Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean

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Abstract

The observed onset of climate change at high northern latitudes has highlighted the need to establish current baseline conditions in the Arctic Ocean, and has raised concern about the potential for the invasion and growth of biota that have warm temperature optima, such as cyanobacteria. In this study, we used 16S rRNA gene sequences as a molecular marker to evaluate the hypothesis that Arctic rivers provide a major inoculum of cyanobacteria into the coastal Arctic Ocean. Surface samples were collected along a transect extending from the Mackenzie River (Northwest Territories, Canada), across its estuary, to 200 km offshore at the edge of the perennial Arctic pack ice (Beaufort Sea). The highest picocyanobacteria concentrations occurred in the river, with concentrations an order of magnitude lower at offshore marine stations. The 16S rRNA gene clone libraries of five surface samples and five strains along this gradient showed that the cyanobacterial sequences were divided into eight operational taxonomic units (OTUs), six OTUs closely related to freshwater and brackish *Synechococcus* and two OTUs of filamentous cyanobacteria. No typically marine *Synechococcus* sequences and no *Prochlorococcus* sequences were recovered. These results are consistent with the hypothesis of an allochthonous origin of picocyanobacteria in the coastal Arctic Ocean, and imply survival but little net growth of picocyanobacteria under the present conditions in northern high-latitude seas.

Introduction

Picocyanobacteria are widely distributed in marine and freshwater environments from the tropics to the polar regions. They are present in extreme environments, including geothermal and sulphide-rich anoxic waters, as well as nutrient-poor open ocean waters (Stockner *et al.*, 2000). However, although cyanobacteria are abundant in Arctic and Antarctic lakes and rivers, they are often poorly represented in the adjacent polar seas (Vincent *et al.*, 2000). There is a decrease in cell concentration of oceanic picocyanobacteria with increasing latitude in the southern hemisphere (Marchant *et al.*, 1987; Fouilland *et al.*, 1999), and *Prochlorococcus*, the most abundant photosynthetic cell type in tropical oceans (Johnson *et al.*, 2006), appears to be largely absent from polar waters, although it has been found in the sub-Arctic as far north as latitude 61°N (Buck *et al.*, 1996).

Picocyanobacteria in the genus *Synechococcus* have been recorded amongst the microbial dominants in the Great Whale River that discharges into Hudson Bay in the eastern Canadian Arctic (Rae & Vincent, 1998), and we hypothesized that these phototrophic picoplankton are also likely to be abundant in the large rivers that discharge into the Arctic Ocean. The largest freshwater input on the North American side is the Mackenzie River, which enters the Beaufort Sea in the western Canadian Arctic. There is considerable interest in the extent of allochthonous inputs of dissolved and particulate materials into Arctic seas from its large riverine inputs, particularly in the context of climate change (Garneau *et al.*, 2006). The Mackenzie River is the largest single input of suspended sediment into the Arctic Ocean. It has been hypothesized recently that there may also be a large allochthonous input of sediment-attached prokaryotes (Wells *et al.*, 2006), although data from clone library analysis show that this is not the case for *Archaea* (Galand *et al.*, 2006). The input of phototrophic organisms by large Arctic rivers has not been addressed to date.

Current models as well as observations indicate the recent onset of rapid climate warming throughout the Arctic Basin (Mueller *et al.*, 2003; Stroeve *et al.*, 2005). Cyanobacteria

generally have growth optima under warmer conditions than ambient polar temperatures, and this component of northern aquatic ecosystems is likely to be highly responsive to climate change (Vincent & Pienitz, 1996). The objectives of this study were therefore to provide a baseline for the assessment of future change in the coastal Arctic Ocean, and to evaluate whether marine picocyanobacteria in this region could have an allochthonous origin. A related aim was to determine whether typically marine *Synechococcus* sequences were present, as was the case in an analogous study in the Southern Ocean (Wilmotte *et al.*, 2002). We addressed these objectives by way of a sampling transect during maximum open water conditions in the Beaufort Sea, from the Mackenzie River and its estuary to the edge of the perennial pack ice.

Materials and methods

Sampling

Water samples (Table 1) were collected in autumn 2002 (22 September to 14 October) in the Mackenzie Shelf and Franklin Bay areas (Table 1) from the icebreaker CCGS *Pierre Radisson*, or from a helicopter or Zodiac (small boat) deployed from the ship, as described in Garneau *et al.* (2006). The sampling encompassed a north–south transect composed of the river stations R1 and R2 (station R1 in the East Channel above Inuvik, 120 km from the river mouth, and station R2 in the Main Channel, 90 km above the river mouth), the two coastal stations Z1 and Z2 (inside the 20-m isobath located at *c.* 69°56′N) and offshore stations (42, 45, 49, 65, 66 and 83). Additional sampling was carried out at station 101 in Amundsen Gulf, station 24 north of Cape Bathurst and station 12 in Franklin Bay. Further hydrographic details for this cruise are given in Garneau *et al.* (2006).

