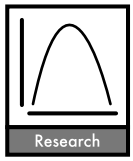

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Isotopic analysis of the sources of organic carbon for zooplankton in shallow subarctic and arctic waters

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Shallow high-latitude lakes and ponds are usually characterized by an oligotrophic water column overlying a biomass-rich, highly productive benthos. Their pelagic food webs often contain abundant zooplankton but the importance of benthic organic carbon versus seston as their food sources has been little explored. Our objectives were to measure the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of pelagic and benthic particulate organic matter (POM) in shallow water bodies in northern Canada and to determine the relative transfer of this material to zooplankton and other aquatic invertebrates. Fluorescence analysis of colored dissolved organic matter (CDOM) indicated a relatively strong terrestrial carbon influence in five subarctic waterbodies whereas the CDOM in five arctic water columns contained mostly organic carbon of autochthonous origin. The isotopic signatures of planktonic POM and cohesive benthic microbial mats were distinctly different at all study sites, while non-cohesive microbial mats often overlapped in their $\delta^{13}C$ signals with the planktonic POM. Zooplankton isotopic signatures indicated a potential trophic link with different fractions of planktonic POM and the non-cohesive mats whereas the cohesive mats did not appear to be used as a major carbon source. The zooplankton signals differed among species, indicating selective use of resources and niche partitioning. Most zooplankton had $\delta^{13}C$ values that were intermediate between the values of putative food sources and that likely reflected selective feeding on components of the pelagic or benthic POM. The results emphasize the likely importance of benthic-pelagic coupling in tundra ecosystems, including for species that are traditionally considered pelagic and previously thought to be dependent only on phytoplankton as their food source.

Shallow lakes and ponds are widely distributed throughout high northern latitudes. These systems freeze to the bottom each winter and melt out for up to three months each summer. They have a high perimeter:area ratio and are therefore likely to be strongly influenced by their surrounding terrestrial environment. A striking feature of these ecosystems is that they contain abundant zooplankton populations despite an oligotrophic water column (Rautio and Vincent 2006). However, the base of these waterbodies is often covered by productive algal mats (Vézina and Vincent 1997, Vadeboncoeur et al. 2003, Bonilla et al. 2005) that could potentially play a role in supporting consumer populations. There is some evidence from clear arctic waters in northern Canada that bottom-growing algae may be a food source for zooplankton (Hecky and Hesslein 1995) and benthic algae have been shown to contribute at least in part to the diet of large copepods in subantarctic lakes (Hansson and Tranvik 2003). In one of the earliest observations of arctic zooplankton, in a small tundra lake near Lake Hazen on Ellesmere Island, McLaren (1958) noted: ''The impression gained in the field was that the very considerable crop of zooplankton in this small lake could not have been supported by the amount of phytoplankton present. It is possible that another food source is available. This shallow, unstratified lake is carpeted thickly with aquatic moss, and the zooplankton may be able to feed on benthically produced organic detritus.''

In the present study we applied isotopic and fluorescence analysis to characterize the organic carbon content and potential food sources for zooplankton in northern high-latitude water bodies. Stable isotope composition is a powerful tool for investigating

zooplankton trophic links as it combines information about long-term food selection and assimilation which may be very different from the responses measured in short-term experiments (Kling et al. 1992, Grey et al. 2001). This approach is especially advantageous for analysing foodwebs that depend on mixtures of carbon sources with different isotopic signals (Vander Zanden and Rasmussen 1999, Barnard et al. 2006). Isotopic analysis has revealed that the ecological importance of non-planktonic carbon such as allochthonous materials or epibenthic algae may be more pronounced in some aquatic systems than has been previously thought (Grey et al. 2001, Hansson and Tranvik 2003, Kritzberg et al. 2004, Pace et al. 2004). As a complement to stable isotope analysis, synchronous fluorescence scans of water can be used to characterize the dissolved organic carbon pool in natural waters. These scans provide insights into the chemical composition of coloured dissolved organic matter (CDOM) and a guide to the relative importance of autochthonous versus allochthonous carbon inputs (McKnight et al. 2001, Belzile et al. 2002). Organic carbon varies in its properties depending on its autochthonous or allochthonous origin and likely influences the whole food web structure in the water body. For instance, bacteria are known to be efficient in using allochthonous organic matter for their growth (Tranvik 1988) and may suppress primary production in lakes via their better nutrient competition abilities (Drakare et al. 2003).

We hypothesized that the zooplankton in high-latitude lake and pond ecosystems are supported by organic carbon sources of benthic origin in addition to planktonic primary producers. We addressed this hypothesis by isotopic analysis of the phytoplankton, the phytobenthos and other organic matter sources in two tundra areas in northern Canada, and evaluated the transfer of these food signatures to different zooplankton species. Zooplankton were analyzed at the species level and in relation to the hypothesis that diet differed according to taxonomic group. We further hypothesized that the importance of terrestrial carbon varies between the subarctic ponds in the forest-tundra region and high arctic ponds in the polar desert catchments, and addressed this hypothesis by synchronous fluorescence analysis of CDOM.

Methods

Study sites

We sampled nine tundra ponds, all <1 m in depth. Five of the sites were located in coastal subarctic northern Quebec (55–56°N, 77–78°W) and four on Cornwallis Island in the Canadian High Arctic (74–75°N, 94–95°W). On Cornwallis Island, in addition to ponds we also sampled 9 m deep Meretta Lake (site A2) in the vicinity of Resolute Bay village. All sites were oligotrophic and had transparent water columns (Table 1). As is typical of tundra ponds and lakes in general, benthic algae and associated heterotrophic organisms formed mat-like structures on the bottom of the water bodies. In some of the ponds the mats formed either dense orange mats up to several millimetres thick, while in others the bottom was covered with loose brown microbial mats. The mats were dominated by filamentous cyanobacteria (mostly oscillatorians, notably *Leptolyngbya* spp. and *Oscillatoria* spp.) but also contained protists and bacteria. In addition to these two main types of mats, some water bodies had green aggregates of filamentous green algae (*Zygnema* sp.) and aquatic mosses. According to our recent data, the benthic community represents $>85\%$ of the total (i.e. planktonic plus benthic) autotrophic biomass per unit area in all the sites, and 60–98% of the total primary production per unit area (Rautio and Vincent 2006). These numbers are in accordance with earlier measurements from Char Lake in Resolute where Welch and Kalff (1974) reported that benthic photosynthesis contributed 80% of the total, lake-wide

Table 1. Environmental characteristics of the five subarctic and five arctic waterbodies. The values for temperature, conductivity and pH are means for 12–17 midday measurements in July for S8–S10 ponds, and 4–5 measurements in late August for A1–A2, and A4–A5 sites. nd = no data, A2 = Meretta Lake.

