Limnol. Oceanogr., 53(2), 2008, 813–823

Microbial community diversity and heterotrophic production in a coastal Arctic ecosystem: A stamukhi lake and its source waters

Pierre E. Galand,[1,2] *Connie Lovejoy, and Jérémie Pouliot*
Département de Biologie et Québec-Océan, Université Laval, Québec, QC G1V 0A6, Canada

Marie-Ève Garneau and Warwick F. Vincent
Département de Biologie et Centre d'études nordiques, Université Laval, Québec, QC G1V 0A6, Canada

Abstract

Stamukhi lakes are vast but little-explored Arctic ecosystems. They occur throughout winter, spring, and early summer near large river inflows along the Arctic coastline, and are the result of freshwater retention behind the thick barrier of rubble ice (stamukhi) that forms at the outer limit of land-fast sea ice. We examined the molecular biodiversity within all three microbial domains (Bacteria, Archaea, and Eukaryota) and the heterotrophic productivity in Lake Mackenzie, a stamukhi lake in the western Canadian Arctic, and made comparative measurements in the freshwater (Mackenzie River) and marine (Beaufort Sea) source waters. Bacterial and eukaryotic communities in the stamukhi lake differed in composition and diversity from both marine and riverine environments, whereas the archaeal communities were similar in the lake and river. Bacteria 16S ribosomal RNA sequences from the lake were mostly within freshwater clusters of *Betaproteobacteria* and *Bacteroidetes* and the Archaea were within the Lake Dagow sediment and Rice cluster-V clusters of *Euryarchaeota*. The eukaryotes were mainly ciliates from the subclass *Choreotrichia*, and there was a notable lack of flagellates. Heterotrophic production rates in the lake were lower than in the river and more similar to those in the sea, despite much higher bacterial concentrations than in either. The lake samples had markedly higher ratios of ^{3}H leucine to ^{3}H thymidine incorporation than in the river and sea, implying some physiological stress. Lake Mackenzie is an active microbial ecosystem with distinct physical and microbiological properties. This circumpolar ecosystem type, vulnerable to the ongoing effects of climate change, likely plays a key functional role in processing riverine inputs to the Arctic Ocean.

The application of molecular microbiological techniques to temperate estuaries has uncovered diverse, active, and spatially variable microbial communities (Crump et al. 1999; Kirchman et al. 2005), reflecting the complex hydrography of such environments. Arctic estuaries are also hydrodynamically complex, but they additionally experience extreme seasonality in their physical structure caused by ice formation and melting, which in turn creates diverse conditions for biological processes (Carmack and Macdonald 2002). Arctic estuarine systems include lagoons that form along the Arctic coastline each year behind the barrier of rubble ice (stamukhi) that is generated by ice convergence at the outer edge of the land-fast sea ice. These lagoons, termed "stamukhi lakes," are widely distributed and are formed especially in the vicinity of large Arctic rivers that push the ice offshore and fill the inshore zone with freshwater (Reimnitz et al. 1978). The ice dams have drafts from 5 to 20 m and are partially grounded, semiporous barriers to water flow. Stamukhi lake ecosystems may persist for over 6 months each year and are common along the coastline of Siberia (Ogorodov et al. 2005) and the Canadian Arctic (Carmack and Macdonald 2002) with their large river inflows. These rivers are major sources of organic carbon entering the Arctic Ocean. Understanding the basic properties of such ecosystems, including their biodiversity and metabolic activity, will be crucial for predicting the consequences of climate change and shifts in the Arctic ice regime. Yet the microbial diversity and productivity of Arctic estuarine systems has been little studied to date, and there are no published accounts of the microbiological properties of stamukhi lakes.

A large stamukhi forms every year near the outflow of the Mackenzie River in the western Canadian Arctic (Carmack and Macdonald 2002) and over the winter a vast lagoon of brackish water called "Lake Mackenzie" (or "Lake Herlinveaux"; both unofficial names) forms behind this stamukhi (Fig. 1). The lake spreads over an area of 12,000 km^2 and contains 70 km^3 of water, placing it within the top 20 or 30 lakes of the world by area or volume, respectively (Macdonald 2000). The stamukhi lake disappears in late June–early July, well after the peak annual

[1] Corresponding author (pgaland@ceab.csic.es).
[2] Present address: Unitat de Limnologia - Departament d'Ecologia Continental, Centre d'Estudis Avançats de Blanes - CSIC, 17300 Blanes, Spain.

Acknowledgments

We thank the officers and crew of the CCGS *Amundsen*, and C. Martineau, S. Roy, R. Terrado, M.-J. Martineau, and M. Rautio for field assistance and analysis, and R. Macdonald (Fisheries and Oceans Canada) for his insightful comments on stamukhi systems. This work was conducted within the Canada Arctic Shelf Exchange Study (CASES) led by L. Fortier. We also thank the anonymous reviewers for helpful comments and suggestions.

Financial support for this study was provided by the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chair program, Fonds québécois de recherche sur la nature et les technologies, and Indian and Northern Affairs Canada.

discharge of the Mackenzie River in late May. As soon as the ice dam begins to melt and break up, the seasonally retained water is released to the sea and the Mackenzie River mouth reverts to more typical estuarine conditions.

The unusual conditions within the stamukhi lake may select for genetically distinct microorganisms specifically adapted to the environment and forming an autochthonous community. Conversely, the stamukhi lake may not be biologically selective, in which case it would contain a mixture of allochthonous microbial assemblages that enter from the inflowing river and through the porous ice dam from offshore marine waters. The microbial diversity and ecology of stamukhi lakes is still unknown and there is little information on the source waters, both marine and fresh, that may contribute to the microbial populations of these seasonal lakes.

The objectives of the present study were first to identify the phylogenetic composition of microbes (Bacteria, Archaea, and small Eukaryota) in the stamukhi zone, which includes the stamukhi lake, the upstream river, and the adjacent sea. The second objective was to measure heterotrophic activity among the three sites to evaluate the contribution of the stamukhi lake toward carbon cycling. We sampled Lake Mackenzie during its period of maximum development in June before the annual loss of the ice dam, and made comparative measurements in the Mackenzie River and the coastal Beaufort Sea. The diversity of Bacteria and Archaea was evaluated by cloning and sequencing of the 16S ribosomal (r)RNA gene, and for eukaryotes the 18S rRNA gene. Heterotrophic production rates were estimated by measuring the rates of leucine (Leu) and thymidine (TdR) incorporation into protein and DNA, respectively.

Material and methods

Field sampling and environmental variables—The study was carried out within the framework of the Canadian Arctic Shelf Exchange Study onboard the icebreaker CCGS *Amundsen*. Samples from the marine site (70°47.35′N, 126°56.02′W) were collected on 19 June 2004 using a Seabird Carousel rosette system of 12-liter polyvinyl chloride bottles equipped with a Seabird 911+conductivity–temperature–depth (CTD) with fluorometer, transmissometer, pH, oxygen, and photosynthetically active radiation probes. Stations in the Mackenzie River (a downstream-flowing freshwater site at 69°15.85′N, 134°05.93′W) and in Lake Mackenzie (69°51.78′N, 132°38.62′W) were reached by ship-based helicopter on 17 June 2004. This is the first sample obtained from the Lake Mackenzie. Earlier data from the area are from samples taken in October (Garneau et al. 2006), and at that time of year Lake Mackenzie did not exist because the ice dam breaks down each summer. Samples were collected with a 5-liter Niskin bottle and salinity and temperature were measured with a SBE-19 CTD profiler. Microbial biomass was collected by filtering 1 liter of seawater through a 47-mm-diameter 0.2-μm Durapore filter (Millipore). Filters were stored in buffer (50 mmol L^{-1} tris, 40 mmol L^{-1} ethylenediamine tetra-acetic acid, and 0.75 mol L^{-1} sucrose) and frozen at −80°C until the nucleic acid was extracted as in Lovejoy et al. (2006).