Samples for epifluorescence analysis were prefiltered through a Nuclepore polycarbonate membrane (pore diameter, 3 μm); this was followed by the filtration of 10-mL (river) and 50-mL (offshore ocean) samples through Anapore filters (diameter, 25 mm; pore size, 0.2 μm) within 4 h of collection. The filters were mounted on glass slides with Aqua-Polymount (Polysciences), sealed and stored frozen until analysis at a magnification of ×1000 under a Zeiss fluorescence microscope. Cells that fluoresced bright red–orange under green light were counted as picocyanobacteria, and those that fluoresced red under blue and green light were counted as picoeukaryotes (Lovejoy *et al.*, 2002). At least 200 picophytoplankton cells were counted in each sample. Cell counts were converted to biovolumes assuming average cell diameters of 0.8 μm for picocyanobacteria and 2 μm for picoeukaryotes.

Representative samples across the gradient of river, inshore and offshore sites were used for the construction of clone libraries: R1, 0 m; Z2, 0 m; 42, 3 m; 42, 59 m; 49, 2 m; 83, 2 m. These samples were prefiltered through a Nuclepore

Table 1. Picophytoplankton cell concentrations in the surface waters of the Mackenzie River and Beaufort Sea, and the percentage contribution of picocyanobacteria to the total picophytoplankton cell concentrations

Station	Latitude N; longitude W	Salinity (pss)	Temperature (°C)	Halocline depth (m)	Picophytoplankton (10^3 cells mL^{-1})	Picocyanobacteria (%)
Mackenzie River						
R1	68°21′; 133°43′	0.2	2.9	–	4.18	84
R2	68°41′; 143°14′	0.2	3.9	–	8.45	79
Mackenzie estuary						
Z1	69°30′; 133°14′	24.5	−0.04	–	0.61	65
Z2	69°31′; 133°14′	25.4	−0.54	–	3.99	58
Beaufort Shelf						
65	70°9′; 133°3′	26.9	−0.32	10	0.87, 1.25	46, 22
65R	70°9′; 133°31′	26.5	−0.45	10	2.26	71
66	70°51′; 133°39′	20.3	−0.61	8	3.35, 1.14	63, 43
83	71°16′; 128°30′	25.2	−1.34	14	2.17	54
24	70°47′; 127°34′	22.7	0.12	6	1.30, 0.86	57, 25
Ice edge						
42	71°32′; 131°53′	25.3, 31.9	−1.29, −1.15	16	0.95, 0.57	59, 14
45	71°35′; 132°54′	26.4	−1.37	20	0.56	71
49	71°27′; 133°4′	26.3	−0.67	20	0.36, 1.91	63, 16
Amundsen Gulf						
101	68°23′; 112°46′	23.9	−0.81	18	3.73, 1.03	35, 24
Franklin Bay						
12	69°50′; 126°10′	23.4	−1.3	30	2.22	64

Where two values are given for a station, the first is for a sample within 5 m of the surface, and the second is for a sample at 1% of the surface irradiance. The halocline depth was defined as the uppermost depth at which the measured salinity increased by 0.5 practical salinity scale (pss) above the values in the surface mixed layer.

Maps of station positions are given in Garneau *et al.* (2006) and Wells *et al.* (2006).

polycarbonate membrane (pore diameter, 3 μm), followed by filtration of 0.5–2 L samples through Supor filters (diameter, 47 mm; pore size, 0.2 μm) (Pall). The filters were transferred to centrifuge tubes containing 2 mL of sterile lysis buffer (Wilmotte *et al.*, 2002), stored frozen at −80 °C and subsequently analysed at the University of Liège, Liège, Belgium.

Isolation of strains

For five samples, cultures were started by inoculation in two liquid culture media: one-tenth BG11 made up in deionized water (as detailed in Vézina & Vincent, 1997) and one-tenth BG11 made up in artificial seawater. The growth of cyanobacteria was observed only in the freshwater BG11 medium. Five strains were isolated from samples R2 (R2S1), Z1 (Z1S1), 49 (49S1), 65 (65S1) and 65R (a repeat sampling at station 65 at the end of a 24-h period at that station) (65RS1). For all strains, sequencing of the 16S rRNA gene was performed with primer 16S1092R (Hrouzek *et al.*, 2005).

