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BENTHIC AND PLANKTONIC ALGAL COMMUNITIES IN A HIGH ARCTIC LAKE: PIGMENT STRUCTURE AND CONTRASTING RESPONSES TO NUTRIENT ENRICHMENT[1]

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We investigated the fine pigment structure and composition of phytoplankton and benthic cyanobacterial mats in Ward Hunt Lake at the northern limit of High Arctic Canada and the responses of these two communities to *in situ* nutrient enrichment. The HPLC analyses showed that more than 98% of the total pigment stocks occurred in the benthos. The phytoplankton contained Chrysophyceae, low concentrations of other protists and Cyanobacteria (notably picocyanobacteria), and the accessory pigments chl c_2, fucoxanthin, diadinoxanthin, violaxanthin, and zeaxanthin. The benthic community contained the accessory pigments chl *b*, chl c_2, and a set of carotenoids dominated by glycosidic xanthophylls, characteristic of filamentous cyanobacteria. The black surface layer of the mats was rich in the UV-screening compounds scytonemin, red scytonemin-like, and mycosporine-like amino acids, and the blue-green basal stratum contained high concentrations of light-harvesting pigments. In a first bioassay of the benthic mats, there was no significant photosynthetic or growth response to inorganic carbon or full nutrient enrichment over 15 days. This bioassay was repeated with increased replication and HPLC analysis in a subsequent season, and the results confirmed the lack of significant response to added nutrients. In contrast, the phytoplankton in samples from the overlying water column responded strongly to enrichment, and chl *a* biomass increased by a factor of 19.2 over 2 weeks. These results underscore the divergent ecophysiology of benthic versus planktonic communities in extreme latitudes and show that cold lake ecosystems can be dominated by benthic phototrophs that are nutrient sufficient despite their ultraoligotrophic overlying waters.

Key index words: **arctic lakes; bioassays; cyanobacteria; HPLC; mats; nutrients; photosynthesis; phytoplankton; pigments**

Abbreviations: **DIC, dissolved inorganic carbon; ELA, Experimental Lakes Area; MAAs, mycosporine-like amino acids; SRP, soluble reactive phosphorus**

Polar and alpine lakes are typically thought of as low-resource ecosystems in which the photosynthetic communities are severely constrained by nutrient supply (Douglas et al. 1994, Vézina and Vincent 1997). The classic work on Char Lake in the Canadian High Arctic drew attention to its ultraoligotrophic characteristics, with phytoplankton populations limited by the meager inputs of phosphorus from its polar desert watershed (Rigler 1978). In nearby Meretta Lake, an increase in nutrient loading from anthropogenic sources resulted in a sharp increase in phytoplankton biomass, confirming the nutrient responsiveness of arctic lakes and their susceptibility to eutrophication (Schindler et al. 1974a, Douglas and Smol 2000). Similarly, in antarctic and alpine lakes, nutrient enrichment bioassays have confirmed the strong limitation of their phytoplankton communities by phosphorus and/or nitrogen availability (Vincent 2000, Dore and Priscu 2001, Goldman et al. 2001).

Shallow lake ecosystems contain two separate algal communities: phytoplankton in the water column and the phytobenthos attached to bottom substrata. The latter attached communities can be a major source of organic carbon for planktonic as well as benthic food webs (Sand-Jensen and Borum 1991, Zimba 1995) and can play a leading role in the production dynamics of the overall ecosystem (Burkholder and Wetzel 1989). The responses to environmental variation have the potential to differ greatly between the plankton and the benthos (Sand-Jensen and Borum 1991, Vinebrooke and Leavitt 1999). Although nutrients may severely limit planktonic production, benthic phototrophs may be subject to quantitatively or even qualitatively different controls. Many arctic and

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antarctic lakes contain luxuriant benthic mosses (Sand-Jensen et al. 1999) or thick algal mats (Vincent et al. 1993, Quesada et al. 1999), implying a different growth strategy and nutrient regime in the benthic zone relative to the overlying water column. In shallow lakes of both polar regions, cyanobacterial mats may achieve high standing stocks and often dominate overall ecosystem productivity (Hawes and Schwarz 1999, Vincent 2000, Hodgson et al. 2004), although this may represent the gradual accumulation of slow-growing species over many seasons (Vincent 2000). Nutrient and isotopic studies on oligotrophic lakes in the Experimental Lakes Area (ELA, north-temperate Canada) have shown that although the dilute phytoplankton may be phosphorus limited, the high biomass and productivity of the periphyton can result in limitation of the benthic phototrophic community by inorganic carbon supply (Turner et al. 1994). Nutrient uptake studies on periphyton of some arctic and antarctic systems have indicated that nitrogen or phosphorus may be regulating factors for certain high latitude benthic communities (Howard-Williams and Vincent 1989, Hullar and Vestal 1989, Hansson 1992, Miller et al. 1992). However, the role of nutrients as controls on algal standing stocks and productivity still remains little explored in polar aquatic environments.

In addition to nutrient constraints, algal communities of the polar regions are subject to large variations in light availability, from total winter darkness to continuous light in summer, coupled with long periods of freezing and a short growing season. During summer ice-out conditions, the phytoplankton and benthic mats can be abruptly exposed to high levels of PAR and UV radiation (Roos and Vincent 1998). The communities therefore require a broad set of pigment strategies embracing both light harvesting and light protection. Previous studies on high latitude microbial mats have shown that they can be vertically structured to optimize the combination of pigments (Howard-Williams and Vincent 1989, Vincent et al. 1993, Quesada et al. 1999, Mueller et al. 2005) and that the harvesting/screening pigment ratios are strongly regulated in response to PAR, UV, and temperature stress (Roos and Vincent 1998). The resultant optimization of growth rates is also likely to increase nutrient requirements (Borchardt 1996) and the potential for nutrient limitation.

We had two objectives in the present study. First, our aim was to describe the pigment characteristics of phototrophic communities in high arctic fresh waters and to determine the array of light-harvesting and light-screening pigments that allow growth and survival under the low temperature continuous light regime of summers in the extreme northern environment. Second, we aimed to determine the extent of nutrient limitation in the thick benthic microbial mats of arctic lakes and to evaluate the hypothesis that these communities differ qualitatively in their nutrient status relative to the overlying plankton.