Phytoplankton biomass was estimated from chlorophyll *a* (Chl *a*) analysis of the total community as described earlier (Garneau et al. 2006). Briefly, samples for total community Chl *a* were filtered onto Whatman GF/F glass fiber filters; samples for Chl *a* in the small-size cell fraction (Chl $a_{<3\ \mu m}$) were first filtered through a Nuclepore 3-μm pore-size filter and then onto a GF/F filter. All samples were stored frozen (−80°C) until pigment extraction in ethanol. Chl *a* concentration was determined by fluorometry before and after acidification using a Cary Eclipse spectrofluorometer standardized with *Anacystis* Chl *a*.

Samples for particulate inorganic (PIM) and organic (POM) material determination were filtered onto precombusted and preweighed Whatman GF/F filters, rinsed with 10 mL of ammonium formate 1% to remove dissolved salts, and stored at −20°C until analysis. In the laboratory, filters were first dried at 60°C for 24 h and weighed, and the filter weight was subtracted to obtain total material (TM). Filters were then combusted at 500°C for 90 min, reweighed, and corrected for the filter weight to give the PIM value. POM was calculated as TM − PIM.

Estimating prokaryote abundance—Water samples (5 to 25 mL) were fixed with formalin (2% final concentration) in the dark at 4°C for 1 to 24 h. After fixation, samples were filtered onto 0.2-μm black 25-mm-diameter polycarbonate filters (Poretics) and stained with 4′,6-diamidino-2-phenylindole (DAPI, 5 μg mL^{-1} final concentration, Sigma) for at least 5 min. Filters were mounted onto slides with nonfluorescent mounting oil (Immersol 518 M) and prokaryotes were enumerated by fluorescence microscopy at ×1000 magnification either onboard the CCGS *Amundsen* using an Olympus BX51 microscope, or subsequently in the laboratory with a Zeiss Axiovert 100. A minimum of 400 DAPI-stained cells was counted on each slide.

Heterotrophic productivity—Heterotrophic production rates were estimated from incorporation of ^{3}H-leucine into protein and ^{3}H-thymidine into DNA (Fuhrman and Azam 1982; Smith and Azam 1992). Briefly, 1.2 mL of samples that had been prefiltered through 3-μm Nuclepore membranes as well as unfiltered samples were dispensed into two sets of microtubes. [4,5-^{3}H]Leucine (Leu; specific activity 5.9 TBq $mmol^{-1}$) was added to the first set and [methyl-^{3}H]thymidine (TdR; specific activity 3.2 TBq $mmol^{-1}$) to the second set, both at a final concentration of 10–15 nmol L^{-1}. Blanks were killed immediately and incubations were terminated by adding trichloroacetic acid (TCA; 5% final concentration). The triplicate samples were incubated in the dark at the approximate in situ temperatures for 4 h. After cold centrifugation and three ice-cold 5% TCA rinses (0°C at 12,000 rpm for 10 min each), 1 mL of Ready Safe scintillation cocktail (Beckman) was added to the tubes and samples were radioassayed within 24 h or kept at −20°C in the 2-mL microtubes for later radioisotopic analysis.

Polymerase chain reaction (PCR) amplification and cloning—Amplification of archaeal 16S rRNA genes by

Table 1. Environmental and sampling characteristics of the three stations: the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea). POM, particulate organic matter; PIM, particulate inorganic matter. Particulate concentrations in parentheses are for the <3-μm fraction.

	Station		
	River	Lake	Sea
Sample depth (m)	0.5	1.5	5
Bottom depth (m)	5	9	260
Temperature (°C)	15.7	1.2	1.1
Salinity	0.13	0.97	30.86
POM (mg L^{-1})	11.8 (2.8)	11.5 (14.8)	1.27 (0.18)
PIM (mg L^{-1})	87.2 (6.9)	15.2 (11.1)	1.74 (1.74)

primers 109F and 934R followed the protocol as described previously (Galand et al. 2006). Bacterial 16S rRNA genes were amplified with the Bacteria-specific primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and universal primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR reaction conditions were 30 cycles of 94°C for 30 s, 50°C for 30 min, and 72°C for 2 min. Eukaryotic 18S rRNA genes were amplified by PCR with eukaryote-specific primers EukA and EukB as described earlier (Lovejoy et al. 2006). PCR products were analyzed by gel electrophoresis, purified with Qiaquick PCR Purification Kit (Qiagen), and cloned with TA cloning kit (Invitrogen) according to the manufacturer's instructions. In total we constructed nine clone libraries: one archaeal, one bacterial, and one eukaryotic library for each the Mackenzie River (river), Lake Mackenzie (lake), and coastal Beaufort Sea (sea). For each library, positive clones were picked and inoculated into LB media in 96-well plates. Bacterial clones were screened by restriction fragment length polymorphism (RFLP) with the restriction enzyme *Hae*III and colonies showing distinct RFLP patterns were sequenced as described earlier (Galand et al. 2006). For the bacteria, we sequenced 32 clones from the river, 30 from the lake, and 33 from the sea. For Archaea and Eukaryotes, clones containing inserts were chosen randomly from each library and sequenced directly, without the fingerprinting step. For Archaea, 64 clones were sequenced from the river, 82 from the lake, and 55 from the sea using the vectors' T7p universal primer. For Eukaryotes, 95 clones were sequenced from the river, 86 from the lake, and 77 from the sea with the Euk 528F primer.

Suspected chimeras were checked by using BLAST with sequence segments separately and then using the Chimera check program at Ribosomal Data Project II (Michigan State University; http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). Between one and seven chimeras were detected and excluded from each library, with highest numbers for the river. The 16S rRNA sequence data obtained in this study have been archived in the GenBank database under accession numbers EF014496 to EF014713.

Sequence analysis and diversity calculations—Sequences were compared with those in the GenBank database using the BLAST server at the National Center for Biotechnology Information. The approximately 800-base pair (bp) sequences were aligned using the CLUSTAL W package (Higgins et al. 1994) and checked manually. The Shannon–Wiener diversity index and the Chao1 nonparametric richness estimator were calculated with the program DOTUR (Schloss and Handelsman 2005) through a Jukes–Cantor corrected distance matrix obtained using the DNADIST program from PHYLIP. The 3% distance level between sequences was used for calculation of diversity estimators. Statistical differences in the composition of clone libraries were determined using the LIBSHUFF program (Singleton et al. 2001).

Results

Environmental conditions—The sampling was just after peak discharge of the Mackenzie River and so Lake Mackenzie was filled to near its maximum freshwater capacity. Both the Mackenzie River and coastal Beaufort Sea were ice-free, but ice cover (1.5 m) remained over Lake Mackenzie. Upper water column temperatures were above 0°C at all sites and much higher in the river than in the lake and sea. The lake salinity was higher than in the river but well below the salinities in the marine waters (Table 1). Concentrations of POM were highest at the river and lake stations and lowest at the marine station. POM was nearly 10 times higher at the river and lake stations than in the sea. The distribution of the small-size fraction (<3 μm) of POM followed a different pattern, with values in the lake much higher than in the river or sea (Table 1). PIM was highest in the river, lower in the lake, and lowest in the sea (Table 1). The small-size fraction (<3 μm) of PIM showed a different trend, with values more than 60 times higher in the lake than in either the river or sea (Table 1). Our observations of the DAPI samples from the lake under epifluorescence microscopy also confirmed the high abundance of small organic (autofluorescent) and abiotic (nonfluorescent) particles, with large numbers in every field at ×1000.