Site	Temp. (°C)	pH	POC (mg L^{-1})	DIC (mg L^{-1})	NO_3-N (μg L^{-1})	TP (μg L^{-1})
S6	14.9	6.0	0.53	nd	3	4
S7	23.5	6.6	1.47	nd	2	12
S8	16.6	7.3	0.35	4	4	9
S9	16.1	7.4	0.44	3.7	4	33
S10	16.8	7.6	0.23	4.2	4	13
A1	3.6	8.5	0.26	22.7	2	5
A2	4.7	8.5	0.26	15.6	<1	5
A3	6.3	9.6	0.86	25.5	1	21
A4	4.5	8.4	0.15	25.4	<1	5
A5	4.1	8.4	0.29	25.4	1	4

POC, particulate organic carbon; DIC, dissolved inorganic carbon; NO_3-N, nitrate nitrogen; TP, unfiltered total phosphorus.

primary production, although primarily by slow-growing mosses (Sand-Jensen et al. 1999) rather than by benthic algal mats.

Sample collection and analysis

The sampling was undertaken in July 2002 in northern Quebec and in August 2002 on Cornwallis Island. For stable isotope analysis, samples of the dominant crustacean zooplankton species and their potential pelagic and benthic food sources were obtained from each water body. Each sample was collected in 2–4 replicates except where the low amount of material did not allow for more than one sample. Water samples for chromophoric dissolved organic matter (CDOM) were collected in acid washed, sample-rinsed bottles.

Up to 10 L of water were collected at each sampling site for seston stable isotope analysis. This volume was prefiltered through 200 μm (POM $<$200 μm) to remove larger material, and then a subsample was filtered through 5 μm (POM $<$5 μm) (all but S6 and S7 ponds). For site S10 a $<$50 μm fraction was also taken. Prefiltration through 200 μm may have removed some large diatoms and *Dinobryon* colonies but this effect was probably minimal as our earlier work has shown that 70% of the total phytoplankton biomass in these water bodies is associated with taxa that are $<$30 μm in size (Rautio and Vincent 2006). In addition, $>$200 μm phytoplankton cells and colonies are too large for many zooplankton to feed on. Each fraction was then filtered through a precombusted (500°C, 1 h) Whatman GF/F filter that was stored frozen until analysis. Two sites (S8, S9) were also sampled for the large colonial algae *Volvox* sp. by transferring individual colonies with a Pasteur pipette to GF/F filtered water and then to an Eppendorf tube.

The dominant microbial mat types were collected from representative sites in all the sampled water bodies, however in Meretta Lake on Cornwallis Island, only mats in the shallow ($<$1 m) bay were sampled. Samples from orange-coloured, cohesive mats were collected with a 10 mm diameter sediment corer (a syringe with the end cut off). The top 1 mm of the core was sectioned with a blade, put in an aluminium foil and frozen until analysis. Brown mats, which were too loose for coring, were sampled by brushing material from submerged stones into a vial. Filamentous mats were brought up from water by hand, dried with absorbent paper, and cut into 1 × 1 cm sections. Aquatic mosses (A1), macrophytes (S7) and surface material from a log (S7) were also collected from single sites.

Zooplankton samples were obtained by horizontal trawls using a 250 μm meshed sized net attached to a long pole. Animals were washed with GF/F filtered water, sorted by hand while still alive and stored frozen in Eppendorf tubes (5–300 individuals depending on species). Altogether 10 different zooplankton species representing 21 populations were collected from the 10 sites studied. The species sampled included cladocerans (*Daphnia middendorfiana*, *Ceriodaphnia quadrangula*, *Scapholeberis mucronata*), copepods (*Leptodiaptomus minutes*, *Hesperodiaptomus arcticus* and 3 unidentified but different cyclopoid species) and fairy shrimps (*Artemiopsis stefanssoni*, *Branchinecta paludosa*). In addition, invertebrate predators and benthic animals including phantom midge larvae (*Chaoborus* sp.), water mites (Hydracarina), water beetles (Dytiscidae), snails, midge larvae (Chironomidae) and seed shrimps (Ostracoda) were sampled when present.

All samples were acidified via fuming with 36% HCl to remove inorganic carbon (Yamamuro and Kayanne 1995, Martineau et al. 2004). This method was selected because it avoids any loss of organic C or N during inorganic C removal. Isotopic analyses were carried out by the Commission Géologique du Canada using an Isotope Ratio Mass Spectrometer (Fisons Instruments, model VG Prism Isotech). The standards for ^{13}C and ^{15}N were PeeDee Belemnite (PDB) and atmospheric N_2, respectively. The precision for both $\delta^{13}C$ and $\delta^{15}N$ was $<$0.2 ‰. The samples were analysed for $\delta^{13}C$ and $\delta^{15}N$, percent carbon and percent nitrogen. Because lipid concentration is high in arctic zooplankton and it is believed to deplete the $\delta^{13}C$ signal (Kling et al. 1992), we removed lipids from five samples representing both cladocerans and calanoids with visible lipid droplets. Samples were washed in a 1:1 methanol:-chloroform solution for three 10 min intervals and then freeze-dried before mass spectrometer analyses.

CDOM was characterized in water samples that were filtered through prerinsed 0.22 μm Sartorius cellulose acetate filters immediately after sampling and stored at 4°C in acid-cleaned, amber glass bottles until analysis. Synchronous fluorescence spectra (Senesi et al. 1991) were recorded with a Shimadzu FR5000 spectrofluorometer (Shimadzu, Kyoto, Japan) used in the synchronous mode with a difference of 14 nm between excitation and emission wavelengths as in Belzile et al. (2002). Fluorescence scans were standardized to quinine sulphate (QSU) and corrected for the absorption within the sample (inner filter effect) according to McKnight et al. (2001) except that the absorption coefficient of the sample was measured by spectrophotometry as in Belzile et al. (2002).