We focused this study on Ward Hunt Lake, a freshwater ecosystem at the northern limit of the Canadian High Arctic (Villeneuve et al. 2001) and a key monitoring region for climate change (Mueller et al. 2003). Like other high latitude lakes and ponds, this body of water contains a low-biomass phytoplankton community overlying a well-developed microbial mat community dominated by cyanobacteria. In our first set of experiments, we evaluated the hypothesis that the benthic mat community was limited in both biomass and productivity by inorganic carbon supply, as has been identified for periphyton in the lower latitude ELA region (Turner et al. 1994). These results in combination with the chemical analysis of mat interstitial water indicated that neither carbon nor other nutrients were likely to be limiting, although the variability among replicates was large and precluded the detection of small differences. To verify these results and to evaluate the hypothesis that the benthos differed qualitatively from the plankton in nutrient limitation, we returned to the lake and undertook a nutrient enrichment bioassay based on pigment analysis by HPLC. This allowed a more detailed analysis of the organization and nutrient responses of the microbial mats, a higher degree of replication, and direct comparison with the phytoplankton community by way of parallel enrichment incubations.

MATERIALS AND METHODS

Study area. The study was performed during summer open water conditions at Ward Hunt Lake (83.1° N, 74.1° W) on Ward Hunt Island in the Canadian High Arctic. This island is located at the northern limit of Nunavut, in Quttinirpaaq National Park. The lake has an area of 0.37 km^2 and a maximum depth of 5.5 m. Most of the lake has a depth of less than 2 m, and these shallow waters are likely to be completely frozen for at least 9 months of the year. Furthermore, at this latitude there is an extreme seasonality in photoperiod, with 147 days of continuous darkness in winter and 147 days of continuous light in summer (Mueller et al. 2005). The growing season for phototrophic organisms at this site is likely restricted to a few weeks each year. Summer air temperatures on Ward Hunt Island rarely exceed 10° C (range, −5 to 15° C), and 50% of the lake surface typically remains covered by ice. Water temperatures at the sampling and incubation sites ranged from 0 to 5° C. The highly transparent waters of the lake contain moderate concentrations of dissolved inorganic carbon (DIC; 15.9 $mg \cdot L^{-1}$), low nutrient concentrations (dissolved total nitrogen, 0.12 $mg \cdot L^{-1}$; soluble reactive phosphorus [SRP], 0.43 $\mu g \cdot L^{-1}$), and planktonic chl *a* concentrations in the ultraoligotrophic to oligotrophic range ($< 0.5\ \mu g \cdot L^{-1}$) (Villeneuve et al. 2001). The parts of the lake that melt out each year contain a well-developed benthic film of cyanobacterial mats with chl *a* up to 105.6 $mg \cdot m^{-2}$ (Villeneuve et al. 2001).

Field sampling. Initial sampling and experiments were conducted in July 1998, and the sampling for HPLC pigment analysis and the second set of experiments were undertaken in July and August 2003. Additional plankton samples were obtained in August 2004 from this site for further HPLC analysis. Samples were taken 1 m from shore in the littoral zone where the water depth was 30 cm. This area was chosen as representative of the open water region of the lake in which the microbial mats form a cohesive benthic

layer 2–5 mm in thickness. Sections of mat (100 × 100 mm) were cut at random from the benthos with a plastic spatula and carefully packed in acid-washed plastic boxes and bubble-wrap to avoid physical disruption of the communities during transport. Subsamples of the mats (9 mm diameter cores obtained with a plastic cut-off syringe) were wrapped in aluminum foil, transferred to Whirlpaks (Fisher Scientific Ltd., Nepean, Ontario, Canada), immediately frozen in an ice–salt mixture, and subsequently transferred to −80° C storage until pigment analysis by reverse-phase HPLC. Phytoplankton samples (100 mL) were taken from the littoral zone with acid-washed 1-L Nalgene bottles (Fisher Scientific) filled 5 cm beneath the lake surface with care to prevent any suspension of benthic material. Phytoplankton samples for HPLC analysis were filtered in the field under low vacuum pressure onto 25 mm diameter GF/F glass-fiber filters (Fisher Scientific) that were frozen and subsequently transferred to a −80° C freezer until further processing. Samples for taxonomic and biomass analysis of the mats and phytoplankton were preserved with a glutaraldehyde/formaldehyde solution (Villeneuve et al. 2001). Samples of mats and phytoplankton for the bioassays were maintained in the dark at around 5° C and transported within 24 h for the beginning of the incubations.

Pigment, mycosporine-like amino acids (MAAs), and taxonomic characterization of mats and phytoplankton. In the laboratory, cores of frozen mats were sectioned by scalpel into surface (black), middle (pink), and bottom (green) layers. Each sample was extracted by grinding for 2 min and then sonicated (twice for 30 s at 10 W) in 4 mL of 90% acetone. The extraction was then continued by incubating overnight in the dark at −20° C under argon gas. This protocol gave an optimal yield of pigment, with values equivalent to those using the serial extraction method described in Vézina and Vincent (1997). The extracts were cleared by centrifugation (10 min, 4000 rpm), and the pellets were washed with 1 mL of 90% acetone to transfer any remaining pigments. The extracts were filtered through 0.2-μm Acrodisc filters (Cole-Palmer, Canada, Inc., Anjou, QC, Canada) and stored under argon gas at 4° C in darkness until the HPLC analysis within 24 h of extraction. Fifty microliters of the mat extracts were injected into a ProStar HPLC (Varian, Palo Alto, CA, USA) equipped with a Symmetry C8 column (3.5 μm pore size, 4.6 × 150 mm, Waters Corporation, Milford, MA, USA). The frozen phytoplankton filters were sonicated in 3 mL of 90% acetone, followed by 24 h extraction, as for the mats. The extracts were cleared by centrifugation, and 100-μL subsamples were then injected into the HPLC system.

The HPLC peaks were detected by diode-array spectroscopy (350–750 nm) set to a slit width of 1 nm. Absorbance chromatograms were obtained at 384 nm (for scytonemin), 440 nm (for chl), and 450 nm (for carotenoids). Chlorophylls were also detected by fluorescence (excitation, 440 nm; emission, 650 nm). Standards for identification and quantification of pigments (chl *a*, *b*, and c_2, β,β-carotene, canthaxanthin, diadinoxanthin, echinenone, fucoxanthin, lutein, myxoxanthophyll, violaxanthin, and zeaxanthin) were obtained from Sigma Inc. (St. Louis, MO, USA) and DHI Water & Environment (Hørsholm, Denmark) to calibrate our HPLC. The antheraxanthin peak areas were converted to concentrations using its published extinction coefficient (235 $L \cdot g^{-1} \cdot cm^{-1}$). A glycoside pigment closely resembling 4-keto-myxoxanthophyll (Schlüter et al. 2004) was commonly found in the mats, and its concentration was calculated based on the conversion factor for myxoxanthophyll. However, the diversity of glycoside pigments is high in cyanobacteria and not always fully resolved by HPLC; thus, some minor carotenoids may not have been detected. Scytonemin was quantified by using its peak area at 384 nm and the extinction coefficient of 112.6 $L \cdot g^{-1} \cdot cm^{-1}$ (Garcia-Pichel et al. 1992). The same quantification procedure was used for a pigment closely resembling red scytonemin. The HPLC solvent protocol followed the procedure of Zapata et al. (2000) based on the gradient dilution with two solvent mixtures: a methanol, acetonitrile, and aqueous pyridine (50:25:25 v/v/v) solution and a methanol, acetonitrile, and acetone (20:60:20 v/v/v) solution. The flow rate was 1 $mL \cdot min^{-1}$ and the equilibration time, 5 min.