Chl a, prokaryotic abundance, and heterotrophic production rates—Total Chl *a* concentrations were similar in the lake and river and about double the concentrations in the sea. The picophytoplankton fraction estimated from Chl $a_{<3\ \mu m}$ followed a different pattern, with very low concentrations (0.04 μg L^{-1}) in the lake and much higher values in the river and the sea. Lake prokaryotic cell concentrations were more than double the sea values and four times greater than in the river (Table 2). In contrast, heterotrophic production rates estimated from both Leu and TdR incorporation were greatest in the river and very little was attributable to the <3-μm fraction. The lake and sea production rate values were similar, with one-third of the total Leu incorporation in the <3-μm fraction. In the lake, the ratio of Leu to TdR incorporation (Leu : TdR) was twice that in either the river or the sea (Table 2).

Bacterial communities—Detailed descriptions of the bacterial sequence data and phylogenetic trees are given in Web Appendix 1 (www.aslo.org/10/toc/vol_52/issue_2/0813al.pdf). The number of bacterial phylotypes in Lake

Table 2. Chlorophyll *a* (Chl *a*) concentrations, abundance of prokaryotic cells, size-fractionated ^{3}H-leucine, and ^{3}H-thymidine incorporation (± SE) and leucine (Leu)-to-thymidine (TdR) ratio at stations from the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea). Chl $a_{<3\ \mu m}$, Chl *a* values for the <3-μm fraction.

	Station		
	River	Lake	Sea
Chl *a* total (μg L^{-1})	1.25	1.17	0.65
Chl $a_{<3\ \mu m}$ (μg L^{-1})	0.247	0.044	0.185
Chl $a_{<3\ \mu m}$ (% total)	20	4	29
Prokaryotes (10^5 cells mL^{-1})	2.25	10.3	4.26
^{3}H-Leu (pmol L^{-1} h^{-1})	25.91 (±0.46)	10.75 (±3.12)	7.54 (±1.47)
^{3}H-Leu <3 μm (pmol L^{-1} h^{-1})	0.43 (±0.02)	3.53 (±0.81)	2.49 (±0.20)
^{3}H-Leu <3 μm (% total)	2	33	33
^{3}H-TdR (pmol L^{-1} h^{-1})	5.27 (±0.30)	1.07 (±0.10)	1.37 (±0.10)
^{3}H-TdR <3 μm (pmol L^{-1} h^{-1})	0.24 (±0.02)	0.83 (±0.03)	1.84 (±0.3)
^{3}H-TdR <3 μm (% total)	5	77	134
Leu : TdR ratio <3 μm	1.79	4.25	1.34
Leu : TdR ratio total	4.92	10.05	5.50

Mackenzie was comparable with the river and slightly higher than in the sea (Table 3). Calculation of the Shannon–Weiner and Chao1 indices showed that diversity of Bacteria was highest in the Mackenzie River, lower in the lake, and lowest in the coastal Beaufort Sea (Table 3).

Clone libraries from Lake Mackenzie included sequences from the five bacterial phyla *Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia*, and *Planctomycetes* (Fig. 2). The most abundant group belonged to *Bacteroidetes*, with sequences mainly related to a cluster of typical freshwater bacteria defined as PRD01a001B. The next most common group was *Betaproteobacteria*, mainly from the freshwater clusters GKS16 and LD28 and the most frequent *Actinobacteria* belonged to the acI-A cluster.

Mackenzie River bacterial sequences were affiliated with *Proteobacteria, Bacteroidetes, Deinococcus, Verrucomicrobia, Actinobacteria, Acidobacteria*, and candidate division OD1. The majority of the sequences retrieved from the river grouped within the *Betaproteobacteria* (Fig. 2). The Mackenzie River *Betaproteobacteria* belonged to clusters that are widely distributed in freshwater systems such as the *Rhodoferax* sp. BAL 47 cluster, *Polynucleobacter necessarius* cluster, GKS16, LD28 cluster, and *Methylophilaceae*. The next most important group of sequences belonged to *Bacteroidetes* and clustered with typically freshwater bacteria.

Beaufort Sea bacterial sequences were affiliated with *Bacteroidetes, Alpha-proteobacteria, Gammaproteobacteria*, and *Betaproteobacteria* (Fig. 2). Half of the sequences belonged to *Alphaproteobacteria* and mostly to the SAR11 clade. The second most abundant group was *Bacteroidetes*, which contained predominantly *Polaribacter* sequences.

Archaeal communities—Detailed descriptions of the archaeal sequence data and phylogenetic trees are given in Web Appendix 1. The archaeal community in Lake and river Mackenzie were similarly diverse, whereas fewer phylotypes were detected in the sea. The Shannon and Chao1 indices of diversity showed similar trends, with much higher diversity in the river and the lake than in the sea (Table 3).

In Lake Mackenzie, the majority of the clones belonged to the *Euryarchaeota*, as in the river and in contrast to the marine sample (*see* below). Among *Euryarchaeota*, the largest groups were part of the Lake Dagow sediment (LDS) and Rice cluster-V (RC-V) clusters (Fig. 3). *Crenarchaeota* represented more than one-third of the clones and formed one single monophyletic group closely related to sequences from freshwater environments. We designated this clade Freshwater Group I.1a and defined it as a subclade of Group I.1a *Crenarchaeota*, containing sequences from freshwater environments (Fig. 3). Freshwater Group I.1a was closely related to the Marine Group I.1a, with nearest similarity between members of the two clusters ranging between 96% and 98%.

Most of the clones retrieved from the river were also affiliated with *Euryarchaeota*. LDS was the most abundant group (50% of the clones), followed by RC-V (Fig. 3).

Table 3. Diversity of Bacteria and Archaea assemblages in the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea). Enumeration of phylotypes and calculation of diversity estimators were done for a 3% distance level between sequences.

	Bacteria			Archaea		
	River	Lake	Sea	River	Lake	Sea
Number of phylotypes	21	21	17.5	22	30	5
Shannon diversity	2.7	2.6	2.3	2.8	2.74	0.44
Chao1	73.5	32	21.7	37	66	6.5
Number of clones	40	47	56	36	56	52

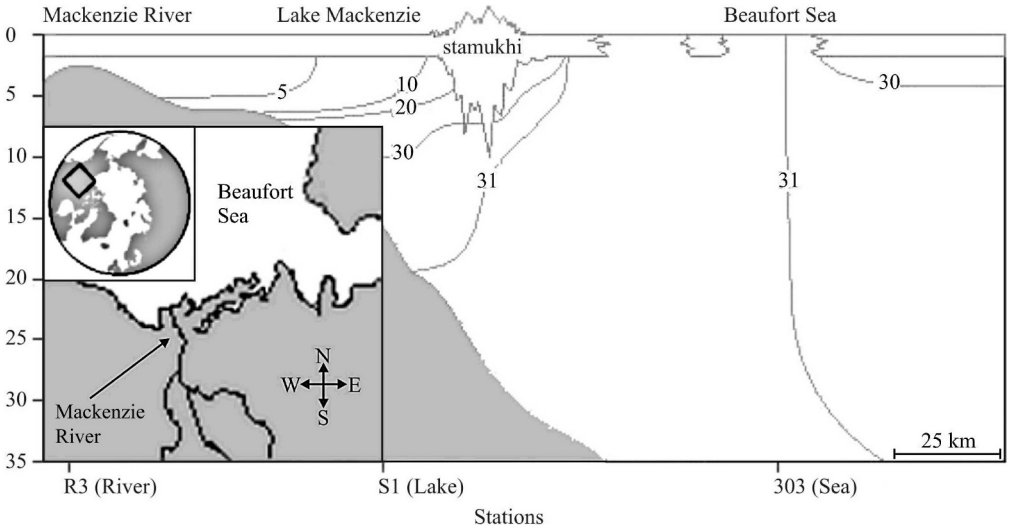

Fig. 1. Schematic diagram showing the inflow from the Mackenzie River trapped near shore behind the stamukhi and forming Lake Mackenzie (modified from AMAP [1998]). Location of the sampling stations R3 (River), S1 (Lake), and 303 (Sea) is indicated.

