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Seasonal Nitrogen Uptake and Regeneration in the Water Column and Sea-Ice of the Western Coastal Arctic

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A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

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by

Steven E. Baer

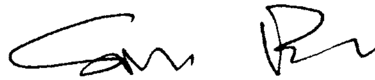
2013

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy



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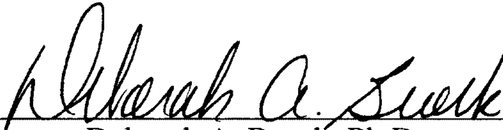

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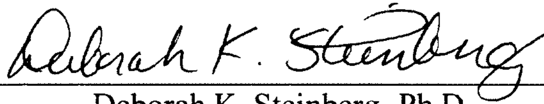

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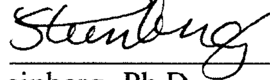

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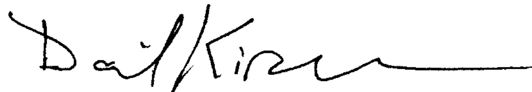

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and maintain a sense of wonder at the natural splendor of everything. I strive to make them proud.

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Abstract

The logistical difficulties of research in extremely low temperatures and lack of access to the Arctic have meant that there is a historic dearth of knowledge of coastal Arctic biogeochemistry, especially during winter when sea ice is present. Recent observations, however, indicate that the Arctic is changing rapidly. Changes include increased temperatures, decreased extent and volume of sea ice, and increased freshwater inputs. How these changes influence biogeochemical cycles is an open question, especially in the highly productive coastal regions of the Chukchi Sea. Here I present nitrogen (N) uptake and regeneration rates for phytoplankton and bacteria measured in the shallow waters and landfast sea ice near Barrow, Alaska. Experiments were performed using tracer-level incubations of stable isotope (^{15}N) ammonium, nitrate, urea, and amino acids during January, April, and August over two successive years (2010 – 2012). Autotrophic versus heterotrophic N uptake was measured with traditional size fractionation. In brief, I found that outside of the spring phytoplankton bloom period, ammonium and amino acids were the preferred N substrates assimilated. Regeneration of N and nitrification were especially high during winter. A high-speed cell sorting flow cytometer was used to distinguish bacterial sub-populations and their uptake rates. Low nucleic acid populations were active in taking up N compounds, although not at quite the same rate as high nucleic acid cells. The difference was less pronounced during winter compared to summer. Additional experiments were designed to artificially warm the samples to demonstrate that ammonium uptake rates increased with temperature and substrate availability, whereas nitrification rates did not. Uptake and regeneration of ammonium and nitrate along with nitrification was also measured in landfast sea ice. This is the first report of N uptake from within the sea ice matrix in the Chukchi Sea. Given the paucity of information on N cycling in the Arctic Ocean, these data can inform modeling efforts to predict future changes in the system and also provide a baseline by which to compare future observations.

Nitrogen Uptake in the Western Coastal Arctic

Chapter 1 – Introduction

The Arctic Ocean has relatively wide continental shelves, substantial freshwater flux from rivers, and significant Pacific Ocean inputs, such that it resembles a large estuary rather than an oceanic basin (Carmack and Wassmann 2006; Dittmar and Kattner 2003; McClelland et al. 2012). Compared to the size of the basin, the Arctic receives extraordinarily large freshwater inputs from rivers and continental runoff relative to the other oceans (Aagaard and Carmack 1989). In the western Arctic, the Chukchi Sea also receives relatively low salinity Pacific water through the Bering Strait (Roach et al. 1995). Surface waters tend to flow northeast past Barrow, Alaska and out into the Beaufort Gyre. In addition to input fluxes from surrounding seas and terrestrial areas, sea ice within the system adds salt during formation and releases fresh water during melt (Carmack 2007). Organisms in the Arctic must tolerate rapid changes in salinity, temperature, and light caused by seasonal changes in sea ice cover. Despite those apparent challenges, secondary production in the Chukchi Sea is a high (Bates et al. 2005; Sakshaug 2004).

Of pressing concern to Arctic science is how the system will react to climate change. Since there is relatively little data on the biogeochemistry of the Arctic, it is difficult to predict the consequences of changes in the marine ecosystem of the region. The goals of this project were to provide a current empirical baseline of N cycling processes along the coastal Chukchi Sea during winter, spring and summer. Field sampling for this dissertation was based out of the former Naval Arctic Research Station in Barrow, Alaska. New facilities located there allowed us access to the landfast sea ice and coastal region during year-round seasonal sampling, along with modern laboratory facilities and walk-in incubators. Experiments performed in the water column and sea ice

provide a unique set of data that shows the importance of regenerated and organic N sources to the coastal Chukchi ecosystem along with information on how these processes may change with future increases in temperature. By way of introduction to the dissertation, below I briefly review information about nutrients and nitrogen cycling, the climate changes occurring, and the unique environment of sea ice.

Nutrients and nitrogen cycling

The Pacific Ocean delivers surface waters with high concentrations of nutrients essential for biological growth, such as nitrate, phosphate, and silica (Codispoti et al. 2005; Codispoti et al. 2009). These nutrients along with those supplied by sea ice retreat (Carmack and Chapman 2003) support intense seasonal pulses of productivity in the Chukchi Sea (Dyda et al. 2009). The spring thaw results in the transport of riverine dissolved organic matter (DOM) to the coastal ocean, which can provide labile material that is likely important to both primary and secondary production (Kirchman et al. 2009; Wickland et al. 2012).

Light and community composition control the timing and fate of the spring production pulse, but the primary control on annual net community production is nitrogen supply (Tremblay et al. 2006; Walsh et al. 2004). Nitrogen plays a central role in ocean biogeochemistry because it is a constituent of amino acids, proteins and nucleic acids, and is therefore fundamental to all living things. Marine organisms mediate transformations among different N forms as part of their metabolism, either for growth or energy. As such, the availability, uptake, and remineralization of N are important

controls on biomass production, and N is subject to fierce competition by autotrophic and heterotrophic organisms. This balance between autotrophs and heterotrophs is a fundamental driver of important ecological parameters in the marine environment and has important implications for food web dynamics and carbon (C) exchange with the atmosphere.

Despite the importance of N to Arctic microorganisms, there are limited studies of N uptake rates in the Arctic generally, and especially in the Chukchi Sea. The Canadian Arctic Shelf Exchange Study (CASES) project produced a high temporal resolution dataset of N pools and uptake in the southeast Beaufort Sea (Simpson et al. 2013; Tremblay et al. 2008), but there is no corresponding information for the Chukchi Sea. The only published reports of N uptake in the Chukchi Sea are offshore during late summer (Lee et al. 2012; Lee et al. 2007). Those studies found N uptake that was heavily reliant on regenerated forms of N, namely NH_4^+. This is in contrast to early studies of other regions of the Arctic (Daly et al. 1999; Garneau et al. 2007; Harrison et al. 1982; Kristiansen et al. 1994; Smith 1993) and implies an especially active microbial loop. So there is some indication that the Chukchi Sea is highly productive and fueled by N inputs, but little corresponding information on rates of N uptake and regeneration. This is especially true along the coast, where landfast sea ice and shallow waters limit the access to icebreakers.

The first goal of this work was to comprehensively understand N uptake and regeneration in the water column of the coastal Chukchi Sea. We focused on NH_4^+, NO_3^-, urea, and an amino acid mixture. In Chapter 2, I present data from ^{15}N incubations done

to measure rates of N uptake and regeneration during January, April, and August of multiple phytoplankton size classes over two successive years. This allowed us to account for conditions of sea ice cover with no irradiance (winter), sea ice cover with high incident irradiance (spring), and high light and no sea ice cover (summer). Complementary data included ambient nutrient measurements of dissolved organic carbon and nitrogen, phosphate and silicate. Collaborators provided data on physical parameters such as temperature and salinity, along with measurements of bacterial production.

In uptake studies, size fractionation is commonly used to separate different phytoplankton size classes and also phytoplankton from bacteria. A drawback to this method is variable but significant bacterial retention on GF/F filters (Gasol and Morán 1999). An alternate method to distinguish phytoplankton from bacteria is to use flow cytometric sorting. In combination with ^{15}N tracer incubations, uptake rates can to be unambiguously aligned with the microorganisms responsible for them (Lipschultz 1995; Lomas et al. 2011). After staining the samples, the bacterial fraction further separates into low nucleic acid (LNA) and high nucleic acid (HNA) groups (Gasol and del Giorgio 2000; Li et al. 1995). These bacterial groups are present whenever bacteria are investigated on a flow cytometer, regardless of region or protocol (Bouvier et al. 2007). Despite their ubiquity, their reason for existence and role in the environment is the subject of fierce debate (e.g Morán et al. 2007). Chapter 3 presents results of ^{15}N uptake after sorting of LNA and HNA populations. This is the first time I am aware of that flow cytometric sorting of the LNA and HNA fractions have been used in combination with

^{15}N uptake. This work provides key insight into the activity of these bacterial subpopulations.

Climate change

Positive feedbacks within the Arctic are serving to accelerate the impacts of warming (Serreze and Francis 2006). Increases in air temperature are already leading to reduced volume and extent of sea ice, accelerated river discharge, rising sea level, thawing of permafrost, and coastal erosion (ACIA 2005; Anisimov et al. 2007). These physical changes in the Arctic are expected to have a profound effect on nutrients, temperature, and light attenuation, which are the fundamental drivers of microbial production. It is already known that changes in temperature and sea ice cover are impacting the timing and extent of community production in the coastal regions of the Arctic (Carmack and Wassmann 2006). What is not understood is how temperature increases will impact biogeochemical rate processes, although theory would predict that enzymatic processes would perform at higher rates in a warmer environment. Chapter 4 describes experiments undertaken to directly test the effect of temperature on rates of NH_4^+ uptake and nitrification of coastal pelagic Arctic microorganisms.

Sea ice

Climate change is causing rapid changes in the sea ice landscape, with the Arctic predicted to become seasonally ice free by 2080 or earlier (e.g. Boé et al. 2009; Stroeve et al. 2007). Sea ice plays an important role in the region. Primary production on the

underside of sea ice may seed spring bloom populations in the spring (Perrette et al. 2011). Sea ice algae are productive both at the ice-water interface and also within the sea-ice matrix (Mock and Gradinger 1999). They provide a significant proportion of primary production in the Arctic Ocean and form the foundation of the food web in the both the pelagic and benthic realms (Brown and Belt 2012a, b; Riedel et al. 2007; Tremblay et al. 1989). Freshly produced organic matter decomposition from the receding ice during spring is also an important mechanism for the supply of surface nutrients (Conover et al. 1999; Cota et al. 1990).

While the effects of nutrient concentrations and light limitation on primary production in sea ice have been studied, the actual rates of N uptake and regeneration have received relatively less focus. In Chapter 5 I directly address the dearth of information on N uptake in Arctic sea ice. I was able to capture information on multiple depths within the ice, where I quantified uptake of NH_4^+ and NO_3^- during January and April sampling trips. These processes are especially important in the highly productive coastal regions where first year landfast ice can support higher sympagic biomass (Gosselin et al. 1997).

Literature cited

Aagaard K, Carmack EC (1989) The role of sea ice and other fresh water in the Arctic circulation. J Geophys Res 94 (C10):14485-14498.

ACIA (2005) Arctic climate impact assessment. Cambridge University Press, New York

Anisimov OA, Vaughan DG, Callaghan TV, Furgal C, Marchant H, Prowse TD, Vilhjálmsson H, Walsh JE (2007) Polar regions (Arctic and Antarctic). Climate Change 2007: The Physical Science Basis. Contribution of Working Group II to

the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Bates NR, Best MHP, Hansell DA (2005) Spatio-temporal distribution of dissolved inorganic carbon and net community production in the Chukchi and Beaufort Seas. Deep-Sea Res Pt II 52 (24):3303-3323.

Boé J, Hall A, Qu X (2009) September sea-ice cover in the Arctic Ocean projected to vanish by 2100. Nature Geoscience 2 (5):341-343.

Bouvier T, del Giorgio PA, Gasol JM (2007) A comparative study of the cytometric characteristics of high and low nucleic-acid bacterioplankton cells from different aquatic ecosystems. Environ Microbiol 9 (8):2050-2066.

Brown TA, Belt ST (2012a) Closely linked sea ice-pelagic coupling in the Amundsen Gulf revealed by the sea ice diatom biomarker IP25. J Plankton Res 34 (8):647-654.

Brown TA, Belt ST (2012b) Identification of the sea ice diatom biomarker IP_{25} in Arctic benthic macrofauna: direct evidence for a sea ice diatom diet in Arctic heterotrophs. Polar Biol 35:131-137.

Carmack E, Chapman DC (2003) Wind-driven shelf/basin exchange on an Arctic shelf: The joint roles of ice cover extent and shelf-break bathymetry. Geophys Res Lett 30 (14):1778.

Carmack E, Wassmann P (2006) Food webs and physical–biological coupling on pan-Arctic shelves: Unifying concepts and comprehensive perspectives. Prog Oceanogr 71 (2-4):446-477.

Carmack EC (2007) The alpha/beta ocean distinction: A perspective on freshwater fluxes, convection, nutrients, and productivity in high-latitude seas. Deep-Sea Res Pt II 54 (23):2578-2598.

Codispoti LA, Flagg C, Kelly V, Swift JH (2005) Hydrographic conditions during the 2002 SBI process experiments. Deep-Sea Res Pt II 52 (24-26):3199-3226.

Codispoti LA, Flagg CN, Swift JH (2009) Hydrographic conditions during the 2004 SBI process experiments. Deep-Sea Res Pt II 56 (17):1144-1163.

Conover RJ, Mumm N, Bruecker P, MacKenzie S (1999) Sources of urea in arctic seas: seasonal fast ice? Mar Ecol Prog Ser 179:55-69.

Cota GF, Anning JL, Harris L, R., Harrison WG, Smith REH (1990) Impact of ice algae on inorganic nutrients in seawater and sea ice in Barrow Strait, NWT, Canada, during Spring. Can J Fish Aquat Sci 47 (7):1402-1415.

Daly KL, Wallace DWR, Smith Jr. WO, Skoog A, Lara R, Gosselin M, Falck E, Yager PL (1999) Non-Redfield carbon and nitrogen cycling in the Arctic: Effects of ecosystem structure and dynamics. J Geophys Res 104 (C2):3185-3199.

Dittmar T, Kattner G (2003) The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. Mar Chem 83 (3-4):103-120.

Dyda RY, Suzuki MT, Yoshinaga MY, Harvey HR (2009) The response of microbial communities to diverse organic matter sources in the Arctic Ocean. Deep-Sea Res Pt II 56 (17):1249-1263.

Garneau M-È, Gosselin M, Klein B, Tremblay J-É, Fouilland E (2007) New and regenerated production during a late summer bloom in an Arctic polynya. Mar Ecol Prog Ser 345:13-26.

Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina 64 (2):197-224.

Gasol JM, Morán XAG (1999) Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. Aquat Microb Ecol 16:251-264.

Gosselin M, Lavasseur M, Wheeler PA, Horner RA, Booth BC (1997) New measurements of phytoplankton and ice algal production in the Arctic Ocean. Deep-Sea Res Pt II 44 (8):1623-1644.

Harrison WG, Platt T, Irwin B (1982) Primary production and nutrient assimilation by natural phytoplankton populations of the eastern Canadian Arctic. Can J Fish Aquat Sci 39:335-345.

Kirchman DL, Morán XAG, Ducklow H (2009) Microbial growth in the polar oceans — role of temperature and potential impact of climate change. Nat Rev Microbiol 7 (6):451-459.

Kristiansen S, Farbrot T, Wheeler PA (1994) Nitrogen cycling in the Barents Sea--Seasonal dynamics of new and regenerated production in the marginal ice zone. Limnol Oceanogr 39 (7):1630-1642.

Lee SH, Joo HM, Liu Z, Chen J, He J (2012) Phytoplankton productivity in newly opened waters of the Western Arctic Ocean. Deep-Sea Res Pt II 81-84:18-27.

Lee SH, Whitledge TE, Kang S-H (2007) Recent carbon and nitrogen uptake rates of phytoplankton in Bering Strait and the Chukchi Sea. Cont Shelf Res 27 (17):2231-2249.

Li WKW, Jellett JF, Dickie PM (1995) DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnol Oceanogr 40 (8):1485-1495.

Lipschultz F (1995) Nitrogen-specific uptake rates of marine phytoplankton isolated from natural populations of particles by flow cytometry. Mar Ecol Prog Ser 123:245-258.

Lomas MW, Bronk DA, van den Engh G (2011) Use of flow cytometry to measure biogeochemical rates and processes in the ocean. Annu Rev Mar Sci 3:537-566.

McClelland JW, Holmes RM, Dunton KH, Macdonald RW (2012) The Arctic Ocean estuary. Estuaries Coasts 35:353-368.

Mock T, Gradinger R (1999) Determination of Arctic ice algal production with a new *in situ* incubation technique. Mar Ecol Prog Ser 177:15-26.

Morán XAG, Bode A, Suárez LÁn, Nogueira E (2007) Assessing the relevance of nucleic acid content as an indicator of marine bacterial activity. Aquat Microb Ecol 46 (2):141-152.

Perrette M, Yool A, Quartly GD, Popova EE (2011) Near-ubiquity of ice-edge blooms in the Arctic. Biogeosciences 8 (2):515-524.

Riedel A, Michel C, Gosselin M (2007) Grazing of large-sized bacteria by sea-ice heterotrophic protists on the Mackenzie Shelf during the winter–spring transition. Aquat Microb Ecol 50:25-38.

Roach AT, Aagaard K, Pease CH, Salo SA (1995) Direct measurements of transport and water properties through the Bering Strait. Journal of Geophysical Research 100 (C9):18443-18457.

Sakshaug E (2004) Primary and secondary production in the Arctic seas. In: Stein R, MacDonald RW (eds) The organic carbon cycle in the Arctic Ocean. Springer Berlin Heidelberg, pp 57-81

Serreze MC, Francis JA (2006) The Arctic amplification debate. Clim Change 76 (3-4):241-264.

Simpson KG, Tremblay J-É, Brugel S, Price NM (2013) Nutrient dynamics in the western Canadian Arctic. II. Estimates of new and regenerated production over the Mackenzie Shelf and Cape Bathurst Polynya. Mar Ecol Prog Ser 484:47-62.

Smith WO, Jr. (1993) Nitrogen uptake and new production in the Greenland Sea: the spring *Phaeocystis* bloom. J Geophys Res 98 (C3):4681-4688.

Stroeve J, Holland MM, Meier W, Scambos T, Serreze M (2007) Arctic sea ice decline: Faster than forecast. Geophys Res Lett 34 (9):L09501, doi:09510.01029/02007gl029703.

Tremblay C, Runge JA, Legendre L (1989) Grazing and sedimentation of ice algae during and immediately after a bloom at the ice-water interface. Mar Ecol Prog Ser 56:291-300.

Tremblay J-É, Michel C, Hobson KA, Gosselin M, Price NM (2006) Bloom dynamics in early opening waters of the Arctic Ocean. Limnol Oceanogr 51 (2):900-912.

Tremblay J-É, Simpson K, Martin J, Miller L, Gratton Y, Barber D, Price NM (2008) Vertical stability and the annual dynamics of nutrients and chlorophyll fluorescence in the coastal, southeast Beaufort Sea. J Geophys Res 113:C07S90, doi:10.1029/2007JC004547.

Walsh JJ, Dieterle DA, Maslowski W, Whitledge TE (2004) Decadal shifts in biophysical forcing of Arctic marine food webs: Numerical consequences. J Geophys Res 109:C05031, doi:05010.01029/02003JC001945.

Wickland KP, Aiken GR, Butler K, Dornblaser MM, Spencer RGM, Striegl RG (2012) Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. Global Biogeochem Cy 26:GB0E03,doi:10.1029/2012gb004342.

Chapter 2 – Seasonal nitrogen uptake and regeneration in the western coastal Arctic

Abstract

The western coastal Arctic is subject to extreme seasonal changes in light, temperature, and nutrient inputs, all of which influence microbial resource competition, and therefore the structure of food webs in the region. This study investigated the relative importance of different nitrogen (N) compounds to a coastal Arctic microbial community. Using a dual ^{15}N and ^{13}C approach, we measured size-fractionated (> 3 μm and 0.7 - 3.0 μm) uptake rates of ammonium (NH_4^+), nitrate (NO_3^-), urea, and amino acids, along with primary productivity in the near shore environment of the Chukchi Sea. We performed incubations on seawater samples collected near Barrow, Alaska, during January, April, and August for two consecutive years. The total dissolved N (TDN) pool was dominated by NO_3^- during the spring and winter (46-78% of TDN) but dropped to < 0.4 μmol N L^{-1} (2-6% of TDN) during the late summer. Of the substrates tested, amino acids supported the bulk of N and C nutrition in both size fractions during ice-covered periods (winter and spring); urea uptake was low during these periods. During summer, absolute uptake of NH_4^+ was highest of the substrates tested, closely followed by urea and amino acids. The Chukchi Sea community is more reliant on NH_4^+ than NO_3^-, and NO_3^- had the lowest relative preference index during any season. This is likely due to the availability of reduced N and the metabolic costs associated with reducing this oxidized form for use, although there is evidence of important late spring drawdown of NO_3^- that coincides with the phytoplankton bloom period. Ours is the first study to investigate the importance of amino acid-derived N to Arctic near shore microbial communities and to compare N-cycling across winter, spring, and summer conditions. Reduced N forms

provide easily assimilated N to the microbial community and cannot be ignored in studies of Arctic N uptake dynamics.

Introduction

Historically, low temperatures were thought to inhibit microbial activity, however, several studies have shown that the Arctic has an abundant and well adapted community of psychrotolerant and psychrophilic (i.e. cold-loving) microorganisms (Connelly et al. 2006; Hodges et al. 2005; Yager et al. 2001), with high overall production near the coast (Bates et al. 2005). Even though microorganisms dominate biomass and production, and are critical to understanding the overall functioning of the ecosystem, microbial processes in the Arctic are currently understudied (Dyda et al. 2009; Kirchman et al. 2009a; Sherr et al. 2009). In the western coastal Arctic, primary and secondary production are principally supported by N inputs from the Pacific Ocean through the Bering Strait, with N drawn down to limiting levels during the summer when primary productivity is highest (Codispoti et al. 2005; Ortega-Retuerta et al. 2012). In contrast, heterotrophic bacterial communities are believed to be C limited throughout the year if algal production does not result in a surplus supply of dissolved organic matter (Kirchman et al. 2009a; Kirchman et al. 2005). Spring supplies of riverine dissolved organic matter may also provide labile material (Letscher et al. 2011) that is likely important to both primary and secondary producers (Kirchman et al. 2009b; Wickland et al. 2012). As such, the availability, uptake, and remineralization of N and C are subject to intense competition by autotrophic and heterotrophic microorganisms (Fouilland et al. 2007). This balance between autotrophs and heterotrophs is a fundamental driver of important ecological parameters in the marine environment and has important implications for food web dynamics and C exchange with the atmosphere.

As the amount of open water in the Arctic increases and freshens, primary productivity, especially along the coasts, is expected to increase because of increased light penetration (Arrigo et al. 2008; Pabi et al. 2008). If nutrient limitation is not alleviated by increased upwelling on the shelves (Carmack and Chapman 2003) or increased delivery of labile terrigenous material, primary production would likely be limited by N supply, thereby reducing the ability of the Arctic Ocean to act as a sink of carbon dioxide (Cai et al. 2010). With the rapid changes observed and predicted in the Arctic, a thorough understanding of N uptake and regeneration is needed to determine how the system is currently structured, and how it may react to changes in nutrient supply and removal processes.

Recent investigations of microbial population dynamics and productivity in the Arctic have shown that heterotrophs remain active during winter, even when they are decoupled from phytoplankton activity (Alonso-Sáez et al. 2008; Garneau et al. 2008). The logistical difficulties of sampling in this remote environment have limited studies of the nutrient concentrations and concomitant uptake supporting growth, especially over a full seasonal cycle, although there are some notable exceptions in the Canadian Arctic (Martin et al. 2012; Simpson et al. 2013a; Simpson et al. 2008; Tremblay et al. 2008). With months of no direct sunlight, temperatures that remain well below freezing for much of the year, and an unstable sea-ice landscape subject to shifting currents and winds, the majority of studies investigating N uptake have focused on areas amenable to research vessels and in the summer only. This project sought to quantify nutrient

inventories and size-fractionated uptake rates of N and C during winter, spring, and summer in the coastal waters of the Chukchi Sea near Barrow, Alaska.

Methods

Field sample collection

Sampling was performed during three seasons over two successive years centered on 71°21' N, 156°41' W, which is approximately 2.5 km northwest of Barrow, Alaska (Figure 1; Table 1). To capture the extreme Arctic light and physical conditions, sampling took place during winter (January 26-30, 2011 and January 16-21, 2012), spring (April 22-25, 2010 and April 26-May 2, 2011) and summer (August 25-29, 2010 and August 15-20, 2011). During winter and spring the sample site was covered by landfast ice and during the summer the site was in open water. Each of the sampling efforts included two trips to the sampling site and the data collected were averaged.

Winter and spring sampling was accomplished by traveling to near the outer edge of the landfast ice by snow machine and then cutting through the ice with an 8" auger to sample the seawater below. Once we accessed the water column, sample collection was performed from a tent placed above the hole. During the summer, when ice was not present at the site, sampling was accomplished from a small boat. Photosynthetically active radiation (PAR) was measured using a LI-193 Spherical Quantum Sensor (LI-COR Biosciences, Lincoln, NE) mounted on an extending arm to measure light levels under the ice (winter and spring) and to avoid shading from the boat during the summer. Depth profiles of temperature, salinity, and oxygen saturation were measured using a hand-deployed water quality sonde (Eureka Environmental, Austin, TX). Water samples

for analysis of depth profiles of chlorophyll a (Chl *a*), bacterial abundance and a suite of ambient nutrients were collected using a hand-deployed 1 L Niskin bottle. Larger volumes necessary for studies of N uptake were collected with a low-pressure submersible electric pump (Johnson Pump model #16004) powered either by a portable generator or a 12V battery. Sampling depths were chosen that were below any fresh water inputs from surface ice melt and above any contaminating inputs from the benthos (see Table 1).