Phycobilins from the mats were extracted using the serial glycerol-water method described in Quesada and Vincent (1993). The absorption by phycocyanin (620 nm), allophycocyanin (650 nm), and phycoerythrin (565 nm) was measured by spectrophotometry and normalized to measurements of turbidity at 750 nm.

The UV-absorbing substances with properties similar to MAAs from the three mat layers were extracted in MeOH 25% as in Tartarotti and Sommaruga (2002) and scanned in a Cary-Varian spectrophotometer (Varian). The absorption peak areas (from 302 to 357 nm) multiplied by the extract volume and normalized to the area of mat (cm^{-2}) were calculated to determined relative concentrations of MAAs. To compare the three layers, data were standardized by calculating the ratios of relative MAAs to chl *a*.

Before taxonomic identification and enumeration, the mat samples were dispersed by passing them through a syringe (first without a needle and after with an 18-gauge needle) and diluted with a known volume of deionized water. Subsamples of the dispersed samples were sedimented in counting chambers and examined using an Axiovert 10 inverted microscope (Zeiss, Jena, Germany) at 400× and 1000× magnification. A total of 400 individuals (1 individual = 1 coccoid or filamentous cyanobacterium, or 1 diatom) per sample was enumerated in fields distributed randomly across the chamber. Fifty-milliliter phytoplankton samples were sedimented in counting chambers for 72 h and then analyzed under the inverted microscope. Cell biovolume per taxon was calculated based on simple geometric shapes and the average of the microscopic dimensions of 10–30 individuals per taxon.

First set of enrichment bioassays. On 30 July1998, samples of algal mats were obtained as described above and transported to a comparably cold, shallow, high arctic lake on Cornwallis Island in the vicinity of Resolute (74.7° N, 94.9° W) for the incubations. Each bioassay included a control (no enrichment) and three enrichment treatments: DIC added at a final concentration of 25 mg bicarbonate-$C \cdot L^{-1}$, a full spectrum of nutrients excluding DIC and based on 10% BG11 culture medium (phosphate, 54 μg $P \cdot L^{-1}$; nitrate, 247 μg $N \cdot L^{-1}$; final concentrations), and the full nutrients plus DIC (F + DIC). This design was adopted given the potential synergistic effects of combined nutrients (Elser et al. 1990). The nutrient concentrations were selected to ensure significant enrichment above known values of DIC and phosphorus for Nordic regions; for example, 0.1–1.9 μg $SRP \cdot L^{-1}$ and 0.1–27.7 mg $DIC \cdot L^{-1}$ in arctic tundra fresh waters (Pienitz et al. 1997) and 0.5–4.8 μg $SRP \cdot L^{-1}$ and 12.1–45.9 mg $DIC \cdot L^{-1}$ in high arctic lake waters at Alert, Ellesmere Island (Antoniades et al. 2003). Three replicates were prepared per treatment and for the control.

To evaluate the photosynthetic response, two different bioassays were conducted with ^{14}C-bicarbonate incubations: long term (15 days, from 30 July to 13 August) and short term (initial 6 hours of enrichment). For the short-term experiments, small cores (5 mm diameter) were cut at random from the larger mat samples with a cut-off plastic syringe. Each core was put in a Whirlpak plastic bag containing 20 mL of unfiltered Ward Hunt Lake water; this contributed a negligible addition of algal biomass and particulate nutrients relative to the mat sample (Villeneuve et al. 2001). Ten μCi of $NaH^{14}CO_3$ was then added to each bag. A series of incubations was also con-

ducted for dark uptake corrections by wrapping a set of bags (three replicates per treatment) in two layers of aluminum foil. All bags were arranged in a metallic support that was fixed in the lake at 30 cm depth and 1 m from the shore and incubated for 6 h. At the end of this incubation, mat samples were each filtered onto a GF/F filter (Whatman, Maidstone, UK) and stored frozen until subsequent preparation and analysis in the laboratory. Five-millimeter diameter cores were used to measure chl *a* by acetone extraction and spectrophotometry as described in Villeneuve et al. (2001).

The long-term photosynthesis assay was conducted with the same treatments and number of replicates, but the measurements were made after 15 days of enrichment. Cores of 50 mm diameter were cut from the 100 × 100-mm mats with a plastic cylinder, placed in Whirlpak plastic bags with Ward Hunt Lake water as above, and incubated in the shallow lake on Cornwallis Island. Five-millimeter diameter cores were then subsampled at T_{final} (day 15) and photosynthetic incubations made as above.

In the laboratory, samples from the photosynthetic incubations were acidified (HCl 1 N, 10 min), homogenized with a glass rod in 5 mL of scintillation cocktail, and then digested with 0.5 mL of Scintigest (Fisher Scientific) for 24 h. Subsamples (0.5 mL) of these solutions were collected, transferred to a scintillation vial, and diluted with 9.5 mL of cocktail. The ^{14}C-content of each sample was then measured in a scintillation counter (LS 6500, Beckman, Fullerton, CA, USA) and the photosynthetic rates calculated as in Lind (1974). The concentrations of DIC used in the calculation were measured in subsamples of the incubation water by Gran titration (Wetzel and Likens 2000).

The long-term bioassay treatments were also subsampled at T_0 and T_{final} for biomass analysis of main taxonomic groups. To detect any major changes in floristic composition, two fixed (glutaraldehyde/formaldehyde solution) replicates per treatment and control were analyzed. Before enumeration, mat subsamples were dispersed by passing them through a syringe and diluted with a known volume of deionized water. Subsamples were counted using a Zeiss Axiovert 10 inverted microscope at 400 × and 1000 × magnification with epifluorescence. A total of 400 individuals per sample was enumerated in random fields and the biovolume calculated as described above.