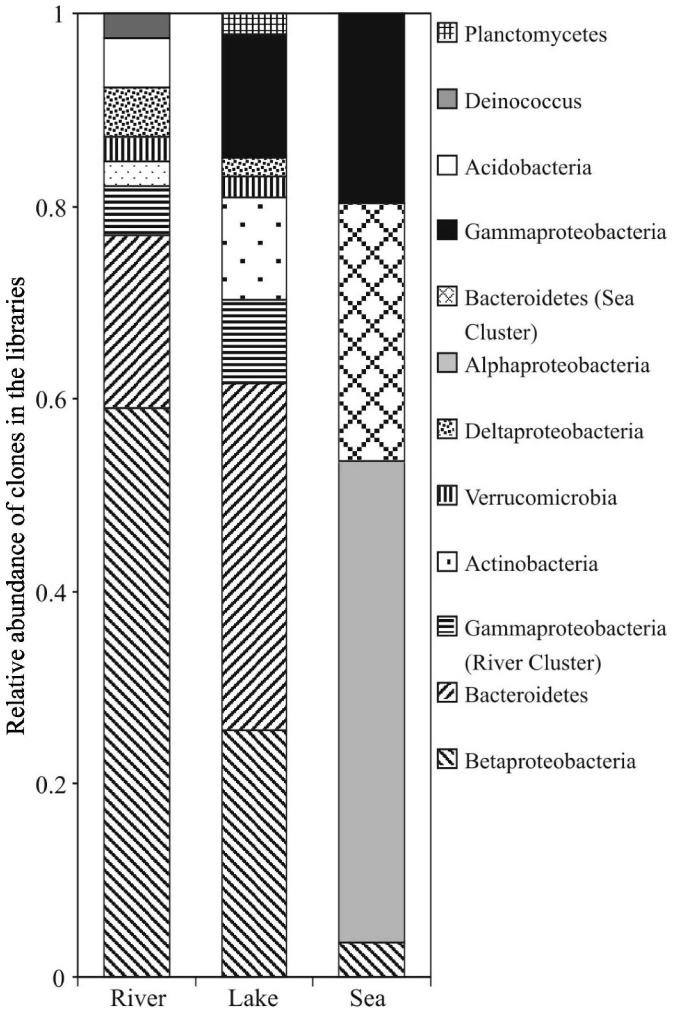

Fig. 2. Bacterial community structure in clone libraries from the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea). Communities are represented by the relative abundances of clones in the different phylogenetic clusters.

Crenarchaeota represented almost one-third of the clones in the river and belonged to the same Freshwater Group I.1a as found in the lake. In the sea, the composition of the clone library differed significantly from the lake and the river, with more than 90% of the clones belonging to the crenarchaeotal Marine Group I.1a (Fig. 3). Few euryarchaeotal sequences were amplified and they belonged to Group II.a and II.b, two clusters that were not detected in the river or the lake.

Eukaryotic communities—We recovered 79, 68, and 59 high-quality sequences (over 750 bp) from the river, lake, and sea respectively. Ciliates made up more than 95% of the sequences from Lake Mackenzie (Table 4) and all but three belonged to the subclass *Choreotrichia*; the three others were affiliated with subclasses *Haptoria*, *Peritrichia*, and class *Colpodea* (Table 4). Our examination of the DAPI filters by epifluorescence microscopy confirmed the presence of abundant small ciliates at this site. We found one sequence from the phototrophic *Cryptophyta* in the Lake Mackenzie library and four diatom sequences. Two of the diatoms were typically freshwater (99% and 100% similar to *Aulacoseira islandia* [AY569572] and *Stephanodiscus niagarae* [DQ45009]) and one was a marine species (*Chaetoceros* sp., DQ310228). We also recovered one sequence that was 98% identical to a Guaymas Vent sequence (AY046808) that groups between diatoms and the marine bolidophytes.

In the river, the most abundant group of sequences was affiliated with Cryptophytes. Stramenopiles affiliated with genera *Spumella* and *Ochromonas* were the next most abundant group, followed by ciliates from the subclass *Choreotrichia*. The river also contained a diverse array of heterotrophic protists from four major groups (Table 4). Diatoms from the river represented two freshwater species with 99% matches to *Diatoma tenue* (DTE535143) and *A. islandica* (AY569572).

In the sea, ciliates belonged predominantly to the subclass *Oligotrichia* and made up the dominant clone

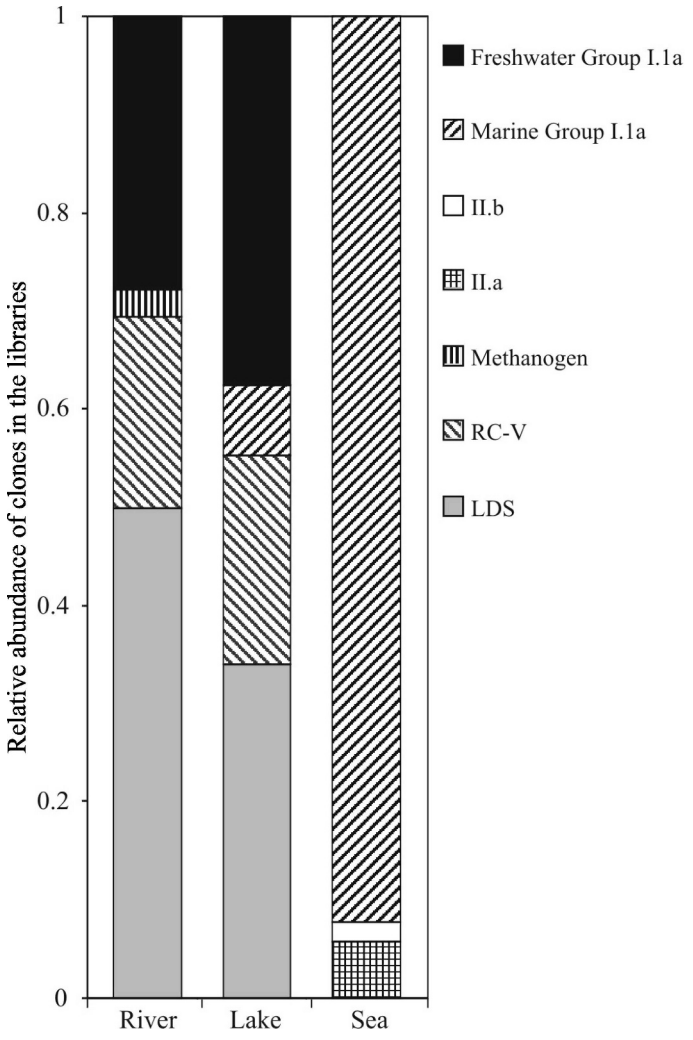

Fig. 3. Archaeal community structure in clone libraries from the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea). Communities are represented by the relative abundances of clones in the different phylogenetic clusters.

type (37% of the clones, Table 4). The second most abundant group in the sea was affiliated with phototrophic class *Prasinophyceae*, which was not recovered from either the river or Lake Mackenzie. The three diatom sequences had a 99% match to the same marine *Chaetoceros* sp. (DQ310228) found in the lake.

