

## 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  $\delta^{13}\text{C}$  values consistent with allochthonous inputs of DOM. The most positive POM  $\delta^{13}\text{C}$  values ( $-27\text{‰}$ ) occurred during winter, when heterotrophic bacteria and nanoflagellates peaked in abundance; the isotopically lighter autotrophic phytoplankton shifted the POM  $\delta^{13}\text{C}$  to  $-29\text{‰}$  in summer. The  $\delta^{13}\text{C}$  values of zooplankton were up to 4‰ more negative than those of POM, most likely because detritus and other nonliving material with higher  $\delta^{13}\text{C}$  values comprised 45–90% of POM. The  $\delta^{13}\text{C}$  of the cladoceran *Daphnia umbra* mirrored the trend of POM  $\delta^{13}\text{C}$ , indicating a dietary shift from heavy dependence on autochthonous carbon in summer to allochthonous or heterotrophic food in winter. The  $\delta^{13}\text{C}$  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 Sävström 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<sup>-2</sup> d<sup>-1</sup>; 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 Sävström 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 <sup>-1</sup> )	POM (mg L <sup>-1</sup> )	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  $\delta^{13}\text{C}$  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 Sävström 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  $\delta^{13}\text{C}$  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 km<sup>2</sup>, max. depth 24 m) were collected eight times between November 2007 and October 2008. The catchment of Lake Saanajärvi (4.6 km<sup>2</sup>) 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  $\mu\text{g L}^{-1}$  and DOC concentrations of 1.8 mg L<sup>-1</sup> (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- $\mu\text{m}$  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<sub>254</sub>). The SUVA<sub>254</sub> parameter is defined as the UV absorbance at 254 nm measured in inverse meters (m<sup>-1</sup>) divided by the DOC concentration (mg L<sup>-1</sup>). 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- $\mu\text{m}$  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  $\mu\text{g mL}^{-1}$  for 1 h, followed by filtration through a 0.2- $\mu\text{m}$  black Nucleopore membrane. Filters were mounted on slides with immersion oil and frozen (-20°C) until examination at 1000 $\times$  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- $\mu\text{m}$  black polycarbonate membrane. Slides were frozen until examination at 1000 $\times$  using a Leica Leitz fluorescence microscope. Green excitation was used to discriminate between colorless heterotrophic nanoflagellates (HNF) and autotrophic cells with plastids 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  $\mu\text{m}^3$  and a volume-biomass conversion factor of 0.308  $\text{pg C } \mu\text{m}^{-3}$  (Fry 1988). Heterotrophic nanoflagellates were enumerated to four different size categories (diameter 2, 2–5, 5–10, and

> 10  $\mu\text{m}$ ) to allow volume estimation and subsequent carbon conversion using a conversion factor of 0.22  $\text{pg C } \mu\text{m}^{-3}$  (Børsheim and Bratbak 1987). Picoautotroph carbon biomass was calculated using the average cell volume of 4.2  $\mu\text{m}^3$  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- $\mu\text{m}$  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  $\delta^{13}\text{C}$  sample, stored in three replicates, when possible, in 2.5-mL vials, and frozen. The samples were lipid extracted because greater lipid content lowers  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$ , where

$$\delta^{13}\text{C} = \left( \frac{{}^{13}\text{C} : {}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C} : {}^{12}\text{C}_{\text{reference}}} - 1 \right) \times 1000 \quad (1)$$