Second set of enrichment bioassays. For the second set of experiments, we modified the design according to the results obtained in our first bioassays. Given the absence of differences between full nutrients and inorganic carbon, we focused on one treatment containing all elements (F + DIC, concentrations as above) plus the control. We adopted a higher number of replicates ($n = 6$) in response to the variability of the community as seen in the first set of experiments. Individual 50-mm diameter cores of the mat were transferred to acid-washed lake water–rinsed Whirlpaks, and 100 mL of unfiltered lake water was added to each bag, as in the 1998 experiment. This second set of incubations was performed in situ at Ward Hunt Lake, between 20 July and 3 August 2003, to eliminate any effects of transport and to provide the best representation of natural conditions. Finally, we incubated a parallel set of Whirlpaks containing 100 mL of lake water without mats, with and without F + DIC enrichment, to compare directly the nutrient response by benthic versus planktonic communities in Ward Hunt Lake. At the end of the 15-day incubation, the phytoplankton bioassay samples were filtered onto 25-mm GF/F filters (Whatman) and stored frozen, and the mat bioassays were subsampled (5-mm diameter cores) and stored as described above. Changes in the community structure of the phytoplankton and phytobenthos were evaluated by HPLC analysis of carotenoids, scytonemins, and chls.

Statistical analysis. One-way analysis of variance or *t*-tests were used (except for biovolume data) to test the null hypothesis that there were no significant differences between treatments and control at the end of each experiment (T_{final}). The data were tested for normality and homoscedasticity (Sokal and Rohlf 1995), and $\log_{10} x + 1$ transformations undertaken if necessary. Analysis of covariance was performed on primary production values of the long-term experiment, using the T_0 values as covariable. Assumptions of normality, homogeneity, and parallelism were also tested. If simple transformations of the data failed, then Kruskal-Wallis one-way analyses on ranks or Mann-Whitney rank sum tests were performed. Because of the low number of replicates for the biovolume data ($n = 2$), the treatment effect on total, Bacillariophyceae, Chlorophyta, and Cyanobacteria cell volumes was evaluated using the nonparametric Kruskal-Wallis statistic H (Kruskal and Wallis 1952, Conover 1980, Legendre and Legendre 1998). We compared the results of the Kruskal-Wallis H analysis from our data to the 95% percentile of the exact distribution of the nonparametric statistic H based on all 2520 possible combinations of the data (four groups with two samples each).

RESULTS

Mat community structure and pigments. Three different morphological, taxonomic, and functional components were identified within the vertical profiles of the benthic mats (Fig. 1). A black layer was irregularly distributed over the surface of a pink layer that was in turn underlain by a blue-green colored basal layer. Microscopic observations revealed that the thick mats (total biovolume, 5.0 $mm^3 \cdot cm^{-2}$) were dominated by cyanobacteria (92.1% of the total biovolume). The black layer was composed of large colonies with radially oriented filaments of *Tolypothrix* sp. The pink layer was dominated by thin filamentous species of cyanobacteria (*Leptolyngbya* sp., *Pseudanabaena* sp., cf. *Tychonema* sp.) and colonies of *Nostoc* spp. Finally, the blue-green layer was composed of diverse filaments,

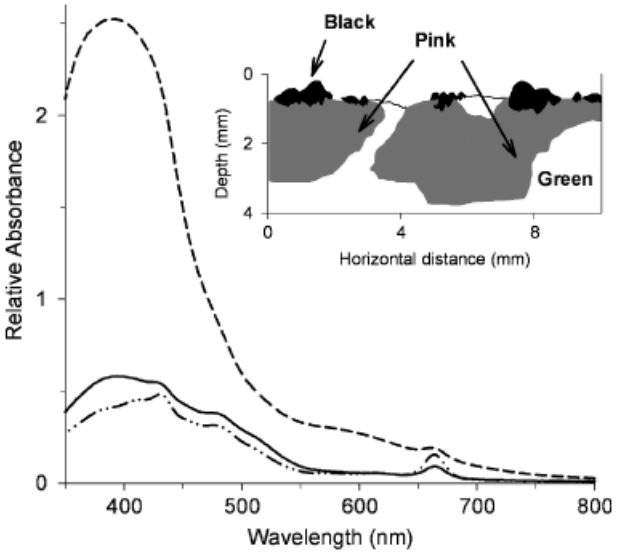

FIG. 1. Absorbance scans of acetone extracts of the three layers: black (broken line), green (broken and dotted line), and pink (continuous line). Inset: Schematic diagram of a vertical section through the mat community.

both narrow and wide taxa from the order Oscillatoriales (*Lyngbya* spp., *Oscillatoria* spp., *Leptolyngbya* sp.). Small diatoms (e.g. *Achnanthes* sp. and *Cymbella* spp.) and Chlorophyta (e.g. *Mougeotia* sp. and *Closterium* sp.) were found in both the pink and blue-green layer.

Scans of the acetone extracts from the three layers showed distinct peaks and differences with depth in the mat (Fig. 1). The black layer had maximal absorbance across a wide band, with its absolute maximum near 390 nm corresponding to the UV-protective pigment scytonemin. The two other layers had maximum absorbances around 430 nm in the chl and carotenoid waveband and at 680 nm corresponding to chl *a*.

The pigment structure determined by HPLC was consistent with the taxonomic observations. Pigment markers indicated the dominance of Cyanobacteria (scytonemin, 4-keto-myxoxanthophyll-like, echinenone, canthaxanthin, myxoxanthophyll, and zeaxanthin) followed by Chromophyta (chl c_2, diadinoxanthin, and fucoxanthin) and Chlorophyta (chl *b*, violaxanthin, and lutein) (Tables 1 and 2, Fig. 2a). The absolute concentrations of scytonemin and its ratio to chl *a* were maximal at the top of the mat; similarly, the ratio of total carotenoid to chl *a* was maximal in the upper mat surface (Table 1). A compound that is likely to be red scytonemin, a degradation product of scytonemin, was also detected, mainly in the black layer (Fig. 2, a and c). The mat samples contained high concentrations of carotenoids that are typical of filamentous cyanobacteria, notably canthaxanthin, echinenone, myxoxanthophyll, and a related glycoside closely resembling 4-keto-myxoxanthophyll (Fig. 2c). The latter was the most abundant carotenoid in the mats (Table 2). The highest concentrations of violaxanthin and chl *b* were found in the basal blue-green layer, indicating the increased importance of Chlorophyta in this lowermost stratum. The hydrophilic pigments included phycobiliproteins. Phycocyanin and allophycocyanin absorbance ratios to 750 nm were highest in the basal stratum (black, 0.79 and 0.61; pink, 0.87 and 0.64; and green layer, 1.96 and 1.38, phycocyanin and allophycocyanin, respectively). No phycoerythrin was detected. The scans of the acetone extracts showed strong absorbance in the waveband range of MAA compounds (300–357 nm), with highest MAAs/chl *a*

TABLE 2. Pigment ratios in the Ward Hunt Lake mats and phytoplankton calculated from data presented in Table 1.

