acquired energy (Smyntek et al. 2008; Müller-Navarra 2008). Paper III took selective feeding further through experimentally evaluating accessibility and nutritional benefit of benthic mats to growth and survival of *Daphnia*. In this way, I and III provide new insight into the source and transfer of FA in pond food webs. The mechanisms driving zooplankton acquisition and retention of FA has implications to how zooplankton populations have evolved to adapt to northern environmental conditions.

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# 4    CONCLUSIONS

This thesis presents a thorough examination of trophic dynamics in high-latitude freshwater ecosystems. Beginning at the base of the food web, the proportional contribution of the putative components of the DOC pool was estimated on an annual scale. The annual scale allowed for a more complete understanding of the seasonal factors driving within lake productivity. The rates of primary production were highly dependent on season and carbon source. Next, the contributions of these sources (benthic, terrestrial, and pelagic) were tracked through to zooplankton consumers. Using both stable isotopes and fatty acids, zooplankton diet sources could be quantified.

Based on hydrology, t-DOC inputs are greatest in early and late winter, and this is confirmed by the highest SUVA values at these times. For the duration of winter, the water column was characterized by a high relative contribution of t-DOC, as light limitation under the ice prohibits photosynthetic production. The zooplankton community showed two different feeding strategies associated with the changing basal carbon pool according to our stable isotope values. Copepods seemed to manage on summer energy reserves stored as fats, whereas cladocerans continued to feed on available heterotrophic food items, which were terrestrial-derived in winter. All zooplankton responded rapidly to summer p-DOC resources, as observed through changed $\delta^{13}C$ values along with p-DOC carbon values. Based on the results t-DOC helps fuel secondary production, and is particularly critical in winter for cladocerans.

Based on algal community composition, stable isotope ratios, and FA composition, benthic- and pelagic–derived carbon are from ecologically distinct communities and can be easily separated in analysis. It was experimentally observed that under limiting food conditions, additions of benthic carbon sources improved survival and increased the fatty acid concentrations of *Daphnia*. The treatment with the longest survival was the benthic addition, albeit benthic material alone was not enough to sustain the population.

Each season has it own set of stress factors that require consumers to adapt rapidly to their changing environment, be it from winter ice cover, spring wash out, or consumer competition in the summer. The ability of zooplankton

to balance individual metabolic needs under environmental stress can be attributed to both behavioural and physiological plasticity in carbon mobilization. Specifically this can involve a pelagic consumer feeding on benthic resources, an example of behavioural plasticity. Additionally, consumers can metabolically select how the acquired energy is sequestered, as shown with preferential retention of PUFA. The results show that the specific retention of unsaturated storage lipids is one mechanism by which zooplankton are resilient to the demanding conditions of arctic winters.

The results showed that overwintering zooplankton have their highest lipid concentrations at the beginning of ice cover, with these storage lipids gradually decreasing throughout the winter months. The active overwintering *Daphnia* retained proportionally more EPA and SDA than the diapausing *Daphnia*. The composition of storage lipids in the active winter *Daphnia* is a direct physiological response to seasonal changes in food availability. The combination of food limitation and low temperatures together act to lower metabolism, thus increasing life span, giving the surviving winter *Daphnia* a distinct advantage to access spring phytoplankton compared to individuals hatching from ephippia. Understanding the changes in zooplankton life histories and the associated trophic interactions as a consequence of seasonal controls can indicate the degree of population plasticity in response to ongoing environmental change.

By studying many of the physical and living components throughout the year, this thesis has given new insight into the quantity, quality and utilization of food resources by crustacean zooplankton and their life history strategies in the harsh, northern environment. Ultimately the driving evolutionary force leading to fitness success, selects for optimum dietary intake in order to maximize individual growth and reproduction. The relationship between evolved traits of species within their physical environment is a critical step to understanding how ecosystems are structured and function. The simple food web of the tundra ponds was a good opportunity to trace the effects of a few potential food sources through to consumers. Both benthic and terrestrial subsidies can affect the plankton community interactions, nutrient cycling, and the stability of food webs (Schindler & Scheuerell 2002). Together the outcomes from this thesis add to our understanding of lake ecosystem functioning, in general.

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Like isotopes, ideas can be traceable. As ideas are shared, passed along between disciplines and researchers, they fractionate into a new ratio of interesting: mundane ideas depending on the researcher or audience. This mixture of ideas becomes our own research signature. My research is the sum of innumerable iteration, inspired from literature, conversations with colleagues, and catalyzed by continually new interactions with people who share a passion for science. Of course, the direction from a handful of key mentors focused my efforts.

Milla Rautio has been an excellent mentor leading by example rather than through direct supervision. While the Atlantic Ocean augmented my independence, Milla's faith in my competence gave room for self-development. Efficient, determined, and directed in her approach to research; tolerant, respectful, and patient as a supervisor. I am grateful for all the opportunities I've been provided because of her leadership. Roger Jones is also an admirable leader. Roger has a knack for transforming complex research questions, messy manuscripts, and/or problematic technicalities into simple, articulate solutions. With years of experience, Roger's advice has continually kept me on the right track. And for this I am indebted!

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My sincerest thanks to all for contributing to my research signature and encouraging me throughout the process.

# YHTEENVETO (RÉSUMÉ IN FINNISH)

### Vuodenaikainen vaihtelu subarktisen eläinplanktonin ravinnonkäytössä

Ympäristöolosuhteet vaihtelevat pohjoisissa järvissä vuodenaikaisesti enemmän kuin valtaosassa maailman muita järviä. Pitkä pimeä talvi ja lyhyt kylmä kesä vaikuttavat ravinnonsaatavuuteen ja eliöiden menestymiseen ja sitä kautta määräävät koko pohjoisen vesiekosysteemin toimimisen ja rakenteen. Väitöskirjani tavoite on selvittää, millä tavalla vuodenaikainen vaihtelevuus vapaan veden, pohjan ja valuma-alueen hiilen saatavuudessa ravinnonlähteenä vaikuttaa eläinplanktonin ravinnonkäyttöön subarktisissa vesissä. Olen selvittänyt ennen kaikkia, miten eläinplankton selviää arktisesta talvesta, jolloin perustuotannosta lähtöisin oleva hiilenlähde on minimissään. Tärkeimpinä menetelminä käytin vakaita isotooppeja ja rasvahappoja. Niiden avulla pystyin arvioimaan kunkin hiilen lähteen osuuden eläinplankton ravinnossa ja mittaamaan rasvavarastojen merkityksen keinona selvitä ravintoköyhistä ajanjaksoista.

Vuodenaikainen vaihtelu ravinnonlähteissä määräytyy talven pituuden ja jäänlähdön mukaan. Bakteerit ja muut toisenvaraiset eliöt ovat runsaita jään alla, kun taas keväällä vallitseva hiilenlähde on valuma-alueelta sulavesien mukana tuleva hiili. Kasviplankton ja muut yhteyttävät eliöt ovat runsain ravinnonlähde järvissä kesällä. Tämä vuodenaikainen vaihtelevuus ravinnonlähteissä heijastui tutkimieni eläinplanktonlajien vakaissa hiili-isotoopeissa. Valuma-alueen hiilen käyttö ravinnonlähteenä muun muassa vaihteli vuodenaikaisesti ja eri eläinplanktonlajien välillä. Samalla tavalla eläinplanktonin rasvahappokoostumus noudatteli vuodenaikaisuutta ja oli erilainen eri lajien kesken. Avovesikaudella kerätyt rasvavarastot mahdollistivat eläinplanktonin selviytymisen yli niukkaravintoisen talven. Kesällä pohjalevät muodostivat 75-97 % tunturilammikoiden eläinplanktonin rasvahapoista ja toimivat tärkeänä lisänä kasviplanktonpohjaiselle ravinnolle. Myös laboratorikokeet osoittivat, että pohjalevät lisäsivät *Daphnia*-vesikirppujen selviytymistä ja rasvahappomäärää silloin kun kasviplanktonin määrä oli rajoittava.

Tutkimukseni on tuonut lisätietoa vesieliöiden käyttämistä sopeutumismekanismeista ympäristössä, jossa olosuhteet muuttuvat paljon vuodenaikaisesti. Vastaavat mekanismit saattavat aktivoitua myös isompien ympäristömuutosten aiheuttamana, esimerkiksi ilmastonmuutoksen vaikutuksesta. Väitöstutkimukseni lisää siten arvokasta tietoa pohjoisten vesiekosysteemien sopeutumiskyvystä muuttuvassa ilmastossa.

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# ORIGINAL PAPERS

# I

## FOOD SOURCES AND LIPID RETENTION OF ZOOPLANKTON IN SUBARCTIC PONDS

by

Heather L. Mariash, Matteo Cazzanelli, Martin J. Kainz & Milla Rautio 2011

# Food sources and lipid retention of zooplankton in subarctic ponds

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**SUMMARY**

1. Subarctic ponds are seasonal aquatic habitats subject to short summers but often have surprisingly numerous planktonic consumers relative to phytoplankton productivity. Because subarctic ponds have low pelagic productivity but a high biomass of benthic algae, we hypothesised that benthic mats provide a complementary and important food source for the zooplankton. To test this, we used a combination of fatty acid and stable isotope analyses to evaluate the nutritional content of benthic and pelagic food and their contributions to the diets of crustacean zooplankton in 10 Finnish subarctic ponds.

2. Benthic mats and seston differed significantly in total lipids, with seston (62.5 $\mu$g mg$^{-1}$) having approximately eight times higher total lipid concentrations than benthic mats (7.0 $\mu$g mg$^{-1}$). Moreover, the two potential food sources differed in their lipid quality, with benthic organic matter completely lacking some nutritionally important polyunsaturated fatty acids (PUFA), most notably docosahexaenoic acid and arachidonic acid.

3. Zooplankton had higher PUFA concentrations (27–67 $\mu$g mg$^{-1}$) than either of the food sources (mean benthic mats: 1.2 $\mu$g mg$^{-1}$; mean seston: 9.9 $\mu$g mg$^{-1}$), indicating that zooplankton metabolically regulate their accumulation of PUFA. In addition, when each pond was evaluated independently, the zooplankton was consistently more $^{13}$C-depleted ($\delta$ $^{13}$C −20 to −33‰) than seston (−23 to −29‰) or benthic (−15 to −27‰) food sources. In three ponds, a subset of the zooplankton (*Eudiaptomus graciloides*, *Bosmina* sp., *Daphnia* sp. and *Branchinecta paludosa*) showed evidence of feeding on both benthic and planktonic resources, whereas in most (seven out of 10) ponds the zooplankton appeared to feed primarily on plankton.

4. Our results indicate that pelagic primary production was consistently the principal food resource of most metazoans. While benthic mats were highly productive, they did not appear to be a major food source for zooplankton. The pond zooplankton, faced by strong seasonal food limitation, acquires particular dietary elements selectively.

*Keywords*: benthic–pelagic coupling, fatty acids, food web, stable isotopes

## Introduction

The ecological importance of benthic-derived material to lake food webs is receiving increasing attention (Rautio & Vincent, 2006; Vander Zanden *et al*., 2006;

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Brett, Müller-Navarra & Persson, 2009). It has been argued that the trophic transfer of benthic-derived primary production is especially important in shallow, clear lakes where a combination of restricted depth and low concentrations of dissolved organic carbon (DOC) allows for high production of benthic material (Vadeboncoeur *et al.*, 2003, 2008; Rautio & Vincent, 2006; Karlsson *et al.*, 2009). Hence, it has been widely suggested that benthic mats, defined here as cohesive microbial layers of algae attached to the substratum, may be important in sustaining high summer zooplankton biomass in oligotrophic, high-latitude ponds (Hobbie, 1980; Rautio & Vincent, 2006; Karlsson *et al.*, 2009). However, direct empirical evidence for the consumption and retention of benthic carbon by pond zooplankton is scarce.

The application of stable isotope analysis (SIA) to food web ecology has improved our ability to identify the resources used by secondary planktonic consumers (e.g. Karlsson *et al.*, 2003). A SIA approach requires different food sources to have distinct isotopic signatures. These signatures (e.g. $\delta^{13}C$ or $\delta^{15}N$) can then be traced through the food web because there is limited isotopic fractionation of $\delta^{13}C$ signatures between resources and consumers (Post, 2002); however, there is an enrichment of $\delta^{15}N$ signatures by *c.* 2–4‰ with each trophic transfer (e.g. Post, 2002; Matthews & Mazumder, 2008; and references therein). This trophic enrichment allows consumers to be placed sequentially into the food chain. In addition to carbon, zooplankton require dietary omega-3 (ω3) and ω6 polyunsaturated fatty acids (PUFA), which are integral parts of their cell membranes, improving somatic growth and helping maintain cell fluidity (Pruitt, 1990). Cell fluidity and fast growth are especially important in these high-latitude ponds where short, cold summers prevail. Zooplankton depend strongly on dietary lipids (e.g. Brett & Müller-Navarra, 1997) because, like other animals, they lack Δ12 and Δ15 desaturases and therefore cannot synthesise ω3 or ω6 PUFA *de novo* (Cook & McMaster, 2002). For this reason, zooplankton are expected to retain dietary PUFA selectively (Kainz, Arts & Mazumder, 2004; Hessen & Leu, 2006). Because dietary sources, in particular algae from different habitats, often exhibit distinct PUFA compositions, PUFA can be used as biomarkers for food sources (Brett *et al.*, 2006). Thus, fatty acid (FA) analysis can be coupled with a stable isotope approach to provide more detailed information on lacustrine food web structure (e.g. Perga *et al.*, 2006).

