Freshwater Biology

Freshwater Biology (2012) **57,** 541–551 doi:10.1111/j.1365-2427.2011.02722.x

Benthic resources are the key to *Daphnia middendorffiana* survival in a high arctic pond

MATTEO CAZZANELLI*, LAURA FORSSTRÖM†, MILLA RAUTIO†, ANDERS MICHELSEN‡ AND KIRSTEN S. CHRISTOFFERSEN*

*Freshwater Biological Laboratory, University of Copenhagen, Hillerød, Denmark

†Department of Environmental Science, University of Jyväskylä, Jyväskylä, Finland

‡Terrestrial Ecology Section, University of Copenhagen, Copenhagen K, Denmark

SUMMARY

1. Shallow arctic lakes and ponds have simple and short food webs, but large uncertainties remain about benthic–pelagic links in these systems. We tested whether organic matter of benthic origin supports zooplankton biomass in a pond in NE Greenland, using stable isotope analysis of carbon and nitrogen in the pond itself and in a ^{13}C-enrichment enclosure experiment. In the latter, we manipulated the carbon isotope signature of benthic algae to enhance its isotopic discrimination from other potential food sources for zooplankton.

2. The cladoceran *Daphnia middendorffiana* responded to the ^{13}C-enrichment of benthic mats with progressively increasing $\delta^{13}C$ values, suggesting benthic feeding. Stable isotope analysis also pointed towards a negligible contribution of terrestrial carbon to the diet of *D. middendorffiana*. This agreed with the apparent dominance of autochthonous dissolved organic matter in the pond revealed by analysis of coloured dissolved organic matter.

3. Daily net production by phytoplankton in the pond (18 mg C m^{-2} day^{-1}) could satisfy only up to half of the calculated minimum energy requirements of *D. middendorffiana* (35 mg C m^{-2} day^{-1}), whereas benthic primary production alone (145 mg C m^{-2} day^{-1}) was more than sufficient.

4. Our findings highlight benthic primary production as a major dietary source for *D. middendorffiana* in this system and suggest that benthic organic matter may play a key role in sustaining pelagic secondary production in such nutrient-limited high arctic ponds.

Keywords: arctic pond, benthic algae, *Daphnia middendorffiana*, terrestrial carbon, zooplankton

Introduction

Shallow lakes and ponds are the dominant freshwater habitats in many arctic regions and are vital for a variety of plants, animals and microorganisms (Smol & Douglas, 2007). As a result of nutrient limitation, production by phytoplankton is characteristically low (Vincent & Laybourn-Parry, 2008). However, despite the poor resources, the biomass of zooplankton often exceeds that of phytoplankton, sometimes by an order of magnitude (Christoffersen *et al.*, 2008).

The conventional view of food webs, in which pelagic primary production is regarded as the major, if not the only, food source for zooplankton, has recently been challenged, and there is growing evidence that additional sources may contribute to the diet of planktonic primary consumers at high latitudes (Karlsson *et al.*, 2003; Rautio & Vincent, 2006). A distinguishing feature of arctic

Correspondence: Matteo Cazzanelli, Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, 3400 Hillerød, Denmark. E-mail: mcazzanelli@bio.ku.dk

Present address: Laura Forsström, Environmental Change Research Unit, Department of Environmental sciences, University of Helsinki, P.O. Box 65, Viikinkaari 1, 00014 Helsinki, Finland.

Present address: Milla Rautio, Département des Sciences Fondamentales & Centre for Northern Studies (CEN), Université du Québec à Chicoutimi, 555 boulevard de l'Université, G7H 2B1 Chicoutimi, QC, Canada.

shallow lakes and ponds is the high production of benthic algae, which may be responsible for over 90% of total primary production (Vadeboncoeur *et al*., 2003; Rautio & Vincent, 2006). Zooplankton can exploit benthic algae by grazing directly on the sediment or on the resuspended particles (Hecky & Hesslein, 1995; Rautio & Vincent, 2006). Further, heterotrophic bacteria can grow on benthic organic matter (Hopkinson *et al*., 1998) and are probably an additional source of carbon for zooplankton (Karlsson & Säwström, 2009). Benthic mats accumulate biomass during the open-water season and, in lakes that do not freeze to the bottom, may provide a reserve of food for zooplankton during the dark, unproductive winter period (Karlsson & Säwström, 2009).

Hitherto, however, no conclusive evidence exists that zooplankton growth is sustained by direct grazing on benthic resources during the growing season. This lack of evidence of benthic–pelagic coupling between zooplankton and benthic algae can be explained by a number of factors. First, the high and stable standing stocks of benthic mats suggest that they are not heavily exploited. Further, zooplankton may find it difficult to access benthic resources, as they are better adapted to feeding on suspended food particles. Third, the quality of benthic mats as food for zooplankton may be poor. A recent study (Mariash *et al*., 2011) on subarctic ponds in Finland showed that benthic mats may lack some of the essential fatty acids required for zooplankton somatic growth, while phytoplankton contained higher concentration of polyunsaturated fatty acid (PUFA) than the benthos.

Although Mariash *et al.* (2011) showed that, in their systems, phytoplankton was superior to benthic mats in supporting zooplankton, their results also demonstrated variability among ponds and zooplankton species, suggesting that benthic mats may sometimes be an important food source. The overall amount of PUFA in the benthic mats exceeded that of the seston, as a result of the higher biomass of benthic algae in the system. Benthic algae may therefore be a lower quality food than phytoplankton but, when resources are scarce, may be significant. This is particularly true of high arctic lakes, where pelagic algal production is restricted not only by poor nutrients but also by the short growing season, low temperature and low light.

We aimed to determine the relative importance of different food sources in supporting the biomass of zooplankton in a high arctic pond. We selected *Daphnia middendorffiana* Fisher, which has a circumpolar distribution and is a key zooplanktonic species in many arctic shallow lakes and ponds (Haney & Buchanan, 1987), as model organism for the present study. We hypothesised that benthic production would provide significant support to secondary production in high arctic ponds during the short polar summer, and we tested this by using carbon and nitrogen stable isotope analysis (SIA). Previous studies of lake food webs using SIA (e.g. Rautio & Vincent, 2007) showed that benthic and sestonic algae may overlap in their isotope signals, and therefore, the relative importance of each to the diet of consumers is difficult to distinguish. Here, we combined, for the first time in the Arctic, *in situ* pond measurements of plankton communities with an enclosure experiment in which we manipulated the isotope signature of benthic algae, aiming to enhance their isotopic discrimination from other potential food sources. Spectroscopic analyses of dissolved organic matter (DOM) were used to characterise the autochthonous or allochthonous origin of organic matter (Helms *et al*., 2008).

