Metabolic diversity of heterotrophic bacterioplankton over winter and spring in the coastal Arctic Ocean

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Summary
Metabolic diversity of heterotrophic bacterioplankton was tracked from early winter through spring with Biolog Ecoplates under the seasonally ice covered arctic shelf in the Canadian Arctic (Franklin Bay, Beaufort Sea). Samples were taken every 6 days from December 2003 to May 2004 at the surface, the halocline where a temperature inversion occurs, and at 200 m, close to the bottom. Despite the low nutrient levels and low chlorophyll a, suggesting oligotrophy in the winter surface waters, the number of substrates used (NSU) was greater than in spring, when chlorophyll a concentrations increased. Denaturing gradient gel electrophorisis analysis also indicated that the winter and spring bacterial communities were phylogenetically distinct, with several new bands appearing in spring. In spring, the bacterial community would have access to the freshly produced organic carbon from the early phytoplankton bloom and the growth of rapidly growing specialist phenotypes would be favoured. In contrast, in winter bacterioplankton consumed more complex organic matter originated during the previous year’s phytoplankton production. At the other depths we tested the NSU was similar to that for the winter surface, with no seasonal pattern. Instead, bacterioplankton metabolism seemed to be influenced by resuspension, advection, and sedimentation events that contributed organic matter that enhanced bacterial metabolism.

Introduction
Arctic Ocean ecosystems are particularly sensitive to climate changes, for example small temperature differences may have large effects on the thickness and extent of sea ice (Smetacek and Nicol, 2005). Snow and ice cover are biologically important, influencing light availability and the timing of phytoplankton production in the Arctic (Carmack et al., 2004). Primary production in oceanic areas constitutes the main organic source for bacterioplankton growth (Bird and Kalf, 1984; Cole et al., 1988), mainly owing to direct bacterial use of dissolved compounds released by growing phytoplankton cells (Baines and Pace, 1991). However, recent evidence suggests that bacteria do not respond immediately to phytoplankton production in cold waters (Bird and Karl, 1999; Pomeroy and Wiebe, 2001; Duarte et al., 2005; Morán et al., 2006). The Arctic coastal ocean is also influenced by the input of large rivers and riverine inputs may also contribute significant amounts of terrigenous organic matter that bacterioplankton could use for substrate (Emmerton, C.A., Lesack, L.F.W. and Vincent, W.F., in press).

The only seasonal study to date of Arctic bacterioplankton under the ice was over the Central Arctic Ocean, where bacterial biomass and production in the upper water column responded strongly to phytoplankton growth in summer and activity was restricted to the upper 60 m (project SHEBA, Sherr and Sherr, 2003; Sherr et al., 2003). This Central Arctic Ocean study measured the bulk production of bacterioplankton, but it did not analyse the possible organic substrates. Moreover, it was conducted from an ice camp that drifted with the pack ice, making it difficult to separate changes due to seasons from those due to different water masses. Despite this pioneering effort, winter bacterial activity under the Arctic ice remains largely unknown, and a detailed view into the seasonal changes in bacterial utilization of carbon sources under ice cover is lacking. The CCGS Amundsen was frozen into first-year pack ice in Franklin Bay (Beaufort Sea) from December 2003 to May 2004 thus providing an excellent platform for detailed sampling throughout the winter. This cruise was part of the Canadian Arctic Shelf Exchange Study (CASES).
Garland and Mills (1991) first used the Biolog plates to evaluate the metabolic potential of a whole microbial community, as they allow simultaneous detection of the utilization of a large number of carbon sources. Biolog plates have found application for the assessment of microbial metabolic diversity in freshwater (Grover and Chrzanowski, 2000; Sinsabaugh and Foreman, 2001), estuarine (Schultz and Ducklow, 2000) and, to a lesser extent, marine bacterioplankton (Holibaugh, 1994; Jellet et al., 1996). The Biolog-Ecoplate contains 31 carbon sources in triplicate (Insam, 1997) and has provided information, not available using other techniques, on the functional diversity of bacterioplankton in marine environments such as western Arctic (Sala et al., 2005a) and the Mediterranean Sea (Sala et al., 2005b; 2006b) and can also be used to estimate bacterial use of dissolved organic nitrogen compounds in seawater (Sala et al., 2006a).