Laboratory incubations

Water was collected into a series of 2 L acid-washed PETG bottles. All samples were run in duplicate and were inoculated with additions of ^{15}N labeled ammonium chloride ($^{15}NH_4Cl$; 98.85% ^{15}N), potassium nitrate ($K^{15}NO_3$; 98%), dual-labeled ^{15}N- and ^{13}C-urea (98%), or ^{15}N and ^{13}C labeled algal amino acid mixture comprised of 16 amino acids (96-99%; Cambridge Isotope Laboratories, Andover, MA). Labeled bicarbonate ($H^{13}CO_3^-$; Cambridge Isotope Laboratories) was also added to the same bottles of $^{15}NH_4Cl$ and $K^{15}NO_3$ incubations. Literature values were used to estimate N tracer additions and alkalinity was used to derive ambient HCO_3^- concentrations. During winter and spring, additions were made of 0.09, 0.4, 0.04, and 0.04 μmol N L^{-1} for NH_4^+, NO_3^-, urea, and amino acids respectively. During summer, all incubations received 0.05 μmol N L^{-1} of the relevant substrate. These additions corresponded to atom percent enrichments of 11% for both NH_4^+ and NO_3^-, 22% for urea, 29% for the amino acid mixture, and 7.3% for HCO_3^- averaged across all seasons. The bottles were surrounded

by ambient seawater, placed in insulated coolers, and brought to the laboratory within one hour of collection to reduce temperature fluctuations. Separate bottles were filled in the same manner and used to track temperature at 60 second intervals with a HOBO TidbiT v2 water temperature data logger; fluctuations were limited to an overall average standard deviation of 0.31°C during all trips.

Samples were incubated for 24 hours at ambient light and temperature conditions in a temperature-controlled chamber. To mimic spectral attenuation and wavelength, light levels were maintained by fluorescent lights covered with GamColor blue films (GAM Products, Inc., Los Angeles, CA) and confirmed using the PAR sensor. At the termination of the incubations, the samples were filtered through 3.0 μm silver (Sterlitech Corporation) or Whatman GF/F (nominal pore size of 0.7 μm) filters. The filters were placed in cryovials and frozen until analysis. During this study, we measured bacterial abundance flow cytometrically before and after filtering through the GF/F filters, as they are known to capture bacteria as well as phytoplankton cells (Kirchman and Wheeler 1998). For analysis of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC), a 40 mL aliquot of filtrate was poured into acid-washed and muffled glass EPA vials and immediately frozen; the remaining filtrate was poured into polypropylene tubes and frozen until analysis of the remaining nutrients.

Sample analyses

Concentrations of Chl *a* were estimated fluorometrically after acetone extraction using a Turner Design Model 10-AU fluorometer (Welschmeyer 1994). Bacterial

abundance was determined from triplicate whole water fixed in the field with additions of paraformaldehyde at a final concentration of 0.2% w/v, kept for 15 minutes at 5°C to ensure complete fixation, and stored at -80°C until laboratory analysis. Each sample was run in duplicate on a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA) after staining with SYBR Green (Invitrogen) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7-2.2um), and then analyzed using FlowJo software (Treestar Inc., San Carlos, CA).

Concentrations of NH_4^+ were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Duplicates of nitrate (NO_3^-), nitrite (NO_2^-), silicate (Si), and phosphate (PO_4^{3-}) were measured on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Dissolved primary amines (DPA), used as a proxy for amino acids (Keil and Kirchman 1991), were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Measurements of TDN and DOC were made in triplicate by high temperature combustion on a Shimadzu TOC-V TNM (Sharp et al. 2002). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N (NH_4^+, NO_3^-, and NO_2^-) from TDN (Bronk et al. 2000); the errors for all the terms were propagated to provide standard error for DON. Urea was measured in duplicate using the manual monoxime method (Price and Harrison 1987). Isotopic measurements for ^{15}N and ^{13}C uptake rate samples were run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler. N uptake rates were calculated as per Dugdale and Goering (1967), and C uptake rates as per Hama et al. (1983). The uptake rates associated with the 0.7 – 3.0 μm size fraction were estimated by subtraction of the

>3.0 μm fraction from rates measured from whole water collected on GF/F filters (nominal pore size = 0.7 μm). At the end of the incubations, we isolated NH_4^+ and NO_3^- by solid phase extraction (Brzezinski 1987; Dudek et al. 1986) and the denitrifier method (Sigman et al. 2001) respectively. This allowed us to correct NH_4^+ uptake rates for isotope dilution (Glibert et al. 1982) and to calculate rates of NH_4^+ regeneration and nitrification. The isolation of NO_3^- also included NO_2^-. As a result, the nitrification rates calculated are for NH_4^+ and NO_2^- oxidation combined.

Statistics

Data analyses were conducted in the open-source statistical software program R, version 2.15.0 for Mac (R Core Team 2012). Mean rate estimates were compared using two-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Method at the 95% confidence level. Linear regressions were plotted in SigmaPlot version 12 .0 (Systat Software, San Jose, CA) and correlation coefficients (R values) ≥ 0.4 were considered significant. As described by McCarthy et al. (1977), the relative preference index (RPI) was calculated for each N substrate by size fraction to determine how the planktonic community responded to the different substrates during the different seasons.

Results

Physical conditions

Winter on the North Slope of Alaska is a time of near 24-hour darkness and the sampling site was fully ice covered, although the coastal landfast ice can be unstable until late winter. During January 2011, sampling took place the week after first sunrise (January 26 - 28). During this period there was less than four hours per day of direct sunlight with approximately two hours of twilight on either side of direct sun. In January 2012, sampling occurred the week prior to the first sunrise of the season (January 16-21). Both years, ice thickness was approximately 1.0 m (Table 1), and irradiance in the water column was < 0.30 μmol quanta^{-1} m^{-2} s^{-1}. The water column was well mixed, as indicated by the constant temperature and salinity in the water column depth profiles (data not shown). Air temperatures ranged from -50 to -10°C, and the water temperature was -1.8°C. Salinity over the depth of the water column varied depending on the day of sampling and had a lower mean in 2011 (range = 30.7 - 31.6) than in 2012 (range = 33.6 – 34.2). Dissolved oxygen saturation averaged 87 ± 8 % during winter sampling.

During the spring sampling expeditions, the site experienced over 20 h d^{-1} of full sun. Air temperatures were higher (-18 to -7°C), but remained below freezing. Sea ice thickness averaged 1.0 m. As observed during the winter, the water column appeared to be well mixed with respect to temperature and salinity. Water temperature averaged -1.7 ± 0.07 °C and salinity ranged from 31.9 – 32.3. At the sampling depth used for all rate measurements, PAR was approximately 5 μmol quanta^{-1} m^{-2} s^{-1} with the exception of our

final April trip in 2011, when the PAR doubled to 11 μmol quanta^{-1} m^{-2} s^{-1} because we sampled at a shallower depth (Table 1).

During the summer expeditions the sampling site experienced 17 – 19 hours d^{-1} of sunlight, and air temperatures ranged from +8 to +19°C. As in other seasons the water column was well mixed with respect to temperature and salinity during August of 2010, with water column temperatures at +6.1°C. In 2011, there was a steady decrease in temperature from the surface (+5.6°C) to the bottom (+3.3°C). Salinity was 29.5 in 2010 and 31.5 in 2011. At the depth of sampling for the N uptake rate studies PAR was over 44 μmol quanta^{-1} m^{-2} s^{-1}, an order of magnitude higher than observed in the spring. Dissolved oxygen was supersaturated during summer, with a mean of 102 ± 1.7 %.

Ambient biological abundance and nutrient concentrations

Biological inventories were low during winter. Mean Chl *a* was 0.02 ± 0.01 μg L^{-1} and bacterial cell counts averaged 2.9 ± 0.3 x10^{8} cells L^{-1} across both sampling efforts. Concentrations of NH_4^+ and NO_3^- were highest during winter (Table 2), with NO_3^- accounting for 46-78% of TDN. The winter of 2012 was the only season when NO_2^- was measurable, and even then was just barely above the detection limit (0.03 μmol N L^{-1}). Most of the DON (3.68 ± 0.89 μmol N L^{-1}) pool was not detected in the specific components that we measured; urea and DPA were only 8.5% of the total DON. With DOC concentrations of 79.2 ± 5.51 μmol C L^{-1} during the winter, the DOC:DON ratio was 22.5 (Figure 2), well above the stochiometric ratio for plankton (Redfield 1934).

Both PO_4^{3-} and Si concentrations were approximately double relative to summer (Table 2).

Springtime algal biomass increased to an average of 0.55 ± 0.61 μg Chl *a* L^{-1}, but was variable from year to year, with 2010 values (range = 0.64 – 1.38 μg Chl *a*) 6 - 23 times higher than 2011 (range = 0.06 – 0.11 μg Chl *a*; Table 1). Bacterial cell counts remained about the same as winter at 2.1 ± 0.1 $x10^8$ cells L^{-1}. Remaining high in spring, NO_3^- accounted for 50-68% of TDN (Table 2). Spring NH_4^+ concentrations were approximately half of winter concentrations. Urea and DPA concentrations remained low in the spring while DON concentrations increased slightly to 4.78 ± 0.54 μmol N L^{-1}. Concentrations of DOC remained approximately the same as in winter on average at 79.0 ± 16.1 μmol C L^{-1}, but were variable over the two years of sampling. The nutrients PO_4^{3-} and Si remained high in spring, although Si varied by over a factor of two from 2010 to 2011.

During summer, Chl *a* increased to 0.63 ± 0.16 μg L^{-1} and bacterial cell counts increased to 14.2 ± 13.0 $x10^8$ cells L^{-1}. Both NO_3^- and NH_4^+ concentrations were reduced compared to the prior season. Exhibiting the opposite pattern, DON was relatively higher during summer (6.59 ± 0.75 μmol N L^{-1}). Urea and DPA concentrations increased in absolute and relative (9.8% of total DON) terms, but remained below 0.5 μmol N L^{-1}. Concentrations of DOC also increased, to 92.3 ± 5.87 μmol C L^{-1}, but not as much relative to DON, such that the DOC:DON ratio fell to 14.1, which is still well above the canonical Redfield ratio, but almost half of the ratio found during winter. Concentrations of PO_4 dropped during summer to ~47% of concentrations present during the ice-covered

seasons. Similarly, Si was even lower relative to other seasons, drawn down to only 25% of winter and spring levels.

Uptake rates

Our check on the size fractionation technique revealed that some bacteria were retained on the GF/F filters, but the percentages of bacterial cells retained differed depending on the season. During summer, 61% of the bacterial cells were retained, and even more (74%) during spring, while winter apparently shifted to smaller cells or less aggregates and the GF/F filters only retained 36% of the bacterial cells. Thus, our N and C uptake rates include variable but incomplete contributions from heterotrophic bacteria. During September in the surface waters of a Baffin Bay polynya, Fouilland et al. (2007) calculated that GF/F filters captured 80% of the total living bacterial community, which roughly aligns with our results for August.

Low biomass in the winter samples resulted in the lowest absolute N uptake rates (Figure 3). Within the winter sampling season, there was no significant difference ($p > 0.05$) between the >3 μm and 0.7-3.0 μm uptake rates of NH_4^+ (Table 3). Even though NO_3^- concentrations were high, total uptake was lower than NH_4^+, and the 0.7-3.0 μm fraction had higher rates than the larger (>3.0 μm) fraction. Urea uptake rates were lower than the other substrates, even though ambient urea concentrations remained essentially unchanged throughout the year. Rates of amino acid derived N (AA_N) uptake were highest of all the substrates measured in both size fractions; again the uptake rates in the smaller size fraction was higher than those in the larger size fraction. The amino acid

mixture also appears to provide energy in the form of C for the smaller size fraction (Figure 4). During the winter season, uptake of C from the amino acid mixture (AA_C) was highly variable between the two years (Table 3). Uptake of C from urea ($Urea_C$) and HCO_3^- uptake was not detected in any of the winter samples.

All of the spring rates were approximately double the winter rates, and exhibited a similar relative pattern to winter. For all of the substrates, there were no statistically significant differences ($p > 0.05$) in the rates between the size fractions (Figure 3). During the spring, NH_4^+ continued to be an important resource, for both the small and large size fractions. As in the winter, even though NO_3^- and urea concentrations were high, uptake rates were still < 0.01 nmol N L^{-1} h^{-1}. Amino acids remained the preferred form of N for uptake, especially for the smaller size fraction that exhibited AA_N uptake rates approximately three times higher than the larger size fraction. Uptake of $Urea_C$ was not detected (Figure 4) and uptake rates of HCO_3^- were not significantly different ($p > 0.05$) between the size fractions. Absolute uptake of AA_C and HCO_3^- were approximately equal in the larger size fraction (>3.0 μm). The highest C uptake was from the smaller size fraction AA_C, although it was highly variable both within a season and from year to year (Table 3).

During summer, absolute uptake rates for all substrates except AA_N were more than an order of magnitude higher than the other two seasons, with NH_4^+ utilization being the highest relative to other substrates (Figure 3). Unlike the ice covered winter and spring seasons, the larger size fraction had higher uptake rates. The uptake of NH_4^+ dominated both the smaller and larger size fractions. The >3.0 μm size fraction jumped

two orders of magnitude for NO_3^- uptake and the 0.7-3.0 μm fraction also increased by the same magnitude, but only during 2011. During 2012, there was no difference between the spring and summer NO_3^- uptake rates for the smaller size fraction. Rates of urea uptake also increased by more than an order of magnitude, and had the second highest rates beside NH_4^+ uptake. Compared to spring AA_C uptake doubled in both size fractions and remained an important N source, but did not have the same relative seasonal increase as the other substrates. Uptake of AA_C remained the highest of the C sources provided. Uptake of HCO_3^- increased by an order of magnitude for both size fractions. $Urea_C$ utilization was only detected during the summer 2010 sampling (Table 3), making the seasonal average highly variable.

It was possible to use the calculated RPI to define the hierarchical preference of various substrates relative to their availability (McCarthy et al. 1977). For both winter and spring, the RPI of AA_N was more than an order of magnitude higher than the other N substrates (Table 4). While this method is known to be sensitive to nutrient concentration (Stolte and Riegman 1996), DPA concentrations remain low year-round, and cannot explain the seasonal difference. The RPI also shows that urea was typically preferred over NO_3^- when it was available.

Nutrient Regeneration

Rates of NH_4^+ regeneration averaged 13.4 ± 7.4 nmol N L^{-1} h^{-1} for all samples, but with large interannual variability during the spring (Table 5). Nitrification was high during the winter and spring (mean of 21.7 ± 10.9 nmol N L^{-1} h^{-1}), but dropped more than

an order of magnitude during summer. During this study, NO_3^- concentrations were high during winter and spring, and no regeneration of NO_3^- was detected.

Discussion

Most Arctic marine N cycling studies have focused on dissolved inorganic N (NH_4^+, NO_3^-) with limited investigations of the role of DON sources such as urea and amino acids. Additionally, studies have generally been conducted in offshore waters and during the summer (Table 6). This is the first study we are aware of that provides comprehensive N uptake rates in Arctic near shore waters during winter, spring, and summer, each of which present a unique set of environmental conditions. In addition to variations in seasonal light and ice coverage, episodic advection can change the nutrient regime rapidly on the coastal shelves (Carmack and Chapman 2003). This leads to high regional (Codispoti et al. 1991) and interannual (Kirchman et al. 2009a) variability in community production, along with dynamic winter to summer changes in community composition (Alonso-Sáez et al. 2008). Corresponding to changes in the physical and biological realms, it would be expected that nutrient inventories are also highly dynamic. Biogeochemical cycles both drive biological variables and respond to changes in the system over the yearly cycle. The seasonal sampling allowed us to quantify N under conditions at the extremes of light and temperature present in the region, as the confounding factors of temperature, irradiance, and nutrient concentrations have historically eluded straightforward description (Smith and Harrison 1991).

Seasonal trends

By January, TDN accumulated to seasonal highs. Although NO_3^- was the most abundant N pool, NO_3^- uptake rates were consistently lower than that of NH_4^+ and AA_N. This is likely due to the metabolic cost needed to reduce NO_3^- to NH_4^+ intracellularly. Amino acids accounted for the majority of uptake for both N and C of the substrates tested, indicating that microbial activity was primarily supported by DON sources during the winter. Interestingly, there was evidence of the larger size fraction also taking up N, but there is no concomitant evidence of HCO_3^- uptake. Given that there was little to no light available, it is not surprising that the smaller size fraction is active, albeit at reduced rates. What is surprising is that the larger size fraction, which presumably consists of large phytoplankton, took up amino acids at all. There have been reports of mixotrophy in this system (Cottrell and Kirchman 2009; Seuthe et al. 2011), and there is mounting evidence that phytoplankton can effectively utilize DON (e.g. Berman and Bronk 2003; Bronk et al. 2007). Alternatively, it could be that particle-attached bacteria and other microheterotrophs (e.g. microflagellates, small ciliates) were collected on the 3.0 μm filters.

Although surface irradiance increased dramatically in April, ice thickness continued to grow toward its yearly apex. The water column community remained light limited, and NO_3^- uptake was low despite near seasonally high NO_3^- concentrations. In fact, NO_3^- utilization was low year round, but especially so during winter and spring. This observation is consistent with earlier work that found NO_3^- uptake to be more sensitive to low temperatures than NH_4^+ (Reay et al. 1999). Our winter and spring NO_3^-

uptake rates are similar to the only other study of N uptake in under-ice plankton of this region, where NO_3^- uptake rates ranged from 0.02 – 0.39 nmol N L^{-1} h^{-1} (Lee et al. 2010; Table 6). Though still cold in spring with relatively low PAR, winter to spring Chl *a* increased by a factor of 23, and rates of NH_4^+ and AA_N uptake increased by over a factor of two overall. These increases in rates occurred despite little difference in the ambient nutrient or temperature regimes from winter to spring, implying light as the critical limiting factor during winter and spring in this system (Terrado et al. 2008; Tremblay et al. 2006).

As Garneau et al. (2007) showed, the changes in uptake rates between ice-free and ice-covered conditions can happen quickly and the difference can be large (Table 6). When light limitation imposed by the sea ice was completely lifted in the summer, the biological community responded with increased biomass and broad usage of all the N substrates measured (NH_4^+, NO_3^-, urea, and AA_N). Ambient DIN was drawn down below 1.0 μmol N L^{-1}, while DON rose in concentration to over 6.0 μmol N L^{-1} (Figure 2). This rapid drawdown of DIN and production of DON, coupled with increases in Chl *a* and NO_3^- uptake rates (Table 3) all point to high levels of productivity over the summer. Uptake rates of all N substrates except AA_N increased by an order of magnitude during the summer. As shown by the RPI results (Table 4), rather than AA_N being the dominant preferred substrate, all of the substrates are utilized to a similar degree during August. The phytoplankton community was finally able to utilize the available light for photosynthesis and incorporate N from NO_3^-, as the cost of reduction may be less than costs of competition for scarce amino acid compounds. In a study spanning spring to late

fall in the Canadian Arctic, Martin et al. (2012) found higher dependence on NO_3^- (relative to NH_4^+) only during early spring. With time-series sampling in the Amundsen Gulf (Canadian Arctic), Simpson et al. (2013a) found that indeed absolute uptake rates of NO_3^- were only greater than NH_4^+ and urea uptake during a bloom (Table 6). Our sampling bracketed the water column spring bloom period. Given the low concentrations of NO_3^- (<0.4 μmol N L^{-1}) that we found in August, it is clear that the period of most intense NO_3^- drawdown and high Chl *a* was over (e.g. Yager et al. 2001) by the time our sampling began. We did not correct our NO_3^- uptake rates for isotope dilution, as effects are generally negligible when ambient concentrations are high (as it was during January and April), although the effect of isotope dilution on NO_3^- uptake is not well understood because there are far fewer measurements available (Harrison et al. 1985).

During late August of both study years, Chl *a* concentrations were relatively low (<1.0 μg/L; Table 2) compared to temperate regions, but there was a 52 – 81% reduction in the concentration of Si from April to August (Figure 2), indicating the presence of diatoms. In addition, as light limitation was lifted by the seasonal cycle and retreat of sea ice, C uptake from AA_C increased by a factor of seven while HCO_3^- uptake increased by a factor of 60 from winter/spring to summer. It should be noted that both size fractions took up HCO_3^- in summer. This could indicate that there are small chemoautotrophs or photoautotrophs (Worden and Not 2008) passing through the 3.0 μm filters. Another potential source of C could be $Urea_C$, but uptake rates from this study are relatively low, which is consistent with the results of Harrison et al. (1985) who found that $Urea_C$ assimilation was highest at more northerly sites (> 77° N) in their study of Baffin Bay.

The coastal area of the Chukchi Sea is highly productive (Bates et al. 2005), with high relative uptake rates of N to support primary and secondary production. During our August sampling, we measured total absolute N uptake (i.e. uptake of NH_4^+, NO_3^-, and urea combined) higher than those found in almost any other N uptake study in the western Arctic (Table 6). Additionally, the general trend of studies from other regions of the western Arctic is that NO_3^- uptake rates are generally equal to or greater than NH_4^+ uptake, with more importance ascribed to "new" as opposed to "regenerated" production (cf. Dugdale and Goering 1967). This study and others in the region (Lee et al. 2012; Lee et al. 2007) are the only consistent results from the western Arctic that show a preference for NH_4^+ over NO_3^- (Table 6). This trend continues during the January and April sampling periods. It is therefore unlikely that it is simply a matter of substrate availability, but rather an indication of a fundamental difference in the metabolic strategies of the plankton community in the coastal Chukchi Sea.

The importance of DON

Many of the earliest studies of N uptake in the Arctic took place during the summer in the large polynyas surrounding Greenland and, as stated above, found that NH_4^+ and NO_3^- uptake were of similar magnitude (Table 6). When measured, urea uptake was generally a small component (~15% of the total uptake) in those regions. This study is the only one we are aware of in this region that measured urea uptake, and found that it accounted for 27% of the uptake (not including AA_N, for a direct comparison to the other studies). The uptake rates reported here are on the upper end of the range reported

by others despite the use of tracer additions. However, Martin et al. (2012) in their studies explicitly investigated the significance of saturating additions with inorganic N in Arctic waters and reported them to be minimal. The relatively high N uptake rates observed in this study may be explained by being conducted in coastal rather than open ocean waters. Coastal systems are known to be highly productive, especially when compared to pelagic systems that are further removed from allochthonous nutrient sources (e.g. rivers, Bering Strait).

In the other studies of the western Arctic, RPIs have indicated low preference for urea even when uptake rates are high (Harrison et al. 1985), although urea concentrations are highly variable throughout the region (Simpson et al. 2008). At this study site in the western Arctic, the community present also appears to favor NH_4^+ and AA_N utilization (Table 4). Only during the summer, when zooplankton and migrating seabirds are a likely localized source of urea via excretion and sloppy feeding (Conover and Gustavson 1999), does the microbial community seem to respond. However, the source is not as important as overall N concentrations, competition for them, and the need to utilize a range of substrates. In fact, urea concentrations are also fairly high during the spring, most likely due to release from landfast ice (Conover et al. 1999), but uptake rates remain low.

During both the winter and spring AA_N was the most preferred form of N (Table 4). This is consistent with previous reports from the Arctic that demonstrated that labile dissolved organic matter constituents including amino acids are an important nutrient resource for prokaryotes (Alonso-Sáez et al. 2008; Kirchman et al. 2007; Nikrad et al.

2012; Rich et al. 1997; Yager and Deming 1999). In marine systems generally, amino acids can support up to 40% of bacterial production (Kirchman 2000) and prokaryotes can assimilate them even at nanomolar concentrations (Cottrell and Kirchman 2000; Ouverney and Fuhrman 2000). Pelagic microorganisms in the Northeast Water polynya (Greenland) had high specific affinities for amino acid mixtures even at subzero temperatures (Yager and Deming 1999). In this study, AA_N uptake was observed year-round, with decreased importance relative to other N and C sources in the summer. During the winter of 2012, the molar C:N uptake ratio of the amino acid mixture was 10.5 ± 5.4 for the whole community, but for all other sampling trips was 2.6 ± 1.0. The mixture added had an initial C:N of 3.7, highlighting the use of the amino acid mixture as a rich N source that the microbial community is able to use efficiently. The fact that AA_N was being taken up by both larger (>3.0 μm) and smaller (0.7 – 3.0 μm) cells lends further credence to the importance of DON as a nutrient source for phytoplankton as well as bacteria, along with potential uptake by microflagellates and ciliates (Bronk et al. 2007; Sanderson et al. 2008).

In the future, the components and lability of Arctic DON is expected to change as more terrigenous material is transported from the tundra to the coastal ocean (Sipler et al. in prep.; Tait et al. submitted). It is therefore important to understand how the concentration of DON components like urea and amino acids are changing over time, as that will have a large impact on the processing of this material. In the Arctic, rivers currently deliver high concentrations of labile organic nutrients (e.g. Dittmar and Kattner 2003; Holmes et al. 2008; McClelland et al. 2012). DON tends to be rapidly consumed

in the marine receiving waters, but can also be locally produced in the Chukchi Sea (Letscher et al. 2013). In this study, urea and AA_N were only a small percentage (<10%) of the overall DON pool, but proved to have high rates of uptake compared to inorganic N. Marine DON consists of a complex mixture of compounds that remain uncharacterized, but urea and amino acids are highly labile fractions (Bronk 2002). Our results highlight the importance of the quality of the DON pool, not just the quantity, when trying to assess the complex overall dynamics of DON production and consumption in the Arctic.

The importance of regeneration

Regeneration of N sources can imply tight coupling within a system. In the summer, when NH_4^+ resources are scarce, we measured relatively high regeneration rates (Table 5), but the relationship between NH_4^+ concentrations and regeneration rates is highly equivocal, with no significant correlation (Figure 5; $R^2 = 0.21$) between them. While the overall trend appears to be positive, regeneration rates during the summer seem to generally confound this trend, such that even though ambient concentrations are low, regeneration rates are relatively high, indicating a tightly coupled system during summer that helps to keep NH_4^+ concentrations low (Clark et al. 2008).

In addition to regeneration, nitrification could oxidize a significant portion of NH_4^+ to NO_3^-, especially during the ice-covered seasons (Christman et al. 2011, Veuger et al. 2013). In the Southern Ocean, Bianchi et al. (1997) measured spring nitrification rates of 1.7 – 3.5 nmol L^{-1} h^{-1}. Our late summer rates (mean = 1.02 ± 0.32 nmol L^{-1} h^{-1};

Table 5) were below that range, but during winter and spring we measured nitrification rates more than 20 times higher than summer, with a mean of 21.7 ± 10.9 nmol L^{-1} h^{-1}. This seasonal disparity aligns with recent work in the same region, where Christman et al. (2011) measured mean nitrification rates of 3.6 and 89.2 nmol L^{-1} h^{-1} during summer and winter respectively. During winter and early spring, Simpson et al. (2013b) calculated a mean nitrification rate of 317 ± 7.2 nmol L^{-1} h^{-1} in the Beaufort Sea. In the seasonally ice covered North Sea (Netherlands), Veuger et al. (2013) measured mean nitrification rates of 93.8 ± 54.1 (range = 43 – 221) nmol L^{-1} h^{-1} during that same time period. It is clear that nitrification is very active during winter relative to summer and could explain the high NO_3^- inventories found generally in the Arctic before the spring bloom period (Tremblay et al. 2008). In addition to the seasonal dynamics in light and temperature that can influence nitrification rates, there is some evidence that the process is sensitive to NH_4^+ concentrations (Ward 2008). In this study nitrification rates were significantly correlated with ambient NH_4^+ concentrations (Figure 5; $R^2 = 0.54$; $p = 0.0005$), in contrast to urea concentrations ($R^2 = 0.18$; $p > 0.05$) which have been proposed as an important source for nitrification in the polar regions (Alonso-Sáez et al. 2012). Nitrification dominates during the low-light seasons, and the NH_4^+ assimilation during summer (Veuger et al. 2013), which is likely a result of light inhibition of nitrification (Ward 2008). Additionally, the low concentration of NH_4^+ during summer could result in phytoplankton and bacteria effectively outcompeting nitrifiers for substrate.

