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The changing Arctic

The Arctic is a miner's canary, sending out early warning signals of a changing global climate. The entire Earth is currently part of a warming trend with surface temperatures increasing most significantly in the Arctic. In 2005, surface air temperatures (SATs) increased by 1.5 to 3.4°C over the entire Arctic, with highest increases over the Arctic Ocean (Arctic Change 2006). The current warming trend began in the late 1970's. However, the Arctic has warmed before. Between 1920 and 1940, the Arctic warmed by 1.7 to 2.2°C and remained warm during the entire period (Bengtsson et al. 2004). The current Arctic warming trend has already surpassed the maximum values of the 1920 to 1940 warming event, and there is little evidence of a reversal in SAT trends happening in the near future (Vinnikov et al. 1999, Bengtsson et al. 2004, Johannessen et al. 2004).

The Arctic warming event which began in the 1920's, is possibly the result of large-scale changes in atmospheric circulation which transported heat from the North Atlantic Current into the Barents Sea (Bengtsson et al. 2004). Concurrent reductions in sea-ice cover created a positive feedback, which enhanced SATs and maintained the warming trend. Anthropogenic forcing appeared unlikely since the warming event occurred when greenhouse gas concentrations were only 20% of late 1990 concentrations.

In comparison, the current warming trend, globally and in the Arctic, is generally considered to be driven by anthropogenic emissions of greenhouse gasses, primarily CO_2 (Vinnikov et al. 1999, Karl & Trenberth 2003, Johannessen et al. 2004). In 2005, atmospheric CO_2 concentrations increased by 2.5 ppm, again rising above the long-term annual CO_2 increase of 1.5 ppm (CCGG 2006). This increase brings atmospheric CO_2 concentrations to over 380 ppm as compared to 280 ppm in the pre-industrialized 1800's (CCGG 2006), providing evidence of a substantial increase in greenhouse gases, which contributes to enhanced global warming (IPCC 2001 for review).

During the current warming trend, there have also been large-scale changes in atmospheric circulation patterns, influencing SAT and winds, which are closely linked to variations in Arctic ice cover (Comiso 2003). An important source of variation is the Arctic Oscillation (AO) which rotates between positive and negative phases, influencing SAT and ice conditions on numerous time scales (e.g. decadal, interannual and seasonal scales; Polyakov & Johnson 2000, Wang & Ikeda 2000, Rigor & Wallace 2004). Between 1989 and 1995 the AO was in a strong positive phase, corresponding to the onset of the current SAT warming trend (Rigor & Wallace 2004). During a positive AO phase, there is low atmospheric sea level pressure over the polar region which changes wind patterns such that warm air enters the Arctic and multi-year sea ice is transported out of the Arctic (Kerr 1999). However, since the late 1990's the AO appears to have had less of an effect on warming and sea-ice trends, with record low sea-ice extent occurring despite a negative phase AO (Stroeve et al. 2005).

The oscillations in atmospheric circulation make it difficult to identify to what extent the changing Arctic temperatures and ice cover is a response to natural cycles in climate variability or to anthropogenic forcing (Polyakov & Johnson 2000, Serreze & Francis 2006, Stroeve et al. 2005). Regardless of the driving forces, the Arctic canary is showing signs of stress. Of greatest concern is the apparent reduction in Arctic sea-ice, which has imminent local and global consequences, and was the motivation for this research.

Arctic sea ice

Sea ice is one of the largest biomes on earth representing 3 to 6% of the Earth's surface. In the Arctic there is enough sea ice to cover all of Canada, with an additional 4 million km^2 to spare. The maximum winter sea-ice extent in the Arctic (excluding adjacent seas) is ~14 x 10^6 km^2 with sea-ice extent decreasing to ~7 x 10^6 km^2 during the Arctic summer (Johannessen et al. 2004). These current values reflect a decrease of ~7% in annual sea-ice area in the Northern Hemisphere from 1978 to 2002. Sea-ice reduction has been greater in the summer than in the winter, with summer melt resulting in a 4 to 9% reduction in the area of multi-year ice cover, per decade (Comiso 2003, Polyakov et al. 2003). The shift from perennial, multi-year to annual first-year sea ice could result in an ice-free arctic summer within the next 100 years (Comiso 2002).

There is a clear trend in decreasing sea-ice extent in the Arctic (Fig. 1) with record minima occurring consistently since 2002 (Stroeve et al. 2005). Sea ice plays a major role in the exchange of energy between the ocean and the atmosphere and thus a reduction in

sea-ice extent may potentially influencing global heat budgets and thermohaline circulation (Aagaard & Carmack 1994). Sea ice typically reflects ~80% of incoming solar radiation such that a reduction in sea-ice cover reduces the Earth's albedo and increases energy absorption by the open Arctic Ocean (Kerr 1999). Therefore, reduced sea-ice cover creates a positive feedback, further enhancing Arctic SATs.

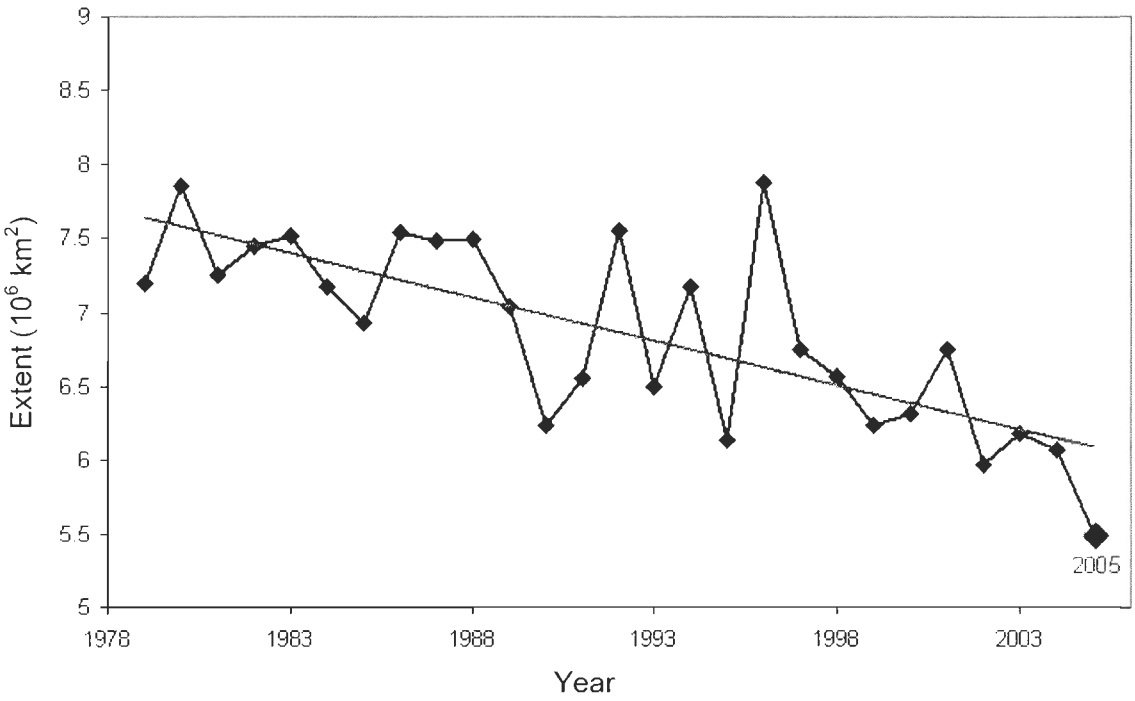

Fig. 1. Decline in Arctic sea-ice extent at the end of summer, from 1978-2005. The September trend from 1979 to 2005, showing a decline of >8% per decade, is shown with the straight line (Arctic Change 2006)

Given the evolution towards increasing concentrations of first-year sea-ice, knowledge of biochemical processes associated with first-year sea ice is essential in order to understand and predict future changes to the Arctic Ocean carbon cycle. Consequently, the Canadian Arctic Shelf Exchange Study (CASES), a large-scale multidisciplinary international oceanographic study, was carried out to assess the physical, chemical, biological and geological processes involved with sea-ice organic carbon cycling on an Arctic shelf. The research presented here was conducted over an entire season of sea ice formation and decline (September 2003 to June 2004) as part of the CASES study. The main objective of this research was to assess the role of heterotrophic microorganisms and exopolymeric substances in the cycling of organic carbon within the sea ice and in surface waters of the Mackenzie shelf.

Ecological role of first-year sea ice

In the Arctic, the formation of first-year sea ice can begin in September when water temperatures reach ca. -1.8°C, with maximum sea-ice thickness being typically attained in late April (Eicken 2003). First-year sea ice can form in association with land (i.e. landfast sea ice on the continental shelves) such that it remains fixed in place, or it can form as free-drifting pack ice (Weeks & Ackley 1982, Carmack & Macdonald 2002). Leads and polynyas are also sites of extensive first-year sea-ice formation throughout the ice covered period (Smith et al. 1990) and are considered to be important areas of biological production (Bradstreet & Cross 1982, Stirling 1997).

During the formation of first-year sea ice, inorganic sediments and microorganisms can be incorporated into the newly formed sea ice, accumulating at concentrations higher than the underlying water column (Reimnitz et al. 1992, Gradinger & Ikävalko 1998). Microorganisms and sediments from the benthos and water column can adhere to ice crystals (i.e. frazil), which form at depth and rise to the surface. A layer of unconsolidated new ice is thus created, which includes the associated microorganisms and sediments (Weeks & Ackley 1982, Garrison et al. 1983, Reimnitz et al. 1992). Cell size and surface characteristics (i.e. stickiness) appear to be involved in the enrichment of microorganisms in newly formed sea ice as compared to surface waters (Grossmann & Dieckmann 1994, Gradinger & Ikävalko 1998, Weissenberger & Grossmann 1998). Microorganisms incorporated during sea-ice formation can survive the dark winter (Zhang et al. 1998), thereby establishing the foundation of the spring sea-ice community.

Arctic sea ice is an important component of local marine ecosystems. For example, the timing and magnitude of pelagic and benthic production are coupled to first-year sea ice cover and melt (Carey 1987, Michel et al. 1996, Carmack et al. 2004). As a platform, first-year sea ice supports the migration, hunting and reproduction of numerous bird and mammal species including polar bears (Bradstreet & Cross 1982, Stirling 1997, 2002). The brine channels and bottom surface of first-year sea ice are also sites of extensive production and biomass accumulation during the spring (Horner & Schrader 1982, Legendre et al. 1992, Michel et al. 1996, Arrigo 2003). These habitats are associated with a diverse community of microorganisms including viruses (Maranger et al. 1994, Wells & Deming

2006), bacteria (Junge et al. 2004) and heterotrophic (e.g. flagellates and ciliates, Sime-Ngando et al. 1997, 1999) and autotrophic (e.g. diatoms and dinoflagellates, von Quillfeldt et al. 2003) protists. In addition, first-year sea ice can also contain high concentrations of dissolved organic carbon (DOC), which could support secondary production throughout the ice covered period (Smith et al. 1997a, Vézina et al. 1997). The release of these carbon sources, along with inorganic nutrients, to the water column during ice melt can act as a catalyst for pelagic production (Spindler 1994, Michel et al. 1996, 2002). During the sea-ice algal bloom and ice melt periods, sea-ice biomass may also sink rapidly to the benthos thereby stimulating increased benthic activity, especially on Arctic shelves (Carey 1987, Renaud et al. 2006).

Algae are the major component of sea-ice communities and have been extensively studied in first-year Arctic sea ice (e.g. Michel et al. 1993, Gosselin et al. 1997, Arrigo 2003, von Quillfeldt et al. 2003, Ban et al. 2006). Dominant characteristics of microbial communities in first-year sea ice include the spring sea-ice algal bloom and the patchy distribution of algal biomass in relation to variable snow cover (Gosselin et al. 1986, Rysgaard et al. 2001, Mundy et al. 2005). Sea-ice algae can be an early and abundant food source for planktonic grazers when other food sources are not available (Michel et al. 1996, Arrigo 2003, Hill & Cota 2005). Other components of communities in first-year sea ice including DOC (Smith et al. 1997a), exopolymeric substances (EPS, Krembs et al. 2002) and bacteria (Smith & Clement 1990, Kaartokallio 2004) can also be important contributors to sea-ice organic carbon cycling. However, the structure and dynamics of heterotrophic

microbial food webs and the role of EPS in microbial interactions are not well established in first-year sea ice (Sime-Ngando et al. 1999, Krembs et al. 2002, Lizotte 2003, Meiners et al. 2003).

Heterotrophic microorganisms

Heterotrophic bacteria are active and abundant components of first-year Arctic sea ice (Bunch & Harland 1990, Smith & Clement 1990, Junge et al. 2002, Kaartokallio 2004) although relatively little is known about their heterotrophic protist counterparts (Laurion et al. 1995, Sime-Ngando et al. 1997). Direct measurements of heterotrophic sea-ice interactions are lacking despite the fact that heterotrophic bacteria and protists are key components of marine microbial food webs (Azam et al. 1983, Sherr & Sherr 1994). For example, there is currently only one study which directly measures bacterivory within first-year, landfast sea ice in the Canadian Arctic (Laurion et al. 1995). Bacterivory is central to microbial food webs as it allows for the transfer of DOC, via bacterial biomass and heterotrophic protist grazing, to higher trophic levels.

The carbon requirements of sea-ice heterotrophic protists may be primarily fulfilled by bacterivory although microflagellates and ciliates have been estimated to consume 15 to 20% of net sea-ice algal production in first-year sea ice (Vézina et al. 1997). Dissolved or colloid organic carbon sources may also be directly utilized by sea-ice heterotrophic protists (Sherr 1988, Tranvik et al. 1993) to satisfy their carbon requirements.

An important result of heterotrophic protist activity is the regeneration of inorganic nutrients, in particular nitrogen (Caron & Goldman 1990, Sherr & Sherr 1994, 2002), which can support regenerative production in marine and sea-ice environments (Thomas et al. 1995, Owrid et al. 2000, Lizotte 2003, Kaartokallio 2004, Wawrik et al. 2004). In addition, heterotrophic bacteria have a dual role in nutrient cycling as they regenerate and directly utilize inorganic nutrients (Kirchman 1994). The study of inorganic nitrogen cycling within first-year sea ice has been limited to its association with autotrophic production (Gosselin et al. 1985, Demers et al. 1989, Cota et al. 1990, Harrison et al. 1990) although the uptake of nitrogen by bacteria was suggested to occur in first-year sea ice in Barrow Strait (Harrison et al. 1990).

Exopolymers

Exopolymeric substances are part of a diverse group of high molecular weight polymers released primarily by bacteria and algal cells in marine environments (Stoderegger & Herndl 1999, Decho 2000, Passow 2002a). These polymers are characterized as gel-like, sticky, sorptive, ubiquitous components of benthic, pelagic and sea-ice environments. As gels, EPS are composed of three-dimensional polymer networks surrounded by water molecules. Free polymers can be as small as 1 nm although most marine polymer gels are macromolecular colloids (~1 μm to 1 mm, Verdugo et al. 2004).

The presence of EPS has been established in Arctic sea ice (Krembs & Engel 2001, Krembs et al. 2002, Meiners et al. 2003). From these studies, it is evident that EPS are

found at high concentrations within first-year sea ice and that these carbon-rich polysaccharides can have important implications for sea-ice organic carbon cycling. Krembs et al. (2002) suggest that EPS have a key role in the cryoprotection of algal cells within first-year Arctic sea ice, while EPS were identified as an important substrate for bacteria in Antarctic pack ice (Meiners et al. 2004).

In pelagic and benthic environments, exopolymers have multiple roles, which may also be important within first-year sea ice. For example, EPS can influence the structure and stability of the surrounding environment and can aid in cellular locomotion (Cooksey & Wigglesworth-Cooksey 1995, Decho 2000). Exopolymers also create microenvironments which can alter microbial cellular processes such as inorganic nutrient cycling, grazing and growth rates (Simon et al. 2002, Mari & Rassoulzadegan 2004). Of great significance is their role in the aggregation of organic matter, due to their sticky characteristics, and subsequent implications for the vertical fluxes of marine organic matter (Alldredge et al. 1993, Passow et al. 2001, Engel 2004). In addition, exopolymers also introduce alternative organic carbon flow pathways in marine systems such as the spontaneous transformations of polymer gels between dissolved and particulate pools (Chin et al. 1998). Current research clearly shows that EPS are directly involved in trophic interactions and organic carbon flux processes; and these substances were thus targeted as important component of this study.

Research objectives

The need for further research on heterotrophic processes and alternative organic carbon pathways in first-year sea ice is particularly important on the extensive Arctic continental shelves (30% of the Arctic Ocean, Macdonald et al. 1998). These shelves are the primary sites of first-year sea ice formation and are also the most productive regions of the Arctic Ocean (Legendre et al. 1992, Sakshaug 2004). In addition, organic carbon cycling associated with first-year sea ice may represent the total contribution of sea-ice to Arctic Ocean carbon cycling in the future, given the continued reduction in multi-year and increased proportion of first-year sea ice (Comiso 2003).

In Chapter 1, the establishment of communities within first-year sea-ice was assessed in newly formed sea ice on Mackenzie shelf and slope. These early communities can be active contributors to the Arctic Ocean carbon cycle in the fall and winter, and also form the basis for the productive spring communities in first-year sea ice. The objectives of Chapter 1 were to assess the variability in the concentration and enrichment of nutrients, EPS and microorganisms in newly formed sea ice, and to evaluate any selectivity based on cell size. In addition, the role of heterotrophic microorganisms in nitrogen regeneration was assessed in sea ice that was formed within a few hours. It was hypothesized that EPS could influence both physical and biogeochemical interactions in the sea ice due to their stickiness and high carbon content, and that any enrichment in dissolved nitrogen in newly formed sea ice would result from heterotrophic activity.

Extensive seasonal (winter to late spring) investigations of organic carbon cycling associated with first-year sea ice on an Arctic shelf are described in Chapters 2 and 3. Chapter 2 focuses on heterotrophic community dynamics and grazing activity. The main objectives of this chapter were to assess the variability of heterotrophic bacteria and protists abundances within the sea ice in relation to high and low snow cover and to compare the composition of heterotrophic bacterial and protist communities in the sea ice and surface waters. Bacterivory by heterotrophic protists was also assessed from the disappearance of fluorescently labeled bacteria to determine if the consumption of bacteria was sufficient to meet the carbon requirements of heterotrophic protists during the sea-ice algal pre-bloom and bloom periods. The abundance of heterotrophic bacteria and protists in the sea ice were expected to be highest under low snow cover, corresponding with highest sea-ice algal biomass.

Chapter 3 focuses on various roles of EPS associated with first-year sea ice. The objectives of Chapter 3 were: firstly, to evaluate EPS concentrations in the sea ice and surface waters thereby quantifying the seasonal contribution of EPS-carbon to sea-ice particulate organic carbon; secondly, to identify relationships between sea-ice EPS, algae and bacteria to identify the primary source of EPS within the sea ice; and thirdly, to assess the influence of EPS on the sinking velocities of sea-ice bacteria and algae. It was hypothesized that EPS concentrations would vary in parallel with sea-ice algal biomass and that high concentrations of sea-ice EPS would favor the aggregation and rapid sinking of sea-ice algae and bacteria.

This research was conducted on the Canadian portion of the Mackenzie shelf in southeastern Beaufort Sea. The Mackenzie shelf represents the largest continental shelf on the North American side of the Arctic Ocean having an area of ca. 6×10^4 km^2 (i.e. 530 km long, 120 km wide, Macdonald et al. 1998). This shelf also receives extensive inputs of inorganic sediments and particulate organic carbon (POC input: 1.8 to 2.1 10^{12} g y^{-1}) from the Mackenzie River (Dittmar & Kattner 2003), which can influence heterotrophic microbial activity (Parsons et al. 1988).

CHAPITRE I

ENRICHMENT OF NUTRIENTS AND MICROORGANISMS IN NEWLY FORMED SEA ICE ON THE MACKENZIE SHELF: SIGNIFICANCE OF HETEROTROPHIC REGENERATION AND EXOPOLYMERIC SUBSTANCES

RÉSUMÉ

La glace de mer nouvellement formée a été échantillonnée à 32 stations réparties sur le plateau continental du Mackenzie, entre le 30 septembre et le 10 novembre 2003. À chaque station, des échantillons de glace de mer et d'eau de surface ont été prélevés afin de déterminer les concentrations et l'enrichissement en nutriments, en substances exopolymériques (EPS, mesurés avec du bleu Alcian), en chlorophylle *a* (chl *a*), en protistes autotrophes et hétérotrophes, ainsi qu'en bactéries hétérotrophes. Des incubations au noir ont été menées afin d'estimer les taux de régénération nette par les hétérotrophes, dans la glace d'une épaisseur < 5 cm. Nos résultats montrent que les protistes autotrophes de grande taille (≥ 5 µm) sont enrichis de façon sélective au cours de la formation de la glace et présentent l'indice d'enrichissement le plus élevé (Is = 62), bien que les protistes hétérotrophes (Is = 19), les EPS (Is = 17), les bactéries (Is = 6), et les nutriments azotés (Is = 3 à 5) soient aussi enrichis dans la glace de mer. Des relations significatives ont été observées entre les concentrations en EPS et celles en chl *a* totale dans la glace ($r = 0.59$, $p < 0.001$) et entre les indices d'enrichissement des EPS et des protistes autotrophes ($r = 0.48$, $p < 0.01$), ce qui suggère que les EPS sont produits par les algues incorporées dans la glace. Ces résultats indiquent aussi que la présence des EPS favoriserait l'enrichissement sélectif des protistes autotrophes de grande taille. La régénération par les hétérotrophes a contribué à l'enrichissement du NH_4 dans la glace, avec un taux de régénération moyen de 0.48 µM N d^{-1}, et en contribuant 67 % des concentrations en NH_4 mesurées dans la glace. La régénération en NH_4 était aussi couplée à la consommation de $Si(OH)_4$ et corrélée de façon significative avec les concentrations en EPS dans la glace. Nos résultats suggèrent que les EPS favorisent la régénération du NH_4 dans la glace, en procurant une source de carbone pour les protistes hétérotrophes et/ou un substrat pour les bactéries.

ABSTRACT

Newly formed sea ice was sampled at 32 stations on the Mackenzie shelf, between 30 September and 19 November 2003. At each station, sea ice and surface water were analyzed to assess the concentration and enrichment of nutrients, exopolymeric substances (EPS, measured with Alcian blue), chlorophyll *a* (chl *a*), autotrophic and heterotrophic protists, and heterotrophic bacteria. Dark incubations were also conducted to estimate net heterotrophic NH_4 regeneration rates in sea ice <5 cm thick. Large (≥5 μm) autotrophs were selectively enriched during sea-ice formation, having the highest average enrichment index (I_S = 62), although heterotrophic protists (I_S = 19), EPS (I_S = 17), bacteria (I_S = 6), and dissolved inorganic nitrogen (I_S = 3 to 5) were also significantly enriched in the sea ice. Significant relationships were observed between sea-ice EPS and total chl *a* concentrations ($r = 0.59$, $p < 0.001$) and between sea-ice EPS and ≥5 μm autotroph enrichment indices ($r = 0.48$, $p < 0.01$), suggesting that EPS were actively produced by algae entrapped in the sea ice. These relationships also suggest that the presence of EPS may enhance the selective enrichment of large autotrophs. Heterotrophic regeneration contributed to the observed enrichment of NH_4 in the sea ice, with an average regeneration rate of 0.48 μM d^{-1}, contributing 67% of sea-ice NH_4 concentrations. In the newly formed ice, NH_4 regeneration was coupled to NO_3 and $Si(OH)_4$ consumption and was significantly related to EPS concentrations ($r = 0.87$, $p < 0.05$). Our data suggests that EPS enhance NH_4 regeneration by acting as a carbon source for sea-ice heterotrophs and/or a substrate for sea-ice bacteria.

1.1 Introduction

High biomasses of algae and other protists can accumulate in Arctic first-year sea ice in the spring (i.e. sea-ice algal blooms in April-May, Smith and Herman 1991, Michel et al. 1996, Melnikov et al. 2002). However, algal cells and other microorganisms are present within the sea ice for several months prior to the beginning of the spring bloom (Gradinger & Ikävalko 1998, Melnikov et al. 2002). Microorganisms from the water column and even the benthos are incorporated in the sea ice during its formation, which occurs primarily between September and December on the Canadian Arctic shelves (Canadian ice services 2002). Organisms incorporated in the sea ice during the fall can overwinter in the sea-ice matrix and are the founding members of the spring bloom community (Zhang et al. 1998).

In the Arctic Ocean, suspension freezing (Campbell & Collin 1958) is the most important process for the accumulation of inorganic sediments within the sea ice (Reimnitz et al. 1992). This same process leads to the accumulation of microorganisms in newly formed sea ice (Garrison et al. 1983, Reimnitz et al. 1993). Suspension freezing occurs under cold turbulent conditions which can lead to a supercooled water column. Once supercooled, frazil sea ice (i.e. suspended ice crystals) forms in the water column and rises to the surface where it accumulates as new ice with columnar ice growth continuing after frazil formation ends (Weeks & Ackley 1982, Eicken 2003).

Inorganic sediments and microorganisms can adhere to individual frazil crystals as they rise through the water column. Frazil formation may occur as deep as 25-30 m so that small particles can be picked up directly from the benthos on shallow shelves (Reimnitz et al. 1992). Large quantities of sediment and benthic microorganisms can also be incorporated into newly formed sea ice when frazil adheres to coarse particles on the sea-floor forming unconsolidated masses of anchor ice (Reimnitz et al. 1992). Under calm conditions the anchor ice, along with entrapped particles, can float to the surface and become incorporated into the newly formed sea ice. The formation of anchor ice and deep frazil can explain the numerous benthic algal species found in Arctic sea ice during the spring bloom (von Quillfeldt et al. 2003).

The harvesting or scavenging of cells by frazil crystals during early stages of ice formation can cause microorganisms to accumulate in the sea ice at concentrations orders of magnitude greater than in the surface water (Garrison et al. 1983, Gradinger & Ikävalko 1998). The extent to which cells become enriched within newly formed sea ice is dependent upon any cell incorporation, growth or loss (e.g. grazing) processes associated with the developing sea ice. The incorporation of microorganisms can be selective with preferences for different cell sizes or taxa (Weissenberger & Grossmann 1998). Enrichment is usually highest for algal cells, as compared to other microorganisms, and a selective enrichment for large-sized cells such as diatoms has been observed in Arctic sea ice (Gradinger & Ikävalko 1998, von Quillfeldt et al. 2003). Small cells such as bacteria can also be enriched within newly formed sea ice. However, bacterial enrichment in newly formed sea ice has not been

consistently observed and is usually associated with high algal enrichment (Grossmann & Dieckmann 1994, Weissenberger & Grossmann 1998).