In high-latitude aquatic ecosystems, such as in subarctic Finland, organisms must cope with a short, ice-free growing season (July to September), low temperature (4–15 °C) and oligotrophy. Each of these environmental constraints increases with altitude, especially above the tree line where terrestrial subsidies are slight. Short summers and high zooplankton population densities imply intense competition for resources during the ice-free months (Van Geest *et al.*, 2007). The primary food source for zooplankton is pelagic algae but, by late summer, zooplankton communities have often exhausted these pelagic sources (Hessen & Leu, 2006). Consumers can cope with environmental constraints and competition either behaviourally by seeking alternative, lower-quality food resources or physiologically by sequestering higher-quality nutrient constituents, like PUFA. High primary benthic productivity in high-latitude ponds is known to make a significant contribution to whole-lake autochthonous production (Vadeboncoeur *et al.*, 2003, 2008) and could serve as a dietary complement to resource-limited zooplankton communities during late summer (Hansson & Tranvik, 2003; Rautio & Vincent, 2006; Karlsson *et al.*, 2009).

We designed a field study to evaluate the dietary role of pelagic and benthic food sources for zooplankton in oligotrophic, high-latitude ponds. We sampled three fractions of the seston, benthic mats and zooplankton in 10 subarctic ponds in northern Finland and analysed food availability and the biochemical quality of pelagic and benthic primary producers. In addition, we examined the relationship between these resources and the lipid composition of the five most prevalent metazoan groups in late summer. Previous experimental studies have shown that zooplankton can access benthic resources (Rautio & Vincent, 2006); however, no previous studies have quantified the quality of benthic mats as food. To our knowledge, this is the first field study to assess the dietary relevance of benthic mats to pond zooplankton. We hypothesised that zooplankton obtain part of their somatic needs from the benthic primary production and tested this using a combination of stable isotope and FA analyses. In addition, we hypothesised that, to compensate for the shorter growing season and lower temperatures at higher altitude, the standing stock of both food sources would have higher quality (in terms

of PUFA), which would be reflected in the primary consumers.

## Methods

Samples were taken during August 2008 from 10 ponds in north-western Finland (69°04′N, 22°45′E). The study area is located within the Scandinavian mountain chain situated in the subarctic region, typically having only three to four ice-free months with a mean summer (June–August) air temperature of 9 °C. A characteristic feature of the area is the tree line of birch (*Betula pubescens* (Ehrh) subsp *czerepanovii* (Orlova), Hämet-Ahti) that approximately follows the 600-m contour. Of the 10 ponds sampled, four were below the tree line and six were above (Table 1). Temperature, conductivity and pH were measured from the upper metre of water in the centre of each pond once (YSI 63 meter; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Water temperature was closely related to daily air temperature; only the deep 7-m pond was stratified (Lampo, pond 8). Water samples for DOC were filtered using 0.2-$\mu$m pore size cellulose acetate filter and analysed on a Shimadzu TOC-Vcph at the North Ostrobothnia Regional Environmental Centre, Finland.

Seston and benthic potential food sources were gathered from each pond. Bulk water samples (5–10 L) from the upper metre in the middle of the pond were strained through a 50-$\mu$m mesh to remove zooplankton while retaining the potentially edible particles (Kainz *et al.*, 2009). Seston was then isolated on pre-weighed, pre-combusted (400 °C for 2 h) glass fibre

filters (Whatman GF/F, 0.7-$\mu$m pore size), from c. 3–5 L, depending on the particulate organic matter (POM) content of the pond. To have two additional fractions of the available resources within the POM, an additional 3–18 L of pond water was passed through a tangential flow filtration device (Millipore Pellicon 2 cassette filter; Millipore, Billerica, MA, U.S.A.); 1 L of the permeate (<0.2 $\mu$m) was retained while the back-wash was a concentrated sample of the original volume that did not pass through the filter (0.2–0.7 $\mu$m). The latter contained the bacterial fraction while the former was the DOC fraction of the seston; both were later freeze-dried and analysed for FA. Benthic cores were taken using a cylinder (diameter 5 cm and length 40 cm) that was hand-pushed into the sediment and closed with airtight caps at both ends before lifting the undisturbed sediment core from the pond. The cores were taken from three locations around the perimeter (20–60 cm depth) and at the centre of the ponds depending on the distribution of rock (epilithic) and sediment (epipelic) algae. The surface layer of each core was carefully sub-sampled using an 8-mm-diameter cut-off syringe (thickness 1 mm) to attain an undisturbed pellet of algae from the sediment–water interface. When epilithic algae were present, algae were scraped off from a fixed area (1–0.5 cm$^2$) using a scalpel from rocks found along the pond's perimeter. Both sample types were pooled in the data analysis to represent a single benthic source.

To assess general biomass and productivity of seston and benthic algae, we measured chlorophyll-*a* (chl-*a*) and the rate of photosynthesis. For seston chl-*a*, each water sample (0.5–1 L) was filtered onto a GF/F

Table 1 Physical and chemical characteristics of the study ponds located below and above the tree line. Numbers in parenthesis refer to pond coding in Rautio (2001)

| | Pond no | Altitude (m) | Depth (m) | Area (m²) | Temp (°C) | pH | Conductivity ($\mu$s cm$^{-1}$) | Dissolved organic carbon (mg L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Below tree line | | | | | | | | |
| Masi (4) | 1 | 500 | 1.3 | 118 | 8.9 | 7.2 | 35.1 | 2.9 |
| Boat (3) | 2 | 500 | 1.5 | 471 | 12.5 | 6.9 | 16.9 | 7.3 |
| Big rock | 3 | 527 | 0.5 | 47 | 15.2 | 4.9 | 21.0 | 6.0 |
| Happy | 4 | 531 | 1.0 | 471 | 11.6 | 6.8 | 20.1 | 6.4 |
| Above tree line | | | | | | | | |
| North Malla | 5 | 545 | 3.0 | 11775 | 11.1 | 7.6 | 22.7 | 3.2 |
| South Malla | 6 | 599 | 2.5 | 7850 | 12.1 | 7.8 | 21.7 | 2.5 |
| Reindeer (11) | 7 | 710 | 1.5 | 1178 | 9.7 | 8.1 | 22.2 | 3.4 |
| Lampo (15) | 8 | 858 | 7.5 | 79 | 11.7 | 7.0 | 3.8 | 1.7 |
| Tuono (14) | 9 | 873 | 1.0 | 550 | 9.4 | 6.5 | 4.2 | 2.8 |
| Straca | 10 | 1000 | 0.5 | 47 | 4.9 | 7.1 | 9.2 | 1.2 |

filter and stored at −80 °C. For the analysis, the thawed sample was solubilised in 5 mL of 90% ethanol, placed in a water bath (75 °C, 5 mins), cooled and then analysed using spectrofluorometry (Yentsch & Menzel, 1963). For benthic chl-*a*, triplicates of benthic particles were freeze-dried prior to analysis to enhance extraction (Hansson, 1988) and were then analysed by spectrophotometry (Nusch, 1980). To estimate the algal proportion from the bulk seston and benthic mats from each site, we converted the chl-*a* concentration with a C/chl-*a* value of 40 : 1, a value obtained from a late summer phytoplankton community survey of Lake Saanajärvi in the vicinity of the sampled ponds (Rautio, Mariash & Forsström, 2011). The carbon values were divided by the concentration of particulate organic carbon (POC) to obtain the proportional algal contribution.

Primary productivity was measured *in situ* using the $^{14}$C-bicarbonate protocol described by Rautio & Vincent (2006). Triplicates of pre-filtered (<50 $\mu$m) pond water (20 mL) were spiked with $^{14}$C-bicarbonate (specific activity 80 $\mu$C mL$^{-1}$) and were incubated using five different light levels (0, 6, 25, 60 and 100% of total solar radiation) to obtain an estimate of the potential maximum pelagic photosynthetic rate ($P_{max}$) of the community. Owing to their heterogeneity, benthic algae were analysed for five replicates but for only three light levels (0, 25 and 100%) that were used to calculate the maximum productivity of the community ($P_{max}$). Filtered (<0.7 $\mu$m) pond water (20 mL) was used to suspend the benthic pellets. Each measurement included two dark bottles for both seston and benthic particles. Incubations were conducted outside under natural light in a 10 °C lake water bath for 2 h and were terminated by filtering the sample onto GF/F filters and freezing. Radioactivity was later determined with a liquid scintillation counter (RackBeta; LKB Wallac, Turku, Finland). Dissolved inorganic carbon titrations with 0.02 N NH$_2$SO$_4$ were made to determine carbon availability for photosynthesis in each pond.

Samples of seston and benthic organic matter were prepared for C and N stable isotope analyses to identify trophic links between zooplankton and different potential dietary sources. To estimate food quality, we quantified FA from both resource pools by taking the dry mass (DM) of benthic pellets and from the difference of seston filters to determine the fatty acid methyl ester (FAME) concentration ($\mu$g mg$^{-1}$ DM). In all localities, six replicates of each resource were taken, seston and benthic food sources were collected in the same way as described earlier, and then freeze-dried, of which three samples were stored at −20 °C for SIA and the remaining three were stored at −80 °C for FA analysis.

Zooplankton were collected using a 210-$\mu$m mesh hand net, sweeping the majority of the pond area. In addition, vertical tows (50-$\mu$m mesh net) were included for ponds more than 2 m deep and were later sieved through a 250-$\mu$m mesh to retain only the adults. Samples were stored in bottles, half of which were shock-frozen on dry ice in the field to minimise the change in FA composition. The other zooplankton were kept alive and stored at 4 °C overnight to allow for gut evacuation. The most abundant species (copepods: *Eudiaptomus graciloides* Lilljeborg 1888, cladocerans: *Bosmina* sp., *Daphnia* sp.; anostracans: *Polyartemia forcipata* Fischer 1851, *Branchinecta paludosa* Müller 1788) were then sorted, counted and compiled into three replicate Eppendorf tubes and kept in the freezer until SIA. Similarly, from the shock-frozen zooplankton, the most abundant species were hand-sorted and retained in triplicates for FA analysis. The number of individuals per replicate varied depending on size and taxon: Anostraca (three-15 individuals), *Daphnia* (20–100), *Eudiaptomus* (60–100) and *Bosmina* (200–500), aiming for a total DM > 0.5 mg.

Zooplankton have a greater lipid content than their food. If not corrected for, these lipids further deplete consumer $\delta^{13}$C values (Syväranta & Rautio, 2010). Lipids were therefore removed from the zooplankton before SIA using a 1-mL wash of chloroform/methanol (2 : 1 v/v), slowly shaken overnight before removing the lipids. The remaining lipid-extracted zooplankton samples were set to dry before being weighed.

Dried seston filters, benthic pellets and zooplankton samples were analysed for $\delta^{13}$C and $\delta^{15}$N signatures using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific Corporation, Waltham, MA, U.S.A.) coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer. Pike (*Esox lucius, L.*) white muscle tissue and potato leaves (*Solanum tuberosum, L.*) were used as internal working standards for zooplankton and for seston and benthic mats, respectively. Standards were inserted in each run after every five samples. Stable isotope ratios are expressed as delta values ($\delta^{13}$C or $\delta^{15}$N) relative to the international

standards for carbon (Vienna PeeDee belemnite) and nitrogen (atmospheric nitrogen). Internal precision for standards was usually <0.3‰ for both C and N in each run.

Lipids were analysed as described by Heissenberger, Watzke & Kainz (2010). In brief, freeze-dried and homogenised samples (0.5–1.5 mg) were extracted using chloroform–methanol. Fatty acids (C14-C24) were analysed as FAME after derivatisation using toluene and $H_2SO_4$–methanol. FAME were identified using a gas chromatograph with a flame ionisation detector (TRACE GC-FID THERMO™), equipped with a temperature-programmable injector and an autosampler. A Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 $\mu$m film thickness) was used for FAME separation. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations. For the quantified FA data, only concentrations >0.5 $\mu$g mg$^{-1}$ DM were included.

Because earlier work has shown that ponds above and below the tree line differ significantly in their physicochemical characteristics and species composition (Rautio, 2001), we grouped the ponds into those lying above ($n$ = 6) and below ($n$ = 4) the tree line to examine the effect of altitude on resource quality.