Library comparisons—The phylogenetic composition of the Lake Mackenzie libraries was clearly different from the Beaufort Sea libraries for the bacterial, archaeal, and eukaryotic communities. The lake libraries contained mostly sequences affiliated with typical freshwater clades, whereas the Beaufort Sea libraries had only marine sequences. Possible differences between the Lake Mackenzie and Mackenzie River libraries for Bacteria and Archaea were less obvious and consequently tested using LIBSHUFF (Singleton et al. 2001). For bacteria, the heterologous coverage of the combined river and lake libraries was different from the homologous coverage of the lake library ($p < 0.05$), indicating that most of the lake sequences differed from the river sequences. However, comparison of the $(C_x - C_{xy})^2$ to the $p = 0.05$ value for $(C_x - C_{xy})^2$ of randomized samples indicates that the lake and river libraries had a different coverage only for an evolutionary distance smaller than 0.13 (Fig. 4A). The heterologous coverage of the combined river and lake archaeal libraries was not significantly different from the homologous coverage of the lake library ($p = 0.679$), indicating that most of the archaeal sequences from the lake were similar to sequences from the river (Fig. 4B).

Discussion

Lake Mackenzie and other stamukhi lake ecosystems likely exert a controlling influence on the adjacent coastal waters of the Arctic Ocean. They delay by weeks to months the arrival of water and heat from large rivers to the open waters of the sea, and they retain organic carbon and other biogeochemically active constituents at the coastal margin, including during the period of peak discharge when solutes and particle concentrations and fluxes are maximal (Retamal et al. 2007). Despite their vast circumpolar occurrence and their key location at the coastal margin, little is known about their environmental properties and the microbiological processes that may operate within these natural holding reservoirs. Stamukhi lakes are logistically difficult systems to access and sample, but increased spatial and temporal observations will be required in the future to fully understand their properties and dynamics.

The observations reported here provide a first-order description of a stamukhi ecosystem, and reveal a number of features that distinguish it from its riverine or marine source waters. At the time of sampling, the brackish surface waters of Lake Mackenzie contained concentrations of Chl *a* that were about twice those in the sea, indicating growth of phytoplankton under the ice cover. Prokaryote concentrations were much higher than in either the river or the sea, implying increased growth rates or decreased losses, for instance decreased nanoflagellate grazing. The particulate inorganic matter concentrations declined abruptly in Lake Mackenzie relative to the river, suggesting that the stamukhi system may act as a decantation lagoon. Contrary to expectation, the POM concentrations were as high as in the river, but shifted from dominance by large particles in the river to small particles in the lake, implying the breakdown of larger POM aggregates and differential loss by sedimentation. Our phylogenetic analyses of the three microbial domains also indicated that this stamukhi lake had distinctive features.

Bacterial communities—Although freshwater *Betaproteobacteria* and *Bacteroidetes* bacterial clusters dominated the river and lake libraries, the bacterial community in Lake Mackenzie was less diverse than and phylogenetically distinct from the riverine community. The differences between the lake and the river communities were seen in the sequence heterogeneity within the most abundant *Betaproteobacteria* and *Bacteroidetes* clusters and in the presence of rare sequences. Among the less abundant

Table 4. Classification of Eukaryotes detected in clone libraries from the Mackenzie River (River), Lake Mackenzie (Lake), and coastal Beaufort Sea (Sea).

Major ranks	Subranks	Detail	No. of clones		
			River	Lake	Sea
Alveolate	Ciliates	*Oligotrichia*	6	0	19
		Choreotrichia	13	59	2
		Tetrahymenina	2	0	0
		Stichotrichida	1	0	0
		Colpodea	2	1	0
		Haptoria	0	1	0
		Peritrichia	0	1	0
	Perkinsida		1	0	0
	Group II	*Amoebophrya*	1	0	0
	Apicomplexa		2	0	0
	Dinoflagellate		1	0	5
Chlorophyta		*Prasinophyceae*	0	0	17
Stramenopiles		Diatoms	5	4	3
		Spumella, Ochromonas	16	0	1
		Bicosoecida	1	0	3
		MAST	0	0	2
Cryptophyta			19	1	0
Katablepharidophyta			1	0	0
Fungi, Metazoa	*Choanozoa*		1	0	4
Apusomonadidae			1	0	0
Lobosea			1	0	0
Rhizaria	*Cercozoa*		5	1	3
Total number of clones			79	68	59

sequences in the lake, some were of terrestrial origin and belonged to *Gammaproteobacteria*. Several of the *Gammaproteobacteria* sequences were related to the methane-oxidizing *Methylobacter* sp. and had closest matches to organisms originally from wetlands, peat, and tundra soils. Such soils characterize the Mackenzie River basin, which indicates a probable allochthonous origin of the sequences. One sequence from the lake was affiliated with *Colwellia rossensis*, a *Gammaproteobacteria* originally retrieved from sea ice (Brinkmeyer et al. 2003), but no true marine bacterial sequences were detected. The absence of marine sequences in the lake indicates relatively little mixing between the sea and the lake, or poor survival of marine species in the lake.

Betaproteobacteria prevailed in both the lake and the river, and were also the dominant group of bacteria that were previously detected by fluorescence in situ hybridization in the Mackenzie River in October 2002 (Garneau et al. 2006). *Betaproteobacteria* are often the dominant bacterioplankton in freshwater lakes (Methé et al. 1998) and represent a consistently large fraction of the microbial community in diverse river types, including the Parker and Ipswich rivers (Crump and Hobbie 2005; Zwart et al. 2002) and the Delaware River (Cottrell et al. 2005). In all riverine studies, including the present study, the proportion of *Betaproteobacteria* was similar, comprising 50% to 60% of the clones. The main *Betaproteobacteria* clusters from the lake and the river all grouped within typical freshwater phylogenetic clades. Some of the clades have representative isolates that could point toward the function of the organisms in the ecosystem. The *P. necessarius* cluster isolates are small-celled, free-living aerobic heterotrophic bacteria (Hahn 2003), whereas the Bal 47 cluster isolates, affiliated with *Rhodoferax* sp., are able to grow phototrophically and by fermentation of fructose (Madigan et al. 2000), or as facultative anaerobes that oxidize acetate (Finneran et al. 2003). *Betaproteobacteria* and *Bacteroidetes* were suggested to share the ability to degrade complex organic macromolecules (Eiler and Bertilsson 2004); however, the true physiological role of those organisms in lakes and rivers remains unclear. Some of our *Betaproteobacteria* sequences were related to clones from soils including the rhizosphere, suggesting a terrestrial source to the communities. Nevertheless, sequences of terrestrial origin were a minor fraction of the clones in the libraries.