Conclusions

The western coastal Arctic is shifting to a regime that is increasingly reliant on pelagic processes (Grebmeier et al. 2006). The contribution of the sea ice community to the ecology of the region is sure to decline along with the sea ice habitat. At least in the short term, pelagic phytoplankton will respond positively to the earlier light penetration through the ice (Arrigo et al. 2012; Lee et al. 2011) and open water conditions (Arrigo et al. 2008). In the future, the Arctic Ocean is generally expected to be heavily influenced by increased thermal stratification (Peterson et al. 2006). However, the near shore site occupied by this study was never thermally stratified, likely due to strong currents, and therefore the physical forcing mechanisms in the shallow coastal areas may respond to other physical processes or not at all. Our work here highlights instead the unique characteristics of the near shore environment of the Chukchi Sea. While there is likely a short-term pulse of NO_3^- uptake that corresponds with the spring bloom period (e.g. Martin et al. 2012), the remainder of the year is heavily dependent on NH_4^+ and dissolved organic matter sources. This highly regenerative system will likely limit benthic coupling and the ability of the coastal region to act as a C sink. By focusing on offshore locations during summer, other studies have not been able to elucidate the unique conditions and activity of the near shore environment and therefore may misrepresent the biogeochemical dynamics of the Chukchi Sea and the Arctic Ocean as a whole. It will be critical to incorporate seasonal uptake rates of N and certain C containing compounds in the western Arctic shallow coastal zone, which display different importance of N compounds than previously studied areas in the Arctic.

Our research indicates that there is a robust planktonic microbial community during winter that has access to large pools of ambient N, but maintains low levels of assimilation until the lifting of light limitation in the summer. The microbial community is able to respond to nutrient availability and utilize multiple forms of both N and C for low levels of growth and maintenance during the ice-covered seasons. We have demonstrated that specific DON components play a large role in the N cycling processes of the coastal western Chukchi Sea. It will be important to understand how future changes in DON delivery and the reduction of sea ice will interplay to drive the productivity of this system.

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Literature cited

Alonso-Sáez, L., O. Sánchez, J. M. Gasol, V. Balagué, and C. Pedrós-Alio. 2008. Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. Environ. Microbiol. **10:** 2444-2454.

Alonso-Sáez, L. and others 2012. Role for urea in nitrification by polar marine Archaea. P. Natl. Acad. Sci. USA **109:** 17989-17994.

Arrigo, K. R. and others 2012. Massive phytoplankton blooms under Arctic sea ice. Science **336:** 1408.

Arrigo, K. R., G. van Dijken, and S. Pabi. 2008. Impact of a shrinking Arctic ice cover on marine primary production. Geophys. Res. Lett. **35:** L19603, doi:19610.11029/12008GL035028.

Bates, N. R., M. H. P. Best, and D. A. Hansell. 2005. Spatio-temporal distribution of dissolved inorganic carbon and net community production in the Chukchi and Beaufort Seas. Deep-Sea Res. Pt II **52:** 3303-3323.

Berman, T., and D. A. Bronk. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat. Microb. Ecol. **31:** 279-305.

Bianchi, M., F. Feliatra, P. Tréuger, M.-A. Vincendeau, and J. Morvan. 1997. Nitrification rates, ammonium and nitrate distribution in upper layers of the water column and in sediments of the Indian sector of the Southern Ocean. Deep-Sea Res. Pt II **44:** 1017-1032.

Bronk, D. A. 2002. Dynamics of DON, p. 153-247. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic Press.

Bronk, D. A., M. W. Lomas, P. M. Glibert, K. J. Schukert, and M. P. Sanderson. 2000. Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. Mar. Chem. **69:** 163-178.

Bronk, D. A., J. H. See, P. Bradley, and L. Killberg. 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences **4:** 283-296.

Brzezinski, M. A. 1987. Colorimetric determination of nanomolar concentrations of ammonium in seawater using solvent extraction. Mar. Chem. **20:** 277-288.

Cai, W.-J. and others 2010. Decrease in the CO_2 uptake capacity in an ice-free Arctic Ocean basin. Science **329:** 556-559.

Carmack, E., and D. C. Chapman. 2003. Wind-driven shelf/basin exchange on an Arctic shelf: The joint roles of ice cover extent and shelf-break bathymetry. Geophys. Res. Lett. **30:** 1778.

Christman, G. D., M. T. Cottrell, B. N. Popp, E. Gier, and D. L. Kirchman. 2011. Abundance, diversity, and activity of ammonia-oxidizing prokaryotes in the coastal Arctic Ocean in summer and winter. Appl. Environ. Microbiol. **77:** 2026-2034.

Clark, D. R., A. P. Rees, and I. Joint. 2008. Ammonium regeneration and nitrification rates in the oligotrophic Atlantic Ocean: Implications for new production estimates Limnol. Oceanogr. **53:** 52-62.

Codispoti, L. A., C. Flagg, V. Kelly, and J. H. Swift. 2005. Hydrographic conditions during the 2002 SBI process experiments. Deep-Sea Res. Pt II **52:** 3199-3226.

Codispoti, L. A., G. E. Friederich, C. M. Sakamoto, and L. I. Gordon. 1991. Nutrient cycling and primary production in the marine systems of the Arctic and Antarctic. J. Mar. Syst. **2:** 359-384.

Connelly, T. L., C. M. Tilburg, and P. L. Yager. 2006. Evidence for psychrophiles outnumbering psychrotolerant marine bacteria in the springtime coastal Arctic. Limnol. Oceanogr. **51:** 1205-1210.

Conover, R. J., and K. R. Gustavson. 1999. Sources of urea in arctic seas: zooplankton metabolism. Mar. Ecol. Prog. Ser. **179:** 41-54.

Conover, R. J., N. Mumm, P. Bruecker, and S. MacKenzie. 1999. Sources of urea in arctic seas: seasonal fast ice? Mar. Ecol. Prog. Ser. **179:** 55-69.

Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. **66:** 1692-1697.

Daly, K. L. and others 1999. Non-Redfield carbon and nitrogen cycling in the Arctic: Effects of ecosystem structure and dynamics. J. Geophys. Res. **104:** 3185-3199.

Dittmar, T., and G. Kattner. 2003. The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. Mar. Chem. **83:** 103-120.

Dudek, N., M. A. Brzezinski, and P. A. Wheeler. 1986. Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments. Mar. Chem. **18:** 59-69.

Dugdale, R. C., and J. J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. **12:** 196-206.

Dyda, R. Y., M. T. Suzuki, M. Y. Yoshinaga, and H. R. Harvey. 2009. The response of microbial communities to diverse organic matter sources in the Arctic Ocean. Deep Sea Research Part II: Topical Studies in Oceanography **56:** 1249–1263.

Fouilland, E., M. Gosselin, R. B. Rivkin, C. Vasseur, and B. Mostajir. 2007. Nitrogen uptake by heterotrophic bacteria and phytoplankton in Arctic surface waters. J. Plankton Res. **29:** 369-376.

Garneau, M.-È., M. Gosselin, B. Klein, J.-É. Tremblay, and E. Fouilland. 2007. New and regenerated production during a late summer bloom in an Arctic polynya. Mar. Ecol. Prog. Ser. **345:** 13-26.

Garneau, M.-È., S. Roy, C. Lovejoy, Y. Gratton, and W. F. Vincent. 2008. Seasonal dynamics of bacterial biomass and production in a coastal arctic ecosystem: Franklin Bay, western Canadian Arctic. J. Geophys. Res. **113:** C07S91, doi:10.1029/2007jc004281.

Glibert, P. M., F. Lispschultz, J. J. McCarthy, and M. A. Altabet. 1982. Isotope dilution models of uptake ammonium by marine plankton. Limnol. Oceanogr. **27:** 639-650.

Grebmeier, J. M., L. W. Cooper, H. M. Feder, and B. I. Sirenko. 2006. Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. Prog. Oceanogr. **71:** 331-361.

Guerrero, M. A., and R. D. Jones. 1996. Photoinhibition of marine nitrifying bacteria I. Wavelength-dependent response. Mar. Ecol. Prog. Ser. **141:** 183-192.

Hama, T. and others 1983. Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. Mar. Biol. **73:** 31-36.

Harrison, W. G., E. J. H. Head, R. J. Conover, A. R. Longhurst, and D. D. Sameoto. 1985. The distribution and metabolism of urea in the eastern Canadian Arctic. Deep Sea Research Part A: Oceanographic Research Papers **32:** 23-42.

Harrison, W. G., T. Platt, and B. Irwin. 1982. Primary production and nutrient assimilation by natural phytoplankton populations of the eastern Canadian Arctic. Can. J. Fish. Aquat. Sci. **39:** 335-345.

Hodges, L. R., N. Bano, J. T. Hollibaugh, and P. L. Yager. 2005. Illustrating the importance of particulate organic matter to pelagic microbial abundance and community structure—an Arctic case study. Aquat. Microb. Ecol. **40:** 217-227.

Holmes, R. M., J. W. McClelland, P. A. Raymond, B. B. Frazer, B. J. Peterson, and M. Stieglitz. 2008. Lability of DOC transported by Alaskan rivers to the Arctic Ocean. Geophys. Res. Lett. **35:** L03402, doi:03410.01029/02007GL032837.

Keene, N. K., W. O. Smith Jr., and G. Kattner. 1991. Nitrogen uptake in two frontal areas in the Greenland Sea. Polar Biol. **11:** 219-225.

Keil, R. G., and D. L. Kirchman. 1991. Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. **71:** 1-10.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria, p. 261-288. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley-Liss.

Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2007. Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol. Oceanogr. **52:** 495-507.

Kirchman, D. L., V. Hill, M. T. Cottrell, R. Gradinger, R. R. Malmstrom, and A. Parker. 2009a. Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. Deep-Sea Res. Pt II **56:** 1237-1248.

Kirchman, D. L., R. R. Malmstrom, and M. T. Cottrell. 2005. Control of bacterial growth by temperature and organic matter in the Western Arctic. Deep-Sea Res. Pt II **52:** 3386-3395.

Kirchman, D. L., X. A. G. Morán, and H. Ducklow. 2009b. Microbial growth in the polar oceans — role of temperature and potential impact of climate change. Nat. Rev. Microbiol. **7:** 451-459.

Kirchman, D. L., and P. A. Wheeler. 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific. Deep-Sea Res. Pt. I **45:** 347-365.

Koroleff, F. 1983. Determination of nutrients, p. 125-187. *In* K. Grasshoff, M. Ehrhardt and K. Kremling [eds.], Methods of seawater analysis. Verlag Chemie.

Kristiansen, S., T. Farbrot, and P. A. Wheeler. 1994. Nitrogen cycling in the Barents Sea--Seasonal dynamics of new and regenerated production in the marginal ice zone. Limnol. Oceanogr. **39:** 1630-1642.

Kristiansen, S., and B. A. Lund. 1989. Nitrogen cycling in the Barents Sea -- I. Uptake of nitrogen in the water column. Deep-Sea Research **36:** 255-268.

Lee, S. H., H. M. Joo, Z. Liu, J. Chen, and J. He. 2012. Phytoplankton productivity in newly opened waters of the Western Arctic Ocean. Deep-Sea Res. Pt II **81-84:** 18-27.

Lee, S. H. and others 2011. Holes in progressively thinning Arctic sea ice lead to new ice algae habitat. Oceanography **24:** 302-308.

Lee, S. H., D. Stockwell, and T. E. Whitledge. 2010. Uptake rates of dissolved inorganic carbon and nitrogen by under-ice phytoplankton in the Canada Basin in summer 2005. Polar Biol. **33:** 1027-1036.

Lee, S. H., and T. E. Whitledge. 2005. Primary and new production in the deep Canada Basin during summer 2002. Polar Biol. **28:** 190-197.

Lee, S. H., T. E. Whitledge, and S.-H. Kang. 2007. Recent carbon and nitrogen uptake rates of phytoplankton in Bering Strait and the Chukchi Sea. Cont. Shelf Res. **27:** 2231-2249.

Letscher, R. T., D. A. Hansell, and D. Kadko. 2011. Rapid removal of terrigenous dissolved organic carbon over the Eurasian shelves of the Arctic Ocean. Mar. Chem. **123:** 78-87.

Letscher, R. T., D. A. Hansell, D. Kadko, and N. R. Bates. 2013. Dissolved organic nitrogen dynamics in the Arctic Ocean. Mar. Chem. **148:** 1-9.

Martin, J., J.-É. Tremblay, and N. M. Price. 2012. Nutritive and photosynthetic ecology of subsurface chlorophyll maxima in the Canadian Arctic waters. Biogeosciences **9:** 5353-5371.

McCarthy, J. J., W. R. Taylor, and J. L. Taft. 1977. Nitrogenous nutrition of plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. **22:** 996-1011.

McClelland, J. W., R. M. Holmes, K. H. Dunton, and R. W. Macdonald. 2012. The Arctic Ocean estuary. Estuaries Coasts **35:** 353-368.

Nikrad, M. P., M. T. Cottrell, and D. L. Kirchman. 2012. Abundance and single-cell activity of heterotrophic bacterial groups in the western Arctic Ocean in summer and winter. Appl. Environ. Microbiol. **78:** 2402-2409.

Ortega-Retuerta, E., W. H. Jeffrey, J. F. Ghiglione, and F. Joux. 2012. Evidence of heterotrophic prokaryotic activity limitation by nitrogen in the Western Arctic Ocean during summer. Polar Biol. **35:** 785-794.

Ouverney, C. C., and J. A. Fuhrman. 2000. Marine planktonic Archaea take up amino acids. Appl. Environ. Microbiol. **66:** 4829-4833.

Pabi, S., G. L. van Dijken, and K. R. Arrigo. 2008. Primary production in the Arctic Ocean, 1998-2006. J. Geophys. Res. **113:** C08005, doi:08010.01029/02007jc004578.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.

Peterson, B. J., J. McClelland, R. Curry, R. M. Holmes, J. E. Walsh, and K. Aagaard. 2006. Trajectory shifts in the Arctic and subarctic freshwater cycle. Science **313:** 1061-1066.

Price, N. M., and P. J. Harrison. 1987. Comparison of methods for the analysis of dissolved urea in seawater. Mar. Biol. **94:** 307-317.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Reay, D. S., D. B. Nedwell, J. Priddle, and J. C. Ellis-Evans. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl. Environ. Microbiol. **65:** 2577-2584.

Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton, p. 177-192. *In* R. J. Daniel [ed.], James Johnstone Memorial Volumes. University Press of Liverpool.

Rich, J., M. Gosselin, E. Sherr, B. Sherr, and D. L. Kirchman. 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. Deep-Sea Res. Pt II **44:** 1645-1663.

Sanderson, M. P., D. A. Bronk, J. C. Nejstgaard, P. G. Verity, A. F. Sazhin, and M. E. Frischer. 2008. Phytoplankton and bacterial uptake of inorganic and organic nitrogen during an induced bloom of *Phaeocystis pouchetii* Aquat. Microb. Ecol. **51:** 153-168.

Seuthe, L., B. Töpper, M. Reigstad, R. Thyrhaug, and R. Vaquer-Sunyer. 2011. Microbial communities and processes in ice-covered Arctic waters of the northwestern Fram Strait (75 to 80°N) during the vernal pre-bloom phase. Aquat. Microb. Ecol. **64:** 253-266.

Sharp, J. H., C. A. Carlson, E. T. Peltzer, D. M. Castle-Ward, K. B. Savidge, and K. R. Rinker. 2002. Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. Mar. Chem. **77:** 239-253.

Sherr, E. B., B. F. Sherr, and A. J. Hartz. 2009. Microzooplankton grazing impact in the Western Arctic Ocean. Deep Sea Research Part II: Topical Studies in Oceanography **56:** 1264–1273.

Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Böhlke. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal. Chem. **73:** 4145-4153.

Simpson, K. G., J.-É. Tremblay, S. Brugel, and N. M. Price. 2013a. Nutrient dynamics in the western Canadian Arctic. II. Estimates of new and regenerated production over the Mackenzie Shelf and Cape Bathurst Polynya. Mar. Ecol. Prog. Ser. **484:** 47-62.

Simpson, K. G., J.-É. Tremblay, and N. M. Price. 2013b. Nutrient dynamics in the western Canadian Arctic. I. New production in spring inferred from nutrient draw-down in the Cape Bathurst Polynya. Mar. Ecol. Prog. Ser. **484:** 33-45.

Simpson, K. J., J.-É. Tremblay, Y. Gratton, and N. M. Price. 2008. An annual study of inorganic and organic nitrogen and phosphorus and silicic acid in the southeastern Beaufort Sea. J. Geophys. Res. **113:** C07016, doi:07010.01029/02007JC004462.

Sipler, R. E., T. L. Connelly, S. E. Baer, Q. N. Roberts, M. P. Sanderson, and D. A. Bronk. in prep. Impact of tundra humics on nitrogen cycling in the coastal Arctic.

Smith, W., Jr., and W. G. Harrison. 1991. New production in polar regions: the role of environmental controls. Deep-Sea Research **38:** 1463-1479.

Smith, W. O., Jr. 1993. Nitrogen uptake and new production in the Greenland Sea: the spring *Phaeocystis* bloom. J. Geophys. Res. **98:** 4681-4688.

---. 1995. Primary productivity and new production in the Northeast Water (Greenland) Polynya during summer 1992. J. Geophys. Res. **100:** 4357-4370.

Smith, W. O., Jr., M. Gosselin, L. Legendre, D. Wallace, K. Daly, and G. Kattner. 1997. New production in the Northeast Water Polynya: 1993. J. Mar. Syst. **10:** 199-209.

Stolte, W., and R. Riegman. 1996. The relative preference index (RPI) for phytoplankton nitrogen use is only weakly related to physiological preference. J. Plankton Res. **18:** 1041-1045.

Tait, Z. S. and others submitted. Increased terrestrial carbon flux from melting permafrost may stimulate bacterial nitrate uptake in the Arctic Ocean.

Terrado, R., C. Lovejoy, R. Massana, and W. F. Vincent. 2008. Microbial food web responses to light and nutrients beneath the coastal Arctic Ocean sea ice during the winter-spring transition. J. Mar. Syst. **74:** 964-977.

Tremblay, J.-É., C. Michel, K. A. Hobson, M. Gosselin, and N. M. Price. 2006. Bloom dynamics in early opening waters of the Arctic Ocean. Limnol. Oceanogr. **51:** 900-912.

Tremblay, J.-É. and others 2008. Vertical stability and the annual dynamics of nutrients and chlorophyll fluorescence in the coastal, southeast Beaufort Sea. J. Geophys. Res. **113:** C07S90, doi:10.1029/2007JC004547.

Veuger, B., A. Pitcher, S. Schouten, J. S. Sinninghe Damsté, and J. J. Middelburg. 2013. Nitrification and growth of autotrophic nitrifying bacteria and Thaumarchaeota in the coastal North Sea. Biogeosciences **10:** 1775-1785.

Ward, B. B. 2008. Nitrification in marine systems, p. 199-262. *In* D. A. Bronk, D. G. Capone, E. J. Carpenter and M. R. Mulholland [eds.], Nitrogen in the Marine Environment. Elsevier Inc.

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr. **39:** 1985-1992.

Wickland, K. P., G. R. Aiken, K. Butler, M. M. Dornblaser, R. G. M. Spencer, and R. G. Striegl. 2012. Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. Global Biogeochem. Cy. **26:** GB0E03,doi:10.1029/2012gb004342.

Worden, A. Z., and F. Not. 2008. Ecology and diversity of picoeukaryotes, p. 159-206. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. John Wiley & Sons, Inc.

Yager, P. L. and others 2001. Dynamic bacterial and viral response to an algal bloom at subzero temperatures. Limnol. Oceanogr. **46:** 790-801.

Yager, P. L., and J. W. Deming. 1999. Pelagic microbial activity in an arctic polynya: Testing for temperature and substrate interactions using a kinetic approach. Limnol. Oceanogr. **44:** 1882-1893.

Yun, M. S., K. H. Chung, S. Zimmerman, J. Zhao, H. M. Joo, and S. H. Lee. 2012. Phytoplankton productivity and its response to higher light levels in the Canada Basin. Polar Biol. **35:** 257-268.

Tables

Table 1. Physical conditions of the individual sampling locations. Exact locations and physical parameters for stations in this study. During ice-covered seasons, sample depth was measured from the bottom of the sea ice. Nd is for no data.

Month	Day	Year	Latitude (W)	Longitude (N)	Water depth (m)	Sample depth (m)	Ice depth (m)	PAR (µmol quanta^{-1} m^{-2} s^{-1})	Temp. (°C)	Salinity
January	26	2011	156°41'40"	71°20'21"	10.4	2.0	0.91	0.30	-2.5	26.2
	28		156°41'40"	71°20'21"	10.4	2.0	0.91	0.30	-2.0	32.9
	16	2012	156°41'00"	71°21'20"	16.5	2.0	1.20	0.08	-1.8	33.5
	21		156°34'33"	71°22'12"	7.0	1.0	0.56	0.12	-1.9	33.8
April	23	2010	156°41'09"	71°21'22"	17.7	8.0	0.55	5.0	nd	nd
	25		156°41'09"	71°21'22"	17.7	8.0	0.55	5.0	nd	nd
	26	2011	156°43'16"	71°18'07"	17.0	6.5	1.50	4.7	-1.6	31.5
	30		156°43'39"	71°18'13"	8.0	4.0	1.30	11	-1.8	32.4
August	25	2010	156°41'09"	71°21'22"	12.5	4.0	0.0	65	6.0	31.4
	28		156°41'09"	71°21'22"	12.5	4.0	0.0	44	6.0	31.6
	15	2011	156°43'16"	71°18'07"	17.0	4.0	0.0	nd	5.9	30.2
	17		156°43'16"	71°18'07"	17.0	8.0	0.0	nd	4.7	30.6
	19		156°43'39"	71°18'13"	8.0	2.0	0.0	nd	4.7	30.2

Table 2. Ambient biological and chemical inventories. For each sampling trip, which consisted of at least two visits to the sampling site, the overall mean and standard deviation of Chl *a*, bacterial abundance, and nutrient concentrations are given for the depth at which uptake incubations were performed (see Table 1).

Month	Year	Chl *a* ($\mu g\ L^{-1}$)	Bacterial Abundance (10^8 cells L^{-1})	NH_4^+ ($\mu mol\ N\ L^{-1}$)	NO_3^- ($\mu mol\ N\ L^{-1}$)	Urea ($\mu mol\ N\ L^{-1}$)	[1]DPA ($\mu mol\ N\ L^{-1}$)	PO_4^{3-} ($\mu mol\ P\ L^{-1}$)	Si ($\mu mol\ Si\ L^{-1}$)	DOC ($\mu mol\ C\ L^{-1}$)	DON ($\mu mol\ N\ L^{-1}$)
January	2011	0.03 ± 0.00	3.0 ± 0.4	2.45 ± 0.60	6.93 ± 1.03	0.25 ± 0.05	0.14 ± 0.04	1.35 ± 0.08	20.0 ± 2.9	75.3 ± 2.24	4.3 ± 0.18
	2012	0.02 ± 0.01	2.8 ± 0.3	0.69 ± 0.24	9.07 ± 3.11	0.17 ± 0.04	0.07 ± 0.01	1.28 ± 0.19	28.4 ± 8.3	83.1 ± 1.81	3.1 ± 0.42
April	2010	1.01 ± 0.52	0.9 ± 0.1	0.69 ± 0.11	5.91 ± 1.25	0.38 ± 0.39	0.15 ± 0.14	0.98 ± 0.04	10.8 ± 1.3	90.4 ± 7.31	5.2 ± 1.1
	2011	0.09 ± 0.03	3.2 ± 0.9	0.86 ± 0.37	9.46 ± 1.69	0.20 ± 0.01	0.10 ± 0.02	1.15 ± 0.17	34.0 ± 7.3	67.6 ± 0.33	4.4 ± 0.24
August	2010	1.38 ± 1.17	5.9 ± 3.8	0.37 ± 0.11	0.16 ± 0.04	0.35 ± 0.16	0.20 ± 0.07	0.59 ± 0.04	5.14 ± 1.1	96.5 ± 3.95	6.1 ± 0.52
	2011	0.52 ± 0.18	19.7 ± 14.7	0.80 ± 0.50	0.35 ± 0.07	0.49 ± 0.33	0.26 ± 0.15	0.52 ± 0.04	6.46 ± 1.9	88.2 ± 6.55	7.11 ± 1.5

[1] Dissolved primary amines

Table 3. Nitrogen and carbon absolute uptake rates. Mean and standard deviations of N and C uptake rates in the large (>3.0 μm) and small (0.7 to 3.0 μm) size fractions. BDL is below detection limit.

Month	Year	Size Fraction	NH_4^+ (nmol N L^{-1} h^{-1})	NO_3^- (nmol N L^{-1} h^{-1})	Urea (nmol N L^{-1} h^{-1})	[1]AA_N (nmol N L^{-1} h^{-1})	[1]AA_C (nmol C L^{-1} h^{-1})	HCO_3^- (nmol C L^{-1} h^{-1})
January	2011	> 3 μm	0.16 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.21 ± 0.14	0.57 ± 0.46	BDL
		0.7-3 μm	0.14 ± 0.09	0.09 ± 0.01	0.01 ± 0.00	0.31 ± 0.13	0.95 ± 0.29	BDL
	2012	> 3 μm	0.07 ± 0.00	0.06 ± 0.02	0.01 ± 0.01	0.11 ± 0.09	0.08 ± 0.11	BDL
		0.7-3 μm	0.06 ± 0.06	0.14 ± 0.02	0.01 ± 0.01	0.34 ± 0.26	3.74 ± 5.50	BDL
April	2010	> 5 μm	0.36 ± 0.00	0.09 ± 0.07	0.18 ± 0.18	0.11 ± 0.13	0.33 ± 0.42	0.71 ± 0.62
		0.2-5 μm	0.20 ± 0.67	0.01 ± 0.02	0.06 ± 0.07	0.09 ± 0.02	0.47 ± 0.67	0.27 ± 0.45
	2011	> 3 μm	0.28 ± 0.16	0.09 ± 0.04	0.02 ± 0.00	0.45 ± 0.37	0.62 ± 0.53	0.13 ± 0.15
		0.7-3 μm	0.61 ± 0.18	0.19 ± 0.07	0.02 ± 0.00	1.27 ± 0.69	2.01 ± 1.09	0.14 ± 0.03
August	2010	> 3 μm	10.7 ± 3.70	2.79 ± 0.06	4.92 ± 2.79	1.42 ± 0.15	2.26 ± 0.83	20.4 ± 0.36
		0.7-3 μm	8.61 ± 6.56	1.25 ± 0.80	3.71 ± 0.83	3.80 ± 1.65	7.25 ± 10.3	4.42 ± 6.25
	2011	> 3 μm	10.5 ± 8.07	1.75 ± 1.48	4.69 ± 4.89	2.93 ± 1.58	9.95 ± 6.59	42.3 ± 25.7
		0.7-3 μm	3.52 ± 4.22	0.17 ± 0.15	1.34 ± 1.89	2.51 ± 1.31	4.26 ± 6.77	4.47 ± 2.92

[1] AA_N and AA_C refers to rates uptake of the nitrogen and carbon component of amino acids, respectively.