Methods

Study site

The investigation took place in August 2009 in 'Pond 19', a freshwater body of *c.* 200 m^2 located at Zackenberg (74°30′N; 21°00′W), Northeast Greenland (Christoffersen *et al*., 2008). The pond is shallow (maximum and mean depths of 0.3 m and 0.2 m, respectively) and normally freezes solid from October to May/June. The bed consists of an organic substratum overlying coarse and fine sand particles and sparsely distributed pebbles. The basin has clear water originating from melted snow and run-off in the catchment area. Part of the pond is covered by the aquatic macrophyte *Hippuris vulgaris* L., and cyanobacteria *Nostoc* spp. are also well established. The vegetation in the catchment area is composed of mosses (*Aulacomnium turgidum* Wahlenberg and *Dicranum* sp.), sedges (*Carex saxatilis* L. and *Eriophorum scheuchzeri* Hoppe) and dwarf shrubs (*Salix arctica* Pall., *Vaccinium uliginosum* L. and *Empetrum nigrum* L.). The zooplankton community in the pond consists almost entirely of the cladoceran *D. middendorffiana*, but small populations of Chydoridae and the tadpole shrimp *Lepidurus arcticus* Pallas are also present. Further details of this and other shallow waterbodies present in the area are provided by Christoffersen *et al.* (2008).

Experimental design, sampling and analyses

The enclosure experiment was performed in cylindrical plastic tanks with closed bottoms (non-toxic, semitransparent PVC containers; height 0.7 m, diameter 0.45 m,

total volume 100 L). We placed three replicate control enclosures and three replicate treatment enclosures in a row in the pond, randomised along a north–south line. We added similar amounts of benthic material, which mostly consisted of loose sediment with detritus and benthic algae, from the pond into the enclosures, forming a bottom layer of *c.* 8 cm in thickness. We then added 50 L of pond water to each enclosure, minimising resuspension as much as possible.

In the treatment enclosures, we manipulated the carbon signature of the benthic mats by adding ^{13}C-enriched dissolved inorganic carbon (DIC). For two consecutive days, a solution containing 42 μmol of $NaH^{13}CO_3$ (^{13}C, 99 atom %; Cambridge Isotope Laboratories, Andover, MA, U.S.A.) was diluted to 1 L with pond water, poured into each treatment enclosure and the water stirred gently. This additions increased total DIC concentration in the treatment enclosures by <1%, and the same increase was applied to the controls with a solution containing 43 μmol of $NaHCO_3$ (natural abundance ^{13}C; Merck, Darmstadt, Germany). Forty-eight hours after the first addition, we replaced the entire water column in each enclosure to maintain the ^{13}C-enriched label only in the benthic mats. Sampling took place before each $NaHCO_3$ addition and every second day after water column replacement.

After replacing the water column, we added *D. middendorffiana* from the pond to the enclosures to a density of 7 L^{-1}, which corresponded to the measured natural density in the pond. At each sampling, we collected *c.* 40 individuals of *D. middendorffiana* from each enclosure with a 200-μm mesh sieve. The cladocerans were then incubated for at least 3 h in Whatman GF/F-filtered pond water for gut evacuation and subsequently moved to pre-weighed Eppendorf vials and stored in a freezer (−18 °C). Daphniids were freeze-dried for biomass determination. Lipid concentration is high in arctic zooplankton and can significantly deplete their δ^{13}C signal (Kling, Fry & O'Brien, 1992; Syväranta & Rautio, 2010). Therefore, we removed lipids from the dried samples by washing them in a 2 : 1 dichloromethane–methanol solution for 30 min. As *D. middendorffiana* samples contained some residual fat after lipid extraction, carbon-to-nitrogen (C : N) ratios ranged between 4.5 and 4.9, but should be as low as *c.* 4 when all lipids were extracted from freshwater zooplankton; following Syväranta & Rautio (2010), we normalised the δ^{13}C of daphniids ($\delta^{13}C_{norm}$; Table 1).

For analysis of sestonic particulate organic matter (POM – the 0.7- to 50-μm fraction), we filtered up to 0.5 L of water through a 50-μm mesh to remove large inedible particles and then through pre-combusted and pre-weighed GF/F filters. The filters were dried at 50 °C for biomass determination, acid-fumed to remove excess inorganic carbon, and the material retained scraped into tin cups for SIA.

For concentration and δ^{13}C analysis of DOM and DIC, we collected the filtrates passing through the GF/F filters in 40 mL TraceClean glass vials equipped with silicone–teflon septa and stored at 4 °C until analysis.

The top 3-mm layer of bulk benthos, including the benthic algae, was collected with a mini core sampler (an 8-mm-diameter syringe with head cut-off) and stored in pre-weighed Eppendorf vials. The samples were freeze-dried for biomass determination and acid-fumed to remove excess inorganic C before SIA (δ^{13}C, δ^{15}N).

At the beginning of the study, we collected samples to determine the natural abundance δ^{13}C and δ^{15}N of zooplankton, benthic mats and POM, and the δ^{13}C of DOM and DIC in the pond. Additionally, we dried the samples of each of the most common plant species in the catchment at 50 °C and finely ground them prior to SIA.

Concentration and carbon stable isotopes of DIC and DOM were analysed at UC Davis stable isotope facility (University of California) using a TOC Analyser (OI Analytical, College Station, TX, U.S.A.) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, U.K.). We performed all other analyses of stable isotopic and elemental ratios of C and N in-house, using a continuous-flow CN analyser (Euro-Vector, Milan, Italy) coupled to an Isoprime IRMS (Micromass-GV Instruments, Manchester, U.K.). The results are expressed as δ values in per mille units (‰) following the equation:

$$\delta = \left(R_{sample}/R_{standard} - 1\right) \times 1000,$$

where R = ^{13}C/^{12}C or ^{15}N/^{14}N. The standards used as reference were materials of known isotopic composition relative to the international standard of Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. The analytical precision of the IRMS was better than 0.2‰.