## Results

All of the ponds in this study fell within expected ranges for oligotrophic, tundra alpine waters in terms of chl-*a* and DOC contents (Kalff, 2001). These ponds also showed clear trends along the altitudinal gradient, with DOC (Table 1) decreasing linearly with altitude ($r^2$ = 0.53, d.f. = 9, $P$ = 0.007) and conductivity values lower in the three highest ponds (<10 $\mu$S cm$^{-1}$, Table 1). In addition, benthic mats were found mainly below the tree line, while higher-altitude ponds had only thin epilithic algal cover. No other physical parameters were directly related to altitude or vegetation cover (Table 1).

Benthic mats had more chl-*a* and higher primary production than seston in all ponds (Table 2). Chl-*a* in benthic mats (0.7–41.8 mg m$^{-2}$) was an order of magnitude higher than in seston (0.3–2.8 mg m$^{-2}$). In five out of the 10 ponds, benthic mats contributed >90% of the potential maximum primary production. Benthic mats had similar proportions of algae compared with the bulk seston (3.5 ± 5% algal carbon in benthos and 2.6 ± 1% phytoplankton carbon in seston).

### Carbon transfer and consumer acquisition

Stable isotope analysis results revealed two distinct food sources (Fig. 1). Mean $\delta^{13}$C values of seston (−23 to −29‰) were significantly more depleted than those of benthic mats (−15 to −27‰; $P$ = 0.001, d.f. = 1). In addition, benthic mats had lower $\delta^{15}$N values than seston for eight out of the 10 ponds (Fig. 1a). The mean $\delta^{15}$N value of all pond benthic mats was 0.2‰, while seston $\delta^{15}$N values were, on average, 1.5 ‰.

**Table 2** Chlorophyll-a (chl-*a*) concentrations (mg m$^{-2}$) and primary production as $P_{max}$ (mg C m$^{-2}$ h$^{-1}$) in the water column and in benthic mats in 10 Finnish subarctic ponds

| | Pond no | Chl-*a* | | | Primary productivity ($P_{max}$) | | | Dissolved inorganic carbon mg C m$^{-3}$ |
|---|---|---|---|---|---|---|---|---|
| | | Seston | Benthic | % Benthic | Seston | Benthic | % Benthic | |
| Below tree line | | | | | | | | |
| Masi | 1 | 1.1 | 8.8 | 88.9 | 1.0 | 40.2 | 97.6 | 2900 |
| Boat | 2 | 2.8 | 41.8 | 93.7 | 8.7 | 7.8 | 47.4 | 7300 |
| Big rock | 3 | 0.9 | 1.8 | 67.1 | 3.7 | 6.4 | 63.6 | 6000 |
| Happy | 4 | 0.8 | 4.8 | 85.9 | 4.9 | 43.2 | 89.9 | 6400 |
| Above tree line | | | | | | | | |
| North Malla | 5 | 1.6 | 7.9 | 83.0 | 1.4 | 33.8 | 95.9 | 2500 |
| South Malla | 6 | 1.0 | 11.7 | 92.1 | 0.3 | 49.6 | 99.5 | 2500 |
| Reindeer | 7 | 1.2 | 8.8 | 88.4 | – | 3.3 | – | 3400 |
| Lampo | 8 | 2.3 | 2.8 | 54.8 | – | – | – | 1700 |
| Tuono | 9 | 1.0 | 3.7 | 78.6 | 0.2 | 11.6 | 98.4 | 2800 |
| Straca | 10 | 0.3 | 0.7 | 70.8 | 3.7 | 2.3 | 38.6 | 1200 |
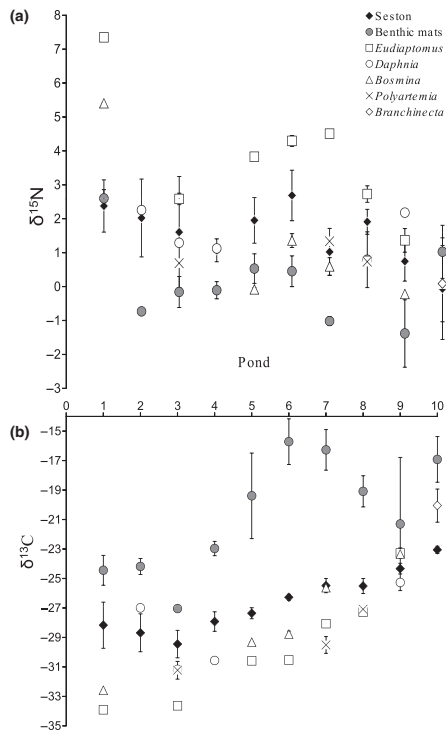
**Fig. 1** Mean (±SE) isotope values of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ for seston, benthic mats and zooplankton taxa for the ponds ordered by increasing altitude (1–10). Ponds 5–10 are above the tree line.

When each pond was evaluated independently, zooplankton $\delta^{13}C$ values (−20 to −33‰) were consistently more depleted than the respective seston values (Fig. 1b). Only four out of 19 populations had a $\delta^{13}C$ value higher than that of seston. The $\delta^{13}C$ values of *Daphnia* in pond 2 and *E. graciloides* and *Bosmina* in pond 9 were intermediate between seston and benthic values, suggesting consumption of both phytoplankton and benthic algae. In all other ponds where *Daphnia* (three ponds), *Bosmina* (four ponds) and *E graciloides* (five ponds) were present, their $\delta^{13}C$ values were lower than the respective seston value, indicating little to no consumption of benthic algae. Most zooplankton had higher $\delta^{15}N$ values than either

potential food source (Fig. 1a), with the exception of *Bosmina* sp., which generally had lower $\delta^{15}N$ values than seston. The enrichment in the zooplankton $\delta^{15}N$ values was especially pronounced in *E. graciloides*, which had a higher $\delta^{15}N$ value than both food sources (by up to 4.9‰ from benthic values).

*Fatty acids and consumer retention*

Analysis of the FA composition revealed distinct differences in resource quality between benthic and pelagic sources. Seston contained eight times more total lipids than benthic mats per unit DM. PUFA were consistently lower in benthic mats (1.2 ± 0.7 $\mu g\ mg^{-1}$ DM) than in seston (9.9 ± 5.7 $\mu g\ mg^{-1}$ DM). Notably, the PUFA docosahexaenoic acid (DHA; 22:6ω3) and arachidonic acid (ARA; 20:4ω6) were entirely absent from the benthic mats, while present in the seston of each pond. From the smaller-size fractions of the seston, the DOC and bacterial fraction contained no PUFA or any ω3 FA (Table 3). In the bacteria fraction, the FA found were mostly non-source specific (e.g. C16:0). Zooplankton FA concentrations differed when compared with their resources. From the suite of PUFA analysed, zooplankton had higher concentrations of the following PUFA than their dietary resources: linoleic acid (LIN; 18:2ω6), α-linolenic acid (ALA; 18:3ω3), stearidonic acid (SDA; 18:4ω3), ARA, eicosapentaenoic acid (EPA; 20:5ω3) and DHA (Table 4). The only monounsaturated fatty acid found at higher concentrations in zooplankton than in seston or benthic mats was vaccenic acid (18:1ω7). The observed high PUFA retention made PUFA the most prevalent group of FA in zooplankton, closely followed by saturated fatty acids (SAFA; Table 4).

Comparing the difference in FA concentrations between zooplankton and their resources provides a means of quantifying consumer lipid retention. Thus, we calculated PUFA retention ratios, by dividing PUFA concentrations of zooplankton by PUFA concentrations of their potential diet [i.e. $(PUFA)_{zooplankton}/(PUFA)_{diet}$], to assess the trophic relationship between dietary PUFA supply and PUFA retention in the consumer. When comparing retention ratios between zooplankton and seston or benthic sources, all zooplankton had the smallest PUFA retention ratios from seston (by a multiple of 10), suggesting that seston supplies more PUFA to herbivorous consumers than does the benthic mat.

**Table 3** Mean concentration ($\mu$g mg$^{-1}$ dry mass) of individual fatty acid food resources: DOC (<0.2 $\mu$m), the bacterial fraction (0.7–0.2 $\mu$m), benthic organic matter and seston (>0.7 $\mu$m) from 10 ponds below and above the tree line in subarctic Finland. Only lipids accounting for >0.5 $\mu$g mg$^{-1}$ are shown. Mean total fatty acid percentages included. Below the tree line $n = 4$, above $n = 6$

| Fatty acids | DOC <0.2 $\mu$m Below | Above (n = 5) | Mean | % | Bacteria (0.2–0.7 $\mu$m) Below (n = 3) | Above (n = 5) | Mean | % | Benthic mats Below | Above | Mean | % | Seston Below | Above | Mean | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SAFA | 0.3 | 0.8 | 0.6 | 58 | 1.1 | 1.7 | 1.5 | 48 | 3.2 | 2.6 | 2.9 | 41 | 34.2 | 36.8 | 35.8 | 57 |
| MUFA | 0.2 | 0.3 | 0.2 | 24 | 1.2 | 1.5 | 1.4 | 45 | 2.4 | 1.8 | 2.0 | 29 | 17.2 | 16.9 | 17 | 27 |
| PUFA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 1.2 | 1.1 | 1.2 | 17 | 8.9 | 10.6 | 9.9 | 16 |
| 20–22C PUFA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0.9 | 0.3 | 0.5 | 7 | 4.6 | 3.3 | 3.8 | 6 |
| LIN | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0.2 | 0.1 | 2 | 2.7 | 2.9 | 2.9 | 5 |
| ALA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0.1 | 0.1 | 0.1 | 1 | 1.7 | 2.8 | 2.3 | 4 |
| SDA | – | – | – | – | – | – | – | – | 3 | 6.5 | 3.8 | 0 | 5.2 | 7.7 | 4.3 | 1 |
| ARA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0.5 | 0.3 | 0.4 | 1 |
| EPA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0.2 | 0.1 | 2 | 1.2 | 1.6 | 1.4 | 2 |
| DHA | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 1.1 | 0.8 | 0.9 | 1 |
| $\omega$-6 | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| $\omega$-3 | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0 | 0.6 | 0.5 | 0.6 | 8 | 4 | 5.3 | 4.8 | 8 |
| Total lipids | 0.7 | 1.3 | 1.0 | 100 | 2.5 | 3.4 | 3.1 | 100 | 7.4 | 6.7 | 7.0 | 100 | 55.9 | 66.8 | 62.5 | 100 |

ALA, $\alpha$-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; LIN, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SDA, stearidonic acid.

**Table 4.** Mean concentration ($\mu$g mg$^{-1}$ dry mass) of individual fatty acids in zooplankton from 10 ponds below and above the tree line in subarctic Finland. Only lipids accounting for >0.5 $\mu$g mg$^{-1}$ are shown. Mean total fatty acid percentages included for the zooplankton

| Fatty acids | Eudiaptomus Below | Above | Mean | % | Daphnia Below | % | Bosmina Below | Above | Mean | % | Polyartemia Below | Above | Mean | % | Branchinecta Above | % | Zooplankton Bulk | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SAFA | 50.4 | 62.5 | 56.4 | 36 | 39.6 | 39 | 39.3 | 40 | 39.9 | 41 | 20.5 | 10.1 | 18.3 | 25 | 13.6 | 23 | 40.6 | 36 |
| MUFA | 22.0 | 23.1 | 22.5 | 14 | 29.4 | 29 | 24.4 | 26 | 25.8 | 26 | 22.0 | 9.8 | 18.3 | 25 | 18.1 | 30 | 24.8 | 22 |
| PUFA | 55.2 | 78.7 | 67.0 | 43 | 29.4 | 29 | 23.8 | 32 | 30.1 | 31 | 33.5 | 19.0 | 32.6 | 45 | 26.8 | 44 | 42.7 | 38 |
| 20–22C PUFA | 33.2 | 49.5 | 41.4 | 26 | 16.9 | 17 | 14.8 | 18 | 17.6 | 18 | 20.5 | 13.4 | 22.1 | 31 | 15.7 | 26 | 26.1 | 23 |
| LIN | 7.0 | 10.4 | 8.7 | 6 | 5.4 | 5 | 6.0 | 5 | 5.3 | 5 | 4.9 | 1.5 | 3.6 | 5 | 4.8 | 8 | 6.4 | 6 |
| ALA | 9.1 | 10.6 | 9.9 | 6 | 5.1 | 5 | 3.0 | 5 | 4.6 | 5 | 7.2 | 1.8 | 5.1 | 7 | 4.9 | 8 | 6.6 | 6 |
| SDA | 8.6 | 4.3 | 3.2 | 2 | 3.8 | 4 | 2.1 | 0 | 8.4 | 6 | 1.8 | 0.4 | 4.6 | 3 | 2.8 | 4 | 2.4 | 4 |
| ARA | 3.0 | 3.4 | 3.2 | 2 | 4.3 | 4 | 5.2 | 6 | 5.8 | 6 | 3.2 | 1.1 | 2.2 | 3 | 2.7 | 4 | 4.1 | 4 |
| EPA | 10.3 | 16.2 | 13.3 | 8 | 11.4 | 11 | 8.7 | 11 | 10.6 | 11 | 12.9 | 7.6 | 13.0 | 18 | 10.7 | 18 | 12.5 | 11 |
| DHA | 17.2 | 25.8 | 21.5 | 14 | 0.2 | 0 | 0.9 | 1 | 1.0 | 1 | 3.3 | 3.8 | 5.0 | 7 | 1.5 | 2 | 7.8 | 7 |
| $\omega$-6 | 1.3 | 1.3 | 1.3 | 1 | 0.4 | 0 | 0.0 | 1 | 0.4 | 0 | 0.5 | 0.0 | 0.1 | 0 | 0.0 | 0 | 0.6 | 1 |
| $\omega$-3 | 37.6 | 53.9 | 45.8 | 29 | 17.0 | 17 | 12.6 | 18 | 16.6 | 17 | 23.8 | 13.4 | 23.4 | 32 | 17.3 | 28 | 27.4 | 24 |
| Total lipids | 133.8 | 179.6 | 156.7 | 100 | 101.3 | 100 | 87.6 | 100 | 98.2 | 100 | 78.9 | 40.8 | 72.3 | 100 | 60.6 | 100 | 113.4 | 100 |

ALA, $\alpha$-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LIN, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SDA, stearidonic acid.

