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Milne Fiord epishelf lake: A coastal Arctic ecosystem vulnerable to climate change[1]

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Abstract: Milne Fiord in the Canadian High Arctic contains the last known ice-dammed fiord lake (epishelf lake) in the Northern Hemisphere. This freshwater ecosystem is retained by the Milne Ice Shelf and is underlain by sea water that is connected to the Arctic Ocean. Using microscopy, photosynthetic pigment analyses, and molecular techniques we examined the planktonic communities present in Milne Fiord to determine the biotic characteristics of the epishelf lake and the sea water below. Net sampling of the water column of Milne Fiord revealed a mixture of marine, freshwater, and brackish zooplankton taxa, and high performance liquid chromatography (HPLC) pigment analysis showed pronounced differences in phytoplankton composition through the highly stratified water column. Chlorophytes dominated in the epishelf lake, prasinophytes prevailed in the halocline, and the bottom layer harboured mainly fucoxanthin-containing groups. Clone libraries of a dark-incubated, concentrated sample from below the halocline (30 m depth) yielded marine Archaea (mainly Crenarchaeota) and known bacterial taxa from the Pacific and Arctic oceans (*e.g.*, *Roseobacter*, *Oleispira*, *Colwellia*). An equivalent sample from the epishelf lake (5 m depth) yielded many bacterial taxa that are characteristic of cold, freshwater habitats (*e.g.*, *Polynucleobacter*, *Variovorax*, *Flavobacterium*), the euryhaline genus *Polaromonas*, and freshwater eukaryotes, notably ciliates. Similarly, denaturing gradient gel electrophoresis (DGGE) analyses of T4-like bacteriophages showed different viral assemblages in the upper and lower water column. This diverse, stratified ecosystem is dependent on the integrity of the bounding ice shelf and is therefore vulnerable to the ongoing effects of climate change in this region.
Keywords: Archaea, Arctic, bacteria, cyanobacteria, extremophiles, phytoplankton, protists, zooplankton.

Résumé : Le fjord Milne dans le Haut-Arctique canadien renferme le dernier lac de barrage glaciaire dans un fjord (lac épi-plate-forme) connu dans l'hémisphère nord. Cet écosystème d'eau douce est retenu par la plateforme de glace Milne et flotte au-dessus d'eau de mer reliée à l'océan Arctique. En utilisant la microscopie, des analyses de pigments photosynthétiques et des techniques moléculaires, nous avons examiné les communautés planctoniques présentes dans le fjord Milne afin de déterminer les caractéristiques biotiques du lac épi-plate-forme et de l'eau de mer sous-jacente. Un échantillonnage à l'aide de filets de la colonne d'eau du fjord Milne a révélé un mélange de taxons zooplanctoniques marins, d'eau douce et d'eau saumâtre et l'analyse de pigments par chromatographie liquide à haute performance a montré des différences prononcées de composition en phytoplancton dans la colonne d'eau fortement stratifiée. Les chlorophytes dominaient dans le lac épi-plate-forme, les prasinophytes prévalaient dans l'halocline et la couche de fond hébergeait principalement des groupes contenant de la fucoxanthine. Les banques de clones d'un échantillon concentré incubé à l'obscurité et provenant de la couche sous l'halocline (profondeur de 30 m) ont révélé des archées marines (principalement des Crénarchées) et des taxons bactériens connus des océans Pacifique et Arctique (par exemple : *Roseobacter*, *Oleispira*, *Colwellia*). Un échantillon équivalent provenant du lac épi-plate-forme (profondeur de 5 m) a révélé plusieurs taxons bactériens caractéristiques d'habitats froids en eau douce (par exemple : *Polynucleobacter*, *Variovorax*, *Flavobacterium*), le genre euryhalin *Polaromonas* et des eucaryotes d'eau douce, notamment des ciliés. Similairement, des analyses d'électrophorèse sur gel en gradient dénaturant des bactériophages de type T4 ont révélé des assemblages viraux différents dans la colonne d'eau supérieure et inférieure. L'existence de cet écosystème stratifié diversifié dépend de l'intégrité de la plate-forme de glace qui le borde pour son existence. Il est donc vulnérable aux effets des changements climatiques qui affectent déjà cette région.
Mots-clés : archée, arctique, bactérie, cyanobactérie, extrémophiles, phytoplancton, protistes, zooplancton.

Nomenclature: Guiry & Guiry, 2011; National Center for Biotechnology Information, 2011.

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Introduction

Ice plays a major role in the limnology of high-latitude aquatic ecosystems, including as a physical barrier that creates ice-dammed lakes. The latter encompass several types of freshwater ecosystems, including epishelf lakes that are contained in fiords or embayments completely blocked by ice shelves. Meltwater derived from ice and snow of the drainage basin accumulates behind these floating ice masses and forms an upper freshwater layer overlying sea water. These ecosystems were first described in Antarctica (Heywood, 1977), where there are many known examples (Gibson & Andersen, 2002; Laybourn-Parry *et al.*, 2006; Smith *et al.*, 2006). However, only one such ecosystem is known to remain in the Arctic, in Milne Fiord, located at the northern limit of High Arctic Nunavut, Canada (Veillette *et al.*, 2008). This lake lies between Milne Glacier at the head of the fiord and the Milne Ice Shelf, which blocks the seaward end of the fiord (Figure 1). Arctic ice shelves are rapidly declining because of climate warming (Copland, Mueller & Weir, 2007; England *et al.*, 2008; Vincent *et al.*, 2009), and their demise has resulted in the contraction and loss of epishelf lakes (Mueller, Vincent & Jeffries, 2003; Veillette *et al.*, 2008). The Milne Ice Shelf is composed of glacial ice and thickened sea ice (Jeffries, 1986), and to date it has not experienced the extensive calving that has occurred elsewhere along this northern coastline.

Fiords or embayments harbouring epishelf lakes are vertically stratified ecosystems in which there may be strong contrasts in environmental properties between the upper freshwater layer and the marine layer underneath. This gradient provides habitats for an unusual combination of freshwater and marine macrobiota in the same water column, as indicated by zooplankton studies (Van Hove *et al.*, 2001). Microbial diversity has been explored in other Arctic ecosystems where contiguous habitats contain distinctly different environmental conditions, including estuaries (Garneau *et al.*, 2006), meromictic lakes (Pouliot *et al.*, 2009); stamukhi lakes (Galand *et al.*, 2008), the Arctic Ocean (Hamilton *et al.*, 2008; Galand *et al.*, 2009a), and the North Water polynya with interleaving water masses (Lovejoy *et al.*, 2002). However, similar investigations of epishelf systems are still lacking.

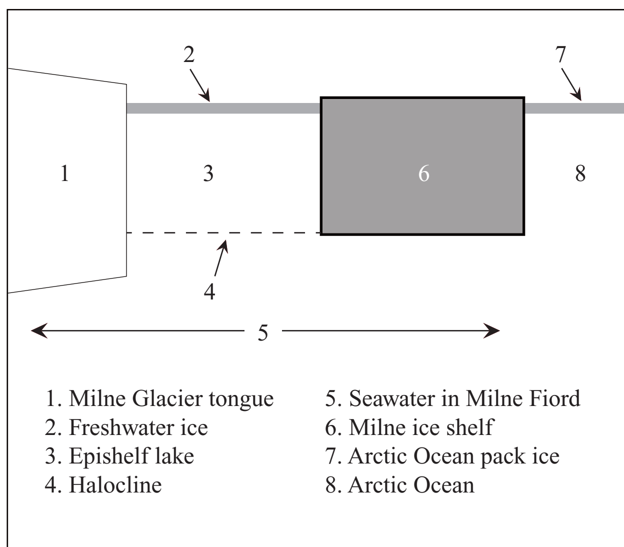

FIGURE 1. Schematic profile diagram of the different water masses and ice types in Milne Fiord, Canadian High Arctic (not drawn to scale). The Milne Glacier tongue floats on the sea at the head of the fiord and Milne Ice Shelf blocks the seaward end of the fiord, retaining the freshwater epishelf lake. The minimum draft of the ice shelf determines the depth of the epishelf lake.

