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ORIGINAL PAPER

The impact of ice melting on bacterioplankton in the Arctic Ocean

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Abstract Global warming and the associated ice melt are leading to an increase in the organic carbon in the Arctic Ocean. We evaluated the effects of ice melt on bacterioplankton at 21 stations in the Greenland Sea and Arctic Ocean in the summer of 2007, when a historical minimum of Arctic ice coverage was measured. Polar Surface Waters, which have a low temperature and low salinity and originate mainly from melted ice, contained a very low abundance of bacteria ($7.01 \times 10^5 \pm 2.20 \times 10^5$ cells ml^{-1}); however, these bacteria had high specific bacterial production (2.40 ± 1.61 fmol C bac^{-1} d^{-1}) compared to those in Atlantic Waters. Specifically, bacterioplankton in Polar Surface Waters showed a preference for utilizing carbohydrates and had significantly higher specific activities of the glycosidases assayed, i.e. β-glucosidase, xylosidase, arabinosidase and cellobiosidase. Furthermore, bacterioplankton in Polar Sea Waters showed preferential growth on some of the carbohydrates in the Biolog Ecoplate, such as D-cellobiose and *N*-acetyl-D-glucosamine. Our results suggest that climate change and the associated melting of Arctic ice might induce changes in bacterioplankton functional diversity by enhancing the turnover of carbohydrates. Since organic aggregates are largely composed of polysaccharides, higher solubilization of aggregates might modify the carbon cycle, weaken the biological pump and have biogeochemical and ecological implications for the future Arctic Ocean.

Keywords Bacteria · Arctic · Global change · Functional diversity · Ectoenzyme · Biolog

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Introduction

Arctic ecosystems are the focus of major environmental concern due to the impact of global warming on the northern polar region. Increased temperatures have decreased the ice volume in the Arctic in the winter by 42% since 2005 (Kwok et al. 2009). This reduced sea ice extent and thickness as well as the increasing temperatures will almost definitely affect planktonic communities, for example by changing the timing and duration of phytoplankton blooms (Carmack et al. 2004), and consequently affect all steps of the microbial food chain in polar marine ecosystems (Vaqué et al. 2009). Furthermore, sea ice melting could lead to changes in the biogeochemistry of the Arctic Ocean by releasing microorganisms living in the ice (Maranger et al. 1994), metals (Tovar-Sánchez et al. 2010), organic carbon (Thomas et al. 1995) and exopolymeric substances (Krembs et al. 2002; Meiners et al. 2003) into the water, and increasing protist mediated bacterial mortality (Boras et al. 2010).

Heterotrophic bacterioplankton play a key role in decomposing organic matter and recycling nutrients in the ocean (Azam et al. 1983). Most of this organic matter is too large for direct uptake, and therefore must first be hydrolysed by ectoenzymes (Chróst 1990). Different patterns of ectoenzymatic activities can be found depending on the quality of the organic matter available in the environment (Sala and Güde 1999, 2004). Little information is available on ectoenzymatic activities in the Arctic Ocean. Vetter and Deming

(1994) found higher chitobiase activities than peptidase activities in the surface waters of the Arctic northeast water polynya. Arnosti (2008), using macromolecular fluorogenic substrates, found a different pattern of polysaccharide degradation in surface waters and sediments in a Svalbard fjord.

In addition to ectoenzymatic activities, studies of bacterial growth on enrichment cultures with selected substrates can show which carbon sources the bacterial community may use (Pinhassi et al. 2006; Sala et al. 2002). Biolog Ecoplates are a miniaturization of enrichment cultures and allow bacterial utilization of 31 carbon sources, mainly monomers, to be assayed (Insam 1997). Bacterioplankton at the surface of the Canadian Arctic (Franklin Bay) preferentially used carbohydrates in spring, while in winter a large spectrum of carbon sources, including polymers, were consumed (Sala et al. 2008). Those previous studies in different locations of the Arctic Ocean indicate that carbohydrates play an important role for the growth of Arctic bacterioplankton.

Our main aim was to study the effects of ice melting on bacterial communities in the Greenland Sea and Arctic Ocean (see also Boras et al. 2010). With this goal in mind, the ATOS-I cruise included ice-free stations and stations with up to 60% ice cover. The ice cover in the studied area changed dynamically. Indeed, the cruise was conducted in July 2007 when unprecedented ice melting occurred in the Arctic (Stroeve et al. 2007), and in September 2007, the ice extent was the lowest it had been since 1970 (Zhang et al. 2008). The study area, the Greenland Sea and the Arctic Ocean, has a complex hydrography (Rudels et al. 2005). Atlantic Water enters the Arctic close to Svalbard, and above the Atlantic Waters it is common to find a layer of Polar Sea Water, with lower temperature and salinity.

Since Arctic sea ice is enriched in polysaccharides (Krembs et al. 2002), which are an important carbon-rich substrate for bacteria (Riedel et al. 2006), we hypothesize that bacterioplankton in Polar Surface Waters, of largely ice melt origin, would be specialized in consuming carbohydrates. We tested the hypothesis by assessing the bacterial utilization of the largest set of carbon sources assayed in marine ecosystems. This comprised the activity of 10 ectoenzymes, 6 of them involved in the hydrolysis of different steps of several polysaccharides, and the utilization of 31 carbon sources, 10 of them carbohydrates, included in the Biolog Ecoplate.

Materials and methods

Study and sampling area

The study was carried out on the ATOS-I cruise in June–July 2007, in the northern Greenland Sea and Arctic Ocean (Fig. 1) on board the R/V BIO-Hespérides. We took samples at ice-free stations and stations with up to 60% ice cover. Seawater samples were collected from the upper 100 m at 21 stations. Generally, 6 depths were sampled below 5 m with a rosette sampler of 12 Niskin bottles mounted on a conductivity-temperature-depth profiler (CTD). Surface seawater samples were collected both at 1 m, with a single Niskin bottle, and at ca. 10 cm with a plastic bucket lowered from a rubber boat.