Data analysis

ANOVAs were used to examine if the average $\delta^{13}C$ and $\delta^{15}N$ values were different among regions (Subarctic, Arctic), food types (POM $<$200 μm, POM $<$5 μm, orange mat, brown mat) and taxonomic groups

(cladocerans, copepods, fairy shrimps, bottom dwellers, invertebrate predators). Variance components were estimated using restricted maximum-likelihood method (VARCOM procedure, Anon. 1999). When a source of variation was significant, a posteriori multiple comparisons (LS means, Anon. 1999) were carried out with the Bonferonni-adjusted level to identify differences.

Results

Between-region variability

Our dataset represents tundra ponds or lakes from both arctic and subarctic areas separated by >2000 km, and it was of interest to determine whether there occurred regional separation in the isotopic values that were used to establish the food web structure. The region effect accounted for 29% of the total variance in $\delta^{13}C$ and 30% in $\delta^{15}N$ (Table 2). In general, the $\delta^{13}C$ values of POM and benthic mats in subarctic sites were more negative than the respective values in arctic sites (Fig. 1). These between-region differences were statistically significant for orange mats and POM <200 μm (Bonferroni's multiple comparison test for $\delta^{13}C$; Fig. 1, Table 2). $\delta^{13}C$ values of POM <5 μm were also generally more negative in the subarctic although the differences were not significant, whereas the $\delta^{13}C$ values for the fine structured loose microbial mat (brown mat) overlapped between the regions. Similarly the $\delta^{15}N$ values of the food types in subarctic ponds were lower than in arctic ponds and the differences were statistically significant between POM <200 μm and between orange mats in the two regions (Bonferroni's multiple comparison test for $\delta^{15}N$, Fig. 1). The isotopic signals of crustacean zooplankton also varied between the regions with subarctic species lighter in both $\delta^{13}C$ and $\delta^{15}N$ than their arctic counterparts (Fig. 2).

The synchronous fluorescence scans provided insights into the chemical composition of the CDOM and the origin of carbon in the water body (Fig. 3). The subarctic pond samples had strong fluorescence peaks around 400–500 nm, indicating the dominance of high molecular weight allochthonous humic and fulvic materials. In the Arctic, the peaks were either highest around 300 nm indicating dominance of autochthonous carbon in the water body, or the magnitude of peaks was invariable between allochthonous and autochthonous wavebands. The ratio of fluorescence at 300 nm to 475 nm ranged from 0.94 at A3 to 0.18 at S6, consistent with the much greater autochthonous carbon in the total DOC pool in the arctic lakes and ponds.

Within-region variability

Within the two regions benthic microbial mats, especially the orange mats were characterized by heavier

Table 2. Summary of ANOVAs showing the effect of region (Subarctic, Arctic), food type (POM <200 μm, POM <5 μm, orange mat, brown mat) and interactions on (a) $\delta^{13}C$, (b) $\delta^{15}N$. For a given region, ANOVA results of the effect of taxonomic groups (cladocerans, copepods, fairy shrimps, bottom dwellers, invertebrate predators) are shown for (c) subarctic $\delta^{13}C$, (d) subarctic $\delta^{15}N$, (e) arctic $\delta^{13}C$, and (f) arctic $\delta^{15}N$. Variance components are estimated using restricted Maximum likelihood method.

Source of variation	DF	MS	F-value	p	% variance
(a) $\delta^{13}C$					
Region	1	222.82	21.09	<0.0001	29
Food	3	205.97	19.46	<0.0001	24
Region × food	3	46.15	4.37	0.0087	17
Error	46	10.57			31
(b) $\delta^{15}N$					
Region	1	62.98	18.24	<0.0001	30
Food	3	14.78	4.28	0.0096	1
Region × food	3	15.12	4.38	0.0086	22
Error	43	3.45			47
(c) Subarctic $\delta^{13}C$					
Taxonomic group	2	0.72	0.14	0.8689	0
Error	24	5.12			100
(d) Subarctic $\delta^{15}N$					
Taxonomic group	2	14.04	27.29	<0.0001	75
Error	21	0.51			25
(e) Arctic $\delta^{13}C$					
Taxonomic group	3	22.10	1.43	0.2628	6
Error	20	15.42			94
(f) Arctic $\delta^{15}N$					
Taxonomic group	3	8.80	8.03	0.0015	61
Error	17	1.49			39

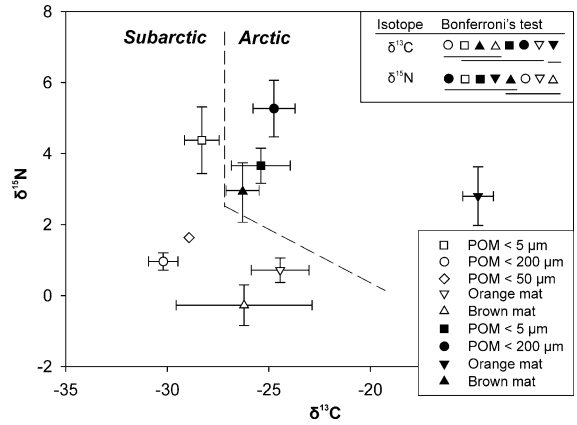

Fig. 1. Mean $\delta^{13}C$ vs mean $\delta^{15}N$ ($\pm$SD) for the putative zooplankton food sources in the sampled high latitude lakes. The dashed line represents the division between the subarctic (open symbols) and arctic (closed symbols) values. Multiple comparisons of stable isotope values among food types are shown in the inserted upper box. Food types are placed in decreasing order of isotopic values (LS means).

$\delta^{13}C$ signals than the planktonic POM fractions (Fig. 1, Fig. 4) although the differences proved to be significant only when the arctic orange mats were compared to all other arctic food sources (Bonferroni's multiple comparison test for $\delta^{15}N$, Fig. 1). There was significant within-region difference among primary producer $\delta^{13}C$ values, while differences in $\delta^{15}N$ values among potential food source types were less pronounced. Of the subarctic sources measured, only POM <5 µm was statistically different. In the arctic region there was even more overlap in the $\delta^{15}N$ values and none of the differences were statistically significant.

Volvox colonies, aquatic mosses and filamentous algae mats were found in few sites and had distinct $\delta^{13}C$ values that differed from both POM <200 and benthic primary producers (Fig. 5). *Volvox* was found only in the subarctic and had $\delta^{13}C$ values that were similar to orange microbial mats. Mosses and filamentous algae occurred in arctic ponds with mosses $\delta^{13}C$ values significantly depleted compared to filaments.