Gradinger & Ikävalko (1998) suggest that cell surface characteristics (e.g. stickiness) can influence cell selectivity during sea-ice formation. Grossmann & Dieckmann (1994) also propose that bacterial incorporation into newly formed sea ice could be mediated through their attachment to the surface of algal cells or their mucus coatings. These interactions indicate a potentially important role for expopolymers in the selectivity and enrichment of sea-ice assemblages. Exopolymers are a diverse group of sticky compounds which include gel-like polysaccharides and glycoproteins, produced by phytoplankton (Passow 2002b), sea-ice algae (Krembs et al. 2002, Meiners et al. 2003, see Chapter 3) and in copious amounts by benthic diatoms (Smith & Underwood 1998). These exopolymers can exist as a gel surrounding cells or as free colloidal substances.

The terminology used to describe these exopolymers varies in the published literature. In the present study, the term exopolymeric substances (EPS) is used to specifically represent particulate (>0.4 µm) exopolysaccharides as measured by the Alcian blue method (Passow & Alldredge 1995). The Alcian blue method was first used to measure transparent exopolymer particles (TEP), which refer primarily to discrete particles in the water column formed abiotically from dissolved precursors (for review: Decho 1990, Passow 2002a). The exopolymers investigated in the present study may not be discrete particles but rather could be associated with cell surfaces in the sea-ice, water column or

benthos during the period of ice formation. Therefore, the particles measured by the Alcian blue method in this study will be referred to as EPS.

Newly formed sea ice can also be enriched in dissolved inorganic and organic nutrients. However, nutrient enrichment in newly formed sea ice is not expected to result from physical incorporation processes but rather from *in situ* nutrient regeneration (Gradinger & Ikävalko 1998). Heterotrophic nutrient regeneration by micro- or nano-sized protists and bacteria (Gilbert 1993), can result in high nitrogen concentrations within the sea ice (Harrison et al. 1990, Arrigo et al. 1995, Conover et al. 1999).

Community dynamics and trophic interactions, including nutrient cycling, within newly formed sea ice are still poorly understood, especially for very thin sea ice (i.e. <10 cm) formed on the extensive Arctic shelves. Therefore, the fall and winter contribution of sea-ice assemblages to Arctic Ocean carbon cycling is largely unknown. In this study, we investigated microbial interactions and assessed the enrichment of nutrients, EPS and microorganisms in newly formed sea ice between 0.1 and 40 cm thick. We hypothesized that EPS could influence both physical and biogeochemical interactions in the sea ice due to their stickiness and high carbon content, and that any enrichment in dissolved nitrogen in newly formed sea ice would result from heterotrophic activity. In order to verify these hypotheses, this study was centered around three main objectives: (1) assess the variability in the concentration and enrichment of nutrients, EPS, autotrophic and heterotrophic protists and bacteria in newly formed sea ice on the Mackenzie shelf, high

Canadian Arctic, (2) evaluate and identify any selectivity based on cell size; and (3) assess the role of heterotrophs in nitrogen regeneration in newly formed sea ice.

1.2 Materials and Methods

1.2.1 Sampling and analyses

This study was conducted on the Mackenzie shelf, Canadian Arctic, from 30 September to 19 November 2003, as part of the Canadian Arctic Shelf Exchange Study (CASES) on board the CCGS *Amundsen*. Newly formed sea ice and underlying surface waters were collected at 32 stations (Fig. 1). The sea-ice samples belonged to one of four stages of ice development, which vary in structure and/or thickness. The stages of ice development, as described in MANICE (Canadian ice services 2005), and the thicknesses observed during this study are as follows: new ice (0.1 to 8.4 cm, n = 6), nilas (0.1 to 9.0 cm, n = 9), young ice (11 to 27.3 cm, n = 13) and thin first-year ice (31.4 to 38.8 cm, n = 4).

Newly formed sea ice <7 cm thick was sampled with a strainer, with the entire ice thickness being retained for analyses. Newly formed congelation sea ice, ≥7 cm thick, was sampled with a manual ice corer (Mark II coring system, Kovacs Enterprise, 9 cm internal diameter), with only the bottom 3 cm of each core kept for analysis. Sea ice was melted in 0.2 μm filtered seawater collected at the surface at the time of sampling, to minimize osmotic stress during the melting process (Garrison & Buck 1986). A separate sea-ice

subsample, to which no filtered seawater was added, was kept in a sterile container for later salinity and nutrient analyses. At each station, seawater just under the ice was also collected with a clean container or Niskin bottle. Sea-ice and surface water subsamples were analysed for salinity, nutrients (NH_4, NO_2, NO_3, $Si(OH)_4$ and PO_4), EPS, chlorophyll *a* (chl *a*), and the abundance of autotrophic and heterotrophic protists as well as heterotrophic bacteria. When necessary, sea-ice concentrations were corrected for the dilution from the addition of seawater during the melting process.

Salinity was determined with an Autosal (model 8400B) analyzer. Nutrient samples were stored at 4°C in the dark and analysed within 24 h on a Bran+Luebbe III Autoanalyser. Chlorophyll *a* concentration was determined fluorometrically (10AU Turner Designs) on duplicate subsamples filtered on Whatman GF/F filters (nominal pore size of 0.7 µm, total chl *a*) and 5 µm Poretics polycarbonate membranes (>5 µm chl *a*), after 24 h extraction in 90% acetone at 5°C in the dark (Parsons et al. 1984).

Triplicate EPS subsamples were filtered onto 47 mm diameter, 0.4 µm, polycarbonate filters and stained with Alcian blue. Concentrations of EPS were measured colorimetrically (787 nm) after a 2 h extraction in 80% H_2SO_4 and were recorded as gum xanthan equivalents (µg xeq. l^{-1}, Passow & Alldredge 1995). The addition of 0.2 µm filtered seawater during the melting process may have introduced dissolved EPS which could reassemble during the filtering process. However, this would represent only a minimal addition of EPS due to low EPS concentrations in the surface water.

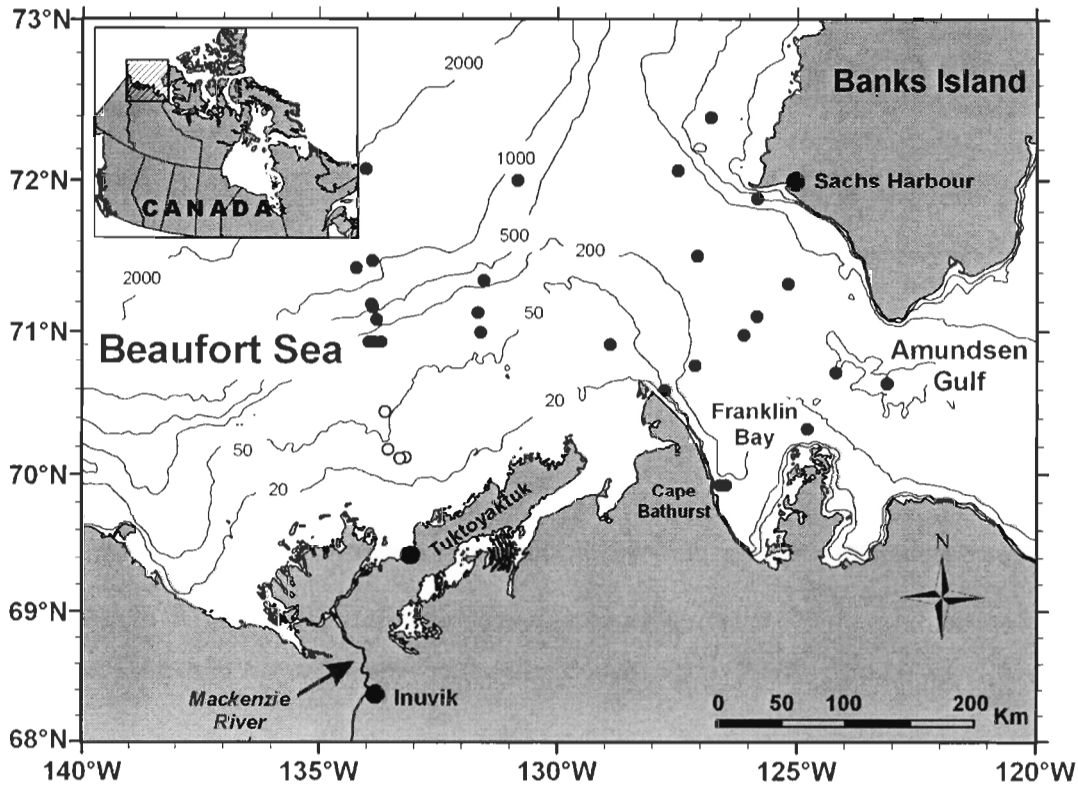

Fig. 1. Location of sampling stations on the Mackenzie shelf, in fall 2003. Open circles indicate stations in closest proximity to the Mackenzie River (see text for details)

Epifluorescence microscopy was used to count free-living and filamentous bacteria as well as autotrophic and heterotrophic protists (Sherr et al. 1993). Bacteria and protist samples were stained with DAPI (4, 6-diamidino-2-phenylindole) at a final concentration of 1 $\mu g\ ml^{-1}$, filtered on 0.2 or 0.8 μm, black Nuclepore filters, respectively, and stored at -80°C. Heterotrophic, free-living and filamentous bacteria were enumerated in ten fields of duplicate subsamples at 1000X magnification. A subsample of 200 bacteria filaments was measured with an image analysis system (Image Pro 5.1) to estimate the average and range of filament length.

Autotrophic and heterotrophic protists were counted at 400X and 1000X magnification. On average, a minimum of 75 cells were counted. Autotrophic cells were separated into two size classes, i.e., <5 μm (mean diameter = 2.5 μm) and ≥5 μm (mean diameter = 27 μm). The abundance of cells ≥5 μm was determined from the average cell counts at the two magnifications, whereas the abundance of cells <5 μm in diameter was obtained from cell counts at 1000X magnification only. Autotrophic cells were identified by chlorophyll autofluorescence (red fluorescence) under blue light excitation. This counting method can not rule out potential mixotrophy in these organisms. The heterotrophic protists were generally ≥5 μm in diameter (mean diameter = 12 μm), and consequently were not separated into size classes.

Semi-quantitative estimates of inorganic sediment abundances in the sea ice were performed from visual inspection of ten fields at 400X magnification. Three categories of sediment abundance were defined based on the number of sediment particles observed in each square of the ocular grid (low: <5, mid: 5 to 10 and high: >10). These semi-quantitative estimates of sediment abundances were used for comparison purposes only, no attempt was made to obtain quantitative estimates of sediment concentrations.

Enrichment indices (I_S) for nutrients, EPS, chl *a*, protists and bacteria were calculated according to Gradinger & Ikävalko (1998). The enrichment calculations include a salinity

factor, since brine loss can alter the concentration of microorganisms and nutrients during sea-ice formation. Enrichment indices are calculated according to equation 1:

$$I_S = (X_i/S_i) \times (S_w/X_w) \quad (1)$$

Where X_i and X_w are the concentrations or abundances of each variable in the ice and water, respectively and S_i and S_w are the salinity of the ice and water, respectively. Enrichment indices indicate the degree to which the concentration or abundance of a variable has increased (e.g. $I_S = 3$ is a 3-fold increase) despite implied losses due to desalinization. An index equal to one indicates a similar change in the variable concentration and salinity. Enrichment indices comprise multiple processes which can influence concentrations or abundances within the newly formed sea ice including: physical incorporation, brine drainage, grazing, reproduction and sampling artifacts (Gradinger & Ikävalko 1998).

1.2.2 Regeneration rates

Net ammonium regeneration ($\mu M\ d^{-1}$) by heterotrophic microorganisms in newly formed sea ice was estimated in seven dark incubation experiments conducted between 30 September and 18 October 2003. The new sea-ice samples were between 0.1 and 3.0 cm thick and were either new ice or dark nilas. Dark incubations of melted samples were conducted for 24 h at 0°C following a 1 h acclimatization period.

For each experiment, the sea ice was partitioned into five subsamples in sterile Whirl-Pak bags (Nasco) and slowly melted in the dark without the addition of filtered seawater, to avoid nitrogen contamination. Slow melting, over a 24 h period, was imperative to protect heterotrophic microorganisms which are sensitive to osmotic stress (Garrison & Buck 1986). Ammonium concentrations were analysed every 6 h, for 24 h, using a different subsample at each time point, thus eliminating potential contamination during the time-series subsampling.

Net ammonium regeneration rates were determined from the slope of the change in NH_4 concentrations over the 24 h experimental period (Model I linear regression, Sokal & Rohlf 1995). In four of the seven experiments, NO_3 and $Si(OH)_4$ consumption rates were also estimated from the slope of the change in the concentrations of these nutrients over the 24 h period. Chlorophyll *a*, bacteria and protist samples were collected at the beginning and end of every experiment to monitor any changes in abundance during the experiments. Nutrient and chl *a* concentrations and bacterial and heterotrophic protist abundances were analysed as described above.

1.2.3 Statistical analyses

Wilcoxon's signed ranks tests were used to determine if enrichment indices were significantly different from one (Sokal & Rohlf 1995). Model II linear regressions (reduced major axis) were used to evaluate relationships between measured variables. This method takes into account that both independent and dependant variables are subject to analytical

measurement errors (Sokal & Rohlf 1995). In order to test for significant differences in biochemical properties between stages of sea-ice development (new ice; nilas; young ice; thin first-year ice) and between classes of sea-ice thickness (<10 cm; 10-20 cm; >20 cm), one-way analyses of variance (ANOVAs) and multiple comparison test of means (Tukey HSD test for unequal sample sizes, Sokal & Rohlf 1995) were performed. For all parametric statistics, the data were log-transformed when necessary in order to meet the assumptions of homogeneity of variance and normality of distribution required for the analyses. All statistical analyses were carried out using JMP (SAS Institute).

1.3 Results

1.3.1 Physical and chemical environment

Salinity, nutrient concentrations and ratios and the abundance of protists and bacteria in the sea ice and surface waters are summarized in Table 1. Bulk salinity was consistently lower in the new ice (4.6 to 22) as compared to surface waters (17 to 34). Surface water salinities were lowest (17 to 21) at the sites in closest proximity to the Mackenzie River (open circles in Fig. 1).

Ammonium was the most abundant source of dissolved inorganic nitrogen within the sea ice and surface waters followed by NO_3 and NO_2 (Table 1). $Si(OH)_4$ concentrations ranged between 0 and 11.7 µM in the sea ice and reached as high as 25.8 µM in the surface waters. NO_3:PO_4 molar ratios averaged 1.3 and 1.0 in the sea ice and surface waters, respectively

(Table 1). These ratios were significantly lower than the Redfield et al. (1963) ratio of 16 N to 1 P (Wilcoxon's signed ranks, $p < 0.001$).

1.3.2 Sea-ice development and thickness

According to one-way ANOVAs, there were no significant differences between the different stages of sea-ice development (i.e. new ice, nilas, young ice and thin first-year ice) or ice thicknesses (i.e. <10 cm, 10-20 cm and >20 cm) for all variables presented in Table 1, except salinity and EPS:total chl *a* ratios. Salinity was significantly higher in sea ice <10 cm as compared to sea ice 10 to 20 cm thick (Tukey HSD, $p < 0.05$). The EPS:total chl *a* ratios were significantly higher in sea ice >20 cm as compared to <10 cm thick (Tukey HSD, $p < 0.05$) and in young ice as compared to nilas ice (Tukey HSD, $p < 0.05$).

The one-way ANOVAs also revealed that the enrichment indices of EPS, total chl *a* and ≥5 μm autotrophic protists were significantly higher in sea ice 10 to 20 cm as compared to <10 cm thick (Tukey HSD, $p < 0.05$) and in young ice as compared to new ice (Tukey HSD, $p < 0.05$). All other enrichment indices in Table 1 were not significantly different between the different stages of sea-ice development or ice thicknesses.

The majority (75%) of sea-ice samples had low inorganic sediment content whereas high sea-ice inorganic sediment content was found at only four stations located in shallow waters (<100 m) north of the mouth of the Mackenzie River estuary (Fig. 1, open circles). At this location, we measured the maximum heterotrophic protist abundance

(172 x 10^3 cells l^{-1}) and the minimum bacterial abundance (0.21 x 10^9 cells l^{-1}) of the entire sampling area. Average sea-ice bacterial abundances were significantly lower (t-test, $p < 0.01$) and average sea-ice heterotrophic protist abundances were significantly higher (t-test, $p < 0.0001$) at these four stations than in the rest of the sampling area.

1.3.3 Sea-ice and surface water assemblages

All samples of newly formed sea ice contained both autotrophic and heterotrophic protists as well as bacteria. Protist abundances may have been slightly underestimated since fragile ciliates are not adequately quantified by our methods. In a concurrent study, M. Różańska (pers. comm.) found that ciliates constituted <5% of total protist abundances. In the sea ice, <5 µm autotrophs were the most abundant protists (average: 256 x 10^3 cells l^{-1}), followed by ≥5 µm autotrophs (average: 177 x 10^3 cells l^{-1}) and heterotrophs (average: 36 x 10^3 cells l^{-1}, Table 1). Autotrophs <5 µm, generally identified as autotrophic flagellates, contributed 58 ± 21% of total autotrophic abundance whereas ≥5 µm autotrophs were generally identified as centric and pennate diatoms and contributed 42 ± 21% of total autotrophic abundance. In the surface water, the abundance of <5 µm autotrophs was, on average, >1 order of magnitude higher than the abundance of ≥5 µm autotrophs (Table 1).

The majority of bacteria in this study were free-living single cells, although filamentous and diatom-attached bacteria were also observed. Free-living, non-filamentous, bacterial abundance in the sea ice and surface waters averaged 1.1 ± 0.25 x 10^9 and

$0.8 \pm 0.13 \times 10^9$ cells l^{-1} (Table 1). Filamentous bacteria occurred in 81% of the sea-ice samples and in 45% of surface water samples. In sea ice and surface waters, filament abundances were two orders of magnitude lower than free-living, non-filamentous bacterial abundances (Table 1). Filamentous bacteria were <1 μm in diameter with an average filament length of 31.28 ± 22.3 μm.

Total chl *a* concentrations ranged between 0.3 and 22 μg l^{-1} and between 0.2 and 4.9 μg l^{-1} in the sea ice and surface waters, respectively, whereas chl *a* concentrations of cells >5 μm ranged between 0.1 and 20 μg l^{-1} and between 0.03 and 3.8 μg l^{-1} in the sea ice and surface waters, respectively (Table 1). The percent chl *a* >5 μm in total chl *a* was, on average, 52 ± 20% and 29 ± 19% in the sea ice and surface waters, respectively.

The concentration of EPS ranged between 28 and 1170 μg xeq. l^{-1} in the sea ice and between 12 and 868 μg xeq. l^{-1} in the surface water with an average EPS:total chl *a* ratio of 138 and 179 in the sea ice and surface waters, respectively (Table 1). Concentrations of EPS were significantly related to chl *a* concentrations in the sea ice (total chl *a*: $r = 0.59$, $p < 0.001$; chl *a* >5 μm: $r = 0.62$, $p < 0.001$, Fig. 2A) and surface waters (total chl *a*: $r = 0.59$, $p < 0.001$; chl *a* >5 μm: $r = 0.60$, $p < 0.001$, results not shown). In the sea ice, the slopes of the relationships between EPS concentrations and >5 μm and total chl *a* concentrations were not significantly different ($p > 0.05$). A weaker yet significant, relationship was also observed between sea-ice EPS concentrations and the abundance of heterotrophic protists ($r = 0.41$, $p < 0.05$; Fig. 2B). Free-living, non-filamentous, bacterial

abundances were not significantly related to sea-ice EPS concentrations ($p = 0.55$, result not shown) but were significantly related to heterotrophic protist abundances ($r = -0.41$, $p < 0.05$; Fig. 2C).

1.3.4 Enrichment

Table 1 presents the enrichment indices for all variables measured during our study. All three forms of dissolved inorganic nitrogen (DIN = NH_4, NO_2, NO_3) were significantly enriched in the newly formed sea ice with average I_S values of 3.4, 2.9 and 3.8 for NH_4, NO_2 and NO_3, respectively (Table 1). $Si(OH)_4$ and PO_4 enrichment indices were not significantly different than one (Wilcoxon's signed-ranks, $Si(OH)_4$: $p = 0.14$, PO_4: $p = 0.33$) with an average I_S values of 1.5 and 2.0, respectively (Table 1).

Table 1. Summary of newly formed sea-ice and surface water variables and enrichment indices (I_S) for newly formed sea ice. Averages and ranges are given and N represents the number of data points for each variable. Note that enrichment values with $N \leq 31$ are due to zero values in ice or surface waters. An asterisk (*) indicates significant enrichment ($p < 0.001$) in the sea ice compared to surface waters. NA = not applicable

Variable	New sea-ice stations			Surface waters			Enrichment		
	Average	Range	N	Average	Range	N	Average	Range	N
Salinity	8.3	4.6-22.2	31	25.5	16.7-33.6	32		NA	
NH_4* μM	0.7	0.2-1.7	29	1.0	0.1-3.5	29	3.4	0.6-21.7	26
NO_2* μM	0.09	0.01-0.3	17	0.2	0-0.4	18	2.9	0.2-14.7	16
NO_3* μM	0.19	0.01-0.4	28	0.3	0.02-1.5	29	3.8	0.4-21.2	26
$Si(OH_4)$ μM	2.5	0-11.7	29	6.0	1.5-25.8	31	1.5	0.1-7.4	27
PO_4 μM	0.3	0-0.8	20	0.8	0.03-2.9	23	2.0	0.1-9.11	14
NO_3:PO_4 mol:mol	1.3	0.2-5.3	16	1.0	0.03-5.6	23		NA	
EPS* μg xeq. l^{-1}	295	27.5-1170	30	126	11.8-868	29	18.0	1.5-61.8	26
Total chl *a** μg l^{-1}	4.0	0.3-22.6	32	0.8	0.2-4.9	32	20.8	0.9-111	31
>5 μm chl *a** μg l^{-1}	2.5	0.1-20.9	32	0.4	0.03-3.8	32	41.0	0.9-185	31
>5 μm:total chl *a* %	52	17-96	32	29	9.7-84	32		NA	
EPS:total chl *a* g:g	138	12.4-544	30	179	22.9-814	29		NA	
Bacteria* 10^9 cells l^{-1}	1.1	0.2-2.0	30	0.8	0.3-1.6	32	5.1	0.3-23.9	29
Filamentous bacteria 10^6 cells l^{-1}	13.7	0-67.0	31	2.1	0-16.0	32		NA	
Hetero. protists* 10^3 cells l^{-1}	35.6	3.1-172	32	16.3	0-108	32	18.7	1.0-93.7	30
≥5 μm Auto. protists* 10^3 cells l^{-1}	177	9.4-577	32	20.5	0-97.4	32	63.0	1.7-409	30
<5 μm Auto. protists* 10^3 cells l^{-1}	256	32.6-1114	32	284	6.9-798	32	4.6	0.6-30.9	31

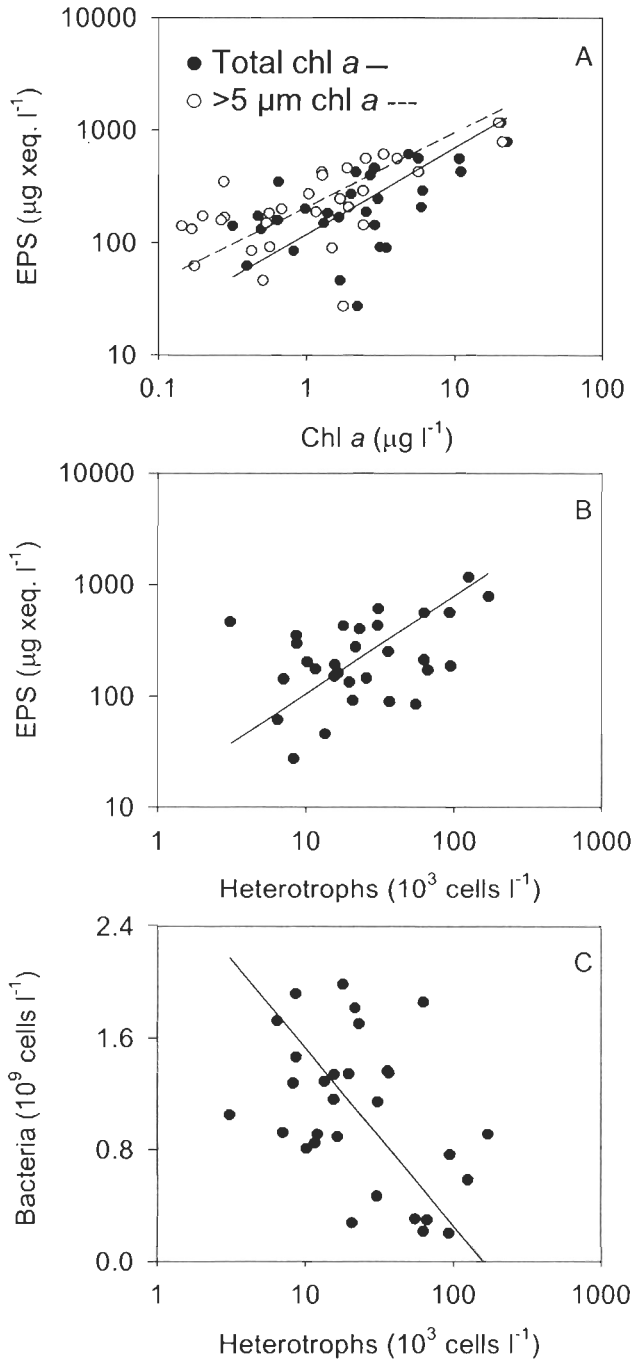

Fig. 2. Relationships between exopolymeric substances (EPS) concentrations and (A) chlorophyll *a* (chl *a* total and >5 µm) concentrations, and (B) heterotrophic protist abundances, and (C) between bacterial and heterotrophic protist abundances for newly formed sea ice on the Mackenzie Shelf. Note logarithmic scales for all variables except bacterial abundances

Total and >5 μm chl *a*, free-living bacteria and heterotrophic and autotrophic protists were significantly enriched in the newly formed sea ice (Wilcoxon's signed-ranks, $p < 0.001$, Table 1). Enrichment indices for filamentous bacteria could not be calculated due to the absence of filamentous bacteria in numerous surface water samples. Amongst the protist groups, autotrophic protists ≥5 μm had the highest average I_S (63), whereas heterotrophic protists and <5 μm autotrophs had an average I_S of 18.7 and 4.7, respectively (Table 1). The high I_S values for ≥5 μm autotrophs are consistent with the higher average I_S for >5 μm chl *a* (41) as compared to total chl *a* (21, Table 1). The enrichment indices of total chl *a* were significantly related to the enrichment indices of ≥5 μm autotrophs ($r = 0.75$, $p < 0.001$; Fig. 3A) but were not significantly related to the enrichment indices of the <5 μm autotrophic protists ($p = 0.38$, result not shown).

Exopolymeric substances were enriched in the newly formed sea-ice samples, with an average I_S of 18.0 (range 1.5 to 62, Table 1). The EPS enrichment indices were significantly related to the enrichment indices of ≥5 μm autotrophs ($r = 0.48$, $p < 0.05$; Fig. 3B). Enrichment indices for free-living bacteria were also significantly related to EPS enrichment indices ($r = 0.57$, $p < 0.01$; Fig. 3C) but were not significantly related to the enrichment indices of ≥5 μm autotrophs ($p = 0.31$, Fig. 3D).