We used the Biolog plates as a way to estimate the metabolic diversity of the heterotrophic bacterioplankton through winter and at the onset of the productive spring season, when phytoplankton were likely to become the main source of dissolved organic matter. Our main aim was to determine changes in the carbon sources potentially used by bacterioplankton during winter and spring at three different layers within the ice-covered water column of the Arctic shelf. Additionally, we also followed the composition of the bacterial community in the surface with denaturing gradient gel electrophoresis (DGGE) in order to assess if eventual changes in metabolic diversity were related to changes in phylogenetic diversity.

Results

Out of the 31 substrates tested, 21 were used by communities in the Arctic water samples. Ten substrates did not show any positive result: the two amines (putrescine and phenylethylamine); three carboxylic acids (α-ketobutyric, D-malic and D-glucosaminic acid), five carbohydrates (β-methyl-D-glucoside, D,L-α-glycerol phosphate, α-D-glactose, D-galactonic acid γ-lactone and i-erythritol). Generally, polymers and carbohydrates were the categories with higher number of positive results (Fig. 1). The most often used substrates (more than 25 positives out of 75 samples) were Tween 80 (a trademark for polysorbate 80), glycogen, D-cellobiose, D-mannitol, N-acetyl-D-glucosamine, and glycyl-L-glutamic acid.

Although the differences were not significant, surface bacteria utilized a greater number of substrates (number of substrates used, NSU) than bacteria at deeper depths. Likewise, most of the substrates had a higher number of positive results at the surface, but this was only significant for three of them (one-way ANOVA): pyruvic acid methyl ester \( (F = 5.26, P = 0.01) \), D-mannitol \( (F = 4.12, P = 0.02) \) and D-cellobiose \( (F = 3.17, P = 0.03) \). Two substrates, Tween 80 and N-acetyl-D-glucosamine showed more positive results at the bottom than at other layers (not significantly different).

Chlorophyll \( a \) concentrations at the surface varied between 0.02 and 0.11 \( \mu \text{g} \) l\(^{-1} \) from December to 15 March. Then, as the ice started thinning, chlorophyll \( a \) increased rapidly, reaching values from 0.20 to 0.36 from 17 April to the end of the study, 21 May (Fig. 2). Bacterial abundance did not show a clear seasonal pattern and varied between \( 1.5 \times 10^{5} \) and \( 5.2 \times 10^{5} \) bacteria ml\(^{-1} \) over the period of study. There was a distinct seasonal pattern in chlorophyll concentrations. Initially, from December to the end of
March, chlorophyll a concentrations were low and started to increase during the first 2 weeks of April. Higher chlorophyll concentrations were present from 17 April to the end of the period of study, 21 May. The DGGE dendogram showed that different bacterial assemblages were present during these two periods. We chose this marked change in composition to separate biologically definable winter and spring periods. The number of substrates used over the entire period showed a trend opposite to that of chlorophyll a concentrations. In winter, the NSU varied between 1 and 12 (mean 6.1), and in spring this was between 1 and 3 (mean 1.9). Utilization of all categories of substrates was low in spring, but especially that of carboxylic acids which did not show any positive results during this period.

Chlorophyll a concentrations at the temperature inversion (TI) depth were even lower than at the TI depth (0.01–0.05 μg L⁻¹) and there was no clear seasonal pattern (Fig. 3). As was the case at the TI depth, bacterial abundances had no seasonal signal. The highest values over the study period and among all three layers were from this deep region, with up to 5.6 × 10^5 in January. There was no pattern in the NSU, but the greatest values were found during a short period between February and March, similar to that of the TI layer.