The objective of the present study was to determine the planktonic biodiversity of Milne Fiord as an extreme High Arctic ecosystem and to characterize its patterns of biological stratification. This remote site is extremely difficult to access, but we were able to sample at this location on 2 visits, one in 2007 and the other in 2009. Given the microbial dominance of polar aquatic ecosystems, we studied all components of the microbiota: viruses, Bacteria, Archaea, and Eukarya, including the phytoplankton. We evaluated their community biomass and structure using a combination of microscopy, flow cytometry, photosynthetic pigment analyses, and molecular techniques. We also characterized the physical and biological environment by salinity–temperature profiling and by analysis of zooplankton species composition within the water column.

Methods

WATER COLUMN PROFILING AND SAMPLING

We sampled Milne Fiord (82° 45' N, 82° 00' W) on the northern coast of Ellesmere Island, Canada (Figure 2) by helicopter from our base camp at Ward Hunt Island, 115 km to its east. The sampling site was in Neige Bay (unofficial name), a bay on the eastern side of the fiord (Figures 3 and 4) with a maximum water depth of 80 m. The climate in this region is characterized by extreme cold, with an average annual air temperature of –18.4 °C at Alert (1951–2005), the closest Environment Canada weather station (www.weatheroffice.gc.ca). Temperatures at Milne Fiord are likely to be slightly colder than at Alert since it is a long fiord facing north and shaded by steep terrain, with the Milne Glacier at its head (Figures 1, 3, and 4). The fiord was covered by ~1.6 m (2007) and ~1.1 m (2009) of ice, but no snow, at the times of sampling, with a top 5- to 10-cm layer of white, candled ice overlying clear, fused ice below. Sampling and profiling was through 22-cm-diameter holes drilled through the ice.

Temperature and salinity profiles of the water column were taken with an XR-620 CTD (conductivity-temperature-depth profiler; RBR Ltd., Ottawa, Ontario, Canada) on 13 July 2007 along with water samples from the freshwater and marine layers (5 and 30 m depth) that were used for pigment, molecular, and microscopic analyses. Water samples were taken using a 6.2-L Kemmerer sampler, transferred to acid-washed opaque 20-L plastic containers, and stored in the dark at 4 °C for a maximum of 8 h until processing. On 4 July 2009, water column profiles were again taken, and additional samples for phytoplankton pigment analyses were collected at 1.6, 5, 10, 14, 16, 18, and 30 m. Sampling depths were selected in relation to the

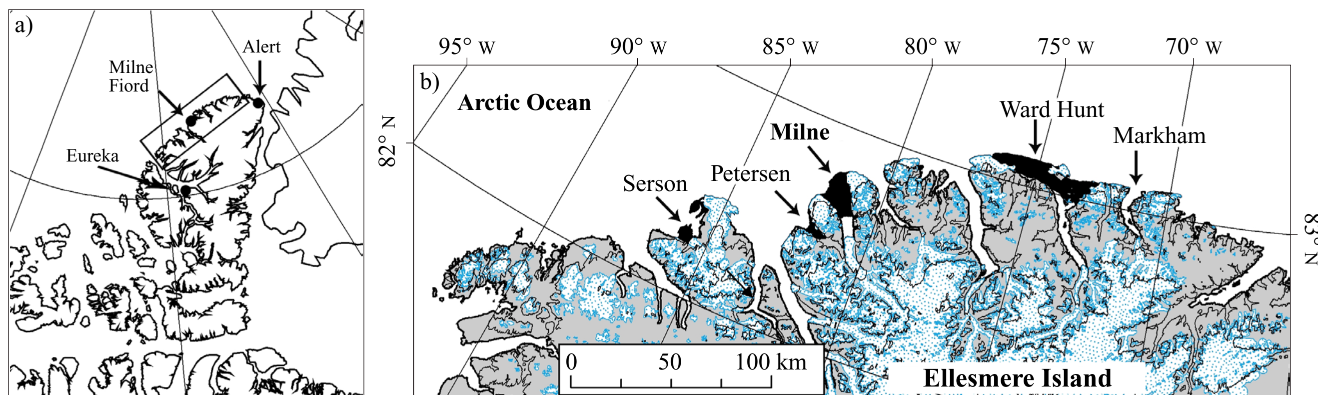

FIGURE 2. Location of Milne Fiord (a) and ice shelves along the northern coast of Ellesmere Island (b). Ice shelves as of September 2008 are indicated in solid black, glaciers are marked in stipple, and the contour lines correspond to 200, 1000, and 1800 m. Disraeli Fiord is located behind the Ward Hunt Ice Shelf.

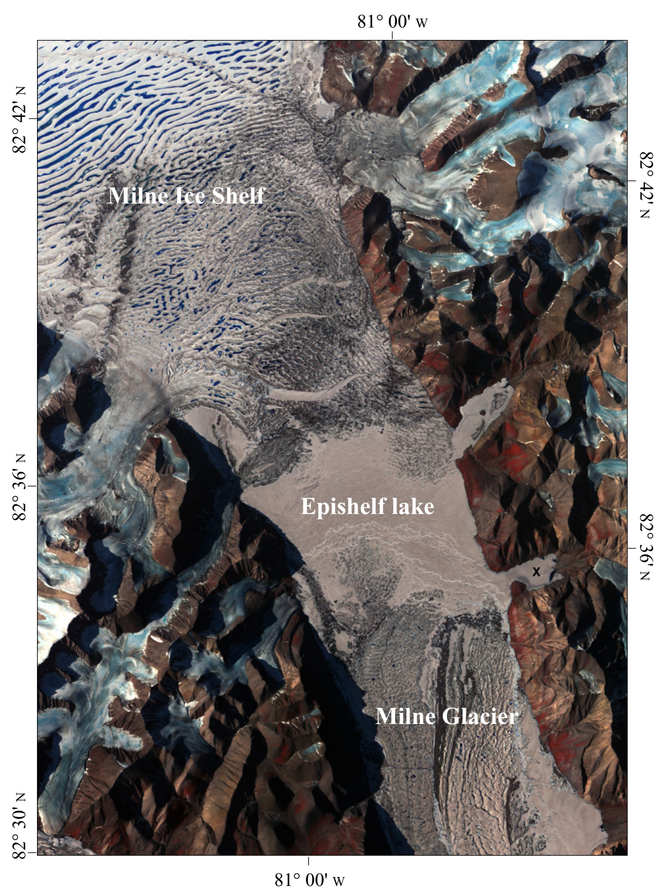

FIGURE 3. A satellite image (ASTER image, acquired 27 August 2009 at 2005 UTC; courtesy of NASA/GSFC/METI/ERSDAC/JAROS and US/Japan ASTER Science Team) showing the location of the Milne epishelf lake (note the brighter freshwater lake ice) in relation to Milne Ice Shelf (to the north) and Milne Glacier (to the south). The sampling site in Neige Bay (unofficial name) is marked with a x.

salinity profile to target the upper epishelf lake, the lower sea water, and, in 2009, the halocline, which is the zone of abrupt salinity change between the fresh water and sea water. Two zooplankton vertical tows (14–0 m then 50–0 m, through the same hole) were made on 4 July 2009 with a 39-cm-long net with a 210-µm mesh size and diameter of 13 cm.