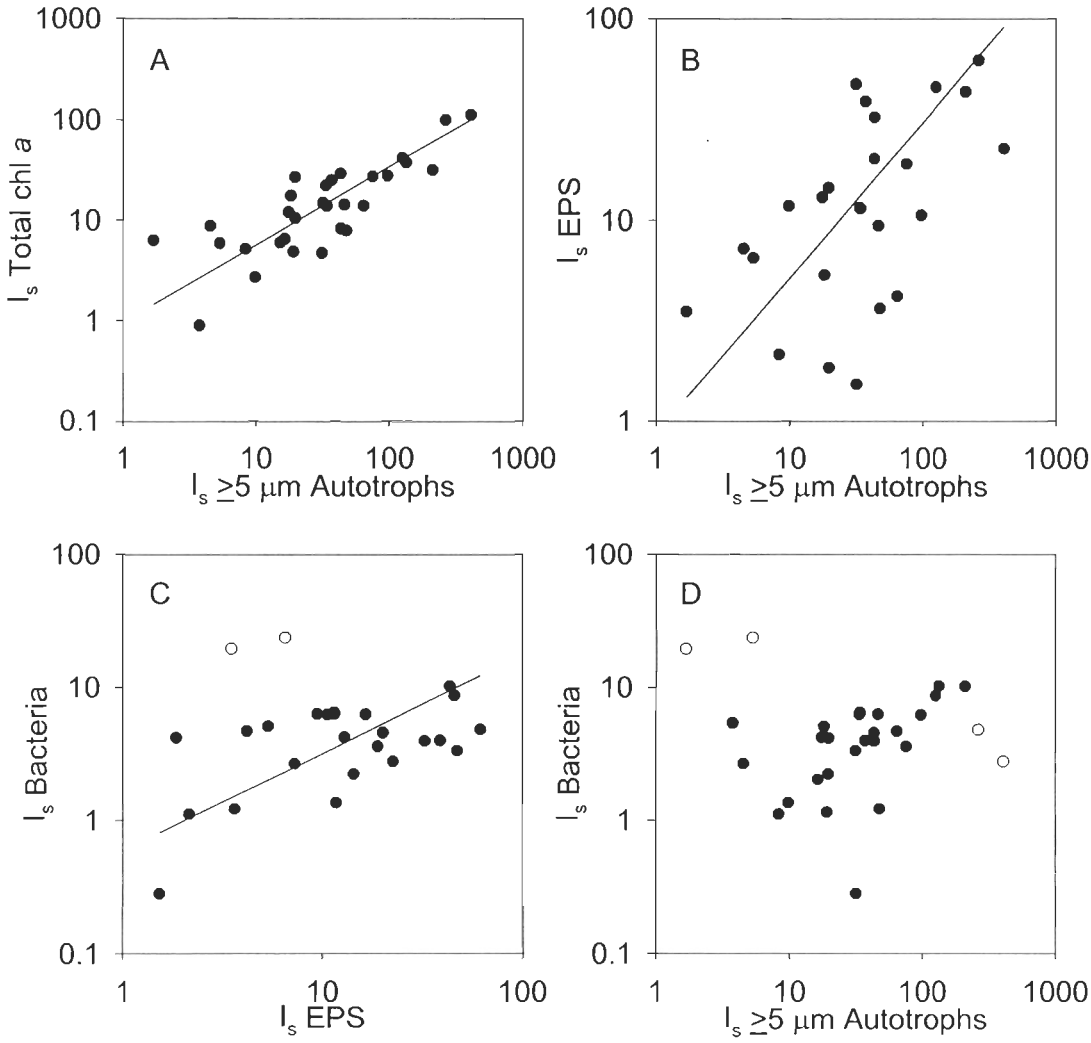

Fig. 3. Relationships between the enrichment indices (I_S) of (A) chlorophyll *a* (chl *a*) and ≥5 µm autotrophs, (B) exopolymeric substances (EPS) and ≥5 µm autotrophs, (C) bacteria and EPS and (D) bacteria and ≥5 µm autotrophs for newly formed sea ice on the Mackenzie shelf. Open circles are not included in the regression analyses. Note logarithmic scales for all variables

1.3.5 Ammonium regeneration rates

Summary results from the NH_4 regeneration experiments are presented in Table 2. During the 24 h incubation, NH_4 concentrations increased in five of the seven experiments, whereas NH_4 concentrations decreased in the other two experiments. The average net regeneration rate estimated from increases in NH_4 concentrations was 0.48 $\mu M\ d^{-1}$, with values ranging between 0.12 and 1.2 $\mu M\ d^{-1}$. The average net consumption rate of NH_4 was 0.25 $\mu M\ d^{-1}$, with values of 0.16 and 0.34 $\mu M\ d^{-1}$ (Table 2).

In the five experiments where NH_4 concentrations increased over the 24 h experimental period, average NO_3 and $Si(OH)_4$ concentrations decreased at an average rate of $0.10 \pm 0.08\ \mu M\ d^{-1}$ and $1.4 \pm 0.4\ \mu M\ d^{-1}$, respectively. In the first experiment where NH_4 concentrations decreased, NO_3 and $Si(OH)_4$ decreased at a rate of 0.01 and 0.02 $\mu M\ d^{-1}$, respectively (3 October, Table 2). In the second experiment, NO_3 increased at a rate of 0.1 $\mu M\ d^{-1}$ whereas $Si(OH)_4$ decreased at a rate of 0.16 $\mu M\ d^{-1}$ (8 October, Table 2). The concentration of chl *a* and abundance of bacteria and heterotrophic protists (t-test, $p = 0.60\text{-}0.80$) did not change significantly over the 24 h duration of any experiment. Ammonium regeneration rates were significantly related with EPS concentrations ($r = 0.87$, $p < 0.05$; Fig. 4A), while there was no clear relationship between NH_4 regeneration rates and the abundances of sea-ice heterotrophic protists ($p = 0.34$, Fig. 4B) or bacteria ($p = 0.26$, Fig. 4C).

Figure 5 presents relationships amongst biological variables from parallel sea-ice samples during the period when the dark incubation experiments were conducted (30 September to 18 October). A significant negative relationship between sea-ice NH_4 concentrations and bacterial abundances was observed ($r = -0.68$, $p < 0.05$; Fig. 5A) whereas NH_4 concentrations were not significantly related to heterotrophic protist abundances ($p = 0.23$, Fig. 5B) but sea-ice EPS concentrations were significantly related to heterotrophic protist abundances ($r = 0.77$, $p < 0.01$; Fig. 5C).

Table 2. Summary of NH_4 regeneration experiments. The concentrations of nutrients and total chl *a* and bacterial and protist abundances provided are those measured at the beginning of each experiment. Nutrient regeneration and consumption rates are estimated from changes in nutrient concentration over the duration of the experiments (24 h). Consumption rates are indicated by a minus sign. All regression coefficients (i.e. the rates) are significantly different from zero ($p < 0.05$).
nd = no data.

Date	Ice type	Ice thickness	Nutrient concentrations			Total chl *a*	Heterotrophic		Regeneration/consumption rates		
			NH_4	NO_3	$Si(OH_4)$		Bacteria	Protists	NH_4	NO_3	$Si(OH_4)$
Date		cm	µM	µM	µM	µg l^{-1}	10^9 cells l^{-1}	10^3 cells l^{-1}	µM d^{-1}	µM d^{-1}	µM d^{-1}
30/09	nilas	0.1	0.83	0.20	9.4	10.7	0.22	63	1.2	nd	nd
30/09	new	3.0	1.1	nd	nd	5.7	0.21	93	0.42	nd	nd
02/10	nilas	0.1	0.60	0.17	2.7	1.4	0.76	95	0.25	-0.16	-1.7
03/10	nilas	2.5	0.62	0.06	0.12	0.82	0.31	55	-0.16	-0.01	-0.02
08/10	new	2.0	0.45	0.07	1.2	0.47	0.85	12	0.44	-0.04	-1.2
08/10	nilas	2.0	0.33	0.01	1.08	0.39	1.7	6.4	-0.34	0.10	-0.16
12/10	new	0.5	0.64	nd	nd	2.9	nd	26	0.12	nd	nd

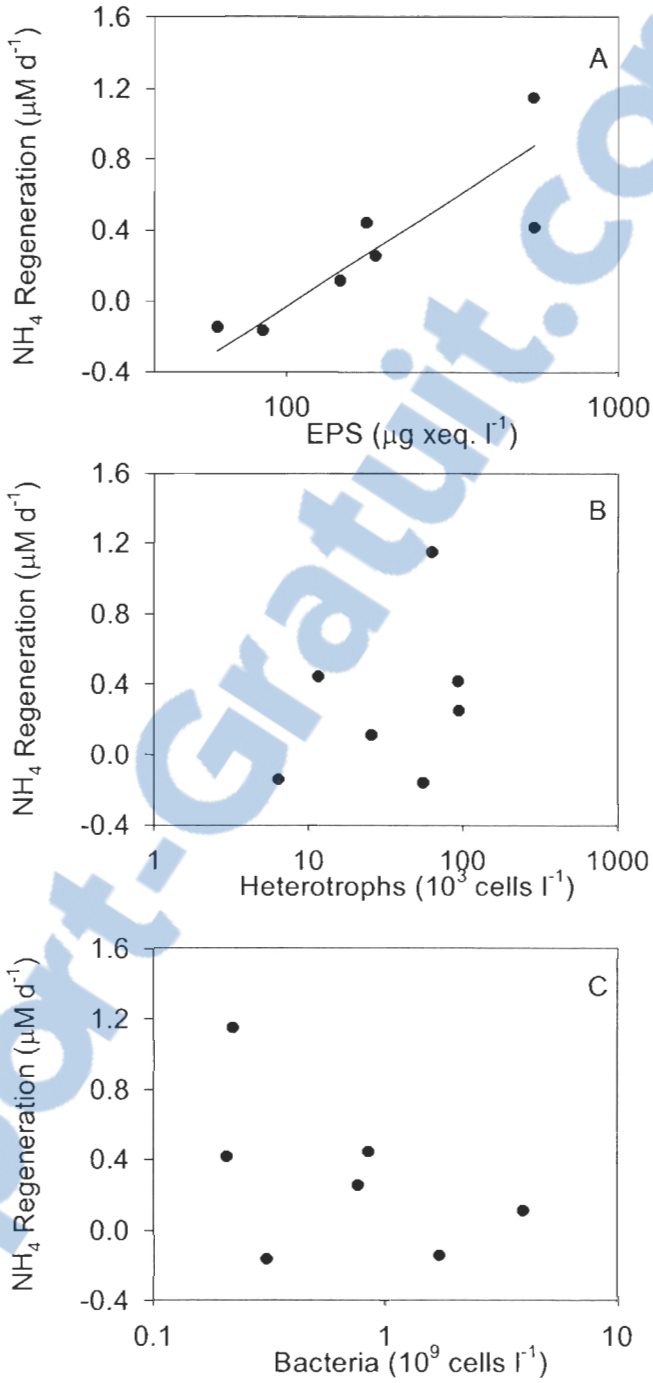

Fig. 4. Relationships between net NH_4 regeneration rates and (A) exopolymeric substances (EPS) concentrations, (B) heterotrophic protist and (C) bacterial abundances in newly formed sea ice on the Mackenzie shelf. Note logarithmic scales for x-axes

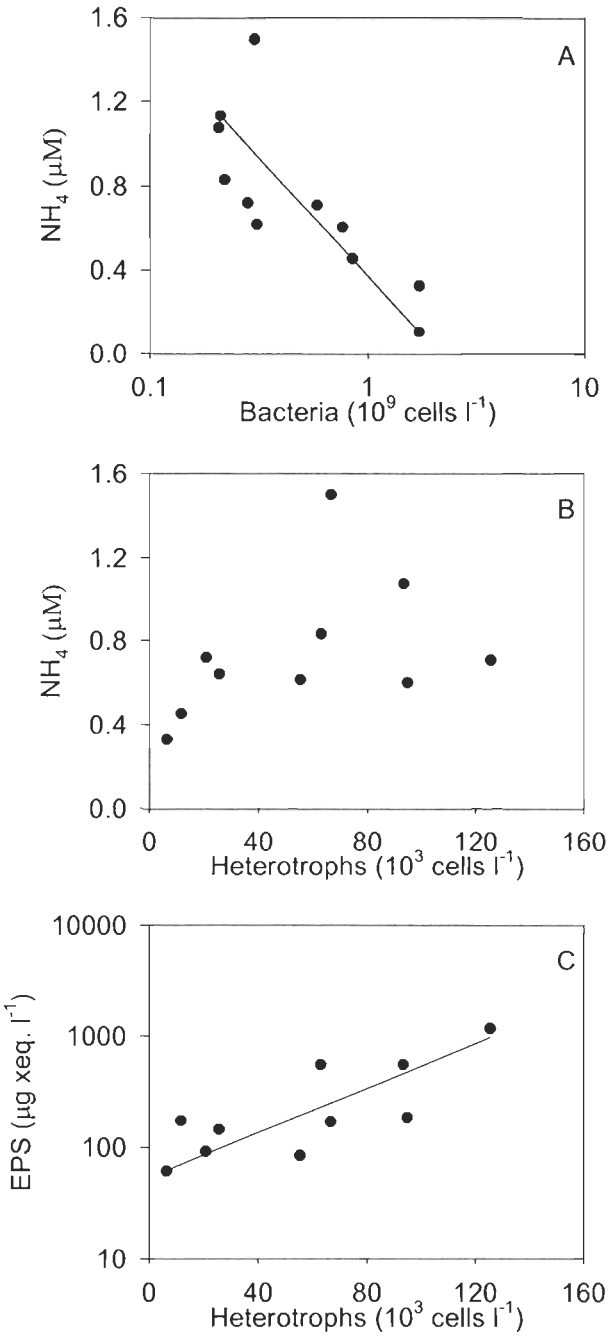

Fig. 5. Relationships between NH_4 concentrations and (A) bacterial and (B) heterotrophic protist abundances and between (C) exopolymeric substances (EPS) concentrations and heterotrophic protist abundances in newly formed sea ice sampled in parallel to the dark incubations. In (A) and (C), note logarithmic scale for bacteria and EPS

1.4 Discussion

This study allowed for the *in situ* collection of very young sea ice. Sea ice can grow up to a thickness of ca. 10 cm within a single day (Eicken 2003), indicating that the majority of our samples were only hours or days old. Therefore, we present results on microbial assemblages and the biological activity of sea-ice communities at very early stages of sea-ice formation on an Arctic continental shelf and slope.

Our newly formed sea-ice samples were collected over a large geographical area with the sea-ice differing in thickness and stage of development. However, these variations in the newly formed sea ice had no effect on measured variables except for ≥5 μm autotrophs and their related variables (i.e. EPS and chl *a*). The sediment-laden Mackenzie River outflow appeared to influence both bacterial and heterotrophic protist abundances within the sea ice, but only within a localized area (Fig. 1, open circles). We thus conclude that the results of this study are generally applicable to the extensive Mackenzie shelf and slope region, and possibly other Arctic shelves.

1.4.1 Sea-ice assemblages

All samples of newly formed sea ice, even those <1 cm thick, contained similar multi-trophic level communities comparable to those observed in a previous study of newly formed sea ice in the Greenland Sea (Gradinger & Ikävalko 1998). These communities generally consist of free-living bacteria, phototrophic flagellates, diatoms and other

autotrophic and heterotrophic protists. In our study, autotrophs <5 μm in diameter were the most abundant protists (Table 1), whereas diatoms represented the largest cells within the sea ice. There was no evidence of large flagellates (e.g. > 70 μm) or meiofauna (e.g. nematodes) which are conspicuous components of multi- and first-year Arctic sea ice (Nozais et al. 2001, Michel et al. 2002).

The majority (>80%) of our sea-ice samples also contained filamentous bacteria, which have not been previously reported in newly formed sea ice of the Arctic but were observed in newly formed pack-ice of Antarctica (Zdanowski 1988). Filamentous bacteria appear to be a temporary component of sea-ice assemblages on the Mackenzie shelf, since they were not observed in any sea-ice samples collected only a few months later (February to June) in the same region (see Chapter 3). For the remainder of this discussion, bacteria will refer to non-filamentous bacteria only.

Concentrations of sea-ice bacteria were lowest at stations in closest proximity to the Mackenzie River (Fig. 1, open circles) despite these stations having the highest concentrations of inorganic sediments in the sea ice and average bacterial concentrations (average of four stations: 0.8×10^9 cells l^{-1}) in the surface water (Table 1). This suggests that inorganic sediments, efficiently scavenged by frazil in the Arctic (Reimnitz et al. 1993), are not a major pathway by which bacteria entered the sea ice. In our samples, bacteria were not observed to be attached to inorganic sediments although this attachment may have been disturbed during sample preparation. Bacteria may also have been grazed

after their incorporation into the sea-ice at stations in closest proximity to the Mackenzie River, since lowest bacterial concentrations were also coupled with highest concentrations of sea-ice heterotrophic protists. The area of the Beaufort shelf near the mouth of the Mackenzie River is characterized by high heterotrophic activity (Parsons et al. 1988) and our study suggests that active heterotrophy may also be occurring within sea ice which forms in this area.

1.4.2 Sea-ice EPS

This study documents the wide-scale presence of EPS in newly formed sea ice on an Arctic shelf (Table 1). During the spring and summer, diatoms are primarily responsible for EPS production in first-year sea ice (Krembs & Engel 2001, Meiners et al. 2003, see Chapter 3). The similarities in slopes of the relationships between EPS and total chl *a* and EPS and >5 chl *a* concentrations (Fig. 2A) point to a tight linkage between EPS and large autotrophic producers in the sea ice during this study. This is also supported by the significant relationship between the enrichment of EPS and ≥5 μm autotrophs (Fig. 3B).

The relationship between EPS and ≥5 μm autotrophs enrichment indices (Fig. 3B) and the fact that EPS occurred in all sea-ice samples only hours old, suggest that EPS were incorporated into the sea ice in association with phytoplankton and/or benthic algal cells scavenged by rising frazil ice crystals. Relatively high concentrations of EPS (i.e. average >300 μg xeq. l^{-1}) were observed in the thinnest sea ice (i.e. <1 cm thick) suggesting that algal cells with their attached EPS were being incorporated at the time of sea-ice formation.

In the fall, phytoplankton cells are likely to be nutrient limited, as indicated by the low NO_3:PO_4 during our study (Table 1), and as observed in other Arctic environments (e.g. Cota et al. 1996, Lewis et al. 1996). Therefore phytoplankton may have been producing elevated amounts of EPS due to nutrient limitation (Magaletti et al. 2004), which could become incorporated in the newly formed sea ice.

Alternatively or additionally, EPS could be produced by algal cells after they are entrapped in the sea ice as indicated by the significantly higher EPS:total chl *a* ratios in young versus nilas sea ice and in >20 cm versus <10 cm thick sea ice (ANOVAs, $p < 0.05$). This suggests that EPS continues to accumulate within the sea ice during its growth, even after scavenging by frazil ends at a sea-ice thickness of ca. 5 cm (Weeks & Ackley 1982). We suggest that entrapped diatoms continue to actively produce EPS within the newly formed sea ice. Exopolymeric substances may be produced in response to altered nutrient conditions (Magaletti et al. 2004) or for cryoprotection against high salinities or growing ice crystals, thereby enhancing the survival of cells within the newly formed sea ice (Krembs et al. 2002). We thus conclude that EPS are incorporated with scavenged algae cells and continue to be actively produced by algal cells within newly formed sea ice of the Arctic.

Sampling conducted in March 2004 in Franklin Bay (Fig. 1) found EPS concentrations at the sea-ice bottom to be, on average 185 μg xeq. l^{-1} (range: 91-388 μg xeq. l^{-1}, see Chapter 3), less than one third of the new sea-ice EPS measured at

the same site at the beginning of November (600 μg xeq. l^{-1}). This may indicate that EPS do not accumulate in sea ice throughout the entire winter period but instead, EPS appear to be lost from, or utilized within the sea ice after the fall period included in this study. Exopolymeric substances were found to be retained within the sea ice during the spring melt on the Mackenzie shelf (see Chapter 3), therefore it is unlikely that EPS would be released from the sea ice during the winter period. Concentrations of EPS in our study did not vary significantly between the different sea-ice thicknesses (i.e. <10 cm, 10-20 cm, >20 cm; ANOVA, $p = 0.26$) suggesting that EPS were not lost from the sea ice due to continued brine drainage during sea-ice formation (Eicken 2003). We thus hypothesize that, during the winter period, sea-ice EPS may be broken down or change chemically such that these EPS would not be detected by our analytical methods, or that EPS may be utilized as a carbon source for bacteria or other heterotrophs present in the sea ice, as reported for similar exopolymers in the water column (Sherr 1988, Tranvik et al. 1993).

1.4.3. Enrichment and selectivity in newly formed sea ice

The newly formed sea ice was significantly enriched in nitrogenous nutrients, chl *a*, EPS, bacteria and protists. The enrichment of EPS has been documented in only one other sea-ice study. An enrichment value of 5.6 was obtained from a single sample of nilas sea ice, 11 cm thick, from the Arctic (Meiners et al. 2003), thus falling at the lower range of EPS enrichment indices observed in our study (Table 1). Enrichment of EPS in the sea ice of the Mackenzie shelf appeared to be associated with the incorporation of algae and bacteria, as discussed below.

Among the microorganisms, enrichment indices were lowest for bacteria and <5 μm autotrophs and highest for ≥5 μm autotrophs (Table 1). The same pattern of enrichment was observed by Gradinger & Ikävalko (1998) in a study of newly formed drift ice near Greenland. It thus appears that during sea-ice formation in the Arctic there is consistent selectivity for larger autotrophic cells. Our results suggest that both cell size and cell-associated EPS are key factors influencing the selection of large autotrophs during sea-ice formation.

Assuming that the effect of cell size on enrichment is consistent for ≥5 μm autotrophic and heterotrophic protists, we calculated the expected enrichment of ≥5 μm autotrophic protists based on the size:enrichment ratio of the heterotrophic protists (i.e. I_S autotrophs = average autotrophs size x average heterotrophs size/I_S heterotrophs). Based on this ratio, we would expect an average enrichment index of 42, instead of the observed enrichment index of 62, for the autotrophic protists ≥5 μm. We surmise that the higher enrichment index observed is caused by the association of EPS with autotrophic cells (Fig. 2A). Algal cells associated with even small amounts of EPS would have sticky surfaces which could increase their probability of attaching to rising frazil ice crystals. Estimates of exopolymer stickiness (e.g. Dam & Drapeau 1995) have been found to be two to four orders of magnitude higher than other particles (Passow 2002a).

Average bacterial enrichment indices in newly formed sea ice on the Mackenzie shelf (average I_S = 5.5) closely matched those from pancake and nilas sea ice <22 cm thick, sampled near Greenland (average I_S = 7, Gradinger & Ikävalko 1998). Bacteria enrichment has been suggested to be an indirect result of attachment to larger cells, in particular algal cells, instead of direct scavenging by rising frazil ice (Grossmann & Dieckmann 1994, Weissenberger & Grossmann 1998). However, the attachment of bacteria to diatoms was observed in only 12.5% of our newly formed sea-ice samples and we did not observe a direct relationship between bacterial enrichment and the enrichment of ≥5 μm autotrophs (Fig. 3D). It is possible that ≥5 μm autotrophs indirectly enhanced the enrichment of bacteria by the production of EPS since bacterial enrichment indices were significantly related to EPS enrichment indices (Fig. 3C). The attachment of bacteria to exopolymers has been directly observed in the pelagic (Passow & Alldredge 1994) and benthic (Underwood et al. 1995) environments.

1.4.4 Nutrient regeneration

The enrichment of nitrogenous nutrients and lack of enrichment for PO_4 or $Si(OH)_4$, are consistent with the results of Gradinger & Ikävalko (1998), who suggested that the enrichment of DIN was a consequence of heterotrophic regeneration within the sea ice. Dark incubation experiments carried out during this study showed that heterotrophic biological activity could contribute, on average, 67% of NH_4 concentrations in the newly formed sea ice on the Mackenzie shelf. Ammonium regeneration rates have not previously been determined for newly formed sea ice in the Arctic. Our estimated net NH_4

regeneration rates (0.1 to 1.2 μM d^{-1}) fall within the same range as NH_4 regeneration rates measured from open water in coastal zone areas using ^{15}N isotopic methods (e.g. 0.17-1.30 μM d^{-1}, Maguer et al. 1999, Molina et al. 2005). The newly formed sea ice appears to be an environment of primarily regenerated nutrients given that NO_3:PO_4 ratios were very low (i.e. <2) and NH_4 was the dominant form of DIN (Table 1). This indicates that heterotrophic regenerative processes are an important component of nutrient cycling in newly formed sea ice of the Arctic. It is likely that regeneration within first-year sea ice continues during the winter period. Reduced metabolic activity of autotrophic cells during the dark winter (Zhang et al. 1998) together with continued heterotrophic regeneration could result in the accumulation NH_4, possibly supporting the onset of the spring sea-ice algal bloom (Conover et al. 1999).

The regeneration of NH_4 and dark consumption of NO_3 and $Si(OH)_4$ were coupled, with no measurable regeneration occurring at low consumption rates (Table 2). It was apparent that the relatively high concentrations of sea-ice NH_4 (Table 1) did not inhibit NO_3 consumption by the sea-ice algae. Ammonium concentrations <1 μM have been observed to inhibit NO_3 uptake and/or assimilation within the water column (Wheeler & Kokkinakis 1990) and possibly in sea ice (Priscu & Sullivan 1998). Dark consumption of NO_3 in our study, can be attributed at least in part to diatom cells specifically, since diatoms are the only group which would also be taking up $Si(OH)_4$ for growth. Dark consumption of NO_3 by sea-ice algae has been previously reported for bottom sea ice of McMurdo Sound Antarctica, with dark consumption being <50% of light-mediated NO_3

consumption (Priscu et al. 1991). Dark NO_3 consumption has been suggested to indicate nitrogen limitation for phytoplankton (Kanda et al. 1989). However, the nutrient status of the sea-ice algae in this study is not known. Denitrification may have also contributed to the observed decrease in NO_3 concentrations since bacterial denitrification can occur in the bottom layer of first-year Arctic sea ice (Rysgaard & Glud 2004).

Dark consumption of NH_4 by the sea-ice algae was likely also occurring during our experiments since NH_4 is a preferred nitrogen source for sea-ice algae (Harrison et al. 1990). Concurrent dark consumption of both NO_3 and NH_4 has been shown to occur in bottom sea-ice of Antarctica (Priscu et al. 1991). In addition, bacterial consumption of NH_4 may have been occurring during our experiments, as indicated by the significant negative relationship between NH_4 concentrations and bacterial abundances in the sea ice (Fig. 5A). Significant consumption of NH_4 by bacteria has been previously suggested for Arctic first-year sea-ice communities in Barrow Strait (Harrison et al. 1990). Therefore, our NH_4 regeneration rates are potentially underestimated due to dark NH_4 consumption by both algae and heterotrophic bacteria.