	Ratios to chl *a* ($\mu g \cdot \mu g^{-1}$)	
Pigment	Mat	Phytoplankton
Chl *b*	0.01	—
Chl c_2	0.02	0.22
Red scytonemin	1.21	—
Scytonemin	21.82	—
Antheraxanthin	—	0.07
β,β-Carotene	0.15	0.10
Canthaxanthin	0.13	—
Diadinoxanthin	0.15	0.25
Echinenone	0.25	—
Fucoxanthin	0.04	1.07
Myxoxanthophyll	0.11	—
Lutein	<0.01	—
Violaxanthin	0.17	0.89
Zeaxanthin	0.05	0.39
4-Keto-Myxo	0.36	—
Total Car	1.42	2.77

Total Car, total carotenoids; 4-keto-myxo, 4-keto-myxoxanthophyll-like.

TABLE 1. Lipid pigment concentrations in late summer (August) benthic mat ($n = 3$) and phytoplankton ($n = 4$) samples from Ward Hunt Lake, determined by HPLC.

	Mat ($\mu g \cdot cm^{-2}$)				Phytoplankton	
	Black	Pink	Green	Mat total	($\mu g \cdot L^{-1}$)	Contribution to total (%)
Chlorophylls						
Chl *a*	2.18	2.15	6.07	10.4	0.39	0.15
Chl *b*	nd	nd	0.14	0.14	nd	0
Chl c_2	0.12	0.02	0.12	0.25	0.08	2.35
Scytonemins						
Red scytonemin	12.48	0.11	0	12.59	nd	0
Scytonemin	197.76	19.21	9.93	226.90	nd	0
Carotenoids						
Antheraxanthin	nd	nd	nd	—	0.03	100
β,β-Carotene	0.34	0.41	0.83	1.59	0.04	0.10
Canthaxanthin	0.71	0.41	0.20	1.32	nd	0
Diadinoxanthin	0.57	0.67	0.30	1.54	0.10	0.25
Echinenone	0.81	0.85	0.99	2.65	nd	0
Fucoxanthin	0.22	0.16	0.00	0.38	0.42	4.19
Myxoxanthophyll	0	0.53	0.61	1.14	nd	0
Lutein	0.04	0	0	0.04	nd	0
Violaxanthin	0.17	0.43	1.20	1.80	0.35	0.76
Zeaxanthin	0.11	0.09	0.31	0.51	0.15	1.18
4-Keto-myxo	1.33	1.45	0.99	3.77	nd	0
Total Car	4.29	5.07	5.44	14.79	1.07	0.35

The mats were split into three strata that differed in color (see Fig. 1). The sum for the layers is shown (Mat total). The contribution of phytoplankton to the total pigment stocks (%) was calculated per unit area assuming an average lake depth of 0.4 m (Villeneuve et al. 2001). Total Car, total carotenoids; 4-keto-myxo, 4-keto-myxoxanthophyll-like; nd, not detected.

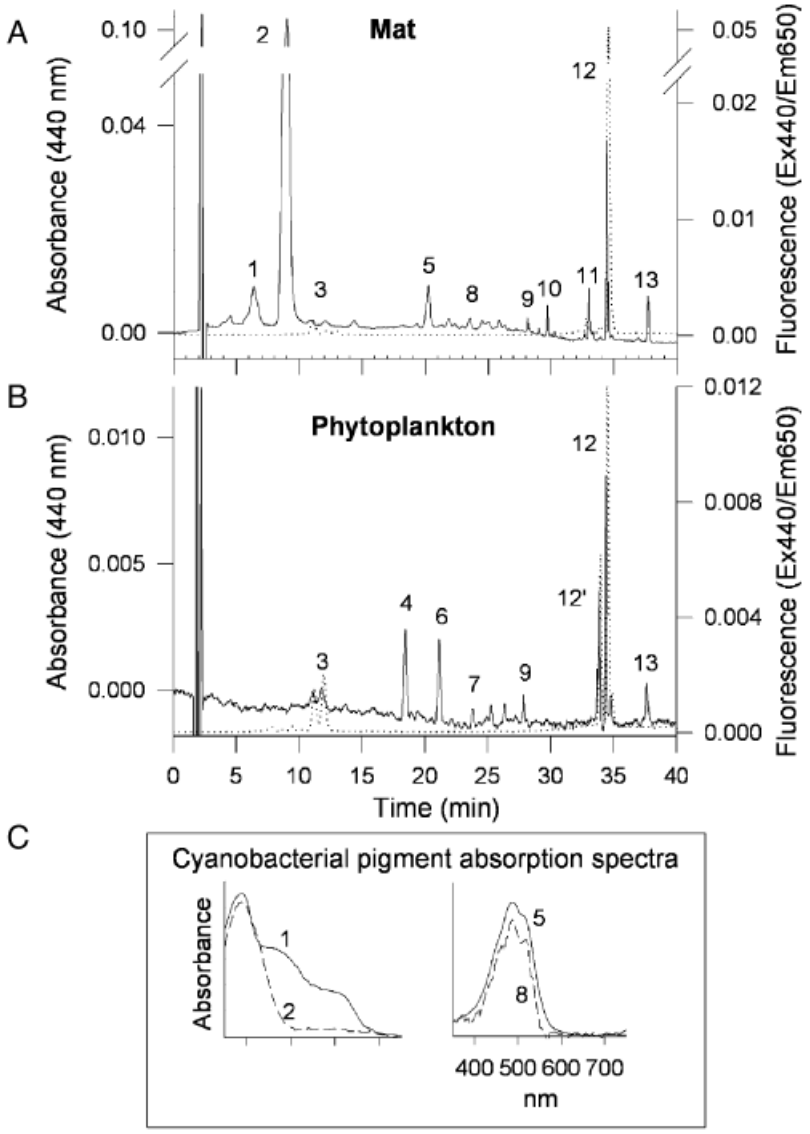

FIG. 2. HPLC chromatograms of the whole mat (a) and phytoplankton (b) communities. Detection was by absorbance at 440 nm (continuous line) and by fluorescence at Ex440/Em650 nm (dotted line). The absorption spectra of some representative pigments (c) found in high abundance in the cyanobacterial mats, sheath pigments (left) and glycoside carotenoids (right). Numbers refer to the pigments as follow: 1, red scytonemin; 2, scytonemin; 3, chl c_2; 4, fucoxanthin; 5, 4-keto myxoxanthophyll; 6, violaxanthin; 7, diadinoxanthin; 8, myxoxanthophyll; 9, zeaxanthin; 10, canthaxanthin; 11, chl *b*; 12, chl *a*; 12′, chl *a* allomer-like; and 13, β,β-carotene.

ratios in the top layer (0.53, 0.49, and 0.15 for black, pink, and green layers, respectively).