$\delta^{13}C$ and $\delta^{15}N$ values of zooplankton differed according to taxonomy. Differences due to taxonomic groupings accounted for 75% of the variance in subarctic $\delta^{15}N$ and 61% of the variance in arctic $\delta^{15}N$ but did not account for a significant component of the variation in $\delta^{13}C$ (Table 2). Stable isotope values used for the analyses were from animals without lipid removal as the measured $\delta^{13}C$ values were not significantly different with and without lipid removal (average difference between treatments 0.18‰, SE 0.09‰). Species representing the same taxonomic orders plotted close to each other on the $\delta^{15}N$-scale

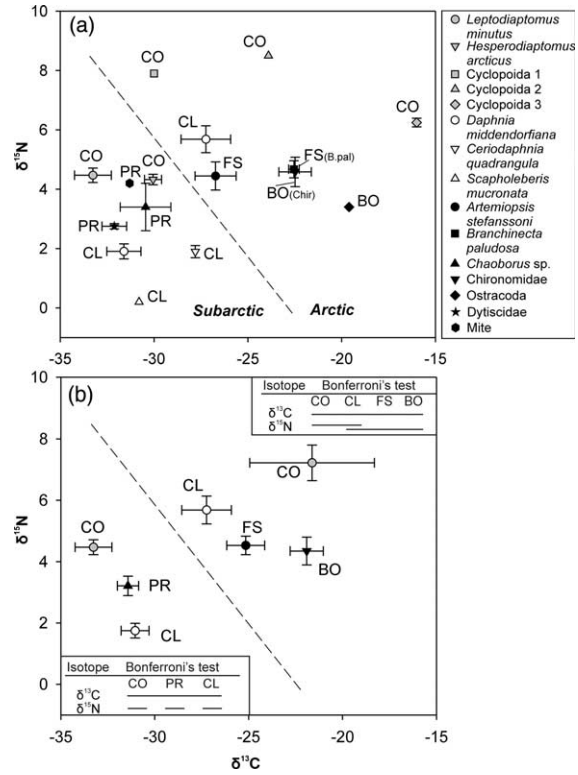

Fig. 2. Scatter plot of $\delta^{13}C$ vs $\delta^{15}N$ signatures (‰) for cladocerans (CL), copepods (CO), invertebrate predators (PR), fairy shrimps (FS) and bottom dwellers (BO). The dashed line represents the division between subarctic and arctic species. (a) Each value is the mean $\pm$ SD for individual species labelled according to the box on the right. (b) Species data aggregated into higher taxonomic groups. The inserted boxes show multiple comparisons of stable isotope values among the groups in the two regions

indicating that closely related species were feeding at the same trophic level (Fig. 2). $\delta^{15}N$ signals of cladocerans were significantly lower than the copepod signals at the subarctic ponds (Bonferroni's test, subarctic region, Fig. 2). The same pattern was observed in the arctic ponds, however the differences were not statistically significant (Bonferroni's test, arctic region, Fig. 2). Fairy shrimps in the Arctic had the lightest $\delta^{15}N$ signals in comparison to other pelagic groups (cladocerans and copepods) but the signals overlapped with the values of the benthic species (Ostracoda, chironomid larvae) and also were not significantly different from the cladoceran $\delta^{15}N$ signals. In the subarctic, invertebrate predators (*Chaoborus* sp., Hydracarina and Dytiscidae) had $\delta^{15}N$ values more positive than the cladocerans but lighter than the copepods and these differences were statistically significant. $\delta^{13}C$ values among taxonomic groups did not differ from each other in either of the regions (Table 2, Fig. 2).

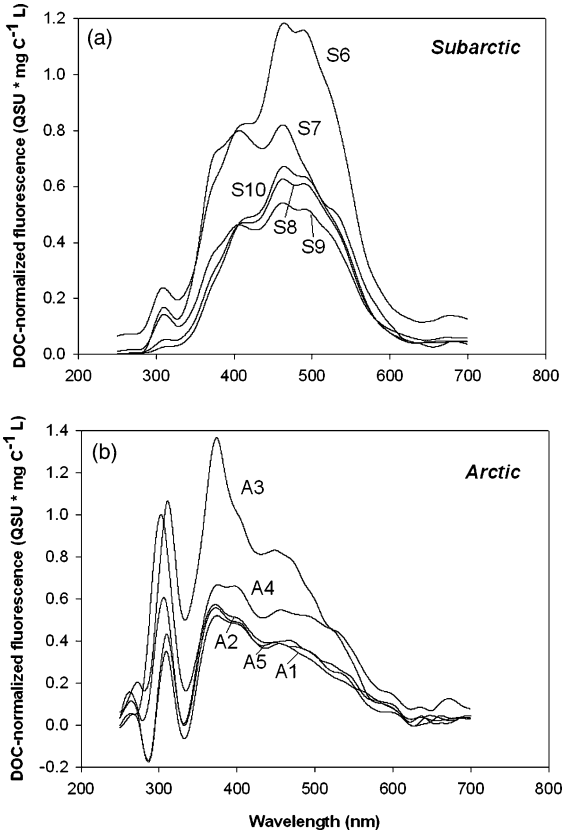

Fig. 3. Synchronous fluorescence spectroscopy scans of chromophoric dissolved organic matter (CDOM) from the (a) subarctic and (b) arctic water bodies. The data have been normalized to the concentrations of dissolved organic carbon (DOC). QSU = quinine sulphate units.