Table 4. Relative preference index of N substrates. For each substrate, the mean and standard deviation for RPI for each season and year. Rates used in the RPI calculation were for the whole community collected on GF/F filters (i.e the 3.0 μm fraction was not subtracted from the overall rate). Means are presented for each season.

Month	Year	NH_4^+	NO_3^-	Urea	AA_N
January	2011	1.3 ± 0.3	0.2 ± 0.0	0.6 ± 0.0	38 ± 8.1
	2012	2.9 ± 1.5	0.3 ± 0.1	0.9 ± 0.3	79 ± 30
	mean	**2.1**	**0.2**	**0.8**	**58**
April	2010	6.2 ± 0.9	0.0 ± 0.0	7.3 ± 1.9	10 ± 11
	2011	3.7 ± 2.5	0.1 ± 0.0	0.8 ± 0.2	76 ± 49
	mean	**4.9**	**0.1**	**4.1**	**43**
August	2010	1.4 ± 0.3	0.7 ± 0.2	0.8 ± 0.0	0.9 ± 0.2
	2011	1.3 ± 0.2	0.3 ± 0.2	0.6 ± 0.3	2.6 ± 1.7
	mean	**1.3**	**0.5**	**0.7**	**1.7**

Table 5. Nitrification and NH_4^+ regeneration rates. Rates listed are the mean and standard deviation for each sampling trip. Nd is for no data.

Month	Year	NH_4^+ Regeneration (nmol N L^{-1} h^{-1})	Nitrification (nmol N L^{-1} h^{-1})
January	2011	17.2 ± 5.67	27.4 ± 8.21
	2012	10.4 ± 0.48	15.8 ± 9.58
April	2010	4.85 ± 1.42	nd
	2011	27.8 ± 1.18	24.9 ± 16.6
August	2010	12.5 ± 9.37	nd
	2011	15.2 ± 3.11	1.02 ± 0.32

Table 6. Uptake rates of NH_4^+, NO_3^-, and urea in the western Arctic water column. Rates are reported as the mean with range in parentheses. Where there are no uptake values reported, that column was left blank. All values are for size fractions greater than 0.7 µm, with the exception of the studies by Kristiansen (Kristiansen et al. 1994; Kristiansen and Lund 1989), who used GF/C filters (nominal pore size = 1.2 µm) and (Simpson et al. 2013a), who corrected their rates for bacterial retention on the GF/F filter. Values determined by integration over the euphotic zone are denoted by Z_e with mean depth in parentheses. SCM stands for subsurface chlorophyll max, with mean depth in parentheses. MIZ stands for marginal ice zone, which includes a mix of ice-free and ice-covered conditions.

Region	Season	Year	Sampling depth	Ice cover?	NH_4^+ nmol N L^{-1} h^{-1}	NO_3^- nmol N L^{-1} h^{-1}	Urea nmol N L^{-1} h^{-1}	Reference
Baffin Bay	Aug – Sep	1978	Z_e (34 m)	no	2.26 (0.87 – 5.86)	3.03 (1.05 – 7.18)		Harrison et al. 1982
	Jul - Aug	1980	Z_e (33 m)	no	2.62 (0.65 – 5.29)	1.30 (0.24 – 5.55)	1.02 (0.26 – 2.17)	Harrison et al. 1985
	Sep	1999	100% light	no	6.88 (0.59 – 17.8)	6.06 (0.57 – 13.6)	1.66 (0.21 – 3.74)	Fouilland et al. 2007
	Sep	1999	Z_e (42 m)	no	10.95 (2.86 – 28.1)	12.62 (4.52 – 36.0)	4.29 (1.43 – 7.38)	Garneau et al. 2007
	Sep[1]	1999	Z_e (45 m)	yes	2.22	3.56	1.11	Garneau et al. 2007

					(0.67 – 7.78)	(0.44 – 12.4)	(0.22 - 4.44)	
	Sep	2006	surface (5 m)	no		1.37 (0.04 – 2.19)		Martin et al. 2012[2]
Barents Sea	Summer	1984	unknown	no	5.5 (2.3 – 9.0)	5.1 (2.3 – 8.5)	1.5 (0.4 – 2.2)	Kristiansen and Lund 1989
	Pre- and post-bloom	1984-1988	surface (<15 m)	MIZ	4.7 (<0.1 – 10.2)	1.8 (0.6 – 4.0)	0.3 (0.3 – 2.3)	Kristiansen et al. 1994
	Bloom	1984-1988	surface (<15 m)	MIZ	1.65 (0.5 – 2.8)	12.5 (11.3 – 13.7)	2.3 (<0.1 – 0.5)	Kristiansen et al. 1994
Beaufort Sea	Apr – Jul	2008	SCM (39 m)	no	3.41 (0.0 – 23.9)	11.14 (0.70 – 67.8)		Martin et al. 2012[2]
	Aug – Oct	2005-2007	SCM (24 m)	no[3]	0.84 (0.02 – 2.78)	2.96 (0.06 – 18.7)		Martin et al. 2012[2]
	Pre- and post-bloom	1984-1988	Z_e (50 m)	no	0.64	0.07	0.95	Simpson et al. 2013a
	Bloom	1984-1988	Z_e (50 m)	no	2.98	3.53	7.11	Simpson et al. 2013a
Canada Basin	Aug – Sep	2002	surface	no	1.94	1.01		Lee and Whitledge 2005
				yes	0.77	0.29		

	Jun - Jul	2005	100% light[4]	yes	0.40 (0.06 – 2.11)	0.08 (0.02 – 0.39)		Lee et al. 2010
	Sep - Oct	2009	100% light	no	0.11 (0.05 – 0.27)	0.02 (0.00 – 0.05)		Yun et al. 2012
Chukchi Sea	Aug	2004	surface	no	13.1 (2.22 – 52.7)	3.69 (0.16 – 24.5)		Lee et al. 2007
shelf	Aug	2008	100% light	no	5.48 (1.23 – 8.82)	0.21 (0.02 – 0.35)		Lee et al. 2012[2]
nearshore	Jan	2011, 2012	2 m	Yes	0.21 (0.08 - 0.35)	0.15 (0.12 – 0.20)	0.01 (0.01 – 0.02)	this study
	Apr	2010, 2011	4 m	Yes	0.71 (0.36 – 1.12)	0.16 (0.07 – 0.31)	0.14 (0.04 – 0.34)	this study
	Aug	2010, 2011	4 m	no	16.7 (5.10 - 28.1)	2.99 (0.44 - 4.65)	7.33 (1.20 – 13.8)	this study
Greenland Sea	May – Jun	1987	100% light	no	14.2 (0.10 – 164)	5.05 (0.70 – 11.6)		Keene et al. 1991
	Apr – May	1989	Z_e (43 m)	no	29.9 (5.44 – 71.8)	46.7 (4.22 – 254)		Smith 1993[2,4]
	Jul – Aug	1992	100% light	no	1.77 (0.21 – 7.49)	1.16 (0.01 – 5.17)		Smith 1995

May – Aug	1993	100% light	no	2.36 (0.16 – 7.24)	0.84 (0.00 - 5.17)	Smith et al. 1997[2]
May – July	1993	Z_e (< 70 m)	no	1.35[(6)] (0.00 – 23.9)	1.94[(6)] (0.00 – 108)	Daly et al. 1999

[1] Garneau et al. make a distinction between early September and late September in their study, as the latter half of the month involved rapidly increasing ice cover at the experimental location.

[2] Rate data provided by personal communication.

[3] Of the 13 Beaufort Sea locations in the Martin et al. study, 3 had some sea ice present.

[4] Normalized to under-ice conditions.

[5] The 1989 cruise (Smith 1993) occurred during a *Phaeocystis* bloom and is the most likely explanation for such high rates.

[6] Daly et al. (1999) report median values.

Figure legends

Figure 1. Sampling location. Samples were taken approximately 2.5 km north of Barrow, Alaska indicated by the black circle. Depth contours are 50 m intervals. See Table 1 for exact locations and bottom depths.

Figure 2. Ambient nutrient comparisons. Values are only shown for the depths at which incubations were performed. The dashed line represents Redfield ratios of C:N:Si:P of 106:16:15:1. Error bars are standard deviation.

Figure 3. Absolute uptake rates of nitrogen. Mean rates of N uptake (two experiments for each of two years) for each substrate tested, with error bars representing the standard deviation (n = 4). Solid bars are >3.0 μm fraction, and hatched bars are GF/F – 3.0 μm fraction. The dashed line in the summer panel corresponds to the uppermost y-axis scale of the winter and spring panels, and is included solely to provide a reference.

Figure 4. Absolute uptake rates of carbon. Mean rates of C uptake for each substrate tested, with error bars representing the standard deviation. Solid bars are >3.0 μm fraction, and hatched bars are GF/F – 3.0 μm fraction. The dashed line in the summer panel corresponds to the uppermost y-axis scale of the winter and spring panels, and is included solely to provide a reference.

Figure 5. Ammonium cycling processes. Concentrations of NH_4^+ plotted against rates of nitrification and NH_4^+ regeneration. The regression lines are for all values in the plot (see text for significance and R^2 values).

Figure 1

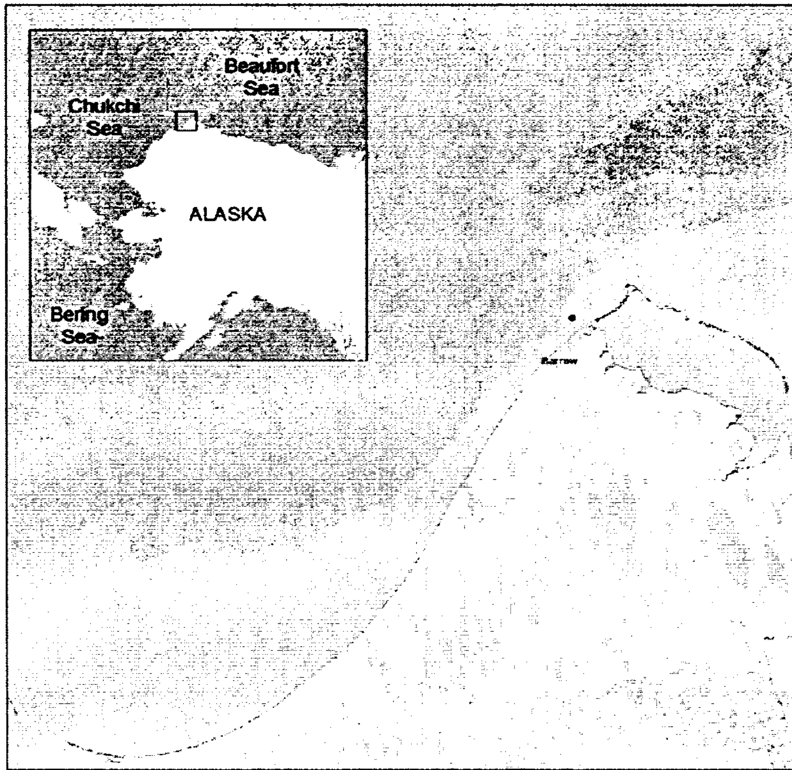


Figure 2

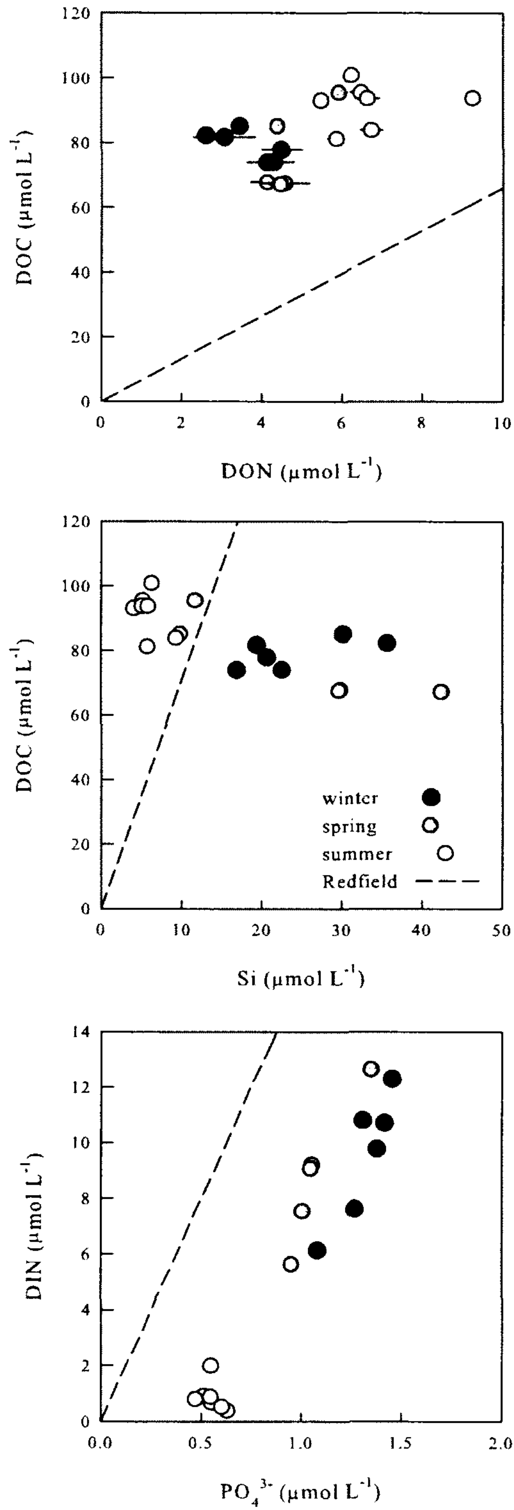


Figure 3

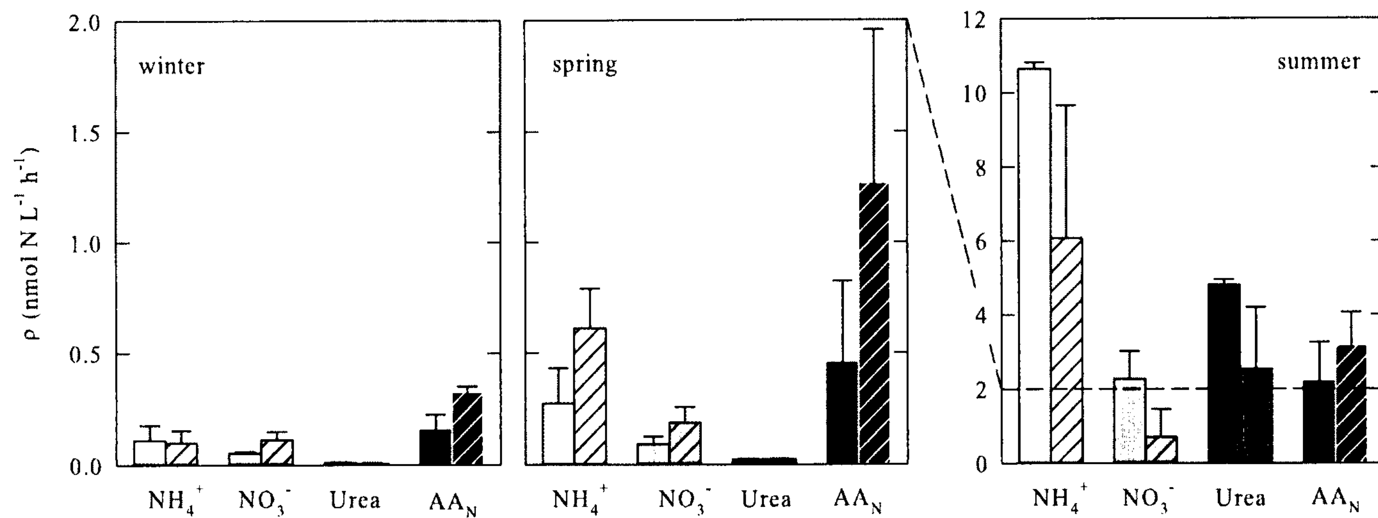


Figure 4

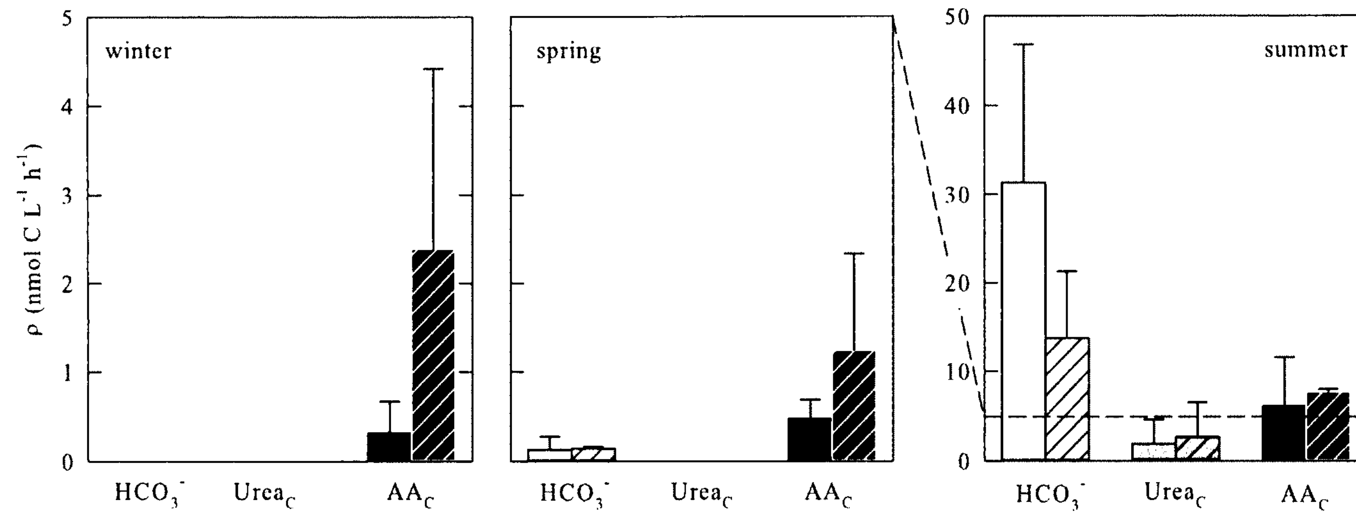
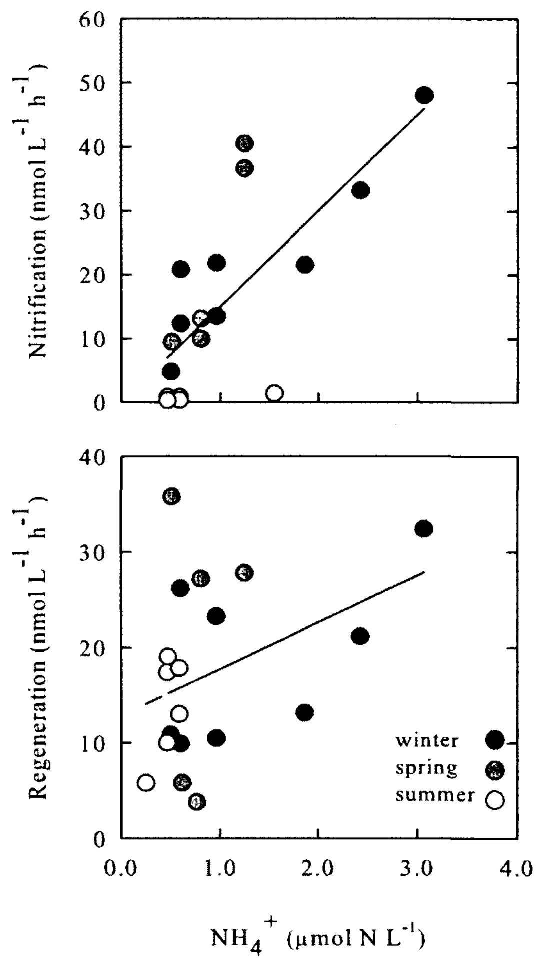


Figure 5



Chapter 3 – Nitrogen uptake rates of low- and high-nucleic acid bacterial subgroups from the coastal Arctic (Chukchi Sea)

Author's Note

The number of samples analyzed for this chapter was small due to limitations in access to a high-speed cell sorter. Additional samples will be processed on a newly acquired BD Influx cell sorter prior to submission of this chapter to a journal.

Abstract

In flow cytometric analyses of marine bacterioplankton, there is a bimodal distribution of subpopulations that are ubiquitous – one with low nucleic acid (LNA) and one with high-nucleic acid (HNA) content. In this study we used nitrogen (N) stable isotope tracer techniques and flow cytometric sorting to determine rates of N uptake of LNA and HNA populations collected in the shallow coastal environment of the Chukchi Sea during winter, spring and summer. To our knowledge this is the first report of N uptake in LNA and HNA populations. In summer, HNA cells comprised a higher percentage of total bacterial abundance compared to winter. These HNA cells had lower cellular N content than LNA cells, but had higher uptake rates of all substrates. Specific and absolute uptake rates of ammonium and an amino acid mixture were higher than for nitrate and urea in both groups. Seasonal differences in the relative abundance and N uptake rates of LNA and HNA fractions indicate that these groups are both active, but they appear to respond differently to environmental signals.

Introduction

In marine waters, phytoplankton and bacteria compete intensely for limited resources to fuel their metabolisms. Traditionally, it has been methodologically difficult to separate microbial populations such as phytoplankton and bacteria, and therefore challenging to ascribe biogeochemical rate measurements to these two major microbial groups. A promising approach is to combine flow cytometric analysis and sorting with ^{15}N stable isotopes techniques (Lipschultz 1995), which allows accurate and unambiguous determination of uptake of N compounds by different identifiable populations.

With a flow cytometer, large numbers of particles can be identified quickly and accurately based on size, density and fluorescent characteristics such as chlorophyll pigments (Gasol & del Giorgio 2000). With the development of a wide variety of fluorescent stains that bind to DNA, organisms that do not have any naturally fluorescent properties can be distinguished from detritus and other particles, leading to quick and accurate bacterial abundance measurements along with the ability to identify and sort cells for further analysis (reviewed in Lomas et al. 2011).

In flow cytometric analyses of aquatic systems, the bacterioplankton (which includes archaea, but will heretofore be referred to as "bacterial") fraction of aquatic samples clusters in two distinct groups, commonly referred to as low nucleic acid (LNA) and high nucleic acid (HNA) fractions (Li et al. 1995, Gasol et al. 1999, Lebaron et al. 2001). This remains true even with samples from a variety of locations and the use of different staining protocols (Bouvier et al. 2007, Wang et al. 2009). Although these

clusters are found universally, there are conflicting reports of their ecological significance or what they indicate about the populations themselves (Bouvier et al. 2007). Initially, HNA and LNA cells were generally considered as the active and the inactive fractions, respectively (Jellett et al. 1996, Gasol et al. 1999, Lebaron et al. 2001, Vaqué et al. 2001). Some studies investigating these two fractions found higher bacterial production in the HNA cells (Lebaron et al. 2001, Lebaron et al. 2002, Servais et al. 2003, Morán et al. 2007, Morán et al. 2011), but others found that the LNA fraction could be at least as active as the HNA fraction (Zubkov et al. 2001, Mary et al. 2006, Wang et al. 2009). There is also conflicting evidence of whether the LNA and HNA groups consist of distinct phylogenetic groups (Zubkov et al. 2001, Schattenhofer et al. 2011, Vila-Costa et al. 2012) as opposed to significant overlap between the two groups (Servais et al. 2003, Longnecker et al. 2005). Differences in results are possibly related to broad ecological parameters, as it has been found that LNA cells are more active in coastal regions relative to the oligotrophic open ocean (Servais et al. 2003, Longnecker et al. 2005, 2006).

Most studies of LNA and HNA populations have taken place in temperate regions, although a few studies have found that these groups are also present in the higher latitudes (Corzo et al. 2005, Belzile et al. 2008, Garzio et al. 2013, Schattenhofer et al. 2011). In Antarctica (Corzo et al. 2005) and the Beaufort Sea in the Arctic (Belzile et al. 2008), HNA cells had increased growth rates in response to the spring phytoplankton bloom, while the LNA cells growth rate was delayed by more than two months. These results imply that the HNA population has greater genetic plasticity and the ability to react more quickly to changing environmental conditions. There is no corresponding

information on LNA and HNA groups in the Chukchi Sea, even though this is an area of known high bacterial productivity (Bates et al. 2005, Kirchman et al. 2009).

Though the measurement of N uptake rates of the LNA and HNA subpopulations could provide detailed empirical information on the activity of the prokaryotic assemblage, no published studies exist. The use of ^{15}N tracers in combination with flow cytometric sorting was pioneered by Lipschultz (1995), using natural phytoplankton in coastal Maine. Further work used this approach to measure inorganic N uptake by phytoplankton in the Sargasso Sea (Casey et al. 2007, Fawcett et al. 2011) and to determine phytoplankton-specific from bacterial-specific inorganic and organic N uptake in the mid-Atlantic Bight (Bradley et al. 2010a), Chesapeake Bay (Bradley et al. 2010b), and a mesocosm study in a Norwegian fjord (Bradley et al. 2010c). Similarly, methionine with isotopically labeled sulfur has been used to investigate amino acid uptake in *Prochlorococcus* (Zubkov et al. 2003, Zubkov & Tarran 2005). Here we use flow cytometric sorting of LNA and HNA populations of the coastal Chukchi Sea to determine N uptake rates within those two groups and to highlight potential seasonal differences in these populations.

Materials and methods

Sampling

Water column samples were taken as part of a larger study investigating N uptake and regeneration in the coastal Arctic; details of the sampling methodology can be found in Chapter 2. Briefly, during April and August of 2010 and 2011 and January of 2011

and 2012, water samples were taken off the coast of Barrow, Alaska with a small, low-pressure pump from beneath the sea ice or a small boat, depending on conditions. Replicate subsamples were incubated with tracer (<10%) additions of ^{15}N-labelled ammonium chloride (98.85% ^{15}N), potassium nitrate (98%), urea (98%) or an amino acid mixture (96-99%; Cambridge Isotope Laboratories). The incubation bottles were placed in an environmental control chamber at ambient light and temperature for 24 hours.