Nutrient concentrations, bacterioplankton abundance and bacterial production reported in this paper were determined at all stations and depths. Ectoenzymatic activities were determined for 17–21 stations depending on the enzyme, and the utilization of carbon sources in the Biolog plate for 16 stations.

Samples were classified according to their temperature and salinity with a complete linkage cluster analysis (Legendre and Legendre 1998). Three main clusters were obtained that revealed three groups of samples characterized by their different temperatures and salinities: Polar Surface Waters (PSW), warm Polar Surface Waters (PSWw), and Atlantic Waters (AW) (Fig. 2).

Nutrients and dissolved organic carbon

Soluble reactive phosphorus (SRP), nitrate and nitrite were measured following a spectrophotometric method (Hansen and Koroleff 1999). Samples were kept frozen until analysed in the lab in a Bran + Luebbe AA3 autoanalyzer. Water samples for determining dissolved organic carbon (DOC) were filtered through precombusted fibreglass filters (Whatman GF/F) and frozen (−18°C) in pre-combusted and acid-washed 20-ml vials until analysis on a Shimadzu TOC-5000 analyzer (Shimadzu Corp. Kyoto, Japan). Standards provided by Dennis A. Hansell and Wenhao Chen (University of Miami, USA) of 2 μM and 45 μM TOC were used to assess the accuracy of the estimates. Ice contained average total organic carbon concentrations of 69.4 ± 7.68 μmol C L^{-1} compared to 93.8 ± 4.5 μmol C L^{-1} in surface seawater and showed relatively high average elemental ratios of 930 C (total organic carbon) : 58 N : 1 P (Tovar-Sánchez et al. 2010).

Bacterioplankton abundance and production

The methodologies used in the study do not allow us to distinguish the contribution of heterotrophic bacteria from that of archaea. Therefore, the terms bacterioplankton or bacteria used throughout this paper refer to both groups. Bacterial abundance was determined on board by flow cytometry. Subsamples of 2 ml were fixed with paraformaldehyde (1% final concentration) and analysed on board

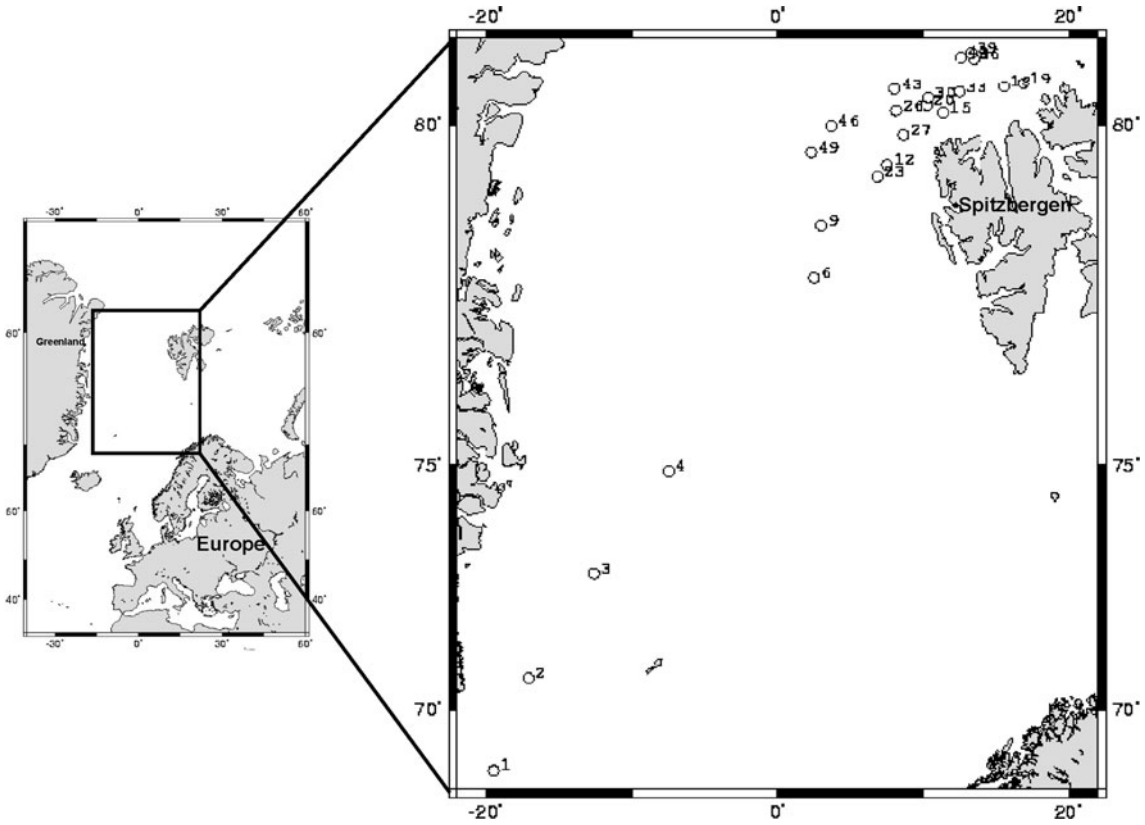

Fig. 1 Location of stations in the study region sampled during the ATOS-I cruise

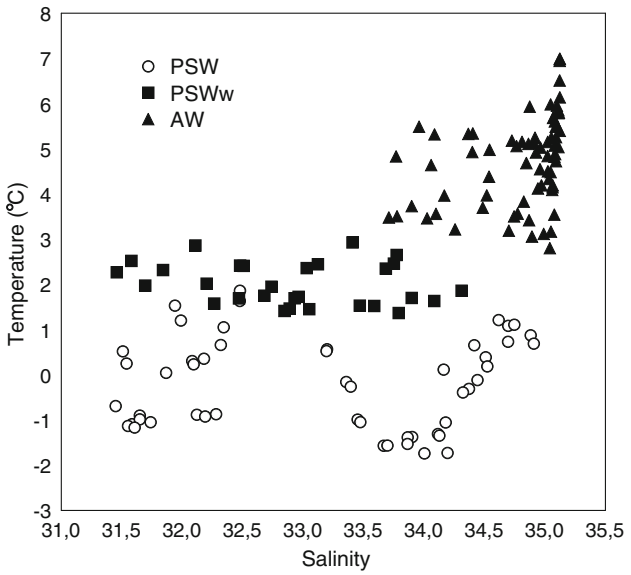

Fig. 2 Temperature and salinity plot of the samples grouped into the three water masses. PSW (Polar Surface Water), PSWw (warm Polar Surface Water) and AW (Atlantic Water)