DNA extractions and amplifications

Total DNA was extracted from the filters using a modified hot phenol method (Wilmotte *et al.*, 2002). A negative control was made by extracting lysis buffer without a filter. DNA amplifications were performed in 50-μL reaction volumes containing 5 μL of 10 × SuperTaq Plus buffer, 5 μL of bovine serum albumin (BSA) (10 mg mL^{-1}), 200 μM of each dNTP (MBI Fermentas, Germany), 25 pmol of each primer 359F (Nübel *et al.*, 1997) and 23S30R (Taton *et al.*, 2003) and 1 U of SuperTaq Plus Polymerase (HT Biotechnology, UK). Amplification was performed using the I-cycler (BIO-RAD), with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation (94 °C, 45 s), annealing (54 °C, 45 s) and extension (68 °C, 2 min), with a final extension step at 68 °C for 7 min. The amplified products were electrophoretically separated in 1% (w/v) agarose gel at 120 V for 1.5 h in Tris-Acetate-EDTA (TAE) buffer, and visualized with UV light after staining in ethidium bromide (0.5 μg mL^{-1}).

Construction of clone libraries of the 16S rRNA gene and sequencing

Cloning was performed using the TOPO TA Cloning Kit (Invitrogen BV, the Netherlands) according to the manufacturer's instructions. The PCR products from three independent reactions were mixed and purified with Quantum Prep PCR Kleen Spin Columns (BIO-RAD). Because the PCRs were performed with a proofreading DNA polymerase, a single adenine base was added to the 3′ ends of the PCR products using the A-Addition Kit (Qiagen, Germany). The recombinant clones were purified by streaking on to solid selective media. Plasmid DNAs were extracted with the Quantum Prep Plasmid Miniprep (BIO-RAD) and sequenced using primer 16S1092R by GenomeExpress (France) on an ABI PRISM system 377.

Sequence alignment and analysis

The 279 partial sequences obtained were corrected manually and aligned. The clone sequences were checked for the presence of chimera with CHIMERACHECK [available on the website Ribosomal DataBase Project II (RDP-II): http://rdp8.cme.msu.edu/html]. Bacterial and plastid sequences, identified by a BLAST search, were handled separately from the cyanobacterial sequences. For the 179 cyanobacterial sequences, a distance matrix was constructed by the software DNADIST using the correction of Jukes and Cantor in the PHYLIP package (http://evolution.genetics.washington.edu/phylip.html). The sequences sharing more than 97% similarity were grouped into operational taxonomic units (OTUs) using the software DOTUR (Schloss & Handelsman, 2005) and the option furthest neighbour [Canadian Arctic Shelf Exchange Study (CASES) OTUs A–H]. One representative sequence per clone library per OTU was selected and used for further analysis. The 20 most similar sequences to each representative were obtained using the tool Sequence Match on RDP-II 9.41 (http://rdp.cme.msu.edu/) and aligned using CLUSTALW in BIOEDIT (Hall, 1999). When quasi-identical sequences came from the same sampling place, only the closest related sequence was kept in the tree. Only strain sequences longer than 510 bp were selected and *Gloeobacter violaceus* PCC8105 was added as outgroup. Six hundred and eighty-one positions (*Escherichia coli* positions 370–1060) were used for the phylogenetic analysis. Genetic distances were calculated and corrected for multiple mutations by the method of Jukes and Cantor. Indels were excluded. A distance tree was constructed by the neighbour-joining method of Saitou & Nei (1987), and a bootstrap analysis was performed with 500 replicates as implemented in the software package TREECON for Windows (Van de Peer & De Wachter, 1994). The OTUs were again calculated as before, but including all the sequences, and indicated on the tree topology. The threshold around 97% was indicated by Stackebrandt & Göbel (1994) as corresponding to a limit for the bacterial species definition; below, the sequences belong to different species and, above, they may or may not belong to the same species.

Results

Distribution of picocyanobacteria

Synechococcus-like picocyanobacteria were recorded by epifluorescence counts in the surface waters at all stations, with highest concentrations in the Mackenzie River

(3503 cells mL^{-1} at R1 and 6713 cells mL^{-1} at R2) and concentrations an order of magnitude lower at the offshore sites near the Arctic pack ice (225 cells mL^{-1} at station 49 and 560 cells mL^{-1} at station 42). The highest concentrations were generally in the euphotic zone and decreased with depth, but an increase in cell concentration was sometimes observed near the bottom of the water column (Fig. 1). Picoeukaryotes were also present at all stations. These populations had comparable densities to the picocyanobacteria in the surface waters, but, because of their larger cell size (on average *c.* 2 μm diameter versus 0.8 μm diameter for the cyanobacteria), their estimated biovolume concentrations were an order of magnitude greater than those of the cyanobacteria (Fig. 1). The analyses for several depth profiles showed that the percentage contribution of cyanobacteria to the total picophytoplankton counts was much greater in the surface waters than beneath the halocline in more saline waters at depth (Table 1). This difference in percentage contribution was highly significant (paired *t*-test, $t = 5.13$, d.f. = 5, $P = 0.004$).