The second most frequent group of sequences in the stamukhi lake and river belonged to the *Bacteroidetes* Cytophaga–Flavobacterium–Bacteroides group. Members of the *Bacteroidetes* are known to degrade the high-molecular-mass fraction of dissolved organic material (Kirchman 2002). Our *Bacteroidetes* sequences were associated with typical freshwater clusters (Zwart et al. 2002) that do not include any cultivated isolates at present. *Bacteroidetes* or *Cytophaga*-like bacteria have been detected in a number of temperate rivers (Cottrell et al. 2005; Crump and Hobbie 2005; Zwart et al. 2002) and thus appear to form the next most abundant group of bacteria in rivers together with *Actinobacteria*, also an important constituent of temperate lake communities (Glockner et al. 2000). *Actinobacteria*, however, were not a significant component in the stamukhi lake and Mackenzie River communities. Similarly, we did not detect any members of the *Alphaproteobacteria* even though they have been reported in rivers (Cottrell et al. 2005; Crump and Hobbie

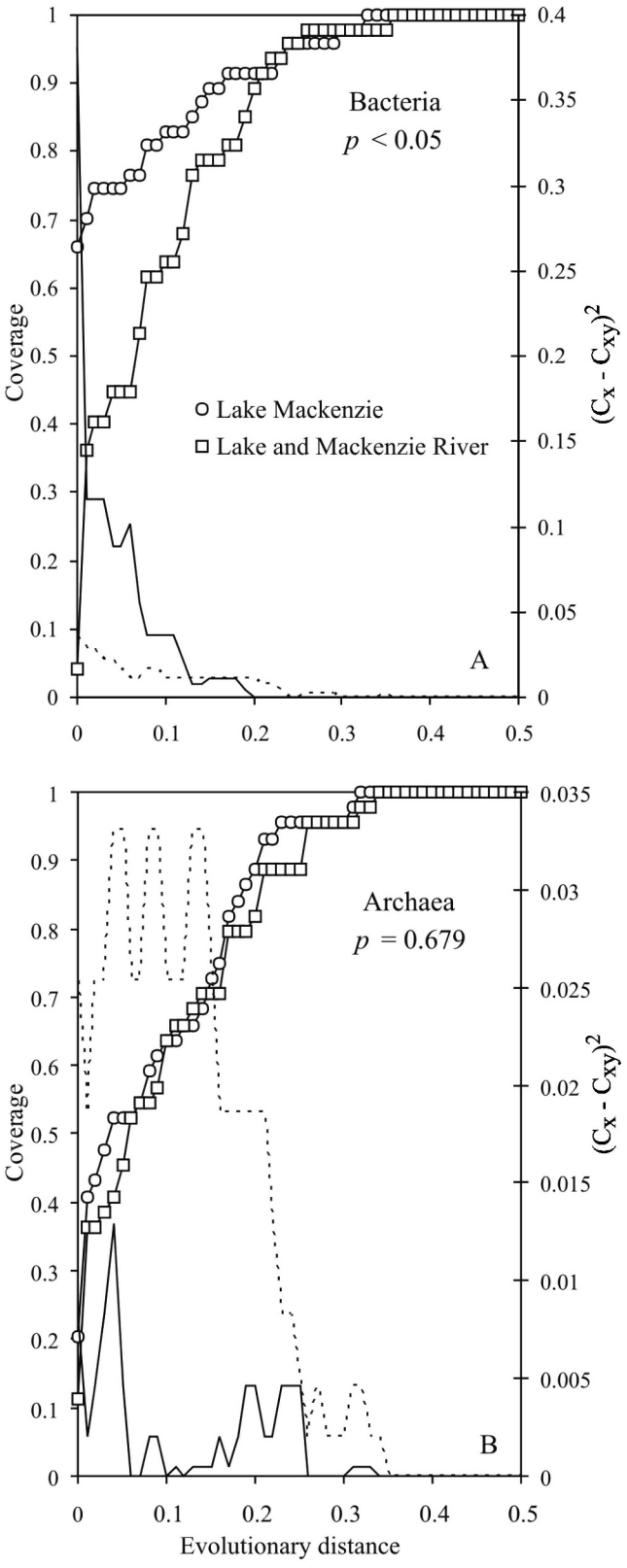

Fig. 4. LIBSHUFF comparison of the homologous and heterologous coverage curves for (A) Bacteria and (B) Archaea. Solid line indicates the value of $(C_x - C_{xy})^2$ for samples at each value of Jukes–Cantor evolutionary distance. Dotted line indicates the $p = 0.05$ value for $(C_x - C_{xy})^2$ of randomized samples.

2005; Garneau et al. 2006). Several rare sequences were present in the Mackenzie River and contributed to the high bacterial riverine diversity. They included *Gammaproteobacteria* and *Clostridium* related to sequences retrieved from soils or peatlands, emphasizing the probable input of terrestrial microorganisms to the riverine bacterial community.

The bacterial community composition and diversity at the marine station differed from the lake and river communities. The diversity and dominant groups, *Alphaproteobacteria*, *Bacteroidetes*, and *Gammaproteobacteria*, in the coastal Beaufort Sea were similar to the Central Arctic (Bano and Hollibaugh 2002). We found sequences from the OM43 clade in the *Betaproteobacteria* in our Beaufort Sea library, but no *Deltaproteobacteria* or *Epsilonproteobacteria*, which had been reported from the deeper Central Arctic Ocean libraries. Our most common sequences belonged to the SAR11 clade (*Alphaproteobacteria*), which is the dominant clade in the surface ocean (Morris et al. 2002). The SAR11 sequences detected in the Beaufort Sea were closely related to clones previously retrieved from the Central Arctic (Bano and Hollibaugh 2002) and to the only cultivated isolate *Pelagibacter ubique* from the Oregon coast (Rappe et al. 2002). The proportion of SAR11 clones (40%) in the Beaufort Sea was similar to the Central Arctic (Bano and Hollibaugh 2002) and the Greenland Sea (Zaballos et al. 2006), confirming SAR11 as a predominant bacterioplankton in the Arctic Ocean. *Roseobacter*, the second most abundant clade of *Alphaproteobacteria* in our sea sample, belonged to *Roseobacter*-clade-affiliated (RCA) cluster. RCA cluster members are thought to be strictly heterotrophic with a bipolar distribution (Selje et al. 2004). The transition from *Betaproteobacteria* in the lake and river to *Alphaproteobacteria* in the Beaufort Sea is consistent with the traditional view on bacterial community shift from fresh- to marine waters.

The second most abundant marine group was *Polaribacter*, phylum *Bacteroidetes*. *Polaribacter* has been often detected in Antarctic and Arctic waters and sea ice (Brinkmeyer et al. 2003), and this genus likely plays a significant role in bacterial production in high-latitude seawater and sea ice (Abell and Bowman 2005). The third most abundant group of sequences belonged to different clusters of *Gammaproteobacteria*. Many are only known from environmental sequences, such as the SAR92 or SAR86 cluster, present in the central Arctic (Bano and Hollibaugh 2002) and well represented in the Greenland Sea (Zaballos et al. 2006). Other *Gammaproteobacteria* belonged to the *Fundibacter/Alcanivorax* clade also detected in the central Arctic (Bano and Hollibaugh 2002) and commonly encountered in a late-winter Antarctic clone library (Grzymski et al. 2006).