Nutrient analysis

Nutrient analyses were performed on sub-samples after filtration and then frozen at -20°C until analysis. Concentrations of ammonium (NH_4^+) were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were measured in duplicate on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Dissolved primary amines (DPA) were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Urea was measured in duplicate using the manual monoxime method (Price & Harrison 1987).

Flow cytometric sorting

At the end of the incubation, samples were concentrated over 0.2 μm Supor® polyethersulfone filters (Pall Corporation) under gentle vacuum pressure (<5 Hg). When a small volume (<30 ml) of sample remained, the filter was gently sparged using a clean pipette, and 10 mL of sample was placed in a 15 mL polypropylene tube. Immediately

before the filter went completely dry, it was removed, placed in the same tube, and gently agitated to recover cells from the filter (see Bradley et al. 2010b for full details on the method). Samples were fixed with an addition of 63 μL of paraformaldehyde (0.2% final concentration), allowed to sit for 10 minutes, and flash frozen in liquid nitrogen. Samples were stored at -80°C until analysis.

Flow-cytometric analyses were performed on a high-speed Influx cell sorter (BD Biosciences) with a blue-light (488 nm) laser operating at 200 mW and a bandpass filter at 531/40 nm (green fluorescence) and a longpass filter at 692/20 nm (red fluorescence). Staining with SYTO 13 green fluorescent nucleic acid stain in DMSO (1:100 final dilution of the commercial stock; Invitrogen) was carried out for 5 minutes in the dark at room temperature. The sample was passed through a 50 μm CellTrics® (Partec) to reduce any chance of clogging the nozzle and run with a sheath fluid of sterilized and 0.2 μm filtered phosphate buffered saline (PBS; Fisher Scientific). A 70 μm nozzle tip was used with a sample pressure of 28.5 PSI and sheath pressure between 29.5 and 30.0. Post-acquisition analysis was performed with FlowJo version X (Tree Star Inc.).

Cells were detected and gated according to relative values of side scatter (SSC) and green fluorescence (FL1), with greater FL1 indicating HNA populations (Gasol & del Giorgio 2000). The Influx sorter was set in "1.0 drop pure" sort mode and maintained a sorting rate of 800 – 1500 events s^{-1}, depending on the density of cells present. The populations were simultaneously sorted into several separate sterilized 0.5 ml polypropylene tubes. To acquire enough cells for isotopic and mass analysis, approximately the entire volume of sample (10 mL) was processed. The biggest

constraint of this study was the number of uptake rates we were able to measure based on the access we had to the flow cytometer we used. Timing is an issue because it takes considerable time to sort enough biomass to get a reliable measurement on the mass spectrometer. An average sort time for each sample was close to four hours. This led to the small number of samples able to be processed. After staining, LNA and HNA populations were fairly distinct (Figure 1). Cell densities were high enough to reliably sort at a target event rate of over 1000 events s^{-1}.

Samples for each given sorted population were mixed and immediately filtered through 0.2 μm silver membrane filters (Sterlitech Corporation). The filter tower was rinsed with 0.2 μm filtered ultrapure water (18.2 MΩ; EMD Millipore) to ensure complete capture of sorted populations. Filters were placed in cryovials and kept at -20°C until analyzed on a Europa Geo 20/20 isotope ratio mass spectrometer with an ANCA autosampler. A ^{14}N carrier addition was made to ensure there was enough mass for analysis. Carrier additions were estimated based on the number of cells sorted and a N biomass conversion of 12 fg N $cell^{-1}$ (Vrede et al. 2002).

Specific uptake rates (V; h^{-1}), which describe the physiological ability of cells to assimilate N (Dugdale & Wilkerson 1986) were calculated as in Lipschultz (1995). We calculated absolute uptake rates (ρ; nmol N L^{-1} h^{-1}) by multiplying the specific uptake rate by the particulate nitrogen in the LNA or HNA bacteria:

$$\rho_{HNA} = V \cdot [PN]_{HNA} \quad (1)$$

where [PN] is the particulate N, estimated by the following equation:

$$[PN]_{HNA} = BA \cdot \%HNA \cdot N\,cell^{-1} \quad (2)$$

where BA is the bacterial abundance (cells L^{-1}) at the start of the incubation, %HNA is the percent of HNA cells determined with the flow cytometer, and N $cell^{-1}$ is the mean mass of N present in a HNA cell, which was determined by dividing the mass of N of the sorted HNA cells by the number of HNA cells sorted.

Results

Environmental conditions

Conditions varied widely between the seasons, although the water column remained well mixed during all sampling, as fully explained in Chapter 2. Briefly, the water column in April had lower ice cover in 2010 than 2011 (0.55 m and 1.4 m thickness respectively). The water column was well mixed with regard to temperature (-1.8°C) and salinity (mean = 32). Photosynthetically active radiation (PAR) averaged 6.4 µmol $quanta^{-1}$ m^{-2} s^{-1}. Chlorophyll *a* (Chl *a*) was higher in 2011 (mean and standard deviation = 1.0 ± 0.52 µg L^{-1}) than 2010 (0.08 ± 0.03 µg L^{-1}), but bacterial abundance exhibited the opposite pattern, with 0.9 ± 0.06 $x10^8$ cells L^{-1} in 2010 and 3.2 ± 0.9 $x10^8$ cells L^{-1} in 2011. Ambient substrate concentrations were all below 1.0 µmol N L^{-1} (Table 1).

August conditions were ice-free and the water temperature increased to a mean of 5.5°C, while salinity decreased slightly (mean = 31). With the lack of ice cover and increased hours of daylight, PAR was much higher than the other seasons, at 55 µmol $quanta^{-1}$ m^{-2} s^{-1}. Chl *a* and bacterial abundance both reached their maximum values during summer. The multiyear mean of Chl *a* was 0.6 ± 0.2 µg L^{-1}. Bacterial abundance was lower in 2010 (5.9 ± 3.8 $x10^8$ cells L^{-1}) than 2011 (20 ± 14 $x10^8$ cells L^{-1})

respectively. Ambient nutrient concentrations reached seasonal lows, with all measured compounds below 0.5 μmol N L^{-1} (Table 1).

January was characterized by moderate ice cover (0.9 m in 2011 and 2012), low temperature (-1.8°C) and PAR (0.2 μmol $quanta^{-1}$ m^{-2} s^{-1}), with salinity (33) slightly higher than the other seasons. Chl *a* reached a seasonal low (0.02 ± 0.01 μg L^{-1}), but bacterial abundance was higher on average (2.9 ± 0.3 $x10^8$ cells L^{-1}) than in April. Ambient NH_4^+ and NO_3^- were high in January, but amino acid concentrations were slightly lower than in other seasons (Table 1).

Characteristics of LNA and HNA clusters

Clusters of LNA and HNA cells could be clearly separated based on their SSC and FL1 characteristics (Figure 1). The ratio of HNA cells to the total (%HNA) ranged from 32 – 91%, with the lowest values consistently found in January (mean ± standard deviation = 50 ± 15%) and the highest in August (78 ± 8.0%; Table 1). Cellular N content was greatest in the LNA fraction during April and August (Table 2). The same was true in January 2011, but in January 2012, however, HNA cells had twice the N content of the LNA cells.

Nitrogen uptake

The HNA fraction had higher NH_4^+ specific uptake than the LNA fraction in all samples except in January 2012 (Table 1). This was more pronounced in the summer sample, when the specific uptake of NH_4^+ in the HNA fraction was 94% greater than the

LNA fraction. In spring and winter, the HNA fraction had NH_4^+ specific uptake rates that were 64% and 53% greater than the LNA fraction, respectively. Similar results were found for absolute uptake of NH_4^+ – all of the samples had higher NH_4^+ absolute uptake in the HNA fraction. Likewise, both specific and absolute uptake of NO_3^- was higher in the HNA fraction, regardless of season. The same holds true for urea, but only a summer sample was analyzed, so there is no comparison for winter or spring. Uptake of amino acids by the HNA fraction was higher in summer, but approximately equal during winter.

When considering NH_4^+ and AA uptake, there is no significant correlation between %HNA and specific or absolute uptake, although they appear to be weakly positive (data not shown). Even when using all of the substrates in the analysis and normalizing for the amount of uptake within each N compound, there is no significant relationship between the %HNA and the percent of specific or absolute uptake (Figure 2). In fact, none of the factors we tested (%HNA, seasons, ambient nutrient concentration) were significant predictors of N uptake, which may have been a function of the small sample size.

Discussion

In the Chukchi Sea, N uptake rates of phytoplankton have been determined by filtration through GF/F filters (nominal pore size = 0.7um), but there has been no accounting for bacterial uptake (Lee et al. 2007, Lee et al. 2010). In order to distinguish bacterial uptake from phytoplankton uptake, it is necessarily to positively identify bacterial groups and determine their uptake rates unequivocally. Other Arctic studies

have attempted to specifically account for bacterial N uptake using size fractionation (Allen et al. 2002), but this technique has limited success due to the overlapping size of bacteria and phytoplankton and variable retention of bacteria on filters (Lee & Fuhrman 1987, Gasol & Morán 1999). Simpson et al. (2013) tried to overcome that limitation by mathematically accounting for bacteria passing through the GF/F filters and then caught on 0.2 μm silver filters. Metabolic inhibitors have also been used (Fouilland et al. 2007), but that technique is notoriously unreliable due to lack of specificity and incomplete inhibition (Oremland & Capone 1988). Thus, we used ^{15}N uptake after flow cytometric sorting to describe the activity of LNA and HNA fractions in coastal Arctic samples.

Seasonal differences

Abundance is not a good indicator of bacterial activity (Yager & Deming 1999, Alonso-Sáez et al. 2008). This also appears to be true within the bacterial nucleic acid groups. There was a difference in %HNA by season, but %HNA had a very weak correlation with different rates of uptake ($R^2 = 0.16$ for specific uptake and 0.01 for absolute uptake). In high latitude waters, it has been found that during winter HNA cells are preferentially grazed (Garzio et al., in press, Gasol et al. 1999, Corzo et al. 2005) and %HNA falls below 50% (Belzile et al. 2008). This seasonal difference appears to be unique to the polar regions. In temperate waters, %HNA was lower in summer compared to winter (Calvo-Díaz & Morán 2006, Morán & Calvo-Díaz 2009, Vila-Costa et al. 2012). It is thought that HNA is an indication of larger genomes, which allows bacterioplankton to be more adaptable to conditions and therefore grow faster in response to changing

environmental variables (Schattenhofer et al. 2011). If this is true, then it would imply that rather than any static measured nutrient or physical parameter, it is the rate of change in that parameter over time that is an important factor determining the activity of the LNA and HNA fractions. Consistent with this idea, Belzile et al. (2008) found that LNA had slower growth rates and a delayed response to the spring phytoplankton bloom in the coastal Beaufort Sea.

The difference between LNA and HNA in both specific and absolute uptake of N was greater during the late summer than winter. This would lend further support to the hypothesis that some feature of the ecosystem is allowing for greater relative activity of the LNA fraction during winter. It is possible that the lack of phytoplankton competition, along with static environmental conditions (e.g. light, temperature) favor the smaller LNA cells. Furthermore, there is little difference between overall bacterial abundance and the uptake rates during different seasons studied. It appears that these coastal Arctic bacterioplankton can maintain their N metabolism at consistent rates throughout the year.

Substrate preference

Each fraction effectively assimilated all of the N substrates tested. The LNA cells are clearly active, but not at the same rate as HNA cells (Table 1). Both LNA and HNA groups are actively taking up NH_4^+ and amino acids at higher rates than NO_3^- and urea. The work done here was part of a larger study of N cycling in the Chukchi, which also found that NH_4^+ and amino acids are the preferred N forms (see Chapter 2). On the other hand, the difference between LNA and HNA uptake rates of all N compounds tested was

reduced during winter. This could be because there are different groups of bacteria that take up amino acids depending on the season (Alonso-Sáez et al. 2008, Nikrad et al. 2012).

It is known that bacteria can effectively compete with phytoplankton for inorganic N (NH_4^+ and NO_3^-) in the Arctic. During conditions of light limitation (i.e. depth and/or ice cover), bacteria assimilate NH_4^+ and NO_3^- (Allen et al. 2002). In the Beaufort Sea, Simpson et al. (2013) found that bacteria were responsible for less than 15% of total N (NH_4^+, NO_3^-, and urea) uptake. On the other hand, Fouilland et al. (2007) found that bacteria could account for more than 50% of total NO_3^- uptake. In this study, specific uptake of NO_3^- was similar in magnitude to NH_4^+ specific uptake, which indicates that the bacteria have equal physiological capability to assimilate both forms of inorganic N.

Conclusions

This is the first report of N uptake by LNA and HNA populations of bacteria. We have shown that both fractions are actively assimilating N, including NO_3^-. We found little difference in uptake rates during different seasons even though the environmental conditions were drastically different. This was true despite the fact that HNA cells were relatively more abundant during late summer as opposed to winter. Further work is necessary to understand whether these are universal features of LNA and HNA bacteria, or whether the patterns we found are specific to this region.

Acknowledgments

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Literature cited

Allen AE, Howard-Jones MH, Booth MG, Frischer ME, Verity PG, Bronk DA, Sanderson MP (2002) Importance of heterotrophic bacterial assimilation of ammonium and nitrate in the Barents Sea during summer. J Mar Syst 38:93-108

Alonso-Sáez L, Sánchez O, Gasol JM, Balagué V, Pedrós-Alio C (2008) Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. Environ Microbiol 10:2444-2454

Bates NR, Best MHP, Hansell DA (2005) Spatio-temporal distribution of dissolved inorganic carbon and net community production in the Chukchi and Beaufort Seas. Deep-Sea Res Pt II 52:3303-3323

Belzile C, Brugel S, Nozais C, Gratton Y, Demers S (2008) Variations of the abundance and nucleic acid content of heterotrophic bacteria in Beaufort Shelf waters during winter and spring. J Mar Syst 74:946-956

Bouvier T, del Giorgio PA, Gasol JM (2007) A comparative study of the cytometric characteristics of high and low nucleic-acid bacterioplankton cells from different aquatic ecosystems. Environ Microbiol 9:2050-2066

Bradley P, Sanderson MP, Frischer ME, Brofft J, Booth MG, Kerkhof LJ, Bronk DA (2010a) Inorganic and organic nitrogen uptake by phytoplankton and heterotrophic bacteria in the stratified Mid-Atlantic Bight. Estuar Coast Shelf Sci 88:429-441

Bradley PB, Lomas MW, Bronk DA (2010b) Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach. Estuaries Coasts 33:971-984

Bradley PB, Sanderson MP, Nejstgaard JC, Sazhin AF, Frischer ME, Killberg-Thoreson LM, Verity PG, Campbell L, Bronk DA (2010c) Nitrogen uptake by phytoplankton and bacteria during an induced *Phaeocystis pouchetii* bloom, measured using size fractionation and flow cytometric sorting. Aquat Microb Ecol 61:89-104

Calvo-Díaz A, Morán XAG (2006) Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. Aquat Microb Ecol 42:159-174

Casey JR, Lomas MW, Mandecki J, Walker DE (2007) *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophys Res Lett 34:L10604, doi:10610.11029/12006GL028725

Corzo A, Rodríguez-Gálvez S, Lubian L, Sobrino C, Sangrá P, Martínez A (2005) Antarctic marine bacterioplankton subpopulations discriminated by their apparent content of nucleic acids differ in their response to ecological factors. Polar Biol 29:27-39

Dugdale RC, Wilkerson FP (1986) The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. Limnol Oceanogr 31:673-689

Fawcett SE, Lomas MW, Casey JR, Ward BB, Sigman DM (2011) Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea. Nature Geoscience 4:717-722

Fouilland E, Gosselin M, Rivkin RB, Vasseur C, Mostajir B (2007) Nitrogen uptake by heterotrophic bacteria and phytoplankton in Arctic surface waters. J Plankton Res 29:369-376

Garzio LM, Steinberg DK, Erickson M, Ducklow HW (2013, in press) Microzooplankton grazing on phytoplankton and bacteria along the Western Antarctic Peninsula. Aquat Microb Ecol

Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina 64:197-224

Gasol JM, Morán XAG (1999) Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. Aquat Microb Ecol 16:251-264

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65:4475-4483

Jellett JF, Li WKW, Dickie PM, Boraie A, Kepkay PE (1996) Metabolic activity of bacterioplankton communities assessed by flow cytometry and single carbon substrate utilization Mar Ecol Prog Ser 136:213-225

Kirchman DL, Hill V, Cottrell MT, Gradinger R, Malmstrom RR, Parker A (2009) Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. Deep-Sea Res Pt II 56:1237-1248

Koroleff F (1983) Determination of nutrients. In: Grasshoff K, Ehrhardt M, Kremling K (eds) Methods of seawater analysis. Verlag Chemie, New York

Lebaron P, Servais P, Agogué H, Courties C, Joux F (2001) Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? Appl Environ Microbiol 67:1775-1782

Lebaron P, Servais P, Baudoux A-C, Bourrain M, Courties C, Parthuisot N (2002) Variations of bacterial-specific activity with cell size and nucleic acid content assessed by flow cytometry. Aquat Microb Ecol 28:131-140

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53:1298-1303

Lee SH, Stockwell D, Whitledge TE (2010) Uptake rates of dissolved inorganic carbon and nitrogen by under-ice phytoplankton in the Canada Basin in summer 2005. Polar Biol 33:1027-1036

Lee SH, Whitledge TE, Kang S-H (2007) Recent carbon and nitrogen uptake rates of phytoplankton in Bering Strait and the Chukchi Sea. Cont Shelf Res 27:2231-2249

Li WKW, Jellett JF, Dickie PM (1995) DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnol Oceanogr 40:1485-1495

Lipschultz F (1995) Nitrogen-specific uptake rates of marine phytoplankton isolated from natural populations of particles by flow cytometry. Mar Ecol Prog Ser 123:245-258

Lomas MW, Bronk DA, van den Engh G (2011) Use of flow cytometry to measure biogeochemical rates and processes in the ocean. Annu Rev Mar Sci 3:537-566

Longnecker K, Sherr BF, Sherr EB (2005) Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. Appl Environ Microbiol 71:7737-7749

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

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH, Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. Aquat Microb Ecol 45:107-113

Morán X-AG, Ducklow HW, Erickson M (2011) Single-cell physiological structure and growth rates of heterotrophic bacteria in a temperate estuary (Waquoit Bay, Massachusetts). Limnol Oceanogr 56:37-48

Morán XAG, Bode A, Suárez LÁn, Nogueira E (2007) Assessing the relevance of nucleic acid content as an indicator of marine bacterial activity. Aquat Microb Ecol 46:141-152

Morán XAG, Calvo-Díaz A (2009) Single-cell vs. bulk activity properties of coastal bacterioplankton over an annual cycle in a temperate ecosystem. FEMS Microbiol Ecol 67:43-56

Nikrad MP, Cottrell MT, Kirchman DL (2012) Abundance and single-cell activity of heterotrophic bacterial groups in the western Arctic Ocean in summer and winter. Appl Environ Microbiol 78:2402-2409

Oremland RS, Capone DG (1988) Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv Microb Ecol 10:285-383

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis, Vol. Pergamon, Oxford

Price NM, Harrison PJ (1987) Comparison of methods for the analysis of dissolved urea in seawater. Mar Biol 94:307-317

Schattenhofer M, Wulf J, Kostadinov I, Glöckner FO, Zubkov MV, Fuchs BM (2011) Phylogenetic characterisation of picoplanktonic populations with high and low nucleic acid content in the North Atlantic Ocean. Syst Appl Microbiol 34:470-475

Servais P, Casamayor EO, Courties C, Catala P, Parthuisot N, Lebaron P (2003) Activity and diversity of bacterial cells with high and low nucleic acid content. Aquat Microb Ecol 33:41-51

Simpson KG, Tremblay J-É, Brugel S, Price NM (2013) Nutrient dynamics in the western Canadian Arctic. II. Estimates of new and regenerated production over the Mackenzie Shelf and Cape Bathurst Polynya. Mar Ecol Prog Ser 484:47-62

Vaqué D, Casamayor EO, Gasol JM (2001) Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquat Microb Ecol 25:163-177

Vila-Costa M, Gasol JM, Sharma S, Moran MA (2012) Community analysis of high- and low-nucleic acid-containing bacteria in NW Mediterranean coastal waters using 16S rDNA pyrosequencing. Environ Microbiol 14:1390-1402

Vrede K, Heldal M, Norland S, Bratbak G (2002) Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Appl Environ Microbiol 68:2965-2971

Wang Y, Hammes F, Boon N, Chami M, Egli T (2009) Isolation and characterization of low nucleic acid (LNA)-content bacteria. ISME J 3:889-902

Yager PL, Deming JW (1999) Pelagic microbial activity in an arctic polynya: Testing for temperature and substrate interactions using a kinetic approach. Limnol Oceanogr 44:1882-1893

Zubkov MV, Fuchs BM, Burkill PH, Amann R (2001) Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. Appl Environ Microbiol 67:5210-5218

Zubkov MV, Fuchs BM, Tarran GA, Burkill PH, Amann R (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. Appl Environ Microbiol 69:1299-1304

Zubkov MV, Tarran GA (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. Aquat Microb Ecol 40:241-249

Tables

Table 1. N uptake rates. The ambient N concentration ([N]), percent of cells in the high nucleic acid cluster (%HNA), specific uptake and absolute uptake for high nucleic acid (HNA) and low nucleic acid (LNA) populations.

Substrate added	Month	Year	[N] (μmol N L^{-1})	Bacterial Abundance (10^8 cells L^{-1})	%HNA	Specific Uptake ($x10^{-4}$ h^{-1}) LNA	Specific Uptake ($x10^{-4}$ h^{-1}) HNA	Absolute Uptake (pmol N L^{-1} h^{-1}) LNA	Absolute Uptake (pmol N L^{-1} h^{-1}) HNA
NH_4^+	April	2010	0.77	1.5	52	1.8	6.5	54	131
		2011	0.81	2.6	73	12	13	686	1121
	August	2010	0.47	3.2	72	1.4	29	67	414
	January	2011	1.9	2.7	49	3.4	5.6	129	166
		2012	0.55	2.7	46	12	9.4	322	548
NO_3^-	August	2010	0.20	3.2	74	1.4	4.9	29	60
	January	2011	5.8	2.7	48	1.8	2.8	41	50
Urea	August	2010	0.18	3.2	70	1.0	1.9	46	83
Amino	August	2010	0.14	3.2	91				
acids[1]						7.4	12	149	401
		2011	0.22	3.6	83	7.4	26	1782	4231
	January	2011	0.09	2.7	82	4.9	5.1	230	223
		2012	0.08	2.7	32	9.1	9.0	291	418

[1] Amino acid mixture consists of 20 amino acids labeled with ^{15}N.

Table 2. Cellular N content. Calculated values of mean and standard deviation of N per cell for each sampling season. Lower case n is for number of samples.

		fg N $cell^{-1}$		
Month	**Year**	**LNA**	**HNA**	**n**
April	2010	55	36	1
	2011	116	67	1
August	2010	74 ± 60	15 ± 9	5
	2011	56	8	1
January	2011	61 ± 49	28 ± 7	4
	2012	29 ± 13	70 ± 26	3

Figures

Figure 1. Flow cytometry dot plot. Characteristic flow cytometry density plot and adjacent histogram of low- and high-nucleic acid content bacterioplankton, labeled as LNA and HNA respectively. Darker colors, along with higher peaks on the histograms, indicate greater density.

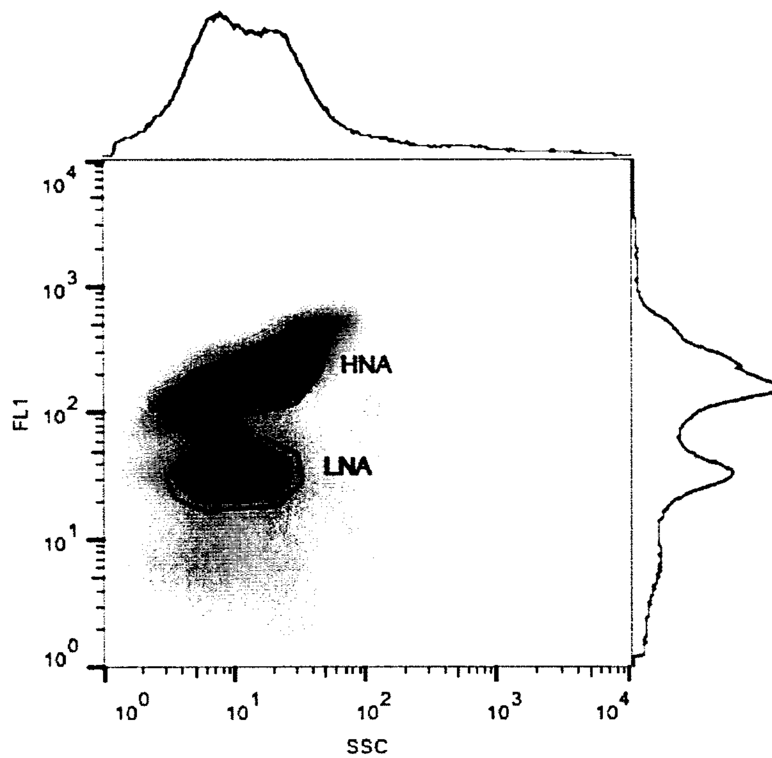
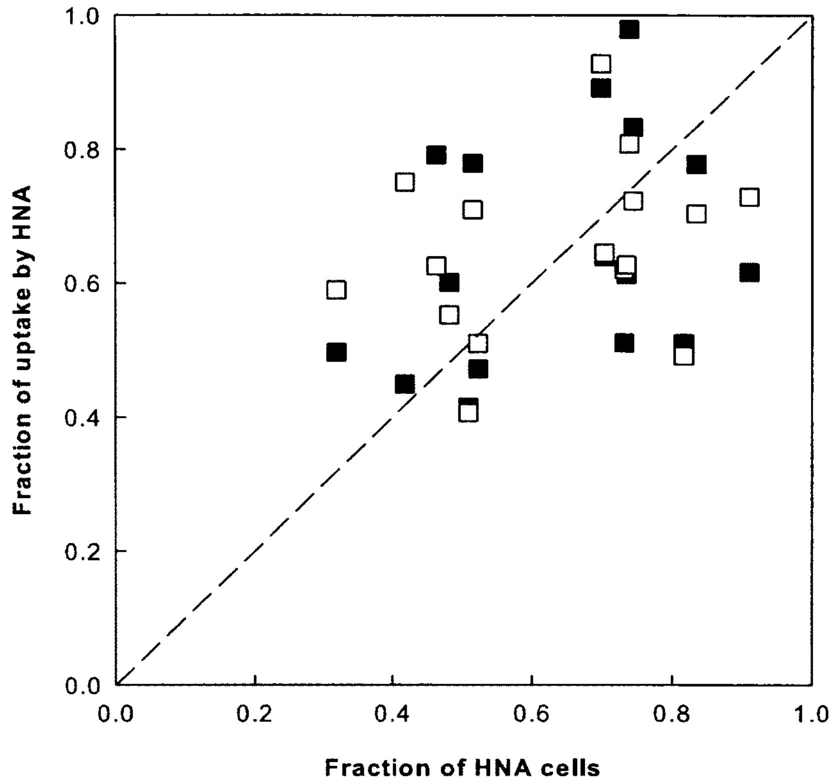


Figure 2. %HNA versus percent of uptake. Comparison of percent of high nucleic acid cells (%HNA) and the percent of specific (filled squares) and absolute (open squares) uptake in the HNA fraction. The dashed line represents 1:1 and is included solely for reference. Data include all substrates and seasons.