We did not observe a clear relationship between NH_4 regeneration and heterotrophic protists in the sea ice (Figs. 4B & 5B). Heterotrophic bacteria can also regenerate NH_4 (Gilbert 1993) although no significant relationship between NH_4 regeneration and bacteria was observed during our study (Figs. 4C & 5A). Ammonium regeneration rates were only significantly related to sea-ice EPS concentrations (Fig. 4A), which were shown to enhance

NH_4 regeneration rates. Exopolymeric substances appear to be directly involved in the process of sea-ice NH_4 regeneration since there was no direct relationship between EPS and NH_4 concentrations in the sea ice ($p = 0.96$). We suggest that EPS stimulate grazing by heterotrophic protists (Figs. 2B & 5C) thereby enhancing NH_4 regeneration in sea ice. Exopolymeric substances may be specifically selected as a carbon source (Sherr 1988, Tranvik et al. 1993) or EPS may facilitate the consumption of bacteria by heterotrophic protists.

Meiners et al. (2004) observed that 100% of EPS in Antarctic pack ice were colonized by bacteria and Passow & Alldredge (1994) observed a similar level of colonization for transparent exopolymers in the water column. Bacteria attached to EPS may be more easily grazed by heterotrophic protists than free-living bacteria since the attached bacteria are part of a larger particle. Bacteria-EPS interactions could therefore support increased grazing rates, explaining the observed negative relationship between bacteria and heterotrophic protists (Fig. 2C), resulting in enhanced NH_4 regeneration rates in the newly formed sea ice on Arctic shelves.

1.5 Conclusions

All groups of microorganisms, including bacteria, were significantly enriched in the newly formed sea ice on the Mackenzie shelf and slope. There was a clear selection for large autotrophic cells, likely due to cell size and the presence of EPS, which greatly

enhances the stickiness of cell surfaces. These larger sized autotrophic cells also appeared to be responsible for the incorporation of bacteria into the newly formed sea ice, with EPS potentially mediating these interactions.

Microorganisms in newly formed sea ice are active, taking up NO_3 and $Si(OH)_4$ and producing NH_4. Heterotrophic regeneration increased the concentration of NH_4 within the newly formed sea ice, whereas $Si(OH)_4$ and PO_4 were not significantly enriched in newly formed sea ice as compared to the surface water. Our experiments showed that NH_4 regeneration could contribute, on average, 67% of the ambient NH_4 concentrations within the newly formed sea ice. Given that (1) there was significant NH_4 regeneration, (2) NH_4 was the dominant source of DIN and (3) low NO_3:PO_4 ratios were observed in sea ice, we conclude that the newly formed sea ice is a regenerative environment and that heterotrophic regeneration constitutes an important component of nutrient cycling in newly formed sea ice of the Arctic. Our results indicate that EPS may be an important substrate stimulating heterotrophic activity in newly formed sea ice.

1.6 Acknowledgements

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CHAPITRE II

EVIDENCE OF AN ACTIVE MICROBIAL FOOD WEB IN SEA ICE AND SURFACE WATERS ON THE MACKENZIE SHELF (CANADIAN ARCTIC) DURING THE WINTER-SPRING TRANSITION

RÉSUMÉ

La dynamique des bactéries hétérotrophes a été étudiée dans la glace de mer et les eaux de surface du plateau du Mackenzie (mer de Beaufort), entre le 5 mars et le 3 mai 2004. À 12 occasions, le broutage par les protistes hétérotrophes sur les bactéries a été mesuré suivant la méthode de disparition de bactéries fluorescentes (fluorescently labeled bacteria) sur des échantillons de glace prélevés sous couvert de neige épais et mince, ainsi que sur des échantillons d'eau de surface. Concurremment, des échantillons de glace et d'eau de surface ont été analysés afin de mesurer les concentrations en carbone organique dissous (DOC), en substances exopolymériques (EPS), et en chlorophylle *a* (chl *a*), ainsi que l'abondance des protistes et des bactéries. L'abondance totale des bactéries était plus élevée dans la glace de mer que dans l'eau de surface ($p < 0.05$). Toutefois, les concentrations en DOC et l'abondance des grosses bactéries (≥ 0.7 µm) n'étaient pas statistiquement différentes entre la glace et l'eau de surface ($p > 0.20$). Le broutage par les hétérotrophes (HP) sur les bactéries représentait en moyenne, 27 % et 35 % de la biomasse bactérienne dans la glace, sous couvert de neige épais et mince, respectivement, et 29 % de cette biomasse dans les eaux de surface. Dans la glace, les taux d'ingestion de la communauté étaient, en moyenne, de 1.9 et 1.7 x 10^3 bactéries HP^{-1} d^{-1}, sous couvert de neige épais et mince, respectivement. Il semble que les fortes concentrations en EPS pendant la période de floraison des algues de glace auraient pu interférer avec l'activité de broutage des protistes hététrotrophes, tel qu'indiqué par les corrélations négatives entre les taux d'ingestion des hétérotrophes >5 µm et les concentrations en EPS ($\tau = -0.46$ $p < 0.01$). Dans les eaux de surface, les taux d'ingestion par la communauté hétérotrophe étaient élevés, en moyenne 7.1 x 10^3 bacteria HP^{-1} d^{-1}, et ce possiblement en raison de la présence de protistes mixotrophes. Le broutage sur les bactéries était suffisant pour combler les besoins en carbone des hétérotrophes ≤5 µm, ce qui n'était pas le cas pour les hétérotrophes >5 µm pendant la période de floraison des algues de glace. Les EPS pourraient constituer une source alternative de carbone pour ces derniers, particulièrement pendant la période de floraison des algues de glace. Cette étude met en évidence la présence d'un réseau microbien hétérotrophe actif dans la glace de mer annuelle, pendant la période précédant, ainsi qu'au cours de la période de floraison des algues de glace. Cette étude met aussi de

l'avant l'importance du DOC et des EPS en tant que composantes intégrales du réseau microbien dans la glace de mer et les eaux de surface du plateau continental arctique.

ABSTRACT

Heterotrophic bacterial dynamics were assessed in the sea ice and surface waters on the Mackenzie shelf (Beaufort Sea), from 5 March to 3 May 2004. On 12 occasions, heterotrophic protist bacterivory was assessed from the disappearance of fluorescently labeled bacteria in sea-ice samples collected from areas of high and low snow cover, and surface water samples collected at the ice-water interface. Concurrently, sea-ice and surface water samples were analyzed for dissolved organic carbon (DOC), exopolymeric substances (EPS) and chlorophyll *a* concentrations, and protist and bacterial abundances. Total bacterial abundances were significantly higher in the sea ice than in surface waters ($p < 0.05$). However, DOC concentrations and abundances of large (≥0.7 μm) bacteria were not significantly higher in the sea ice as compared to surface waters ($p > 0.20$). This suggests that DOC was being released from the sea ice, potentially supporting the growth of large bacteria at the ice-water interface. Heterotrophic protist (HP) bacterivory averaged 27% and 35% d^{-1} of bacteria standing stocks in the sea ice under high and low snow cover, respectively, and 29% d^{-1} in surface waters. Ingestion rates averaged 1.9 and 1.7 x 10^3 bacteria HP^{-1} d^{-1} in the sea ice under high and low snow cover, respectively. High concentrations of EPS during the sea-ice algal bloom may have interfered with the grazing activities of heterotrophic protists as indicated by the significant negative correlations between ingestion rates of >5 μm heterotrophs and EPS concentrations ($\tau = -0.46$, $p < 0.01$). In surface waters, heterotrophic ingestion rates were high, averaging 7.1 x 10^3 bacteria HP^{-1} d^{-1}, possibly due to the presence of mixotrophic protists. Bacterivory satisfied the carbon requirements of heterotrophs ≤5 μm but did not satisfy the carbon requirements of heterotrophs >5 μm during the sea-ice algal bloom period. Exopolymeric substances may have been an alternative carbon source for the heterotrophs >5 μm, especially during the sea-ice algal bloom period. This study provides evidence of an active heterotrophic microbial food web in first-year sea ice, prior to and during the sea-ice algal bloom. This study also highlights the significance of DOC and EPS as integral components of the microbial food web within the sea ice and surface waters of Arctic shelves.

2.1 Introduction

Bacteria and protists are significant components of marine carbon cycling as they regulate the transfer of dissolved organic carbon to higher trophic levels (Azam et al. 1983). These microbial food webs are also active in Arctic sea ice (Laurion et al. 1995, Kaartokallio 2004) as the sea ice contains high concentrations and diverse assemblages of heterotrophic and autotrophic protists and bacteria, in addition to abundant diatom assemblages (Horner & Schrader 1982, Maranger et al. 1994, Ikävalko & Gradinger 1997, Sime-Ngando et al. 1997).

Diatoms are generally the major component of Arctic sea-ice assemblages with bacterial carbon biomass being ~3% of algal carbon biomass, and bacterial secondary production being <10% of ice-algal production in landfast sea ice during the spring/summer period (Smith et al. 1989, Smith & Clement 1990). In Arctic pack ice or in landfast sea ice during winter, bacteria can be an important contributor to total sea-ice carbon biomass, potentially surpassing algal or heterotrophic protist carbon biomass (Gradinger & Zhang 1997, Gradinger et al. 1999a, Kaartokallio 2004) and bacterial secondary production can surpass primary production when sea-ice algae are light limited in thick pack ice (Grossmann & Dieckmann 1994).

Bacteria in landfast Arctic sea ice can be much larger than bacterial cells in the surface waters (Bunch & Harland 1990, Laurion et al. 1995). In addition, sea-ice bacteria in

Arctic pack ice appear to be more active than pelagic bacteria (Junge et al. 2002) and are able to remain metabolically active even at very low temperatures (e.g. -20°C) and at extreme salinities which can occur within the sea-ice brine channels (e.g. >200 ppt, Junge et al. 2004). Large bacteria and high bacterial activity within the sea ice may be a result of high concentrations of dissolved organic carbon (DOC) within the sea ice compared to surface waters (Bunch & Harland 1990, Thomas et al. 1995, Smith et al. 1997), or high concentrations of inorganic and organic particles within the sea ice, since particle-associated bacteria have been found to be more active than free-living bacteria (Sherr et al. 1999).

Few studies have assessed the fate of sea-ice bacteria and the seasonal dynamics of sea-ice heterotrophic protists in the Arctic (e.g. Laurion et al. 1995, Sime-Ngando et al. 1997). Grazing by heterotrophic protists is known to be an important factor contributing to bacteria mortality and nutrient regeneration in marine systems (for review see Sherr & Sherr 2002). In the central Arctic Ocean, heterotrophic protists were important grazers of phytoplankton and bacteria, and heterotrophic protists <10 μm in size were the greatest consumers of bacteria (Sherr et al. 1997). Bacterivory in Arctic sea ice has been assessed in only two studies in the highly productive Resolute Passage (high Canadian Arctic) and Saroma-ko lagoon (Okhotsk Sea), showing heterotrophic microprotists to be an active component of microbial food webs within Arctic sea ice and surface waters (Laurion et al. 1995, Sime-Ngando et al. 1999). To better understand organic carbon cycling in the Arctic Ocean, further research on the microbial transformation of organic carbon within the sea ice

is needed, especially over the continental shelves where sea-ice production is coupled with both pelagic and benthic production (e.g. Michel et al. 1996, Renaud et al. in press).

This study investigated the grazing of bacteria by heterotrophic protists within the sea ice and surface waters on the Mackenzie shelf, Canadian Arctic, during the winter to spring transition. The objectives of this study were to assess the seasonal dynamics of bacteria and protists within the sea ice and surface waters and to determine if bacterivory was sufficient to meet the carbon requirements of heterotrophic protists during the sea-ice algal pre-bloom and bloom periods.

2.2 Materials and Methods

2.2.1 Sampling and analyses

Sea-ice and surface water sampling was conducted in an area of first-year, landfast sea ice in Franklin Bay, Northwest Territories (Fig. 1), every three to five days between 5 March and 3 May 2004. All samples were collected 1.5 km northeast of the overwintering site of the CCGS *Amundsen* (70°04'N, 126°26'W; water depth: ~250 m) as part of the Canadian Arctic Shelf Exchange Study (CASES). Sample analyses and grazing experiments were conducted in laboratories on board the ship shortly after sample collection.

Ice cores were collected with a manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprise) within an area approximately 0.01 km^2, with consecutive ice coring conducted within ~10 m of previous coring sites. On each sampling day, ice cores were collected at two sites representative of high and low snow cover to account for some of the horizontal patchiness of sea-ice biomass (Gosselin et al. 1986, Rysgaard et al. 2001).

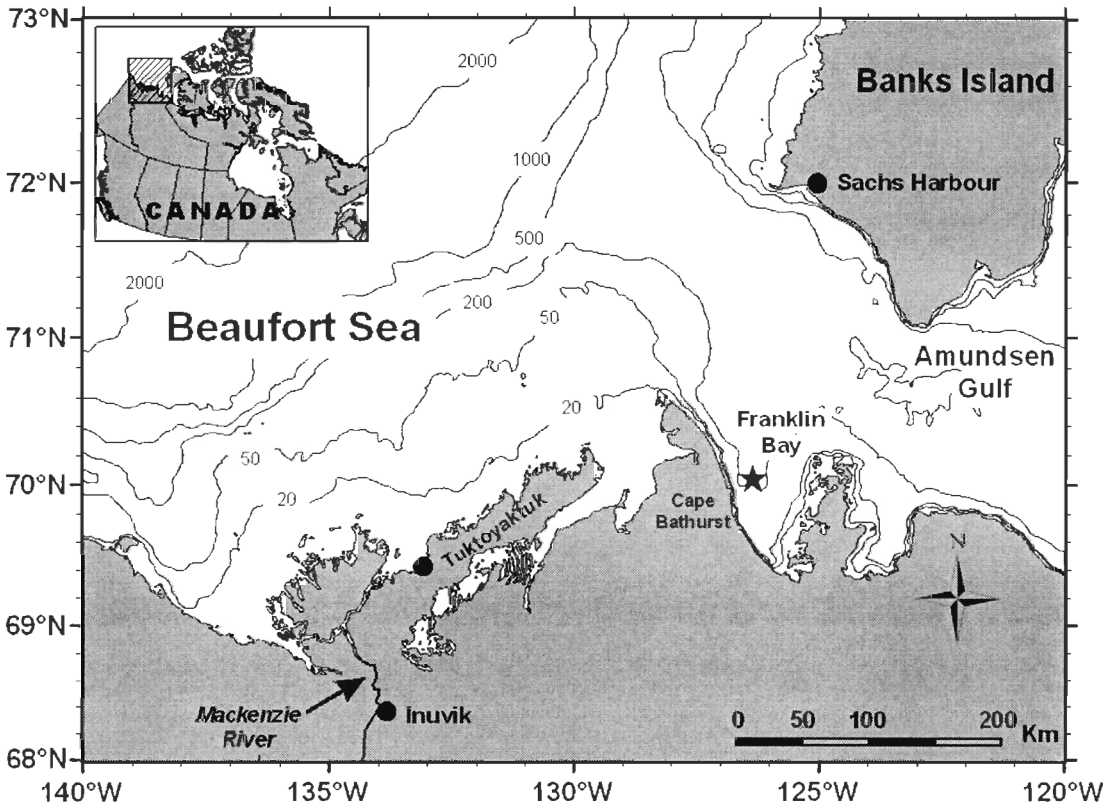

Fig. 1. Location of the sampling station in Franklin Bay (Mackenzie shelf), Canadian Arctic, as indicated by the star

At the high and low snow cover sampling sites, multiple cores were collected for routine measurement of DOC, exopolymeric substances (EPS), chlorophyll *a* (chl *a*), protist and bacterial abundances, and for fluorescently labeled bacteria (FLB) grazing experiments. The bottom 4 cm of three to five ice cores was pooled together in an isothermal container, to obtain one sample for each snow cover depth. Sterile gloves were worn at all times during the manipulation of the cores. On each sampling day, water from the ice-water interface was also collected using a hand pump. One subsample of this surface water was analyzed for DOC, EPS, chl *a* and cells, a second was used for FLB grazing experiments and a third subsample was filtered on polycarbonate 0.2 µm filters and added to the ice core samples to minimize osmotic stress during the melting process (Garrison & Buck 1986). A separate ice core, kept in a sterile Whirl-Pak bag, was melted without the addition of filtered surface water for the analysis of sea-ice DOC. When necessary, sea-ice concentrations of measured variables and experimental results were corrected for the dilution from the addition of seawater during the melting process.

2.2.2 Chlorophyll *a*, EPS and DOC

Chlorophyll *a* was determined fluorometrically (10AU Turner Designs) on duplicate subsamples filtered on Whatman GF/F filters, after 24 h extraction in 90% acetone at 5°C in the dark (Parsons et al. 1984). Triplicate EPS subsamples were filtered on 47 mm 0.4 µm Nuclepore filters and stained with Alcian blue. Exopolymeric substances, operationally defined as >0.4 µm acidic exopolysaccharides, were measured colorimetrically (787 nm) after a 2 h extraction in 80% H_2SO_4 (Passow & Alldredge 1995). Concentrations of EPS

were recorded as μg gum xanthan equivalents (xeq.) l^{-1}. Duplicate DOC subsamples were filtered through precombusted Whatman GF/F filters using acid-washed syringe filters, acidified with 50% H_3PO_4 and stored at 4°C until analysis using a Tekmar/Dohrman (Apollo 9000) analyzer.

2.2.3 Bacteria and protists

Subsamples for bacteria and protist counts were preserved with buffered formaldehyde (1% final concentration), stained with DAPI (4, 6-diamidino-2-phenylindole) at a final concentration of 1 μg ml^{-1} and filtered on 0.2 and 0.8 μm black Nuclepore filters, respectively (Sherr et al. 1993). A minimum of 200 free-living bacteria was counted in ten fields from duplicate subsamples using epifluorescent microscopy. Total bacterial abundances represent the sum of small (i.e. <0.7 μm) spherical and large (≥0.7 μm) rod-shaped bacteria. When present, diatom-attached bacteria were also counted. In this study, bacteria refer to heterotrophic cells only.

Autotrophic and heterotrophic protists, excluding pennate and centric diatoms, were counted at 400X and 1000X magnification with, on average, a minimum of 100 cells counted. Cells were separated into two size classes with the abundance of cells >5 μm in diameter determined from the average cell counts at the two magnifications and the abundance of cells ≤5 μm in diameter obtained from cell counts at 1000X only. Autotrophic cells were identified by chlorophyll autofluorescence under blue light

excitation. Autotrophic and heterotrophic protists will hereafter be referred to as autotrophs and heterotrophs.

The size of each autotrophic and heterotrophic protist cell counted was measured using image analysis software (Image Pro 5.1). Biovolumes were determined based on the nearest geometric shape (Hillebrand et al. 1999) and carbon biomass was calculated according to the carbon to volume relationship of Menden-Deuer & Lessard (2000), $pg\ C\ cell^{-1} = 0.216 \times (\mu m^3\ cell^{-1})^{0.939}$. A subsample of 600 bacteria cells was also measured using the same image analysis system and bacterial carbon was estimated using the carbon to volume relationship of Simon & Azam (1989) as modified by Norland (1993), $pg\ C\ cell^{-1} = 0.12 \times (\mu m^3\ cell^{-1})^{0.7}$.

2.2.4 FLB grazing experiments

Bacterial grazing rates from sea ice under high (n = 11) and low (n = 12) snow cover and surface waters (n = 12) were estimated from the disappearance of FLB added to surface water and diluted sea-ice subsamples in sterile Whirl-Pak bags (1 l experimental volume). The sea-ice subsamples were pre-filtered through 200 µm or 425 µm mesh to exclude protist predators (e.g. copepods) or bacterivorous meiofauna (e.g. nematodes). The FLB were stained with dichlorotriazinylamino fluorescein (DTAF) according to Sherr & Sherr (1993) and were added at a final concentration of 3 to 41% (average 12%) of natural sea-ice bacterial abundances. The FLB were prepared from >1 µm, cultured bacteria, isolated from first-year sea ice of the Chukchi Sea (Strain 11B5, provided by K. Junge & J. Deming).

The sea-ice and surface water subsamples were incubated at sub-ice light conditions from under high snow cover (2.2 ± 0.32 μE m^{-2} s^{-1}), as measured with a Li-1923B underwater 2π PAR (photosynthetically available radiation, 400-700 nm) sensor, and at near *in situ* temperature (0°C). The subsamples were incubated for 96 or 72 h prior to 13 April, after which the experimental duration was decreased to 48 h as the abundance of potential grazers (i.e. heterotrophic protists) increased within the sea ice. Duplicate subsamples for flow cytometry and microscopic counts were taken every 2 to 12 h at which time the bags were gently mixed.

Uptake rates of FLB were calculated from the linear decrease in FLB concentrations over time (Model I linear regression, Sokal & Rohlf 1995) and community grazing rates (10^6 bacteria l^{-1} d^{-1}) were calculated using the initial and final ratios of FLB and naturally occurring bacteria in the experimental subsamples (Salat & Marrasé 1994). Community grazing rates and the abundances of ≤5 and >5 μm heterotrophs on each sampling date were used to calculate community (i.e. total) and size-fractionated (i.e. ≤5 and >5 μm) heterotrophic protist ingestion rates (bacteria HP^{-1} d^{-1}), assuming that grazing by heterotrophs was the only factor responsible for the disappearance of FLBs. The calculation of size-fractionated heterotrophic protist ingestion rates assumes that all protists consume bacteria at a similar rate, and therefore does not account for species specific differences in grazing rates.

Naturally occurring bacteria were counted microscopically at the beginning and end of each FLB grazing experiment as described above. The FLB subsamples were preserved with paraformaldehyde (1% final concentration), stored at -80°C and later analyzed using an Epics Altra flow cytometer (Beckman-Coulter) fitted with a 15 mW, 488 nm laser. DTAF fluorescence was measured at 525 ± 5 nm and FLB were identified from their typical signature in plots of side scatter (SSC) versus green fluorescence. For each FLB subsample, ten thousand events were acquired and the weight of the subsample before and after each analysis was measured to calculate the number of FLB ml^{-1}.

In conjunction with each grazing experiments, 250 ml sea-ice and surface water subsamples were incubated in the dark at 0°C to assess net changes in NH_4 concentrations over a 24 h period. NH_4 concentrations in these dark incubations were analyzed every 6 h using the salicylate-hypochlorite method (Bower & Holm-Hansen 1980). Net ammonium regeneration rates were determined from the slope of the change in NH_4 concentrations over the 24 h period (Model I linear regression, Sokal & Rohlf 1995). Chlorophyll *a* concentrations at the beginning and end of each FLB grazing experiment were also assessed. Total chl *a* concentrations were determined as described above and size-fractionated chl *a* concentrations (>5 μm) were determined using 5 μm Poretics polycarbonate membranes.

2.2.5 Statistical analyses

Kendall's coefficients of rank correlation (τ) and partial correlation coefficients ($p\tau$) were used to infer the strength of associations between two variables (Sokal & Rohlf 1995). Wilcoxon's signed-ranks tests were used to compare paired variates from the high and low snow cover sites and from the FLB grazing experiments. Statistical analyses were carried out using JMP (SAS Institute).

Carbon requirements (pg C HP^{-1} d^{-1}, Laurion et al. 1995) for ≤ 5 µm and >5 µm heterotrophs during the pre-bloom (5 March to 3 April) and sea-ice algal bloom (8 April to 3 May) periods of this study were assessed as follows:

$$\text{Carbon requirement} = f \times g/GE \quad (1)$$

where f is the carbon content of the cell (pg C $cell^{-1}$), g is the growth rate (d^{-1}) and GE is the growth efficiency (0.33, Hansen et al. 1997). Specific growth rates were estimated according to $(\ln N_t - \ln N_0)/t$. N_t and N_0 are the final and initial heterotrophic protist biomass (HP, µg C l^{-1}), respectively, after t days.

2.3 Results

Sea-ice thickness increased from 1.5 m to a maximum of 1.9 m under high snow cover and from 1.6 m to a maximum of 1.9 m under low snow cover from 5 March to

3 May, in Franklin Bay. Seasonally-averaged snow depth at the high and low snow cover sites was 14.8 ± 3.0 and 3.6 ± 1.6 cm, respectively.

Chlorophyll *a* concentrations averaged 23.8 μg l^{-1} (range 0.3 to 74.7 μg l^{-1}) and 137.5 μg l^{-1} (range 3.1 to 496.4 μg l^{-1}) at the bottom surface of the sea ice under high and low snow cover. In the surface water, chl *a* concentrations averaged 0.28 μg l^{-1} and ranged between 0.10 and 0.64 μg l^{-1}. Chlorophyll *a* concentrations were significantly higher in sea ice under low snow cover than high snow cover (Wilcoxon's, $p < 0.01$). Sea-ice EPS concentrations ranged between 91 and 781 μg xeq. l^{-1} and between 101 and 7480 μg xeq. l^{-1} in the sea ice under high and low snow cover, respectively, whereas surface water EPS concentrations ranged from below detection to 59 μg xeq. l^{-1}. Concentrations of EPS were significantly higher in sea ice under low snow cover than high snow cover (Wilcoxon's, $p < 0.05$). A detailed description of seasonal chl *a* and EPS concentrations is presented elsewhere (see Chapter 3), with the onset of the sea-ice algal bloom being defined by a rapid increase in chl *a* concentrations beginning ca. 3 April.

Sea-ice DOC concentrations averaged 88 μM and 239 μM under high and low snow cover, respectively, with concentrations rapidly increasing after 3 April (Fig. 2A), corresponding with the onset of the sea-ice algal bloom. Surface water DOC concentrations averaged 133 μM and ranged between 100 and 267 μM (Fig. 2B). Concentrations of DOC in the sea ice under low snow cover and in surface waters were both significantly higher than DOC concentrations in the sea ice under high snow cover (Wilcoxon's, $p < 0.05$).

There was no significant difference between DOC concentrations in sea ice under low snow cover and in surface waters (Wilcoxon's, $p = 0.90$).

Significant correlations were observed between sea-ice DOC and chl *a* concentrations (high and low snow: $\tau = 0.73$, $p < 0.01$, Fig. 2C) and between sea-ice DOC and EPS concentrations (high snow: $\tau = 0.70$, $p < 0.01$, low snow: $\tau = 0.88$, $p < 0.001$, Fig. 2D). In surface waters, DOC concentrations were not significantly correlated to either chl *a* ($p = 0.09$) or EPS ($p = 0.53$) concentrations.

Average and range abundances of autotrophic and heterotrophic protists in the sea ice and surface waters are summarized in Table 1. In sea ice under high and low snow cover, autotrophs >5 μm were, on average, the most abundant protist whereas heterotrophs ≤5 μm were least abundant. In the surface water, autotrophs ≤5 μm were, on average, most abundant whereas the heterotrophs >5 μm were least abundant.