Fatty acid retention varied significantly between zooplankton taxa (Fig. 2). While all taxa had higher PUFA concentrations than either benthic or seston resources, cladocerans (*Daphnia* and *Bosmina* sp.) retained lower concentrations of DHA relative to their pond-specific seston source (Fig. 2). Of all PUFA,
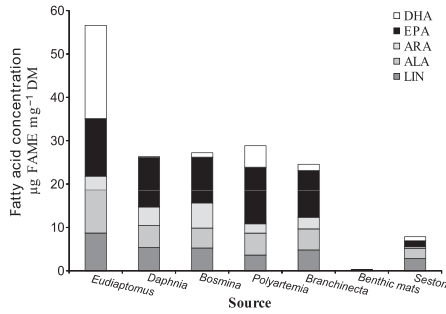
**Fig. 2** Mean concentrations of essential fatty acid in *Eudiaptomus graciloides*, *Daphnia* sp., *Bosmina* sp., *Polyartemia forcipata* and *Branchinecta paludosa* and in their two potential food sources, benthic mats and seston. DHA, docosahaexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; ALA, α-linolenic acid; LIN, linoleic acid. Benthic mats contained 0.2 $\mu$g mg$^{-1}$ DM (the detection limit) only of LIN, ALA and EPA.

cladocerans retained EPA most efficiently (11 ± 2.4 $\mu$g mg$^{-1}$ DM). For *E. graciloides*, a third of all their PUFA was DHA (21.5 ± 2.6 $\mu$g mg$^{-1}$ DM; Fig. 2), which is substantially higher than the average zooplankton DHA values excluding *E. graciloides* (mean 1.9 ± 2.1 $\mu$g mg$^{-1}$ DM).

*Elevation gradient*

The altitudinal gradient contributed to differences in $\delta^{13}$C values of seston, which increased with increasing altitude ($r^2 = 0.68$, $P = 0.002$, d.f. = 9). This trend was mirrored by zooplankton $\delta^{13}$C values that also increased with altitude ($r^2 = 0.68$, $P < 0.001$, d.f. = 17).

All PUFA, except SDA, were absent from benthic mats below the tree line. In mats above the tree line, SDA doubled and trace amounts of ALA, LIN and EPA were present, although, nevertheless, the overall FA concentration was very low (Fig. 3a). For seston, there were only slight increases in PUFA concentrations above the tree line, although ARA and DHA decreased (Fig 3b). Altitude, specifically the tree line, affected FA concentrations of benthic mats more than it did for seston (Table 3).

The taxonomic composition of zooplankton and their FA retention also changed with altitude. Cladocerans were primarily found in ponds below the tree line, and *B. paludosa* only above the tree line. *Eudiaptomus graciloides,* which was the only taxon found in ponds both above and below the tree line, contained significantly higher total lipid concentrations (Fig. 3c, Table 4) in ponds above the tree line (Wilcoxon signed-rank test: $P < 0.05$, d.f. = 5). In
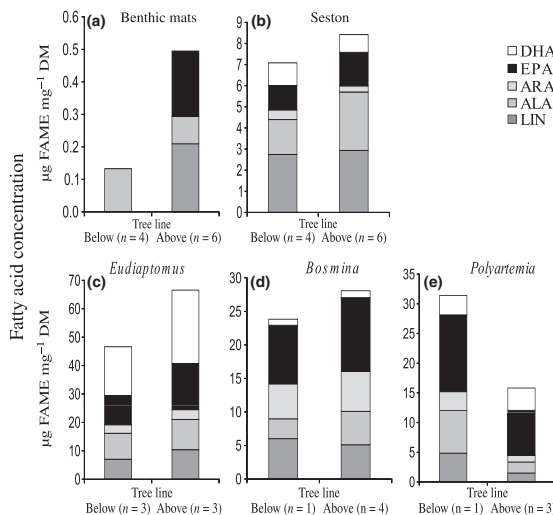


**Fig. 3** Mean essential fatty acid concentrations in (a) benthic mats, (b) seston and in three prevalent zooplankton taxa: (c) *Eudiaptomus* (d) *Bosmina* and (e) *Polyartemia* from ponds above ($n = 6$) and below ($n = 4$) the tree line. Note the different vertical scales. Docosahexaenoic acid: 22:6ω3; eicosapentaenoic acid: 20:5ω3; arachidonic acid: 20:4ω6; α-linolenic acid: 18:3ω3; linoleic acid: 18:2ω6.

addition, not only the amount of PUFA in *E. graciloides* and *Bosmina* increased above the tree line but highly unsaturated fatty acids were retained 2–16 times more, relative to the resource from which they were acquired (Fig. 3c,d. *Polyartemia* was the only taxon to show a lower PUFA concentration above the tree line (Fig. 3e).

## Discussion

Our results suggest that metazoan zooplankton from these subarctic ponds obtain their diet predominantly from pelagic resources. In general, benthic mats had extremely low concentrations of FA, with DHA and ARA lacking entirely. According to FA analysis, the pelagic resource was more likely to provide zooplankton with essential FAs than the benthic mats. Similarly, $\delta$ $^{13}C$ values showed that zooplankton carbon signals were more similar to those of seston than of the benthic mats. Only four out of a total of 20 zooplankton populations showed any sign of benthic feeding. Although highly productive and with a high biomass, benthic mats showed no clear nutritional advantage over the pelagic resources for supplying metazoan consumers, based on the carbon isotope and FA analyses. Our results indicate therefore that benthic mats are less important as a food source than the low-density phytoplankton, than suggested elsewhere (Hansson & Tranvik, 2003; Rautio & Vincent, 2006, 2007).

Stable isotope analysis was able to differentiate between benthic mats and pelagic seston. Benthic mats had heavier isotopic carbon signals than seston, probably due to a boundary layer effect in less-turbulent bottom waters with the resultant $CO_2$ limitation causing less $^{13}C$ fractionation (higher $\delta^{13}C$ values) during photosynthesis (France, 1995; Hecky & Hesslein, 1995). Microscopic examination of phytoplankton and benthic mats in these ponds has shown that the communities are distinct, with benthic taxa rarely found in suspension (L. Forsström, unpubl. data). A similar distinction has been reported for other northern ponds (Bonilla, Rautio & Vincent, 2009). If appreciable resuspension of benthic taxa or sedimentation of planktonic taxa was present in our ponds, the physical coupling between the benthic and pelagic environments would have been reflected in more similar stable isotope signatures for the two resources.

Zooplankton isotopic signals were consistently more depleted than either bulk potential food resource, indicating preferential feeding on the isotopically light, photosynthetic portion of the POM (Kankaala *et al.*, 2006; Rautio & Vincent, 2007). *Branchinecta*, regarded as a benthic feeding species (Bertilsson *et al.*, 2003), had a carbon signal indicating that pelagic algae provided up to 50% of its diet (Fig. 1b; Pond 10). The FA composition of *B. paludosa* also suggests that this benthic feeder used sestonic food; EPA found in *B. paludosa* was absent in benthos, although it is not known whether *B. paludosa* can convert precursor FA to EPA. Since there were no pelagic grazers found in this pond, sedimentation of pelagic algae is a plausible explanation of how *Brachinecta* was able to access pelagic resources. Consistent with the $\delta^{13}C$ results, the $\delta^{15}N$ value of *Brachinecta* is intermediate between seston and the benthic mats, indicating a mixed diet; however, the bulk nature of measured samples may mask the $\delta^{15}N$ value of the specific food items. Overall, it was clear from the $\delta^{15}N$ values that most zooplankton had a 1–4‰ trophic enrichment from the seston, indicating pelagic resource selection.

Using mixing models with the bulk data to determine the relative mixture of the resources to zooplankton diets was not possible for all communities, because most of the consumers lay outside either food source isotope value. We acknowledge that the mixture of edible and non-edible carbon sources in bulk resource samples causes inherent problems in linking consumer and food source isotope values. Calculating the edible proportion of the seston requires a calculation of $\delta^{13}C_{algae}$, but this is highly sensitive when bulk seston contains <20% POC (Marty & Planas, 2008). In the end, bulk seston entailed fewer assumptions than the algal correction calculations for linking consumers to food source.

Fortunately, by incorporating FA analysis, a more comprehensive determination of food web interactions could be obtained. Zooplankton accumulated dietary PUFA efficiently, as was clear from the higher concentrations of PUFA in zooplankton biomass than in either resource. In contrast, SAFA concentrations of zooplankton were in general similar than in resources, indicating no trophic accumulation. Our values of zooplankton FA content values are comparable with ranges compiled from several studies and summarised by Brett *et al.* (2009). The only non-PUFA that was

retained at higher concentrations in all the consumers compared with the food sources was 18:1ω7, a FA found in heterotrophic bacteria (Vainshtein, Hippe & Kroppenstedt, 1992). For physiological reasons that are not clear, the retention of 18:1ω7 in zooplankton suggests that the consumers preferentially retained this FA over others detected in bacteria. It is likely that the bacteria of subarctic ponds contribute some energy to zooplankton, as has been shown with SIA and direct feeding experiments for waterbodies ranging from humic lakes (Taipale *et al.*, 2008) to subarctic ponds (Rautio & Vincent, 2006). However, it is unlikely that bacteria supply dietary PUFA that are required for cell membranes and somatic growth of zooplankton (Brett *et al.*, 2009). The lack of PUFA in the bacterial and DOC fraction of the seston indicates that the FA found in zooplankton are derived from POM (0.7–50 $\mu$m).

Since FA composition differed among zooplankton species, the potentially growth-limiting substance may have also differed from species to species (Persson & Vrede, 2006; Smyntek *et al.*, 2008). Both those studies showed that cladocerans preferentially retain EPA, while copepods accumulate more DHA than EPA. Similarly in our study, the main taxon-specific difference in FA retention was between *Daphnia* and *Eudiaptomus*, showing that *Daphnia* did not retain DHA, yet contained nearly twofold higher concentrations of SAFA, EPA and ARA than in Persson & Vrede's (2006) study of high-latitude ponds. Conversely, *E. graciloides* contained 20 times more DHA than the concentration in the seston. In addition, *Eudiaptomus* had high concentrations of EPA, but to a lesser extent than *Daphnia*. This preference for specific FA is based on differences in life history strategies and evolved physiological adaptations (Brett *et al.*, 2006; Persson & Vrede, 2006). For instance, species have particular adaptations to cope with certain environmental conditions (e.g. cold), which is the impetus to regulate metabolically different concentrations of specific FA (Smyntek *et al.*, 2008).

The differences in FA profiles between seston and benthic mats may be attributed to differences in FA composition of algal populations. Flagellates, including Chrysophyta, Cryptophyta and Dinophyta, dominate the phytoplankton of northern Finland (Forsström, Sorvari & Korhola, 2005). However, benthic mats are known to be composed primarily of Cyanobacteria, Chlorophyta and Dinophyta (L. Forsström, pers. comm), which are also frequently found in benthic mats of other regions (Bonilla *et al.*, 2009). Cyanobacteria synthesise very little EPA or DHA (Brett *et al.*, 2006, 2009). In addition, our FA and $\delta^{15}N$ value results from benthic mats are both consistent with the presence of nitrogen-fixing cyanobacteria; neither DHA nor ARA was detected in the benthic mats, and near-zero $\delta^{15}N$ values of the benthos are consistent with values for nitrogen-fixing cyanobacteria (Vincent, 2000). Zooplankton are known to discriminate against cyanobacteria (Sarnelle, Gustaffsson & Hansson, 2010). Diatoms and cryptophytes readily synthesise PUFA, whereas green algae and cyanobacteria produce only shorter-chain PUFA (Brett *et al.*, 2009).