Cloning and sequencing of the 16S rRNA gene

Between 19 and 93 clone sequences were obtained for the five surface samples and five strain sequences from samples R2, Z1, 49, 65 and 65R. The nonidentical sequences were submitted to GenBank and received accession numbers DQ524823–DQ524845, DQ851567, and DQ989218–DQ989224. Using CHIMERACHECK software, the sequence of clone R1-42, that is the only clone of CASES OTU F, appeared to be a chimera with a break point around position 870 (*E. coli* position). The first segment was 98.6% similar to *Synechococcus* PS845 (AF448070), whereas the second part belonged to CASES OTU C, and thus did not represent additional diversity. For further analysis, only the first fragment of 490 positions was used. It is the unique clone representative of CASES OTU F and the corresponding organisms do really exist in our sample.

DOTUR analysis revealed that 179 (174 sequences of clones and five additional sequences of strains) of the 279 obtained sequences were clustered into eight cyanobacterial OTUs. In addition, 62 sequences were 95–99% similar to sequences of

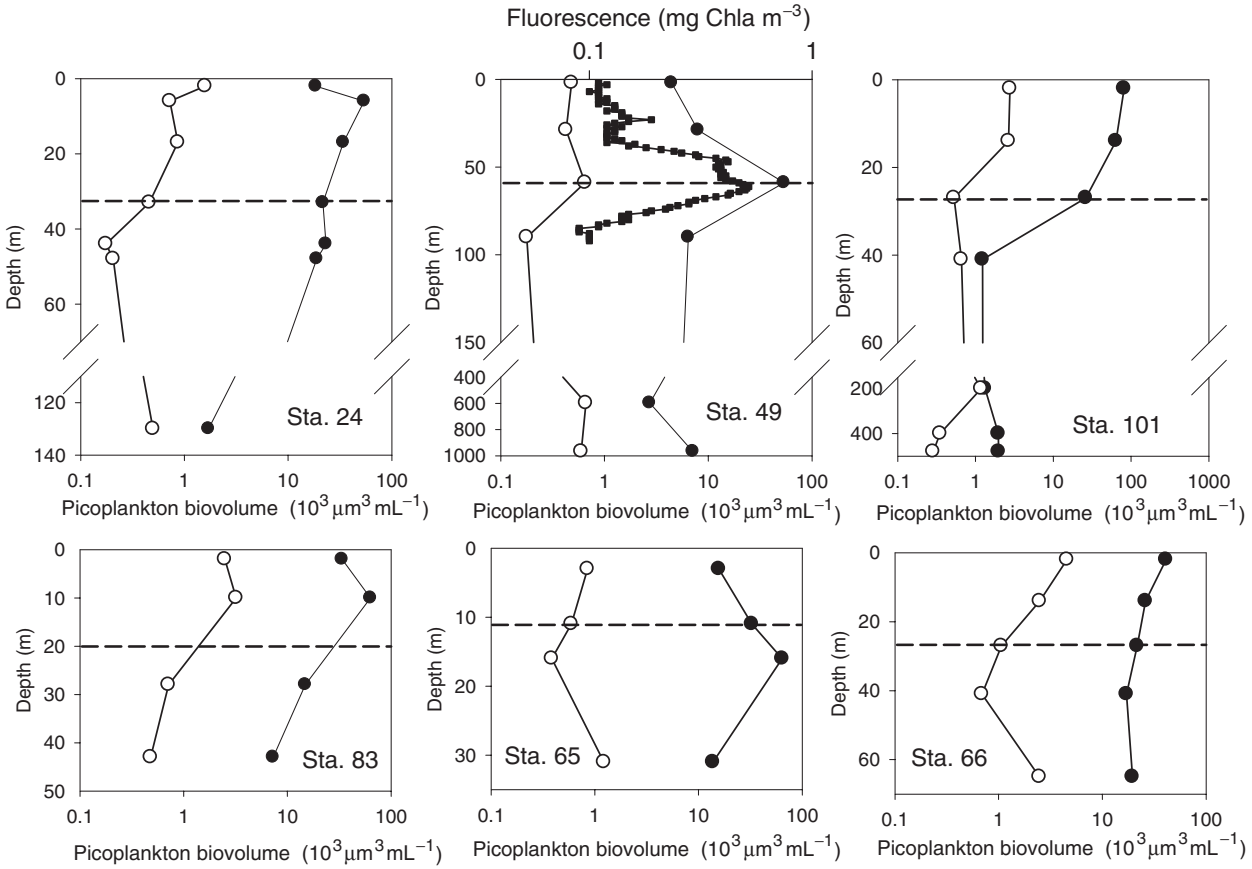

Fig. 1. Depth profiles of picocyanobacteria (open circles) and picoeukaryotes (filled circles) at three deep-water stations in the Beaufort Sea and three shallow-water stations over the Beaufort Shelf. The cell concentrations are expressed in terms of biovolume on a logarithmic scale. The broken line marks the depth of 1% of surface irradiance. The distribution of phytoplankton as measured by *in vivo* chlorophyll fluorescence is also given for station 49 (small filled squares), showing the deep chlorophyll maximum that was sampled for one of the clone libraries.

plastids from three algal families (Table 2). In a clone library from station 42 at a depth of 59 m, only bacterial sequences were found, specifically three groups affiliated to the *Chlorobium/Fibrobacter* lineage (Gordon & Giovannoni, 1996). This probably reflects a low abundance or absence of cyanobacterial DNA, with the cyanospecific primers annealing on the more abundant bacterial DNA.