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

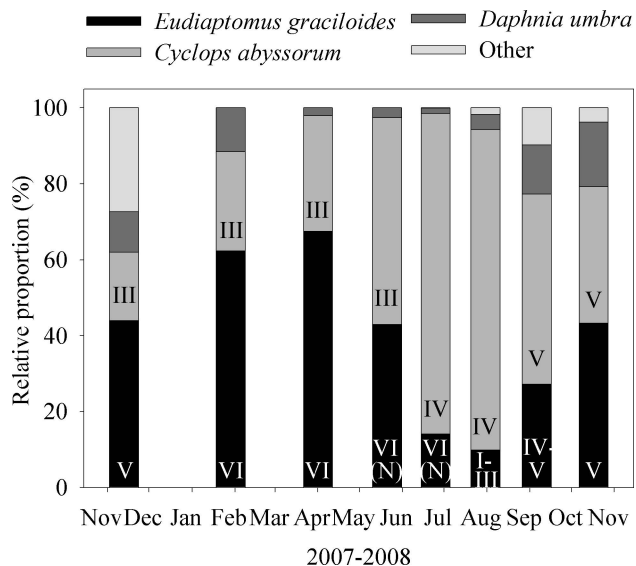


Fig. 1. The composition of zooplankton ( $> 200 \mu\text{m}$ ) in Lake Saanajärvi in 2007–2008. The dominant developmental stage in the sample is shown for *Eudiaptomus graciloides* and *Cyclops abyssorum*: N = nauplia, I–III = copepodites, IV–V = copepodites, and VI = adults. The presence of nauplia is expressed in parentheses because they were not encountered in the samples taken with a  $200\text{-}\mu\text{m}$  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  $\delta^{13}\text{C}$  of POM from the surface waters (0–10 m) showed pronounced seasonal variation during the study period (Fig. 2e):  $\delta^{13}\text{C}$  of POM ranged from  $-26.7\text{‰}$  in late winter to  $-28.7\text{‰}$  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  $\delta^{13}\text{C}$  values responded to the abundance of bacteria, HNF, and the proportion of heterotrophs in POM, with POM  $\delta^{13}\text{C}$  values increasing from  $-28.5\text{‰}$  to  $-26.8\text{‰}$ , along with the increasing proportion of heterotrophic and decreasing proportion of autotrophic organisms in the water column (Table 1; Fig. 2d). The  $\delta^{13}\text{C}$  values of POM from the bottom waters (10–20 m) were similar to the surface  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  of autotrophic organisms found more frequently in the summer epilimnion.

The  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  of *Daphnia umbra* was closely associated with the large-scale seasonal changes in  $\delta^{13}\text{C}$  POM (Fig. 3). During the winter, the values became more positive, mirroring the trend in  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  from  $-26.7\text{‰}$  to  $-30.4\text{‰}$ , which occurred concurrently with the increase of autotrophic algae in the POM. During the summer, the *D. umbra*  $\delta^{13}\text{C}$  value stayed between  $-30.5\text{‰}$  and  $-31.4\text{‰}$  and was negatively correlated with the changes in  $\delta^{13}\text{C}$  POM (correlation coefficient:  $-0.995$ ,  $p < 0.05$ ). In contrast, the carbon isotope ratios of the two copepod species showed a different pattern, where  $\delta^{13}\text{C}$  values stayed constant from the beginning to the end of winter in both *E. graciloides* ( $-29.4\text{‰}$  to  $-30.0\text{‰}$ ) and *C. abyssorum* ( $-29.3\text{‰}$  to  $-29.9\text{‰}$ ) (Fig. 3). Therefore, they diverged from the trend that was seen for POM  $\delta^{13}\text{C}$  (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