immediately after fixation on a FACSCalibur (Becton and Dickinson) flow cytometer. Samples were stained with SYTO-13 and run at low speed using 50 µl of 0.92 µm yellow-green latex beads as the internal standard (Del Giorgio et al. 1996).

Bacterial production (BP) was estimated from radioactive 3H-leucine incorporation using the method described by Kirchman et al. (1985), with the modifications suggested by Smith and Azam (1992) for microcentrifuge vials. Samples of 1.2 ml were taken from each depth and dispensed into four 2-ml vials plus two TCA-killed control vials. Next, 48 µl of a 1 µM solution of 3H-leucine was added to the tubes providing a final concentration of 40 nM (which was found to be saturating in these waters). Incubations were run for 4 h at in situ temperature in thermostatic incubation chambers, and stopped with TCA (50% final concentration). Tubes were spun in a microcentrifuge for 10 min at $16{,}000 \times g$. Liquid was sucked out with a Pasteur pipette connected to a vacuum pump. Pellets were rinsed with 1.5 ml of 5% TCA, and vortexed and spun in the microcentrifuge. Supernatant was removed and 0.5 ml of scintillation cocktail was added. Finally, the tubes were counted within standard 20-ml vials in a Beckman scintillation counter. Bacterial production is shown as ^{3}Hleucine incorporation in pmol leucine l^{-1} h^{-1}. Further details of the technique can be found in Vaqué et al. (2009).

Ectoenzymatic activities

The activity of 10 ectoenzymes was measured fluorometrically using non-fluorescent substrates, which emit fluorescence after hydrolytic cleavage (Hoppe 1983) according to the modifications outlined in Sala et al. (2001). The substrates assayed targeted six glycosidases: 4-MUF-β-D-glucoside (β-glucosidase), 4-MUF-α-D-glucoside (α-glucosidase), 4-MUF-β-D-xyloside (β-xylosidase), 4-MUF-*N*-acetyl-α-D-glucosaminide (chitobiase), 4-MUF α-L-arabinopyranoside (arabinosidase), and 4-MUF β-D-cellobioside (cellobiosidase); three esterases: 4-MUF-butyrate (esterase), 4-MUF-acetate (lipase), and 4-MUF-phosphate (alkaline phosphatase); and a peptidase: L-leucine-7-amido-4-methyl-coumarin (leu-aminopeptidase). Briefly, 0.9 ml subsamples were amended with 0.1 ml substrate (final concentration 100 μM) as determined by shipboard substrate-saturation experiments. After between 15 min and 4 h (depending on the substrate) of incubation in the dark, the fluorescence of the samples was measured on a Turner Designs model 10-005-R fluorometer (365 excitation and 446 emission wavelengths). Fluorescence units were converted to activity with a standard curve prepared with the end product of the reactions. Specific activities, i.e. activities per cell, were calculated by dividing each activity by the bacterial cell concentration.

Utilization of sole carbon sources

Biolog EcoplateTM (Biolog Inc.) microplates were used to assess the utilization of carbon sources by the bacterial communities. Biolog Ecoplates are 96-well microtiter plates containing 31 carbon sources in triplicate and 3 blanks without a carbon source. A redox dye was added to each well as an indicator of oxidation of the carbon source. After inoculation of the samples in the wells, carbon source oxidation could be observed due to the formation of insoluble formazan in the well, which can be measured spectrophotometrically. Biolog plates were inoculated with 150 μl of sample in each well and the plates were incubated for 7–15 days at 4°C. During this period, the absorbance of each plate was measured regularly on board at a wavelength of 590 nm using a spectrophotometric microplate reader (ELX800 BIOTEK Instruments Inc. Winooski, Vermont, USA). The plate measurement was considered optimum when the highest total absorbance of the plate was reached, and this absorbance measurement was used for further analysis. In order to allow an appropriate incubation time, Biolog plates were not inoculated at the last 5 stations visited and data is restricted to stations 1–33. One plate per sample was inoculated, and samples were generally taken at 2 or 3 depths for each station (10 cm, the depth of the chlorophyll maximum, and often also at 5 m).

The utilization of each carbon source was expressed as the average substrate colour development (ASCD): First, the mean absorbance of the blanks was subtracted from the absorbance of each well. Second, the total absorbance of the plate was calculated by adding the absorbance of each well. Third, the percentage absorbance of each well was calculated from the total absorbance of the plate. Finally, negative values were set at 0, and the ASCD was calculated as the mean of the percentage absorbance for each of the three wells for each substrate. Further details on the methodology and calculations can be found in Sala et al. (2005b).

The 31 substrates used in the plate can be grouped into 6 categories: carbohydrates, amino acids, polymers, amines, phenolic compounds and carboxylic acids. A list of the substrates and categories used in the Biolog Ecoplate can be found in Table 1.

Calculations and statistical analysis

We classified the samples according to their temperature and salinity with a complete linkage cluster analysis (Legendre and Legendre 1998). One-way ANOVA and the Tukey–Kramer HSD test were used to evaluate the differences between the three types of water masses. A Pearson correlation analysis was performed to determine the relationships between the measured parameters. All statistical analyses were performed using the Statistica program.