FIGURE 4. View towards the west at the sampling site in Neige Bay (unofficial name), Milne Fiord, 4 July 2009.

PHOTOSYNTHETIC PIGMENT ANALYSES

Water samples (1 L in 2007, 0.5 L in 2009) for pigment analyses were filtered onto 25-mm-diameter GF/F glass fibre filters (approximate pore size 0.7 µm) that were frozen immediately in the field in a Dry Shipper (–196 °C) and subsequently stored in a –80 °C freezer until analysis. Pigments were extracted from the frozen phytoplankton filters by sonication in 2.5 mL of 95% methanol, cleared by centrifugation, and filtered with PTFE syringe filters (pore size 0.2 µm) into HPLC vials. The extracts were then put under argon and kept at 4 °C in the dark in the HPLC autosampler to prevent pigment degradation. Shortly following extraction, 100 µL of phytoplankton pigment extracts were injected into a Varian ProStar HPLC system equipped with a Symmetry C8 column. The solvent protocol followed that of Zapata, Rodriguez, and Garrido (2000). Chlorophylls were detected by fluorescence (excitation, 440 nm; emission, 650 nm), and carotenoids were detected by photodiode-array (PDA) spectroscopy (350–750 nm) with a slit width of 2 nm. Absorbance chromatograms were obtained at 450 nm. Standards for identification (based on PDA spectra

and retention times) and quantification (using calibration coefficients) of pigments were obtained from Sigma Inc. (St. Louis, Missouri, USA) (chl *a*, chl *b*, β,β-carotene) and DHI Water & Environment (Hørsholm, Denmark) (alloxanthin, astaxanthin, canthaxanthin, chl c_2, diadinoxanthin, echinenone, fucoxanthin, lutein, myxoxanthophyll, violaxanthin, zeaxanthin) to calibrate our HPLC. Standards for identification of chl c_3, MgDVP, neoxanthin, peridinin, and prasinoxanthin were part of a mixed standard solution also obtained from DHI Water & Environment, and these carotenoids were quantified using extinction coefficients from the literature (Jeffrey, Mantoura & Wright, 1997; Latasa *et al*., 2004) since they were not available in a sufficient quantity to make calibration curves. Prasinophyte pigments (antheraxanthin, micromonal, micromonol, uriolide) were identified from a culture of Arctic *Micromonas* sp. (strain CCMP2099; West Boothbay Harbor, Maine, USA; Lovejoy *et al*., 2007) and quantified using extinction coefficients from the literature (Latasa *et al*., 2004) and the extinction coefficient of β,β-carotene for micromonol. Chlorophyllide *a* and pheophytin *a* were identified based on their retention time in the fluorometer chromatogram (Zapata, Rodriguez & Garrido, 2000). Concentrations of unknown chlorophylls were calculated using the calibration coefficient for chl *a*, and concentrations of unknown carotenoids were calculated using the calibration coefficient for β,β-carotene.

DNA concentration and extraction

Water from 5 and 30 m depth (~16 L) was pre-filtered through 43-μm mesh Nitex net and concentrated using tangential flow. The > 0.2 μm material (Bacteria, Archaea, and eukaryotes) was concentrated *ca* 100-fold by the use of a 0.2-μm Vivaflow 200 tangential flow module, and the remaining virus-size material (< 0.2 μm) was concentrated *ca* 175-fold by use of a 30 000 MWCO Vivaflow 200 tangential flow module. The concentrates were stored at 4 °C in the dark for 3 weeks until further analysis. This incubation period resulted in a shift in the community, and the taxa identified in the clone libraries should be considered an enriched subset of the original communities (see Massana *et al*., 2006). The > 0.2-μm concentrate was filtered onto a 0.2-μm filter and stored in sterile lysis buffer at –80 °C within 3 weeks, and extracted 2 months later. DNA extraction for the bacterial, archaeal, and eukaryotic analyses was adapted from Diez, Pedrós-Alió, and Massana (2001) and Aljanabi and Martinez (1997). In brief, cells were treated with lysozyme (final concentration, 1 $mg \cdot mL^{-1}$), proteinase K (0.21 $mg \cdot mL^{-1}$) and sodium dodecyl sulfate (0.01%). DNA was precipitated in 70% ethanol and NaCl with a final ethanol wash step (Galand *et al*., 2009c). Viral concentrate subsamples (250 μL) were extracted within 2 weeks using a viral RNA/DNA extraction kit (Qiagen, Mississauga, Ontario, Canada).

PCR and cloning of Bacteria, Archaea, and Eukarya

PCR amplification of cyanobacterial, bacterial, and archaeal 16S rRNA genes and eukaryotic 18S rRNA genes was performed using group-specific primers (Table I). PCR products were purified with a kit (Qiagen, Mississauga, Ontario, Canada) and cloned using a Strataclone cloning kit (Stratagene, Cedar Creek, Texas, USA) or TOPO TA cloning kit (Invitrogen, Carlsbad, California, USA) following the manufacturer's instructions. Correct size amplicons were then selected and sequenced as in Galand, Lovejoy, and Vincent (2006) for Archaea, Galand *et al*. (2009c) for Bacteria, and Potvin and Lovejoy (2009) for eukaryotes at the Centre de recherche du Centre hospitalier de l'Université Laval with an ABI 3730xl system (Applied Biosystems, Foster City, California, USA). All sequences were verified and edited using Chromas software (Technelysium Pty Ltd, Brisbane, Australia) and compared with sequences in GenBank by BLASTn (Altschul *et al*., 1990). Chimera detection for bacterial sequences was done using the Chimera Detection program from the Ribosomal Database Project II. Eukaryote sequences were also submitted to the KeyDNATools website (http://www.pc-informatique.fr/php-fusion/news.php) to analyze sequence affinities and possible chimeras, which were then investigated by blasting separate sections of the suspect sequences. Operational taxonomic units (OTUs) were defined as > 97% similar for Archaea and Bacteria and > 99% similar for eukaryotes using the program Dotur (http://www.bio.umass.edu/micro/schloss/software/dotur.html). All sequences were archived in GenBank, under accession numbers HQ230047 to HQ230050 and HQ230052 to HQ230102.

PCR and DGGE of viruses

PCR amplification was performed using primers MZIA1bis and MZIA6 (Filée *et al.,* 2005), and amplification products were analyzed by agarose gel electrophoresis. The gels were stained with SYBR Gold and visualized under conditions of UV illumination. A second-round PCR amplification using primers with a GC clamp was conducted on eluted DNA from each lane of agarose containing amplified DNA. Second-round amplification products were confirmed by agarose gel electrophoresis, and amplicons were then subjected to denaturing gradient gel electrophore-

TABLE I. Primers used to target and sequence the main phylogenetic groups of microbiota, and the total number of clones sequenced (includes RFLP repeats) with the correct size insert for each depth sample.