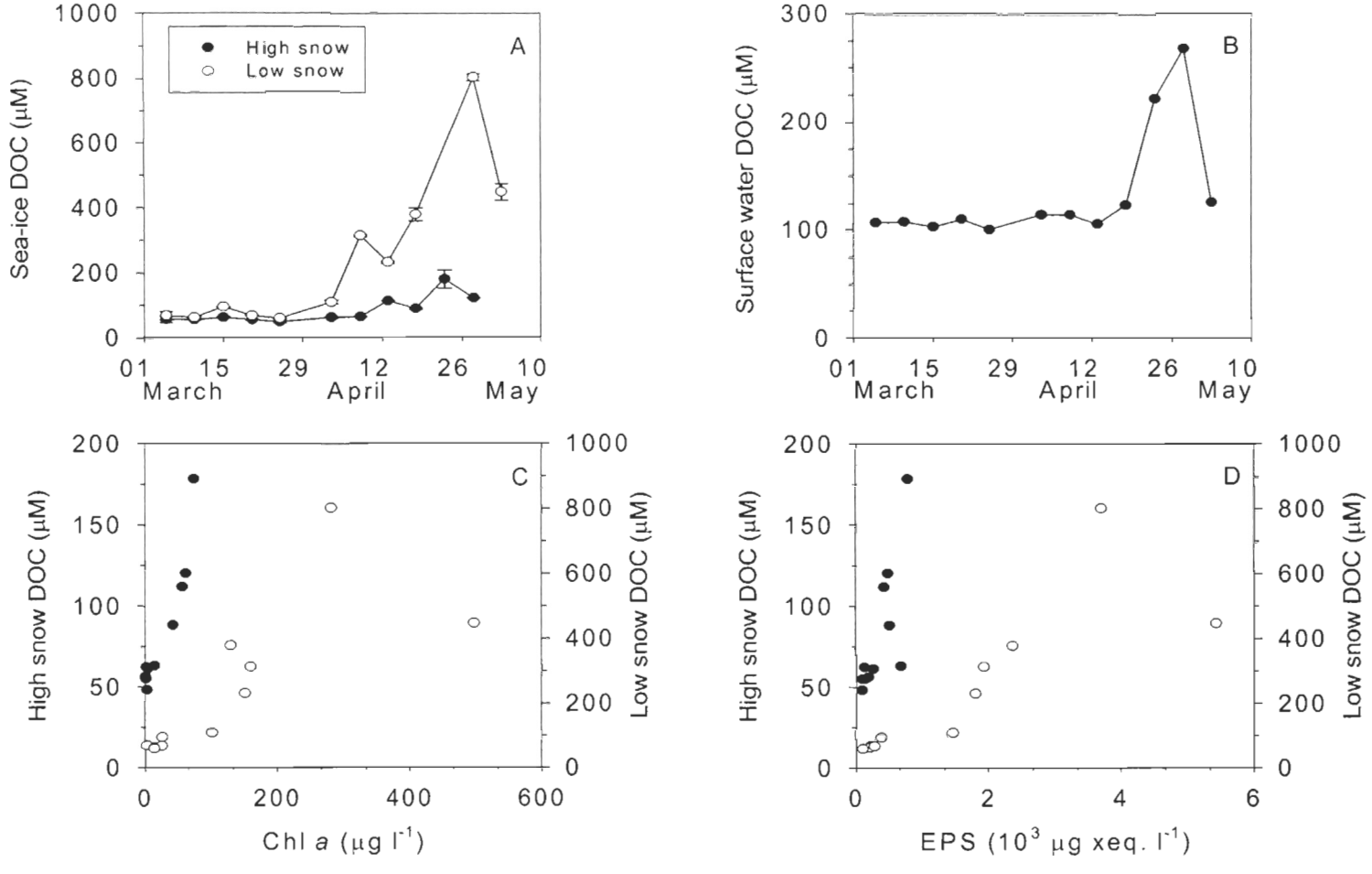

Fig. 2. Seasonal trends in the concentrations of (A) sea-ice dissolved organic carbon (DOC) and (B) surface water DOC and the relationships between (C) DOC and chlorophyll *a* concentrations and between (D) DOC and exopolymeric substances (EPS) concentrations in the sea ice under high and low snow cover, March to May 2004. In (A), means ± SD are shown

Sea-ice protist abundances gradually increased starting in April and the abundance of all protists (auto- and heterotrophs) >5 μm remained high for the rest of the sampling period (Fig. 3A, C). In April, the abundance of all protists >5 μm and autotrophs ≤5 μm rapidly increased in surface waters (Fig. 3A-C). The abundance of heterotrophs ≤5 μm did not show any clear seasonal trend in surface waters (Fig. 3D). In the sea ice, the abundance of protists under high snow cover was not significantly different than the abundance of protists under low snow cover (calculated for each ≤5 and >5 μm autotrophic and heterotrophic group separately: Wilcoxon's, $p = 0.44\text{-}0.72$).

Total free-living bacterial abundances varied between 0.4 and 2.6 x 10^9 cells l^{-1} and between 0.3 and 2.7 x 10^9 cells l^{-1} under high and low snow cover, respectively (Table 1, Fig. 4A). In surface waters, total free-living bacterial abundances ranged between 0.2 and 0.9 x 10^9 cells l^{-1} (Table 1, Fig. 4A). Total sea-ice bacterial abundances were not significantly different between high and low snow cover (Wilcoxon's, $p = 0.56$). However, total sea-ice bacterial abundances under high (Wilcoxon's, $p < 0.05$) and low (Wilcoxon's, $p < 0.05$) snow cover were significantly higher than bacterial abundances in surface waters.

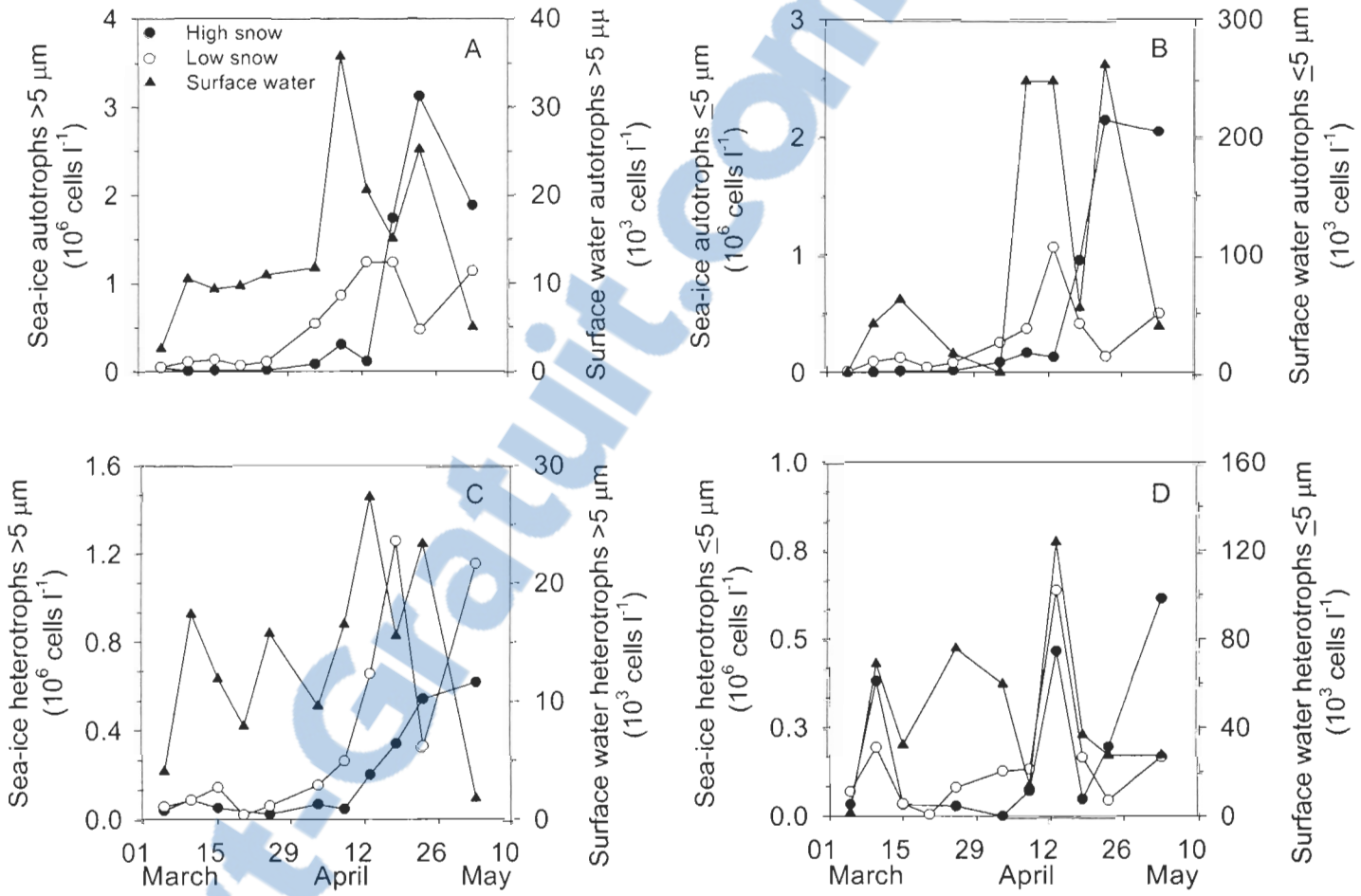

Fig. 3. Seasonal trends in the abundances of (A) >5 µm and (B) ≤5 µm autotrophs and (C) >5 µm and (D) ≤5 µm heterotrophs in the sea ice under high and low snow cover and in surface waters, March to May 2004

The abundance of large sized bacteria (i.e. ≥0.7 μm, average volume 3.7 μm^3) averaged 0.43, 0.57 and 0.26 x 10^9 cells l^{-1} under high and low snow and in surface waters, respectively (Table 1, Fig. 4B). The large sea-ice bacteria contributed, on average, 28% (range 4 to 70%) and 33% (range 3 to 76%) of total bacterial abundances under high and low snow cover, respectively. In surface waters, large bacteria contributed an average of 36% (range 8 to 63%) of total bacterial abundances. The abundance of large bacteria and their contribution to total bacterial abundances in the sea ice were not significantly different between high and low snow cover (Wilcoxon's, $p = 0.76$-0.85). There was also no significant difference in the abundance of large bacteria and their percent contribution to total bacterial abundances between the sea ice and surface waters (Wilcoxon's, high snow: $p = 0.27$-0.44; low snow: $p = 0.24$-0.64).

From 8 April onward, diatom-attached bacteria were observed in the sea ice only. The diatom-attached bacteria were consistently large rods, which varied in abundance between 0.6 and 305 x 10^6 cells l^{-1}. Highest concentrations occurred in the sea ice under low snow cover (data not shown).

Figure 5 shows linear-log correlations observed between total free-living bacterial abundances and the abundances of protists in the sea ice. Total sea-ice bacterial abundances were significantly correlated with the abundance of autotrophs >5 μm under high ($\tau = 0.67$, $p < 0.05$) and low snow cover ($\tau = 0.60$, $p < 0.05$, Fig. 5A), and with the abundance of

autotrophs ≤5 μm under high ($\tau = 0.54$, $p < 0.05$) and low snow cover ($\tau = 0.60$, $p < 0.05$, Fig. 5B).

Total sea-ice bacterial abundances were not correlated with the abundance of heterotrophs >5 μm ($p = 0.10$) or ≤5 μm ($p = 0.67$) under high snow cover, and were only weakly correlated with the abundance of heterotrophs >5 μm ($\tau = 0.53$, $p < 0.05$) and ≤5 μm ($\tau = 0.49$, $p < 0.05$) under low snow cover (Fig. 5C, D). In the surface waters, total bacterial abundances were not correlated with the abundance of heterotrophs >5 μm ($p = 0.81$) or ≤5 μm ($p = 0.59$), neither autotrophs >5 μm ($p = 0.31$) or ≤5 μm ($p = 0.93$, results not shown).

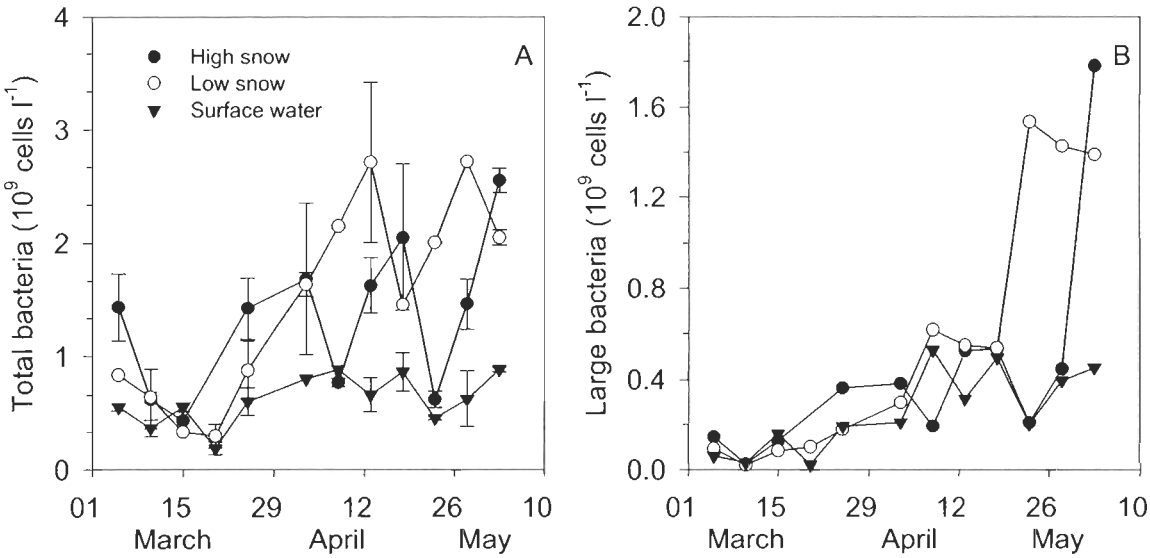

Fig. 4. Seasonal trends in (A) total bacterial and (B) large (≥0.7 μm) bacterial abundances in the sea ice under high and low snow cover and in surface waters, March to May 2004. In (A), means ± SD are shown

Table 1. Abundance of bacteria and autotrophic and heterotrophic protists in the sea ice under high and low snow cover, and in surface waters on the Mackenzie shelf, March to May 2004 (n = 11). Total bacteria represent free-living cells only

Variable	Sea ice				Surface waters	
	High snow		Low snow			
	Average	Range	Average	Range	Average	Range
Total bacteria 10^9 cells l^{-1}	1.3	0.4-2.6	1.5	0.3-2.7	0.6	0.2-0.9
Large bacteria 10^9 cells l^{-1}	0.4	0.03-1.8	0.6	0.02-1.5	0.3	0.02-0.5
≤5 μm autotrophs 10^3 cells l^{-1}	554	0-2145	279	6.9-1061	97.0	0-261.4
>5 μm autotrophs 10^3 cells l^{-1}	730	6.8-3118	541	47.9-1237	14.2	2.6-35.7
≤5 μm heterotrophs 10^3 cells l^{-1}	187	0-614	150	4.3-637	46.7	1.5-124
>5 μm heterotrophs 10^3 cells l^{-1}	201	22.4-618	379	19.1-1257	13.7	1.8-27.4

Total bacterial abundances were significantly correlated with DOC ($\tau = 0.48$, $p < 0.05$) and EPS ($\tau = 0.45$, $p < 0.05$) concentrations under low snow cover but not under high snow cover. In the sea-ice under high and low snow cover, the abundances of heterotrophs >5 μm were correlated with DOC (High snow: $\tau = 0.56$, $p < 0.05$; Low snow: $\tau = 0.54$, $p < 0.05$) and EPS (High snow: $\tau = 0.56$, $p < 0.05$; Low snow: $\tau = 0.64$, $p < 0.01$) concentrations. However, partial correlation analyses found that these individual correlations were not substantiated due to intercorrelations between DOC, EPS and chl *a* concentrations. The correlations between total bacterial abundances and DOC concentrations and between total bacterial abundances and EPS concentrations under low

snow cover dropped from $\tau = 0.48$ to $p\tau = 0.14$ and from $\tau = 0.45$ to $p\tau = -0.002$, respectively, when the effects of chl *a* were controlled. Correlations between sea-ice heterotrophs >5 μm and DOC were not substantiated when the effects of EPS were controlled, and the same result was observed for the correlation between sea-ice heterotrophs >5 μm and EPS when the effects of DOC were controlled.

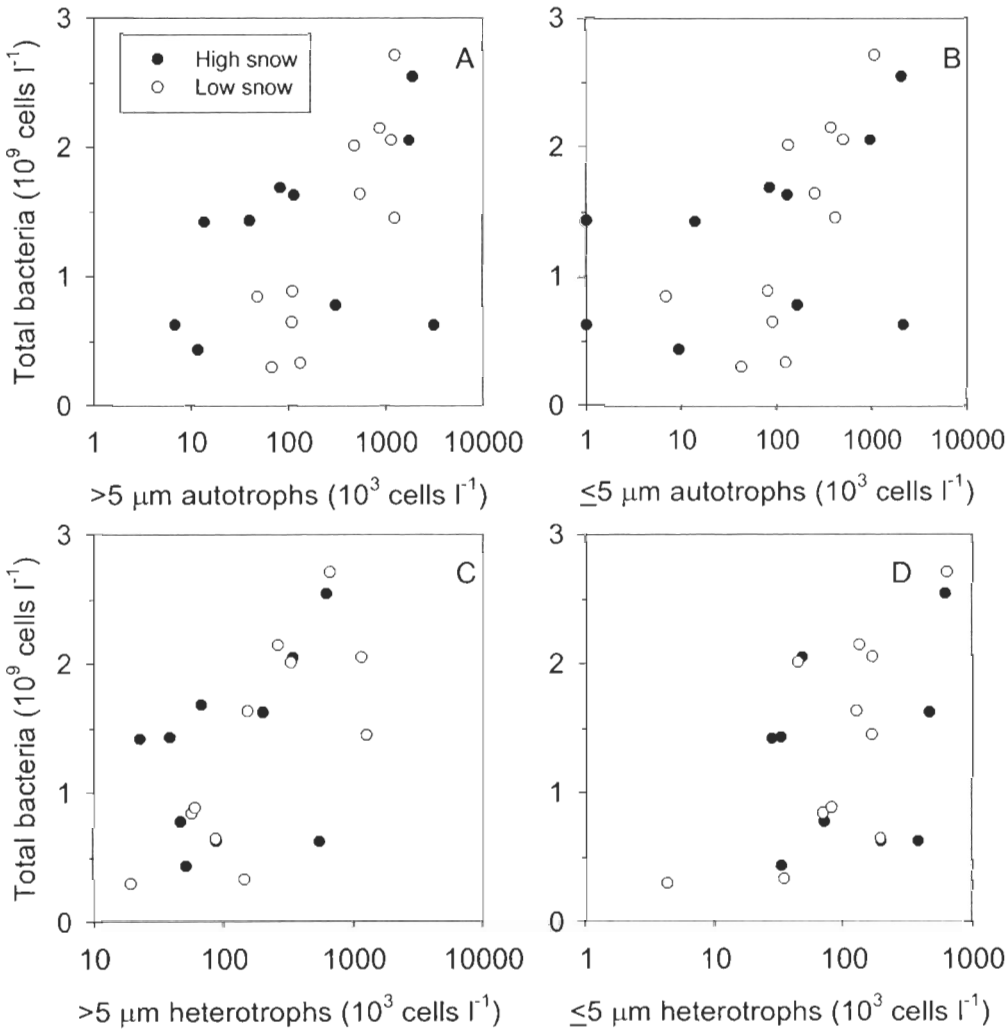

Fig. 5. Relationship between total bacterial abundances and the abundance of (A) >5 μm and (B) ≤5 μm autotrophs and (C) >5 μm and (D) ≤5 μm heterotrophs in the sea ice under high and low snow cover. Note: horizontal axes with log(x) + 1 scale

2.3.1 Experimental conditions

The rate of change in net sea-ice NH_4 concentrations over a 24 h period was significantly different from zero in only two high snow and four low snow cover experiments. In the two high snow cover experiments there was an average net NH_4 regeneration rate of 0.48 μM d^{-1} and in the four low snow cover experiments there was an average net NH_4 consumption rate of 1.68 μM d^{-1}. Net changes in NH_4 concentrations could not be determined in surface waters since the majority of NH_4 concentrations were under the limit of detection (<0.05 μM).

During the grazing experiments, total and > 5 μm chl *a* concentrations in the sea ice and surface waters generally decreased. However, total and >5 μm chl *a* concentrations were not significantly different between the beginning and end of any sea-ice or surface water FLB grazing experiment (Wilcoxon's, $p = 0.25$-0.73).

The abundance of large, free-living bacteria significantly increased during high snow cover (average rate: 0.30 d^{-1}, Wilcoxon's, $p < 0.01$), low snow cover (average rate: 0.46 d^{-1}, Wilcoxon's, $p < 0.01$) and surface water (average rate: 0.15 d^{-1}, Wilcoxon's, $p < 0.05$) FLB grazing experiments. The abundance of small, free-living bacteria did not change significantly during any of the FLB grazing experiments (Wilcoxon's, $p = 0.31$-0.65).

2.3.2 Grazing rates

Significant linear decreases in FLB concentrations were observed over the entire incubation period for all grazing experiments except under high snow cover on 28 April. In this one experiment, a linear decrease was observed during the first 10 h of the experiment, with no further decrease for the remainder of the 48 h experiment. The result of this experiment was excluded from our dataset. For all grazing experiments, the regression coefficients (i.e. slopes) were significantly different from zero ($p < 0.05$).

Community grazing rates of bacteria were high with no clear seasonal trends observed (Fig. 6A). Community grazing rates varied between 81.6 and 626 x 10^6 bacteria l^{-1} d^{-1} and between 3.8 and 1570 x 10^6 bacteria l^{-1} d^{-1} in the sea ice under high and low snow, respectively, and between 12.7 and 534 x 10^6 bacteria l^{-1} d^{-1} in surface waters (Fig. 6A). Between 9 and 74% and 0.4 and 81% of total, free-living, bacterial assemblages in the sea ice under high and low snow cover, respectively, and between 2 and 60% of total, free-living, bacterial assemblages in the surface water could be grazed each day according to the observed grazing rates. Sea-ice community grazing rates were not significantly different between high and low snow cover (Wilcoxon's, $p = 0.73$, Fig. 6A) and community grazing rates in sea ice under high (Wilcoxon's, $p = 0.06$) and low (Wilcoxon's, $p = 0.95$) snow cover were not significantly different than community grazing rates in surface waters (Fig. 6A).

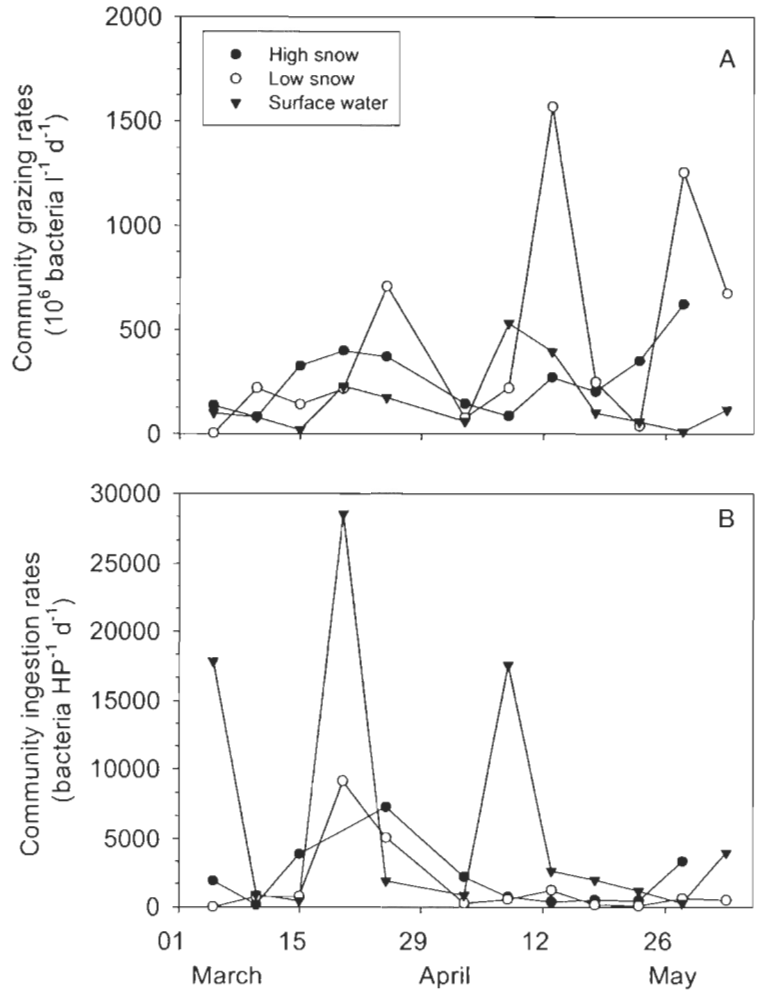

Fig. 6. Seasonal trends in community (A) grazing and (B) ingestion rates of bacteria by heterotrophic protists (HP) in the sea ice under high and low snow cover and in surface waters, March to May 2004

Community ingestion rates were higher prior to than following the onset of the sea-ice algal bloom (i.e. 3 April, Fig. 6B). Community ingestion rates varied between 173 and 7320 bacteria HP^{-1} d^{-1}, 31.2 and 9144 bacteria HP^{-1} d^{-1} and between 0.5 and 28.5 x 10^3 bacteria HP^{-1} d^{-1} in the sea ice under high and low snow and in surface waters,

respectively (Fig. 6B). Community sea-ice ingestion rates were not significantly different between high and low snow cover (Wilcoxon's, $p = 0.75$, Fig. 6B). However, community sea-ice ingestion rates under low snow cover were significantly lower than community ingestion rates in the surface water (Wilcoxon's, $p < 0.05$, Fig. 6B). Size-fractionated ingestion rates are presented in Table 2.

2.3.3 Heterotrophic carbon requirements

Growth rates and carbon requirements of heterotrophs ≤5 μm and >5 μm in the sea ice, under high and low snow cover, and in surface waters are summarized in Table 2 for the sea-ice algal pre-bloom and bloom periods. Carbon requirements for sea-ice heterotrophs ≤5 μm under high snow cover could not be assessed during the pre-bloom period due to the negative growth rate estimate (see Table 2). In the sea ice and surface waters, bacterivory satisfied heterotrophic carbon requirements, except for heterotrophs >5 μm during the sea-ice algal boom period (Table 2). Ingestion rates of sea-ice heterotrophs >5 μm (Table 2) were significantly negatively correlated with EPS concentrations (high and low snow combined: $\tau = -0.46$, $p < 0.01$, Fig. 7). Significant correlations with EPS were not observed for sea-ice heterotrophs ≤5 μm or for heterotrophs ≤5 μm and >5 μm in surface waters ($p = 0.11$-0.53).