Archaeal communities—The archaeal community composition and diversity were similar in Lake Mackenzie and the Mackenzie River even though environmental factors such as temperature and salinity differed between the two habitats. We did find rare sequences such as Marine Group I.1a sequences present in the lake only, but such rare sequences did not influence the outcome of the LIBSHUFF

test. Our analyses of Archaea in the river and the lake confirm that the Mackenzie River system is home to an extremely diverse archaeal community. The majority of the lake and river clones belonged to the LDS cluster and the RC-V cluster. These groups were also predominant in the Mackenzie River during autumn (Galand et al. 2006) and may be a seasonally constant component of the archaeal community. In contrast, sequences from the newly delimited Freshwater Group I.1a were rare in our earlier Mackenzie River clone libraries (Galand et al. 2006), which could indicate a seasonal distribution of that group. Freshwater Group I.1a was related to sequences previously detected in lake water and sediments, confirming the freshwater characteristic of the cluster. The diversity reported here for the Mackenzie River was somewhat lower than previously described from October (Galand et al. 2006), and this may be a result of seasonal change or an artifact of the lower number of clones analyzed here (36 vs. 98 in the October analysis).

The archaeal community in the coastal Beaufort Sea had a very low diversity and most of the sequences belonged to *Crenarchaeota* Group I.1a, which has been previously reported in Arctic surface waters (Bano et al. 2004; Galand et al. 2006). Group I also dominated libraries at all depths in the Southern Ocean (Massana et al. 2000), and in nonpolar libraries *Crenarchaeota* are generally more abundant in the mesopelagic and pelagic environments (Massana et al. 2000; Herndl et al. 2004). *Euryarchaeota* (Groups II.a and II.b) represented less than 10% of the archaeal clones, in contrast to our Beaufort Sea library from October 2002 that was dominated by Group II.a (Galand et al. 2006), suggesting that at that time the surface Beaufort Sea was more similar to other oceans.

It has been hypothesized earlier that Archaea in Beaufort Shelf waters could have an allochthonous origin (Wells et al. 2006). However, the phylogenetic composition of the Beaufort Sea libraries was clearly different from both the Lake Mackenzie and Mackenzie River libraries. Those results confirm that Archaea are not derived from the river but rather are an autochthonous marine community, as reported earlier (Galand et al. 2006). However, freshwater clones of picocyanobacteria have been recovered from offshore using cyanobacterial primers, indicating the persistence of some prokaryotes along the river–sea continuum (Waleron et al. 2007).

Eukaryotic communities—Many of the microbial eukaryote features of Lake Mackenzie underscored the distinctive characteristics of this ecosystem relative to its source waters. The predominance of *Choreotrichia* ciliate sequences in our stamukhi lake libraries was striking, and their abundance was confirmed by epifluorescence microscopy. This ciliate community was less diverse than that in the river, and also differed greatly from the sea, in which most of the ciliates were *Oligotrichia*. The presence of diatoms in the lake is consistent with the moderate concentrations of Chl *a*, and its distribution in mostly the larger cell fraction. This assemblage appeared to contain an interesting mixture of marine and freshwater taxa. Protists in the green algal class *Prasinophyceae* were notably absent from the lake sequences. These are ubiquitously distributed throughout Arctic seas (Lovejoy et al. 2006) and were also the most common clones among the Beaufort Sea sequences in the present study. Another remarkable feature of the stamukhi lake was the lack of representation by heterotrophic nanoflagellates. In contrast, phagotrophic choanoflagellates and stramenopiles occurred in the river (*Spumella, Ochromonas*) and sea (marine stramenopiles, MAST), and their apparent absence from the lake may reflect strong grazing pressure by the abundant *Choreotrichia*. This in turn would reduce the grazing pressure on bacteria, and could be one factor contributing to the much higher bacterial cell concentrations in the lake relative to the river and the sea. These results are limited in extent and require corroboration with more intensive sampling, but they imply that the stamukhi ecosystem differs in its microbial food web structure, and thereby functionally, from the adjacent freshwater and marine ecosystems.

Heterotrophic productivity—The Leu and TdR incorporation assays showed that the stamukhi lake was heterotrophically active, but with properties that differed from those in the river and offshore ocean. The production rates were lower than in the river and more similar to those in the sea, despite much higher bacterial concentrations than in either. The fractionation assay showed that much of the bacterial productivity estimated by both Leu and TdR incorporation in the stamukhi lake was associated with the less-than-3-μm fraction, and more similar to the sea than the river. However, the Leu : TdR ratios were greater in both fractions of the stamukhi lake community and were well above the river and sea ratios. Leu incorporation is considered a proxy for biomass production and TdR incorporation a measure of cell replication. Although an elevated ratio has been attributed to environmental stress and microbial adjustments toward survival rather than population growth (Shiah and Ducklow 1997), the interpretation of Leu : TdR ratios is not clear and may be due to other factors as well (Ducklow 2000; Longnecker et al. 2006). In the case of the stamukhi lake, the frigid temperatures, rising salinities, and nutrient and substrate depletion during the prolonged residence time may have caused some physiological stress.

There were striking contrasts in bacterial production rates between the two end members of the stamukhi system, with three- to fourfold higher rates of TdR and Leu incorporation in the Mackenzie River relative to the coastal ocean. The Mackenzie River rates were higher than those reported earlier from the same site in October 2002 (Garneau et al. 2006) but well below values reported in large Siberian rivers (Meon and Amon 2004) and in temperate rivers (Schultz et al. 2003; Crump and Hobbie 2005). Much greater freshwater relative to marine bacterial activities have been recorded in other estuarine systems in the Arctic and in temperate regions (Del Giorgio and Bouvier 2002), and likely result from greater organic carbon substrate availability in rivers relative to the sea (Meon and Amon 2004) and, for the Arctic, the much lower temperatures of the sea. The Mackenzie River and coastal ocean samples also differed in the importance of large particles, with the TdR incorpora-

tion mostly associated with particles >3 μm in freshwater, shifting entirely to the <3-μm fraction in the sea. Particle-attached bacterial communities are often more productive than free-living communities in other riverine systems and this may be especially pronounced in the Mackenzie River with its high suspended particulate matter concentrations (Retamal et al. 2007).

In summary, our results show that Lake Mackenzie is a heterotrophically active ecosystem, despite its frigid ice-covered waters, with distinct microbial communities in the three domains of life. The bacterial and eukaryotic communities in the lake, as well as properties such as salinity, suspended particulate matter, particle size, picoplankton concentrations, and heterotrophic production, differed from both the river and the sea. The lake was, however, strongly influenced by the river, as shown by the presence of a riverine archaeal community and freshwater clusters of bacteria. Our results suggest that this vast ecosystem type could constitute an active and diverse microbial reaction system in which key functional processes transform the river-derived organic materials before their discharge into the Arctic Ocean. There is increasing evidence of the onset of climate change effects in many parts of the Arctic, and global circulation models predict that there will be a complete loss of Arctic summer sea-ice cover within the next few decades (Holland et al. 2006). The stamukhi ecosystem depends entirely on thick sea ice for its formation and integrity, and this coastal processing zone will be especially vulnerable to the accelerated effects of climate warming.

References

Abell, G. C. J., and J. P. Bowman. 2005. Colonization and community dynamics of class Flavobacteria on diatom detritus in experimental mesocosms based on Southern Ocean seawater. FEMS Microbiol. Ecol. **53:** 379–391.

AMAP. 1998. Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP).

Bano, N., and J. T. Hollibaugh. 2002. Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. Appl. Environ. Microbiol. **68:** 505–518.

———, S. Ruffin, B. Ransom, and J. T. Hollibaugh. 2004. Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. Appl. Environ. Microbiol. **70:** 781–789.

Brinkmeyer, R., K. Knittel, J. Jurgens, H. Weyland, R. Amann, and E. Helmke. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol. **69:** 6610–6619.

Carmack, E. C., and R. W. Macdonald. 2002. Oceanography of the Canadian shelf of the Beaufort Sea: A setting for marine life. Arctic **55:** 29–45.