Target group	Forward primer	Reverse primer	Sequencing primer	Reference	Clones	
					5 m depth	30 m depth
Bacteria	8F	1492R	T7p	Galand *et al.*, 2008	14	58
Cyanobacteria	27F1	809R	T7p	Jungblut *et al.*, 2005	0	0
Archaea	A109F	A934R	A109F	Grosskopf, Janssen & Liesack, 1998	0	96
Eukaryotes	NSF 4/18	NLR 204/21	528F	Elwood, Olsen & Sogin, 1985; Hendriks *et al.*, 1989; Van der Auwera, Chapelle & Dewachter, 1994	177	0

sis (DGGE). The DGGE gel (6 to 7% polyacrylamide) was run for 20 h at 120 V and 60 °C, stained with 0.1x SYBR green solution (Invitrogen) for 3 h, and visualized and photographed with an Alpha Imager 3400 system (Alpha Innotech, CA, USA).

MICROSCOPY ENUMERATIONS

Protist samples were immediately preserved with a mix of buffered paraformaldehyde (0.1% final concentration) and glutaraldehyde (1% final concentration) in duplicate 50-mL polypropylene centrifuge tubes and stored at 4 °C in the dark until analysis. Protists were counted and identified using a combined system of fluorescence, Nomarski interference, and Utermöhl sedimentation (FNU) (Lovejoy *et al*., 1993). Sixteen millilitres of samples were sedimented in Utermöhl chambers over 24 h, and DAPI was then gently added (0.1 $\mu g \cdot mL^{-1}$) and left for at least 1 h prior to examination with a Zeiss Axiovert 100 inverted epifluorescence microscope. Cells > 2 μm in diameter were enumerated at 400 and 1000× magnification. Cells were identified to genus wherever possible, and their size was recorded. Cells were classified as heterotrophs when no chloroplast was observed. The microscopy detection limit was 2.67×10^3 cells$\cdot L^{-1}$. A minimum of 400 cells and 15 fields were counted wherever possible. The zooplankton samples were preserved with formaldehyde (final concentration 10%), and all individuals were then counted and identified to species level using a binocular microscope.

ENUMERATION OF BACTERIA AND VIRUSES

Flow cytometry (FC) was used to determine heterotrophic prokaryote abundance (this method does not distinguish between Bacteria and Archaea) and viral abundance, the latter determined separately. Duplicate subsamples (1.8 mL) were fixed with 0.5% glutaraldehyde (EM-grade), frozen in liquid nitrogen and stored at -80 °C. The samples were stained with SYBR Green I (Invitrogen) and then enumerated by FC (FACSCalibur, Becton-Dickinson) as previously described (Marie *et al*., 1999; Brussaard, 2004; Payet & Suttle, 2008).

Results

WATER COLUMN PROPERTIES

Milne Fiord had a highly stratified water column at both times of sampling (Figure 5). Salinity and temperature profiles from 2009 were very similar to those taken in 2007, although there were some subtle differences. The thickness of the epishelf layer was slightly less in 2009 than in 2007, with the position of the halocline at 14.3 m depth

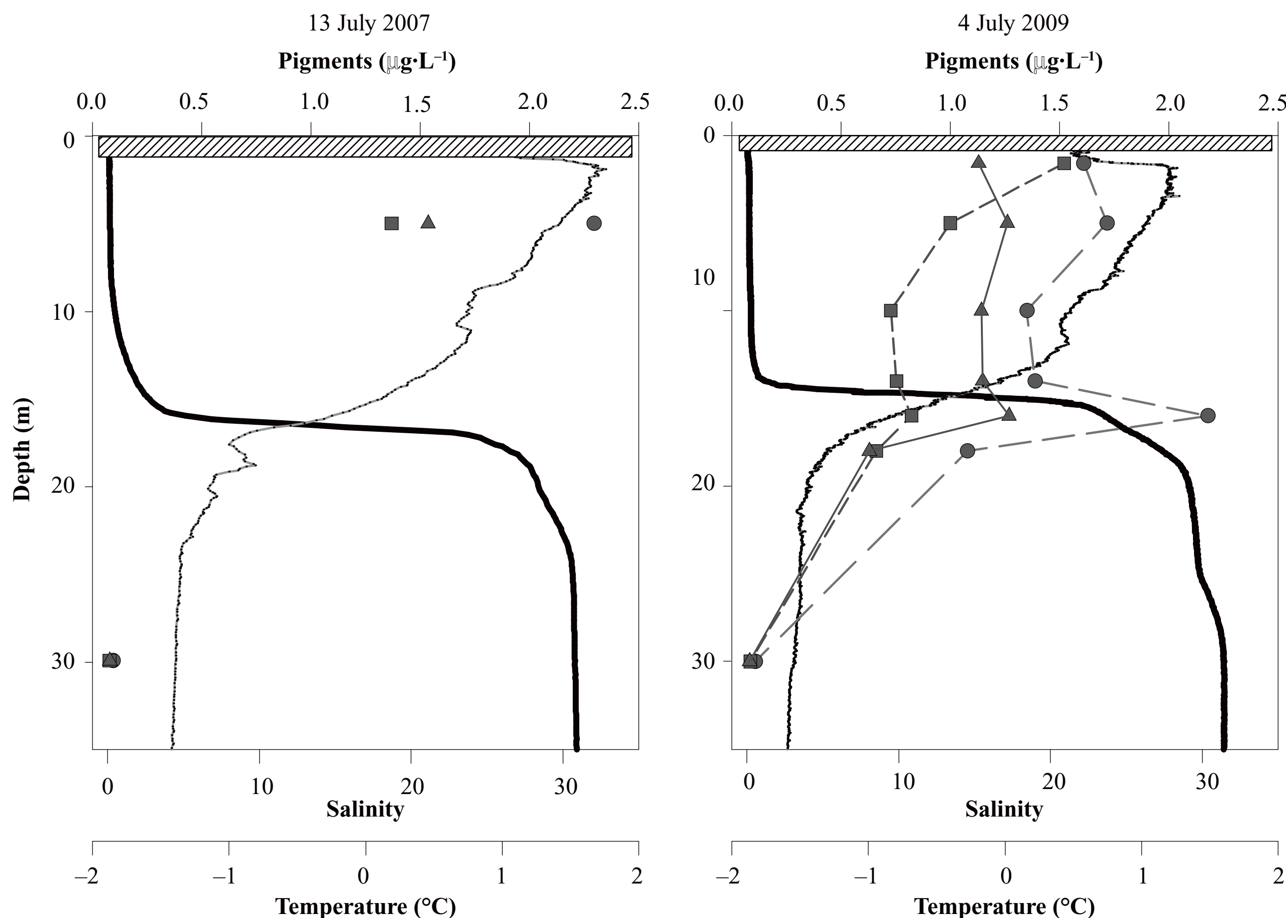

FIGURE 5. Salinity (dark line), temperature (grey line), and pigment profiles from Milne Fiord water column taken 13 July 2007 and 4 July 2009. The hatched box indicates the 1.60 m of ice cover on 13 July 2007 and 1.13 m on 4 July 2009. Circles = total chlorophylls. Squares = total carotenoids. Triangles = chlorophyll *a*.

(2009) *versus* 16.6 m depth (2007). Moreover, the transition to marine water was sharper in 2009. Profiles from 2007 indicated that salinity increased from < 1 to 3 practical salinity units over a 3-m interval (*i.e*., 12.2–15.2 m) before a sharp increase to full seawater values; in 2009 this increase occurred over only 40 cm (*i.e*., 13.9–14.3 m). High concentrations of mineral particles were observed by microscopy in the 5 m depth sample from 2007, indicating the input of glacial meltwater.