Chapter 4 – The effect of temperature on rates of ammonium uptake and nitrification in the western coastal Arctic Ocean during winter, spring, and summer

Abstract

Uptake and regeneration of ammonium (NH_4^+) play a key role in the structure and productivity of ecosystems. When nitrogen (N) is limiting, microbial community structure and function depend in part on whether NH_4^+ is assimilated directly into biomass or instead converted to nitrate (NO_3^-) via nitrification. It is not clear how these relative rates will change as the climate warms, especially at high latitudes. Here we present data on the sensitivity of NH_4^+ uptake and nitrification rates to short-term warming. Samples were collected from the Chukchi Sea off the coast of Barrow, Alaska during winter, spring, and summer and incubated for 24 hours in the dark with tracer-level additions of $^{15}NH_4^+$ at -1.5, 6, 13, and 20°C. Rates of NH_4^+ uptake and nitrification were measured in conjunction with bacterial production. In all seasons, NH_4^+ uptake rates were highest at 6 or 13°C, temperatures similar to current summertime conditions, but dropped off with increased warming, indicative of psychrophilic (i.e. cold-loving) microbial communities. In contrast, nitrification rates were less sensitive to temperature and were higher in winter and spring compared to summer. Contrary to expectations, a greater warming response (Q_{10} in excess of 2) was observed under higher in situ N substrate concentrations. These findings suggest that as the Arctic coastal ecosystem continues to warm, NH_4^+ assimilation may become increasingly important relative to nitrification, potentially reducing the NO_3^- available to primary producers.

1. Introduction

The Chukchi Sea receives significant nutrient inputs from the North Pacific Ocean [*Codispoti et al.*, 2005], and its coastal shelves are regions of high nitrogen (N) turnover [*Bates et al.*, 2005]. The western coastal Arctic is known for its high rates of denitrification [*Devol et al.*, 1997] and overall N limitation of phytoplankton growth [*Codispoti et al.*, 2009]. In the very shallow, nearshore area, there is a lack of established baseline data for N uptake rate measurements, especially during the dark and frigid winter months [*Codispoti et al.*, 2005].

In the euphotic zone, light is thought to inhibit nitrification [*Ward et al.*, 1984], and therefore diminish its importance, but recent evidence suggests this to be highly equivocal [*Yool et al.*, 2007]. Even if nitrification is inhibited by light, it may depend on which wavelength [*Guerrero and Jones*, 1996] or whether the dominant taxa are archaeal or bacterial nitrifiers [*Merbt et al.*, 2012]. Tremblay et al. [2008] hypothesized that winter nitrification, rather than physical processes could be the cause of consistent high NO_3^- concentrations in the Arctic during winter. Although rates for pelagic nitrification in the Arctic are limited [*Deal et al.*, 2011], there are numerous reports of nitrifying organisms in Arctic waters [*Alonso-Sáez et al.*, 2012; *Bano and Hollibaugh*, 2000; *Galand et al.*, 2009; *Kalanetra et al.*, 2009]. The one study that has reported a seasonal comparison of nitrification measurements in coastal Arctic waters found much higher rates during winter than summer and attributed this to the chemoautotrophic potential of the prokaryotic community during the cold and dark conditions under sea ice [*Christman et al.*, 2011].

There is an urgent need to understand N uptake and nitrification in the Arctic, as the region is warming faster than almost anywhere else on the planet [e.g. *Serreze and Francis*, 2006]. This warming has already caused reductions in sea ice extent and volume [*Stroeve et al.*, 2007], freshening of the surface ocean [*Yamamoto-Kawai et al.*, 2009], and numerous other impacts on the overall ecology of the system [*Grebmeier*, 2011; *Wassmann et al.*, 2011]. Additionally, the nutrient regime of the Arctic is changing, with decreasing Pacific nutrient inputs [*Codispoti et al.*, 2009] and increasing terrestrial runoff [*Peterson et al.*, 2002]. Less well understood is how these large-scale physical changes will impact the biogeochemistry of the Arctic.

As the Arctic continues to warm and the ice-free season expands, our understanding of how the rates of NH_4^+ uptake and nitrification may change is not well constrained, especially during seasons other than summer. It is generally expected that rate processes respond positively to temperature increases, although a recent meta-analysis indicated that bacterial activity is not more sensitive to temperature increases in the polar regions as compared to the temperate zone; rather, the increased dissolved organic matter supply in the Arctic can explain most of the large increase in rates reported at low temperatures [*Kirchman et al.*, 2009]. Synergy between temperature sensitivity and substrate concentration in Arctic marine systems has been reported for organic N compounds [*Wiebe et al.*, 1992; *Yager and Deming*, 1999], but there is limited information for inorganic N substrates. In culture, there is evidence of NO_3^- but not NH_4^+ uptake by psychrophiles being hampered at low temperatures [*Reay et al.*, 1999]. Antarctic sea ice algae had maximum uptake rates of inorganic N between 0.5 and 3.0°C,

below which both NO_3^- and NH_4^+ uptake decreased by at least half [*Priscu et al.*, 1989]. In this study, we tested the sensitivity of NH_4^+ uptake and nitrification to warming in nearshore Chukchi Sea waters by incubating winter, spring, and summer seawater samples under a range of temperatures. This study provides a current baseline for these processes along with insight into how they may change under the specter of anthropogenic warming.

2. Materials and Methods

Sampling and analytical methods were part of a larger study (see Chapter 2) investigating overall N uptake and regeneration in the coastal Chukchi Sea near Barrow, Alaska. Briefly, coastal seawater was sampled during January, April, and August of 2011, and again during January of 2012. Experiments were performed multiple times during each trip, with the exception of the first winter, and each sampling was given a station identifier (SID; Table 1). All of the sampling was done at approximately the same location (71°21'N, 156°41'W) with a bottom depth of 17 m, except for the very sampling event (WIN3), when dangerous ice conditions forced us to move approximately 1 km northeast to a shallower site (71°21'N, 156°34'W; 6 m maximum depth).

Winter and spring samples were collected by traveling to near the outer edge of the fast ice by snow machine and then drilling through the ice to sample the seawater below. Summer samples were collect from a small boat. Once we accessed the water column, a low pressure electric bilge pump (Johnson Pump) was used to gently draw water through acid-washed and seawater-seasoned 1.25" i.d. Tygon® tubing (Saint

Gobain Performance Plastics) into 500 ml acid-washed PETG bottles, and then inoculated with additions 0.2 μmol N L^{-1} of ^{15}N labeled ammonium chloride (98.85% ^{15}N NH_4Cl; Cambridge Isotope Laboratories) and 170 μmol C L^{-1} ^{13}C labeled sodium bicarbonate (99% ^{13}C $NaHCO_3^-$; Cambridge Isotope Laboratories). An additional set of incubations was performed with 0.2 μmol N L^{-1} of ^{15}N labeled sodium nitrite (98% ^{15}N $NaNO_2$; Cambridge Isotope Laboratories) added. Though we did not know the ambient concentrations at the time the incubations were set up, we used literature values to estimate a target addition at or below 10% of ambient concentrations; the $^{15}NH_4^+$ additions averaged 18.2% over all seasons, while nitrite (NO_2^-) additions were essentially 100% due to the general lack of measurable NO_2^- in the water column (see Results). To prevent freezing during collection and transport back to the laboratory, the bottles were placed in styrofoam freeze safes that were then filled with ambient seawater (see Table 1 for ambient water temperatures). The *in situ* mean water temperature in summer was 4.7°C so the lowest temperature incubation (i.e. -1.5°C) actually represents cooling of the sample. Temperature changes during transport were limited to less than 0.3°C during all seasons. Upon return to the lab, duplicate bottles were placed in dark water baths for 24 h at -1.5, 6, 13, and 20°C; digital thermometer probes were used to continuously monitor incubation temperatures. At the end of the incubation, samples were filtered through Whatman GF/F (0.7 μm nominal pore size) filters, which retained 61, 74, and 34% of the bacterial cells during summer, spring, and winter respectively (see Chapter 2). The filters were placed in cryovials and the filtrate into polypropylene tubes and frozen until analysis.

Bacterial production was measured using the leucine incorporation method [*Ducklow*, 2000; *Kirchman*, 2001; *Smith and Azam*, 1992], and was measured on the initial sample and following each treatment by incubating triplicate 1.5 ml aliquots with tritiated leucine (^{3}H-leu; specific activity of 144 Ci mmol^{-1}) at a final concentration of 25 nM for 4 h in the dark. Incubations were terminated by adding 0.1 ml of 100% trichloroacetic acid (TCA) to each sample tube. Samples were centrifuged and protein was extracted by rinsing the samples again with 1 ml of ice-cold TCA and then by rinsing with 1 ml of 80% ethyl alcohol, with centrifugation between each rinse. After placement in a fume hood overnight to dry, 1 ml of UltimaGold™ scintillation cocktail (PerkinElmer) was added to each tube and the radioactivity was measured on a liquid scintillation counter. Killed controls were performed by adding the ^{3}H-leu after killing the cells with the addition of TCA. Rates for the WIN1 experiment were anomalously inflated, but it is unclear why; Q_{10} results of that experiment are listed in Table 2, but not shown in Figure 3. During spring sampling, bacterial production from the same incubation bottles as the ^{15}N tracer was only measured at one station (SPR1) . In both cases (WIN1 and SPR1), we use the bacterial production results from a parallel set of incubations performed on the same water but without ^{15}N tracer added. In August 2011, no radioisotope was available, but bacterial production rates were measured at the same site during August 2010, and are used here for comparison to the seasonal production rates. In all three instances, they are denoted by italics (Table 2) and dashed lines (Figure 3).

Bacterial abundance was measured on the initial water sample, prior to incubation, and then again following incubation of the different treatments. Triplicate 3.6 ml samples were fixed with 400 μl of formaldehyde and refrigerated at 6°C for 15 minutes to allow complete fixation. They were subsequently frozen at -80°C until analysis. After staining with SYBR Green (Invitrogen) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7-2.2 μm), samples were run in duplicate on a FACScalibur flow cytometer (Becton Dickinson) and analyzed using FlowJo software (Treestar Inc.).

Concentration of NH_4^+ was measured in triplicate using the phenol-hypochlorite method [*Koroleff*, 1983]. Using a Lachat QuikChem 8500 autoanalyzer [*Parsons et al.*, 1984], concentrations of NO_3^- and NO_2^- were measured in duplicate. All ^{15}N and ^{13}C uptake samples were run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler. We used the calculations of Dugdale and Goering [1967] and Hama et al. [1983] for N and carbon (C) uptake rates respectively. Solid phase extraction [*Brzezinski*, 1987; *Dudek et al.*, 1986] was used to isolate the NH_4^+ pool so that the NH_4^+ atom % could be determined and used to correct for isotope dilution in calculations [*Glibert et al.*, 1982]. The denitrifier method [*Sigman et al.*, 2001] was used to determine the atom% of the NO_3^- pool. Rates of nitrification were calculated by tracing the labeled N from NH_4^+ into the NO_2^- and NO_3^- pools (NO_x) collectively.

$$nitrification = \frac{atom\%\ NO_x}{atom\%\ NH_4^+ \times time} \times [NO_x] \quad (1)$$

The atom percent (atom%) of each substrate was corrected to atom% normal by subtraction of 0.3667. Time in this equation is the total time of the incubation and the NO_x concentration is at the end of the incubation.

To assess the impact of temperature on the rates, Q_{10} values – which are a measure of the factor by which a biological rate is increased by a 10°C rise in temperature – were calculated for each positive change in rate at each temperature difference, by the following equation:

$$Q_{10} = \left(\frac{rate_2}{\text{rate}_1}\right)^{\frac{10}{T_2 - T_1}} \tag{2}$$

[*Segal*, 1975] where the rate is the measured rate at a specific temperature, T is temperature of the incubation, and the subscripts refer to two distinct temperature incubations.

Data were analyzed using two-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Method. Differences were considered significant at a p-value < 0.05. Due to the limited freezesafe space, we were only able to incubate a small number of replicates for each experiment.

3. Results

3.1. Ambient conditions

Water temperature during winter and spring was consistently -1.8°C. In summer of 2010, water temperature averaged 6.1°C, while in summer 2011, it was 4.7°C. Nutrient concentrations were highly variable from season to season and year to year in this extremely dynamic coastal environment. During January 2011, for example, the

ambient NH_4^+ concentration was three times higher than during January 2012 (Table 1). The seasonal minimum in both NH_4^+ and NO_3^- concentrations occurred during the summer, and there was a much smaller range in NH_4^+ concentrations than NO_3^- concentrations. The NO_3^- concentrations during the summer were only 3% of those during winter and spring (Table 1). There was high variability in the concentration of these N compounds, even within a season, potentially indicating the influence of different water masses passing through the study area. In our study NO_2^- was only detectable (limit = 0.03 μmol N L^{-1}) during one winter station (WIN3), and even then it was only 0.05 μmol N L^{-1}. As an intermediary in the nitrification process, the presence of NO_2^- can indicate active net NH_4^+ oxidation. Its absence, however, can indicate tightly coupled NH_4^+ and NO_2^- oxidation or an absence of nitrification all together. We hypothesize that the low or undetectable NO_2^- concentrations in this study indicate the former.

During January of 2011, bacterial abundance in the initial experimental samples from 2 m were $4.6 \pm 0.2 \times 10^8$ cells L^{-1}, whereas in January of 2012 abundances were lower (2.5 - 2.9 $\times 10^8$ cells L^{-1}; Table 1). During April, bacterial abundance was similar or slightly elevated (2.1 - 3.9 $\times 10^8$ cells L^{-1}) compared to January, three to eight times higher (15 - 18 $\times 10^8$ cells L^{-1}) in August.

3.2. Uptake of nitrogen and carbon

During January of 2011, when the ambient NH_4^+ concentration was three times higher, the uptake rate at *in situ* temperature (-1.5°C) was more than double that of 2012

for all temperatures except 13°C during WIN3 (Figure 1). The response to warming was similarly enhanced by the higher NH_4^+ concentrations in 2011, with higher absolute rates, and a greater increase with warming. The rates peak at 6°C and plateau to a statistically equal value at 13°C, with a slight decrease at 20°C. Calculations of Q_{10} (Table 2) reflect a strong sensitivity to temperature (3.1) from -1.5 to 6°C, and a Q_{10} of 1 (indicating no additional temperature response) for 6 to 13°C. The January 2012 experiments (WIN2 and WIN3) both had maxima at 13°C with rates otherwise lower or equal among the other temperature ranges. The Q_{10} values reflect this strong temperature sensitivity with a 6 to 13°C $Q_{10} > 4$ at WIN3, although WIN2 had a Q10 of 2.

During spring, one of the stations (SPR3) had a strikingly similar pattern of NH_4^+ uptake to winter 2012 (WIN2 and WIN3), but at slightly reduced rates. Peaks in uptake rate were at 13°C, with a steep decline at 20°C, where the rate was less than those at -1.5 and 6°C. Uptake rates at SPR1 and SPR2 peaked at statistically equal rates in the 6 and 13°C incubations.

During the summer, uptake rates were significantly higher and the effect of warming was similar. The peak NH_4^+ uptake rate was measured at 13°C and was significantly different from -1.5°C ($p < 0.001$). The Q_{10} values were above 2 for the -1.5 to 6°C increase during SUM2, but lower than 2 for the 6 to 13 and overall -1 to 13°C temperature increases. Again showing sensitivity to higher ambient NH_4^+ concentrations, both the absolute rates and Q_{10} values were higher for all temperature increases during SUM1.

There was no increase in bacterial abundance or particulate N (data not shown) at the different temperature incubations. Cell-specific uptake rates of NH_4^+ (data not shown) had a similar relative pattern to the whole community rates, indicating that the change in rates is likely a physiological response, and not due to any short-term increases in biomass.

Ambient NO_2^- was below our detection limit in all but a one set of samples (Table 1) and uptake rates were extremely low. The mean uptake rates at ambient temperature were 0.004 ± 0.002, 0.003 ± 0.000, and 0.043 ± 0.032 nmol L^{-1} h^{-1} for winter, spring, and summer, respectively. There was no discernible pattern to NO_2^- uptake with changes in temperature, although the mean values for winter stations show a potential peak at 13°C, and the summer stations peak at either 13 or 20°C, but are not significantly different from the other temperatures.

Similarly, dark uptake of HCO_3^- was low and only measurable during the summer. HCO_3^- uptake displayed two maxima at the lowest and highest temperatures, with a minimum at the intermediate temperatures near *in situ* (4.7°C). Mean rates for the summer were 1.57 ± 0.46, 0.82 ± 0.18, 0.89 ± 0.07, and 1.58 ± 0.87 nmol C L^{-1} h^{-1} for the -1.5, 6, 13, and 20°C treatments, respectively. Since incubations were performed in the dark, HCO_3^- uptake rates that we did find are likely due to a delay in the shutdown of the C fixation process.

3.3. Nitrification

During the ice-covered seasons of winter and spring, nitrification rates were much higher than NH_4^+ uptake rates. Nitrification rates had no statistically significant temperature maxima for any season (Figure 2). Overall, nitrification rates were two orders of magnitude higher during winter and spring than summer, and appear to be sensitive to high concentrations of NH_4^+, as WIN1 and SPR3 both had much higher relative rates that correlate to higher ambient NH_4^+ (Table 1). Consistent with a lack of effect, Q_{10} values for nitrification were approximately 1 for all temperature differences during winter and spring (Table 2). Although one summer station (SUM1) had a slight peak at 13°C, and the Q_{10} values are slightly higher for the lower two temperature ranges, the actual rate of nitrification during the summer was so small that any increase would likely have little impact on ecological processes. When we calculate the assimilation and nitrification rates over the time period of the incubation, there would still be enough NH_4^+ in the bottles to measure increased nitrification rates at all temperatures, and we conclude that there is no ceiling on the rate.

3.4. Bacterial production

Leucine incorporation rates followed a similar pattern as NH_4^+ uptake rates. During winter and spring, there is a peak at 13°C after which the rate falls, although the difference during spring is less pronounced (Figure 3). The Q_{10} values for the -1.5 to 6°C range during winter and spring averaged 3.2 ± 0.5 and 2.7 ± 0.3 for each of those seasons, respectively (Table 2). The next temperature range (6 to 13°C), at an average Q_{10} of 2.0

± 0.9 and 1.7 ± 0.6, were also not significantly different. At their respective ambient temperatures, bacterial production at station WIN3 was only slightly less than the spring mean. This is an interesting contrast to the results for the NH_4^+ uptake and nitrification, and may be due to sampling at a shallower site closer to the Beaufort Sea, which had higher dissolved organic C (DOC) concentrations. We found no stimulation of bacterial production compared to our controls that had no ^{15}N substrates added (data not shown).

4. Discussion

Microbes have adapted to many extreme environments that seem to test the limits of life [*D'Amico et al.*, 2006]. In extremely cold regions, the uptake of substrates at low temperatures (-1.5 to 4°C) can be significantly reduced due to decreased cell membrane fluidity [*Nedwell*, 1999]. To counteract this limitation, an increase in substrate concentrations can overcome the negative effect of low temperature on both mesophilic and psychrotolerant bacteria [*Wiebe et al.*, 1992], although this effect is not always observed [*Kirchman et al.*, 2005; *Kirchman et al.*, 2009; *Yager and Deming*, 1999]. In this study, we sought to quantify the extent to which Arctic microplankton in their natural setting can utilize NH_4^+ over a seasonal cycle, and how they may respond to warming.

4.1. Ammonium uptake

The uptake of NH_4^+ displayed a distinct pattern of a prominent temperature effect, with an optimum at 13°C (Figure 1). Laboratory studies of pure cultures report microbes with low temperature optima, but found that NH_4^+ uptake was not temperature sensitive

[*Reay et al.*, 1999]. However, using a meta-analysis of environmental samples of dark NH_4^+ uptake, Smith and Harrison [1991] found Q_{10} values greater than 2 for polar regions. Additionally, Antarctic sea ice algae were found to have low temperature maxima for N uptake and an extraordinarily high Q_{10} of 15.7 for NH_4^+ uptake between 2.0 and 3.0°C [*Priscu et al.*, 1989]. Our results in the spring and summer consistently show the greatest increase for the -1.5 to 6°C temperature change, with a range of Q_{10} range of 1.4 - 3.2 (Table 2). During the winter, the same relationship holds in 2011, but in the 2012 season, the maximum sensitivity was found between the 6 to 13°C incubations.

While the community as a whole responds to increases in temperature, there is also an underlying signal of an enhanced response to increased ambient NH_4^+ concentrations, with a potential for synergistic responses if both factors change in the future. Both the NH_4^+ absolute uptake rates and the rate of increase with temperature (as measured by Q_{10}) were positively correlated with higher NH_4^+ concentrations at the beginning of the incubations. Therefore additive effects of increased nutrient supply and temperature should be considered when projecting future biogeochemical scenarios of the coastal Arctic. These factors are already coupled, as temperature increases are reducing ice extent and volume, which could lead to earlier increases in nutrient supply from the receding ice edge, changing the timing of phytoplankton blooms, and increasing and extending the time of dependence on regenerated production [*Wassmann and Reigstad*, 2011].

4.2. Nitrification

Consistent with other cold oceanic regions, nitrification rates were much higher than NH_4^+ uptake rates during the ice-covered seasons of winter and spring. In the North Sea over a winter cycle, Veuger et al. [2013] found that nitrification accounted for 89% of NH_4^+ sinks (defined as nitrification and NH_4^+ assimilation), which was much higher than rates from temperate areas where nitrification and assimilation were measured simultaneously [*Lipschultz et al.*, 1986; *Ward*, 2005]. At our site, winter and spring nitrification was even more dominant, accounting for 99% of NH_4^+ consumption. This relationship holds even though there was a large difference in the year-to-year rate measured in winter at this study site. These differences in rates are likely due to higher NH_4^+ concentrations during 2011 (see below). In fact, even our highest measured rates are on the low end of a North Sea winter experiment, where the conditions included higher temperatures and NH_4^+ concentrations than our study, and measured nitrification rates ranging 41 – 221 nmol N L^{-1} h^{-1} [*Veuger et al.*, 2013]. Similarly, our summer rate measurements are lower than nitrification rates measured in the perennially cold waters of the Southern Ocean [*Bianchi et al.*, 1997]. During summer, the situation reverses, with NH_4^+ assimilation representing 91% of consumption processes. While we found nitrification rates that were exceedingly low and unchanging with temperature, NH_4^+ uptake is significantly higher ($p < 0.05$) during the summer compared to the other seasons.

Psychrophiles are known to nitrify at rates comparable to temperate mesophiles, but strong relationships between nitrification and environmental or ecological factors in the water column have yet to be established [*Ward*, 2008]. Phytoplankton can

outcompete nitrifiers for NH_4^+ in well-lit ocean layers [*Ward*, 2005], and that is likely the case during the polar summer. During the winter, when primary production is light limited, there is reduced competition from bacterial and archaeal N demand. In addition to light, temperature is often suggested to be a controlling environmental factor, at least for organic N [*Pomeroy et al.*, 1990; *Pomeroy et al.*, 1991; *Wiebe et al.*, 1992]. Conversely, polar microorganisms are generally cold-adapted and polar bacteria only sometimes exhibit sensitivity to organic substrate availability as temperature decreases [*Yager and Deming*, 1999]. Uptake of inorganic N may exhibit a similar sensitivity. A recent set of experiments in the Puget Sound where incubations found no change in nitrification rates with the different temperature incubations ranging from 8 – 20°C [*Horak et al.*, accepted]. In the Southern Ocean, Bianchi et al. [1997] also found no correlation between nitrification rate and temperature nor ambient NH_4^+ concentrations. Our results confirm that N uptake at low substrate concentrations is less sensitive to warming than when substrate concentrations are higher. This result may indicate that the more cold-tolerant members of polar microbial communities require higher substrate [e.g. *Wiebe et al.*, 1992], whereas the true psychrophiles are less sensitive to substrate concentrations [*Yager and Deming*, 1999].

While there are no other studies that we know of testing the sensitivity of nitrification to temperature in polar waters, one ammonia oxidizer capable of growth down to -5°C has been isolated from southern Alaskan waters (sub-Arctic Pacific) but even when acclimated to low temperatures, this organism had an optimum temperature for nitrification of 22°C [*Jones et al.*, 1988]. Nitrification rates have been subjected to

warming experiments in Arctic marine sediments and terrestrial soils, the latter of which are warming at an alarming rate. In Arctic marine sediments, optimum temperatures for nitrification are very low and rates decrease markedly when subjected to experimental warming [*Thamdrup and Fleischer*, 1998]. In Arctic soils, nitrifiers only responded to temperature above 10°C [*Nadelhoffer et al.*, 1991], at which point there was a two-fold increase in N mineralization. Avrahami and Conrad [2003] found subtle changes in bacterial community structure with increases in temperature. Much like our study, these temperatures are above the current normal range, but could portend a future in which nitrifier community shifts trigger rapid changes in NH_4^+ assimilation rates.

The microbial community itself is known to change over the seasonal cycle. Bacterial nitrifiers have been found during summer in Arctic surface waters [*Hollibaugh et al.*, 2002]. During the Arctic winter, there is high crenarchaeal abundance [*Alonso-Sáez et al.*, 2008] and these organisms are known nitrifiers both in the Arctic Ocean [*Christman et al., 2011*] and in other oceanic realms [*Wuchter et al.*, 2006]. Crenarchaeota have been found with high affinities for NH_4^+ at both low [*Martens-Habbena et al.*, 2009] and high [*Morris et al.*, 2010] substrate concentrations, and have even recently been shown to use urea as both a C source and substrate for nitrification in the Arctic [*Alonso-Sáez et al.*, 2012]. It has been proposed that Crenarchaea are responsible for high winter rates seen in this region [*Christman et al.*, 2011], but this supposition has not been proven, as Kalanetra et al. [2009] found no genetic mechanism for nitrification (i.e. it lacked an ammonium monooxygenase gene) in central Arctic archaea, and abundance was not related to activity in another study of nitrification in high

latitude marine waters [*Veuger et al.*, 2013]. On the other hand, Christman et al. [2011] found ammonium monooxygenase (which encodes for the enzyme responsible for the first step in nitrification process) rRNA copy numbers to be positively correlated with both the winter season and increasing NH_4^+ concentrations. While not tested directly in our study, the artificial lifting of these inhibitory or competitive conditions through dark incubations did not increase the rates, indicating that the native coastal bacterial community is sufficiently different during the summer season to respond. Another possible explanation is simply the difference in sampling location between the coastal and central Arctic, which may have distinct populations and metabolic capabilities [*Kirchman et al.*, 2007].