Table 2. Summary of the carbon requirements for sea-ice and surface water heterotrophs during the sea-ice algal pre-bloom (5 March to 3 April) and bloom (8 April to 3 May) period of this study. Required ingestion rates are based on the consumption of large (≥0.7 μm) bacteria. * indicates that heterotrophic carbon requirements were met by the consumption of bacteria. NA = not available

	Sea ice				Surface waters	
	High snow		Low snow			
	Pre-bloom	Bloom	Pre-bloom	Bloom	Pre-bloom	Bloom
Heterotrophic growth rate						
≤5 μm heterotrophs (d^{-1})	-0.02	0.12	0.003	0.07	0.05	0.01
>5 μm heterotrophs (d^{-1})	0.08	0.09	0.05	0.07	0.006	0.009
Heterotrophic carbon requirement						
≤5 μm heterotrophs (pg C $cell^{-1}$ d^{-1})	NA	2.98	0.07	1.92	0.94	0.26
>5 μm heterotrophs (pg C $cell^{-1}$ d^{-1})	116	128	73.4	131	5.93	8.55
Required ingestion rate						
≤5 μm heterotrophs (bacteria HP^{-1} d^{-1})	NA	32.2	0.76	20.7	10.1	2.78
>5 μm heterotrophs (10^3 bacteria HP^{-1} d^{-1})	1.25	1.39	0.79	1.42	0.06	0.09
Observed ingestion rate						
≤5 μm heterotrophs (bacteria HP^{-1} d^{-1})	1318	210*	904*	182*	1658*	3173*
>5 μm heterotrophs (10^3 bacteria HP^{-1} d^{-1})	1.8*	0.27	1.78*	0.38	7.0*	2.3*

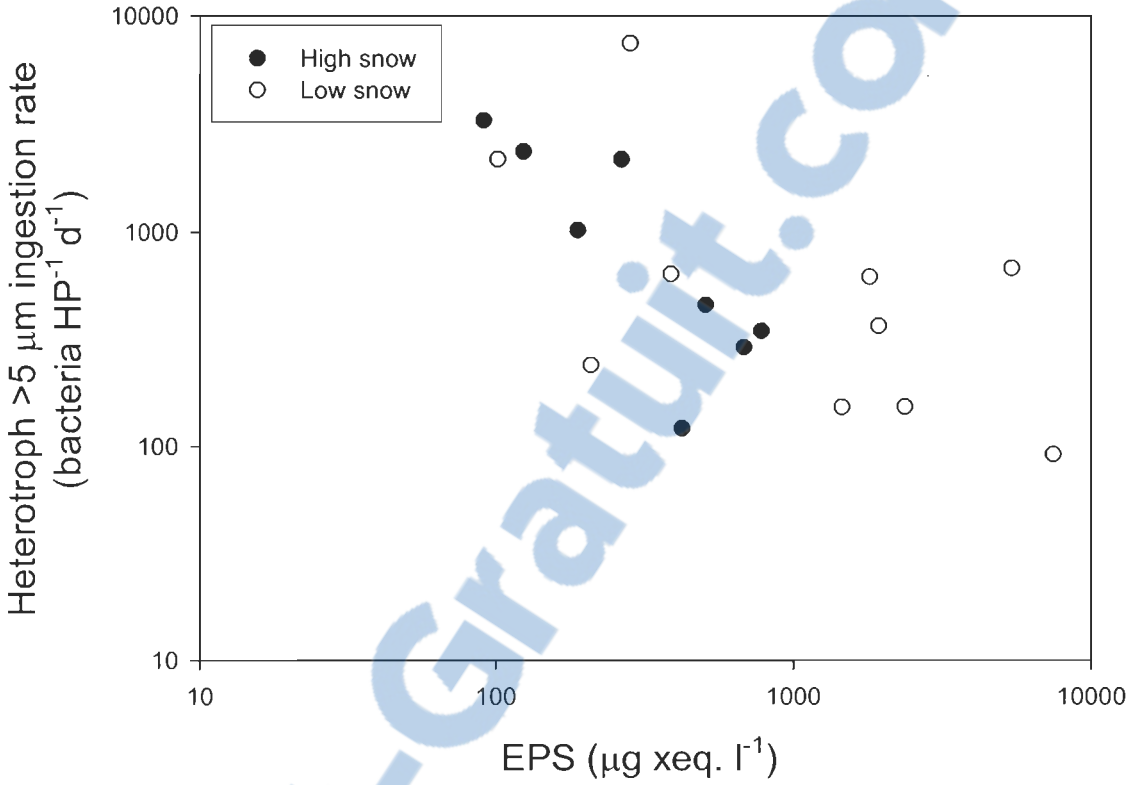

Fig. 7. Relationship between ingestion rates of heterotrophs (HP) >5 µm and exopolymeric substances (EPS) concentrations, in the sea ice under high and low snow cover. Note logarithmic scale on both axes

2.4 Discussion

Arctic sea ice appears to be a favorable growth environment for heterotrophic bacteria, as compared to the pelagic, as indicated by high bacterial abundances and large cell sizes observed in this study. Bacterial abundances in the sea ice on the Mackenzie shelf were within the range previously observed for Antarctic (Growing et al. 2004 and references therein) and Arctic sea ice (Bunch & Harland 1990, Gradinger & Zhang 1997). Bacterial abundances from the ice-water interface were also comparable with previous estimates for surface waters in the same area (Garneau et al. 2006).

High abundances of large-sized bacteria in the sea ice during this study (Fig. 4B) also agree with previous studies of landfast sea ice in the Arctic (Bunch & Harland 1990, Laurion et al. 1995, Kaartokallio 2004). The study by Laurion et al. (1995) did not report the presence of large bacteria in surface waters collected only 5 cm from the bottom of the sea ice. However, in our study, the abundance of large bacteria in surface waters near the ice-water interface was not significantly different than sea-ice bacterial abundances. This suggests that, on the Mackenzie shelf, there may be an exchange of large sized bacteria between the sea ice and interfacial waters or that the interfacial water also constitutes a favorable growth environment for bacteria, possibly in relation to high DOC concentrations (Fig. 2B).

The distribution of sea-ice biomass is notoriously patchy due to small scale variation in snow cover thickness, which influences light availability at the bottom of the sea ice (Gosselin et al. 1986, Mundy et al. 2005). This variability in bottom ice irradiance results in heterogeneous distributions of sea-ice algae and associated variables for example, chl *a* and DOC concentrations (Smith et al. 1997). In this study, both sea-ice chl *a* and DOC concentrations were highest under low snow cover. It was expected that bacterial abundances would follow a similar pattern since sea-ice bacterial abundances have been significantly correlated with chl *a* and DOC concentrations (Smith et al. 1989, Bunch & Harland 1990, Gradinger et al. 1999b, Junge et al. 2004). Interestingly, in the present study, sea-ice bacterial abundances were not significantly different between high and low snow cover even with higher chl *a* and DOC concentrations under low snow cover (Table 1, Fig. 4A, B).

It is possible that sea-ice bacterial production differed between high and low snow cover, although we did not measure bacterial production during this study. Here we discuss the potential use of different carbon sources by sea-ice bacteria and the role of protozoan bacterivory in shaping the observed seasonal and spatial patterns in bacterial abundance.

2.4.1 DOC and EPS

As observed in a previous study of landfast sea ice of Resolute Passage (Smith et al. 1997), seasonal trends in sea-ice DOC concentrations paralleled those in sea-ice algal biomass, with a rapid increase in DOC concentrations following the onset of the sea-ice

algal bloom, especially under low snow cover (3 April, Fig. 2A). The significant correlations between DOC and chl *a* concentrations, under high and low snow cover, indicate that sea-ice algae are significant contributors to sea-ice DOC concentrations (Fig. 2C).

Sea ice and surface waters on the Mackenzie shelf contained high concentrations of DOC, with values being on average seven times higher than DOC concentrations in mixed shelf waters of the Arctic Ocean (0 to 25 m average 34.4 ± 4.0 μM, Wheeler et al. 1997). During our study, DOC concentrations at the ice-water interface were similar to sea-ice DOC concentrations under low snow cover. However, contrary to the sea ice, DOC concentrations in surface waters were not correlated with surface water chl *a* concentrations. Low surface water chl *a* concentrations and the lack of coupling between surface water DOC and chl *a* concentrations indicate that DOC may have been released from the sea ice to the interfacial waters, resulting in the high surface water DOC concentrations observed during this study.

Exopolymeric substances represent a carbon-rich substrate and algae, particularly diatoms, are suggested to be the major producers of EPS within the sea ice (Krembs & Engel 2001, Meiners et al. 2003, see Chapter 3). Our results suggest that similar processes are involved in the algal production of sea-ice DOC and EPS as indicated by the significant correlations between DOC and EPS concentrations under high and low snow cover (Fig. 2D). Due to methodological procedures, DOC (filtered through 0.7 μm nominal pore

size) and EPS (retained on 0.4 μm filters) operationally overlapped within a narrow range of particle size (0.4 to 0.7 μm) in this study. However, DOC and EPS are expected to represent essentially different types of particles since the method used for measuring EPS concentrations specifically applies to acidic polysaccharides (Passow & Alldredge 1995) while DOC measurements include all types of organic matter within its size range.

Although DOC is recognized as the main carbon source for bacterial production, EPS has also been suggested to enhance bacterial growth by providing a carbon-rich substrate or favoring bacterial attachment to particles in the sea ice (Meiners et al. 2004) and water column (i.e. marine snow, Muller-Niklas et al. 1994). In our study, sea-ice bacterial abundances were significantly correlated with EPS concentrations under low snow cover only. However, partial correlation analyses indicated multiple intercorrelations between total bacterial abundances, DOC, EPS and chl *a* concentration such that there was no clear evidence of a preferred bacterial carbon source or preferred site of attachment.

2.4.2 Grazing rates

Community grazing rates, estimated by the disappearance of FLB in this study, are high in comparison to published results from other marine systems (e.g. Arctic Ocean, Sherr et al. 1997). Changes in environmental structure (i.e. ice brine channels versus melted samples) or salinity may have enhanced grazing rates in melted samples. The addition of FLB may have also enhanced grazing since larger prey can be preferentially selected and stimulate protistan grazing (Andersson et al. 1986). However, the average size of FLB

(1.8 μm) added was similar to the average size of observed bacteria (1.1 μm) in the sea-ice samples. The FLB were also added in tracer concentrations (average 12% of natural sea-ice bacterial abundances) in order to limit the stimulation of grazing activity (McManus & Okubo 1991). Non-grazing losses of FLB would have also resulted in an overestimation of FLB disappearance. Losses which could be taken into consideration include viral lysis, abiotic FLB breakdown and the attachment of FLB to particles.

Our measurement of bacterivory by heterotrophs averaged 31% d^{-1} and 29% d^{-1} of bacterial standing stocks in the sea ice (high and low snow sites combined) and surface waters, respectively. These results closely match those of short term FLB uptake experiments from the sea ice (average 36% d^{-1}) and water column (average 24% d^{-1}) in Saroma-ko lagoon, Okhotsk Sea (Sime-Ngando et al. 1999). Despite evidence of active grazing throughout our study, there was only a weak indication of net heterotrophic NH_4 regeneration in the sea ice. On the Mackenzie shelf, NH_4 regeneration was measured (average 0.48 μM N d^{-1}) in newly formed sea ice which had sea-ice heterotrophic abundances an order of magnitude lower than in this study (see Chapter 1). Therefore, we expected that NH_4 regeneration would also be occurring during this study as a result of increased heterotrophic abundance and grazing activity. The lack of detectable net NH_4 regeneration indicates concurrent dark uptake of NH_4 by sea-ice bacteria and possibly algae during the present study. Dark NH_4 uptake by bacteria and algae was also apparent in the newly formed sea ice on the Mackenzie shelf (see Chapter 1).

Sea-ice DOC concentrations were highest under low snow, suggesting that sea-ice bacterial production may also be higher under low snow than high snow cover. We hypothesized that if sea-ice bacterial production was indeed higher under low snow cover, higher heterotrophic bacterivory would be required to explain the fact that sea-ice bacteria abundances were not significantly different between high and low snow cover. However, our results do not support this hypothesis since community sea-ice grazing rates were not significantly different between high and low snow cover. This suggests that sea-ice bacterial production rates were similar under high and low snow cover, unless loss factors not assessed in this study (e.g. release of sea-ice bacteria to surface waters) were significantly higher under low than high snow cover.

2.4.3 Ingestion rates

Community (average: 7.1 x 10^3 bacteria HP^{-1} d^{-1}) and sized-fractionated heterotrophic ingestion rates were very high at the ice-water interface (Fig. 6B, Table 2). These high ingestion rates suggest that heterotrophic protist abundances in surface waters (Fig. 3C, D) may have been underestimated due to preservation or counting methodologies. For example, the abundance of surface water ciliates may have been underestimated due to preservation with buffered formalin (Karayanni et al. 2004). Alternatively, community and size-fractionated ingestion rates of heterotrophs in surface waters may have been overestimated due to the consumption of bacteria by mixotrophic protists (Nygaard & Tobiesen 1993, Keller et al. 1994), which would have been identified as autotrophs in this study. In a concurrent study of water column protists in our study area, high abundances of

mixotrophic prymnesiophytes were observed (M. Estrada pers. comm.). We can not quantify mixotrophy from our estimates. However, it appears that mixotrophy in the sea ice and/or water column can bring an additional and unknown source of error in carbon flow estimates which are routinely based on heterotrophic specific grazing or ingestion rates.

Heterotrophic ingestion rates of bacteria in the sea ice of this study were, on average, higher than the maximum recorded heterotrophic nanoflagellates (i.e. <20 μm, HNAN) ingestion rate (1.5×10^3 bacteria $HNAN^{-1}$ d^{-1}) from first-year sea ice of Resolute Passage during the spring sea-ice algal bloom (Laurion et al. 1995). If ingestion rates of heterotrophic protists ≤5 μm and >5 μm are considered separately (Table 2), the ingestion rates of the heterotrophs >5 μm during the sea-ice algal bloom period of this study are within a similar range of the HNAN ingestion rates observed by Laurion et al. (1995). This potentially suggests that NHAN were the primary grazers amongst the heterotrophs >5 μm size class, especially during the sea-ice algal bloom period, despite the presence of sea-ice heterotrophs >20 μm (maximum diameter of heterotrophs >5 μm = 63 μm).

Community (Fig. 6B) and size-fractionated ingestion rates (Table 2) were consistently lower during the sea-ice algal bloom period as compared to the pre-bloom period, except for heterotrophs ≤5 μm in the surface water. Community and size-fractionated ingestion rates decreased during the sea-ice algal bloom despite increasing abundances of bacteria (Fig. 4A, B). We propose that increased EPS concentrations during the sea-ice algal bloom (see Chapter 3) contributed to lower heterotrophic ingestion rates

by interfering with heterotrophic grazing activity, as evidenced by the significant negative correlations between the ingestion rates of sea-ice heterotrophs >5 μm and EPS concentrations under high and low snow cover (Fig. 7). Heterotrophic protist ingestion rates have been shown to be inversely correlated with the concentration of transparent exopolymer particles (TEP) under experimental conditions (Mari & Rassoulzadegan 2004) and high concentrations of exopolymers can decrease grazing rates of herbivorous (Liu & Buskey 2000) and bacterivorous (Mari & Rassoulzadegan 2004) ciliates. High concentrations of EPS within the sea ice may interfere with the mobility and feeding of heterotrophs by coating or clogging feeding appendages (Liu & Buskey 2000), thereby decreasing ingestion rates.

2.4.4 Carbon sources for heterotrophic protists

The consumption of bacteria provided the minimal carbon requirements for the observed net population growth of heterotrophs ≤5 μm but was insufficient for the growth of heterotrophs >5 μm during the sea-ice algal bloom (Table 2). The requirement of an additional carbon source for heterotrophic protist growth was also observed in Arctic sea ice by both Laurion et al. (1995) and Sime-Ngando et al. (1999).

Our results provide evidence that the growth of heterotrophs in the sea-ice and surface waters of Arctic shelves involves multiple carbon sources. We recognize that several carbon sources could potentially be used by heterotrophs >5 μm during this study including: (1) consumption of algae-attached bacteria (Caron 1987), (2) consumption of

algal cells (Michel et al. 2002), (3) consumption of other heterotrophic protists (Sherr & Sherr 2002), (4) direct utilization of EPS (Sherr 1988, Tranvik et al. 1993) and possibly (5) direct utilization of DOC (Decho & Lopez 1993). During our study, limited consumption of algae by heterotrophs (i.e. herbivory) was suggested by direct observations of ingested diatoms in the largest (ca. 130 x 10^3 μm^3) sea-ice heterotrophs from under low snow cover only. The negative correlations between the ingestion rates of sea-ice heterotrophs >5 μm and EPS concentrations (Fig. 7) may also suggest that during the sea-ice algal bloom, EPS could be used as a carbon source in addition to or instead of bacteria. The low bacterivory rates observed during the sea-ice algal bloom period may be partially explained by the potentially preferential utilization of EPS by the sea-ice heterotrophic protists. The high concentrations of EPS during the sea-ice algal bloom period may facilitate the use EPS by the heterotrophs. However, further study is needed to verify the direct uptake of EPS and any associated organic matter by sea-ice heterotrophs.

2.5 Conclusions

Mackenzie shelf sea ice and associated surface waters are carbon-rich environments supporting high bacterial abundances and the growth of large sized bacteria. Bacterial growth and heterotrophic bacterivory are closely linked to the concentrations of DOC, EPS and chl *a* although intercorrelations among the variables limit our ability to discern direct relationships.

Our study describes an active heterotrophic microbial food web within the sea ice and surface waters on the Mackenzie shelf, which persisted throughout the sea-ice algal pre-bloom and bloom period. Bacterial and heterotrophic protist abundances and rates of bacterivory appear to be relatively consistent component of sea-ice assemblages regardless of differences in snow cover, which strongly affect chl *a* and EPS concentrations. Heterotrophic bacterivory was an important source of bacterial mortality and contributed significantly to the carbon requirements of heterotrophs ≤5 µm in the sea ice and surface waters. To meet the carbon requirements of heterotrophs >5 µm during the sea-ice algal bloom, alternative carbon sources such as EPS may have been used, in addition to bacteria and algal cells. It is evident that complex microbial food webs are associated with first-year sea ice and that DOC and EPS are integral components of heterotrophic processes within the sea-ice and surface waters of Arctic shelves.

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CHAPITRE III

SEASONAL STUDY OF SEA-ICE EXOPOLYMERIC SUBSTANCES ON THE MACKENZIE SHELF: IMPLICATIONS FOR THE TRANSPORT OF SEA-ICE BACTERIA AND ALGAE

RÉSUMÉ

Des échantillons provenant de la couche inférieure de la glace de mer, à deux sites représentatifs d'un couvert de neige mince et épais, ainsi que d'eau de surface, ont été prélevés à 21 occasions, entre le 24 février et le 20 juin 2004, dans la baie de Franklin (plateau continental du Mackenzie). Ces échantillons ont été traités pour analyse des substances exopolymériques (EPS), du carbone organique particulaire (POC) et de la chlorophylle *a* (chl *a*). Les concentrations en EPS ont été mesurées sur des échantillons de glace fondue, suite à une coloration au bleu Alcian. Les vitesses de chute de la chl *a* et des bactéries ont aussi été évaluées en utilisant des colonnes à sédimentation, afin de déterminer le rôle potentiel des EPS sur le transport de la biomasse associée à la glace. Les concentrations en EPS dans la couche inférieure de la glace étaient faibles au cours du mois de mars (moyenne de 185 µg xeq. l^{-1}) et ont augmenté par la suite, pour atteindre des valeurs maximales de 4930 et 10500 µg xeq. l^{-1} sous couvert de neige épais et mince, respectivement. Les concentrations en EPS dans les eaux de surface se sont maintenues à des valeurs de deux ordres de magnitude plus faibles que celles observées dans la glace. Dans la glace, les concentrations en EPS étaient corrélées de façon significative avec les concentrations en chl *a* ($\tau = 0.70$, $p < 0.01$). Les algues de glace étaient principalement responsables de la production de EPS dans la glace; la contribution des bactéries à cette production était mineure. Le carbone associé aux EPS contribuait, en moyenne, à 23 % des concentrations en POC dans la glace, avec une valeur maximale de 73 % au cours de la période de fonte. Les vitesses de chute médianes de la chl *a* étaient de 0.11 et 0.44 m d^{-1} sous couvert de neige épais et mince, respectivement. Aucun effet significatif des EPS sur les vitesses de chute de la chl *a* n'a été démontré. Toutefois, les vitesses de chute des bactéries auraient été influencées par la présence de EPS, soit en association avec des diatomées, ou comme particules libres dans la glace. La présence de EPS sur les diatomées favoriserait l'attachement des bactéries sur les algues, et augmenterait ainsi les vitesses de chute des bactéries, alors que les vitesses de chute de bactéries associées avec des particules libres de EPS, dont la flotabilité est positive, seraient réduites. Les substances exopolymériques contribuent de façon significative à la biomasse en carbone dans la glace de mer, et influencent la sédimentation de cette biomasse. Ces résultats mettent en évidence l'importance du rôle des EPS dans le cycle du carbone sur le plateau continental arctique.

ABSTRACT

Bottom sea ice, from under high and low snow cover, and surface water samples were collected in Franklin Bay (Mackenzie shelf) on 21 occasions between 24 February and 20 June 2004 and analyzed for exopolymeric substances (EPS), particulate organic carbon (POC) and chlorophyll *a* (chl *a*). Concentrations of EPS were measured using Alcian blue staining of melted ice samples. Chlorophyll *a* and bacterial sinking velocities were also assessed with settling columns, to determine the potential role of EPS in the transport of sea-ice biomass. Concentrations of EPS in the bottom ice were consistently low in March (avg. 185 μg xeq. l^{-1}), after which they increased to maximum values of 4930 and 10500 μg xeq. l^{-1} under high and low snow cover, respectively. Concentrations of EPS in the surface water were consistently two orders of magnitude lower than in the sea ice. Sea-ice EPS concentrations were significantly correlated with sea-ice chl *a* biomass ($\tau = 0.70$, $p < 0.01$). Sea-ice algae were primarily responsible for EPS production within the sea ice, whereas bacteria produced insignificant amounts of sea-ice EPS. EPS-carbon contributed, on average, 23% of POC concentrations within the sea ice, with maximum values reaching 72% during the melt period. Median chl *a* sinking velocities were 0.11 and 0.44 m d^{-1} under high and low snow cover, respectively. Exopolymeric substances had little effect on chl *a* sinking velocities. However, bacterial sinking velocities did appear to be influenced by diatom-associated and free EPS within the sea ice. Diatom-associated EPS could facilitate the attachment of bacteria to algae thereby increasing bacterial sinking velocities, whereas the sinking velocities of bacteria associated with positively buoyant, free EPS, could be reduced. Exopolymeric substances contributed significantly to the sea-ice carbon pool and influenced the sedimentation of sea-ice biomass, which emphasizes the important role of EPS in carbon cycling on Arctic shelves.

3.1 Introduction

Arctic sea ice supports a diverse community of organisms, ranging from viruses (e.g. Maranger et al. 1994, Gowing et al. 2004) to metazoa (e.g. Gradinger 1999, Nozais et al. 2001). Sea-ice algae are a major component of biomass in Arctic first-year sea ice (e.g. Smith et al. 1990, Melnikov et al. 2002) with growth being limited primarily by light and nutrient availability (e.g. Gosselin et al. 1985, Cota et al. 1987, Smith et al. 1997). Sea-ice algae are an early source of carbon for water column grazers (Michel et al. 1996, 2002) and can contribute 25% or more to total Arctic primary production (Legendre et al. 1992, Gosselin et al. 1997).

Recent studies have found high concentrations of exopolymeric substances (EPS) in Arctic sea ice (Krembs et al. 2002), with values 1 order of magnitude higher than in the surface water, during the summer and autumn (Krembs & Engel 2001, Meiners et al. 2003). Exopolymeric substances encompass a diverse mixture of polysaccharides and glycoproteins with gel-like characteristics. They are produced by both bacteria and algae, with diatoms being the primary source of EPS in the sea ice (Meiners et al. 2003, Mancuso Nichols et al. 2004). These exopolymers can be found as thick gels around bacteria and algal cells (Myklestad 1995, Underwood et al. 1995), as free colloidal organic matter (Decho 1990, Passow 2000), or as part of large particles and aggregates (Alldredge et al. 1993, Passow et al. 2001). Exopolymeric substances surrounding algal cells, as well as free EPS, have been observed in Arctic and Antarctic sea ice (Krembs et al. 2002, Meiners et al.

2004). In the present study, EPS refer specifically to particulate (>0.4 μm) acidic exopolysaccharides as measured by the Alcian blue method (Passow & Alldredge 1995). The Alcian blue method was first used to measure transparent exopolymer particles (TEP), which refers primarily to discrete particles in the water column formed through the coagulation of dissolved precursors. Exopolymeric substances in the sea ice are often not discrete particles (i.e. they are cell-associated) and their formation processes are largely unknown. Therefore, the particles measured in this study will be referred to as EPS instead of TEP.

Copious amounts of EPS can be produced by benthic diatoms (Smith & Underwood 1998) and by phytoplankton during or following the bloom period (Alldredge et al. 1993). Algal-produced EPS can help protect cells against harsh environmental conditions as well as assist in cell adhesion and motion (Cooksey & Wigglesworth-Cooksey 1995). Exopolymeric substances constitute a carbon-rich substrate for bacteria, potentially supporting bacterial production and metabolic activity (Simon et al. 2002). The release of exopolymers by algae or bacteria further influences carbon cycling by: (1) directly contributing to the organic carbon pool with concentrations potentially equivalent to those of particulate organic carbon (POC) in pelagic environments (Mari 1999, Engel & Passow 2001), (2) influencing sedimentation rates through aggregation (Turner 2002, Azetsu-Scott & Passow 2004), and (3) bypassing microbial-mediated POC production by the abiotic formation of large EPS-containing particles or aggregates (Passow 2002a, Thornton 2002).

Sea-ice EPS have not been previously investigated on the extensive Canadian Arctic shelves. Within first-year sea ice of the eastern Chukchi Sea, large amounts of EPS were observed surrounding algal cells, suggesting an important role in cellular protection against high salinities and low temperatures (Krembs et al. 2002). Sea-ice EPS can be a significant source of carbon, contributing 14 to 32% of integrated POC values in Arctic and Antarctic sea ice of varying ages (Meiners et al. 2003, 2004). The EPS also constitute an important substrate for sea-ice bacteria (Meiners et al. 2004), potentially supporting increased microbial activity.

Our research investigated the seasonal dynamics of EPS in first-year sea ice on the Mackenzie shelf, Canadian Arctic, during an extensive winter to spring study. The general objectives of this research were to (1) evaluate EPS concentrations in bottom sea ice, under high and low snow cover, and identify relationships with sea-ice algal biomass and bacterial abundance, (2) characterize seasonal trends in EPS concentrations in the sea ice and water column and the contribution of EPS to sea-ice POC, during the complete period of sea-ice algae development and decline, and (3) assess the influence of EPS on the sinking velocities of sea-ice bacteria and algae. We hypothesized that EPS concentrations would be highest under low snow cover due to increased light availability for sea-ice autotrophic production (Gosselin et al. 1990) and that EPS concentrations in sea ice would increase seasonally with increasing sea-ice biomass. We also expected high concentrations of sea-ice EPS to favour aggregation and rapid sinking of sea-ice biomass.