Zooplankton communities

The net sampling of the water column of Milne Fiord revealed the presence of marine, freshwater, and brackish zooplankton taxa, but there was no clear difference between the shallow (14–0 m) and deep (50–0 m) tows (Table II). A total of 67 individuals were collected, with the majority (40) from the shallow tow. Eight copepod species were identified, and both tows were numerically dominated by the marine species *Pseudocalanus minutus*, including stages C III-IV-V and adults.

Phytoplankton communities

Photosynthetic pigment analyses revealed large differences in phytoplankton concentration and composition down the water column of Milne Fiord (Figure 5; Table III). Samples from the same depths in 2007 and 2009 (5 and 30 m) were similar, with slightly higher pigment concentrations at 5 m depth in 2007 compared to 2009. The detailed chlorophyll *a* profile from 2009 (Figure 5) showed similar concentrations in the 5 upper depths from the surface to the bottom of the halocline (1.10–1.26 $\mu g \cdot L^{-1}$). The chlorophyll *a* concentrations were lower in the sea water (0.63 $\mu g \cdot L^{-1}$ at 18 m depth and 0.08 $\mu g \cdot L^{-1}$ at 30 m depth) compared to the epishelf lake. Total chlorophyll concentrations increased in the deep chlorophyll maximum at 16 m depth due to high concentration of chlorophyll *b*, consistent with the presence of prasinophytes at this depth (see below). Chlorophyll derivatives (allomers, epimers, chlorophyllide *a*, and pheophytin *a*) were detected in Milne Fiord but represented only 4–6% of total chlorophylls in each sample,

TABLE II. Zooplankton in Milne Fiord, sampled 4 July 2009. The 2 final columns give the abundance integrated over the water column (individuals$\cdot m^{-2}$) of each species and life-cycle stage of zooplankton found in the net tow from 14 or 50 m depth to the surface (2009). C: copepodite. Habitat type refers to the environments in which these taxa are typically observed; FW: freshwater, B: brackish, M: marine, —: not present.

Species (Habitat type)	Stage	Abundance	
		14 m tow	50 m tow
Eurytemora herdmani (FW,B)	C III	79	—
	Nauplius	476	—
Limnocalanus macrurus (FW,B)	C V	—	79
	Female adult	159	159
Metridia longa (M)	C I	79	79
	Male adult	79	79
Microcalanus pusillus (M)	C IV	—	79
	C V	159	79
Oithona similis (M)	C V	—	79
	Female adult	—	79
Oncaea borealis (M)	C V	79	—
	Female adult	79	—
Pseudocalanus minutus (M)	C III	159	317
	C IV	1032	635
	C V	476	317
	Male adult	79	—
	Female adult	159	79
Scolecithricella minor (M)	C IV	79	79

TABLE III. Photosynthetic pigment concentrations (in bold) and mass ratios relative to Chl *a* in phytoplankton samples from different depths in Milne Fiord, determined by HPLC. ND: Not detected. Values in parentheses indicate uncertain pigment identity.

Pigments	2007		2009						
	5 m	30 m	1.6 m	5 m	10 m	14 m	16 m	18 m	30 m
Chl *a* ($\mu g \cdot L^{-1}$)	**1.535**	**0.079**	**1.099**	**1.248**	**1.131**	**1.137**	**1.260**	**0.628**	**0.081**
CHLOROPHYLL RATIOS									
Chl *b*	0.462	0.114	0.326	0.306	0.112	0.160	0.596	0.533	0.236
Chl c_2	0.014	0.060	0.061	0.022	0.029	0.023	0.043	0.093	0.061
MgDVP	ND	ND	0.009	0.008	0.008	0.004	0.050	0.054	0.011
Total chlorophylls ($\mu g \cdot L^{-1}$)	**2.296**	**0.094**	**1.610**	**1.718**	**1.350**	**1.388**	**2.179**	**1.080**	**0.108**
CAROTENOID RATIOS									
Alloxanthin	0.077	ND	ND	0.105	0.065	0.057	0.083	0.033	ND
Antheraxanthin-like	0.019	ND	0.009	ND	(0.006)	ND	ND	ND	ND
ß,ß-Carotene	0.018	ND	0.119	0.080	0.063	0.043	0.052	0.018	ND
Diadinoxanthin	0.038	ND	0.188	ND	(0.034)	ND	ND	ND	ND
Fucoxanthin	0.024	0.928	(0.054)	ND	(0.053)	(0.104)	ND	0.719	0.897
Lutein	0.385	0.048	0.394	0.285	0.091	0.150	0.141	0.032	0.149
Micromonal-like	ND	ND	ND	ND	ND	ND	0.022	(0.026)	ND
Micromonol-like	ND	ND	ND	ND	ND	ND	0.015	ND	ND
Neoxanthin	0.012	ND	(0.025)	(0.015)	(0.006)	(0.010)	0.041	0.028	ND
Peridinin	ND	ND	0.068	ND	ND	ND	ND	ND	ND
Prasinoxanthin	ND	ND	ND	ND	ND	ND	0.078	0.071	ND
Uriolide-like	ND	ND	ND	ND	ND	ND	0.022	0.046	ND
Violaxanthin	0.245	ND	0.212	0.271	0.280	0.245	0.133	0.029	ND
Zeaxanthin	0.056	ND	0.040	0.025	0.012	0.024	0.030	ND	ND
Total carotenoids ($\mu g \cdot L^{-1}$)	**1.369**	**0.077**	**1.521**	**1.000**	**0.727**	**0.754**	**0.822**	**0.661**	**0.085**
Total pigments ($\mu g \cdot L^{-1}$)	**3.665**	**0.171**	**3.131**	**2.717**	**2.077**	**2.142**	**3.001**	**1.742**	**0.193**

and their contribution did not increase with depth. Total carotenoid concentrations decreased down the water column from 1.52 μg·L^{-1} underneath the ice cover to 0.09 μg·L^{-1} at 30 m depth but were at similar values in the depth range 10 m to 18 m.

In the epishelf lake and halocline, the relative contribution of chlorophyte-related pigments (chl *b*, lutein, neoxanthin, violaxanthin) was much greater than in the sea water layer. There were only low concentrations of zeaxanthin (found in cyanobacteria and green algae) in the epishelf lake and halocline, while pigments indicative of dinoflagellates (chl c_2, peridinin, diadinoxanthin) were only present in the sample from immediately beneath the ice cover. Cryptophytes (chl c_2, alloxanthin) and eustigmatophytes (violaxanthin) may also have contributed to the epishelf lake and halocline algal communities. Prasinophyte pigments (chl *b*, MgDVP, uriolide, neoxanthin, micromonol, micromonal, prasinoxanthin, violaxanthin, lutein) were a conspicuous feature of the deep chlorophyll maximum within the halocline and were not detected in the epishelf lake nor in the sea water below. Fucoxanthin groups (diatoms, chrysophytes, and potentially prymnesiophytes that contain fucoxanthin and chl c_2) were better represented in the marine layer compared to the epishelf lake and halocline.