Phytoplankton community structure and pigments. Plankton samples from 1998 and 2004 showed the dominance of Chrysophyceae, notably *Dinobryon sociale* Ehrenberg, but also the presence of some other colonial taxa and pennate diatoms (e.g. *Navicula* spp.). Consistent with these observations, the HPLC analysis of phytoplankton pigment samples from the lake in 2003 and 2004 showed chl *a* and chl c_2 and the carotenoids fucoxanthin, diadinoxanthin, violaxanthin, and zeaxanthin (Table 1, Fig. 2b). Fucoxanthin, a pigment signature for Chromophyta, was the most important carotenoid in the pelagic samples (Table 2, Fig. 2b). The phytoplankton biomass, as measured by chl *a*, was at extremely low concentrations in both years of sampling (0.0080–0.015 $\mu g \cdot cm^{-2}$), and comparison with the benthic chl *a* stocks showed that it represented only a minor fraction (0.08%–0.15%) of the total autotrophic biomass (Table 1). Similarly, all other pigments detected in the phytoplankton, with the exception of antheraxanthin that was not found in the mats, represented only 0.1 to 4.2% of the total stocks per unit area.

First set of mat bioassays. Neither the addition of inorganic carbon (DIC) nor enrichment with a full spectrum of nutrients had any significant effect on photosynthesis by the benthic microbial mats (Fig. 3). This absence of effect on photoincorporation of carbon was found in the short-term (analysis of variance, $F = 0.216$; $P = 0.883$) as well as long-term experiments (analysis of covariance, $F = 0.514$; $P = 0.305$). Similarly, the long-term experiment showed no significant difference in the total biovolume of the mat or in the relative biovolume of the three major taxonomic groups, with or without full nutrient and DIC enrichment (Kruskal-Wallis test for total biovolume, $H = 2.500$, $P = 0.600$; Cyanobacteria, $H = 2.500$,

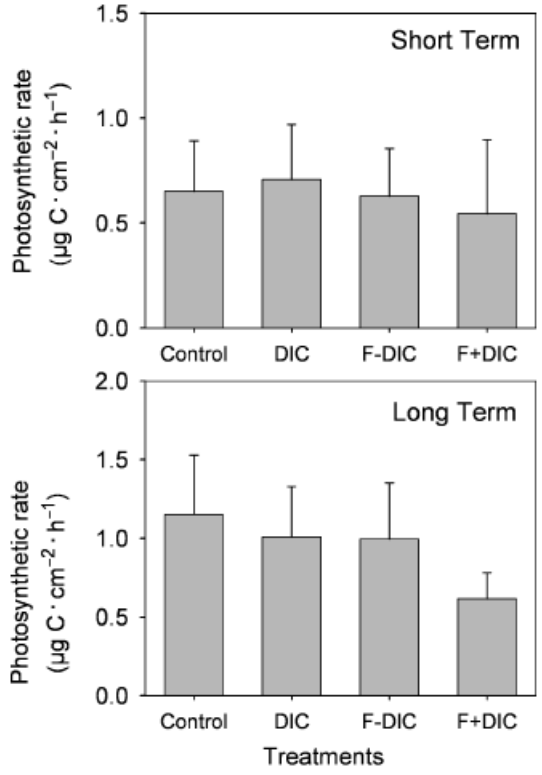

FIG. 3. Primary production in the short-term (6 h) and the first set of long-term (15 day) bioassays of the mat community. The treatment codes are as follows: DIC, dissolved inorganic carbon; F-DIC, full set of nutrients excluding DIC; F + DIC, full set of nutrients and dissolved inorganic carbon. Values are the means ($n = 3$) ± SE.

$P = 0.600$; Bacillariophyceae, $H = 0.177$, $P = 0.991$; and Chlorophyta, $H = 1.177$, $P = 0.714$).

Second set of bioassays, benthos versus phytoplankton. Contrasting results were obtained between the mat and phytoplankton enrichments (Fig. 4). For the benthic community, no significant changes of total biomass ($\mu g\ chl\ a \cdot cm^{-2}$), pigment structure, or pigment ratios were observed (Table 3), consistent with our results from the first set of bioassays. A detailed analysis of the individual HPLC-quantified components showed no significant differences for chl *a*, chl *b*, chl c_2, total carotenoids, and scytonemins (Table 3). Moreover, no differences were found for any of the measured carotenoids (Mann-Whitney, for alloxanthin, $T = 31.5$, $P = 0.24$; fucoxanthin, $T = 27.500$, $P = 0.065$; lutein, $T = 27.500$, $P = 0.065$; myxoxanthophyll, $T = 39.00$, $P = 1.00$; *t*-test for β,β-carotene, $t = -2.097$, $P = 0.062$; canthaxanthin, $t = 0.104$, $P = 0.91$; diadinoxanthin, $t = -0.145$, $P = 0.887$; zeaxanthin, $t = -2.213$, $P = 0.051$; 4-keto-myxoxanthophyll, $t = 0.327$, $P = 0.751$; violaxanthin, $t = 0.653$, $P = 0.528$). Marginally significant higher values of echinenone were found at T_{final} in the N + DIC treatment (*t*-test, $t = -2.422$, $P = 0.036$). The highest concentrations of scytonemin were registered in the initial (T_0) samples, probably due to the irregular distribution of the black layer on the lake surface.

In contrast, nutrient enrichment resulted in strongly significant effects on the phytoplankton community (Fig. 4). The concentration of planktonic chl *a* increased 19.2-fold by the end of the incubation (T_f) in the F + DIC treatment (2.71 ± 0.98 $\mu g \cdot chl\ a\ L^{-1}$, mean ± SD) in comparison with the control (0.14 ± 0.06 $\mu g \cdot chl\ a\ L^{-1}$, mean ± SD) (*t*-test, $t = 10.96$, $P < 0.001$). Significant increases in the concentration of chl c_2 (*t*-test, $t = 21.00$, $P = 0.002$) were also found at T_{final} in the enriched treatment. Chlorophyll *b* and some carotenoids (β,β-carotene and diadinoxanthin) were near our limits of detection at T_0 and in the control but were detectable in small concentrations after enrichment. In terms of total chl *a* standing stocks, the resulting phytoplankton community at the end of nutrient enrichment (mean, 0.11 $\mu g \cdot cm^{-2}$) represented 1.03% of the total phototrophic community (phytobenthos plus phytoplankton) per unit area.