We measured temperature, pH, total phosphorus (TP) and total nitrogen (TN) during the investigation both in the pond and enclosures. Temperature data were recorded from the pond and one enclosure every second hour using a mini data logger (VEMCO, Halifax, Canada). For chlorophyll-*a* analysis of phytoplankton, up to 0.5 L of water was filtered onto GF/F filters and wrapped in aluminium foil. Samples for benthic mat chlorophyll-*a* analysis were collected in Eppendorf vials using a mini core sampler. All samples for chlorophyll analysis were stored frozen and analysed spectrophotometrically after ethanol extraction (Jespersen & Christoffersen, 1987). We

Table 1 Equations and calculations of key parameters used in the study

Parameter	Equation	References
Lipid-normalisation of *Daphnia* $\delta^{13}C$ values	1) $\delta^{13}C_{norm}$ (‰) = $\delta^{13}C + 7.95\times[(C:N - 3.8)/C:N]$ C : N → C to N ratio of *Daphnia* samples	Syväranta & Rautio (2010)
CDOM – calculation of absorption coefficients	2) a_λ (m^{-1}) = 2.303 × A(λ)/L A(λ) → optical density for wavelength λ; L → cell path (m)	Kirk (1994)
CDOM – nonlinear regression of absorption spectrum from 300 to 650 nm	3) a_λ (m^{-1}) = $a_{320} \times e^{S(\lambda-320)}$ a_{320} → absorption coefficient at 320 nm; S → spectral slope coefficient	Jerlov (1968); Lundgren (1976); Bricaud *et al.* (1981)
Daphnia carbon demand	4) C_d (μg C L^{-1} day^{-1}) = (d × CW × g)/[(1−e) × (1−r) × a] d → *Daphnia* density in Pond 19 (=7 individuals L^{-1}); CW → mean carbon weight of *Daphnia* in Pond 19 (=60 μg $individual^{-1}$); g → growth rate (=0.1 day^{-1}); e → investment in production of eggs (=20%); r → energy loss by respiration (=50%); a → assimilation efficiency (=60%)	Green (1954); Yurista (1999); Elser *et al.* (2000); Yurista & O'Brien (2001); Van Geest *et al.* (2007)
Proportion of live algae in seston or benthos	5) p_{algae} = (Chlorophyll-*a* × DW:Chl)/DW_{bulk} DW : Chl → DW to chlorophyll-*a* ratio (=66) DW_{bulk} → DW of bulk samples from seston or benthos	Reynolds (1984); Riemann *et al.* (1989)
Maximum potential $\delta^{13}C$ of POM owing to uptake by phytoplankton of residual ^{13}C-enriched DIC	6) $\delta^{13}C_{POM\_dic}$ (‰) = $[(1-p_{algae\_seston}) \times \delta^{13}C_{POM\_ctrl}] + p_{algae\_seston} \times [\delta^{13}C_{POM\_ctrl} + (\delta^{13}C_{DIC\_treat} - \delta^{13}C_{DIC\_ctrl})]$ $\delta^{13}C_{POM\_ctrl}$ → measured isotopic value of POM in the control; $\delta^{13}C_{DIC\_treat}$ and $\delta^{13}C_{DIC\_ctrl}$ → mean $\delta^{13}C$ of DIC in treatment and control enclosures	

CDOM, coloured dissolved organic matter; DIC, dissolved inorganic carbon; POM, particulate organic matter.

determined the concentration of TP and TN in unfiltered lake water following the method described by Greenberg *et al.* (2005).

To estimate the autochthonous versus allochthonous origin of carbon in the pond and enclosures, coloured dissolved organic matter (CDOM) was analysed from water samples collected on the last sampling date. Water was filtered through pre-rinsed 0.22-μm cellulose acetate filters and stored at 4 °C in acid-rinsed and combusted 100-mL amber glass bottles. Absorption of CDOM was measured using a Cary 300 UV-Vis spectrophotometer (Varian Inc., Walnut Creek, CA, U.S.A.). Measurements were carried out every 1 nm over the wavelength (λ) range 250–850 nm. From the absorption data, we calculated the absorption coefficients and spectral slopes (a_λ, Table 1). We chose a spectral range of 300–650 nm to reflect general trends in CDOM, but also calculated spectral slopes for shorter ranges, 275–295 and 350–400 nm, and their ratio (S_R), to obtain more detailed information on DOM quality (Helms *et al.*, 2008). Additionally, we used the absorption at 320 and 440 nm as a measure of CDOM concentration and colour, and dissolved organic carbon (DOC)-specific a_{320} as a proxy of the degree of DOM colour. Specific UV absorbance (SUVA) at 254 nm corresponds to the absorbance at 254 nm divided by the DOC concentration and is an indication of the origin of carbon in the system (Weishaar *et al.*, 2003).

We compared the effect of $NaH^{13}CO_3$ additions in the enclosures on DIC, DOM, benthic mats, POM and *D. middendorffiana* using analysis of variance with repeated measurements (rmANOVA). Unless otherwise stated, the level of statistical significance used was $P < 0.05$. We performed the statistical analyses with Prism (ver. 5.0; GraphPad, La Jolla, CA, U.S.A.).

Results

Carbon stable isotope ratios

In the treatment enclosures, the $\delta^{13}C$ values of DIC changed significantly following addition of the ^{13}C-enriched $NaHCO_3$ solution (Fig. 1a). Values of $\delta^{13}C$ increased from a natural background of −6.9 ± 0.2‰ (mean ± SE) to a maximum level of 231.8 ± 11.7‰ before water column replacement. After the water was replaced, the isotopic value of DIC dropped towards the natural abundance, but was still moderately enriched (mean ± SE of all dates after water replacement, 6.7 ± 2.8‰), whereas in the controls, the $\delta^{13}C$ of DIC remained virtually unchanged (−8.3 ± 0.3‰).

Addition of DIC caused a significant enrichment of the $\delta^{13}C$ of benthic mats in the treatment enclosures (−23.8 ± 0.1‰) compared to the controls (−24.8 ± 0.3‰; Fig. 1d). This difference persisted and was statistically significant even after water column replacement.