The amount of accumulated benthic algae varied between ponds; highly productive, thick filamentous mats were mainly found in ponds below the tree line. Despite their high biomass, there was no indication of more consumer reliance on their benthic mats than in those above the tree line. Rather, zooplankton $\delta^{13}C$ values reflected changes in seston rather than in the benthic mats along the altitudinal gradient. Similarly, Rautio & Vincent (2007) showed that, in Canadian subarctic and arctic ponds, zooplankton often had a $\delta^{13}C$ value closer to the seston than to the benthos, although feeding on both food resources was more common in the communities they studied.

As we hypothesised, both food resources have higher-quality FA at higher altitudes. PUFA concentration is inversely correlated with trophic status (Müller-Narvarra *et al.*, 2004), which implies that phytoplankton will have more PUFA at higher altitudes, as is evident in our study. Increasing altitude accentuates seasonal constraints, making it necessary, also for consumers, to maximise their retention of high-quality carbon. Coping with seasonal constraints is directly related to the need to maintain fluidity in cold temperatures (Schlechtriema, Arts & Zellmer, 2006) and maintain efficient growth and reproduction in a shorter growing season, which requires EPA (Müller-Narvarra *et al.*, 2000). Therefore, the higher PUFA concentration found in zooplankton at higher altitudes is partly due to the food sources having higher quality and partly from consumers' selective retention.

Zooplankton have evolved the ability to balance their individual metabolic needs in harsh environ-

mental conditions for optimal success. Specifically, this could involve selective feeding (i.e. whether they access benthic resources), an example of behavioural plasticity. Additionally, consumers can metabolically select how the energy acquired is sequestered within the soma (Müller-Navarra, 2008; Smyntek *et al.*, 2008). This metabolic plasticity could be the cause of the higher zooplankton PUFA concentrations above the tree line. Higher concentrations of EPA, DHA and SDA relative to resources in *E. graciloides* demonstrated preferential retention of these PUFA, and this was most pronounced in ponds above the tree line. These biochemical adaptations for selecting and retaining desired FA offer a mechanism by which consumers can enhance their survival. Further understanding of energy acquisition and selective PUFA retention is needed to follow more accurately the dynamics of subarctic food web structure and function. For example, consumer competitive relationships and species composition are affected by resource quality, particularly when the acquisition of nutritional requirements has consequences for somatic growth and reproductive success of consumers (Persson & Vrede, 2006).

In conclusion, our field study has shown that the phytoplankton in the seston probably provides zooplankton in subarctic ponds with essential dietary lipids in sufficient quantities to meet demand, even in late summer. The pelagic resource contained the dietary PUFA required in greater concentrations than benthic resources. In addition, higher retention of PUFA and ω3 FA in *E. graciloides* in ponds above the tree line provides field evidence of metabolic regulation of these physiologically important lipids.

## Acknowledgments

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# II

# SEASONAL SHIFTS BETWEEN AUTOCHTHONOUS AND ALLOCHTHONOUS CARBON CONTRIBUTIONS TO ZOOPLANKTON DIETS IN A SUBARCTIC LAKE

by

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# Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake

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*Abstract*

We addressed the extent to which autochthonous and allochthonous carbon sources contribute to zooplankton diet throughout the year in oligotrophic subarctic Lake Saanajärvi. Optical measures of dissolved organic matter (DOM) indicated high water discharge and associated terrestrial DOM during winter and low inputs in summer. Bulk particulate organic matter (POM) showed $\delta^{13}$C values consistent with allochthonous inputs of DOM. The most positive POM $\delta^{13}$C values ($-27‰$) occurred during winter, when heterotrophic bacteria and nanoflagellates peaked in abundance; the isotopically lighter autotrophic phytoplankton shifted the POM $\delta^{13}$C to $-29‰$ in summer. The $\delta^{13}$C values of zooplankton were up to 4‰ more negative than those of POM, most likely because detritus and other nonliving material with higher $\delta^{13}$C values comprised 45–90% of POM. The $\delta^{13}$C of the cladoceran *Daphnia umbra* mirrored the trend of POM $\delta^{13}$C, indicating a dietary shift from heavy dependence on autochthonous carbon in summer to allochthonous or heterotrophic food in winter. The $\delta^{13}$C of the most abundant zooplankton in Lake Saanajärvi, *Eudiaptomus graciloides* and *Cyclops abyssorum*, remained low and constant in winter, suggestive of little or no food intake during the 8 months of winter. There are significant differences in the zooplankton utilization of nonphytoplankton carbon in different seasons and among species. Although autochthonous carbon seemed to be mainly responsible for the growth of zooplankton, the ability to utilize allochthonous carbon-fueled microorganisms explains the survival of *Daphnia* through winter in northern lakes.

Phytoplankton-derived carbon and lipids are known to be essential for the somatic growth and reproduction of zooplankton and fish (Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000). For decades, aquatic food webs were taken as systems where carbon transfer was linear from phytoplankton to zooplankton to fish. From the discovery of the microbial loop (Azam et al. 1983) to key advances in methods for studying carbon sources (Porter and Feig 1981; de Souza Sierra et al 1997; Gannes et al. 1997), the role of nonphytoplankton carbon in the aquatic food webs has gained attention (Salonen and Hammar 1986; Hessen et al. 1990; Pace et al. 2004). Phytoplankton, although the primary source of certain essential fatty acids (EFA), is therefore no longer considered to be the sole carbon source for higher trophic levels. The mechanisms by which this nonphytoplankton carbon accumulates in zooplankton have been argued to range from direct absorption of dissolved organic carbon (DOC) by the consumer (Sorokin and Wyshkwarzev 1973) to trophic transfer whereby zooplankton feed on heterotrophic microbes that have been fueled with nonphytoplankton carbon (Jones 1992). There is growing evidence of substantial nonphytoplankton carbon accumulation in higher organisms in a variety of lakes (Grey et al. 2001; Cole et al. 2006; Karlsson and Säwström 2009). Particulate organic matter (POM) of terrestrial origin (allochthonous carbon) has been argued to be the key factor controlling whole-lake productivity in lakes where phytoplankton productivity is low (4.4–13.1 mg C m$^{-2}$ d$^{-1}$; Carpenter et al. 2005; Ask et al. 2009). In shallow lakes, the high production of benthic algae and associated heterotrophic organisms may provide an alternative carbon source for zooplankton (Hansson and Tranvik 2003; Rautio and Vincent 2006).

Lakes that are governed seasonally by light, ice cover, and, in consequence, primary productivity also show seasonal shifts in higher organisms. The classic paradigm of polar ecosystems holds that most biological processes slow down or cease during the dark polar winter. Many zooplankton have been observed to be strict diapausing species that disappear from the water column into sediments during winter (Nilssen and Elgmork 1977; Ventura and Catalan 2005; Larsson and Wathne 2006). There are, however, deviations from this pattern. In the 1970s, Rigler and MacCallum (1974) showed that the population density of *Limnocalanus macrurus* peaked in February under the ice in Char Lake (74°N) in High Arctic Canada. Subsequently, further reports appeared about high densities of winter zooplankton, including copepods and cladocerans (e.g., Rautio et al. 2000; Larsson and Wathne 2006). Evidence from oligotrophic lakes suggests that nonphytoplankton carbon would be especially important in sustaining zooplankton in winter, when phytoplankton are scarce in the water column (Grey et al. 2001; Karlsson and Säwström 2009). Although a complete understanding of lake carbon cycles is highly dependent on all seasons, winter remains a little explored season in limnology, and direct evidence regarding the

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Table 1. Ice thickness, temperature, dissolved organic carbon (DOC), particulate organic matter (POM), and composition of seston POM in the 0–10-m depth water layer. The carbon contribution of bacteria, heterotrophic nanoflagellates (HNF), and autotrophic algae is expressed as percentage (%) of POM.

| Date | Snow and ice thickness (cm) | Temperature (°C) | DOC (mg L$^{-1}$) | POM (mg L$^{-1}$) | Bacteria (%) | HNF (%) | Autotrophs (%) |
|---|---|---|---|---|---|---|---|
| 13 Nov 07 | 5; 13 | 0.6 | 2.2 | 125 | 5.5 | 5.9 | 7.6 |
| 29 Jan 08 | 25; 50 | 0.6 | 1.4 | 85 | 6.7 | 1.8 | 2.4 |
| 01 Apr 08 | 5; 70 | 0.9 | 1.6 | 83 | 11.9 | 13.7 | 2.4 |
| 21 May 08 | 5; 100 | 1.4 | 1.6 | 90 | 15.3 | 19.1 | 10.0 |
| 25 Jun 08 | 0; 0–20* | 2.5 | 1.9 | 125 | 13.5 | 29.9 | 23.2 |
| 31 Jul 08 | | 10.5 | 2.1 | 125 | 12.9 | 12.1 | 26.4 |
| 06 Sep 08 | | 7.6 | 2.2 | 205 | 10.8 | 6.2 | 18.8 |
| 17 Oct 08 | | 2.5 | 2.2 | 185 | 15.9 | 1.5 | 13.0 |

* Half of the lake was still covered in ice up to 20 cm thick.

feeding ecology of zooplankton in winter has remained elusive. Whatever zooplankton feed on in winter, the food source itself should not depend on light and photosynthesis, and zooplankton should be able to persist without this production for extended periods of time. Primary productivity in winter is zero or very low in lakes located north of the Arctic Circle (Kalf and Welch 1994). During this dark period, the potential food for zooplankton includes heterotrophic bacteria, nanoflagellates and ciliates, and mixotrophic algae that can switch from autotrophy to heterotrophy when there is a lack of light (Sanders and Porter 1988). The carbon source that supports the growth of the lower trophic levels in winter is thought to be dominated by allochthonous subsidies (Grey et al. 2001). In the absence of phytoplankton, zooplankton may also turn to feeding on benthic POM by grazing directly on the lake bottom or on resuspended benthic filaments (Hansson and Tranvik 2003; Rautio and Vincent 2007).

Stable isotopes have increasingly been used to describe food web structure, and in many systems, they have proved a powerful tool to relatively easily define feeding patterns. Differences in the isotopic signal between allochthonous and autochthonous carbon (Grey et al. 2001), and between autotrophic and heterotrophic food sources (Kankaala et al. 2006), have been used to trace the source and flow of carbon from one system and trophic level to another in the food web. Optical properties of dissolved organic matter (DOM) can be further used to characterize the origin of the carbon pool. Originally developed for studying ultraviolet radiation (UVR) attenuation in lakes, specific UV absorbance (SUVA) can also provide insights into the chemical composition of carbon and be used as a guide to the relative importance of autochthonous versus allochthonous carbon inputs to a lake (Weishaar et al. 2003; Jaffé et al. 2008).

We studied the autochthonous and allochthonous sources of carbon, and the relationships between zooplankton $\delta^{13}$C and the composition of POM in Lake Saanajärvi, located in subarctic Finland. Because of its northern location, there is great seasonal variability in autotrophic and heterotrophic productivity of the lake. To our knowledge, there exist no previous studies on the season-ality of DOM sources in northern lakes using optical properties of water. Furthermore, studies on seasonality of POM and zooplankton isotope signatures are still rare, especially in high-latitude lakes (Gu et al. 1999; Karlsson and Säwström 2009), which likely exhibit some of the largest seasonality of environmental variables in aquatic systems globally. The objectives of this study were therefore (1) to estimate seasonal shifts in the autochthonous and allochthonous carbon quantity in Lake Saanajärvi; (2) to measure variability in the putative autotrophic and heterotrophic zooplankton food sources; (3) to assess the relative contribution of these different food sources to $\delta^{13}$C of POM in different seasons and thus estimate the proportion of water-column allochthonous POM that is consumed by zooplankton; and (4) to examine interspecific differences in feeding strategies among zooplankton in Lake Saanajärvi.

Methods

Samples from Lake Saanajärvi (0.7 km², max. depth 24 m) were collected eight times between November 2007 and October 2008. The catchment of Lake Saanajärvi (4.6 km²) has vegetation dominated by low dwarf shrubs, mosses, grasses, and sedges. The lake is an oligotrophic clear-water lake with annual mean total phosphorus concentration < 3 $\mu$g L$^{-1}$ and DOC concentrations of 1.8 mg L$^{-1}$ (mean for 2007–2008). The lake has one main inlet stream, but several seasonal streams enter the lake as well, especially in early summer and late fall. Stream flow usually begins in late May and quickly reaches its peak flow. Mean annual precipitation is 459 mm, of which 60% occurs as snow (Drebs et al. 2002). Due to its northern location (69°05′N, 20°87′E), Lake Saanajärvi is ice covered for 8 months of the year, and it usually becomes ice free and thermally stratifies in early July for about 2 months (Sorvari et al. 2000). Ice cover, which may exceed 1 m, forms in late October–early November. Five of the eight sampling dates were during winter (Table 1). Bulk water samples were collected from the deepest point at the middle of the lake at 1-m intervals between 0 and 10 m using a 2-liter Limnos sampler and were mixed in equal parts to produce a single integrated sample. This water layer

represented the epilimnion during summer stratification. Another bulk sample was collected from 10–20 m, but these results are only briefly discussed here.