Inside the cyanobacterial sequences, six OTUs were obtained from the river station and five from the estuary. There was a shift between the most abundant groups, from OTU D (48%) in the river to OTU A (67%) in the estuary. Offshore, only clones from OTU A could be found, but in lower proportions than plastid sequences. The representative of OTU D was 99% similar to a clone from a Dutch lake and a *Synechococcus* from an Austrian lake (Zwart *et al.*, 2002; Crosbie *et al.*, 2003). It was positioned in one OTU containing both freshwater and brackish strains. OTU A's representative was 99% similar to a *Synechococcus* from a German lake (Ernst *et al.*, 2003). The five strains isolated belonged to different *Synechococcus* OTUs and, in one case, strain 65RS1, the sequence was distinct from the clones, thus adding additional diversity (OTU E). The river isolate R2S1 was identical to the sequence of *Synechococcus* sp. MW99B6 from Lake Mondsee, Austria, in OTU F that contains sequences from both freshwater and marine habitats. The 16S rRNA gene sequence of strain Z1S1 was 99% similar to *Synechococcus* sp. BS5 isolated from brackish waters of the Baltic Sea, and belongs to OTU C with other brackish and freshwater strains. Strain 49S1 had a sequence that was identical to *Synechococcus* MW73B4, and belonged to OTU A, which only contains sequences from freshwater habitats. Clones belonging to OTU A were also retrieved in station 49. The 65S1 sequence was 99.7% similar to a number of strains in OTU D, including *Cyanobium* sp. PCC6904 and *Synechococcus* PCC7009, B0984127, B08806, B08801, BS20, BGS171 and PCC6307, that come from brackish and coastal marine biotopes. Interestingly, two clones from coastal Antarctic lake microbial mats (H-D20 and RJ105) also share the same sequence similarity. The sequence from strain 65RS1 was identical to *Synechococcus* sp. PS840 isolated from the marine coast of Russia and to the clone H-D13 from microbial mats in Lake Heart, Larsemann Hills, east Antarctica. Amongst the filamentous cyanobacteria, OTU G sequences were identical to *Planktothrix* CYA127 sequences from a Finnish lake, clearly a freshwater planktonic genus. OTU H's representative was identical to *Limnothrix redekei* CCAP 1443/1 and was similar (99%) to the *Phormidium* E18 isolated from Meretta Lake, in the Canadian Arctic, again two freshwater strains. In offshore stations, mainly 16S rRNA gene sequences from plastids of three algal families (*Prasinophyceae*, *Chlorophyceae*, *Cryptophyceae*) were found (Table 2). Fifty-nine plastid sequences were found in the marine samples, whereas only three sequences (*Prasinophyceae* and *Chlorophyceae*) were found in the clone library of the Mackenzie River estuary and none in the river (Table 2).

Discussion

Cyanobacterial abundance

The picophytoplankton at all stations contained picocyanobacteria, but picoeukaryotes were also an important component. The latter populations had comparable densities to the picocyanobacteria in the surface waters, but, because of their larger cell size (on average *c.* 2 μm diameter versus 0.8 μm diameter for the cyanobacteria), their estimated biovolume concentrations were an order of magnitude greater than those of the cyanobacteria (Fig. 1). The

Table 2. 16S rRNA gene-based operational taxonomic units (OTUs), and the numbers of sequences retrieved for each location on the gradient

		River		Estuary		Sea				
						49*,†	42*	65†	65R†	83*
Affiliation	CASES group	R1*	R2†	Z1†	Z2*	2 m	3 m	3 m	2 m	2 m
Cyanobacteria	OTU A	14			46	4	6			3
	OTU B	19			4					
	OTU C	11		1	9			1		
	OTU D	45			8					
	OTU E								1	
	OTU F	1	1							
	OTU G				2					
	OTU H	3								
Plastids	*Prasinophyceae*				2	29	16			12
	Cryptophyceae									1
	Chlorophyceae				1					1

CASES, Canadian Arctic Shelf Exchange Study.
*Used for the construction of a clone library.
†Samples were sources of isolated strains.

contribution of picocyanobacteria to the total picophytoplankton biovolume in the surface waters (Table 1) dropped from $22.4 \pm 3.6\%$ in the river to $9.3 \pm 1.6\%$ in the estuary, $8.7 \pm 3.2\%$ on the shelf and $10.5 \pm 2.5\%$ in the ice edge surface samples. The analyses for several depth profiles showed that the contribution of cyanobacteria to the total picophytoplankton counts was significantly greater in the surface waters than beneath the halocline in more saline waters at depth (Table 1). Our sequencing of 16S rRNA genes from marine sites as well as freshwater samples confirmed that picocyanobacteria were present at all locations, although such DNA could potentially come from nonliving cells. Analysis by fluorescence microscopy showed the presence of intact, fluorescent picocyanobacteria throughout the region. Furthermore, culture enrichments showed that at least a subset of these cells was viable: five picocyanobacterial strains were isolated into culture from our samples: two of these strains came from marine station 65, and one from station 49, well offshore.