Eukaryotic communities

Microscopic observations revealed a diverse and abundant (10^6 cells·L^{-1}) eukaryotic community (> 2 μm) at 5 m depth, but no cells were detected in the 30 m depth sample (Table IV). Cryptophytes, chlorophytes, and diatoms could be distinguished, but the vast majority of the cells (89%) in the 5 m depth sample were unidentified autotrophs (pigment-containing cells) between 5 to 10 μm diameter. The heterotrophic community included dinoflagellates and choanoflagellates. Additionally, 15- to 20-μm-long ciliates (Euplotida, *Balanion*-like) were observed outside counted fields. All taxa identified by microscopy were in the nanoplankton size class, except for *Gymnodinium* sp. (20–50 μm).

We recovered 177 clones from the 5 m depth 18S rRNA gene clone library for eukaryotes and the sequences grouped into 11 operational taxonomic units (OTUs; Table V). Ciliates represented over 90% of the clones, and they mainly belonged to taxa within the Euplotida (Oligotrichea). Other ciliate taxa included Choreotrichia (Oligotrichea), Stichotrichia (Oligotrichea), Scuticociliata (Oligohymenophorea), and Protosmatea. Stramenopiles composed 7% of the sequences, with the classes Chrysophyceae, Eustigmatophyceae, and Dictyochophyceae represented. We also found 1 sequence each of Euglenozoa and Fungi.

Bacterial communities

Bacterial abundance in the epishelf lake (at 5 m depth) was 6.4 × 10^8·L^{-1}, while that in the sea water layer was 4.1 × 10^8·L^{-1} (at 30 m depth). The taxonomic composition of the 2 communities from the dark-incubated enrichment cultures differed completely (Table VI), and no sequences were found at both depths. Clones from 5 m depth belonged to phylogenetic clusters within the Betaproteobacteria, Actinobacteria, and Bacteroidetes, while clones from 30 m depth belonged to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Planctomycetes. The majority of clones at 5 m depth had the highest BLASTn match to *Polaromonas* sp. (Betaproteobacteria). The other clones retrieved from 5 m depth had closest BLASTn matches to sequences previously reported from a variety of freshwater environments from polar, temperate, and alpine lakes, as well as from glaciers and polar lake ice (Table VI). All of the clones from 30 m depth had highest similarity to sequences identified from marine environments such as Pacific and Arctic ocean waters (Table VI) and were related to cultured isolates that require sea salts to grow or are halotolerant. Gammaproteobacteria were the most common group at 30 m depth, with the majority of clones matching to *Colwellia* sp., a marine psychrophilic genus (Methé *et al.*, 2005). No cyanobacteria were amplified from either depth with our cyanobacteria-specific probes (Table I).

Archaeal communities

Archaea sequences were retrieved in clone libraries only at 30 m depth. Almost all of the clones (94 out of 96) were marine Crenarchaeota (MGI.1a), with the remaining 2 clones classified as Euryarchaeota MG II.a.

TABLE IV. Abundance of protists (10^3 cells·L^{-1}) and percentage of each group observed in a phytoplankton sample from 5 m depth in Milne Fiord (2007).

Group	Details	Abundance	%
Bacillariophyceae	Centric (2–5 μm)	9	0.88
Chlorophyceae	*Chlamydomonas* sp. (5–10 μm)	9	0.88
Cryptophyceae	*cf. Rhodomonas* sp. (5–10 μm)	27	2.64
	Cryptophyceae (5–10 μm)	18	1.76
Dinophyceae	*Gymnodinium* sp. (10–20 μm)	9	0.88
	Gymnodinium sp. (20–50 μm)	3	0.29
	Peridinium sp. (10–20 μm)	3	0.29
Choanoflagellates	*cf. Monosiga* sp. (5–10 μm)	3	0.30
Unidentified autotrophs	Non-flagellate (2–5 μm)	24	2.35
	Non-flagellate (5–10 μm)	895	88.56
	Flagellate (2–5 μm)	9	0.88
Unidentified heterotrophs	Flagellate (5–10 μm)	3	0.29
Total		1012	

TABLE V. Eukaryote sequences retrieved in the 5 m depth clone library (2007). RFLP repeats represent the number of clones that were related to each taxon.

Major ranks	Subranks	Details	RFLP repeats
Ciliate	Oligotrichea	Choreotrichia	14
		Euplotida	144
		Stichotrichia	2
	Oligohymenophorea	Scuticociliata	1
	Protosmatea		1
Stramenopile	Chrysophyceae	*Paraphysomonas*	4
		Ochromonadales	3
	Eustigmatophyceae	*Nannochloropsis*	4
	Dictyochophyceae	Pedinellales	2
Euglenozoa	Kinetoplastida	Bodonidae	1
Fungi	Basidiomycota	Pucciniomycotina	1
Total			177

TABLE VI. Identity of sequenced bacterial clones from Milne Fiord. MF5 and MF30 indicate clones from 5 m and 30 m depth, respectively (2007). Highest cultured match was also included when the highest BLASTn match was an uncultured clone from GenBank with > 97% similarity.

Representative clone	Nearest neighbours (source); GenBank accession number	% identity
ALPHAPROTEOBACTERIA		
MF30b8	Clone s99 (surface water, Northern Bering Sea); GQ452879	99.5
	Roseobacter sp. ANT909 (Antarctic pack ice, Weddell Sea, Southern Ocean); AY167254	98.7
BETAPROTEOBACTERIA		
MF5b3	Clone 3C002860 (Chesapeake Bay, USA); EU801543	99.8
	Polaromonas sp. GM1; EU106605	99.1
MF5b4	Clone PIB-27 (freshwater lake, Austria); AM849438	98.3
	Polynucleobacter necessarius subsp. *asymbioticus* QLW-P1DMWA-1; CP000655	98.3
MF5b5	Clone DP10.5.23 (lake water, Dongping Lake, China); FJ612443	99.9
	Variovorax sp. LaGso27k (soil, Roopkund Glacier, India); EU934231	98.6
MF30b1	Clone Nit2A0620_18 (water at 20 m, salt-stratified fiord-lake Nitinat, Canada); EU265945	98.1
	Methylotenera mobilis JLW8; CP001672	95.3
MF30b18	Clone YU201C05 (freshwater sample, Yukon River, Alaska, USA); FJ694630	99.3
	Pigmentiphaga kullae strain K24 (soil); AF282916	95.3
GAMMAPROTEOBACTERIA		
MF30b9	Clone ARKICE-28 (Arctic sea ice, northern Fram Strait); AF468310	99.5
	Colwellia sp. ANT8258 (Antarctic pack ice, Weddell Sea, Southern Ocean); AY167266	99.3
MF30b11	*Moritella* sp. JAM-GA22 (Mariana Trench, Pacific Ocean); AB526345	99.5
MF30b14	*Oleispira antarctica* strain RB-9 (Rod Bay, Ross Sea, Antarctica); AJ426421	98.4
ACTINOBACTERIA		
MF5b6	Clone ST11-18 (Lake Stechlin, Germany); DQ316362	99.6
BACTEROIDETES		
MF30b2	Bacterium ARCTIC40_B_04 (Arctic); EU795091	99.2
	Polaribacter irgensii (Antarctic marine waters); M61002	98.3
MF30b19	Bacterium ARK10272 (Arctic sea water or sea ice, northern Fram Strait); AF468432	99.7
	Flavobacterium sp. isolate V1-3 (sediment, Konsfjord region, Svalbard); FN377738	99.3
MF30b21	Clone 130H27 (sediment, northern Bering Sea); EU925904	96.5
MF5b1	Clone ZWB1-13 (Zoige alpine wetland, China); FJ801173	98.4
	Flavobacterium sp. strain WB 4.4-14 (hard water creek, Westharz Mountains, Germany); AM177634	98.4
PLANCTOMYCETES		
MF30b20	Clone J8P41000_2E05 (water at 1000 m, Eastern North Pacific Ocean); GQ351240	98.0

VIRAL ASSEMBLAGES

Viral abundance in the 2007 samples was slighly higher at 5 m depth, with 2.2×10^9 virus-like particles (VLP)·L^{-1}, compared to 1.4×10^9 VLP·L^{-1} at 30 m depth. The DGGE community profile for T4-like bacteriophages showed different patterns between the 2 depths. The 5 m depth sample yielded 8 visible bands, while 12 visible bands were obtained for the sample from 30 m depth, and no band was shared between these 2 depths (Figure 6).