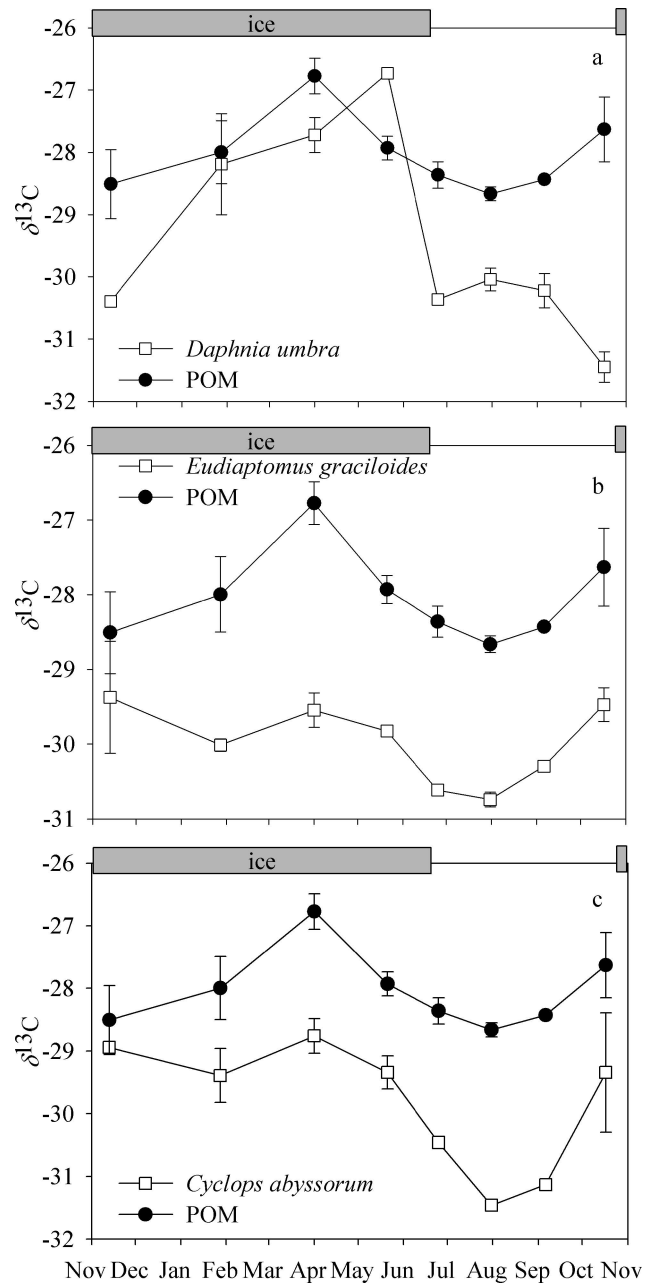
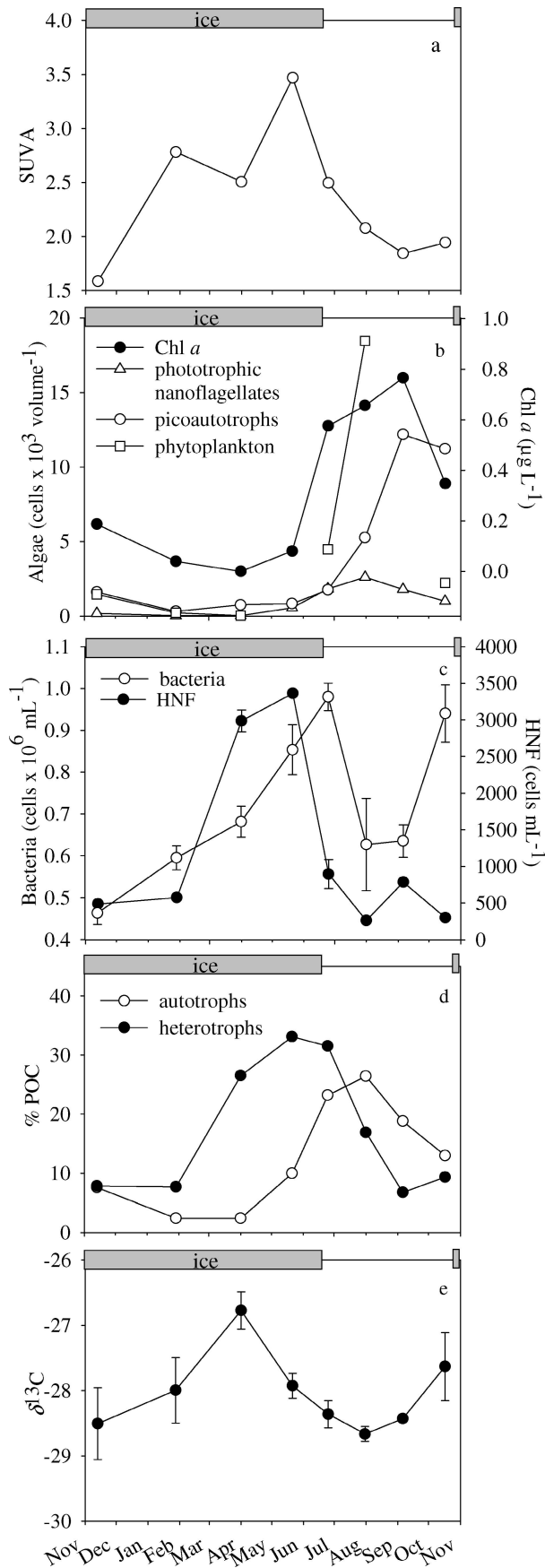


Fig. 3. Seasonal variation of  $\delta^{13}\text{C}$  values of POM and  $\delta^{13}\text{C}$  values of (a) *Daphnia umbra*, (b) *Eudiaptomus graciloides*, and (c) *Cyclops abyssorum* (mean  $\pm$  SE).