For DOM analysis, two 50-mL subsamples of water were filtered through 0.2-$\mu$m cellulose acetate filters, which were first rinsed with sample water to remove possible carbon leaching from the filter. Samples were stored at 4°C in acid-cleaned, glass bottles. One sample was used for determination of DOC concentration using a Shimadzu Total Organic Carbon analyzer (model TOC-Vcph) at the North Ostrobothnia Regional Environmental Centre. The other sample was used for optical quality measures of DOM. In the latter, the absorption was measured every 1 nm over the wavelength ($\lambda$) range 250–850 nm using a 10-cm quartz cuvette in a Cary 300 UV-Vis spectrophotometer (Varian) to determine the specific UV absorbance (SUVA) at 254 nm (SUVA$_{254}$). The SUVA$_{254}$ parameter is defined as the UV absorbance at 254 nm measured in inverse meters (m$^{-1}$) divided by the DOC concentration (mg L$^{-1}$). Increasing SUVA indicates greater contribution of terrestrially derived DOM (Hood et al. 2003, 2005).

POM was concentrated from 2–6-liter samples that were passed through a 50-$\mu$m sieve to remove zooplankton, and it was then collected on precombusted and preweighed 47-mm Whatman GF/F filters and freeze dried. Chlorophyll $a$ (Chl $a$) was determined fluorometrically after filtering a 500-mL subsample of water through a Whatman GF/F filter and extracting the Chl $a$ in 95% ethanol. Another 500-mL subsample was preserved with Lugol's iodine for phytoplankton counting using Utermöhl sedimentation and inverted microscopy. Water samples for bacterial enumeration were preserved in 2% (final concentration) formaldehyde. Sample preparation for microscopy involved staining 4 mL of sample with nucleic acid–staining fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) with a final concentration of 5 $\mu$g mL$^{-1}$ for 1 h, followed by filtration through a 0.2-$\mu$m black Nucleopore membrane. Filters were mounted on slides with immersion oil and frozen (−20°C) until examination at 1000× magnification with UV excitation. Picophytoplankton and nanoflagellates were counted from a sample that was prepared for microscopy by filtering 20–50 mL of DAPI-stained water through a 0.6-$\mu$m black polycarbonate membrane. Slides were frozen until examination at 1000× using a Leica Leitz fluorescence microscope. Green excitation was used to discriminate between colorless heterotrophic nanoflagellates (HNF) and autotrophic cells with plasts and pigments. All water analyses were carried out in triplicates, except for Chl $a$ and phytoplankton counts, which were based on single samples.

POM was converted to carbon biomass using the weight difference of the filter before and after the POM addition, and the percentage of carbon in the sample was obtained from the combustion and elemental analysis stage that precedes stable isotope analysis (SIA) in the mass spectrometer. Bacterial carbon biomass was calculated using an average measured cell volume of 0.1 $\mu$m³ and a volume–biomass conversion factor of 0.308 pg C $\mu$m$^{-3}$ (Fry 1988). Heterotrophic nanoflagellates were enumerated to four different size categories (diameter 2, 2–5, 5–10, and

> 10 $\mu$m) to allow volume estimation and subsequent carbon conversion using a conversion factor of 0.22 pg C $\mu$m$^{-3}$ (Børsheim and Bratbak 1987). Picoautotroph carbon biomass was calculated using the average cell volume of 4.2 $\mu$m³ and the same carbon conversion factor as for nanoflagellates. Due to a loss of some phytoplankton samples, Chl $a$ was used to estimate the carbon biomass in autotrophic organisms, instead of phototrophic nanoflagellate and phytoplankton counts. Algal volumes in July phytoplankton gave a C : Chl $a$ value of 40, and this conversion factor was applied to all Chl $a$ data to calculate the carbon in the phytoplankton community.

Crustacean zooplankton were collected in bulk by vertical tows through 0–10-m and 0–20-m depths using a 200-$\mu$m net, and individuals were counted with a binocular microscope to obtain relative species composition. The sample was left overnight in tap water to allow for gut evacuation. Live zooplankton were then sorted manually using a fine pipette and needle-sharp forceps, and individual species were separated. Approximately 100 *Eudiaptomus graciloides*, 150 *Cyclops abyssorum*, and 50 *Daphnia umbra* specimens were collected for each $\delta^{13}$C sample, stored in three replicates, when possible, in 2.5-mL vials, and frozen. The samples were lipid extracted because greater lipid content lowers $\delta^{13}$C values, which can mask the carbon signature of the diet, and this is especially critical for species that accumulate lipid storages during the growing season to cope with winter, as is the case with the zooplankton studied here (Syväranta and Rautio 2010). After lipid extraction, the samples were oven dried at 60°C before analysis for stable isotopes.

Carbon isotopic analysis was carried out using a FlashEA 1112 elemental analyzer (Thermo Fisher Scientific) coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer. POM samples collected on Whatman GF/F filters were scraped away from the glass fiber before combustion. Pike (*Esox lucius L.*) white muscle tissue was used as an internal working reference with a known relation to the international standard of PeeDee belemnite for carbon. Results are given as $\delta^{13}$C, where

$$\delta^{13}C = ((^{13}C : ^{12}C_{sample}/^{13}C : ^{12}C_{reference}) - 1) \times 1000 \quad (1)$$

Internal precision was usually < 0.2‰.

## Results

Zooplankton in Lake Saanajärvi were dominated for most of the year by the calanoid copepod *Eudiaptomus graciloides*, followed by the cyclopoid *Cyclops abyssorum*, and the cladoceran *Daphnia umbra* (Fig. 1); this is consistent with previous studies of Lake Saanajärvi plankton (Rautio et al. 2000). In Lake Saanajärvi, *E. graciloides* has a 1-yr life cycle, with adults maturing in autumn and copulating in early winter; the eggs are hatched between April and May, and soon after the adults die. In contrast, *C. abyssorum* in the lake has a 2-yr life cycle, with adults maturing in early summer and reproducing in midsummer; they pass their first winter as early
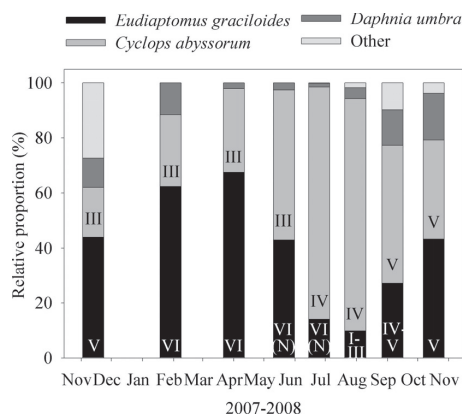
Fig. 1. The composition of zooplankton (> 200 μm) in Lake Saanajärvi in 2007–2008. The dominant developmental stage in the sample is shown for *Eudiaptomus graciloides* and *Cyclops abyssorum*: N = nauplia, I–III = copepodites, IV–V = copepodites, and VI = adults. The presence of nauplia is expressed in parentheses because they were not encountered in the samples taken with a 200-μm net. Group "other" is made of cladocerans *Bosmina* sp., *Holopedium gibberum*, and *Polyphemus pediculus*.

copepodites, which grow to late copepodite stages during the second summer and pass the second winter in this stage before maturing to adults during the following summer. Since our sampling was conducted during the first winter and second summer of the *C. abyssorum* life cycle, we only had copepodites in our samples (Fig. 1). *D. umbra* individuals in Lake Saanajärvi reproduce parthenogenetically throughout the year, including winter, and ephippia are found only rarely; the population density peaks in late summer.

Characteristics of the DOM pool of Lake Saanajärvi allowed us to estimate the autochthonous versus allochthonous origin of carbon in the lake. The optical index used, SUVA, showed clear seasonal shifts (Fig. 2a), with high values under the ice, indicating a greater influence of allochthonous carbon. The highest value (3.5) was measured in late May at the time of extensive spring melting in the catchment area.

The autotrophic organisms exhibited a distinct seasonality, which is typical for lakes where light milieu shows strong annual variability. Because of light limitation, Chl *a* and autotrophic algal abundance were very low under the ice in winter, but values grew steadily during spring (starting in May, a month before ice out) and summer to a single peak in September, and then declined rapidly (Fig. 2b). In contrast, heterotrophic bacteria and nanoflagellates had an annual maximum under the ice in April–June (Fig. 2c). Conversion of bacteria, heterotrophic nanoflagellate and picophytoplankton biovolumes, and larger phytoplankton Chl *a* to carbon biomass (Table 1) allowed us to estimate their contribution to POM, thereby

giving an estimation of the potential food composition of zooplankton on a seasonal basis. Throughout the year, POM was dominated by detritus and other nonliving material, with a contribution that varied between 45% and 90%. Living cells were especially sparse in the POM in early and midwinter, when the autotrophic cells made up 2–8% of the POM and the heterotrophic cells about 8% (Fig. 2d). In terms of zooplankton feeding, the situation was most favorable in mid- and late summer, when the algal community was at its maximum and autotrophic cells contributed > 25% to the POM.

The $\delta^{13}C$ of POM from the surface waters (0–10 m) showed pronounced seasonal variation during the study period (Fig. 2e):$\delta^{13}C$ of POM ranged from $-26.7‰$ in late winter to $-28.7‰$ during the maximum algal abundance in summer (Fig. 2c). During the open-water period, the changes were correlated with the seasonal change of Chl *a* (Pearson correlation coefficient: $-0.914$), although this correlation was not statistically significant ($p = 0.124$). During early and midwinter (November to April), the POM $\delta^{13}C$ values responded to the abundance of bacteria, HNF, and the proportion of heterotrophs in POM, with POM $\delta^{13}C$ values increasing from $-28.5‰$ to $-26.8‰$, along with the increasing proportion of heterotrophic and decreasing proportion of autotrophic organisms in the water column (Table 1; Fig. 2d). The $\delta^{13}C$ values of POM from the bottom waters (10–20 m) were similar to the surface $\delta^{13}C$ values during winter, but they were approximately two units more positive during the growing season between May and October (data not shown), suggestive of carbon derived primarily from heterotrophic organisms and detritus and lacking the lighter $\delta^{13}C$ of autotrophic organisms found more frequently in the summer epilimnion.

The $\delta^{13}C$ values of zooplankton samples were the same for the surface (0–10 m) and whole-water-column samples (0–20 m) (*t*-tests, $p > 0.1$ for all species) and were therefore pooled. The $\delta^{13}C$ of *Daphnia umbra* was closely associated with the large-scale seasonal changes in $\delta^{13}C$ POM (Fig. 3). During the winter, the values became more positive, mirroring the trend in $\delta^{13}C$ POM (Pearson correlation coefficient: 0.546) and reflecting the increase of bacteria and HNF in the food pool. From May to June, *D. umbra* exhibited a 3.7 unit decrease in $\delta^{13}C$ from $-26.7‰$ to $-30.4‰$, which occurred concurrently with the increase of autotrophic algae in the POM. During the summer, the *D. umbra* $\delta^{13}C$ value stayed between $-30.5‰$ and $-31.4‰$ and was negatively correlated with the changes in $\delta^{13}C$ POM (correlation coefficient: $-0.995$, $p < 0.05$). In contrast, the carbon isotope ratios of the two copepod species showed a different pattern, where $\delta^{13}C$ values stayed constant from the beginning to the end of winter in both *E. graciloides* ($-29.4‰$ to $-30.0‰$) and *C. abyssorum* ($-29.3‰$ to $-29.9‰$) (Fig. 3). Therefore, they diverged from the trend that was seen for POM $\delta^{13}C$ (correlation coefficients: 0.008 and 0.483 for *E. graciloides* and *C. abyssorum*, respectively), suggestive of copepod discrimination against the POM food pool. Only at the beginning of the growing season, when autotrophic algae increased in the POM, did the copepods respond to the changes in POM
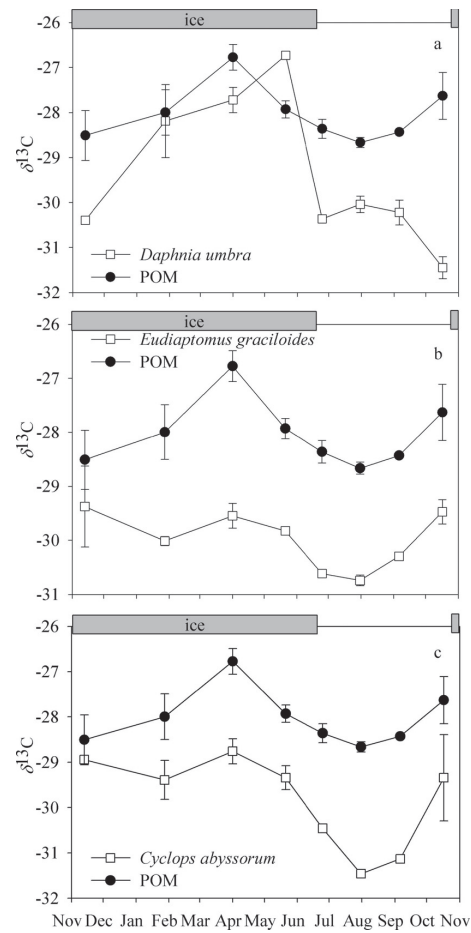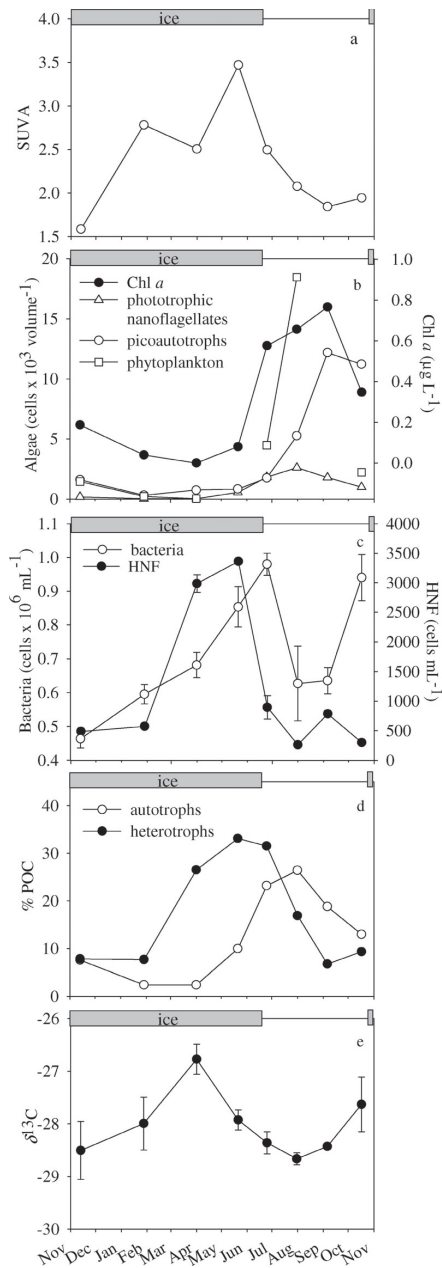
Fig. 3. Seasonal variation of $\delta^{13}C$ values of POM and $\delta^{13}C$ values of (a) *Daphnia umbra*, (b) *Eudiaptomus graciloides*, and (c) *Cyclops abyssorum* (mean ± SE).