The eight substrates with greatest number of positive results over the period of study were chosen for a detailed analysis (Fig. 5). At the surface, most of the substrates were used quite regularly in winter and only occasionally in spring. Five substrates showed significantly more positive results in winter than in spring: pyruvic acid methyl ester (P = 0.001), glycogen (P = 0.005), Tween 40 (P = 0.02), α-cyclodextrin (P = 0.03), and N-acetyl-D-glucosamine (P = 0.04). A very different pattern was found for both the TI and the bottom layers, with a period of positive results concentrated in 5 weeks from the third week of February to the second of March and only a few positive results for the rest of the period. This period of positive results was found for most of the substrates. However, different patterns were also observed for pyruvic acid methyl ester, used only during December.

At the deepest layer just above the bottom, chlorophyll a concentrations were even lower at the TI depth (0.01–0.05 μg L⁻¹) and there was no clear seasonal pattern (Fig. 4). As was the case at the TI depth, bacterial abundances had no seasonal signal. The highest values over the study period and among all three layers were from this deep region, with up to 5.6 × 10^5 in January. There was no pattern in the NSU, but the greatest values were found during a short period between February and March, similar to that of the TI layer.

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and N-acetyl-d-glucosamine, preferentially used towards spring. Taking all depths together, most of the substrates were used more in winter than in spring. A t-test revealed significant differences for pyruvic acid methyl ester ($t = 3.61, P = 0.001$), glycogen ($t = 3.11, P = 0.003$), α-cyclodextrin ($t = 2.83, P = 0.006$), glycyl-glutamic acid ($t = 2.84, P = 0.006$), Tween 40 ($t = 2.22, P = 0.029$), D-cellobiose ($t = 2.22, P = 0.030$), D-xylose ($t = 2.16, P = 0.03$), D-mannitol ($t = 2.07, P = 0.04$). The only substrate that showed higher utilization in spring was Tween 80.

Bacterial community composition was tracked using DGGE. The image and cluster analysis of a DGGE gel (Fig. 6). The first cluster defined the winter samples and the second cluster the spring. The spring samples had new bands that did not appear in winter. Denaturing gradient gel electrophoresis gels were also carried out for TI and bottom samples. However, cluster analyses did not show a clear grouping (data not shown).

**Discussion**

The waters at the sampling station were covered by sea ice during the period of this study, December to May, with ice break up only at the middle of May. In April, chlorophyll $a$ concentrations increased markedly, and maximal values were found much later in summer (Gameau, M.E., Roy, S., Lovejoy, C., Gratton, Y., Vincent, W.F.). Based on chlorophyll $a$ and DGGE, we divided this period into winter and spring and studied the metabolic abilities of bacterioplankton at three different layers (surface, TI depth and bottom).

Biolog-Ecopelates have proven to be an efficient means of assessing microbial metabolic diversity in a variety of habitats (Insam, 1997; Sala et al., 2005). Biolog-Ecopelates provide many simultaneous enrichment cultures with individual single carbon sources. Such an approach, although limited by long incubations owing to bacterial slow growth at *in situ* temperatures and possibility of selective culturing (Smalla et al., 1998) provides information on the potential metabolic abilities of bacterioplankton. In the Arctic, in Resolute Bay, Biolog-Ecopelates have indicated higher NSU in marine than in freshwater samples (Tam et al., 2003). However, these authors did not provide information of the carbon sources used. Most (21 out of 31) of the carbon sources in the Biolog-Ecopleate were used in our study. Polymers and carbohydrates were the most frequently used substrate types at all depths, similar to earlier results from Mediterranean harbours (Sala et al., 2006b). The carboxylic acid pyruvic acid methyl ester and the amino acid glycyl-L-glutamic were also used often. In contrast to the Mediterranean samples (Sala et al., 2006b) or the Bedford Basin (Jellet et al., 1996) but similar to the Antarctic environment (Sala et al., 2005a), amino acids were rarely used. These results support the evidence of a low amino acid uptake in samples from Resolute Bay in the Canadian Arctic (Pomeroy et al., 1990). The amino acid used most often, and more in winter than in spring, was glycyl-L-glutamic acid, a combination of glycine and glutamic acid, two amino acids that are selectively preserved in particulate organic matter (POM) in polar waters (Hubberten et al., 1995). This might explain the higher utilization in winter than in spring. Utilization of glucose-1-P was even lower than utilization of amino acids in surface waters, similar to the findings by Alonso-Sáez, L., Sánchez, O., Gasol, J.M., Balagué, V., Pedrós-Alió, C. (submitted) through microautoradiography. Tan (1997) reported on the substrates used by oligotrophic bacteria isolated from Arctic and Antarctic environments using Biolog GN plates. Some substrates are common to Eco and GN plates and allow a comparison with our results. Similar to our observations, only some of their isolates metabolized amino acids. Among our most commonly used substrates, Tween 40, Tween 80, pyruvic acid and mannitol were used by all these isolated polar bacteria. There were differences to our results in that none of the strains used α-cyclodextrin and glycyl-L-glutamic. It might be that bacteria using those substrates were unable to grow in their enrichment cultures.
The majority of the carbon sources were most often used at the surface layer. However, N-acetyl-D-glucosamine, found in chitin and bacterial peptidoglycan, was more often used at the bottom layer. Davis and Benner (2005) experimentally determined that chitin was biodegraded at high rates in Arctic waters, suggesting that it is not likely accumulated in POM or dissolved organic matter (DOM). As bacteria are generally three orders of magnitude more abundant in the surface layer of sediments than in the water column (Sala and Gude, 2006), resuspension events might have enriched the bottom layer with peptidoglycan, and our results showing the higher utilization of N-acetyl-D-glucosamine in the bottom layer, could reflect the ability to degrade the peptidoglycan in bacterial cell walls.