Previous studies in the Arctic Ocean have drawn attention to the generally low concentrations of picocyanobacteria in these waters and their low contribution to planktonic biomass stocks and carbon fluxes. Our results show somewhat higher concentrations in the Beaufort Sea than have generally been observed elsewhere in Arctic seas; however, our biovolume analyses are consistent with earlier observations (Gradinger & Lenz, 1995) and more recent analyses (Lovejoy *et al.*, 2007), showing the much greater importance of picoeukaryotes in Arctic marine ecosystems.

Cyanobacterial molecular diversity

Phylogenetic analysis of cyanobacterial 16S rRNA gene sequences revealed a high similarity (at least 97%) of our sequences with temperate cyanobacteria isolated from freshwater, brackish and coastal marine biotopes. These Arctic cyanobacterial sequences did not form clusters separated from the sequences of warmer regions (Fig. 2). As a result of the conservation of 16S rRNA gene sequences, we cannot expect the organisms in one OTU to share identical physiological and genomic properties, as shown for marine *Synechococcus* strains differing by less than 3% (Johnson *et al.*, 2006). However, such OTU members should share a common genomic core, and we compare here the distributions of our clones and strains with those from GenBank. *Synechococcus* OTUs A and B only contain GenBank strain sequences from freshwater habitats, although we observed clone sequences of OTU A in the estuary and in offshore stations, and isolated one such strain from station 49 offshore. Therefore, it seems likely that this strain is halotolerant. In *Synechococcus* OTUs C–F, the origin of sequences includes temperate freshwater lakes (e.g. in Austria, Germany and Japan), lakes and ponds in polar regions (Arctic, Antarctic), and brackish and coastal marine habitats. Marine sequences came from strains isolated from the North and Baltic Seas, Atlantic and Pacific Oceans or Russian and American coasts. Although we applied a protocol (Wilmotte *et al.*, 2002) that was used successfully in the Southern Ocean to retrieve typical marine *Synechococcus* sequences of subcluster 5.1 (Fuller *et al.*, 2003), only OTU F included such sequences (WH8101 and WH7803), but with a rather low similarity level to the Arctic members (around 97%). In contrast with unicellular taxa, the filamentous taxa found in the river and estuary were typically freshwater taxa, and likely to have been flushed out of their normal habitat by the river current.

In several instances (OTUs A–F), our CASES sequences inserted into groups already recognized by Ernst *et al.* (2003) and Crosbie *et al.* (2003).

Origin of cyanobacteria in the Beaufort Shelf region

Several lines of evidence indicate that the picocyanobacterial populations observed in the western Canadian Arctic Ocean are largely derived from allochthonous inputs of microbiota from the Mackenzie River and other nearby inflows, such as the Horton River. This has been hypothesized for *Archaea* on the basis of cell count data (Wells *et al.*, 2006), but has not been supported by subsequent molecular analyses (Galand *et al.*, 2006). This study confirmed the high concentrations of picocyanobacteria entering the Beaufort Shelf region via the Mackenzie River, and their persistence offshore. If these values are representative, the total annual discharge of this river ($330\,km^3$) would provide an inoculum of the order of 10^{18} picocyanobacterial cells into the coastal ocean each year. Seasonal variation should also be considered, but this first-order calculation illustrates the potential magnitude of importance of this input. The surface freshwater influence extends well offshore, at least to the edge of the Arctic ice pack, as shown by oceanographic markers, such as salinity and temperature (Garneau *et al.*, 2006); however, this need not imply the advection of viable riverine cells. The most compelling evidence of a freshwater origin of the cyanobacteria in this coastal marine ecosystem comes from the molecular analyses. We observed 12 clone sequences in the offshore waters, as well as the one strain sequence from station 49, that clearly belong to freshwater OTU A. Moreover, OTU A was dominant in the clone library of the estuarine samples and was also present in the river sample.