DISCUSSION

As in many lakes of the polar regions (Elster et al. 1999, Vadeboncoeur et al. 2003, Hodgson et al. 2004), the Ward Hunt Lake ecosystem contained sparse concentrations of phytoplankton, and most of its phototrophic biomass was located in the benthos. Our HPLC analyses showed that for almost all individual algal pigments, more than 98% of their standing stocks per unit area of lake occurred in the benthic microbial mats. The absorption spectra and HPLC pigment signatures for the mats were characteristic of a phototrophic community dominated by cyanobacteria, with high concentrations of light-harvesting phycobiliproteins, protective carotenoids, and UV-screening compounds such as scytonemin. This ecosystem dominance by benthic cyanobacteria is common to many lakes, ponds, and streams in the Arctic and Antarctica and is likely to be the result of their broad tolerances to physical extremes rather than to an ability to grow rapidly in the ambient polar environment (Tang et al. 1997, Vincent 2000). The arctic mats analyzed here also contained allophycocyanin and phycocyanin C but no evidence of phycoerythrin, consistent with the observations of cyanobacterial mats from shallow antarctic lakes (Vincent et al. 1993).

The higher ratios of chl c_2 and fucoxanthin to chl *a* in the phytoplankton reflected the planktonic abundance of *Dinobryon*, a genus that seems to be of widespread importance in arctic and subarctic lakes, including the Svalbard Archipelago (Laybourn-Parry and Marshall 2003). The production of resistant spores by Dinobryon (Villeneuve et al. 2001) and its known mixotrophic capabilities (Laybourn-Parry and Marshall 2003) may allow it to survive prolonged winter darkness. The water column also contained cyanobacteria as indicated by microscopic analyses (cf. *Synechococcus* sp. and *Anabaena* sp.) and high ratios of zeaxanthin to chl *a*.

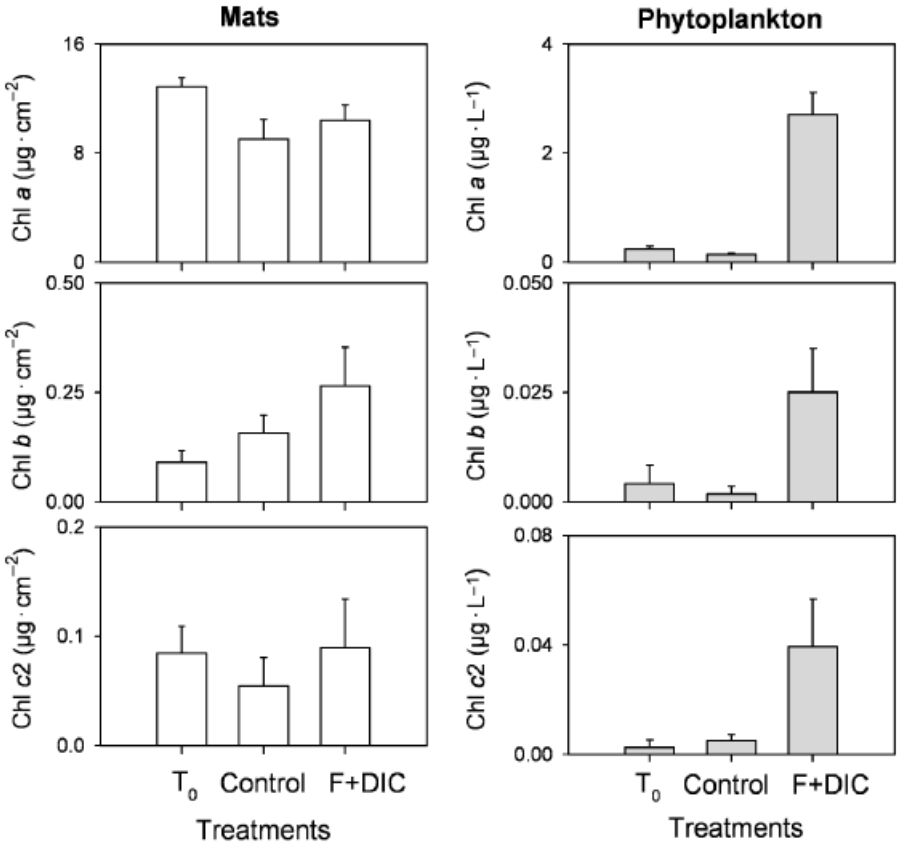

FIG. 4. Pigment responses of the Ward Hunt Lake phototrophic communities to nutrient enrichment in the 15-day *in situ* bioassays. Chlorophyll data for the benthic mats is given in the left column graphs and phytoplankton on the right. The treatment codes are as follows: T_0, initial time; F + DIC, full set of nutrients including dissolved inorganic carbon. The values are means ($n = 6$) ± SE (except for the phytoplankton at T_0, $n = 4$).

Contrasting photoprotective pigment strategies appear to be evident in the planktonic versus benthic communities. The phytoplankton had higher total carotenoid-to-chl *a* ratios, whereas the cyanobacterial mats contained high concentrations of scytonemin in their surface layer, comparable with the amounts found in cyanobacterial mats growing on arctic ice shelves (Vincent et al. 2004) and antarctic cyanobacterial mats (Vincent et al. 1993). The surface black layer in the Ward Hunt Lake mats was dominated by colonies of *Tolypothrix* sp. that had dark-colored sheaths containing the UV-screening pigment scytonemin (Garcia-Pichel and Castenholz 1991). The synthesis of scytonemin appears to be induced by exposure to UV under conditions of restricted metabolism (Castenholz and Garcia-Pichel 2000) and may be favored in arctic waters by the combined stress of UV and cold temperatures. Another UV-absorbing compound, a pigment likely to be red scytonemin, was also present in the black layer in high concentrations. Red scytonemin is stable over long time scales and is formed under anoxic reducing conditions (Garcia-Pichel and Castenholz 1991). Such conditions may occur during the long polar winter at Ward Hunt Lake, as seen in shallow water lakes on Ross Island, Antarctica during prolonged winter freeze-up (Schmidt et al. 1991) and in melt-pond mats growing on ice shelves in the Canadian Arctic (Vincent et al. 2004). Mycosporine-like amino acid re-

TABLE 3. Effects of nutrient enrichment on mat pigment concentrations ($\mu g \cdot cm^{-2}$).