4.3. Bacterial production

It has been proposed that bacterial production in the polar regions depends more on DOM supply than temperature [*Kirchman et al.*, 2009]. In this experiment, where we artificially warmed the incubations on short time scales, the strongest bacterial response occurred in the first step warmer from ambient temperature (i.e. -1.5 to 6°C during winter/spring, and 6 to 13°C during summer), while the optimum temperature was 13°C for winter and spring. The bacterial community is therefore cold-loving or psychrophilic by traditional definitions [*Morita*, 1966; 1975]. Production increases were more pronounced in the winter of 2011 and the shallow site sampled in winter 2012 (WIN3). DOC was higher during winter than spring, and the highest concentration outside of summer was measured at WIN3, which could provide an explanation for the heightened

production during winter. A companion study performed during the summer of the prior year found a consistent rise in bacterial production with temperature, with an optimum at 20°C [*Connelly et al.*, in preparation-a], which is the same relative increase from ambient temperature as the winter and spring incubations in our study (Figure 3). It is clear that the microbial community reacts to temperature increases the same way year-round while DOC concentrations are also potentially influential.

It is not surprising that we were unable to quantify C fixation during the winter and spring periods, as autotrophic production would ostensibly be very limited in the absence of light. On the other hand, chemoautotrophic organisms are likely present under sea ice, and one study reports HCO_3^- uptake during the dark Arctic winter [*Alonso-Sáez et al.*, 2010]. The fact that we did not observe dark C fixation in our study could indicate its absence or could be due to bacterial cells passing through the GF/F filters or that we did not add enough ^{13}C label to discern a signal. Evidence for the latter is found in a companion study [*Connelly et al.*, in preparation-b] where HCO_3^- uptake was only measurable during winter in incubations with greater concentration of labeled HCO_3^- and termination on smaller pore size filters (0.2 μm).

5. Conclusions

The future of the Arctic marine ecosystem is unknown, but the region is certainly changing. Rising air and water temperatures have already been recorded, along with subsequent losses in sea ice volume [e.g. *Stroeve et al., 2012*] While there is some evidence that chlorophyll *a* in the coastal Arctic has been declining during the past

century [*Boyce et al.*, 2010], it is expected that there will be increased annual phytoplankton production [*Arrigo et al.*, 2008] and associated increased importance of DOC and bacterial activity [*Kirchman et al.*, 2009]. In Arctic freshwater systems, higher bacterial and viral activity have already been observed [*Säwström et al.*, 2007], and the marine system could be expected to follow the same trend. Based on the results of our study, we would expect further increases in NH_4^+ uptake during each season in the future, especially if the region warms faster during the summer months than winter and spring [*Wang et al.*, 2012]. As light limitation is lifted earlier in the spring [*Maslanik et al.*, 2007], nitrification processes will be at an even further disadvantage, driving the system closer to dependence on NH_4^+ supplies, rather than any NO_3^- built up in the system during the ice-covered seasons. We have shown a large disparity in the rate of nitrification during the seasons sampled, with a potential dependence on nutrient supply. Even though we tested a wide range of temperatures, no changes were seen in the nitrification rates. Uptake of NH_4^+ on the other hand is highly sensitive to warming and our results suggest that the biogeochemistry and ecology of the western coastal Arctic will be impacted in the coming decades as the region warms.

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Literature cited

Alonso-Sáez, L., O. Sánchez, J. M. Gasol, V. Balagué, and C. Pedrós-Alio (2008), Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes, *Environ. Microbiol.*, *10*(9), 2444-2454.

Alonso-Sáez, L., P. E. Galand, E. O. Casamayor, C. Pedrós-Alió, and S. Bertilsson (2010), High bicarbonate assimilation in the dark by Arctic bacteria, *ISME J.*, *4*(12), 1581-1590.

Alonso-Sáez, L., et al. (2012), Role for urea in nitrification by polar marine Archaea, *P. Natl. Acad. Sci. USA*, *109*(44), 17989-17994.

Arrigo, K. R., G. van Dijken, and S. Pabi (2008), Impact of a shrinking Arctic ice cover on marine primary production, *Geophys. Res. Lett.*, *35*, L19603, doi:19610.11029/12008GL035028.

Avrahami, S., and R. Conrad (2003), Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures, *Appl. Environ. Microbiol.*, *69*(10), 6152-6164.

Bano, N., and J. T. Hollibaugh (2000), Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the β subdivision of the class proteobacteria in the Arctic Ocean, *Appl. Environ. Microbiol.*, *66*(5), 1960-1969.

Bates, N. R., M. H. P. Best, and D. A. Hansell (2005), Spatio-temporal distribution of dissolved inorganic carbon and net community production in the Chukchi and Beaufort Seas, *Deep-Sea Res. Pt II*, *52*(24), 3303-3323.

Bianchi, M., F. Feliatra, P. Tréuger, M.-A. Vincendeau, and J. Morvan (1997), Nitrification rates, ammonium and nitrate distribution in upper layers of the water column and in sediments of the Indian sector of the Southern Ocean, *Deep-Sea Res. Pt II*, *44*(5), 1017-1032.

Boyce, D. G., M. R. Lewis, and B. Worm (2010), Global phytoplankton decline over the past century, *Nature*, *466*(7306), 591-596.

Brzezinski, M. A. (1987), Colorimetric determination of nanomolar concentrations of ammonium in seawater using solvent extraction, *Mar. Chem.*, *20*(3), 277-288.

Christman, G. D., M. T. Cottrell, B. N. Popp, E. Gier, and D. L. Kirchman (2011), Abundance, diversity, and activity of ammonia-oxidizing prokaryotes in the

coastal Arctic Ocean in summer and winter, *Appl. Environ. Microbiol.*, *77*(6), 2026-2034.

Codispoti, L. A., C. N. Flagg, and J. H. Swift (2009), Hydrographic conditions during the 2004 SBI process experiments, *Deep-Sea Res. Pt II*, *56*(17), 1144-1163.

Codispoti, L. A., C. Flagg, V. Kelly, and J. H. Swift (2005), Hydrographic conditions during the 2002 SBI process experiments, *Deep-Sea Res. Pt II*, *52*(24-26), 3199-3226.

Connelly, T. L., K. Sines, and P. L. Yager (in preparation-a), A three-season study of heterotrophic microbial activity in a near-shore environment off Barrow Alaksa and the response of these communities to short-term warming.

Connelly, T. L., S. E. Baer, B. Wawrik, and D. A. Bronk (in preparation-b), Species-specific bicarbonate uptake during the dark Arctic winter as determined by stable isotope probing.

D'Amico, S., T. Collins, J.-C. Marx, G. Feller, and C. Gerday (2006), Psychrophilic microorganisms: challenges for life, *EMBO reports*, *7*, 385-389.

Deal, C., M. Jin, S. Elliott, E. Hunke, M. Maltrud, and N. Jeffery (2011), Large-scale modeling of primary production and ice algal biomass within Arctic sea ice in 1992, *J. Geophys. Res.*, *116*, C07004, doi:07010.01029/02010JC006409.

Devol, A. H., L. A. Codispoti, and J. P. Christensen (1997), Summer and winter denitrification rates in western Arctic shelf sediments, *Cont. Shelf Res.*, *17*(9), 1029-1050.

Ducklow, H. (2000), Bacterial production and biomass in the oceans, in *Microbial Ecology of the Oceans*, edited by D. L. Kirchman, pp. 85-120, Wiley-Liss, New York.

Dudek, N., M. A. Brzezinski, and P. A. Wheeler (1986), Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments, *Mar. Chem.*, *18*(1), 59-69.

Dugdale, R. C., and J. J. Goering (1967), Uptake of new and regenerated forms of nitrogen in primary productivity, *Limnol. Oceanogr.*, *12*(2), 196-206.

Galand, P. E., C. Lovejoy, A. K. Hamilton, R. G. Ingram, E. Pedneault, and E. C. Carmack (2009), Archaeal diversity and a gene for ammonia oxidation are coupled to oceanic circulation, *Environ. Microbiol.*, *11*(1), 971-980.

Glibert, P. M., F. Lispschultz, J. J. McCarthy, and M. A. Altabet (1982), Isotope dilution models of uptake ammonium by marine plankton, *Limnol. Oceanogr.*, *27*(4), 639-650.

Grebmeier, J. M. (2011), Shifting patterns of life in the Pacific Arctic and sub-Arctic seas, *Annu. Rev. Mar. Sci.*, *4*(1), 63-78.

Guerrero, M. A., and R. D. Jones (1996), Photoinhibition of marine nitrifying bacteria I. Wavelength-dependent response, *Mar. Ecol. Prog. Ser.*, *141*(1), 183-192.

Hama, T., T. Miyazaki, Y. Ogawa, T. Iwakuma, M. Takahashi, A. Otsuki, and S. Ichimura (1983), Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope, *Mar. Biol.*, *73*(1), 31-36.

Hollibaugh, J. T., N. Bano, and H. W. Ducklow (2002), Widespread distribution in polar oceans of a 16S rRNA gene sequence with affinity to *Nitrosospira*-like ammonia-oxidizing bacteria, *Appl. Environ. Microbiol.*, *68*(3), 1478-1484.

Horak, R. E. A., W. Qin, A. Schauer, E. V. Armbrust, A. E. Ingalls, J. W. Moffett, D. A. Stahl, and A. H. Devol (accepted), Ammonia oxidation kinetics and temperature sensitivity of a natural marine community dominated by Archaea, *ISME J.*

Jones, R. D., R. Y. Morita, H.-P. Koops, and S. W. Watson (1988), A new marine ammonium-oxidizing bacterium, *Nitrosomonas cryotolerans* sp. nov., *Can. J. Microbiol.*, *34*(10), 1122-1128.

Kalanetra, K. M., N. Bano, and J. T. Hollibaugh (2009), Ammonia-oxidizing archaea in the Arctic Ocean and Antarctic coastal waters, *Environ. Microbiol.*, *11*(9), 2434-2445.

Kirchman, D. (2001), Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments., *Methods in microbiology*, *30*(2), 227-237.

Kirchman, D. L., R. R. Malmstrom, and M. T. Cottrell (2005), Control of bacterial growth by temperature and organic matter in the Western Arctic, *Deep-Sea Res. Pt II*, *52*(24-26), 3386-3395.

Kirchman, D. L., X. A. G. Morán, and H. Ducklow (2009), Microbial growth in the polar oceans — role of temperature and potential impact of climate change, *Nat. Rev. Microbiol.*, *7*(6), 451-459.

Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell (2007), Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean, *Limnol. Oceanogr.*, *52*(2), 495-507.

Koroleff, F. (1983), Determination of nutrients, in *Methods of seawater analysis*, edited by K. Grasshoff, M. Ehrhardt and K. Kremling, pp. 125-187, Verlag Chemie, New York.

Lipschultz, F., S. C. Wofsy, and L. E. Fox (1986), Nitrogen metabolism of the eutrophic Delaware River ecosystem, *Limnol. Oceanogr.*, *31*(4), 701-716.

Martens-Habbena, W., P. M. Berube, H. Urakawa, J. R. de la Torre, and D. A. Stahl (2009), Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria, *Nature*, *461*(7266), 976-969.

Maslanik, J., C. Fowler, J. Stroeve, S. Drobot, H. Zwally, D. Yi, and W. Emery (2007), A younger, thinner ice cover: increased potential for rapid, extensive ice loss, *Geophys. Res. Lett.*, *34*, L24501, doi:24510.21029/22007GL032043.

Merbt, S. N., D. A. Stahl, E. O. Casamayor, E. Martí, G. W. Nicol, and J. I. Prosser (2012), Differential photoinhibition of bacterial and archaeal ammonia oxidation, *FEMS Microbiol. Lett.*, *327*(1), 41-46.

Morita, R. Y. (1966), Marine psychrophilic bacteria, *Oceanogr. Mar. Biol. Annu. Rev.*, *4*, 105-121.

Morita, R. Y. (1975), Psycrophilic bacteria, *Bacteriological Reviews*, *39*(2), 144-167.

Morris, R. M., B. L. Nunn, C. Frazar, D. R. Goodlett, Y. S. Ting, and G. Rocap (2010), Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction, *ISME J.*, *4*(5), 673-685.

Nadelhoffer, K. J., A. E. Giblin, G. R. Shaver, and J. A. Laundre (1991), Effects of temperature and substrate quality on element mineralization in six arctic soils, *Ecology*, *72*(1), 242-253.

Nedwell, D. B. (1999), Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature, *FEMS Microbiol. Ecol.*, *30*(2), 101-111.

Parsons, T. R., Y. Maita, and C. M. Lalli (1984), *A manual of chemical and biological methods for seawater analysis*, Pergamon, Oxford.

Peterson, B. J., R. M. Holmes, J. W. McClelland, C. J. Vörösmarty, R. B. Lammers, A. I. Shiklomanov, I. A. Shiklomanov, and S. Rahmstorf (2002), Increasing river discharge to the Arctic Ocean, *Science*, *298*(5601), 2171-2173.

Pomeroy, L. R., S. A. Macko, P. H. Ostrom, and J. Dunphy (1990), The microbial food web in Arctic seawater: concentration of dissolved free amino acids and bacterial abundance and activity in the Arctic Ocean and in Resolute Passage, *Mar. Ecol. Prog. Ser.*, *61*(1), 31-40.

Pomeroy, L. R., D. Deibel, R. J. Thompson, G. T. Rowe, and J. D. Pakulski (1991), Bacterial responses to temperature and substrate concentration during the Newfoundland spring bloom, *Mar. Ecol. Prog. Ser.*, *75*(2), 143-159.

Priscu, J. C., A. C. Palmisano, L. R. Priscu, and C. W. Sullivan (1989), Temperature dependence of inorganic nitrogen uptake and assimilation in Antarctic sea-ice microalgae, *Polar Biol.*, *9*(7), 443-446.

R Core Team (2012), R: A language and environment for statistical computing, edited, R Foundation for Statistical Computing, Vienna, Austria.

Reay, D. S., D. B. Nedwell, J. Priddle, and J. C. Ellis-Evans (1999), Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at

suboptimal temperatures in both algae and bacteria, *Appl. Environ. Microbiol.*, *65*(6), 2577-2584.

Säwström, C., J. Laybourn-Parry, W. Granéli, and A. M. Anesio (2007), Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments, *Polar Biol.*, *30*(11), 1407-1415.

Segal, I. H. (1975), *Enzyme kinetics*, John Wiley and Sons, New York.

Serreze, M. C., and J. A. Francis (2006), The Arctic amplification debate, *Clim. Change*, *76*(3-4), 241-264.

Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Böhlke (2001), A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater, *Anal. Chem.*, *73*(17), 4145-4153.

Smith, D. C., and F. Azam (1992), A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine, *Marine Microbial Food Webs*, *6*(2), 107-114.

Smith, W., Jr., and W. G. Harrison (1991), New production in polar regions: the role of environmental controls, *Deep-Sea Research*, *38*(12), 1463-1479.

Stroeve, J., M. M. Holland, W. Meier, T. Scambos, and M. Serreze (2007), Arctic sea ice decline: Faster than forecast, *Geophys. Res. Lett.*, *34*(9), L09501, doi:09510.01029/02007gl029703.

Stroeve, J. C., M. C. Serreze, M. M. Holland, J. E. Kay, J. Malanik, and A. P. Barrett (2012), The Arctic's rapidly shrinking sea ice cover: a research synthesis, *Clim. Change*, *110*(3-4), 1005-1027.

Thamdrup, B., and S. Fleischer (1998), Temperature dependence of oxygen respiration, nitrogen mineralization, and nitrification in Arctic sediments, *Aquat. Microb. Ecol.*, *15*(2), 191-199.

Tremblay, J.-É., K. Simpson, J. Martin, L. Miller, Y. Gratton, D. Barber, and N. M. Price (2008), Vertical stability and the annual dynamics of nutrients and chlorophyll fluorescence in the coastal, southeast Beaufort Sea, *J. Geophys. Res.*, *113*, C07S90, doi:10.1029/2007JC004547.

Veuger, B., A. Pitcher, S. Schouten, J. S. Sinninghe Damsté, and J. J. Middelburg (2013), Nitrification and growth of autotrophic nitrifying bacteria and Thaumarchaeota in the coastal North Sea, *Biogeosciences*, *10*, 1775-1785.

Wang, M., J. E. Overland, and P. Stabeno (2012), Future climate of the Bering and Chukchi Seas projected by global climate models, *Deep-Sea Res. Pt II*, *65-70*, 46-57.

Ward, B. B. (2005), Temporal variability in nitrification rates and related biogeochemical factors in Monterey Bay, California, USA, *Mar. Ecol. Prog. Ser.*, *292*, 97-109.

Ward, B. B. (2008), Nitrification in marine systems, in *Nitrogen in the Marine Environment*, edited by D. A. Bronk, D. G. Capone, E. J. Carpenter and M. R. Mulholland, pp. 199-262, Elsevier Inc.

Ward, B. B., M. C. Talbot, and M. J. Perry (1984), Contributions of phytoplankton and nitrifying bacteria to ammonium and nitrite dynamics in coastal waters, *Cont. Shelf Res.*, *3*(4), 383-398.

Wassmann, P., and M. Reigstad (2011), Future Arctic Ocean seasonal ice zones and implications for pelagic-benthic coupling, *Oceanography*, *24*(3), 220-231.

Wassmann, P., C. M. Duarte, S. AgustÍ, and M. K. Sejr (2011), Footprints of climate change in the Arctic marine ecosystem, *Global Change Biol.*, *17*(2), 1235-1249.

Wiebe, W. J., W. M. Sheldon, and L. R. Pomeroy (1992), Bacterial growth in the cold: Evidence for an enhanced substrate requirement, *Appl. Environ. Microbiol.*, *58*(1), 359-364.

Wuchter, C., et al. (2006), Archaeal nitrification in the ocean, *P. Natl. Acad. Sci. USA*, *103*(33), 12317-12322.

Yager, P. L., and J. W. Deming (1999), Pelagic microbial activity in an arctic polynya: Testing for temperature and substrate interactions using a kinetic approach, *Limnol. Oceanogr.*, *44*(8), 1882-1893.

Yamamoto-Kawai, M., F. A. McLaughlin, E. C. Carmack, S. Nishino, K. Shimada, and N. Kurita (2009), Surface freshening of the Canada Basin, 2003–2007: River runoff versus sea ice meltwater, *J. Geophys. Res.-Oceans*, *114*(C1), C00A05, doi:10.1029/2008JC005000,.

Yool, A., A. P. Martin, C. Fernández, and D. R. Clark (2007), The significance of nitrification for oceanic new production, *Nature*, *447*(7147), 999-1002.

Tables

Table 1. Chemical and biological parameters. Ambient water temperature, and nutrient and plankton concentrations at the time of water collection for each experiment and season. The station ID (SID) is as defined in the text. "Depth" is the depth sample taken. BDL is for below detection limit (0.03 μM).

Date	SID	Depth (m)	Ambient Temp. (°C)	NH_4^+ (μmol N L^{-1})	NO_2^- (μmol N L^{-1})	NO_3^- (μmol N L^{-1})	DOC (μmol C L^{-1})	Chl *a* (ug L^{-1})	Bacterial Abundance (10^8 cells L^{-1})
2011									
Jan 30	**WIN1**	2	-1.9	3.07	BDL	7.66	74.0	0.03	4.6 ± 0.2
Apr 26	**SPR1**	6.5	-1.6	0.81	BDL	8.41	67.9	0.11	2.6 ± 0.1
Apr 28	**SPR2**	6.5	-1.6	0.51	BDL	8.57	67.6	0.10	2.1 ± 0.2
Apr 30	**SPR3**	4	-1.8	1.25	BDL	11.4	67.2	0.06	3.9 ± 0.5
Aug 17	**SUM1**	4	+4.7	0.59	BDL	0.32	93.8	0.37	18 ± 0.5
Aug 18	**SUM2**	2	+4.7	0.47	BDL	0.33	93.7	0.62	15 ± 0.3
2012									
Jan 16	**WIN2**	2	-1.8	0.60	BDL	11.7	82.3	0.03	2.5 ± 0.1
Jan 19	**WIN3**	1	-1.8	0.96	0.05	9.86	85.7	0.01	2.9 ± 0.5

Table 2. Q_{10} values. Q_{10} values for NH_4^+ uptake, nitrification, and bacterial production rates for each experiment. Values do not include the 20°C treatment, as all experiments had equal or decreased rates at that temperature. The station ID (SID) is as described in the text. Bacterial production Q_{10} data in italics for SUM1, SPR1, and parenthetically for WIN1 are from separate experiments performed during the same season, as explained in the Methods. n.d. is for no data.

	2011						2012	
	spring			summer		winter	winter	
	SPR1	**SPR2**	**SPR3**	**SUM1**	**SUM2**	**WIN1**	**WIN2**	**WIN3**
NH_4^+ uptake								
-1.5 to 6°C	3.0	2.2	1.4	3.2	2.3	3.1	1.8	1.8
6 to 13	2.1	1.0	1.8	2.4	1.3	1.0	2.2	4.1
-1.5 to 13	2.5	1.5	1.6	2.7	1.7	1.8	2.0	2.7
Nitrification								
-1.5 to 6°C	0.9	1.1	0.9	n.d.	1.2	1.2	1.0	1.1
6 to 13	1.1	1.0	1.2	1.3	1.0	0.8	1.0	1.1
-1.5 to 13	1.0	1.1	1.0	n.d.	1.1	1.0	1.0	1.1
Bacterial production								
-1.5 to 6°C	*2.5*	n.d.	2.9	*1.6*	n.d.	3.7 *(2.7)*	3.1	3.5
6 to 13	*2.1*	n.d.	1.3	*3.4*	n.d.	1.6 *(1.6)*	3.5	1.5
-1.5 to 13	*2.2*	n.d.	2.0	*2.6*	n.d.	2.3 *(2.0)*	3.3	2.0

Figures

Figure 1. Ammonium uptake rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Note different y-axis scales. The SUM1 -1.5°C treatment was not corrected for isotope dilution, as indicated in the dashed line.

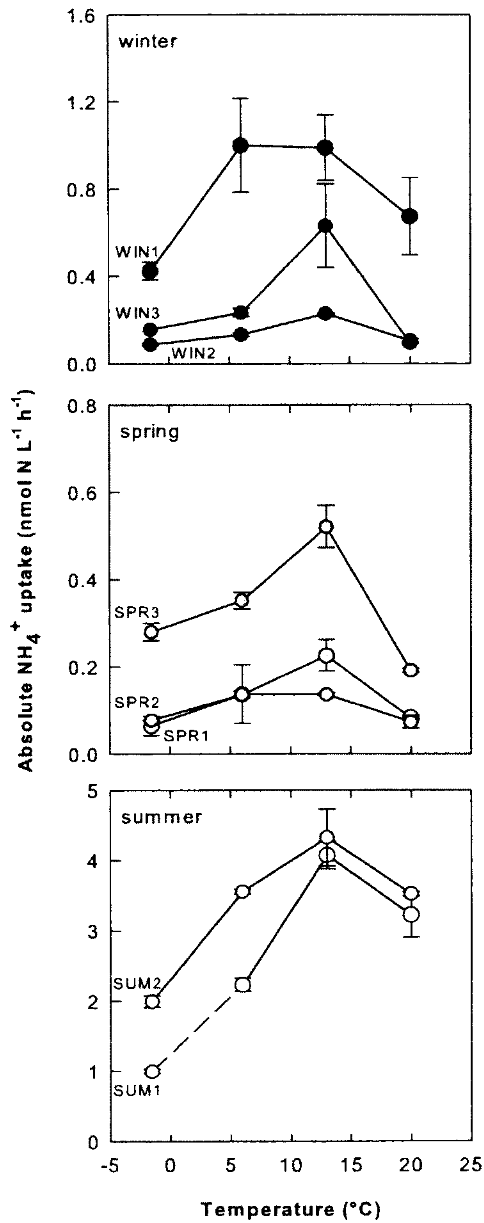


Figure 2. Nitrification rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. SUM1 has no data for the -1.5°C treatment, as explained in the results section. Note that the y-axis scale for the summer plot is two orders of magnitude lower than the winter and spring. Error bars are standard deviation.

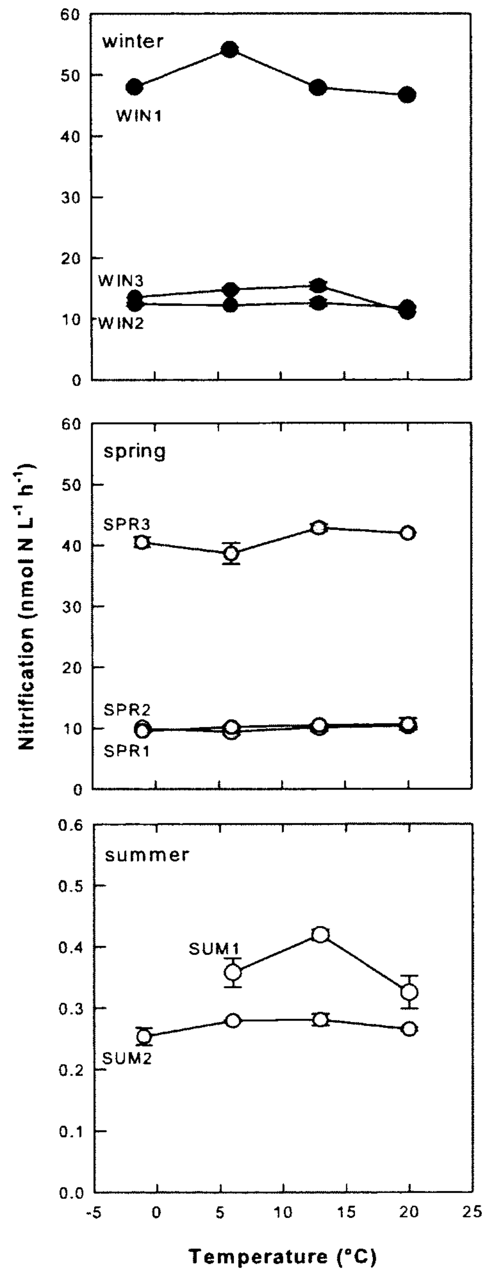
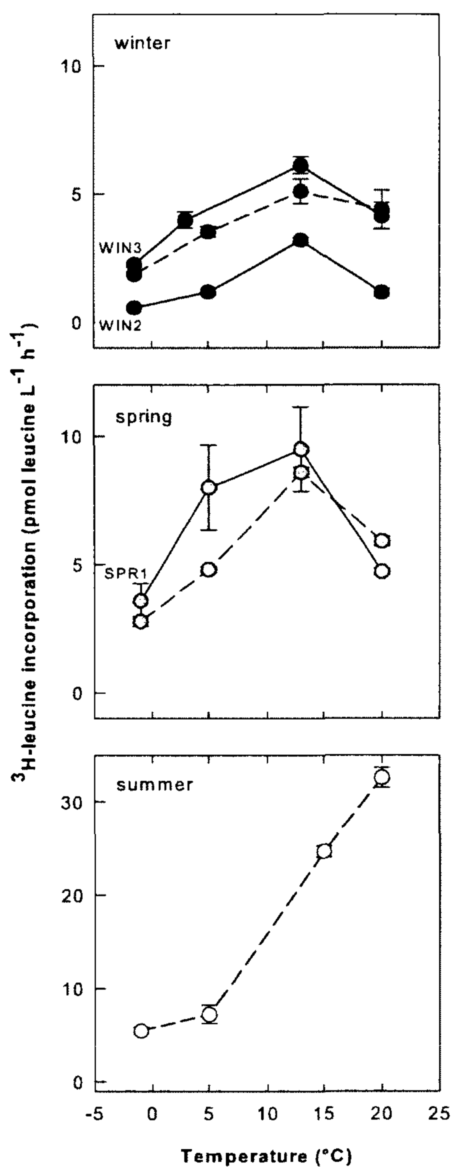


Figure 3. Bacterial production rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Plots with dashed lines are for bacterial production experiments performed in separate incubations (see methods section), and are not labeled with an SID. Note the different y-axis scale for summer. Error bars are standard deviation.