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)  $\delta^{13}\text{C}$  values of POM from Lake Saanajärvi (mean  $\pm$  SE). Algal abundance is given in cells  $\text{mL}^{-1}$  for phytoplankton and cells  $\text{L}^{-1}$  for other autotrophs.

$\delta^{13}\text{C}$  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

$$\begin{aligned} & \% \text{ allochthonous carbon} \\ &= \frac{(\delta^{13}\text{C}_{\text{zpl}} - F - \delta^{13}\text{C}_{\text{auto}})}{(\delta^{13}\text{C}_{\text{POM}} - \delta^{13}\text{C}_{\text{auto}})} \times 100 \end{aligned} \quad (2)$$

where  $F$  is the isotopic fractionation between a consumer and its food, and  $\delta^{13}\text{C}_{\text{zpl}}$ ,  $\delta^{13}\text{C}_{\text{POM}}$ , and  $\delta^{13}\text{C}_{\text{auto}}$  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  $^{13}\text{C}$  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  $\delta^{13}\text{C}_{\text{auto}}$ . First, we assumed that, in the POM, only phytoplankton is autochthonous carbon, i.e.,  $\delta^{13}\text{C}_{\text{auto}} = \delta^{13}\text{C}_{\text{algae}}$ . Second, we assumed that a certain proportion of heterotrophic bacteria, and subsequently HNF, grows on autochthonous DOC originating from phytoplankton, i.e.,  $\delta^{13}\text{C}_{\text{auto}} = \delta^{13}\text{C}_{\text{algae}, x(\text{bact}+\text{HNF})}$ , 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  $49\% \pm 24\%$  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  $\delta^{13}\text{C}_{\text{auto}}$  signature based on POM and autochthonous carbon proportion in POM. The following mixing model was used

$$\delta^{13}\text{C}_{\text{auto}} = (\delta^{13}\text{C}_{\text{POM}} - (1-x) \times (\delta^{13}\text{C}_{\text{allo}}))x^{-1} \quad (3)$$

where the  $x$  represents the portion of autochthonous carbon in the POM. The  $\delta^{13}\text{C}_{\text{allo}}$  value was calculated as a mean  $\delta^{13}\text{C}_{\text{POM}}$  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  $0.2 \mu\text{g L}^{-1}$  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  $\delta^{13}\text{C}$  values. However, Eq. 3 provides unrealistically low estimations for  $\delta^{13}\text{C}_{\text{auto}}$  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  $\delta^{13}\text{C}_{\text{auto}}$  ( $-33.2\%$  for  $\delta^{13}\text{C}_{\text{algae}}$ ,  $-31.2\%$  for  $\delta^{13}\text{C}_{\text{algae}, 49\%(\text{bact}+\text{HNF})}$ ,  $-32.1\%$  for  $\delta^{13}\text{C}_{\text{algae}, 25\%(\text{bact}+\text{HNF})}$ , and  $-30.6\%$  for  $\delta^{13}\text{C}_{\text{algae}, 73\%(\text{bact}+\text{HNF})}$ ), 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  $\delta^{13}\text{C}_{\text{algae}}$ .

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  $\delta^{13}\text{C}$  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