3.2 Materials and Methods

Routine sampling was conducted on first-year landfast ice in Franklin Bay, Northwest Territories (Fig. 1), on 21 occasions between 24 February and 20 June 2004. All samples were collected 1.5 km northeast of the overwintering site of the CCGS *Amundsen* (70°04'N, 126°26'W; water depth: ~250 m) as part of the Canadian Arctic Shelf Exchange Study (CASES). Sample analyses were conducted in laboratories on board the ship shortly after sample collection. This area was expected to have sea-ice algal biomass concentrations comparable to those in the greater Beaufort Sea region (i.e. 1 to >30 mg chl *a* m^{-2}; Horner & Schrader 1982, Horner 1985).

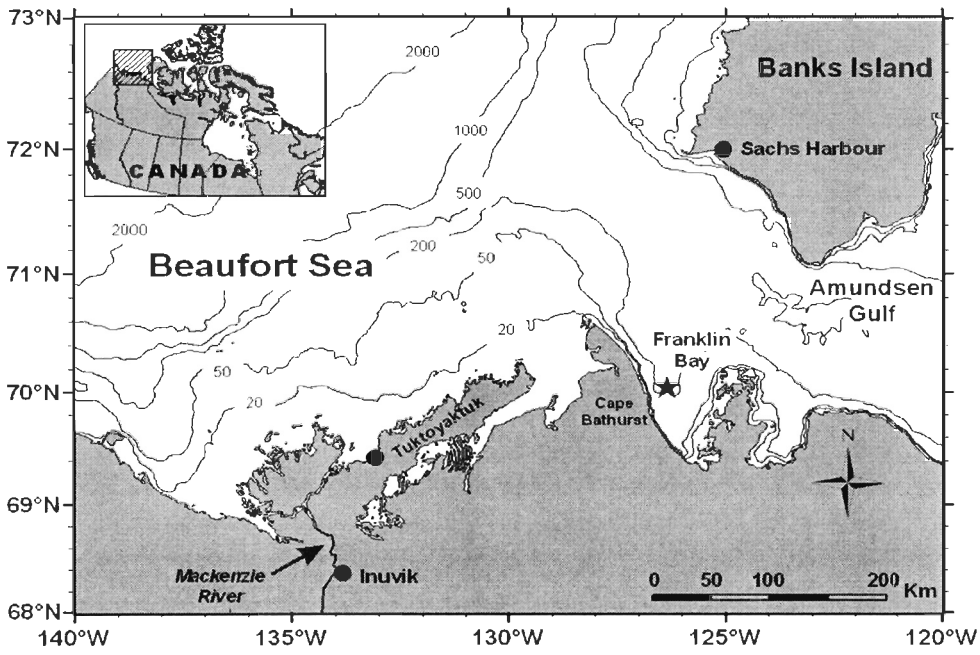

Fig. 1. Sea-ice study station on the Mackenzie shelf (Canadian Arctic), indicated by star in Franklin Bay

Multiple ice cores were collected with a manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprise) within an area of approximately 0.01 km^2 with consecutive coring conducted within ca. 10 m of previous coring sites. On each sampling day, a total of ten to 16 ice cores were collected at two sites representative of high and low snow cover to account for horizontal patchiness of sea-ice biomass (Gosselin et al. 1986, Rysgaard et al. 2001). On the two last sampling dates (14 and 20 June), only low snow cover sites remained due to snow melt.

At the high and low snow cover sampling sites, ice cores were collected for routine measurement of salinity, EPS, POC, chlorophyll *a* (chl *a*) and sinking velocities of chl *a* and bacteria. The bottom 4 cm of each core was cut and put in an isothermal container. Bottom ice cores were pooled together to obtain one sample for each snow cover depth. Sterile gloves were worn at all times during the manipulation of the cores. On each sampling day, water from the ice-water interface was also collected using a hand pump. A subsample of this surface water was analyzed for salinity, EPS and chl *a* and another subsample was filtered on polycarbonate 0.2 µm filters and added to the ice core samples to minimize osmotic stress during the melting process (Garrison & Buck 1986). Sea-ice concentrations of all measured variables were corrected for the dilution arising from the addition of seawater during the melting process.

Sea-ice and surface water salinity were determined with an Orion salinometer. Chlorophyll *a* and pheopigments were determined fluorometrically (10AU Turner Designs)

on duplicate subsamples filtered on Whatman GF/F filters, after 24 h extraction in 90% acetone at 5°C in the dark (Parsons et al. 1984). Duplicate POC subsamples were filtered on pre-combusted Whatman GF/F filters, stored at -80°C and later analyzed with a Perkin-Elmer Model 2400 CHN analyzer.

Triplicate EPS subsamples were filtered on 47 mm 0.4 µm polycarbonate filters and stained with Alcian blue. The EPS were measured colorimetrically (787 nm) after a 2 h extraction in 80% H_2SO_4 (Passow & Alldredge 1995). Concentrations of EPS were recorded as µg gum xanthan equivalents (xeq.) l^{-1} and converted to carbon equivalents according to Engel (2004).

Duplicate subsamples for bacterial counts were stained with DAPI (4, 6-diamidino-2-phenylindole) at a final concentration of 1 µg ml^{-1} and filtered on 0.2 µm black Nuclepore filters (Sherr et al. 1993). A minimum of 200 free-living bacteria was counted in ten fields from replicate samples using epifluorescent microscopy. Bacterial carbon concentrations were estimated using a conversion factor specific to Arctic bacteria (0.03 pg C $bacteria^{-1}$; Gradinger & Zhang 1997).

At the beginning of the ice algal bloom (9 April to 4 May), the sinking velocities (m d^{-1}) of chl *a* and bacteria from sea ice under high (n = 5) and low (n = 6) snow cover were estimated every 5 d using settling columns (SETCOLs), according to Bienfang (1981). These measurements did not continue throughout the decline of the sea-ice algae

bloom as the ship left the overwintering location in Franklin Bay and a stable platform was no longer available. The SETCOLs consisted of a 0.47 m high Plexiglas cylinder equipped with ports for subsample removal at the top (0.30 l), middle (1.8 l) and bottom (0.33 l) sections of the column. The column was filled to just below the top, to avoid the attachment of sea-ice diatoms to the Plexiglas cap.

To determine sinking velocities, the bottom of two ice cores were melted in 2.5 l of 0.2 µm filtered surface water. Homogeneously mixed subsamples of the diluted sea ice were allowed to settle in the SETCOL for 2 h at 0°C in the dark, after which all three sections of the column were collected and analyzed for pigment concentration and total bacterial abundance, according to the above methods. The percentage of diatom-attached bacteria was also assessed in the settled material (bottom section) of the column by counting the number of diatom-attached and free-living bacteria in an additional ten fields.

Sinking velocities (SV) were calculated according to the following equation:

$$SV = (B_s/B_t) \times l/t \quad (1)$$

Where B_s is the settled biomass in the bottom section of the column, B_t is the total biomass, l is the SETCOL height and t is the settling period in days (Bienfang 1981). Negative sinking velocities indicate the presence of buoyant biomass. The replicability of SETCOL trials were tested on sea-ice subsamples collected on 3 May; the coefficients of variation of

two replicate trials for chl *a* and bacterial sinking velocities were 0.98 and 11.5%, respectively.

The data were analyzed with nonparametric rank statistics (Sokal & Rohlf 1995). Wilcoxon's signed-ranks tests were used to compare paired variates from the high and low snow cover sites and from the SETCOLs. Kendall's coefficient of rank correlation (τ) was computed to infer the relationship between two variables. Statistical tests were performed with JMP (SAS Institute).

3.3 Results

3.3.1 Spatial and seasonal trends

In Franklin Bay, sea-ice thickness increased from 1.3 m to a maximum of 2.0 m during the sampling period (Fig. 2). A significant decrease in ice thickness began in early June, coinciding with the appearance of melt ponds. Seasonally-averaged snow depth at the high and low snow cover sites was 16.2 ± 3.5 and 3.4 ± 2.5 cm, respectively. On the last two sampling dates (14 and 20 June, Fig. 3) there were no high snow sites remaining for sampling due to the spring snow melt. Salinity averaged 9.5 (range 5.9 to 12.8) and 29.7 (range 5.4 to 34.4) in the sea ice and surface water, respectively. Sea-ice salinity remained relatively constant throughout the sampling period. In the surface water there was a rapid decline in salinity during the melt period with values decreasing from 31.0 to 5.4 between 29 May and 20 June (data not shown).

The low snow cover site consistently showed higher bottom sea-ice chl *a* concentrations than the high snow site (except on 28 May, Fig. 3A). From February to April, bottom sea-ice chl *a* concentrations under low snow cover were, on average, 5.5 times higher than values under high snow cover. This difference dropped to ca. 2 from May to June (Fig. 3A, Table 1). In the surface water, chl *a* concentrations remained below 1 $\mu g\ l^{-1}$, about three orders of magnitude lower than in the sea ice, for the entire sampling period (Fig. 4A). Maximum bacterial abundance also occurred under low snow cover (Table 1). However, bacterial abundance under high and low snow cover showed similar seasonal increases with median abundance increasing by 40 and 43% under high and low snow cover, respectively, between the February to April and May to June periods (Table 1).

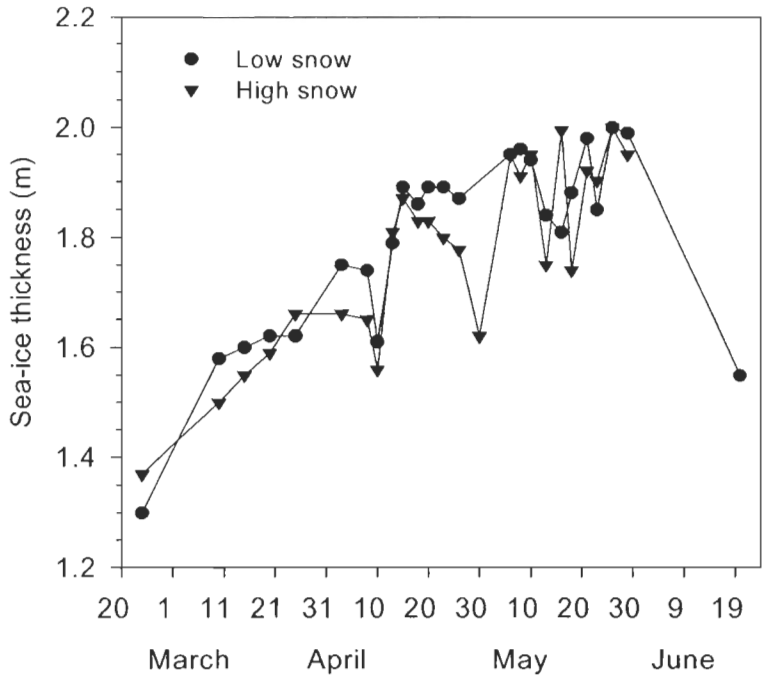

Fig. 2. Seasonal trend in sea-ice thickness, at high and low snow cover sampling sites, from February to June 2004

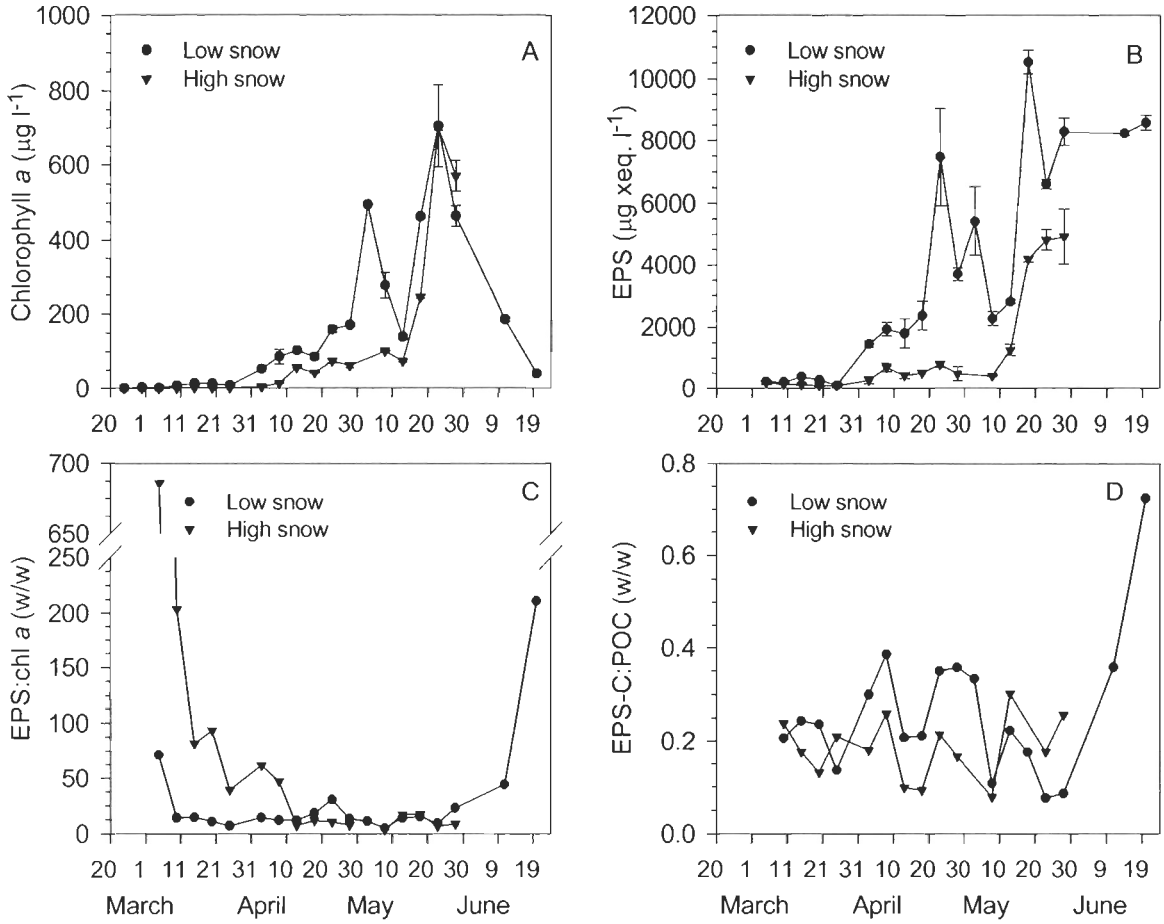

Fig. 3. Seasonal trends in the concentration of (A) sea-ice chlorophyll *a* (chl *a*) and (B) sea-ice exopolymeric substances (EPS) and in sea-ice ratios of (C) EPS:chl *a* and (D) EPS-carbon:POC (EPS-C:POC) under high and low snow cover from February to June 2004. In (A) and (B), averages ± SD are shown

Concentrations of EPS in the bottom sea ice were consistently low during the month of March (high snow: 128 ± 40 µg xeq. l^{-1}; low snow: 241 ± 105 µg xeq. l^{-1}). Thereafter, EPS concentrations increased, reaching maximum values of 4930 and 10 500 µg xeq. l^{-1} under high and low snow cover, respectively (Fig. 3B). Maximum EPS concentrations in the bottom ice were observed on 18 and 28 May at the low and high snow sampling sites,

respectively. Concentrations of EPS in the surface water were two orders of magnitude lower than those measured in the bottom sea ice, ranging from below detection to a maximum of 80 μg xeq. l^{-1} (Fig. 4B). Mid-way through the sampling period (23 to 28 April), EPS concentrations in the surface water dropped to values below detection. This trend was not observed within the sea ice under high or low snow cover.

Table 1. Summary of sea-ice variables under high and low snow cover, during early (February to April 2004) and late (May to June 2004) sampling season. All data represents the combination of the entire sampling season and both snow cover sites. Median and range values are given; sample size in parentheses

Parameter	February-April		May-June		All data
	High snow	Low snow	High snow	Low snow	
EPS	265 (11)	1460 (11)	4190 (5)	6620 (9)	1360 (36)
(μg xeq. l^{-1})	91-781	101-7480	411-4930	2280-10500	91-10500
Chl *a*	4.3 (11)	102 (11)	246 (5)	424 (9)	88.4 (36)
(μg l^{-1})	0.3-74.4	3.2-281	74.9-700	40.6-711	0.3-711
POC	1290 (10)	3110 (10)	7700 (4)	13400 (9)	3310 (33)
(μg l^{-1})	273-3450	471-13500	2640-17200	7470-61000	273-61000
EPS-C	167 (11)	918 (11)	2640 (5)	4170 (9)	857 (36)
(μg l^{-1})	57.4-492	64.0-4710	259-3110	1430-6640	57.4-6640
EPS:chl *a*	47.1 (11)	14.2 (11)	8.6 (5)	13.7 (9)	14.3 (36)
(w/w)	7.4-685	7.2-71.4	4.0-17.1	5.0-211	4.0-685
EPS-C:POC	0.18 (10)	0.24 (10)	0.22 (4)	0.22 (9)	0.21 (33)
(w/w)	0.09-0.26	0.14-0.39	0.08-0.30	0.08-0.72	0.08-0.72
Bacteria	1.5 (12)	1.4 (12)	2.1 (5)	2.0 (8)	1.7 (37)
(10^9cells l^{-1})	0.4-2.1	0.3-2.7	0.5-2.6	1.3-4.3	0.3-4.3

Under high and low snow cover, sea-ice EPS:chl *a* ratios (w/w) ranged between 4.0 and 685 and between 5.0 and 211, respectively (Fig. 3C, Table 1). The maximum EPS:chl *a* ratio under high snow cover was observed at the very beginning of the sampling period (5 March, Fig. 3C). In comparison, the maximum EPS:chl *a* ratio under low snow cover was observed on the last sampling date (20 June) during the melt period. The EPS:chl *a* ratios were similar under both snow cover areas during the period of ice algal growth (Fig. 3). However EPS:chl *a* ratios were significantly higher (Wilcoxon's, $p < 0.01$) under high compared to low snow cover during the first part of the season (5 March to 8 April, Fig. 3C, Table 1). In the surface water, the median EPS:chl *a* ratio was 81.6, with values ranging between 0 and 456 (Fig. 4C). Wilcoxon's signed-ranks tests indicated that this ratio was significantly higher ($p \leq 0.05$) in the surface water than in the sea ice under low snow cover but not significantly different ($p = 0.27$) under high snow cover.

Estimated EPS-carbon ranged from 57.4 to 3110 and 64.0 to 6640 μg C l^{-1} in the sea ice under high and low snow cover, respectively (Table 1) and from below detection to 44.0 μg C l^{-1} in the surface water (data not shown). EPS-carbon:POC ratios were variable in the sea ice and no clear seasonal trends were observed except for large increases on the last two sampling dates, with values increasing to 0.36 and 0.72 on 14 and 20 June, respectively (Fig. 3D). These increases paralleled increases in the EPS:chl *a* ratios on the same dates (Fig. 3C). The median EPS-carbon:POC ratio was lower under high snow compared to low snow cover during February to April (Wilcoxon's, $p < 0.05$), whereas it was similar under both snow covers during May and June (Wilcoxon's, $p = 0.7$, Table 1).

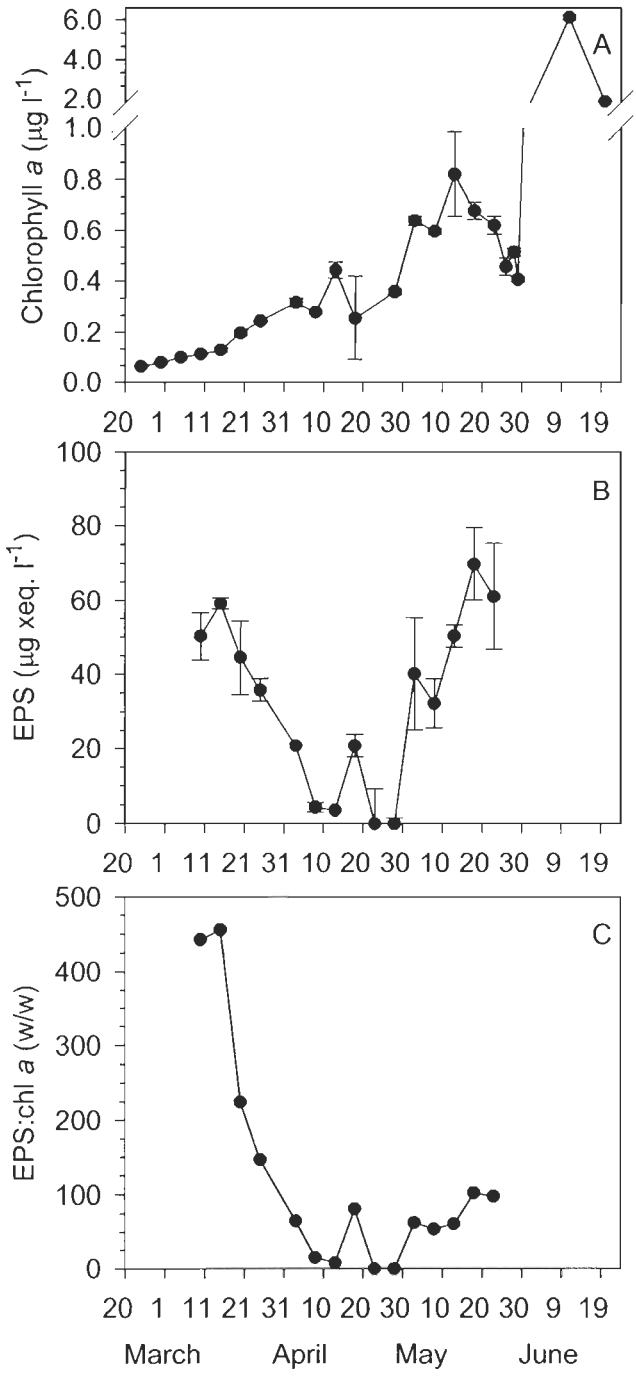

Fig. 4. Seasonal trends in the concentration of (A) chlorophyll *a* and (B) exopolymeric substances (EPS), and in (C) EPS:chl *a* ratios in surface waters, from February to June 2004. In (A) and (B), averages ± SD are shown

EPS concentrations in the surface water were not significantly correlated with surface or sea-ice chl *a* concentrations (Figs. 3A & 4A, B). However, sea-ice EPS concentrations were significantly correlated with sea-ice chl *a* under high ($\tau = 0.65$, $p < 0.01$, Fig. 5A) and low ($\tau = 0.70$, $p < 0.01$, Fig. 5B) snow cover. The positive correlation under high snow cover (Fig. 5A) was driven primarily by parallel increases in both EPS and chl *a* concentrations at the end of the sampling period (18 to 28 May, Fig. 3A, B).

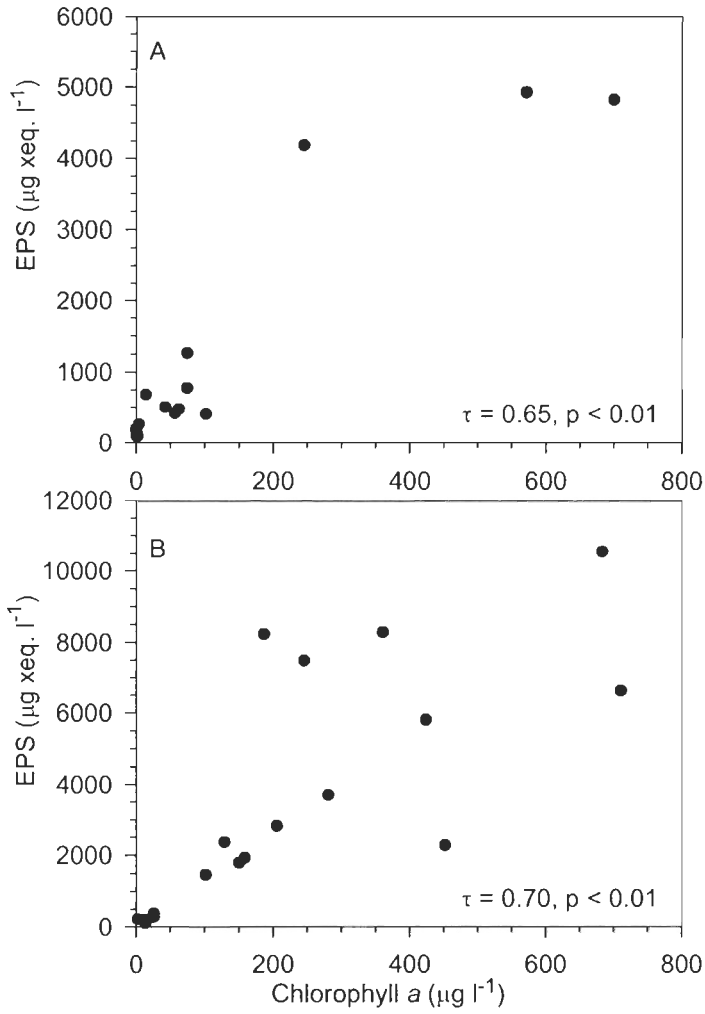

Fig. 5. Correlations between sea-ice exopolymeric substances (EPS) and chlorophyll *a* concentrations under (A) high and (B) low snow cover

3.3.2 Sinking velocities

During this study, chl *a* was the main pigment in the bottom section (i.e. settled pigments) of the SETCOLs. It accounted for 91 ± 10 and 89 ± 11% of the total (i.e. chl *a* + pheopigments) settled pigments in sea ice collected under high and low snow cover, respectively. The sinking velocities of total pigments and chl *a* sinking velocities were not significantly different under high (Wilcoxon's, $p = 0.53$) or low (Wilcoxon's, $p = 0.75$) snow cover.

Table 2 summarizes chl *a* sinking velocities estimated from the SETCOLs. Chlorophyll *a* sinking velocities varied throughout the study with a general increasing trend following 14 April, under high snow cover. Chlorophyll *a* sinking velocities were consistently higher for samples collected under low snow than under high snow (Wilcoxon's, $p < 0.05$), with median values of 0.11 and 0.44 m d^{-1} under high and low snow, respectively. No clear relationships were observed between chl *a* sinking velocities and chl *a* or EPS concentrations under high or low snow cover.

Table 2. Sinking velocities of chlorophyll *a* and bacteria from sea ice under high and low snow cover and the percentage of diatom-attached bacteria in the settled material of the settling columns. Median and range values are given; sample size in parentheses. One chl *a* sinking velocity (14 April) was excluded due to erroneous initial chl *a* measurements

	Sinking velocity (m d^{-1})		Diatom-attached
	Chlorophyll *a*	Bacteria	bacteria (%)
High snow	0.11 (4)	-0.12 (5)	8.9 (5)
	-0.03 to 0.41	-0.25 to 0.41	0 to 17.6
Low snow	0.44 (5)	0.03 (6)	19.8 (6)
	0.24 to 0.68	-0.17 to 0.32	8.0 to 30.3

Sinking velocities of total bacteria ranged between -0.25 and 0.41 m d^{-1} and between -0.17 and 0.32 m d^{-1}, under high and low snow cover, respectively (Table 2). Diatom-attached bacteria were observed in the settled material of all SETCOLs from the low snow cover site (median: 20% of total bacteria, range: 8 to 30%), and in four of the five SETCOLs from the high snow cover site (median: 9% of total bacteria, range: 0 to18%, Table 2). Bacterial sinking velocities were significantly correlated with the percent diatom-attached bacteria under low snow cover ($\tau = 0.80$, $p < 0.05$, Fig. 6) whereas there was no correlation between these two variables under high snow cover.