Cottrell, M. T., L. A. Waidner, L. Yu, and D. L. Kirchman. 2005. Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environ. Microbiol. **7:** 1883–1895.

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. **65:** 3192–3204.

———, and J. E. Hobbie. 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol. Oceanogr. **50:** 1718–1729.

Del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnol. Oceanogr. **47:** 471–486.

Ducklow, H. 2000. Bacterial production and biomass in the oceans, p. 85–120. *In* D. Kirchman [ed.], Microbial ecology of the oceans. Wiley–Liss.

Eiler, A., and S. Bertilsson. 2004. Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ. Microbiol. **6:** 1228–1243.

Finneran, K. T., C. V. Johnsen, and D. R. Lovley. 2003. *Rhodoferax ferrireduce*ns sp nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). Int. J. Syst.Evol. Microbiol. **3:** 669–673.

Fuhrman, J. A., and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Mar. Biol. **66:** 109–120.

Galand, P. E., C. Lovejoy, and W. F. Vincent. 2006. Remarkably diverse and contrasting archaeal communities in a large arctic river and the coastal Arctic Ocean. Aquat. Microb. Ecol. **44:** 115–126.

Garneau, M. E., W. F. Vincent, L. Alonso-Sáez, Y. Gratton, and C. Lovejoy. 2006. Prokaryotic community structure and heterotrophic production in a river-influenced coastal arctic ecosystem. Aquat. Microb. Ecol. **42:** 27–40.

Glockner, F. O., E. Zaichikov, N. Belkova, L. Denissova, J. Pernthaler, A. Pernthaler, and R. Amann. 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. Appl. Environ. Microbiol. **66:** 5053–5065.

Grzymski, J. J., B. J. Carter, E. F. Delong, R. A. Feldman, A. Ghadiri, and A. E. Murray. 2006. Comparative genomics of DNA fragments from six Antarctic marine planktonic Bacteria. Appl. Environ. Microbiol. **72:** 1532–1541.

Hahn, M. W. 2003. Isolation of strains belonging to the cosmopolitan Polynucleobacter necessarius cluster from freshwater habitats located in three climatic zones. Appl. Environ. Microbiol. **69:** 5248–5254.

Herndl, G. J., T. Reinthaler, E. Teira, H. Van Aken, C. Veth, A. Pernthaler, and J. Pernthaler. 2004. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. **71:** 2303–2309.

Higgins, D., J. Thompson, T. Gibson, J. D. Thompson, D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680.

Holland, M. M., C. M. Bitz, and B. Tremblay. 2006. Future abrupt reductions in the summer Arctic sea ice. Geophys. Res. Lett. **33**, L23503, doi:23510.21029/22006GL028024.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol. **39:** 91–100.

———, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2005. Biogeography of major bacterial groups in the Delaware Estuary. Limnol. Oceanogr. **50:** 1697–1706.

Longnecker, K., B. F. Sherr, and E. B. Sherr. 2006. Variation in cell-specific rates of leucine and thymidine incorporation by marine bacteria with high and with low nucleic acid content off the Oregon coast. Aquat. Microb. Ecol. **43:** 113–125.

Lovejoy, C., R. Massana, and C. Pedrós-Alió. 2006. Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas. Appl. Environ. Microbiol. **72:** 3085–3095.

MACDONALD, R. W. 2000. Arctic estuaries and ice: A positive-negative estuarine couple, p. 383–407. *In* E. Lewis, E. Jones, P. Lemke, T. Prowse and P. Wadhams [eds.], The freshwater budget of the Arctic Ocean. Kluwer.

MADIGAN, M. T., D. O. JUNG, C. R. WOESE, AND L. A. ACHENBACH. 2000. Rhodoferax antarcticus sp nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an Antarctic microbial mat. Arch. Microbiol. **173:** 269–277.

MASSANA, R., E. F. DELONG, C. PEDRÓS-ALIÓ, A. E. MURRAY, AND C. M. PRESTON. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl. Environ. Microbiol. **66:** 1777–1787.

MEON, B., AND R. M. W. AMON. 2004. Heterotrophic bacterial activity and fluxes of dissolved free amino acids and glucose in the Arctic rivers Ob, Yenisei and the adjacent Kara Sea. Aquat. Microb. Ecol. **37:** 121–135.

METHÉ, B. A., W. D. HIORNS, AND J. P. ZEHR. 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. Limnol. Oceanogr. **43:** 368–374.

MORRIS, R. M., M. S. RAPPE, S. A. CONNON, K. L. VERGIN, W. A. SIEBOLD, C. A. CARLSON, AND S. J. GIOVANNONI. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature **420:** 806–810.

OGORODOV, S., A. KAMALOV, G. ZUBAKIN, AND Y. GUDOSHNIKOV. 2005. The role of sea ice in coastal and bottom dynamics in the Pechora Sea. Geo-Mar. Lett. **25:** 146–152.

RAPPE, M. S., S. A. CONNON, K. L. VERGIN, AND S. J. GIOVANNONI. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature **418:** 630–633.

REIMNITZ, E., L. TOIMIL, AND P. BARNES. 1978. Arctic continental-shelf morphology related to sea-ice zonation, Beaufort Sea, Alaska. Mar. Geol. **28:** 179–210.

RETAMAL, L., W. F. VINCENT, C. MARTINEAU, AND C. L. OSBURN. 2007. Comparison of the optical properties of dissolved organic matter in two river-influenced coastal regions of the Canadian Arctic. Estuar. Coast. Shelf Sci. **72:** 261–272.

SCHLOSS, P. D., AND J. HANDELSMAN. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl. Environ. Microbiol. **71:** 1501–1506.

SCHULTZ, G. E., E. D. WHITE, AND H. W. DUCKLOW. 2003. Bacterioplankton dynamics in the York River estuary: Primary influence of temperature and freshwater inputs. Aquat. Microb. Ecol. **30:** 135–148.

SELJE, N., M. SIMON, AND T. BRINKHOFF. 2004. A newly discovered *Roseobacter* cluster in temperate and polar oceans. Nature **427:** 445–448.

SHIAH, F. K., AND H. W. DUCKLOW. 1997. Bacterioplankton growth responses to temperature and chlorophyll variations in estuaries measured by thymidine:leucine incorporation ratio. Aquat. Microb. Ecol. **13:** 151–159.

SINGLETON, D. R., M. A. FURLONG, S. L. RATHBUN, AND W. B. WHITMAN. 2001. Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl. Environ. Microbiol. **67:** 4374–4376.

SMITH, D. C., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3} H-leucine. Mar. Microb. Food Webs **6:** 107–114.

WALERON, M., K. WALERON, W. F. VINCENT, AND A. WILMOTTE. 2007. Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. FEMS Microbiol. Ecol. **59:** 356–365.

WELLS, L. E., M. CORDRAY, S. BOWERMAN, L. M. MILLER, W. F. VINCENT, AND J. W. DEMING. 2006. Archaea in particle-rich waters of the Beaufort Shelf and Franklin Bay, Canadian Arctic: Clues to an allochthonous origin? Limnol. Oceanogr. **51:** 47–59.

ZABALLOS, M., AND OTHERS. 2006. Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. FEMS Microbiol. Ecol. **56:** 389–405.

ZWART, G., B. C. CRUMP, M. AGTERVELD, F. HAGEN, AND S. K. HAN. 2002. Typical freshwater bacteria: An analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat. Microb. Ecol. **28:** 141–155.

Received: 24 April 2007
Accepted: 9 October 2007
Amended: 26 October 2007