The epifluorescence count data indicate relatively high concentrations of picocyanobacteria in the Mackenzie River. These organisms were also abundant in the estuary, but decreased dramatically in the offshore samples. A low concentration or even absence of picocyanobacteria has

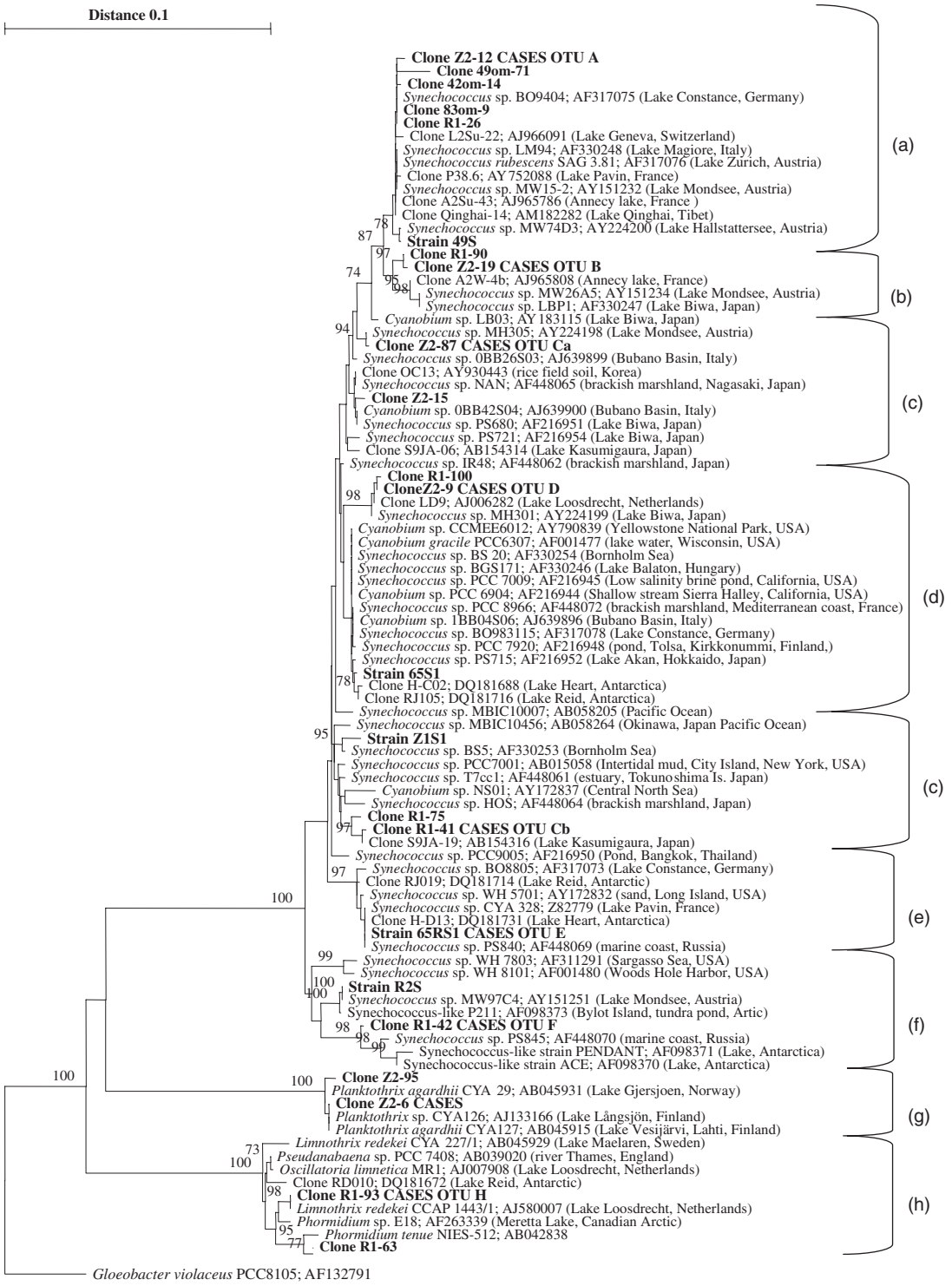

Fig. 2. Distance tree based on partial 16S rRNA gene sequences (681 positions) showing the relationships of Arctic sequences with those from databases. A tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) using the software TREECON (Van de Peer & De Wachter, 1994). The significance of the nodes was evaluated by bootstrap analysis with 500 replicates. The bootstrap values equal or higher than 70% are shown next to the node concerned. Sequences from this study are indicated in bold, and identified as Canadian Arctic Shelf Exchange Study operational taxonomic units.

already been observed in the Arctic Ocean by molecular methods (Brown & Bowman, 2001; Bano & Hollibaugh, 2002) and microscopic counts (Melnikov *et al.*, 2002), as well as in the Southern Ocean by flow cytometry (Fouilland *et al.*, 1999) and a molecular approach using the same protocol as in this study (Wilmotte *et al.*, 2002). However, in most molecular studies in which Arctic bacterioplankton have been analysed, universal primers for bacteria have been used (Bano & Hollibaugh, 2002), and these do not provide an accurate guide to the cyanobacteria present. In this study, we used primers targeting the cyanobacteria specifically, which give much better results than more general protocols. For example, in a single sample from an Antarctic lake (Lake Fryxell, McMurdo Dry Valleys), bacterial universal primers yielded only two cyanobacterial sequences, whereas the use of a specific primer gave a high cyanobacterial diversity (Brambilla *et al.*, 2001; Taton *et al.*, 2003).