Pigments	T_0	Control (T_{final})	F + DIC (T_{final})	*t*-test, Control vs. F + DIC	
				t	*P*
Chl *a*	12.85 ± 0.68	9.03 ± 1.44	10.41 ± 1.11	−0.860	0.410
Chl *b*	0.09 ± 0.03	0.18 ± 0.04	0.26 ± 0.09	−1.093	0.300
Chl c_2	0.08 ± 0.02	0.05 ± 0.03	0.09 ± 0.04	−0.641	0.536
Scytonemin	156.48 ± 28.34	19.39 ± 4.02	18.43 ± 7.12	0.866	0.407
Red scytonemin	11.31 ± 9.28	0.11 ± 0.21	0.19 ± 0.28	−0.520	0.615
Total Car	14.20 ± 2.12	6.05 ± 0.85	8.31 ± 1.43	−1.165	0.271

Values are means ($n = 6$) ± SE. T_0, initial time; F + DIC, treatment with addition of full set of nutrients including dissolved inorganic carbon; Total Car, total carotenoids. The *t*-test results (Control vs. F + DIC at final time) are shown; no values were statistically significant ($P > 0.05$).

lated compounds were also located in the surface layer and likely contributed to UV-screening of the total community, as found in cyanobacterial mat communities in Antarctica (Quesada et al. 1999) and elsewhere (Stal and Caumette 1994).

Glycosidic carotenoids, notably myxoxanthophyll, and its metabolic derivatives, cf. 4-keto-myxoxanthophyll (Schlüter et al. 2004) or 4-keto-myxol-methylpentoside (Francis et al. 1970), were common in the mats. This group of compounds is known to be characteristic of filamentous cyanobacteria (Goodwin 1980) and can play photosynthetic as well as protective roles (Hischberg and Chamovitz 1994). In this respect, the Ward Hunt Lake mats resemble those from Antarctica that contain myxoxanthophyll and myxol-2′-O-methyl-methylpentoside among their most important carotenoids (Vincent et al. 1993). It has been shown that low temperature can increase carotenoid concentrations in general in cyanobacteria (Roos and Vincent 1998) and myxoxanthophyll concentrations specifically (Várkonyi et al. 2002).

Deeper within the mat profile in the Ward Hunt Lake phytobenthos, in the blue-green basal layer the pigment assemblage contained a diverse set of carotenoids and a shift toward increasing concentrations of chl *a* and phycobiliproteins. This indicates a functional shift from light screening to light harvesting to exploit the reduced solar energy supply in the shade environment deep within the mat. This basal layer rich in light-capturing pigments is a feature previously described for cyanobacterial mats in Antarctica (Howard-Williams and Vincent 1989, Quesada et al. 1999).

The large benthic biomass of phototrophs in Ward Hunt Lake and the sparse phytoplankton in the overlying water column would seem to imply a much greater availability of nutrients for growth in the benthos relative to plankton. Consistent with this supposition, previously reported analyses of the nutrient concentrations of the interstitial waters of the mats in Ward Hunt Lake have shown that SRP and inorganic dissolved nitrogen concentrations are 2.5 to 43 times higher than in the lake water (Villeneuve et al. 2001). However, these higher concentrations need not imply an absence of nutrient limitation in the phytobenthos given that its nutrient demand would also be very much higher than in the plankton. The lack of stimulation of mats, both in the short- and long-term incubations, provides strong evidence of nutrient sufficiency and shows that C, N, P, and micronutrients were not limiting factors. The lack of response to carbon addition is consistent with the high measured DIC in the mat profiles (Villeneuve et al. 2001) and likely reflects the carbonate-rich dolomite in the catchment (Trettin 1991). Contrasting results were reported by Turner et al. (1994), who found DIC limitation of periphytic communities but in ELA lakes that lie on carbonate-poor granitic basins in the Precambrian Shield. Shallow high latitude lakes freeze to the bottom each year, and the resultant freeze-concentration of solutes (Schmidt et al. 1991, Belzile et al. 2002) is likely to force dissolved carbon and nutrients into the mats. This freeze concentration from the overlying water column may be a key process enhancing nutrient supply to the benthos.

Factors other than nutrients are likely to control the benthic cyanobacterial biomass in Ward Hunt Lake and probably many other cold polar and alpine lakes. Physical factors such as ice cover, light, and temperature may be of greater importance in these environments. For example, the cyanobacterial community physiognomy, diversity, and pigments of 56 lakes and ponds in Antarctica were strongly correlated with lake depth and light availability but not with other environmental factors (Sabbe et al. 2004). However, nutrient limitation of periphyton has been reported in some arctic rivers (Hullar and Vestal 1989, Miller et al. 1992) and alpine lakes (Vinebrooke and Leavitt 1998) and may depend on the chemical characteristics of the basin, the algal biomass, community structure, and the extent to which the benthic community is able to retain and recycle nutrients.

Under warmer conditions, nutrient recycling processes may be unable to keep pace with demand, and there is evidence of nutrient limitation in microbial mats from lower latitudes. For example, stimulation of the primary production was observed for coastal and estuarine mats with the addition of inorganic nitrogen (Paerl et al. 1993). The growth of cyanobacteria and diatoms in intertidal mats appeared to be nitrogen limited, and the relative abundance of the two groups depended on N and P supply (Pinckney et al. 1995). There is additional evidence of P limitation for mats in other coastal environments (Stal 2000).

The results of our enrichment assay of the Ward Hunt Lake phytoplankton are consistent with the classic studies on high arctic Char and Meretta Lakes that showed a strong response to increased nutrient load (Schindler et al. 1974b, Douglas and Smol 2000). The nutrient addition resulted in a rapid increase of Ward Hunt phytoplankton biomass (as measured by pigments) to chl *a* levels that were higher than those reported in enriched Meretta Lake (Markager et al. 1999). Although the nutrient enrichment had a large effect on phytoplankton biomass, the contribution to the total stocks of chl *a* rose to only 1.03% and thus had little effect on the overall dominance of the benthic mats. Unlike the benthos, the phytoplankton occupies a dynamic habitat, in which they can exhibit rapid fluctuations in abundance and composition in response to environmental change (Reynolds 1984). Because of its shallowness and water transparency, light is not a limiting factor for the pelagic community in Ward Hunt Lake during open water conditions in summer; however, persistent low temperatures are likely to constrain phytoplankton growth rates. Zooplankton net hauls have not detected crustacean zooplankton in this extreme northern lake (M. Rautio, personal communication), although benthic grazers such as chironomids occur. Grazing controls on phytoplankton and phytobenthos have yet to be evaluated in this ecosys-

tem. In other mat systems elsewhere in the Arctic, there was no indication of biomass control by benthic grazers (Vadeboncoeur et al. 2003).

The results presented here draw attention to the contrasting properties of the plankton and benthos in high arctic lakes and probably of other cold, clear, freshwater ecosystems. The dilute plankton was dominated by different algal phyla and had different light-capturing and protective pigment strategies relative to the biomass-rich benthos. The plankton was severely limited by nutrient supply, whereas the benthic cyanobacteria mats were nutrient sufficient. Such lakes are typically described as ''ultraoligotrophic.'' However, this term refers only to the water column and may be a misleading guide to the total productivity and nutrient status of lake ecosystems that are largely driven by their phytobenthos.

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