The $\delta^{13}C$ of *D. middendorffiana* introduced to the treatment enclosures following water column replacement increased progressively, increasing $\delta^{13}C$ values (from

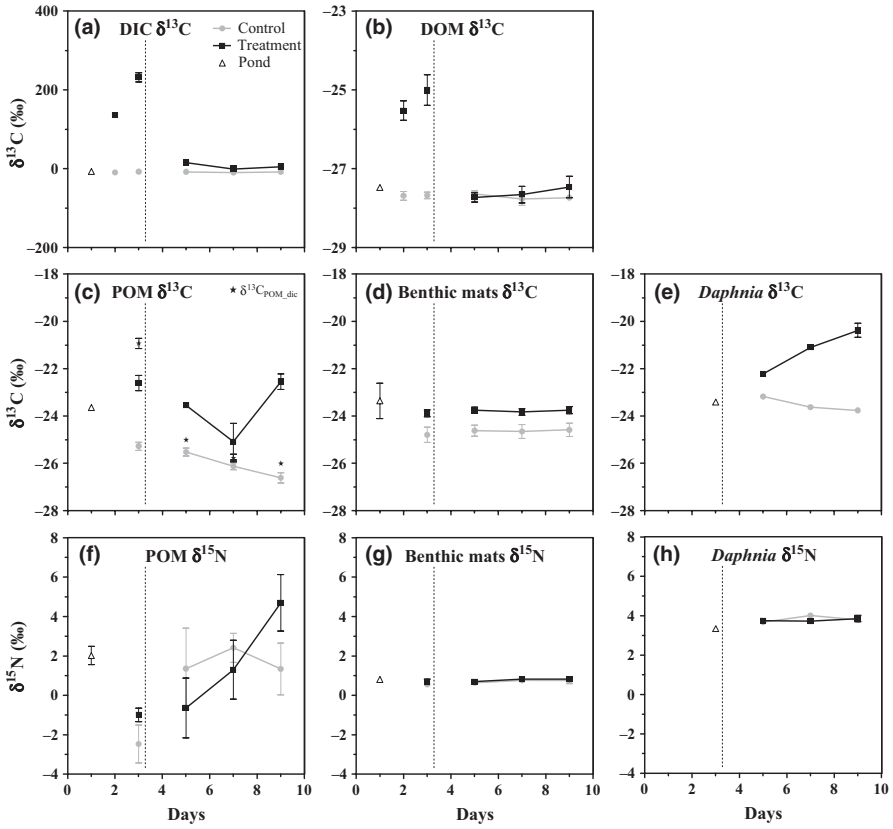

Fig. 1 Carbon and nitrogen stable isotope signatures of (a) dissolved inorganic carbon, (b) dissolved organic matter, (c, f) particulate organic matter, (d, g) benthic mats and (e, h) *Daphnia middendorffiana* (mean ± SE, n = 3) in Pond 19 and enclosures, August 2009. Dashed lines indicate the time at which the water column was replaced in the enclosures. Note different scales. When no SE is visible, the SE is smaller than the symbol.

−23.4 ± 0.1‰ to −20.4 ± 0.3‰; Fig. 1e). The δ^{13}C of daphniids in the control enclosures was progressively slightly depleted (down to −23.8 ± 0.1‰). Both time and ^{13}C-additions had a significant effect on the observed differences between treatment and control.

Particulate organic matter δ^{13}C values in the treatment enclosures were rather variable after water column replacement (ranging from −25.1 ± 0.8‰ to −22.6 ± 0.3‰), but were significantly enriched compared to controls (between −26.6 ± 0.2‰ and −25.5 ± 0.2‰; Fig. 1c). The δ^{13}C signal of DOM increased significantly in the enriched enclosures during the additions (up to −25.0 ± 0.4‰), but declined (−27.6 ± 0.1‰) after water replacement and almost reached the values of the controls (−27.7 ± 0.1‰; Fig. 1b).

Nitrogen stable isotope ratios

None of the parameters measured for δ^{15}N differed significantly between treatment and control enclosures. POM δ^{15}N varied during the experiment, with values ranging between −0.6 ± 1.5‰ and 4.7 ± 1.4‰ (mean ± SE) in the treatment and between 1.3 ± 1.3 and 2.4 ± 0.7‰ in the control after water column replacement (Fig. 1f). The δ^{15}N signal of benthic mats was very stable throughout the study period and averaged 0.8 ± 0.1‰ in the pond, 0.8 ± 0.1‰ in the treatment enclosures and 0.7 ± 0.1‰ in the controls (Fig. 1g). The δ^{15}N of *D. middendorffiana* also remained stable, with mean values of 3.4 ± 0.1‰ in the pond and 3.8 ± 0.1‰ in both treatment and control enclosures (Fig. 1h).

Natural abundance stable isotopes

The vegetation in the catchment area had δ^{13}C (range: −31.6 to −26.2‰) and δ^{15}N (−5.9–0.5‰) signatures that were consistently depleted compared to seston POM (δ^{13}C = −23.6‰, δ^{15}N = 2.0‰) and benthic mats (δ^{13}C = −23.4‰, δ^{15}N = 0.8‰) in Pond 19 (Fig. 2). The isotope ratios of DOM (δ^{13}C = −27.5‰, δ^{15}N = 0.8‰) indicated that both autochthonous and allochthonous sources contributed to the DOM pool. *Daphnia middendorffiana* had the highest δ^{15}N value (3.4‰), consistent with these cladocerans representing the highest trophic level in Pond 19. The δ^{13}C of *D. middendorffiana* (−23.6‰) was close to the carbon isotope value of autochthonous primary producers.

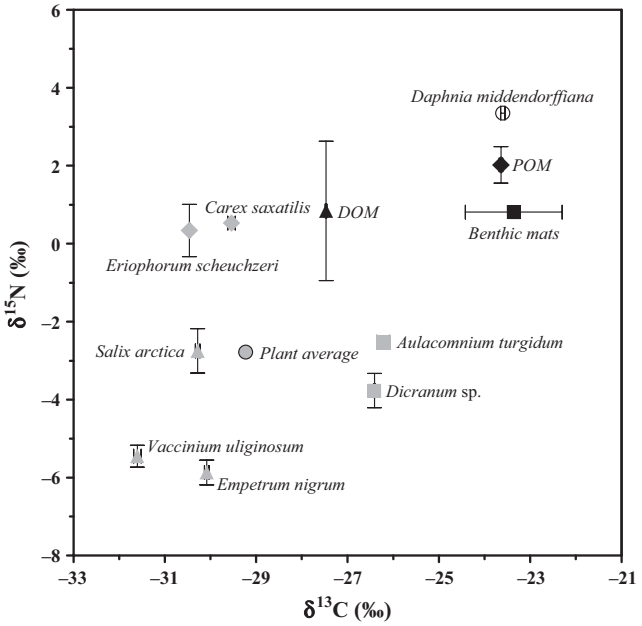

Fig. 2 Natural abundance of carbon and nitrogen stable isotopes (mean ± SE, n = 3) of *Daphnia middendorffiana*, benthic mats, particulate organic matter, dissolved organic matter and vegetation in the catchment, which comprises mosses (*Aulacomnium turgidum* and *Dicranum* sp.), sedges (*Carex saxatilis* and *Eriophorum scheuchzeri*) and dwarf shrubs (*Salix arctica*, *Vaccinium uliginosum* and *Empetrum nigrum*).