Results

Environmental parameters

We sampled 21 stations in an area comprised between the Fram Strait and north of Svalbard (Fig. 1) at a maximum of 7 depths at each station. A cluster analysis based on temperature and salinity values defined three different groups of samples (Fig. 2): Polar Surface Water samples, PSW ($N = 51$), characterized by low temperatures (mean 0.26°C) and salinities (mean 33.2); Atlantic Water samples, AW ($N = 70$), characterized by high temperatures (mean 4.8°C) and salinities (mean 34.8); and warm Polar Surface Water samples, PSWw ($N = 29$), with similar salinities to those of the PSW samples (mean 32.9), but a higher temperature (mean 2.03°C). For the temperature and salinity ranges of each water mass, see Fig. 2. AW was generally found below PSW or PSWw; however, in three stations (St. 42, 46 and 49) PSW was found in the upper 80 m sampled.

DOC concentrations ranged between 50.7 and 137.7 μM and showed a negative correlation with salinity (Table 2). Inorganic nutrients showed positive correlations with both temperature and salinity, and concentrations ranged between 0 and 0.67 μM for soluble reactive phosphorus

Table 1 List of compounds in the Biolog Ecoplate grouped into substrate categories

Compound	Category	PSW	PSWw + AW	*P*
L-Arginine	Amino Acid	1.12	0.63	0.35
L-Asparagine	Amino Acid	1.89	1.68	0.64
L-Phenylalanine	Amino Acid	0.79	0.83	0.83
L-Serine	Amino Acid	1.23	1.08	0.82
Glycyl-L-Glutamic Acid	Amino Acid	6.90	8.47	0.56
L-Threonine	Amino Acid	1.75	2.04	0.81
Phenylethylamine	Amine	0.48	0.98	0.45
Putrescine	Amine	0.05	0.83	0.10
D-Mannitol	Carbohydrate	7.42	4.77	0.12
Glucose-1-Phosphate	Carbohydrate	1.33	0.34	**0.02**
D,L-α-Glycerol Phosphate	Carbohydrate	0.79	1.33	0.39
β-Methyl-D-Glucoside	Carbohydrate	0.43	1.28	0.68
D-Galactonic Acid γ-Lactone	Carbohydrate	0.00	0.35	0.24
i-Erythritol	Carbohydrate	7.42	4.77	**0.00**
D-Xylose	Carbohydrate	0.48	1.38	0.38
N-Acetyl-D-Glucosamine	Carbohydrate	5.23	2.78	0.09
D-Cellobiose	Carbohydrate	6.43	3.86	0.08
α -D-Lactose	Carbohydrate	2.01	1.28	0.40
D-Glucosaminic acid	Carboxylic acid	0.55	0.64	0.90
D-Malic Acid	Carboxylic acid	0.21	0.70	0.45
Itaconic acid	Carboxylic acid	3.00	3.58	0.67
Piruvic Acid Methyl Ester	Carboxylic acid	6.24	8.01	0.51
D-Galacturonic acid	Carboxylic acid	4.98	4.09	0.60
Ketobutyric acid	Carboxylic acid	1.97	0.80	**0.02**
γ -Hydroxybutyric Acid	Carboxylic acid	10.78	8.52	0.53
2-Hydroxy Benzoic Acid	Phenolic compound	0.36	0.71	0.60
4-Hydroxy Benzoic Acid	Phenolic compound	0.05	0.13	0.68
Tween 80	Polymer	6.30	6.46	0.93
Tween 40	Polymer	12.99	17.39	0.26
α -Cyclodextrin	Polymer	5.57	7.70	0.34
Glycogen	Polymer	6.89	7.89	0.73
Amino acids		13.68	14.33	0.83
Amines		0.53	1.81	0.16
Carbohydrates		27.22	17.58	**0.03**
Carboxylic acids		27.73	26.35	0.70
Phenolic c.		0.41	0.84	0.57
Polymers		31.76	39.43	0.08

Average substrate colour development in polar waters ($n = 8$) and the other non-polar waters ($n = 35$), and p values of the t test. Significant values ($P < 0.05$) are highlighted

(SRP), and 0.01 and 14.7 μM for nitrate + nitrite. The three water masses showed no significant differences in DOC concentration, but differences among water masses were found for inorganic nutrients (Fig. 3). The ANOVA test ($F = 13.82$, $P = 0.000$) and post-hoc Tukey test revealed significant differences between all water masses ($P < 0.05$), with higher SRP concentrations in AW samples. Significant differences were also found for nitrate + nitrite concentrations with ANOVA ($F = 14.74$, $P = 0.000$), and the post-hoc Tukey test revealed differences among the three water masses ($P < 0.05$).

Bacterial abundance and production

Bacterial abundance varied between 1.42×10^5 and 2.91×10^6 cells ml^{-1} (Fig. 4) and was positively correlated with temperature and salinity ($P < 0.000$, Table 2). Comparison of water masses showed that PSW and PSWw samples had clearly lower bacterial abundances.

Bacterial production (range: 0.17–208 pmol leu l^{-1} h^{-1}), in contrast, did not correlate with temperature and salinity, and differences among water masses were not detected.