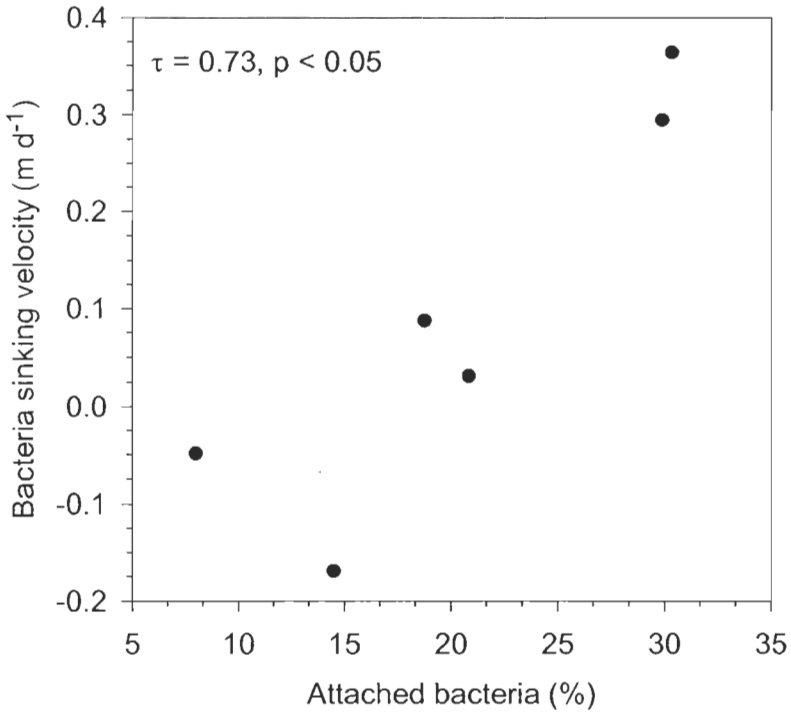

Fig. 6. Correlation between bacterial sinking velocity and percentage of diatom-attached bacteria in the settled material of the settling columns from sea-ice samples collected under low snow cover

We hypothesized that increases in sea-ice EPS concentration and percent diatom-attached bacteria could enhance bacterial sinking velocities. An increase in sea-ice EPS concentrations was also expected to enhance the percentage of diatom-attached bacteria. Correlation analysis was used to test these relationships for high and low snow sites combined. Bacterial sinking velocities were only significantly correlated with the percentage of diatom-attached bacteria ($\tau = 0.54$, $p < 0.05$). Bacterial sinking velocities were not correlated with free-living bacterial abundance ($\tau = 0.36$, $p = 0.15$) or sea-ice EPS concentrations ($\tau = 0.09$, $p = 0.72$). In addition, during the period of SETCOL measurements, EPS concentrations were not correlated with the percentage of diatom-attached bacteria ($\tau = 0.38$, $p = 0.10$).

3.4 Discussion

In pelagic systems, exopolymeric substances in the form of TEP have been recognized as a key component of the carbon cycle, directly contributing to the carbon pool and influencing sedimentation and small-scale microbial processes such as nutrient uptake and bacterial productivity (see Passow 2002a). Previous studies of first-year pack ice in Antarctica (Meiners et al. 2004) and first-year, landfast and pack ice in the Arctic (Krembs & Engle 2001, Krembs et al. 2002, Meiners et al. 2003) have found high concentrations of EPS in the interior and bottom of the sea ice. The current study found EPS to be a significant contributor to sea-ice carbon on Canadian Arctic shelves, with concentrations reflecting spatial and temporal variations in the sea-ice community. Key relationships were

identified between EPS, chl *a* and bacteria, showing that EPS can influence carbon cycling within the sea ice and upon the release of sea-ice biomass into the water column at the time of ice melt.

3.4.1 Producers of EPS in sea ice

During our study, sea-ice EPS concentrations were on average three times lower under high snow than low snow cover. Still, EPS concentrations under high snow cover were two orders of magnitude higher than in the surface water. It is apparent that the sea ice of the Mackenzie shelf contains substantial amounts of EPS with the maximum EPS concentration measured during our study (10500 μg xeq. l^{-1} under low snow) surpassing the highest concentration of EPS previously reported in first-year Arctic sea ice (7710 μg xeq. l^{-1}, Krembs et al. 2002). We may have slightly overestimated sea-ice EPS concentrations due to reassembly of EPS from the addition of 0.2 μm filtered water during the melting process. However, this source of EPS would be minimal due to the very low EPS concentrations in surface waters (Fig. 4B).

Chlorophyll *a* concentrations in the bottom sea ice were consistently lower under high snow compared to low snow cover, suggesting that light limitation did influence sea-ice algal growth under high snow cover. The spatial (i.e. high snow vs. low snow) and seasonal trends in EPS and chl *a* concentrations were very similar (Fig. 3A, B), reflecting the significant correlation between EPS and chl *a* concentrations (Fig. 5).

The significant correlations between EPS and chl *a* concentrations under high and low snow cover (Fig. 5) indicate that sea-ice algae were primarily responsible for the production of EPS in the sea ice. Concentrations of EPS in pack ice of the Laptev Sea (Krembs & Engel 2001) and Fram Strait (Meiners et al. 2003) were also significantly correlated with chl *a* and diatom abundances. Pennate diatoms of the genus *Nitzschia* have been found to be the most important producers of EPS within sea ice (Krembs & Engel 2001, Meiners et al. 2003). Pennate diatoms of the genera *Nitzschia* and *Navicula*, were a dominant component of the ice algal communities during our study. However, species composition and form (i.e. solitary cells vs. colonies) varied between high and low snow cover sites (M. Różańska pers. comm.). Such variation in species composition and form could contribute to the spatial variation in sea-ice EPS concentrations on the Mackenzie shelf. Laboratory experiments have shown that the production of exopolysaccharides varies widely among marine algal species, both in amount and chemical structure (Myklestad 1995, Passow 2002b), and is dependent upon their growth rates (Waite et al. 1995).

Bacteria can also be important producers of EPS (Mancuso Nichols et al. 2005). Bacteria isolates from Antarctic sea ice were found to produce EPS, with highest yields at lower temperatures (i.e. -2 to 10°C, Mancuso Nichols et al. 2004). Pelagic bacteria also produce exopolymers but in low amounts relative to algal exopolymer production (Schuster & Herndl 1995, Passow 2002b) and the cell-specific production rate has been estimated to be only 4 amol C $cell^{-1}$ d^{-1} (Stoderegger & Herndl 1999). Using this pelagic production rate for bacterial exopolymers, Meiners et al. (2004) estimated that total bacterial production of

EPS (2.5 μg C l^{-1} d^{-1}) was two orders of magnitude lower than their estimated algal EPS production (195 μg C l^{-1} d^{-1}) in Antarctic pack ice. We estimated total sea-ice bacterial production of EPS to be, on average, 0.1 μg C l^{-1} d^{-1} during the beginning of the ice algal bloom, also using the production factor of Stoderegger & Herndl (1999). When corrected for low temperatures within the sea ice, our estimate could be as high as 3 μg C l^{-1} d^{-1}. Thus, our estimates of EPS production by sea-ice bacteria are orders of magnitude lower than the average concentration of sea-ice EPS-carbon (1600 μg C l^{-1}) during the same period, showing that sea-ice bacteria are not important contributors of sea-ice EPS. These results strongly suggest that EPS were produced primarily by algae, with a potentially minor contribution from bacteria, within the sea ice of the Mackenzie shelf.

Estimates of EPS-carbon in the bottom sea ice had a median value of 857 μg C l^{-1} and ranged from 57.4 to 6640 μg C l^{-1}. While these values represent an approximation of sea-ice EPS-carbon, as the conversion factor was derived from laboratory experiments of TEP originating from diatoms only (Engel 2004), they indicate that EPS can contribute, on average, 22.8% (range 21.7 to 23.9%) of the bottom ice POC on the Mackenzie shelf. In comparison, sea-ice bacterial carbon contributed, on average, 2.3% of bottom ice POC in our study. Our values for the contribution of EPS-carbon to POC could be overestimated due to differences in filtration pore size (EPS is filtered on 0.4 μm polycarbonate membrane, whereas POC is filtered on glass-fiber filter with a nominal porosity of 0.7 μm). However, our results are similar to results from the pack ice in Fram Strait, where EPS-carbon was estimated to account for 24% of sea-ice POC (Meiners et al. 2003). This

indicates that EPS represents a widespread source of carbon in Arctic sea ice. In the context of Arctic climate warming, sea ice reduction may thus impinge on the roles of EPS in carbon cycling and food web dynamics (e.g. Salcher et al. 2005) in sea ice.

3.4.2 EPS seasonal dynamics

Sea ice

Our study began in February, well before the development of the ice algal bloom, and extended throughout the bloom and its termination, the latter evidenced by the rapid decrease in sea-ice chl *a* at the end of the sampling period (28 May to 20 June, Fig. 3A). In this study, sea-ice EPS concentrations remained low (ca. 185 µg xeq. l^{-1}) until April, as compared to sea-ice concentrations >1000 µg xeq. l^{-1} found in early March near Barrow, Alaska (Krembs et al. 2002).

Sea-ice EPS concentrations increased seasonally, following the trend in sea-ice algal biomass (chl *a*). However, sea-ice EPS and chl *a* concentrations became decoupled at the end of the season when we observed a rapid decline in chl *a* concentrations, but stable and high EPS concentrations (Fig. 3A, B). Consequently, the sea-ice EPS:chl *a* ratios greatly increased upon the termination of the ice algal bloom (Fig. 3C). Concurrently, the EPS-carbon:POC ratio rapidly increased, with EPS-carbon accounting for a maximum of 72% of sea-ice POC on 20 June. This suggests that algal biomass and associated POC were released from the sea ice at a faster rate than EPS. A corollary to this is that not all EPS

were attached to particles within the sea ice, and that free EPS would remain longer in the sea ice than particle-attached EPS.

Alternatively, if EPS were exported from the sea ice at the same rate as particulate organic material, the high EPS:chl *a* and EPS-carbon:POC ratios observed at the end of the season would only be explained by increased *in situ* EPS production. Under-ice sediment traps adjacent to our sampling station confirmed that sea-ice algae, which were mainly diatoms at the peak of the sea-ice algal bloom, were being released from the sea ice at the time of ice melt (T. Juul-Pedersen pers. comm.). This confirms that the low sea-ice chl *a* concentrations observed at the end of season represented a reduced abundance of diatoms rather than a decrease in the chl *a* content of algal cells. Assuming limited EPS production by bacteria, as previously discussed, the remaining algal community on the last sampling day would have had to increase EPS production by, on average, 15 fold to produce the observed EPS concentration. Such increases in EPS production are unlikely. Increases of only 1.5 to 5 fold in diatom extracellular carbohydrate production have been reported due to nutrient or environmental stress (Urbani et al. 2005, Abdullahi et al. 2006). Therefore, we conclude that increased *in situ* production of EPS did not likely explain the high EPS-carbon:POC and EPS:chl *a* ratios observed at the end of the sampling period, suggesting that free EPS were retained within the melting sea ice. The EPS retained in the sea ice could supply a pulse of organic carbon into surface waters after the majority of sea-ice biomass has been released into the water column.

Surface waters

Seasonal EPS dynamics in surface waters did not follow the same trend as those in the sea ice. In March, surface EPS concentrations declined as surface chl *a* concentrations increased (Fig. 4A, B). In April, EPS concentrations fell to below detection (23 to 28 April) and then concurrently increased with increasing surface chl *a* concentrations during the sea-ice algal bloom period.

The average EPS:chl *a* ratio in surface waters was over two times higher than in the sea ice (i.e. 140 vs. 58). The surface EPS:chl *a* ratios in our study were similar to TEP:chl *a* ratios found during a spring phytoplankton bloom in the subarctic Pacific (max. 120 to 190, Ramaiah et al. 2001) and surpassed TEP:chl *a* ratios from the Ross Sea (avg. 85, Hong et al. 1997) and Atlantic Ocean (avg. 49-104, Engel 2004). It is possible that, in our study, phytoplankton produced high amounts of EPS due to light limitation and/or low water temperatures. However, the decoupling of surface EPS and chl *a* concentrations, as well as the increasing surface EPS:chl *a* ratios during the sea-ice algal bloom, suggest that surface EPS may have been supplemented with sea-ice EPS.

If EPS were released from the sea ice during the sampling period, then EPS did not accumulate at high concentrations in surface waters. The EPS released to the surface waters may have been quickly degraded by biological or physical processes. Alternatively, any EPS entering or produced within the surface waters may be advected (Krembs & Engel

2001) or sink, thereby influencing the downward export of sea-ice and water column carbon.

3.4.3 Biogeochemical roles of EPS in sea ice

We hypothesized that high concentrations of EPS in the sea ice would enhance the sinking velocities of sea-ice biomass. However, EPS were not significantly correlated with chl *a* sinking velocities in the sea ice of the Mackenzie shelf. Chlorophyll *a* sinking velocities measured during this study were within the lower range of expected sinking rates for individual phytoplankton cells (<1 to 10 m d^{-1}; Culver & Smith 1989) and two to three orders of magnitude lower than those measured for diatom-containing, marine snow aggregates (16 to 368 m d^{-1}; Turner 2002). Therefore, despite very high concentrations of EPS within the sea ice of the Mackenzie shelf, EPS did not appear to favour the formation of aggregates within the sea ice, which would sink at high rates once released into the water column.

The apparent absence of sea-ice aggregates agrees with the study of Meiners et al. (2003) in which diatom-EPS aggregates were not observed during microscopic examinations of EPS in Arctic sea ice from the Fram Strait. However, Riebesell et al. (1991) observed rapid aggregation after algae was released from the sea ice and suggested that diatom-EPS aggregates would have also been present within the brine channels of the Antarctic sea ice.

The sea ice on the Mackenzie shelf would be expected to contain both free and particle-attached EPS, similar to observations of other Arctic first-year sea ice (Krembs et al. 2002). During the SETCOL measurements, all forms of EPS would have been released from the sea ice into a dilute, turbulence-free environment. Our low sinking velocities suggest the absence of large aggregate formation within the sea ice and the absence of spontaneous aggregation of EPS and algal cells, under non-turbulent conditions. The absence of wind-induced mixing of the surface waters due to ice cover may also have limited aggregate formation in Franklin Bay. However, currents and/or shear at the ice-water interface could enhance aggregate formation after EPS is released from the sea ice (Riebesell et al. 1991). We thus conclude that EPS from the sea ice of the Mackenzie shelf does not directly enhance the sinking rate of sea-ice algae released to the water column.

Bacterial sinking velocities were hypothesized to be positively related to bacterial abundances, EPS concentrations and bacterial attachment to sea-ice diatoms. Our results show that bacterial sinking velocities did increase with increased percentage of attached bacteria, but were not significantly correlated with the abundance of free-living bacteria. Also, contrary to our hypothesis, there was no direct correlation between EPS concentrations and bacterial sinking velocities. However, EPS may facilitate the attachment of bacteria to sea-ice diatoms and could be involved in the upward transport of sea-ice bacteria.

In Antarctic sea ice, 100% of free EPS were observed to be colonized by bacteria (Meiners et al. 2004). Bacteria may utilize this free EPS as a site of attachment, possibly protecting them from grazers (Sälcher et al. 2005), or as a carbon-rich substrate which could enhance bacterial production. Similar levels of colonization by bacteria have been observed for TEP in the water column (Passow & Alldredge 1994). These discrete particles of exopolymeric substances have been observed to be positively buoyant and the direction of transport for cells associated with these particles was dependent on the relative proportions of solid matter, interstitial water and exopolymers (Azetsu-Scott & Passow 2004). These exopolymers could ascend in association with attached bacteria and/or larger organic or inorganic particles that would sink in their absence (Azetsu-Scott & Passow 2004).

Negative bacterial sinking velocities were observed in our SETCOLs under high and low snow cover. The negative sinking velocities suggest that at least a portion of the bacterial community was positively buoyant. The majority of free-living, sea-ice bacteria in this study were small ($\leq$1 μm long) and would not have moved significantly through the water column on their own. Therefore, any upward transport of bacteria was likely due to buoyant EPS in association with free-living bacteria. Under high snow cover, 60% of the bacterial sinking velocities were negative and the negative sinking velocities increased with increasing sea-ice EPS concentrations. It thus appears that, irrespective of snow cover, free EPS may enhance the ascent of free-living bacteria.

Bacterial sinking velocities also appeared to be influenced by diatom-associated EPS. Under low snow cover only, bacterial sinking velocities significantly increased with the proportion of bacteria attached to diatoms (Fig. 6). Our results suggest that bacterial attachment to diatoms could be favoured by EPS surrounding algal cells, as indicated by the positive relationship between EPS concentrations and the proportion of diatom-attached bacteria. It is not known why a higher percent of diatom-attached bacteria was found under low snow cover, thus having a greater influence on bacterial sinking velocities. Both bacterial abundances and EPS concentrations were within the same range under high and low snow cover during the SETCOL measurements. However, algal species-specific differences between snow cover sites may have altered the form of sea-ice EPS produced (Myklestad 1995), thereby influencing bacteria-EPS interactions.

3.5 Conclusions

This study represents the most complete seasonal study of EPS in Arctic sea ice to date. For the first time, EPS concentrations under variable snow cover conditions were assessed, showing local spatial variations in addition to seasonal changes in EPS concentrations. We found that the roles of EPS under high snow cover may represent winter and early spring dynamics of sea-ice EPS in general, with more complex interactions occurring only after sufficiently high concentrations of EPS and algal biomass develop within the sea ice. Exopolymeric substances contributed significantly to the sea-ice carbon pool, with EPS-carbon contributing up to 72% of sea-ice POC during the melt

period. High EPS-carbon:POC and EPS:chl *a* ratios during the melt period indicated that EPS can be retained within the sea ice whereas sea-ice algae and other particulate carbon sources are more easily released to the water column during the melt period.

High concentrations of EPS within the sea ice, under high or low snow cover, had very little effect on the downward transport of sea-ice algae when released into dilute, turbulent-free conditions of the SETCOLs. Despite EPS concentrations two orders of magnitude higher than the surface water, we found no evidence indicating the formation of rapidly sinking aggregates within the sea ice. Exopolymeric substances did appear to influence the upward and downward transport of sea-ice bacteria. The buoyant properties of EPS could have enhanced the upward transport of free-living bacteria resulting in negative sinking velocities, while EPS also appeared to mediate the attachment of bacteria to sea-ice diatoms, thereby increasing their sinking velocities. This study has shown that EPS not only contributes directly to the carbon pool in first-year sea ice but also influences carbon cycling within the sea ice and the fate of sea-ice carbon once released to the water column.

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CONCLUSION GÉNÉRALE

In this study, heterotrophic microorganisms and exopolymeric substances (EPS) in newly formed and first-year sea ice were shown to have multiple roles in the cycling of organic carbon on the Mackenzie shelf. An extensive seasonal characterization of heterotrophic bacterial and protist communities is provided, including the assessment of heterotrophic bacterivory and nutrient regeneration. This study was conducted on the Mackenzie shelf which represents a large area of first-year sea ice in the Arctic. The results presented here are generally applicable to sea-ice organic carbon cycling on the extensive circumpolar shelves and potentially new areas of first-year sea-ice formation that could develop as a result of Arctic warming (Comiso 2002, 2003, Polyakov et al. 2003).

In the first Chapter of this thesis, it was shown that newly formed sea ice is enriched in nitrogenous nutrients, EPS and microorganisms as compared to surface waters, thus providing evidence that sea-ice assemblages on Arctic shelves are established early during sea-ice formation. The results of Chapter 1 clearly demonstrate the presence of an active microbial assemblage within sea ice only hours or days old. Ammonium regeneration was assessed for the first time in newly formed sea ice. Net ammonium regeneration occurred at an average rate of 0.48 $\mu M\ d^{-1}$, and as hypothesized, nitrogen enrichment was enhanced by heterotrophic regeneration within the sea ice. Thus, heterotrophic microorganisms in newly formed sea ice can provide nutrients for sea-ice autotrophic production in the fall. The

heterotrophic microorganisms in newly formed sea ice also contributed to secondary production in the fall, and potentially over the entire winter season on the Mackenzie shelf.

This research was the first to evidence the ubiquitous presence of EPS in newly formed sea ice in the Arctic. Exopolymeric substances appeared to be actively produced by algae entrapped in the sea ice, supporting the hypothesis that the cryoprotective characteristics of EPS may be instrumental in cell survival in the rapidly growing sea ice (Krembs et al. 2002). The presence of EPS also appeared to enhance the selective enrichment of large autotrophs (≥5 μm) and potentially favored the incorporation of bacteria in newly formed sea ice. The results presented in the first Chapter also suggest that EPS may be utilized by microorganisms as an organic carbon source during the winter period, at a time when autotrophic production is limited by light availability. Therefore, EPS appear to play a significant role in the establishment and survival of newly formed sea-ice assemblages, which in turn constitute the foundation of the highly productive spring sea-ice community on Arctic shelves.

Information on heterotrophic processes in Arctic sea ice is still very limited (Laurion et al. 1995, Sime-Ngando et al. 1999) and therefore aspects of heterotrophic production in the cycling of organic carbon within the sea ice are poorly understood. In Chapter 2, bacterial dynamics and bacterivory by heterotrophic protists were assessed in a seasonal study of first-year sea ice. As observed in other studies of first-year sea ice (e.g. Gosselin et al. 1986, Laurion et al. 1995), bottom sea-ice chl *a* concentrations on the Mackenzie shelf

were significantly higher in areas of low snow, as compared to high snow cover. However, contrary to what was hypothesized, bacterial and heterotrophic protist abundances did not follow similar spatial trends as chl *a* since their abundances were not significantly different between high and low snow cover sites. These unexpected results suggest a spatial decoupling between heterotrophic and autotrophic processes in Arctic first-year sea ice.

In Chapter 2, heterotrophic protists were identified as an important source of bacterial mortality in first-year sea ice and surface waters on the Mackenzie shelf, such that bacterivory satisfied the carbon requirements of small heterotrophic protists (i.e. $\leq$5 μm) during the sea-ice algal pre-bloom and bloom period. However, it was shown that alternative organic carbon sources were required for the growth of >5 μm heterotrophic protists during the sea-ice algal bloom period. Interestingly, bacterial ingestion rates of $\leq$5 μm and >5 μm heterotrophic protists were lower during the sea-ice algal bloom period as compared to the pre-bloom period. Therefore, this result points to a temporal decoupling, in addition to the previously mentioned spatial decoupling, between sea-ice heterotrophic and autotrophic processes. The results presented in Chapter 2 suggest that high concentrations of EPS may have physically interfered with protist grazing activities within the sea ice. Alternatively, EPS, other heterotrophic protists and/or sea-ice algae, may have been utilized as a carbon source by heterotrophic protists during the sea-ice algal bloom period, resulting in lower rates of bacterial ingestion.

The results of Chapter 2 also confirmed the presence of high sea-ice DOC concentrations, which would create a carbon-rich environment favorable for bacterial growth. This was supported by the presence of large size free-living and diatom-associated bacteria in the sea ice. Large-sized bacteria are routinely found in the bottom of first-year, landfast ice (Smith et al. 1989, Bunch & Harland 1990, Laurion et al. 1995, Kaartokallio 2004) but do not appear to be as prevalent in multi-year pack ice (Gradinger & Zhang 1997) of the Arctic. Therefore, a potential shift from multi-year to first-year sea ice, in response to warming temperatures in the Arctic, could increase the contribution of sea-ice bacterial biomass and associated processes (e.g. bacterivory) to the Arctic Ocean carbon cycle.

The results presented in Chapter 3 support the conclusions of recent studies (Krembs & Engel 2001, Krembs et al. 2002, Meiners et al. 2003, 2004) that EPS are important components of the sea-ice organic carbon cycle. Sea-ice EPS concentrations on the Mackenzie shelf were two orders of magnitude higher than in surface waters and EPS contributed significantly to sea-ice POC, especially during the sea-ice algal bloom period (up to 72% of sea-ice POC). In agreement with the results of Krembs & Engel (2001) and Meiners et al. (2003), EPS and chl *a* were significantly correlated, indicating that sea-ice algae were primarily responsible for the production of EPS within the sea ice. However, at the end of the sampling period (period of ice melt), sea-ice EPS:chl *a* and EPS-carbon:POC ratios rapidly increased, indicating that EPS were retained within the sea ice during the melt period after most of the sea-ice biomass had been released into surface waters.

Consequently, EPS could represent a significant source of organic carbon to surface waters after the release of particulate material from the sea ice. This novel contribution of organic carbon to surface waters remains unaccounted for in current carbon budgets and flux estimates, even though it can have important implications for small and large scale biogeochemical processes on Arctic shelves.

Contrary to the original hypothesis, results from Chapter 3 show that the high concentrations of EPS within the sea ice did not enhance the sinking velocities of sea-ice algae measured experimentally, under conditions of reduced turbulence as compared to *in situ* conditions. However, the results presented in Chapter 3 suggest that bacterial interactions with free EPS or with diatom-associated EPS may decrease or increase bacterial sinking velocities, respectively. In the water column, EPS can enhance the formation of aggregates, which are significant components of organic carbon sinking fluxes and are sites of increased microbial activity (Simon et al. 2002). Therefore, it would be important for future investigations to determine the fate of EPS after their release from the sea ice, which apparently occurred at a date subsequent to the end of the present investigation. Microscopic examinations of EPS at the ice-water interface or in under-ice sediment traps at the end of the melt period could demonstrate that EPS aggregates do indeed form under natural turbulent/shear conditions, with consequences for the cycling and vertical export of organic carbon.

In conclusion, our results show that heterotrophic microorganisms in the sea ice and surface waters significantly contribute to organic carbon and inorganic nutrient cycling via microbial food web processes (i.e. bacterivory and nutrient regeneration, Azam et al. 1983, Sherr & Sherr 2002) on Arctic shelves. Significant intercorrelations between the concentrations of DOC, EPS, chl *a* and the abundances of heterotrophic microorganisms were identified, indicating intricate microbial interactions within first-year sea ice under high and low snow cover. Therefore, alternative organic carbon transfer pathways, such as those involving EPS, should also be recognized as elements of sea-ice and pelagic organic carbon cycling on Arctic shelves.

Further study is required to clearly understand the relationships between EPS, DOC, bacteria and heterotrophic protists in sea-ice microbial food webs. For example, experimental evidence of the direct uptake of EPS by heterotrophic protists is necessary in order to verify potentially new organic carbon pathways in sea-ice microbial food webs. Future evidence of the aggregation of sea-ice EPS in surface waters would also have extensive implications for the sedimentation of organic carbon and microbial processes on Arctic shelves.

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