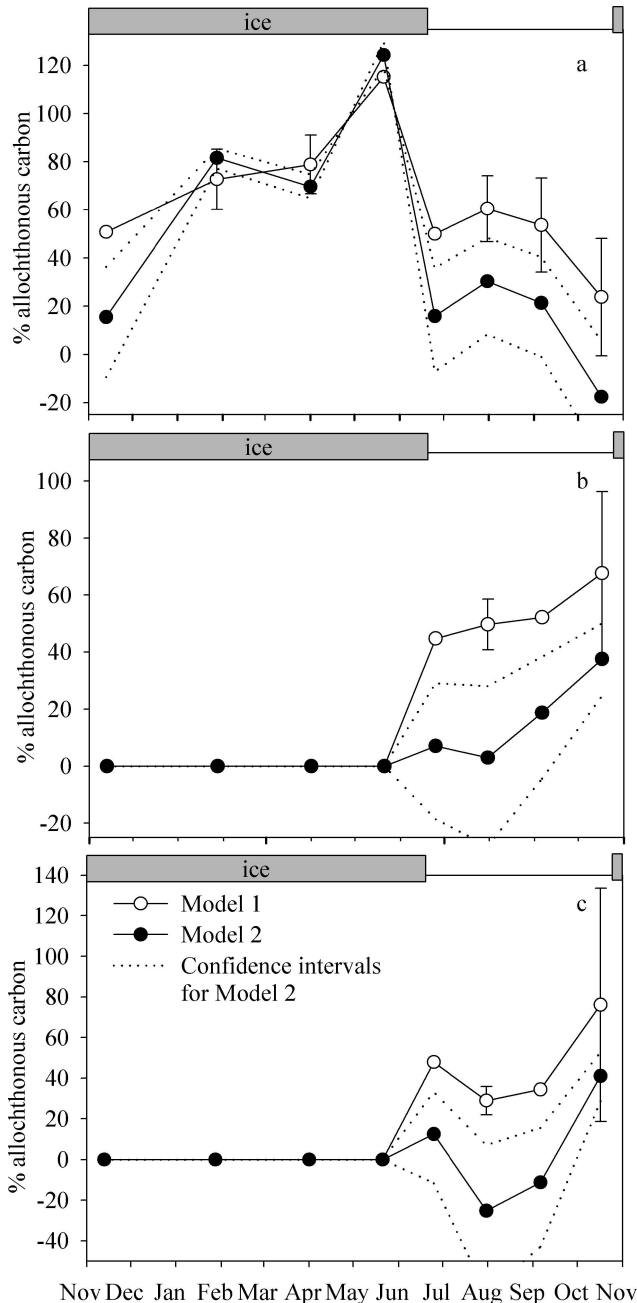


Fig. 4. Percentage contribution of allochthonous carbon to total body carbon of (a) *Daphnia umbra*, (b) *Eudiaptomus graciloides*, and (c) *Cyclops abyssorum* from Lake Saanajärvi, 2008–2009, estimated from mixing models. Model 1 is based on assumption that  $\delta^{13}\text{C}_{\text{auto}} = \delta^{13}\text{C}_{\text{algae}}$ , while model 2 considers 49% of heterotrophic bacteria and HNF to grow on autochthonous carbon,  $\delta^{13}\text{C}_{\text{auto}} = \delta^{13}\text{C}_{\text{algae}, 49\%(\text{bact}+\text{HNF})}$ , with a confidence interval  $\pm 24\%$ .

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  $\delta^{13}\text{C}$  of POM, allochthonous carbon dominated Lake Saanajärvi in winter. The values were between  $-26\%$  and  $-28\%$  in midwinter, i.e., close to the terrestrial  $\delta^{13}\text{C}$  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*  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $0.07 \mu\text{m}^3$  per cell in July and a coefficient of  $308 \text{ fg C } \mu\text{m}^{-3}$  (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  $\delta^{13}\text{C}$ , 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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  POM, and the zooplankton responses to them, were more important than overlapping carbon signatures in detecting trophic links. The similarity in the shape of  $\delta^{13}\text{C}$  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  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  that was close to the  $\delta^{13}\text{C}$  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 Sävström 2009). There is some evidence of this from a Swedish lake located 100 km SW from Lake Saanajärvi, where zooplankton  $\delta^{13}\text{C}$  values became heavier and closer to the benthic community carbon signature in winter. The benthic community  $\delta^{13}\text{C}$  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 ( $49\% \pm 24\%$ ; 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  $\delta^{13}\text{C}_{\text{auto}}$  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  $\delta^{13}\text{C}$  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;  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  turnover in zooplankton. Differences in  $\delta^{13}\text{C}$  between *D. umbra* and POM in winter may well be an indicator of slow  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values from winter. Copepods that feed only in summer, when they also grow quickly, did not show such a slow isotopic turnover rate. Their  $\delta^{13}\text{C}$  value was highly and significantly correlated with the changes in the  $\delta^{13}\text{C}$  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,  $\delta^{13}\text{C}$ , 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 *Leptodiatomus 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%  $\pm$  15% of body carbon was of allochthonous origin, and that *E. graciloides* consumed less (25%  $\pm$  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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