Table 2 Correlation coefficients among variables and temperature and salinity

Variable	Temperature	Salinity
SRP	**$r = 0.225$ $n = 139$**	**$r = 0.686$ $n = 139$**
	$P = 0.008$	**$P = 0.000$**
Nitrate and nitrite	**$r = 0.267$ $n = 139$**	**$r = 0.647$ $n = 139$**
	$P = 0.001$	**$P = 0.000$**
DOC	$r = -0.056$ $n = 97$	**$r = -0.357$ $n = 97$**
	$P = 0.587$	**$P = 0.000$**
Sp. Aminopeptidase	$r = -0.025$ $n = 116$	**$r = -0.212$ $n = 117$**
	$P = 0.790$	**$P = 0.022$**
Sp. Alkaline phosphatase	$r = -0.020$ $n = 115$	$r = -0.138$ $n = 116$
	$P = 0.831$	$P = 0.139$
Sp. Esterase	$r = -0.015$ $n = 116$	$r = -0.151$ $n = 117$
	$P = 0.877$	$P = 0.105$
Sp. Lipase	$r = -0.104$ $n = 63$	**$r = -0.243$ $n = 63$**
	$P = 0.422$	**$P = 0.006$**
Sp. Chitobiase	**$r = -0.178$ $n = 108$**	**$r = -0.278$ $n = 108$**
	$P = 0.065$	**$P = 0.004$**
Sp. Xylosidase	**$r = -0.338$ $n = 108$**	**$r = -0.331$ $n = 108$**
	$P = 0.000$	**$P = 0.000$**
Sp. β-Glucosidase	**$r = -0.342$ $n = 108$**	**$r = -0.375$ $n = 108$**
	$P = 0.000$	**$P = 0.000$**
Sp. Cellobiosidase	**$r = -0.497$ $n = 61$**	**$r = -0.324$ $n = 61$**
	$P = 0.000$	**$P = 0.011$**
Sp. α-Glucosidase	**$r = -0.598$ $n = 29$**	$r = -0.186$ $n = 29$
	$P = 0.001$	$P = 0.343$
Sp. Arabinosidase	**$r = -0.539$ $n = 59$**	**$r = -0.472$ $n = 59$**
	$P = 0.000$	**$P = 0.000$**

Significant correlations ($P < 0.05$) are highlighted

Ectoenzymatic activities

Ectoenzymatic activities showed a large range of variation in the study area (Table 3). Among the 10 ectoenzymes studied, glycosidases generally showed higher values of bulk activities in PSW (Table 3). The trend observed for bulk activities could also be detected for specific activities, i.e. activities per cell (Fig. 5). Significant negative correlations ($P < 0.05$) with both temperature and salinity were found for the specific activities of several glycosidases: β-glucosidase, xylosidase, cellobiosidase and arabinosidase (Table 2). A negative correlation with salinity was found for specific activities of aminopeptidase, lipase and chitobiase, and with temperature for α-glucosidase. Comparison among the water masses (Fig. 5) showed lower specific activities for all glycosidases in AW samples, and these differences were significant (ANOVA and post-hoc Tukey test) for β-glucosidase, cellobiosidase, xylosidase and arabinosidase.

Utilization of sole carbon sources

In our study area, the highest substrate category used was generally polymers, with the highest values for Tween 40. The only category that showed different use among water masses was carbohydrates that were significantly more used in PSW (Fig. 6). Among the carbohydrates, a higher, significant utilization in polar waters was observed for glucose-1-phosphate and *i*-erythritol, and higher but not significant for D-cellobiose, α-D-lactose, *N*-acetyl-D-glucosamine and D-mannitol (Table 1).

Discussion

Our study area, the Greenland Sea and Arctic Ocean, is an area of intensive exchange of water masses (Rudels et al. 2002). We found three water masses: the less dense and colder water comprises the Polar Surface Water (PSW); water in the same density range but with a higher

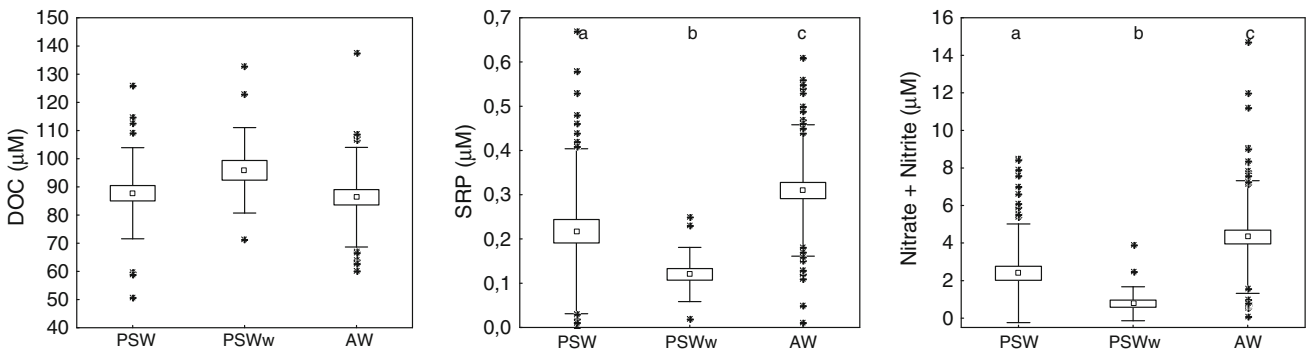

Fig. 3 Distribution (box-plots) of dissolved organic carbon (DOC), soluble reactive phosphorus (SRP) and nitrate + nitrite concentrations in each water mass: PSW (Polar Surface Water), PSWw (warm Polar Surface Water) and AW (Atlantic Water). *Square* mean; *Box* standard deviation; *Whisker* standard deviation; *Asterisks* extremes (values higher/lower than: the upper/lower value of the box + 3 times the height of the box). Values for the regions with the same letter are not significantly different ($P > 0.05$) according to a pair-wise, post-hoc Tukey test and analysis of variance (ANOVA)

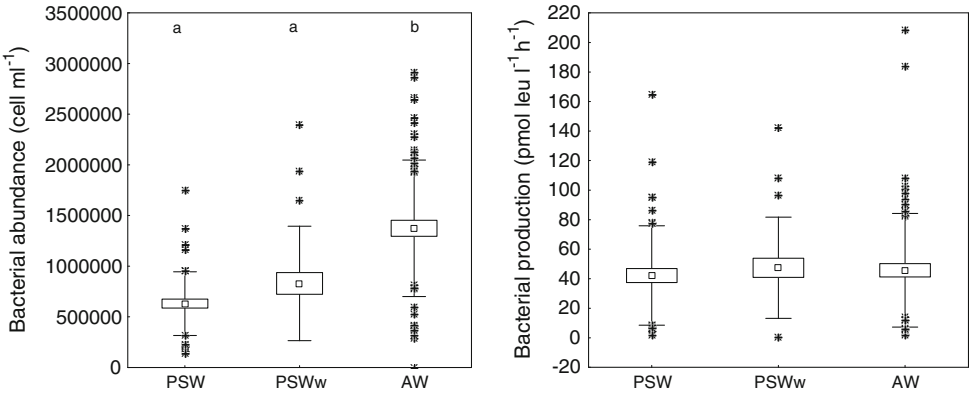

Fig. 4 Bacterial abundance and bacterial production for each water mass. PSW (Polar Surface Water), PSWw (warm Polar Surface Water) and AW (Atlantic Water). *Square* mean, *Box* standard deviation, *Whisker* standard deviation, *Asterisks* extremes (values higher/lower than: the upper/lower value of the box + 3 times the height of the box)