←

Fig. 2. Seasonal variation of (a) DOM quality as determined by optical property SUVA, (b) autotrophic, and (c) heterotrophic organisms of POM, (d) carbon composition of POM, and (e) $\delta^{13}C$ values of POM from Lake Saanajärvi (mean ± SE). Algal abundance is given in cells mL$^{-1}$ for phytoplankton and cells L$^{-1}$ for other autotrophs.

$\delta^{13}C$ pattern, likely reflecting algal food intake (correlation coefficients for the open-water period: 0.956 and 0.971 for *E. graciloides* and *C. abyssorum*, respectively; $p < 0.05$ for both species).

The relative contribution of allochthonous carbon to zooplankton biomass was calculated using a two end-member mixing model

% allochthonous carbon

$$= \frac{(\delta^{13}C_{zpl} - F - \delta^{13}C_{auto})}{(\delta^{13}C_{POM} - \delta^{13}C_{auto})} \times 100 \qquad (2)$$

where F is the isotopic fractionation between a consumer and its food, and $\delta^{13}C_{zpl}$, $\delta^{13}C_{POM}$, and $\delta^{13}C_{auto}$ are the isotopic signatures of zooplankton *D. umbra*, *E. graciloides*, or *C. abyssorum*, the POM, and the phytoplankton, heterotrophic bacteria, and nanoflagellates growing on autochthonous carbon, respectively. Fractionation of dietary isotopes to the tissues of a consumer typically results in enrichment of $^{13}C$ on the order of 0–1.0‰ (Michener and Schell 1994). For this study, we applied an enrichment factor of 0.4‰, as has been suggested for oligotrophic lakes (Grey et al. 2001). The model was run with two assumptions for the composition of $\delta^{13}C_{auto}$. First, we assumed that, in the POM, only phytoplankton is autochthonous carbon, i.e., $\delta^{13}C_{auto} = \delta^{13}C_{algae}$. Second, we assumed that a certain proportion of heterotrophic bacteria, and subsequently HNF, grows on autochthonous DOC originating from phytoplankton, i.e., $\delta^{13}C_{auto} = \delta^{13}C_{algae,\, x(bact+HNF)}$, where x is the proportion of bacteria and HNF growing on autochthonous carbon. The proportion of heterotrophic production by bacteria using autochthonous carbon has been estimated to be 49% (Ask et al. 2009), with 30–65% variation in boreal lakes (Kritzberg et al. 2004) and 25–73% variation in subarctic lakes (Ask et al. 2009). We applied the 49% ± 24% proportion for x in our data, because Lake Saanajärvi and the lakes studied by Ask et al. (2009) are located in the same area in subarctic Scandinavia. Because starvation experiments and lipid data have shown that *E. graciloides* and *C. abyssorum* do not feed in winter (M. Rautio unpubl. data), allochthony calculations were applied to these species only for the open-water dates. We estimated the $\delta^{13}C_{auto}$ signature based on POM and autochthonous carbon proportion in POM. The following mixing model was used

$$\delta^{13}C_{auto} = (\delta^{13}C_{POM} - (1-x) \times (\delta^{13}C_{allo}))x^{-1} \qquad (3)$$

where the x represents the portion of autochthonous carbon in the POM. The $\delta^{13}C_{allo}$ value was calculated as a mean $\delta^{13}C_{POM}$ in winter, assuming that POM contains only allochthonous carbon when Chl *a* concentration is zero. In Lake Saanajärvi, Chl *a* concentration varied between 0 and 0.2 $\mu g\ L^{-1}$ in midwinter, when autotrophs made up about 2% of POM, but because the calculated value (−27‰) was the same as the typical allochthonous signal for a boreal ecoregion (Lajtha and Michener 1994), we used it in the calculations despite the small contribution of algae to the POM $\delta^{13}C$ values. However, Eq. 3 provides unrealistically low estimations for $\delta^{13}C_{auto}$ when the proportion of

autochthonous carbon is < 20% (Marty and Planas 2008). Therefore, in the mixing model (Eq. 2), we applied a constant value for $\delta^{13}C_{auto}$ (−33.2‰ for $\delta^{13}C_{algae}$, −31.2‰ for $\delta^{13}C_{algae,\, 49\%(bact+HNF)}$, −32.1‰ for $\delta^{13}C_{algae,\, 25\%(bact+HNF)}$, and −30.6‰ for $\delta^{13}C_{algae,\, 73\%(bact+HNF)}$), which was calculated as the mean from the open-water samples when autotrophic algae, or autotrophic algae and a given proportion of bacteria and HNF, were present in the water column with > 20% proportion. During this time, the algal community was rather stable, with Chlorophyceae and Bacillariophyceae dominating (L. Forsström unpubl. data), and we would expect little seasonal changes in $\delta^{13}C_{algae}$.

According to these mixing models, allochthonous carbon accounted for close to 100% of *D. umbra* carbon in winter, but the contribution declined during the ice out to 0–50%, and to 0–24% at the end of summer (Fig. 4), which is the time of maximum algal production. The high variability in the estimates derives from the model assumptions (Eq. 2). Highest values for allochthony result from the assumption that only phytoplankton are autochthonous in a lake, while the values decrease with increasing portions of bacteria and HNF, which are assumed to grow on autochthonous carbon. Allochthonous carbon contributed a maximum of 66% (*E. graciloides*) and 76% (*C. abyssorum*) of the carbon of copepods. When high proportions (73%) of bacteria and HNF were assumed to be autochthonous, these maximum values dropped to 25% and 29%, respectively. The maximum values were measured in early September, right after the autumn overturn, when the nonalgal material, abundant in hypolimnion, was mixed into the whole water column. During the other sampling times, allochthonous carbon did not contribute to zooplankton carbon at all (Fig. 4), when 73% of heterotrophic bacteria production was estimated to be autochthonous carbon based.

## Discussion

Here, we have presented evidence of differential contribution of autochthonous versus allochthonous carbon in a subarctic lake ecosystem between summer and winter, and its seasonal utilization among zooplankton, thereby increasing our understanding of the role of allochthonous carbon in lake food webs. Our DOM results suggest that hydrological inputs of allochthonous matter to Lake Saanajärvi were greatest in early and late winter. For the duration of winter, the water column was characterized by a high relative contribution of allochthonous carbon. Moreover, the changes in the dissolved organic carbon pool were mirrored in the seasonally changing $\delta^{13}C$ of POM, and hence in the putative zooplankton food pool. Most interestingly, copepods seemed to discriminate against the carbon in seston in winter with very little or no food intake, while cladocerans (*D. umbra*) continued to feed on available heterotrophic food items, which in winter were fueled by allochthonous carbon.

The optical characteristics of DOM changed seasonally. The DOM quality parameter, SUVA, was anchored at high values in winter and low values in summer. These differences are likely the result of higher water discharge and associated terrestrial DOM input from the catchment
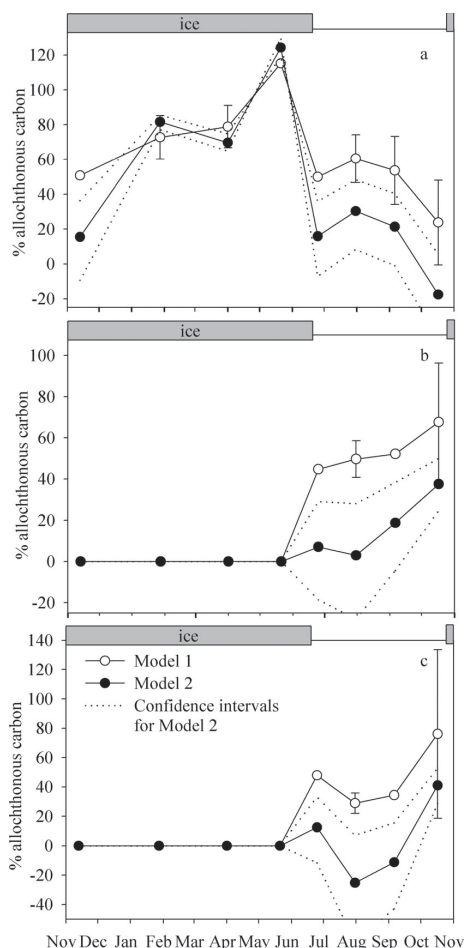
Fig. 4. Percentage contribution of allochthonous carbon to total body carbon of (a) *Daphnia umbra*, (b) *Eudiaptomus graciloides*, and (c) *Cyclops abyssorum* from Lake Saanajärvi, 2008–2009, estimated from mixing models. Model 1 is based on assumption that $\delta^{13}C_{auto} = \delta^{13}C_{algae}$, while model 2 considers 49% of heterotrophic bacteria and HNF to grow on autochthonous carbon, $\delta^{13}C_{auto} = \delta^{13}C_{algae, 49\%(bact+HNF)}$, with a confidence interval ± 24%.

during late autumn–early winter and spring compared to smaller inputs in summer (Hood et al. 2003). The differences were further enhanced through variation in seasonal primary production, with consistently increasing phytoplankton contribution to the DOM pool during the course of the summer. Jaffé et al. (2008) showed similar seasonal changes in DOM quality parameters, which

changed with primary productivity and hydrological processes along an elevation gradient. They showed that SUVA and the associated terrestrial component of the DOM decreased with decreasing elevation, while phytoplankton contribution to the DOM pool increased comparatively.

In addition, according to the $\delta^{13}C$ of POM, allochthonous carbon dominated Lake Saanajärvi in winter. The values were between −26‰ and −28‰ in midwinter, i.e., close to the terrestrial $\delta^{13}C$ in the boreal ecoregion (−27‰; Lajtha and Michener 1994). Bacteria, flagellates, and ciliates, i.e., the putative zooplankton food, were likely supported by allochthonous DOM in winter and subsequently transferred this terrestrial carbon to *D. umbra*, as evidenced with heavier *D. umbra* $\delta^{13}C$ values in winter and the estimations of the mixing models. Such microbial loop–mediated trophic transfer from allochthonous carbon to higher consumers has been suggested to be the main channel to couple terrestrial and aquatic carbon (Jones 1992; Cole et al. 2000). Bacteria and heterotrophic flagellates have long been known to ingest allochthonous carbon molecules (Sherr et al. 1988; Tranvik 1988), although it has also been shown that bacteria grow better with autochthonous carbon (Kritzberg et al. 2004). The POM carbon values in Lake Saanajärvi became more negative only in late spring, when autotrophic algal biomass increased. Shifts in $\delta^{13}C$ of POM are commonly observed when changes in phytoplankton biomass occur (Yoshioka et al. 1994; Gu et al. 1999).