There were distinct patterns in metabolic diversity over the period of study. Bacterioplankton production was assumed to rely mainly on the phytoplanktonic production during spring and summer. We therefore distinguished two main phases in our cycle according to chlorophyll a concentration and bacterial community composition as revealed by DGGE: December to 16 April and 17 April to 21 May.

High chlorophyll a concentrations were found at the surface in spring whereas in winter these were very low. Unexpectedly, bacterial metabolic diversity was markedly lower in spring than in winter. Primary production of phytoplankton in spring provides new DOM to the system. Davis and Benner (2005) suggested that DOM produced in situ, with high amino acid and amino sugar content, was an important source of bioreactive organic matter in the western Arctic Ocean. This increased DOM concentration might have favoured the growth of bacterial species specialized in the use of carbon sources derived from phytoplankton. Possibly, the arrival of fresh algal DOM might have selected for rapidly growing specialist bacteria. Indeed, the DGGE analysis shows a clear shift in the composition of bacterial species in May, with the appearance of new bands not found in the DGGE lanes of the surface winter samples. Likewise, FISH counts showed an increase in the percentage of cells hybridizing with the EUB probe, suggesting a transition from a community of low activity in winter to a more active one in spring (Alonso-Sáez, L., Sánchez, O., Gasol, J.M., Balagué, V., Pedrós-Alió, C., submitted).