Table 3 Range of ectoenzymatic activities (nmol l^{-1} h^{-1}) in the sampling area, and mean and standard deviation in the different water masses

Enzyme activity	Range	PSW	PSWw	AW
Lipase	0–423	67.2 ± 23.4	58.9 ± 17.4	77.1 ± 41.0
Esterase	0–207	22.5 ± 10.7	17.9 ± 4.8	34.4 ± 18.9
Alkaline phosphatase	0–47.8	4.97 ± 4.29	3.10 ± 4.39	6.22 ± 6.04
Aminopeptidase	0.13–24.1	2.97 ± 1.12	3.88 ± 1.75	4.51 ± 2.67
Glycosidases				
Chitobiase	0.002–6.42	0.85 ± 0.51	0.47 ± 0.40	0.75 ± 0.58
Xylosidase	0.002–1.23	0.28 ± 0.17	0.19 ± 0.45	0.18 ± 0.12
β-Glucosidase	0.002–0.86	0.17 ± 0.11	0.11 ± 0.06	0.11 ± 0.06
Cellobiosidase	0.007–0.53	0.12 ± 0.06	0.043 ± 0.02	0.083 ± 0.05
Arabinosidase	0.008–0.29	0.093 ± 0.04	0.044 ± 0.02	0.042 ± 0.01
α-Glucosidase	0–0.20	0.074 ± 0.03	0.030 ± 0.01	0.063 ± 0.03

temperature forms the warm Polar Surface Water (PSWw); and warm, salty Atlantic Water (AW) is generally found below PSW and PSWw in the upper 100 m.

The terrestrial influence of the large Siberian rivers is considered to be minor in the surface waters of the Fram Strait, which have strong local influences, such as melting ice floes (Bussmann 1999). The increased DOC concentrations in Arctic sea ice (Thomas et al. 1995) suggest that melted ice represents a large DOC input to surrounding waters. However, contrary to our expectations, the DOC concentration in PSW was not higher than in AW.

Bacterial abundances recorded in the present study were similar or slightly higher than values found in the Arctic by other authors (Anderson and Rivkin 2001; Vaqué et al. 2008). However, Polar Surface Waters had the lowest bacterial abundance, which was around half that of the mean abundance in AW. In contrast, bacterial production was comparable among groups of stations. As a consequence, the bacteria in Polar Surface Waters had the highest specific bacterial production in the area (PSW: 2.40 fmol C bac^{-1} d^{-1}; AW: 1.19 fmol C bac^{-1} d^{-1}). Both values are in the highest range of specific bacterial productions found in the ocean (Baltar et al. 2009), but within the range of values found in a seasonal cycle in the North Sea (Reinthaler et al. 2005) or in depth profiles in the Ross Sea (Celussi et al. 2009).

Measuring the ectoenzymatic activities expressed by bacteria in a given environment provides clues to the substrate utilization pattern in that environment, which cannot be determined from leucine uptake rates (Hoppe 1983). Using fluorogenic tracers of ectoenzymatic activity is now a widespread technique in aquatic environments. However, in the ocean, most studies have been performed in temperate zones and in coastal environments (e.g. Williams and Jochem 2006; Alonso-Sáez et al. 2008; Taylor et al. 2009), mainly because fluorometers need to be extremely sensitive for the analysis. However, lately more sensitive spectrofluorometers have allowed ectoenzymatic activities to be measured in very oligotrophic areas, such as the deep sea (Baltar et al. 2009).

Ectoenzymatic activities are very suitable for evaluating the bacterial response to ice melt waters, since ectoenzymes respond rapidly, directly or through changes in microbial community composition, to changes in nutrients and substrate availability (Cunha et al. 2001). However, measuring a single ectoenzymatic activity is limited to