CDOM

The CDOM absorption coefficient at 320 nm, an indicator of CDOM concentration, was lowest in pond water (9.1 m^{-1}) and slightly higher in the control enclosures compared to the treatment enclosures (average 13.8 and 11.4 m^{-1}, respectively). DOC-specific absorption at 320 nm was low, from 1.1 in pond water to 1.7 in control enclosures, indicating minimal allochthonous inputs (Morris *et al.*, 1995). The spectral slope coefficient for 300–650 nm varied from 18 μm^{-1} in pond water to 13.2 μm^{-1} in control enclosures. The ratio between $S_{275–295}$ and $S_{350–400}$ (S_R) was >1 in all samples (Table 2), indicating low CDOM and dominance of autochthonous material (Helms *et al.*, 2008). $SUVA_{254}$, another parameter describing DOM quality, varied from 1.5 in pond water to 1.8 L in treatments and 2.0 L in controls. Such low values represent primarily autochthonous DOM.

Biomass and production in the pond

Pond 19 is an example of a shallow high arctic freshwater ecosystem, with low mean water temperature, poor nutrient concentration, scarce phytoplankton biomass but relatively high quantity of benthic algae (Table 3; Christoffersen *et al.*, 2008). Primary producers in the pond were clearly dominated by benthic algae, which accounted for 99 and 89%, respectively, of total algal biomass and production in the pond (Fig. 3). The biomass of phytoplankton was only 20% the biomass of *D. middendorffiana*, whereas biomass of benthic algae was over 20 times that of daphniids. We calculated that a minimum daily food ration of 175 $\mu g\ C\ L^{-1}$ (or 35 $mg\ C\ m^{-2}$) is required to sustain the population of *D. middendorffiana* in Pond 19 (C_d; Table 1). This amount corresponds to about half of the daphniid biomass present in the pond (400 $\mu g\ C\ L^{-1}$).

Benthic algae were composed of diatoms (>90% in volume) and cyanobacteria, whereas the phytoplankton was dominated by small flagellates. The estimated fraction of live algae (as percentage of total DW) was *c.* 3% in the benthic mats and 5% in POM (p_{algae}; Table 1).

Discussion

In our enclosure experiment, *D. middendorffiana* responded strongly to the carbon isotopic manipulation. The ^{13}C-signature of daphniids increased progressively over time in the treatment (over 3‰ enrichment compared to control and pond values), a clear indication that they were feeding on a ^{13}C-enriched food source. The measured $\delta^{13}C$-enrichment of benthic mats following water column replacement was about 1‰, but the actual

Table 2 Mean values (±SE) of parameters related to DOM quantity and quality in Pond 19 and enclosures, August 2009: a_{320}, absorption coefficient of DOM at 320 nm (m^{-1}); DOC-specific a_{320}, a_{320} divided by the DOC concentration; S, spectral slope for light absorption by DOM calculated on wavebands 300–650 nm, 275–295 nm and 350–400 nm (μm^{-1}); S_R, ratio of $S_{275–295}$ to $S_{350–400}$; $SUVA_{254}$, UV absorbance at 254 nm measured in inverse metres divided by the DOC concentration

	a_{320} nm (m^{-1})	DOC-specific a_{320}	$S_{300–650}$ (μm^{-1})	$S_{275–295}$ (μm^{-1})	$S_{350–400}$ (μm^{-1})	S_R	$SUVA_{254}$
Pond	9.1	1.1	18.0	20.7	18.3	1.1	1.5
Control	13.8 ± 0.3	1.7 ± 0.1	13.2 ± 0.0	17.9 ± 0.2	14.1 ± 0.9	1.3 ± 0.1	2.0 ± 0.1
Treatment	11.4 ± 1.0	1.4 ± 0.1	14.7 ± 1.3	18.9 ± 0.6	15.5 ± 1.2	1.2 ± 0.1	1.8 ± 0.1

DOC, dissolved organic carbon; DOM, dissolved organic matter.

Table 3 Mean values (±SE) of temperature (Temp.), TP, TN, DIC, DOM, as well as seston and benthic chlorophyll-*a* (Chl-*a*) measurements in Pond 19 and two sets of enclosures in the pond, August 2009

	Tempature (°C)	TP (μg L^{-1})	TN (mg L^{-1})	DIC (mg C L^{-1})	DOM (mg C L^{-1})	Chl-*a* seston (μg L^{-1})	Chl-*a* seston (μg cm^{-2})	Chl-*a* benthos (μg cm^{-2})
Pond	4.6 ± 0.2	15.1 ± 0.0	0.66 ± 0.02	2.7 ± 0.0	8.6 ± 0.2	2.4 ± 0.3	0.05 ± 0.01	5.9 ± 1.4
Control	4.7 ± 0.2	32.0 ± 4.0	0.73 ± 0.03	2.0 ± 0.0	8.4 ± 0.1	2.8 ± 0.3	0.06 ± 0.01	9.0 ± 0.9
Treatment	4.7 ± 0.2	35.3 ± 2.9	0.78 ± 0.03	2.0 ± 0.1	8.2 ± 0.0	2.3 ± 0.2	0.05 ± 0.00	8.1 ± 0.9

DIC, dissolved inorganic carbon; DOM, dissolved organic matter; TN, total nitrogen; TP, total phosphorus.