The relative abundances of autotrophic and heterotrophic organisms in POM in different seasons were based on microscope counts and various carbon conversion factors in our study. The values must therefore be taken with some caution, because the choice of carbon conversion factor has a strong effect on the values obtained. Bacteria abundances in our study were converted to biomass using a measured biovolume of 0.07 $\mu m^3$ per cell in July and a coefficient of 308 fg C $\mu m^{-3}$ (Fry 1988). However, bacteria biovolumes may change on a seasonal basis; in nearby ephemeral ponds, the volumes increase 25% between spring and midsummer (T. Roiha unpubl.). Similarly, the proportion of algal carbon in POM is influenced by the ratio of carbon to Chl *a*, which may vary greatly according to season, irradiance, and productivity (Leavitt and Carpenter 1990), but it is usually about 35 for oligotrophic lakes (Riemann et al. 1989), and for Lake Saanajärvi phytoplankton, it was calculated as 40. Nevertheless, and keeping these cautions in mind, we argue that the very pronounced seasonal shifts we calculated for autotrophic and heterotrophic organisms in POM are real and give a good estimation of the changes in composition of POM in different seasons.

A point of contention relates to the way in which to accurately discriminate between the natural mixture of edible and nonedible carbon in the putative food sources. Microbial fractionation of carbon from diet to consumer is thought to be between 0‰ and 1‰ (Michener and Schell 1994). In Lake Saanajärvi, there was a 0.5‰ to > 3‰ difference between POM and zooplankton carbon signatures. Such differences could indicate that zooplankton may not feed on POM in times when there is a large difference

between zooplankton and POM $\delta^{13}C$, but rather this difference highlights the challenges in the use of stable isotopes in oligotrophic systems (Marty and Planas 2008). When algae make up < 20% of the bulk POM, a common situation in northern water bodies (Alexander et al. 1980; Rautio and Vincent 2006; Mariash et al. in press), the $\delta^{13}C$ POM does not provide a good estimation of the phytoplankton carbon, as opposed to systems where pelagic food webs are driven by phytoplankton (del Giorgio and France 1996). In situations where algae contribute little to POM, the $\delta^{13}C$ of POM is an indicator of a baseline food source that is a mixture of different organic particles with detritus dominating (Martineau et al. 2004; Rautio and Vincent 2006). In such systems, the true food source $\delta^{13}C$ values may be masked by values of inedible particles, and hence the identification of trophic links becomes challenging.

Our seasonal isotope data, however, allowed us to bypass the influence of detritus dominance in POM because the seasonal shifts in $\delta^{13}C$ POM, and the zooplankton responses to them, were more important than overlapping carbon signatures in detecting trophic links. The similarity in the shape of $\delta^{13}C$ curves of POM and *D. umbra* indicates a strong coupling between the two throughout the year. Further, the seasonal DOM quality analyses, microscopic biomass counts, and the applied mixing models indicate that *D. umbra* grazed in summer mainly on autochthonous organisms and shifted to a allochthonous carbon–supported bacterial and flagellate diet in winter. Daphnids need a constant supply of food (Lampert and Muck 1985), and in lakes where they do not produce resting eggs in winter (Larsson and Wathne 2006), they must change diet according to its availability. The unselective feeding behavior of daphnids (Monakov 2003) is likely one key element to their survival and to staying active in winter. *Daphnia* populations that exhibit a life history with winter dormancy may be less dependent on allochthonous carbon as a supplementary diet. For example, *Daphnia hyalina* in Loch Ness has been reported to grow primarily in summer and autumn and derive almost 100% body carbon from algal sources (Grey et al. 2001).

*C. abyssorum* and its subspecies are known to have different feeding habits that vary among lakes, from herbivory (Whitehouse and Lewis 1973; Hopp and Maier 2005) to predation (Fryer 1957) to cannibalism (Vandenbosch and Santer 1993). In Lake Saanajärvi, both *C. abyssorum* and *E. graciloides* were herbivorous/omnivorous and occupied the same trophic level, as indicated by their $\delta^{15}N$ stable isotope signatures (M. Rautio unpubl. data). During summer, their carbon signatures indicated reliance on POM, and, according to the mixing models, they were selectively feeding on the autochthonous component of the POM. Autochthonous carbon accounted for 47–100% of *E. graciloides* carbon and 53–100% of *C. abyssorum* as a summer mean, respectively. Lowest values, i.e., highest reliance on allochthonous carbon, were obtained when the model assumed phytoplankton to be the only autochthonous component in the POM, while highest values, i.e., 100% reliance on autochthonous carbon, were obtained when 73% of heterotrophic bacteria production was assumed to be based on autochthonous carbon. Putative food sources, heterotrophic flagellates and ciliates, were present in Lake

Saanajärvi in winter, but both copepod species apparently did not show any sign of feeding during winter. The carbon signatures of both species were constant during the 8 months of winter, displaying in each winter sample a value of $\delta^{13}C$ that was close to the $\delta^{13}C$ of POM at the beginning of winter. Starvation experiments and lipid data from these copepods also suggest that they survive the whole winter with no food, with very low metabolism, and using the storage lipids accumulated during the open-water period for reproduction that occurs in winter (M. Rautio unpubl.). Active grazing during the long period of food scarcity would have increased the metabolic rate and perhaps resulted in an unfavorable cost–benefit situation in grazing effort versus gained energy.

Subarctic copepods have been suggested to shift to a benthic diet in winter (Karlsson and Säwström 2009). There is some evidence of this from a Swedish lake located 100 km SW from Lake Saanajärvi, where zooplankton $\delta^{13}C$ values became heavier and closer to the benthic community carbon signature in winter. The benthic community $\delta^{13}C$ value in Lake Saanajärvi ranges from $-14.5$‰ in the littoral zone to $-24.6$‰ at a depth of 24 m (M. Rautio unpubl. data), and no evidence of benthic feeding was observed in our data set. This suggests that the life history and feeding strategy of zooplankton may vary greatly among lakes, even in the same climatological region. The life history of copepods in Lake Saanajärvi appears to alternate between summer feeding and winter fasting phases. Similar life histories are known for subarctic and arctic marine plankton as an adaptation to long periods of food scarcity (Lee et al. 2006; Böer et al. 2007), but to our knowledge, they have not been reported for freshwater zooplankton.

Recent evidence from carbon stable isotopes has demonstrated that an important portion (up to 50%) of lake POM and zooplankton carbon is derived from terrestrial sources (Meili et al. 1996; Grey et al. 2001; Carpenter et al. 2005). These high values have been obtained using mass balance calculations that consider all carbon that does not contain Chl *a* as allochthonous carbon (Pace et al. 2004; Taipale et al. 2007). We take a more conservative approach here and argue that in certain situations, the models estimate autotrophic and heterotrophic rather than autochthonous and allochthonous carbon reliance. For instance, heterotrophic flagellates and bacteria lacking Chl *a* may use DOC with autochthonous origin directly or indirectly as their energy source (Kritzberg et al. 2004; Hobbie and Laybourn-Parry 2008). When they were added to the mixing model as an autochthonous carbon, with proportions (49% ± 24%; Ask et al. 2009) known to exist in similar subarctic lakes as Lake Saanajärvi, the zooplankton reliance on allochthonous carbon substantially decreases. However, when assuming that a large proportion (> 50%) of heterotrophic bacteria grows on autochthonous carbon, the mixing model provides negative values, suggesting that heterotrophic bacteria production in Lake Saanajärvi is likely not highly autochthonous carbon based. In general, depending on the definition of $\delta^{13}C_{auto}$ in the mixing model, the model gives for most of the year very different estimations for the percent allochthonous carbon used by zooplankton. This clearly illustrates how sensitive mixing models are to the way $\delta^{13}C$ POM is partitioned into

autochthonous and allochthonous components. The seasonal changes in the relative use of autochthonous versus allochthonous carbon are most likely true, but the actual values vary among models and depend on the availability and use of autochthonous carbon by heterotrophic bacteria, and this may vary largely on a seasonal basis and among lakes (Kritzberg et al. 2004; Ask et al. 2009).

In addition, a cold-temperature influence on stable isotope turnover rates needs to be considered, and the subsequent influence on the mixing model results. The cold temperatures of winter slow consumer metabolism, growth, and the rate at which the isotopic signature changes following a dietary change (Frazer et al. 1997; Kaufman et al. 2008; Buchheister and Latour 2010). Arctic crustaceans have been reported to have a temperature-dependent turnover in stable isotopes; $\delta^{13}C$ half-life = 14 d at 4°C and 19 d at 1°C (Kaufman et al. 2008), while Antarctic krill had replaced < 30% of their carbon in 10 weeks at 1.5°C (Frazer et al. 1997). In Lake Saanajärvi, temperatures in winter were < 1.5°C and likely slowed down the $\delta^{13}C$ turnover in zooplankton. Differences in $\delta^{13}C$ between *D. umbra* and POM in winter may well be an indicator of slow $\delta^{13}C$ turnover rate in *D. umbra* and may have had an influence on the mixing model estimations, especially on the unrealistic > 100% values obtained for May samples. During that time, *D. umbra* may have already been feeding on the increasing phytoplankton component of the POM but still, because of slow isotopic turnover, displayed the $\delta^{13}C$ values from winter. Copepods that feed only in summer, when they also grow quickly, did not show such a slow isotopic turnover rate. Their $\delta^{13}C$ value was highly and significantly correlated with the changes in the $\delta^{13}C$ POM.

The cool ambient conditions of winter also slow zooplankton feeding rates and secondary production, albeit winter did not preclude the large standing stock of secondary production under the ice. With light limitation, low temperatures, and slow nutrient cycling, primary productivity is reduced, which in turn limits secondary production. However, the rate-limiting response of secondary production is species-specific. For instance, copepods and cladocerans have different metabolic means to cope in cold temperatures. Copepods are able to change their cellular components to more highly unsaturated fatty acids in winter, while cladocerans rely on different fatty acids to maintain cellular fluidity (Smyntek et al. 2008; Mariash et al. in press). These physiological adaptations ultimately affect diet and feeding rates of cold-tolerant species, which was also the case in this study, where copepods in cold winter temperatures stopped feeding but *D. umbra* continued feeding on heterotrophic food items.

Several studies have indicated that lake phytoplankton production is insufficient to support aquatic food webs (Salonen et al. 1992; Pace et al. 2004; Christoffersen et al. 2008). Lake Saanajärvi is a relatively large clear-water lake where the high variation in ambient air temperature largely controls the timing and volume of terrestrial runoff, causing two distinctive seasonal peaks. The first is at the time of snowmelt in spring, and another occurs in early winter, when air temperatures fluctuate below and above zero for some weeks, causing fresh snow to flush into the lake carrying constituents from the catchments area. The changes in hydrology together with seasonally changing biomass of primary producers were detectable in our results of SUVA, $\delta^{13}C$, and microscope counts. All these parameters indicate less influence of allochthonous carbon on lake food webs in summer than in winter, but they do not rule out the possibility that a part of the heterotrophic productivity in the lake was fueled by DOC or detritus originating from autochthonous carbon. As a consequence of the lack of algae in the water column, the *D. umbra* population was dependent on the nonphytoplankton carbon during winter.

Meili et al. (1996) and Pace et al. (2007) have reported a similar high dependence of *Daphnia* on nonphytoplankton carbon. According to their calculations, > 30% of *Daphnia* diet came from allochthonous carbon, while the copepod *Leptodiaptomus minutus* was almost completely supported by autochthonous carbon (Pace et al. 2007). Meili et al. (1996) found that in the cladocerans *Daphnia cristata* and *Bosmina coregoni*, 40% ± 15% of body carbon was of allochthonous origin, and that *E. graciloides* consumed less (25% ± 20%) allochthonous carbon–derived food than cladocerans. According to the mixing models, a similar ranking of cladocerans and copepods occurred also in Lake Saanajärvi, with *D. umbra* carbon originating more often from allochthonous carbon than the carbon of the copepods.

Arctic lake zooplankton need to react rapidly to summer resources in order to gain their growth and essential fatty acids during the few months in summer when phytoplankton diet is available. All the zooplankton in Lake Saanajärvi were highly responsive to shifts in POM carbon signatures in summer, which were driven by the increase of phytoplankton biomass. Secondary aquatic consumers are dependent on the dietary highly unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are obtained mainly from phytoplankton (Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000). Because EPA and DHA cannot be synthesized de novo in zooplankton or in other higher organisms, the importance of allochthonous carbon in lake food webs has been criticized (Brett et al. 2009). However, *Daphnia* sp. has been shown to grow and produce offspring even when supported only by allochthonous carbon, although much less so than with a phytoplankton diet (Brett et al. 2009). In light of our results, we argue that the cladocerans in Lake Saanajärvi could not have survived the 8 winter months under the ice if they had not been able to utilize food sources driven by allochthonous carbon. We further argue that when autochthonous carbon is very low or absent, allochthonous carbon–fueled secondary food sources become critical for the survival of aquatic organisms. This occurs especially in northern lakes during winter, but possibly in many other lakes too, such as in boreal humic lakes that are dominated by allochthonous carbon and where strong light absorption inhibits phytoplankton productivity.

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