Through summer and fall, labile organic matter originating from the previous year primary production in the Arctic is exhausted (Cota et al., 1996; Rich et al., 1997). Organic matter remaining from previous blooms is less bioreactive.
than freshly produced matter in the western Arctic (Davis and Benner, 2005) and probably more complex. The high NSU found in the surface in winter might be a response of the community to expand the metabolic pathways to be able to exploit the scarce and complex carbon sources available at this time. Using the Biolog approach, Sala and colleagues (2005a) found that bacteria did not rely on phytoplanktonic production in Antarctic waters in summer. Moreover, a negative correlation between NSU and chlorophyll a concentrations was found for surface NW Mediterranean waters (Sala et al., 2006b). These results suggest that bacterial assemblages in oligotrophic systems, such as the surface layer of the Beaufort Sea in winter, have a higher number of metabolic pathways and are able to exploit a wide variety of dissolved organic carbon (DOC) molecules present at low concentrations. Bacterial NSU showed a similar pattern at the T1 and bottom layers, very different from that at the surface. At both layers, chlorophyll a concentrations were close to undetectable, without a clear pattern and bacterial NSU and bacterial composition (not shown here) showed no differences between spring and winter. However, it was remarkable to find a period of 5 weeks for the T1 and 3 weeks for the bottom layer, between February and March, of high metabolic diversity. As chlorophyll a concentration at those depths was very low, organic matter was not freshly produced but derived from different sources: deepening of surface water, resuspension of sediment or advection from neighbouring water masses. Several of these events occurred over the winter. Specifically: (i) a deepening of the surface isotherm (−1.8°C) occurred from 1 March to 25 March (Forest et al. 2007), indicating a movement of surface water into the T1 layer. (ii) Sediment resuspension events were detected with transmissivity data (Forest et al. 2007) in early winter at the bottom layer. Sediment resuspension at our station increased both the concentration of polymeric organic matter and of bacteria attached to particles (Wells and Deming, 2006). (iii) Advection probably occurred during winter. Thus, Forest et al. (2007) observed frequent episodes of increased suspended matter in all the water column between January and March, probably owing to turbulence on the shelf and slope induced by Pacific-derived eddies and convective mixing during ice growth. All these three types of events would have resulted in episodic inputs of aged organic matter and with different degrees of recalcitrance and complex composition. These pulses of increased substrate utilization did not occur at the surface, suggesting that surface waters may have a more constant supply of organic matter from the ice algae, the flushing of ice brines, or inputs of riverine water. Our study is the first to show the metabolic capacities of bacterioplankton under the sea ice of the Arctic Ocean. In principle, the oligotrophic conditions prevailing in the Arctic water column during winter would suggest a scenario of low bacterial activity compared with spring conditions. Contrary to what was expected, bacterioplankton in the Arctic winter had higher NSU than in spring at the surface, and high NSU could also be found at deeper layers sporadically. Probably, the low NSU in spring were due to the
arrival of fresh algal DOM that selects for rapidly growing phenotypes, as indicated by the new bands found in the DGGE gel. Sala and colleagues (2006b) reported that bacterial assemblages in the oligotrophic Mediterranean Sea are forced to adapt to changing inputs of organic matter, and therefore express a high plasticity in metabolic pathways in order to exploit the changing and scarce carbon sources available for growth. The present study suggests that this pattern is applicable to other marine oligotrophic environments such as the Arctic Ocean.

**Experimental procedure**

The study was carried out within the framework of the CASES, http://www.quebec-ocean.ualaval.ca/cases/welcome.asp. The CCGS Amundsen research icebreaker overwintered at a fixed position in Franklin Bay (70°03′N, 126°18′W). Samples were collected weekly from three depths: surface (3 m), depth of TI (around 20–50 m) and bottom (220 m). Water from depths below 10 m was collected with a CTD connected to a rosette through the moon pool. In order to avoid ship contamination, surface water (3 m) was collected with Niskin bottles from a hole dug in the ice around 500 m away from the ship. Analysis of surface currents, temperature and salinity indicated that this sampling spot was located within the same water mass as the ship, but away from its influence.

Chlorophyll a concentrations were estimated according to Yentsch and Menzel (1963). Water samples (250–500 ml) were filtered onto 25 mm diameter Whatman GF/F filters and stained with DAPI (4,6-diamidino-2-phenylindole) final concentration 5 µg ml⁻¹. Bacteria on filters were counted under an Olympus-BX40-102/E epifluorescence microscope.

Biolog-Eco plates were used to determine patterns of sole carbon source utilization. Biolog-Eco plates (Biolog) are microtiter plates in which each well contains an individual carbon source, 31 carbon sources in total and a blank with no carbon source available for growth. The present study suggests that this pattern is applicable to other marine oligotrophic environments such as the Arctic Ocean.

Filter and the same water was then filtered through a 0.2 µm pore size polycarbonate filter. The 0.2 µm filters were conserved in crioivials with 2 ml of lysis buffer at −80°C until extraction. DNA extraction and 16S rDNA gene PCR amplification followed the same protocol as Pinhasi and colleagues (2004). Denaturing gradient gel electrophoresis was carried out using a DGGE 2401-Rev B model (CBS Scientific Company). Image analysis was carried out with the Quantity One software (Bio-Rad). The cluster analysis was constructed applying the unweighted pair group average (UPGMA) algorithm with the software PAST (Ryan et al., 1995).

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**References**