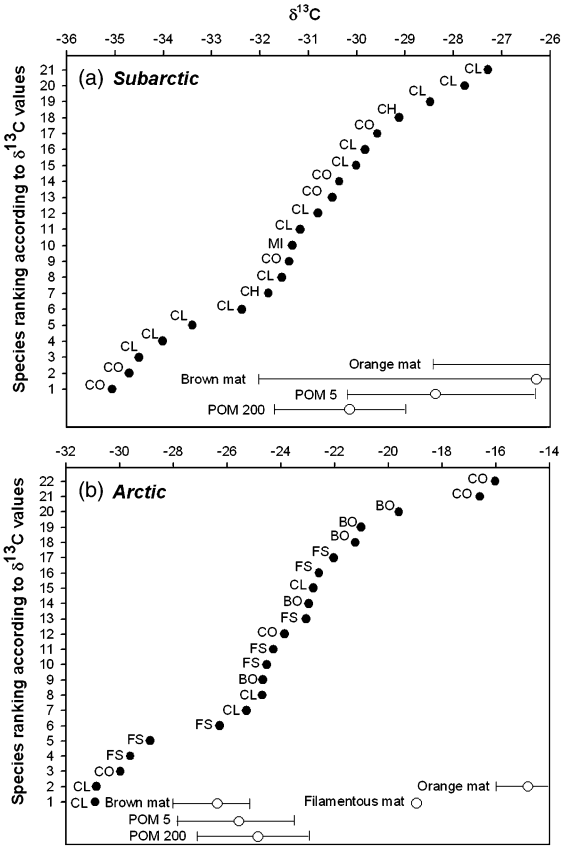

Fig. 4. Carbon isotopic ranking of (a) subarctic and (b) arctic zooplankton and their potential food sources. Each species point is a mean of many (5–300) individuals. The range of carbon values for each putative food source is marked with a line at the bottom of the figure showing the mean, 75th and 25th percentiles. Species labels in the upper (a) panel: CO = calanoids, CL = cladocerans, CH = *Chaoborus*, MI = mites. Species labels in the lower (b) panel: CL = *Daphnia*, CO = cyclopoids, FS = fairy shrimps, BO = bottom dwellers.

Signals of food transfer

The carbon signatures of the different zooplankton species plotted relative to those of the food sources indicated the transfer from these sources to consumers (Fig. 4). Zooplankton $\delta^{13}C$ in the subarctic ranged from -27 to $-36‰$ whereas in the arctic the signals between species were even more variable (-16 to $-32‰$). Figure 4 shows that for the ensemble of data from the subarctic ponds, the mats and POM overlapped in their carbon signals and therefore the relative importance of these two potential food sources in the diet of the majority of the zooplankton species could not be distinguished. In the arctic the orange and green filamentous microbial mats stood apart from the other potential food sources and from the zooplankton, indicating these communities were avoided by most of the animal species as a carbon source.

Given the large site-to-site variability, the data specific to each water body provided a more precise guide to trophic relationships (Fig. 5). Site-specific $\delta^{13}C$ isotopic signals of the crustacean zooplankton, pelagic POM and benthic microbial mats indicate a potential occurrence of both pelagic and benthic feeding strategies, and also a mixed feeding strategy in these northern zooplankton communities. 44.1% of all species were pelagic feeders as indicated by a zooplankton $\delta^{13}C$ signal lighter than or equal to the pelagic POM <200 μm signature. 14.7% of all species were potential benthic feeders, and 41.2% had a $\delta^{13}C$ isotopic signal that was intermediate between the pelagic and benthic POM values.

The enrichment of $\delta^{15}N$ between POM and zooplankton was often very small ($<1‰$), and sometimes negative (Fig. 5a, j). The $\delta^{15}N$ difference between microbial mats and zooplankton reflected more the commonly accepted $\delta^{15}N$ enrichment between trophic levels than the isotopic $\delta^{15}N$ difference between POM

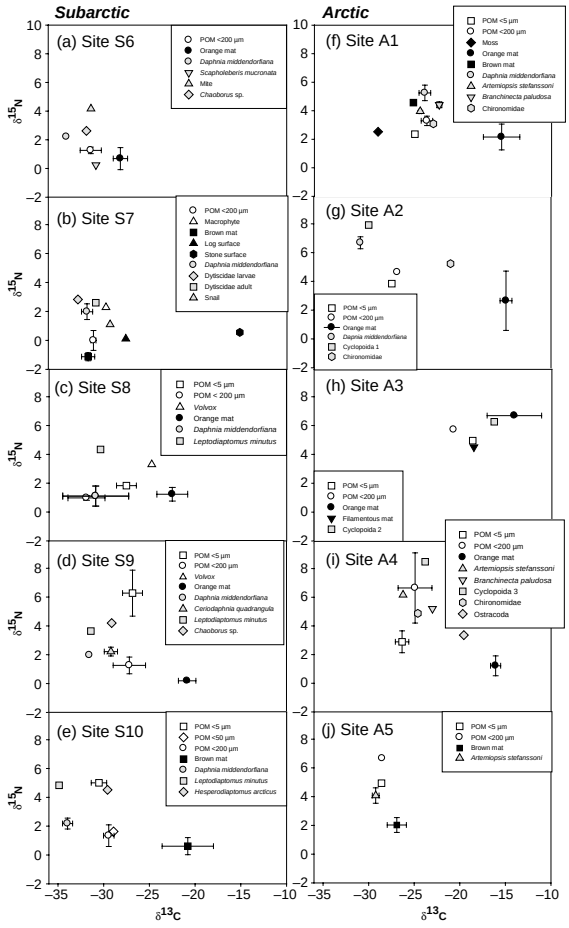

Fig. 5. Isotope plot for pelagic POM, benthic mats and animals in subarctic and arctic ponds. Potential pelagic food sources, including macrophytes, are marked with open symbols, benthic food sources with black symbols, and all organisms with grey symbols.

and zooplankton, however in these cases the $\delta^{13}C$ signal indicated POM feeding. The invertebrate predators *Chaoborus* sp., water mites and water beetles in our system had the highest $\delta^{15}N$ value among the animals, which was likely the result of feeding on small cladocerans in the ponds such as *Scapholeberis mucronata* in S6 and *Ceriodaphnia quadrangula* in S9.

Discussion

In this study we grouped the putative zooplankton carbon sources into four categories: water column seston in two size categories (POM <200 μm and <5 μm) and two benthic mat types that differed in colour and structure. We recognize that some important carbon types incorporated by zooplankton may be masked by the mixture of individual sources in the bulk signal, but this division provided a first indication of feeding preferences. The $\delta^{13}C$ and $\delta^{15}N$ characteristics in these potential zooplankton food sources varied first as a function of the geographical region precluding any data pooling between the subarctic and arctic despite their similarities in ecosystem structure (such as the much greater biomass in the benthos relative to the phytoplankton), and secondly within each region as a function of planktonic versus benthic origin, size fraction of POM and microbial mat type.