The low abundance of cyanobacteria in polar seas contrasts with their widespread distribution in polar freshwater environments. Their poor performance in Arctic seas and the Southern Ocean may be a result of their slow, temperature-depressed growth rates that are unable to keep pace with grazing by the many species of nanoflagellates, ciliates and other fine-particle collectors in marine environments (Tang *et al.*, 1997; Vincent *et al.*, 2000). Marine ecosystems, such as the coastal Arctic Ocean, have a much broader functional array of microbial grazers than freshwaters, including appendicularians, heliozoans, foraminiferans and tintinnids, as well as diverse communities of heterotrophic and mixotrophic flagellates (Lovejoy *et al.*, 2006). The persistence of picocyanobacteria in the low population densities observed offshore suggests a high tolerance (but little net growth) in adverse conditions, and perhaps a decrease to cell concentrations that are below the threshold for regular encounter by microbial food web grazers. Our results provide an interesting comparison with observations by Gradinger & Lenz (1995) in the Greenland Sea and elsewhere in the Arctic Ocean. They found that picocyanobacteria occurred in high concentrations in northward-flowing Atlantic water (up to 5470 cells mL^{-1}, a similar magnitude to that in the Mackenzie River), and concluded that the advective input from this source, combined with the high survival potential of these cells, controlled the variable abundance of picocyanobacteria in the Greenland Sea. Our results similarly support advection (but from freshwater in this western Arctic region) and a lack of net growth, yet survival.

Gradinger & Lenz (1995) noted that picocyanobacteria were not confined to particular depths. Similarly, in this study, we found picocyanobacteria throughout the water column, although generally in higher abundance in the surface mixed layer. Our molecular analyses, however, were restricted to surface samples, and it may be that other transport mechanisms contribute to the deeper picocyanobacterial communities, for example the advection of Pacific water into the Arctic Basin. The presence of fluorescent cells, even at around a depth of 1000 m (station 49, Fig. 1), provides further evidence of the persistence of the cellular and pigment integrity of cyanobacteria despite adverse conditions that preclude phototrophic growth.

Plastids

We also obtained plastid sequences that were 95–99% similar to *Prasinophyceae* [*Pyramimonas parkae* (AF393608), *Mantoniella squamata* (X90641) and clone OCS162 (AF001659)], *Chlorophyceae* (*Koliella spiculiformis*) and *Cryptophyceae* [*Gemminifera cryophila* (AB073111)]. Except for *Koliella spiculiformis*, which grows in freshwater or on the surface of alpine glaciers (Yoshimura *et al.*, 1997), and has also been found in a highly eutrophic and acidic lake (Lessmann *et al.*, 2000), the other two algal groups are marine taxa that are found in polar and temperate oceans (Rappé *et al.*, 1998; Díez *et al.*, 2001, 2004; Lovejoy *et al.*, 2002, 2006). Three plastid sequences were found in the clone library of coastal station Z2, but most of such sequences (59 of 62) were for the offshore stations (Table 2). In both cases, the stations have quite similar salinities and temperatures (Table 1). These results are in agreement with chlorophyll *a* analysis of the total community and of the picophytoplankton fraction from these samples. Total chlorophyll *a* concentrations in the surface waters were low, but twofold higher in marine- than in river-influenced stations (Garneau *et al.*, 2006).

The microscopic counts of the picophytoplankton in river and marine stations revealed that the number of picocyanobacterial cells per millilitre was higher than the number of picoeukaryotic cells in river and coastal stations and almost similar in offshore stations (Table 1). However, the biovolume concentrations were an order of magnitude higher for the picoeukaryotes. The dominance of plastid sequences in samples from offshore stations is probably a result of the differences in copy number of 16S rRNA genes between cyanobacteria and eukaryotic cells. Although most unicellular cyanobacterial genomes contain one to two 16S rRNA gene copies, eukaryotic algae may contain several plastids, each with multiple genome copies. For the chrysophyte *Ochromonas*, total genome numbers ranging from seven to more than 1000 per cell have been recorded (Maguire *et al.*, 1995).

Conclusions

Earlier observations have drawn attention to the striking dichotomy between the importance of picocyanobacteria in polar lakes and rivers and their poor representation in the polar oceans (Vincent, 2000). In this study, we found

picocyanobacteria at all stations and depths, although generally in low concentrations. Our ecological and culture observations suggest that they were largely derived from allochthonous sources, specifically the Mackenzie River. The molecular analyses strongly support this conclusion: no typically marine genotypes were detected in our clone libraries, and all phylotypes were affiliated most closely with freshwater or brackish water strains.

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