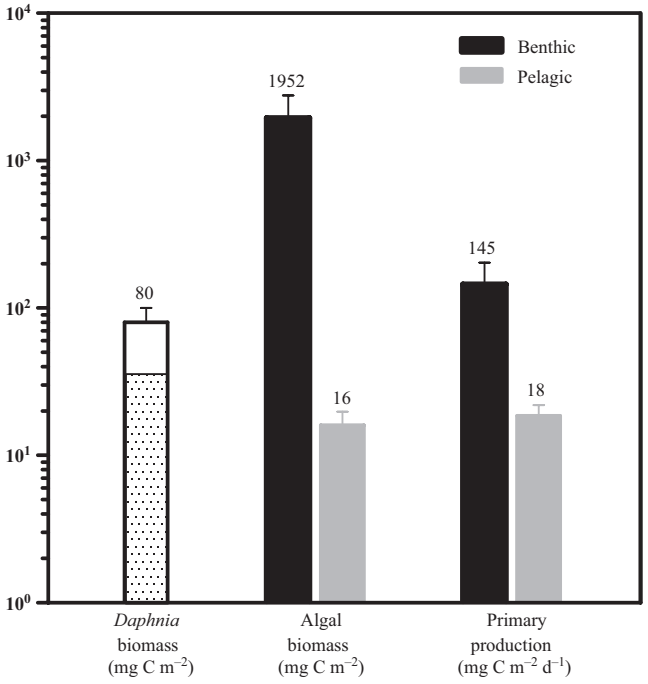

Fig. 3 Biomass of *Daphnia middendorffiana* and primary producers as well as algal primary production in benthic mats and water column of Pond 19 (mean ± SD). The dotted portion of the white column defines the minimum daily energy requirements of *D. middendorffiana* (35 mg C m^{-2} day^{-1}). Primary production values are from Vadeboncoeur *et al.* (2003), who investigated the pond in August 1999. Note the logarithmic scale.

enrichment of photosynthesising benthic algae in the treatment enclosures may have been much higher. This inference is based on the fact that only a small fraction (*c.* 3%) of the bulk benthos consisted of photosynthesising benthic algae, whereas the rest was composed of organic detritus and sediment. The latter did not take up the enriched DIC label and thus probably reduced the potential increase in δ^{13}C values of benthic mats.

However, benthic algae were not the only potential food source for *D. middendorffiana*, showing a ^{13}C-enrichment following replacement of the water column. Indeed, we unexpectedly detected a significant enrichment in the POM fraction as well. This enrichment may well have reflected the occurrence of heterotrophic bacteria in the water column relying on ^{13}C-enriched DOM released by photosynthesising benthic algae. Since bacteria in arctic ponds are typically >1 μm in diameter (Roiha *et al.*, *in press*), they are retained on GF/F filters and therefore contribute to the POM isotopic signal. Recent studies have shown that the DOM released by benthic algae can be exploited efficiently by pelagic heterotrophic bacteria and passed up the food web (Hopkinson *et al.*, 1998; Kamjunke, Bohn & Grey, 2006; Ask *et al.*, 2009; Karlsson & Säwström, 2009). We did not observe any enrichment in the δ^{13}C-signature of DOM after the water column was replaced, but this does not exclude the fact that benthic DOM exudates may have been released into the water column. It is more likely to indicate that such a labile fraction of DOM (Kritzberg *et al.*, 2004; Kamjunke *et al.*, 2006) was readily exploited by pelagic bacteria, whose production was probably boosted by the increased concentration of phosphorus and nitrogen in the enclosures. Such nutrient enrichment was possibly the result of sediment disturbance during the addition of water to the enclosures.

The possibility that phytoplankton could have taken up residual ^{13}C-enriched DIC after water replacement, and caused the observed enrichment in the POM fraction, is unlikely. When replacing the water column, a small fraction (*c.* 5%) of ^{13}C-enriched water remained in the enclosures, as it would have been impossible to drain all the water without removing some of the benthic mats sitting on the top of the sediment. However, the residual-enriched DIC in the water would have been sufficient to promote only a marginal increase in the δ^{13}C values of POM in the treatment enclosures. This inference is supported by our calculation of the maximum potential ^{13}C-enrichment of the POM fraction as a result of uptake of enriched DIC by phytoplankton ($\delta^{13}C_{POM_dic}$; Table 1; Fig. 1c). Even in the unlikely event of all phytoplankton in the treatment enclosure having an enrichment equivalent to the observed difference between control and treatment δ^{13}C of DIC, the resulting POM enrichment would have averaged only up to 0.5‰, much lower than the measured

difference between control and treatment (2.4‰). Moreover, the calculated $\delta^{13}C_{POM\_dic}$ values probably represent an overestimation, because of the likely occurrence of photosynthetically inactive pigments (therefore not fixing the enriched DIC) in such cold, nutrient-poor waters (Markager, Vincent & Tang, 1999). This suggests that the observed differences between treatment and control values of POM are mainly because of reasons other than ^{13}C-labelling of phytoplankton.

Tundra ponds are exposed to wind-induced mixing that may bring loose benthic material into resuspension and make it available as a potential food for pelagic grazers (Rautio & Vincent, 2007). In our enclosure experiment, the sediment may also have been disturbed during sampling operations, leading to potential resuspension of benthic algae that could have increased the $\delta^{13}C$ of POM in the treatment enclosures. However, microscopical analysis revealed that benthic algae were dominated (>90% in volume) by large diatoms, which if resuspended, would sink rather quickly. On the other hand, the pelagic community was composed mostly of small flagellated algae, while diatoms were virtually absent. Hence, the hypothesis that the high $\delta^{13}C$ POM values are caused by suspension of benthic algae into the water column can be refuted.

Another potential cause of enrichment of the $\delta^{13}C$ values of POM may be related to release of organic carbon by *D. middendorffiana* grazing on ^{13}C-enriched benthic mats. Lampert (1978) showed experimentally that between 10 and 17% of the carbon consumed by daphniids is secreted as DOM and by leaching from their faeces. *Daphnia middendorffiana* grazing on benthic algae may have released ^{13}C-enriched DOM, which, in turn, may have been recycled by pelagic bacteria, increasing the $\delta^{13}C$ values of POM.

The results suggest, therefore, that the observed enrichment in $\delta^{13}C$ of *D. middendorffiana* is related to feeding either directly on benthic algae or on bacteria recycling organic matter of benthic origin, or a combination of the two. In any case, the benthic algae probably play an important ecological role in supporting the energy demand of *D. middendorffiana*. During the study, we regularly observed in both pond and enclosures a large number of daphniids swimming to the bottom and apparently either grazing directly on the surface sediment (as described by Rautio & Vincent, 2006) or rotating quickly just above it. The latter may resuspend food particles from the sediment, as confirmed by Horton *et al.* (1979) who showed that *D. magna* Straus and *D. pulex* De Geer in an aquarium normally used suspension feeding. Our observation supports the hypothesis that at least part of the food intake by *D. middendorffiana* may have been obtained through direct grazing on the benthic surface.