Determination of the baseline ^{13}C isotope signatures in the two regions

Regional variations in autotrophic and baseline $\delta^{13}C$ values have been attributed to differences in light availability (Durako and Hall 1992), temperature (Fontugne and Duplessy 1981), photosynthetic mechanisms (Hecky and Hesslein 1995), species composition (Gu et al. 1999) and lake metabolism (Bade et al. 2004). It is also likely that pH played a role in determining regional isotopic differences in the lakes we studied, both directly and through effects on algal species composition. The arctic water bodies (pH range 8.4–9.6) on Cornwallis Island were situated on carbonate-containing sedimentary rock catchments while the subarctic water bodies (pH range 6.0–7.6) in northern Quebec were on Precambrian granites, hence the regional differences between POM $\delta^{13}C$ values were likely explained by a pH-controlled differential availability of $CO_{2(aq)}$ and HCO_3^- between subarctic and arctic. HCO_3^- is isotopically heavier than $CO_{2(aq)}$ and was the dominant inorganic carbon form in arctic waters, consistent with our observation that the primary producers were isotopically heavier in the arctic than in the subarctic. The linear relationship between pH and the $\delta^{13}C$ values for POM <200 μm ($R^2 = 0.61$) further supports the likely importance of pH as a control on algal signatures in the two regions. Other factors that covary with pH probably also contributed to the patterns observed. The subarctic lakes were surrounded by moist, acidic peatlands, also indicated by the CDOM scans, and the respiration of terrestrially derived organic matter in these wetland sites should decrease the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) pool entering the ponds via surface and subsurface water flow (Bade et al. 2004). It is also possible that regional $\delta^{13}C$ differences were enhanced by differential ^{13}C fractionation among the algal species (Bade et al. 2006). The subarctic water bodies were $>10^{\circ}C$ warmer than the lakes in the arctic, with larger species such as *Dinobryon* sp., *Volvox* sp., large diatoms and desmids, whereas in the arctic small single cell species dominated. Pel et al. (2003) and Vuorio et al. (2006) have shown that co-occurring

phytoplankton taxa can differ by $>10‰$ in $\delta^{13}C$. However, other studies have shown that $\delta^{13}C$ is similar between all algal species in individual lakes (Gu et al. 1994, Yoshioka et al. 1994), with the exception of *Volvox* that is enriched by $2–5‰$ in comparison to other algal species. The latter offset was also evident in this study. Recently Riebesell et al. (2000) have suggested that different growth-rate-limiting resources are the principal cause for differences in carbon acquisition among phytoplankton species. Concentrations of DIC in the subarctic were substantially lower than in the arctic. Low DIC can be an indicator of carbon limitation in the system which leads to reduced ^{13}C fractionation and more positive $\delta^{13}C$ values (Smith and Walker 1980). However, the POM $\delta^{13}C$ values in subarctic sites were highly negative (mean $-31.4‰$) and we therefore suggest that the regional baseline differences in $\delta^{13}C$ values were better explained by pH than carbon limitation. Carbon limitation may have played a role, however, in determining baseline ^{13}C differences within sites.

Isotopic characteristics and the origin of the potential zooplankton food sources

Despite the regional differences in absolute isotopic values there was some consistency in the within-region $\delta^{13}C$ values, with the heaviest values found in the orange benthic mats in both regions. These measurements are in accordance with the results reported by France (1995) and Hecky and Hesslein (1995) who compared ^{13}C differences between benthic and pelagic algae in extensive data sets and concluded that, as a consequence of smaller surface area:volume ratio and the presence of thick boundary layers in less turbulent bottom waters, benthic algae are CO_2 limited and thus exhibit less ^{13}C fractionation (thus higher ^{13}C content) during carbon fixation than do phytoplankton. Using this finding Hecky and Hesslein (1995) were able to show that benthic algae contributed to pelagic foodwebs in arctic lakes. However, in our dataset, despite the distinct $\delta^{13}C$ for the orange mats at the heavy end of the carbon scale, there was a considerable overlap between many potential carbon sources. In particular the brown mats had overlapping $\delta^{13}C$ values with values of different POM fractions and in the subarctic these values were also intermediate between POM and orange mats. As brown mats were loose and fine structured they were probably subjected to more rapid exchange between the interstitial water and the overlying water column. Similar decreases in boundary layer effect due to increased turbulence are common in riverine benthic algae (France 1995) although there is evidence that diffusion resistance is not the only factor influencing $\delta^{13}C$ (France and Cattaneo 1998). Furthermore, thick microbial mats in some environments, for example in the High Arctic, have been shown to be replete in all nutrients including inorganic carbon (Bonilla et al. 2005).

The similarity in $\delta^{13}C$ values between the POM fractions in the water column and the brown mat may indicate sloughing and resuspension of the benthos. Tundra ponds in general, including the sampled sites, are shallow and are exposed to vigorous wind-induced mixing that could readily resuspend loose bottom material and make it available as a potential food source to zooplankton higher in the water column. However even without frequent mixing events, at least some zooplankton species have shown to have an ability to access benthic food. Hansson and Tranvik (2003) reported that the cladoceran *Alona* sp. was always attached to benthic algae and never observed swimming in the water column. *Daphnia magna* and *D. pulex* are known to collect algae at the bottom when the algal concentration in the benthos is particularly high (Fryer 1987).

The water column and benthic POM measured in this study contained a mixture of primary producers, microbial food web components and detritus. Therefore the $\delta^{13}C$ signature of POM may not match that of the putative resource users, the zooplankton, if these animals select only a subset of the multiple POM constituents. In our tundra pond systems, the potential non-algal origins of POM were watershed-derived plant and soil materials, benthic animals and detritus, detritus in seston, and bacterioplankton and protist grazers in the water column. The subarctic ponds were characterized by CDOM fluorescence peaks indicating the dominance of allochthonous humic and fulvic materials in the water body whereas the arctic ponds were characterized more with autochthonous-like molecules of CDOM which showed fluorescence peaks at 293–308 nm. These results suggest a relatively small allochthonous input to arctic ponds and the dominance of CDOM that is derived from primary production (pelagic and benthic), in keeping with the sparse vegetation in their polar desert catchments. In contrast the results imply a strong terrigenous influence on subarctic tundra ponds and their seston, as has been observed in similar ecosystems in Alaska (Schell 1983).