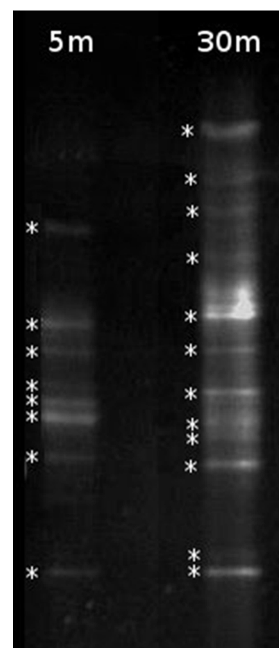

FIGURE 6. Image of denaturing gradient gel of PCR-amplified *g23* gene fragments of T4-like bacteriophages at 5 and 30 m depth in Milne Fiord (2007). Bands are marked with asterisks.

Discussion

Milne Fiord showed the typical structure of an epishelf lake, with a sharp transition at the halocline from the surface freshwater layer to the underlying seawater (Figures 1 and 5). This salinity-driven stratification appears to have been relatively stable since 1983, with reported epishelf lake thicknesses ranging from 17.5 m to 14.3 m (Veillette *et al*., 2008; this study). This *ca* 3-m variation in thickness may be the result of tidal cycles or tidally driven internal waves (Veillette *et al*., 2008), although it might also be due to a thinning of the ice dam, as previously observed at Disraeli Fiord before the Ward Hunt Ice Shelf broke up, causing complete loss of its epishelf lake (Mueller, Vincent & Jeffries, 2003). The Milne Fiord halocline depth in 2009

(14.3 m) was the shallowest on record, and may indicate the thinning of the Milne Ice Shelf during the extremely warm summer of 2008 (Vincent *et al*., 2009). There were also several shoulders in the salinity profiles of 2007 and 2009 that were accompanied by slight temperature increases. These could be related to warmer freshwater inflows laden with glacial sediment that may sink into the marine zone due to greater density (Retelle & Child, 1996; Gilbert, 2000).

Previous studies in epishelf lakes have shown that their animal communities are vertically separated as a consequence of the sharp salinity gradient; *e.g*., different species of zooplankton in Disraeli Fiord (Van Hove *et al*., 2001) and marine fish species at depth in analogous Antarctic systems (Heywood & Light, 1975; Cromer, Williams & Gibson, 2005) at different depths. Our net sampling of the water column of Milne Fiord revealed that there were marine, freshwater, and brackish zooplankton taxa in the fiord, but there was no clear trend between the shallow (14–0 m) and deep (50–0 m) tows (Table II). The choice of the 14 m depth limit for the first vertical tow was based on the 2007 data, when the halocline was at 16 m depth. However, given the shallower freshwater layer in 2009, the tow would have collected any marine species congregating at the bottom of the deep chlorophyll maximum on the sharp halocline, and more detailed sampling will be required to assess the vertical community structure of the Milne Fiord zooplankton. The dominant copepod in our samples, *Pseudocalanus minutus,* has broad thermal and salinity tolerances, is mainly herbivorous, and is widespread in coastal waters of the Arctic Ocean (Corkett & McLaren, 1978; Darnis, Barber & Fortier, 2008). Other marine species that were collected included *Oncaea borealis*, which has been previously reported from Disraeli Fiord and from Tuborg Lake (Greely Fiord system, ~250 km south of Milne Fiord on Ellesmere Island) (Bowman & Long, 1968; Van Hove *et al*., 2001), and *Oithona similis*, which was also found in Disraeli Fiord at the time it contained an epishelf lake (Van Hove *et al*., 2001). A few specimens of *Limnocalanus macrurus* and *Eurytemora herdmani* were recovered, and these have both been reported previously in freshwater or brackish environments elsewhere in the Arctic (Bowman & Long, 1968; Van Hove *et al*., 2001; Darnis, Barber & Fortier, 2008).

Phytoplankton biomass concentrations (up to 1.5 μg Chl*a*$\cdot$L^{-1}) in the Milne Fiord freshwater layer were in the oligotrophic range for lakes (Wetzel, 2001). However, the values were much higher than that previously reported from the Disraeli Fiord epishelf lake (0.27 μg$\cdot$L^{-1}; Van Hove *et al*., 2006) and Antarctic epishelf lakes such as Beaver Lake (< 1 μg$\cdot$L^{-1}; Laybourn-Parry *et al*., 2006), Moutonnée, and Ablation lakes (0.65 and 0.50 μg$\cdot$L^{-1}, respectively; Heywood, 1977). Concentrations of chlorophyll *a* in Milne Fiord were also high compared to other nearby Arctic lakes; for example, in meromictic Lake A at 5 m depth, values were 0.23 μg$\cdot$L^{-1} in late May (Antoniades *et al*., 2009) and 0.34 μg$\cdot$L^{-1} in July (J. Veillette, unpubl. data).

The HPLC pigment results from the epishelf lake, the halocline, and the marine layer provided compelling evidence of a vertically stratified phytoplankton community in Milne Fiord (Table III), as observed in meromictic polar lakes (McKnight *et al*., 2000; Antoniades *et al*., 2009). The pigment profiles showed that picoprasinophytes were a major constituent of the deep chlorophyll maximum at the halocline. This group is ubiquitous throughout Arctic seas, and is the major constituent of deep chlorophyll maxima throughout the North West Passage and Beaufort Sea (Lovejoy *et al*., 2007; Tremblay *et al*., 2008). Their higher abundance in this deep stratum is likely the result of increased nutrient availability at depth, combined with sufficient irradiance for growth, the factors thought to favour deep chlorophyll maxima in general in Arctic seas (Carmack, Macdonald & Jasper, 2004; Tremblay *et al*., 2008) and polar lakes (Lizotte & Priscu, 1992). Fucoxanthin groups (diatoms, chrysophytes, and prymnesiophytes) were better represented in the marine layer of Milne Fiord compared to the epishelf lake and halocline. The permanent ice-cover of the fiord and lack of turbulent mixing would not favour diatoms with heavy siliceous frustules.

The only possible cyanobacterial pigment in Milne Fiord was zeaxanthin, which was in low concentrations and could also be derived from chlorophytes. This accords with our molecular results, which failed to detect any cyanobacterial taxa, even using cyanobacteria-specific primers. This is in contrast to the abundant *Synechococcus* populations in many Arctic lakes and rivers (Vincent, 2000; Waleron *et al*., 2007), including the epishelf lake that existed prior to 2001 in Disraeli Fiord behind the Ward Hunt Ice Shelf (Van Hove *et al*., 2008). However, it is consistent with the scarcity of picocyanobacteria in Arctic seas (Waleron *et al*., 2007).