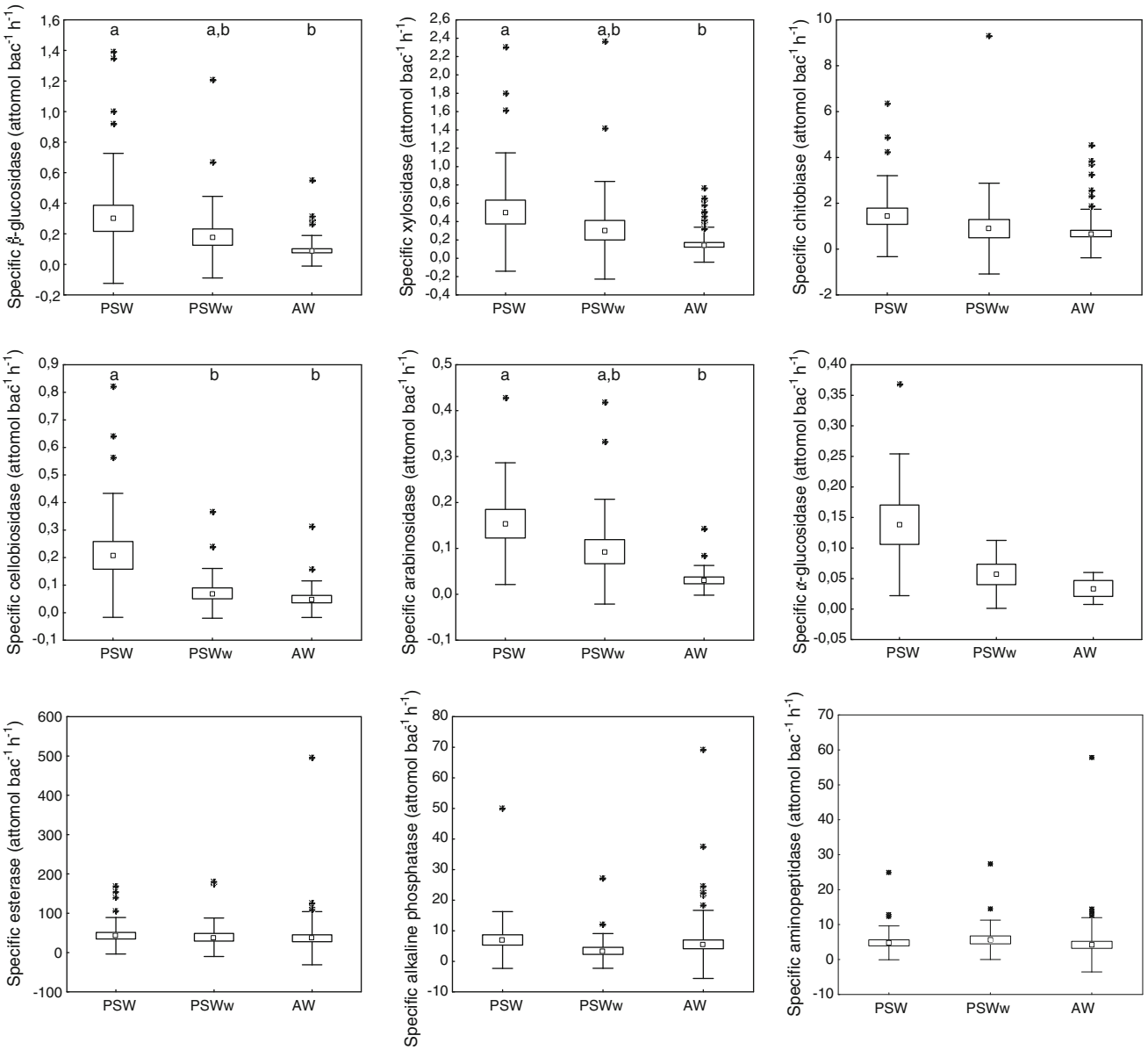

Fig. 5 Specific activities of 9 ectoenzymes investigated in each water mass: PSW (Polar Surface Water), PSWw (warm Polar Surface Water) and AW (Atlantic Water). *Square* mean; *Box* standard deviation; *Whisker* standard deviation; *Asterisks* extremes (values higher/lower than: the upper/lower value of the box + 3 times the height of the box)

represent the behaviour of entire classes of compounds (Arnosti et al. 2005). Therefore, in order to find functional patterns in the bacterioplankton community, in this study we determined the activities of the largest set of ectoenzymes assayed in marine environments. Most of the studies in the ocean have focused on leu-aminopeptidase and β-glucosidase activities as indicators of proteolysis and glucolysis. The ranges of leu-aminopeptidase activity found in our study are clearly lower than those found in two distinct Antarctic regions: the Ross Sea, (0–480 nM h^{-1}) (Celussi et al. 2009), and western Antarctic waters (0–500 nmol l^{-1} h^{-1}) (Sala et al. 2005a). In contrast, the range of β-glucosidase activities in the Arctic (0.002–0.86 nmol l^{-1} h^{-1}) is clearly lower than the range found in the same study in the Ross Sea (0–20 nmol l^{-1} h^{-1}). Several studies have used the ratio between leu-aminopeptidase and β-glucosidase activities (LAPase/BGase) as an indicator of proteolysis vs glucolysis. The LAPase/BGase ratio in our study (55–86) falls between the ratios for the Equator (0.28) and those for the Southern Ocean (mean 593) found by Christian and Karl (1995), and is higher than those obtained in a transect across the Indian Ocean (<15) (Misic et al. 2006). Within our sampling area, the ratio LAPase/BGase showed lower values in PSW (17.2) than in PSWw (36.7) or AW (41.6), which suggests that glucolysis plays a large role in Polar Surface Waters.

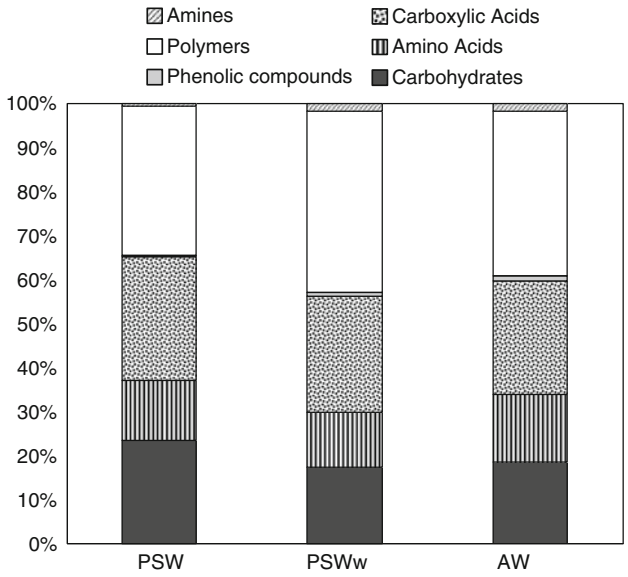

Fig. 6 Mean absorbance of the total plate absorbance of each substrate category in each water mass: PSW (Polar Surface Water), PSWw (warm Polar Surface Water) and AW (Atlantic Water)

Our data of specific ectoenzymatic activities fall in the lower range of the specific activities measured in the ocean for leu-aminopeptidase, alkaline phosphatase, α-glucosidase and β-glucosidase (see Table 2 in Baltar et al. 2009), and are comparable to specific activities of those enzymes in the Baltic Sea in summer (Nausch et al. 1998) and in the Arctic northeast water polynya (Vetter and Deming 1994). In spite of their low number of water stations sampled and the variable depths, their data range is similar to the data range found in this study for lipase (0.1–4 amol bac^{-1} h^{-1}), chitobiase (0.001–1 amol bac^{-1} h^{-1}) and leu-aminopeptidase (0.2–11 amol bac^{-1} h^{-1}).