The phytoplankton contribution to the seston has been previously estimated for the waters sampled in the present study from measurements of phytoplankton biovolume and comparison with chemical analyses of seston particulate organic carbon (POC). For these ponds, the phytoplankton were calculated to contribute $<2–11\%$ of POC (Rautio and Vincent 2006). These values are in accordance with the 5–13% range of living seston material (algae and bacteria) reported in tundra ponds at Barrow (Stross et al. 1980). It is evident that pond seston has a large non-algal

component, and its $\delta^{13}C$ signature may be a poor guide to food transfer if the zooplankton selectively feed on live algae that comprise only a small fraction of the total POC.

Pelagic, benthic and mixed feeding strategies

Given the high degree of overlap in $\delta^{13}C$ values among the different potential zooplankton food sources, it was not possible to identify a single carbon source for the zooplankton. However, the data provide information that can be used to infer the relative importance of the different carbon sources. The detrital component of POM is likely to be enriched in ^{13}C relative to algae, resulting in pelagic algal grazers that have a lower $\delta^{13}C$ signal than the bulk POM (del Giorgio and France 1996). Low zooplankton $\delta^{13}C$ signals may also results from lipid storage that is common for zooplankton inhabiting subarctic and arctic waters (Kling et al. 1992). Lipids are ^{13}C depleted and if zooplankton accumulate large amounts of lipids, this alone may shift their isotopic signature. However, in this study ^{13}C values were equal for zooplankton with and without lipids. Regardless of lipid influence, a zooplankton $\delta^{13}C$ signature that is lighter than that of pelagic POM may reflect a pelagic feeding source, while zooplankton values between pelagic POM and microbial mats values would reflect either mixed or benthic feeding. By these criteria, a pelagic feeding strategy appears to be the most common strategy among the zooplankton species sampled here. True benthic feeders (Ostracoda and chironomid larvae) were higher than the microbial mat in their $\delta^{13}C$ signal by several parts per thousand (Fig. 5g, i). This enrichment is greater than the commonly recognized 1‰ or less for each carbon trophic transfer (Peterson and Fry 1987), but other authors have recently noted that carbon enrichment between trophic levels can vary between -2.7 and 3.4 ‰ (McCutchan et al. 2003). In our study the zooplankton $\delta^{13}C$ signal was at maximum offset from the potential food by >5‰ which seems too high for non-selective, omnivorous feeding on any of the measured food sources. This offset likely indicates that zooplankton were feeding selectively on just a portion of the pelagic POM or benthic mats with a distinct isotopic signal that differed from the bulk properties of the organic matter. Among-species variation was observed in nearly all sites in the zooplankton isotopic signals, and this further supports selective feeding, as well as differences in food choice among species. Such large disparities between bulk measurements of food and zooplankton have been shown for other types of aquatic ecosystem in which there is selective feeding on a minor constituent of the total POM pool (Martineau et al. 2004, and references therein). Pel et al. (2003) have similarly shown that up to 10‰ differences in $\delta^{13}C$ in co-occurring phytoplankton taxa can be reflected in the zooplankton as the result of preferential grazing or selective digestion.

The $\delta^{15}N$ analyses provided additional information about potential food sources and feeding strategies. Algae exhibit highly variable fractionation depending on growth rates, species composition, ambient nutrient concentrations, and nitrogen substrate (Waser et al. 1998). For example, low ^{15}N values indicate the presence of nitrogen fixation since biologically fixed nitrogen has a $\delta^{15}N$ value of only -2 to 0‰ (Yoshioka et al. 1994). The $\delta^{15}N$ values are typically enriched in consumers by 3–5‰, thereby providing a quantitative guide to trophic level (Cabana and Rasmussen 1996). In the present study the apparent enrichment from food to consumers was highly variable; for example, between POM <200 and zooplankton the enrichment ranged between -3‰ and $+3.5$‰. Although enrichment at the base of the foodweb can be more variable than the commonly assumed 3–5‰ (Minawaga and Wada 1984, Adams and Sterner 2000), our results indicate that the ^{15}N signal in the food was being averaged out in bulk samples. The large difference in $\delta^{15}N$ values between POM <200 and POM <5 suggests that the pelagic organic matter available to zooplankton was made of components with variable $\delta^{15}N$ values, and according to zooplankton ^{15}N values different species must have been feeding selectively on different subcomponents of the pelagic and benthic organic nitrogen pools. Grey et al. (2001) showed an example of such selectivity in the oligotrophic Loch Ness where a diatom community had on average 4‰ lower $\delta^{15}N$ value than total POM and the zooplankton had a $\delta^{15}N$ signature that corresponded well with the diatoms with a >3‰ trophic enrichment. Some of the deviations between the putative food sources and zooplankton signals can also be explained by phytoplankton seasonality, hence zooplankton signals may have been partly derived from phytoplankton that had peaked earlier in the season. However, the variation among lakes appears to be generally greater than the variation over time within a lake (Bade et al. 2004).

Conclusions

Our detailed sampling of tundra pond food webs revealed considerable variation in the diet of individual zooplankton species inhabiting these water bodies, reflecting a trophic dependence of the community on multiple resources and niche partitioning of those resources. Different fractions of POM and loose brown microbial mats with their similar $\delta^{13}C$ signatures were often the most important potential carbon sources for the zooplankton. Whether brown mats were consumed

as resuspended material in the water column or directly from benthos requires further evaluation and would benefit from additional indicators to improve discrimination, for example lipid profiles (including algal pigments) and sulphur isotope analysis. The most distinctive benthos type in northern ponds, the cohesive orange microbial mat, proved to be less important as a food source for zooplankton. The highly cohesive structure of these mats probably prevents zooplankton from accessing them as a food source and is an effective defence against grazing pressure, as well as from losses by resuspension to the water column.

Our results support the view that shallow lakes and ponds in the tundra and polar desert are more complex systems that their apparently simple pelagic trophic structure would suggest. Stable isotope analysis provides a valuable tool to reveal some of the unexplored trophic links in these ecosystems. More accurate measurements of the variation in isotopic composition of individual subcomponents of suspended and benthic POM are required to determine more precisely which primary producers or other components of the resource pool are carbon and nitrogen food resources for each consumer species in these zooplankton-rich ecosystems.

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References

Adams, T. S. and Sterner, R. W. 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. – Limnol. Oceanogr. 45: 601–607.

Anon. 1999. The SAS system for Windows. Release 8.02 ed. – SAS Inst., Cary, NC.

Bade, D. L. et al. 2004. Controls of $\delta^{13}C$-DIC in lakes: geochemistry, lake metabolism, and morphometry. – Limnol. Oceanogr. 49: 1160–1172.