The results from nitrogen stable isotope analyses provide further evidence of *D. middendorffiana* feeding on benthic organic matter. The $\delta^{15}N$ values of benthic mats and of the daphniids remained stable throughout the experiment and matched well when taking into account the trophic fractionation of *c.* 3‰ occurring between food source and consumer (Minagawa & Wada, 1984; Kling *et al.*, 1992). On the other hand, the $\delta^{15}N$-signature of POM varied during the experiment, with values increasing from -0.6 ± 1.5 to 4.7 ± 1.4‰ in the treatment enclosures. The lack of any corresponding change in the isotopic signature of daphniids indicates that phytoplankton was not the main food source for *D. middendorffiana* in the studied system.

We calculated that pelagic primary production in Pond 19 was sufficient to satisfy only up to about half of the estimated daily energy requirement of *D. middendorffiana*, implying that the daphniids have an additional food source. Benthic algae clearly dominated primary production in the pond, as is typical of oligotrophic arctic lakes (Vadeboncoeur *et al.*, 2003; Vincent & Laybourn-Parry, 2008). Algal biomass and production in the benthos were over 100 times and eight times greater, respectively, than pelagic values. If *Daphnia* is able to feed on benthic algae, as previous studies suggest (Horton *et al.*, 1979; Rautio & Vincent, 2006, 2007), the latter may well represent a large food reserve for the daphniids.

One might ask why benthic resources are not grazed more heavily by the zooplankton. Benthic mats, as opposed to phytoplankton, may be deficient in part of the essential fatty acids required for zooplankton somatic growth (Mariash *et al.*, 2011). This suggests that, while arctic zooplankton may grow on benthic algae, their performance is improved if they can also consume even small quantities of phytoplankton. Additionally, filter-feeding zooplankton are well adapted to feeding on suspended particles and may find it difficult to access benthic algae. Therefore, a switch to benthic resources may happen only when the concentration of phytoplankton drops below a certain threshold (Siehoff *et al.*, 2009).

Benthic algae contribute progressively more to total area-based primary production with decreasing depth, as irradiance at the sediment surface increases while the water volume declines (Whalen, Chalfant & Fischer, 2008). Nevertheless, primary production in clear-water arctic lakes can be dominated by benthic algae even in systems as deep as 10 m (Rautio & Vincent, 2007; Whalen *et al.*, 2008), lending support to the notion that benthic organic matter may be important for pelagic secondary production in

waterbodies deeper than the one evaluated here. However, lakes that do not freeze solid during winter may host fish, which can greatly reduce the biomass of zooplankton and especially large cladocerans such as *Daphnia* (Rautio & Vincent, 2006; Christoffersen *et al*., 2008). In such systems, pelagic primary production may be sufficient to support zooplankton growth, and benthic algae may play only a minor role as food. Exploitation of benthic resources by zooplankton will also decrease with increasing nutrient concentrations in the lakes, as eutrophication can promote a switch from benthic to pelagic dominance of primary production (Vadeboncoeur *et al*., 2003). Therefore, we suggest that, at lower latitudes, top-down control of zooplankton by fish and a greater supply of nutrients may reduce the contribution of benthic resources to pelagic secondary production. The switch from benthic to pelagic sources may be further reinforced by the combined effect of a longer growing season, higher temperature and higher solar radiation at lower latitudes.

Previous studies have also discussed the importance of terrestrial carbon in fuelling the food webs of arctic lakes. Karlsson *et al*. (2003) claimed that allochthonous carbon may subsidise up to 80% of secondary production in subarctic Swedish lakes during the summer, while Rautio, Mariash & Forsström (2011) calculated that 0–50% of zooplankton food in a Finnish subarctic lake originated from allochthonous carbon in summer and 100% in winter for certain zooplankton species. Organic carbon fixed by plants in the catchment can enter the lakes and be recycled via the microbial loop, which, in turn, may constitute an energy source for zooplankton. Carbon isotope values in Pond 19 seem to indicate that allochthonous organic matter may have made a greater contribution to the DOM pool than autochthonous producers. However, this approach may well underestimate the importance of autochthonous input of DOM into the food web, as autochthonous DOM is labile and used by bacteria selectively over allochthonous DOM. The latter fraction is comprised of high molecular weight compounds that are refractory to microbial use (Kritzberg *et al*., 2004; Kamjunke *et al*., 2006). Therefore, the measured $\delta^{13}C$ values of DOM may well be a better representation of what is left by heterotrophic bacteria, rather than an indication of actual contribution of autochthonous and allochthonous inputs. In contrast to carbon isotope values, DOM spectroscopy provides a clear indication that the DOM in Pond 19 is mainly derived from autochthonous pelagic and benthic processes. The idea that the terrestrial input may have been of lesser importance in the present system is further supported by SIA of primary consumers, as *D. middendorffiana* had an isotopic signature that indicated reliance on pond primary production, rather than allochthonous organic matter.

In conclusion, results from SIA in the enclosure experiment show that organic matter of benthic origin probably constituted a major energy source for *D. middendorffiana* in this system. Primary production by benthic algae was sufficient to satisfy the daily energy demand of the daphniids, whereas algal production in the water column was not. Stable isotope ratios point to little or no contribution of allochthonous carbon to the diet of the daphniids, which agrees with the indication of dominance by autochthonous organic matter in the pond provided by CDOM analysis. We also suggest that benthic algal exudates may be recycled by heterotrophic bacteria in the water column and in turn provide an additional food source for the cladocerans. Future studies should address the importance of the bacteria-mediated benthic–pelagic coupling in supporting secondary production in arctic lakes and ponds.

Acknowledgments

This work was funded by the University of Copenhagen. L. Forsström and M. Rautio were supported by the Academy of Finland (grants 119205 and 140775). We thank Roger I. Jones (University of Jyväskylä) for advice during the planning of the enclosure experiment. Nina Reuss and Trine W. Perlt (University of Copenhagen) are acknowledged for valuable comments to earlier versions of the manuscript and N. John Anderson (Loughborough University) for linguistic support.

References

Ask J., Karlsson J., Persson L., Ask P., Byström P. & Jansson M. (2009) Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology*, **90**, 1923–1932.

Bricaud A., Morel A. & Prieur L. (1981) Absorption by dissolved organic matter of the sea (yellow substance) in the UV and visible domains. *Limnology and Oceanography*, **26**, 43–53.

Christoffersen K.S., Amsinck S.L., Landkildehus F., Lauridsen T.L. & Jeppesen E. (2008) Lake flora and fauna in relation to ice-melt, water temperature and chemistry at Zackenberg. In: *High-Arctic Ecosystem Dynamics in a Changing Climate. Ten Years of Monitoring and Research at Zackenberg* (Eds H. Meltofte, T.R. Christensen, B. Elberling, M.C. Forchhammer & M. Rasch), *Advances in Ecological Research*, 40, 371–389.