Most of the eukaryotic taxa observed in Milne Fiord epishelf lake have been reported in polar freshwater lakes elsewhere (Laybourn-Parry *et al*., 2006; Galand *et al*., 2008; Bonilla, Rautio & Vincent, 2009). The different analytical techniques produced complementary information, although no groups were identified using all 3 methods. For example, chrysophytes were detected by the clone library and the pigment analyses, while chlorophytes and cryptophytes were detected by both pigment analyses and microscopy. The inability to detect all groups using all techniques, and the lack of detection of any protists by the clone library and microscopy in the marine layer, may be explained by the different limits of detection and scope of each method. We were limited to a small volume after partitioning water samples between all the analyses, and the oligotrophic nature of the fiord meant that cell concentrations were very low. The large proportion of unidentified autotrophs (Table IV) may include chlorophytes, chrysophytes, cryptophytes, dictyochophytes, and eustigmatophytes. Vertical differences in the distribution of microbial eukaryotes across salinity gradients are well known from other polar aquatic ecosystems, for example Antarctic meromictic lakes (Vincent, 1981; Roberts *et al*., 2000).

The main protist in the clone library analysis was a ciliate within the Euplotida (Oligotrichea), and this was likely the result of the dark pre-incubation. Most of the bacterial clones were similar to previously isolated bacteria, also likely reflecting the pre-incubation, enrichment conditions that selected for taxa that are amenable to culture, including the dominant taxon *Polaromonas* sp. This was first described from Antarctic waters in association with sea ice, with the species *P. vacuolata* (Irgens,

Gosink & Staley, 1996). However, other species of the genus *Polaromonas* were subsequently described from freshwater environments: *P. naphthalenivorans* from coal-tar-contaminated freshwater sediment (Jeon *et al*., 2004) and *P. aquatica* isolated from tap water (Kämpfer, Busse & Falsen, 2006). Moreover, other studies of isolates from cold freshwater environments such as unfrozen subglacial environments (Foght *et al*., 2004) and moraine lakes and glacial meltwaters (Liu *et al*., 2006) have reported sequences that cluster with *P. vacuolata*. This bacterial genus has also been detected in snow along the northern Ellesmere Island coastline (Harding *et al*., 2011). The dominant group of proteobacteria in each sample corresponded to its typical habitat salinity range, as Betaproteobacteria frequently predominate in freshwater environments, whereas Alphaproteobacteria and Gammaproteobacteria prevail in marine environments (Methé, Hiorns & Zehr, 1998; Garneau *et al*., 2006; Galand *et al*., 2008). This clear dichotomy between bacteria colonizing freshwater and marine habitats has been reported in other studies (Zwart *et al*., 2002; Hahn, 2006). Some clones from Milne Fiord, such as those matching to *P. vacuolata* and *Polaribacter irgensii*, produce gas vesicles that may be important in establishing their vertical position in the stratified water column (Irgens, Gosink & Staley, 1996; Gosink, Woese & Staley, 1998).

The bacterial abundance (including Archaea) we observed in the Milne Fiord epishelf lake ($6.4 \times 10^8 \cdot L^{-1}$) was close to those reported for a diverse set of Arctic and Antarctic inland waters ($0.8 \times 10^9 \cdot L^{-1}$ and $1.5 \times 10^9 \cdot L^{-1}$, respectively; Säwström *et al*., 2008) but greater than that recorded in epishelf Beaver Lake, Antarctica. Bacterial concentrations in the latter ranged from 9 to 14×10^7 cells$\cdot L^{-1}$, and heterotrophic nanoflagellates imposed a significant grazing pressure on these populations (Laybourn-Parry *et al*., 2006). The bacterial concentration of the 30 m depth seawater sample from Milne Fiord ($4.1 \times 10^8 \cdot L^{-1}$) was similar to that reported for the coastal Beaufort Sea ($2 \times 10^8 \cdot L^{-1}$; Garneau *et al*., 2006).

Almost all of the Archaea clones in the 30 m depth sample were Crenarchaeota, and this group is well known from thoughout the Arctic Ocean (Bano *et al*., 2004; Galand *et al*., 2008; 2009b). In the Southern Ocean, Crenarchaeota have also been found to dominate surface archaeal assemblages (Massana, DeLong & Pedrós-Alió, 2000). Recent studies have pointed to the key role of ammonia-oxidizing group I Crenarchaeaota in nitrogen cycling in oceanic and lacustrine Arctic environments (Galand *et al*., 2009a; Pouliot *et al*., 2009), and related sequences were detected in Milne Fiord. Our inability to detect archaeal sequences at 5 m depth in Milne Fiord might be explained by the low volume of water analyzed rather than a complete absence, since Archaea are widespread in freshwater environments (Auguet & Casamayor, 2008; Pouliot *et al*., 2009). Strong vertical differences in archael community diversity and composition in a High Arctic meromictic lake were related to the sharp salinity gradient (Pouliot *et al*., 2009).

Although no gene is universally conserved in viruses, there are a number of genes that are found within specific groups; hence, the approach has been to develop PCR primers that target specific subsets of the viral assemblages (Fuller *et al*., 1998; Filée *et al*., 2005; Short & Suttle, 2005). Primer sets that amplify the major capsid protein genes (*g23*) in T4-like bacteriophages (Myoviridea) have been developed to target tailed phages, which represent important members of aquatic viral assemblages (Filée *et al*., 2005), and our results from Milne Fiord extend the range of ecosystem types that these viruses have now been reported from. The pronounced dissimilarity in the DGGE community pattern suggested that there were major differences in viral assemblages between the freshwater and marine waters in Milne Fiord, as found elsewhere (Short & Suttle, 2005; Chénard & Suttle, 2008), and that T4-like phage richness was greater in the marine stratum. The abundance of viruses in the epishelf lake was at the lower end of the range reported for Arctic and Antarctic lakes ($0.53–94 \times 10^9 \cdot L^{-1}$; Säwström *et al*., 2008). Similarly, viral abundance in the marine layer was low compared with abundances from the southern Beaufort Sea Shelf ($0.13–23 \times 10^9 \cdot L^{-1}$; Payet & Suttle, 2008). Viruses may play a significant role in the fiord microbial food webs, as viral lysis of bacterioplankton (including Archaea), phytoplankton, or other protists releases organic matter back into the dissolved organic matter pool and shunts the flow to heterotrophic nanoflagellates and zooplankton (Suttle, 2007).

Conclusion

Our results show that Milne Fiord is a highly stratified microbial ecosystem and that despite its extreme north polar location, it provides habitats for diverse microbial communities of Eukarya, Bacteria, Archaea, and viruses. Similarly, the zooplankton community down the water column contains freshwater, brackish, and marine taxa. Milne Fiord provides habitats for an unusual co-existence of freshwater and marine microbiota that are vertically segregated within the same water column. The microbial assemblages in the epishelf lake are distinct from the sea water below, likely associated with the combination of differences in salinity, nutrients, and irradiance, with low temperature and the provenance of water masses also playing a role. Epishelf lakes are sensitive indicators of climate change due to their dependence on ice-dam integrity, and the physical and chemical factors that contribute to biological stratification in Milne Fiord are all linked to the High Arctic climate regime. These observations underscore the rich microbial biodiversity of extreme polar ecosystems and the vulnerability of Arctic ecological resources to ongoing climate change.

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