Bade, D. L. et al. 2006. Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models? – Aquat. Sci. 68: 142–153.

Barnard, C. et al. 2006. Trophic position of zebra mussel veligers and their use of dissolved organic carbon. – Limnol. Oceanogr. 51: 1473–1484.

Belzile, C. et al. 2002. Colored dissolved organic matter and dissolved organic carbon exclusion from lake ice: implications for irradiance transmission and carbon cycling. – Limnol. Oceanogr. 47: 1283–1293.

Bonilla, S. et al. 2005. Benthic and planktonic algal communities in a high arctic lake: pigment structure and contrasting responses to nutrient enrichment. – J. Phycol. 41: 1120–1130.

Cabana, G. and Rasmussen, J. B. 1996. Comparison of aquatic food chains using nitrogen isotopes. – Proc. Nat. Acad. Sci. USA 93: 10844–10847.

del Giorgio, P. A. and France, R. L. 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. – Limnol. Oceanogr. 41: 359–365.

Drakare, S. et al. 2003. Relationships between picophytoplankton and environmental variables in lakes along a gradient of water colour and nutrient content. – Freshwater Biol. 48: 729–740.

Durako, M. J. and Hall, M. O. 1992. Effects of light on the stable carbon isotope composition of the seagrass *Thalassia testudinum*. – Mar. Ecol. Prog. Ser. 86: 99–101.

Fontugne, M. R. and Duplessy, J. 1981. Organic carbon isotopic fractionation by marine plankton in the temperature range -1 –$31^{\circ}C$. – Oceanol. Acta 4: 85–90.

France, R. L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. – Mar. Ecol. Prog. Ser. 124: 307–312.

France, R. L. and Cattaneo, A. 1998. $\delta^{13}C$ variability of benthic algae: effects of water colour via modulation by stream current. – Freshwater Biol. 39: 617–622.

Fryer, G. 1987. The feeding mechanisms of the Daphniidae (Crustacea: Cladocera): recent suggestions and neglected considerations. – J. Plank. Res. 9: 419–432.

Grey, J. et al. 2001. Seasonal changes in the importance of the source of the organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. – Limnol. Oceanogr. 46: 505–513.

Gu, B. et al. 1994. Stable carbon and nitrogen isotope analysis of the plankton food web in a subarctic lake. – Can. J. Fish. Aquat. Sci. 51: 1338–1344.

Gu, B. et al. 1999. Seasonal and interannual variability of plankton carbon isotope ratios in a subarctic lake. – Freshwater Biol. 42: 417–426.

Hansson, L.-A. and Tranvik, L. 2003. Food webs in sub-Antarctic lakes: a stable isotope approach. – Polar Biol. 26: 783–788.

Hecky, R. E. and Hesslein, R. H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analyses. – J. North Am. Benthol. Soc. 14: 631–653.

Kling, G. et al. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. – Ecology 73: 561–566.

Kritzberg, E. S. et al. 2004. Autochthonous versus allochthonous carbon sources of bacteria: results from whole-lake 13C addition experiments. – Limnol. Oceanogr. 49: 588–596.

Martineau, C. et al. 2004. Primary consumers and particulate organic matter: isotopic evidence of strong selectivity in the estuarine transition zone. – Limnol. Oceanogr. 49: 1679–1686.

McCutchan, J. H. Jr et al. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulphur. – Oikos 102: 378–390.

McKnight, D. M. et al. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. – Limnol. Oceanogr. 46: 38–48.
McLaren, I. A. 1958. Annual report and investigators' summaries. – J. Fish. Res. Bd. Can. 15: 27–35.
Minawaga, M. and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. – Geochim. Cosmochim. Acta 48: 1135–1140.
Pace, M. L. et al. D. 2004. Whole-lake carbon-13 additions reveal terrestrial support to aquatic food webs. – Nature 427: 240–243.
Pel, R. et al. 2003. Using the hidden isotopic heterogeneity in phyto- and zooplankton to unmask disparity in trophic carbon transfer. – Limnol. Oceanogr. 48: 2200–2207.
Peterson, B. J. and Fry, B. 1987. Stable isotopes in ecosystem studies. – Annu. Rev. Ecol. Syst. 18: 293–320.
Rautio, M. and Vincent, W. F. 2006. Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. – Freshwater Biol. 51: 1038–1052.
Riebesell, U. et al. 2000. Carbon isotope fractionation by a marine diatom: dependence on the growth-rate-limiting resource. – Mar. Ecol. Prog. Ser. 193: 295–303.
Sand-Jensen, K. et al. 1999. Slow growth and decomposition of mosses in arctic lakes. – Can. J. Fish. Aquat. Sci. 56: 388–393.
Schell, D. M. 1983. Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: delayed production from peat in arctic food webs. – Science 219: 1068–1071.
Senesi, N. et al. 1991. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. – Soil Sci. 152: 259–271.
Smith, F. R. and Walker, N. A. 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3 and to carbon isotopic discrimination. – New Phytol. 86: 245–259.
Stross, R. G. et al. 1980. Zooplankton: communities, life cycles and production. – In: Hobbie, J. E. (ed.), Limnology of tundra ponds. Hutchinson and Ross, pp. 251–296.
Tranvik, L. J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of different humic content. – Microbial Ecol. 16: 311–322.
Vadeboncoeur, Y. et al. 2003. From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. – Limnol. Oceanogr. 48: 1408–1418.
Vander Zanden, M. J. and Rasmussen, J. B. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. – Ecology 80: 1395–1404.
Vézina, S. and Vincent, W. F. 1997. Arctic cyanobacteria and limnological properties of their environment: Bylot Island, Northwest Territories, Canada (73°N, 80°W). – Polar Biol. 17: 523–534.
Vuorio, K. et al. 2006. Taxon-specific variation in the stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of lake phytoplankton. – Freshwater Biol. 51: 807–822.
Waser, N. A. D. et al. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. – Limnol. Oceanogr. 43: 215–224.
Welch, H. E. and Kalff, J. 1974. Benthic photosynthesis and respiration in Char Lake. – J. Fish. Res. Bd. Can. 31: 609–620.
Yamamuro, M. and Kayanne, H. 1995. Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediment with a Yanaco MT-5 CHN analyser. – Limnol. Oceanogr. 40: 1001–1005.
Yoshioka, T. et al. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. – Ecology 75: 835–846.