Elser J.J., O'Brien W.J., Dobberfuhl D.R. & Dowling T.E. (2000) The evolution of ecosystem processes: growth rate

and elemental stoichiometry of a key herbivore in temperate and arctic habitats. *Journal of Evolutionary Biology*, **13**, 845–853.

Green J. (1954) Size and reproduction in *Daphnia magna* (Crustacea: Cladocera). *Proceedings of the Zoological Society of London*, **124**, 535–545.

Greenberg A.E., Clesceri L.S., Rice E.W. & Franson M.H. (2005) *Standard Methods for Examination of Water & Wastewater*, 21st edn. American Public Health Association, Washington, DC.

Haney J.F. & Buchanan C. (1987) Distribution and biogeography of *Daphnia* in the Arctic. *Memorie dell'Istituto Italiano di Idrobiologia*, **45**, 77–105.

Hecky R.E. & Hesslein R.H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society*, **14**, 631–653.

Helms J.R., Stubbins A., Ritchie J.D., Minor E.C., Kieber D.J. & Mopper K. (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, **53**, 955–969.

Hopkinson C.S., Giblin A.E., Garritt R.H., Tucker J. & Hullar M.A.J. (1998) Influence of the benthos on growth of planktonic estuarine bacteria. *Aquatic Microbial Ecology*, **16**, 109–118.

Horton P.A., Rowan M., Webster K.E. & Peters R.H. (1979) Browsing and grazing by Cladoceran filter feeders. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **57**, 206–212.

Jerlov N.G. (1968) *Optical Oceanography*. Oceanography Series 5. Elsevier, Amsterdam.

Jespersen A.M. & Christoffersen K. (1987) Measurements of chlorophyll-*a* from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie*, **109**, 445–454.

Kamjunke N., Bohn C. & Grey J. (2006) Utilisation of dissolved organic carbon from different sources by pelagic bacteria in an acidic mining lake. *Archiv für Hydrobiologie*, **165**, 355–364.

Karlsson J. & Säwström C. (2009) Benthic algae support zooplankton growth during winter in a clear-water lake. *Oikos*, **118**, 539–544.

Karlsson J., Jonsson A., Meili M. & Jansson M. (2003) Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography*, **48**, 269–276.

Kirk J.T.O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*, 2nd edn. Cambridge University Press, Cambridge.

Kling G.W., Fry B. & O'Brien W.J. (1992) Stable isotopes and planktonic trophic structure in arctic lakes. *Ecology*, **73**, 561–566.

Kritzberg E.S., Cole J.J., Pace M.L., Graneli W. & Bade D.L. (2004) Autochthonous versus allochthonous carbon sources of bacteria: results from whole-lake ^{13}C addition experiments. *Limnology and Oceanography*, **49**, 588–596.

Lampert W. (1978) Release of dissolved organic-carbon by grazing zooplankton. *Limnology and Oceanography*, **23**, 831–834.

Lundgren B. (1976) *Spectral Transmittance Measurements in the Baltic*. Report no. 30, Institute of Physical Oceanography, University of Copenhagen, Copenhagen, 38 pp.

Mariash H.L., Cazzanelli M., Kainz M.J. & Rautio M. (2011) Food sources and lipid retention of zooplankton in subarctic ponds. *Freshwater Biology*, **56**, 1850–1862.

Markager S., Vincent W.F. & Tang E.P.Y. (1999) Carbon fixation by phytoplankton in high Arctic lakes: implications of low temperature for photosynthesis. *Limnology and Oceanography*, **44**, 597–607.

Minagawa M. & Wada E. (1984) Stepwise enrichment of ^{15}N along food-chains – further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135–1140.

Morris D.P., Zagarese H., Williamson C.E., Balseiro E.G., Hargreaves B.R., Modenutti B. *et al.* (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography*, **40**, 1381–1391.

Rautio M. & Vincent W.F. (2006) Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. *Freshwater Biology*, **51**, 1038–1052.

Rautio M. & Vincent W.F. (2007) Isotopic analysis of the sources of organic carbon for zooplankton in shallow subarctic and arctic waters. *Ecography*, **30**, 77–87.

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

Reynolds C.S. (1984) *The Ecology of Freshwater Phytoplankton*. Cambridge University Press, Cambridge.

Riemann B., Simonsen P. & Stensgaard L. (1989) The carbon and chlorophyll content of phytoplankton from various nutrient regimes. *Journal of Plankton Research*, **11**, 1037–1045.

Roiha T., Tiirola M., Cazzanelli M. & Rautio M. (in press) Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. *Aquatic Sciences*.

Siehoff S., Hammers-Wirtz M., Strauss T. & Ratte H.T. (2009) Periphyton as alternative food source for the filter-feeding cladoceran *Daphnia magna*. *Freshwater Biology*, **54**, 15–23.

Smol J.P. & Douglas M.S.V. (2007) Crossing the final ecological threshold in high Arctic ponds. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 12395–12397.

Syväranta J. & Rautio M. (2010) Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1721–1729.

Vadeboncoeur Y., Jeppesen E., Vander Zanden M.J., Schierup H.H., Christoffersen K. & Lodge D.M. (2003) From Greenland to

green lakes: cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, **48**, 1408–1418.

Van Geest G.J., Spierenburg P., Van Donk E. & Hessen D.O. (2007) *Daphnia* growth rates in Arctic ponds: limitation by nutrients or carbon? *Polar Biology*, **30**, 235–242.

Vincent W.F. & Laybourn-Parry J. (2008) *Polar Lakes and Rivers*. Oxford University Press, Oxford.

Weishaar J.L., Aiken G.R., Bergamaschi B.A., Fram M.S., Fujii R. & Mopper K. (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology*, **37**, 4702–4708.

Whalen S.C., Chalfant B.A. & Fischer E.N. (2008) Epipelic and pelagic primary production in Alaskan Arctic lakes of varying depth. *Hydrobiologia*, **614**, 243–257.

Yurista P.M. (1999) Temperature-dependent energy budget of an Arctic cladoceran, *Daphnia middendorffiana*. *Freshwater Biology*, **42**, 21–34.

Yurista P.M. & O'Brien W.J. (2001) Growth, survivorship and reproduction of *Daphnia middendorffiana* in several Arctic lakes and ponds. *Journal of Plankton Research*, **23**, 733–744.

(Manuscript accepted 10 November 2011)