Differences among water masses revealed a clear pattern in which PSW (and often also PSWw) exhibited significantly higher specific activities of some of the glycosidases assayed: β-glucosidase, cellobiosidase, xylosidase and arabinosidase. These four enzymes are involved in different steps of the hydrolysis of two polymers, cellulose (β-glucosidase and cellobiosidase) and hemicellulose (xylosidase and arabinosidase), the main polymers found in plant cell walls. Since no significant differences among chlorophyll concentrations were found between the water masses (data not shown), the increased glycosidic activities of bacterioplankton cannot be attributed to a higher phytoplanktonic biomass in Polar Surface Waters.

Utilization of sole carbon sources

Although initially created for bacterial identification, Biolog plates have been used to assess the functional diversity of microorganisms in the environment since they were first proposed by Garland and Mills (1991). Later, Insam (1997) introduced Biolog Ecoplates, which are more suitable for ecological purposes, since they contain the carbon sources considered most relevant to the environment. Biolog plates are miniaturized enrichment experiments in which bacterial growth on a sole carbon source is tested simultaneously for 31 carbon sources in triplicate. Although the methodology has certain limits, which are critically reviewed in Preston-Mafham et al. (2002), Biolog Ecoplates have been successfully used to asses the utilization of carbon sources in freshwater (Sinsabaugh and Foreman 2001; Christian and Lind 2006; Comte and Del Giorgio 2009) and marine environments (Tam et al. 2003, Sala et al. 2005a, b, 2006a, b, 2008).

In our study area, we found that carbohydrates and polymers play a major role in bacterial metabolism in the Greenland Sea and the Arctic Ocean, which has also been found in other polar regions such as the Canadian Arctic (Sala et al. 2008) and western Antarctica (Sala et al. 2005a), but which is not so apparent in a temperate sea such as the NW Mediterranean (Sala et al. 2006a). Tween 40 and Tween 80 were found to be greatly used by bacteria in other polar regions (Sala et al. 2005a, 2008). Both are polyols which can be hydrolyzed by polar bacterial strains (Tan and Rüger 1999). Polyols were probably found in polar waters because they accumulate in sea-ice algae, which help the algae tolerate low temperatures and high salinities (Wynn-Williams 1990). As in the Canadian Arctic (Pomeroy et al. 1990; Sala et al. 2008), amino acids were relatively little used in our study area. The carbohydrates most used were *i*-erythritol, *n*-acetyl-D-glucosamine and D-cellobiose. Higher utilization of *n*-acetyl-D-glucosamine and D-cellobiose agrees with the higher specific ectoenzymatic activities of their related enzymes involved in the hydrolysis of chitin (chitobiase) and of cellulose molecules (β-glucosidase and cellobiosidase), respectively. Both D-cellobiose and *n*-acetyl-D-glucosamine are common in marine environments, the first in phytoplankton cell walls, and the second in chitin and bacterial peptidoglycan. Similarly, Antarctic water masses of low temperature and salinity revealed also higher utilization of carbohydrates, especially of *N*-acetyl-D-glucosamine, D-cellobiose and glucose-1-phosphate (Sala et al. 2005a).

Arctic sea ice contains a large pool of organic matter (Thomas et al. 2001), and ice melt contributes DOC and exopolymeric substances to polar seawater (Krembs et al. 2002; Meiner et al. 2003, 2004) which constitute an important carbon-rich substrate for bacteria (Riedel et al. 2006). Based on the negative correlations between carbohydrates and salinity, freshwater inputs could be an important source of carbohydrates in the Arctic Ocean (Wang et al. 2006). Carbohydrates are particularly relevant

for microbial metabolism in the ocean, since they are major constituents of phytoplankton (Parsons et al. 1961) and marine particles (Cowie and Hedges 1984). The composition of carbohydrates in the Arctic Ocean shows that glucose is the main carbohydrate at the surface (Engbrodt and Kattner 2005). Monosaccharides were found to contribute only 10% in a transect through the Arctic Ocean (Rich et al. 1997), which suggests that glycosidases probably play an important role in that ecosystem.

The DOC pool in the ocean is a black box with a large variety of molecules with different lability for bacterial growth. In the Arctic Ocean, the DOC lability controls bacterial growth (Middelboe and Lundsgaard 2003; Kirchman et al. 2009). For example, rapid degradation of fresh algal DOC from an ice floe, especially of arabinose, galactose, glucose and xylose, was observed in a decomposition experiment (Amon and Benner 2003). It is very likely then that the composition and lability of the DOC pool varies among the water masses sampled and that ice melt might have enriched Polar Surface Waters with labile carbohydrates very suitable for bacterioplankton growth.

It is necessary to elucidate if these differences in activity and substrate utilization in bacterioplankton could be due to an input of bacteria probably of ice origin to Polar Surface Waters since, for example, only a few strains isolated from Arctic sea ice have been found to be able to hydrolyse carbohydrates (Yu et al. 2009). The analysis of bacterial phylogenetic diversity in our samples will provide a further clue to the possible influence of ice bacteria to the increased carbohydrate utilization in Polar Sea Waters.

Conclusion

Polar Surface Waters, largely composed of ice melt water, contain a low abundance of highly productive bacterial community specialized in the utilization of carbohydrates, with higher specific glycosidic ectoenzymatic activities and preferential growth on the carbohydrates in the Biolog Ecoplate. Our results suggest that Arctic ice melt, enhanced by global warming, may enhance polysaccharides degradation and have potential effects on the cycle of organic carbon in the Arctic Ocean.

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