Chapter 5 – Nitrogen uptake dynamics in landfast sea-ice of the Chukchi Sea

Abstract

In the Arctic, multiyear ice is likely being replaced by seasonal first year ice, and changes in community composition and biogeochemical cycling are projected to occur as a result. The coastal Chukchi Sea has a particularly productive landfast ice ecosystem, which could be a model for future scenarios of ice ecology throughout the Arctic, but is currently lacking direct measurements of nitrogen (N) cycling. Using stable isotopic tracers, we measured uptake and regeneration of ammonium (NH_4^+), nitrate (NO_3^-), and bicarbonate (HCO_3^-) along with incorporation of tritiated leucine at three depths within landfast sea ice in the Chukchi Sea near Barrow, Alaska during April 2011 and January 2012. Both inorganic and organic nutrient concentrations in the ice were generally higher than the water column, with the exception of phosphate (PO_4^{3-}), which may limit production in certain sections of ice. Primary production per liter at all ice depths was higher than in the water column during April, but was below the detection limit in January. Bacterial production in the bottom ice was consistently higher than the water column across seasons. Absolute uptake of NH_4^+ was highest in the bottom ice (0-10 cm from the ice-water interface), and higher than absolute uptake of NO_3^- at all depth horizons except upper ice during January (30-40 cm). While N uptake rates were higher than the water column, nitrification rates were lower. Regeneration of NH_4^+ and NO_3^- far exceeded uptake of those compounds. The high N uptake and regeneration rates in landfast ice, especially compared to the water column, highlights the importance of the biological sea ice community, and could provide an indication of how the biogeochemistry of the region will change in the future.

Introduction

The marine cryosphere is a critical driver of the Arctic ecosystem, with an established and active microbial community (Junge et al. 2004; Stoecker et al. 2000) despite the extreme seasonal fluctuations of light, temperature, and nutrient regimes within the ice. Primary production of the algae on the underside of sea ice seeds pervasive ice edge blooms (Brown and Arrigo 2013; Perrette et al. 2011) and provides a large seasonal pulse of primary production (Arrigo et al. 2010; Dupont 2012) that is a critical food source for heterotrophic protists (Riedel et al. 2007a) and pelagic zooplankton (Brown and Belt 2012a), thus supporting the short food webs in the Arctic (Hobson and Welch 1992). Organic matter decomposition from the receding ice during spring is an important mechanism for the supply of surface nutrients, which support regenerative production in the water column (Conover et al. 1999; Cota et al. 1990). Whatever organic matter escapes zooplankton and microbial heterotrophy in the water column ultimately sinks out to provide nutritional inputs to the benthos (Brown and Belt 2012b; Grebmeier et al. 2006; Renaud et al. 2007; Tremblay et al. 1989).

Sea ice organisms must deal with low temperatures and high salinity (Eicken 2003; Jones et al. 2012) and quickly adjust to changing light, temperature, and nutrient conditions (Krembs et al. 2011; Krembs et al. 2000). The sympagic (i.e. ice associated) community, along with physical mechanisms and characteristics such as freezing and brine drainage, along with ice age, help determine the chemical environment of the ice through biological processes like uptake and regeneration of nutrients. While there is low

primary and secondary productivity during dark winter months (Forest et al. 2011), ice algae still represent 17 to more than 25% of total Arctic primary production on an annual basis (Gosselin et al. 1997; Loose et al. 2011), and a vigorous ice bacterial community that is presumed to rapidly remineralize organic compounds (Arrigo and Thomas 2004; Harrison et al. 1990; Sala et al. 2010), thus providing a robust feedback and supporting greater biomass than would be expected in such an extreme environment. Even during the dark winter months, microorganisms remain active (Junge et al. 2004) although there is evidence that some cells merely survive due to lack of predation (Collins et al. 2010). As light limitation is lifted in the spring, bottom ice algae thrive by taking advantage of high nutrient surface waters and relatively high irradiance (Lee et al. 2008; Różańska et al. 2009), resulting in the formation of blooms and the rapid drawdown of inorganic nutrients (Cota et al. 1990; Smith et al. 1997).

While the effects of nutrient concentrations and light limitation on primary production in sea ice have been studied, the actual rates of N uptake and regeneration has received relatively less focus, especially in overall ice profiles and during winter (Mock and Gradinger 1999; Mock and Thomas 2005). There are only a limited number of reports of N uptake in Arctic sea ice, and those that do exist are spread out in both space and time (Harrison et al. 1990; Krell et al. 2003). The Arctic is highly variable over both of these scales. The Chukchi Sea in particular is a unique environment with high Pacific nutrient inputs, tight pelagic-benthic coupling, and a relatively large indigenous human population that relies on traditional whaling and is therefore directly connected to the food web of coastal northern Alaska (Moore and Huntington 2008).

With high nutrient concentrations uncoupled from changes in algal biomass, it has been hypothesized that nutrient regeneration must be quite active in sea ice (Arrigo and Thomas 2004; Gradinger and Ikävalko 1998; Thomas et al. 1995). Regeneration of ammonium (NH_4^+) is especially important, as it potentially reduces the flux of nutrients to the benthos and is made available to ice-algae. Additionally, the different ecology of first year compared to multiyear ice may become more relevant. As climate warming causes rapid changes in the sea ice landscape, the Arctic is predicted to become seasonally ice free by 2080 or earlier (e.g. Boé et al. 2009; Serreze and Francis 2006; Stroeve et al. 2007; Stroeve et al. 2012).

In this study we quantified primary production, nutrient uptake and N regeneration in natural sea ice communities using tracer-level stable isotope additions of NH_4^+, nitrate (NO_3^-), and bicarbonate (HCO_3^-), along with bacterial production via tritiated leucine (^{3}H-Leu). Here we present the first data of N uptake and regeneration rates in landfast Arctic sea ice during spring and winter. These processes are especially important in the highly productive coastal regions where first year landfast ice can support higher sympagic biomass (Gosselin et al. 1997).

Materials and Methods

Sampling

On April 30, 2011 and January 19, 2012, a Kovacs Mark V coring system was used to retrieve multiple 14 cm diameter ice cores approximately 2.5 km off the coast of Barrow, Alaska (see Chapter 2 for a map and further description of the study site). The

sampling site was on landfast sea ice overlying 17 m of water, within 50 m of a pressure ridge that prevented further access offshore. Cores were taken in a small (<10 m^2) area to minimize variability, with great care taken to avoid trampling the surface. Two initial cores were used to determine temperature and salinity. For temperature, a small hole was drilled into the ice every 5 cm and a digital probe inserted. Salinity measurements were obtained by slicing 10 cm sections of ice and each was placed in a plastic bag. After melting, salinity was determined by a single-operator multiparameter metabolic analyzer-coulometry system (Johnson et al. 1987). Brine channel volume was subsequently calculated by the equation of Frankenstein and Garner (1967) and brine salinity by the equation of Cox and Weeks (1983). Four additional cores were taken, each placed on an acid washed HDPE cutting board and sliced with a stainless steel saw to obtain replicates of three depths each: 0-10, 15-25, and 30-40 cm up from the ice-water interface, heretofore referred to as bottom, middle, and upper sections of ice respectively. Each of these sections were transferred to salt water seasoned and acid washed 4 L polycarbonate jars, mixed with equal volumes of 0.2 μm filtered artificial seawater (ASW) with a salinity of 35 to minimize osmotic shock (Garrison and Buck 1986). They were subsequently melted in a dark 5°C water bath over 24 hours.

Upon complete melting, corresponding sections from two cores were combined for a total of two replicates per substrate. Each sample was gently mixed and 2 L was transferred to PETG bottles for tracer incubations, while the remainder was used for ambient measurements of Chl *a*, bacterial abundance, nutrient concentrations, and

bacterial production measurements, as outlined below. These ambient parameters were adjusted for dilution with added ASW by the following equation:

$$C_{ice} = (C_{tot} \cdot V_{tot} - C_{sw} \cdot V_{sw})/V_{ice}$$

where C is substrate concentration and V is sample volume of ice (ice), filtered seawater (sw), and the total mixture (tot), respectively. The C_{ice} value was then multiplied by the brine volume fraction to get values of brine-normalized concentrations.

Biomass, abundance, and production

Concentrations of chlorophyll a (Chl *a*) were measured fluorometrically on sub-samples from the melted sections after extraction in 90% acetone on a Turner Design Model 10-AU fluorometer (Parsons 1984). Phaeophytin was subsequently measured on the same instrument after addition of hydrochloric acid. For bacterial abundance, triplicate whole water samples were fixed in the field with 0.5% additions of formaldehyde, stored for 15 minutes at 5°C to ensure complete fixation, and stored at -80°C until bacterial abundance analysis. Each sample was run in duplicate on a FACScalibur flow cytometer after staining with SYBR Green (Life Technologies) and the addition of 2.0 μm reference beads (Spherotech), and then analyzed using FlowJo cytometry analysis software (Treestar).

Bacterial community composition was determined by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH; Pernthaler et al. 2002). Duplicate 15 mL water samples were fixed with 0.75 mL of 0.22 μm filtered formaldehyde and fixed at 4-5°C for 24 hours. Samples were gently (<5 mm Hg) filtered

onto an Isopore™ polycarbonate filter (Millipore) using a cellulose nitrate filter (Whatman) as a backer. After being allowed to dry for 5 minutes, the filters were placed in a plastic scintillation vial and stored at -80°C until analysis. Cells were embedded in 0.1% MetaPhor™ agarose (Lonza), dried at 35°C for 15 minutes, and then removed after the addition of ethanol. Filters were incubated in 2 mL lysozyme solution (Sigma-Aldrich) for 60 minutes at 37°C (for archaea, this was followed directly with achromopeptidase for 30 minutes at 37°C), and then washed in alternated baths of 95% ethanol and Milli-Q water. Cut sections of each filter were placed in a mixture of hybridization buffer and oligonucleotide probes (Table 1) and placed on a rotation shaker at 10 rpm at 35°C for 12 hours. After hybridization, each filter section was placed in 2 mL washing buffer and incubated at 37°C for 40 minutes. Amplification reagent (Perkin Elmer) was added, incubated at room temperature in the dark for 5 minutes, washed in PBS for 30 minutes at room temperature in the dark, rinsed briefly in Milli-Q water, then in ethanol, and air dried. Filter sections were mounted on a slide in a 4:1 mix of Citifluor (Citifluor Ltd) and Vecta Shield (Vector Laboratories, Inc.) containing 1 μg/mL of DAPI.

Bacterial production incubations were performed prior to ^{15}N additions by incubating triplicate 1.5 mL aliquots with ^{3}H-leu (specific activity of 144 Ci per mmol) at a final concentration of 25 nM for 4 hours in the dark. Incubations were terminated by adding 0.1 mL of 100% trichloroacetic acid (TCA) to each sample tube. Protein was extracted by rinsing the samples again with 1 mL of ice-cold TCA and then by rinsing with 1 mL of 80% ethyl alcohol, with centrifugation between each rinse. After drying overnight, 1 mL of scintillation cocktail (UltimaGold) was added to each tube and the

radioactivity was measured on a liquid scintillation counter. Killed controls were performed by adding the ^{3}H-leu only after killing the cells with the addition of TCA. No ^{3}H-leucine incorporation was detected in the added ASW. To convert to units of carbon, we used a conversion factor of 1.5 kg C per mole of incorporated leucine, which is an empirically derived mean for the water column in the western Arctic (Kirchman et al. 2009).

Nutrient concentrations

Nutrient analysis was performed on sub-samples from the melted sections taken before the addition of labeled substrates (ambient values) after filtration by GF/F filters (0.7 μm nominal pore size; Whatman), and then frozen at -20°C until analysis. Concentrations of NH_4^+ were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Duplicates of nitrate (NO_3^-), nitrite (NO_2^-), silicate (Si), and phosphate (PO_4) were measured on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Urea was measured in duplicate using the manual monoxime method (Price and Harrison 1987). Dissolved primary amines (DPA), used as a proxy for amino acids (Keil and Kirchman 1991), were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Measurements of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) were made in triplicate by high-combustion on a Shimadzu TOC-V TNM (Sharp et al. 2002). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N

(NH_4^+, NO_3^-, and NO_2^-) from TDN (Bronk et al. 2000); the errors for all the terms were propagated to provide standard error for DON.

Uptake rates

Replicate subsamples of the melted cores were incubated with tracer (<10%) additions of ^{15}N-labelled ammonium chloride (98.85% ^{15}N) or potassium nitrate (98% ^{15}N), along with ^{13}C-labelled sodium bicarbonate (99% $H^{13}CO_3^-$; Cambridge Isotope Laboratories). Dual-labeled urea (98% ^{15}N and 99% ^{13}C) was added to the bottom ice layer during April of 2011, but not repeated in any other sections or season due to sea ice sampling limitations. The incubation bottles were placed in an environmental control chamber at ambient light (defined as the light levels measured in the water column immediately below the ice) and temperature for 24 hours (see Chapter 2 for complete details).

At the termination of the incubations, the samples were filtered through pre-combusted Whatman GF/F filters and frozen at -20°C until analyzed on a Europa Geo 20/20 isotope ratio mass spectrometer with an ANCA autosampler. Absolute uptake rates were calculated as per Dugdale and Goering (1967) and Hama et al. (1983) for N and C respectively. Specific uptake rates, which are simply the absolute rate divided by particulate N, remove any normalization to biomass. These rates describe the physiological ability of cells to assimilate N (Dugdale and Wilkerson 1986) and can give an indication of cells ability to assimilate N regardless of how many cells are present. The NH_4^+ and NO_3^- pools were isolated at the end of the incubations by solid phase

extraction (Brzezinski 1987; Dudek et al. 1986) and the denitrifier method (Sigman et al. 2001) respectively. The atom percent enrichment of the isolated pools were used to correct the NH_4^+ and NO_3^- uptake rates for isotope dilution, calculate NH_4^+ and NO_3^- regeneration (Glibert et al. 1982), and nitrification rates (Ward 2008).

Results

Physical properties

During spring (April) sampling, the daily light cycle consisted of 20 hours of sunlight, clear skies, low wind, and air temperatures that hovered near -10°C. The water column was well mixed at a temperature of -1.8°C and salinity of 32; further details on water column conditions and N uptake are explored in Chapter 2. Ice depth was 130 cm with very little (<3 cm) snow covering the site. Temperatures in the ice core decreased linearly from the bottom of the core to the top, while brine salinity increased from a low of 41 located 5 cm from the ice-water interface to a high of 123 at the top of the ice (Figure 1). Based on the brine volume percent calculated from these parameters, there is a large amount of interstitial space at the bottom of the core (Golden et al. 1998), with permeability of the brine extending almost 80 cm up from the ice-water interface, excepting one point at 35 cm.

During January of 2012, the sun had remained below the horizon for 2 months prior to our sampling. Each day consisted of approximately 4 hours of twilight. In the days immediately preceding sampling, there were strong winds with daily temperatures ranging from -40 to -60°C. There was a brief respite on the day we obtained the ice cores,

with air temperatures reaching a low of -25°C. The water column remained well mixed during the winter, with water temperatures of -1.8°C and salinity of 34. The ice was only 60 cm, approximately half the depth of the spring sampling. Temperature in the core again reflected an essentially linear decline from the water to the air temperatures (Figure 1). The brine was much saltier than in April, ranging from 72 to 210. By 25 cm, the sea ice was impermeable to flow within the brine (Golden et al. 1998), which is interesting because our incubations were performed on sections to either side of that percolation barrier. There was visible sediment present in the bottom ice, but this was not quantified.

Community composition

In April, Chl *a* was low throughout the profile with the exception of the bottom ice layer, where there was a visible band of ice algae. The ice algae band measured 67.7 ± 2.9 and 415 ± 17.8 μg Chl *a* L^{-1} in the bulk and brine-normalized ice, respectively (Figure 2). Mean Chl *a* in the remainder of the sampling depths in bulk sea ice was 0.11 μg Chl *a* L^{-1}, which was similar to Chl *a* found in the water column. The chlorophyll *a*-to-phaeophytin ratio (chl *a*:phaeo) can be used to give an indication of the physiological state of phytoplankton, with higher values indicating cells with fewer degradation products, and lower values indicating a state of degradation due to stress (Yentsch 1965). In the water column during April, the mean chl *a*:phaeo was 0.66, and bottom, middle, and upper sections of ice were 1.58, 0.95, and 0.64, respectively. Bacterial abundance generally mirrored Chl *a*, with a peak in the bottom ice, and a reduction further up the ice core. Bulk ice bacterial abundance was almost an order of magnitude lower than in the

water column, but higher by almost the same amount when calculating abundance as a function of the brine channel volume.

During January, there remained a Chl *a* peak in the bottom ice, and whether in the bulk ice or brine-normalized, Chl *a* in the sea ice was greater than the water column (Figure 2). While greatly reduced from April, there was still a community with Chl *a* pigment present, although more degraded than in spring. The mean water column chl *a*:phaeo was 0.33, and was 0.62, 0.37, and 0.15 in the bottom, middle, and upper sections of ice respectively. Bacterial abundance in the water column was not significantly different from spring. In the January sea ice, however, bacterial abundance was higher than spring and the water column at all depths. Once accounting for brine volume, the bacterial abundance in the sea ice is not significantly different between the three depth horizons.

Unlike the community present during April in the water column (Galand et al. 2009; Kirchman et al. 2010), Betaproteobacteria were well represented in the ice (Figure 3). In equal proportion were Alphaproteobacteria and Flavobacteria, with a smaller contribution of Gammaproteobacteria. A miniscule percentage (<1%) of probes were positive for crenarchaeota in the middle and upper ice, while not statistically significant from zero in the bottom ice. Winter CARD-FISH samples were not analyzed.

Primary and bacterial production

During April, absolute uptake of HCO_3^- (μmol C L^{-1} h^{-1}) in the bottom ice was more than 1000 times greater than in the water column, and close to two orders of

magnitude greater than in the upper two sections of ice (Table 2). Bacterial production was also highest in the bottom ice, but the bacterial to primary production ratio (BP:PP) was actually highest in the middle ice. Water column HCO_3^- uptake was particularly low, while bacterial production in the water column was slightly higher than the middle and upper ice. This corresponds to an unusually high BP:PP of 7.0, although both BP and PP were very low in absolute terms.

In January we were unable to detect any primary production in the water column or the ice. Bacterial production was approximately an order of magnitude lower during winter than in spring, but still highest in the bottom ice. The middle and upper ice were not significantly different from each other, and again slightly lower than bacterial production measured in the water column.

Nutrients – water column and bulk ice

With the exception of urea and DPA, the water column had relatively high nutrient concentrations (see Chapter 2) compared to the bulk sea ice (Figure 4). In April, the organic components were at or below water column concentrations in the middle and upper ice but higher in the bottom ice. While NH_4^+, urea and overall DON all had maxima in the bottom ice, amino acids (i.e. DPA) and DOC are especially pronounced. It is interesting to note that the highest urea concentrations anywhere in the profile occur in the upper ice. The DOC:DON ratio is lower in the ice than the water column, but still above the Redfield ratio (Redfield 1934). The essential nutrients PO_4^{3-} and Si were

almost zero in the ice. While PO_4^{3-} is low in the water column also, water column Si concentrations are over 40 μM.

Winter concentration profiles of the ice and water column displayed a strikingly similar pattern to the spring (Figure 4), with a few caveats. In the bottom ice, NO_3^- concentrations were not as low as during the spring, and actually increased from the bottom to the top of the ice core. Urea concentrations did not increase to the same extent, and the DOC concentration was lower in the ice than in the water column. During winter, the DOC:DON ratio in the water column was almost double that of spring, and while lower overall in the ice compared to the water column, this ratio increased from the ice-water interface to the upper section of ice.

Nutrients – brine normalized

Sympagic organisms live in the brine channels of the sea ice, and therefore are not subject to the dilution effects of melted ice. Instead, they are exposed to much higher concentrations of ambient nutrients than the bulk ice described above and in Figure 4. When accounting for the brine channel volume, the relative pattern within the sea ice remains, but the absolute concentrations increased dramatically once the actual volume of the living space present in the ice is accounted for.

In April, NH_4^+ and PO_4^{3-} concentrations within the brine channels declined from the bottom to upper ice, while NO_3^- and Si concentrations increased in that upper section (Figure 5). In particular, PO_4^{3-} dropped from 4.8 μmol P L^{-1} to nearly zero (0.06 ± 0.08). At a mean of 8.3, the ratio of NO_3^-:PO_4^{3-} was below the Redfield ratio at every depth in

the water column and ice, with the exception of the middle ice. Organic constituents were especially high in the bottom ice layer. Concentrations of DOC reached 893 ± 8.9 μmol C L^{-1}, while DON peaked at 87.8 ± 1.2 μmol N L^{-1}. Both of these bulk organic pools declined further up the ice, keeping the DOC:DON ratio between 8.3 and 10.2. Urea and DPA combined comprised 26.7, 26.1 and 46.0% of DON in the bottom, middle, and upper sections respectively; DPA accounted for the majority of DON in the bottom ice, and urea in the other two sections.

As described above, the brine channels of upper ice were not connected to the lower sections of ice during winter (Figure 1). The nutrient profiles appear to reflect this lack of connection, with a "closed" system leading to higher nutrient concentrations in the upper ice (Figure 5). This is especially pronounced for NO_3^-, DOC, and Si. While this is also true for NH_4^+, DON, and PO_4^{3-}, the increases in upper ice concentrations were not nearly as substantial. Urea and DPA did not follow this pattern, and we did not observe the great increase from upper to bottom ice section seen during spring. Like spring, however, urea and DPA together accounted for a large percentage of the overall DON pool (51.9, 62.1 and 42.0% of total DON in the bottom, middle, and upper ice respectively), with urea providing the bulk of that. DOC:DON ratios were higher in January than in April at all three depths, and increased higher up in the core to a maximum of 15.0.

Nitrogen uptake and regeneration rates

During April, specific uptake of NH_4^+ and NO_3^- were at maxima at the middle depth, but not significantly different between the bottom and upper ice (Figure 6). The middle ice section had a pronounced maximum of NO_3^- specific uptake in the middle depth, with a value almost 4 times greater than the other two sections. Since biomass (Figure 2) was high in the bottom layer, absolute uptake rates of both NH_4^+ and NO_3^- were highest there, dropping an order of magnitude in the two upper sections. These latter measurements are within range of water column absolute uptake of NH_4^+ (0.89 ± 0.34 nmol N L^{-1} h^{-1}) and actually higher than water column NO_3^- absolute uptake (0.28 ± 0.04 nmol N L^{-1} h^{-1}). Urea uptake was only measured in the bottom ice during April of 2011 and had a specific uptake of 0.0027 ± 0.0007 d^{-1} and absolute uptake of 1.98 ± 0.54 nmol N L^{-1} h^{-1}. In the water column, specific uptake of urea (0.0018 ± 0.0003 d^{-1}) was not significantly different from the ice, and in both cases accounted for less than 5% of the measured specific uptake rates for all the substrates tested. Uptake of C from urea was below the detection limit.

As expected for the dark winter period, both specific and absolute uptake rates for NH_4^+ and NO_3^- were considerably lower than in April (Figure 6). Specific uptake of NH_4^+ was not significantly different between depths in the January core. However, there is a trend of increasing NO_3^- specific uptake with increased depth in the profile. These rates are near the limit of what can be reliably measured, and therefore have more substantial error than for the spring rates. In comparison to the water column, specific uptake of NH_4^+ at the 1 m depth is within range (0.00026 ± 0.00017 h^{-1}) of the ice, and

specific uptake of NO_3^- is higher in the water ($0.00030 \pm 0.00017\ h^{-1}$). Similar to April, absolute uptake of NH_4^+ was highest in the bottom ice layer, while the two upper sections were not significantly different from each other and less than 25% of the bottom ice rate. For absolute uptake of NO_3^- it was again lower in the bottom and middle ice, but slightly higher than absolute uptake of NH_4^+ in the upper ice.

Regeneration of NH_4^+ was highest in the bottom ice during spring. It was almost twice NH_4^+ absolute uptake in that same section, while also an order of magnitude higher than NH_4^+ regeneration in the other sections (Figure 6). Regeneration of NO_3^- was also approximately double NO_3^- absolute uptake in the bottom ice, but still only half the rate of NH_4^+ regeneration. With the exception of NH_4^+ regeneration in the upper ice, the bottom ice NH_4^+ regeneration and all of the NO_3^- regeneration rates were higher than the water column (NH_4^+ regeneration = 27.8 ± 1.18 nmol N $L^{-1}\ h^{-1}$ and NO_3^- regeneration was below detection limit). During January, regeneration of NH_4^+ was variable but not statistically different from zero in the bottom ice. In the water column, NH_4^+ regeneration rates were 13.8 ± 4.76 nmol N $L^{-1}\ h^{-1}$. All of the NO_3^- regeneration rates during January were also highly variable, with a mean of 13.2 nmol N $L^{-1}\ h^{-1}$ for all depths, which was only slightly lower than the mean for April (14.9 nmol N $L^{-1}\ h^{-1}$).

Nitrification, an important process during spring and winter in the water column of this area (Christman et al. 2011), was lower in the sea ice (Table 3) than the water column during both seasons. Rates were highest in the bottom ice during spring, and became progressively lower in the middle and upper ice. During January, nitrification was lowest in the bottom ice, and slightly higher in both the middle and upper ice.

Discussion

Most prior studies of sea ice biogeochemistry have focused on C cycling in bottom ice and investigated N cycling in the context of nutrient limitation. There is limited information on N uptake and regeneration in coastal regions (Cota et al. 1990; Cozzi 2008; Harrison et al. 1990; Kaartokallio 2001; Krell et al. 2003; Kuparinen et al. 2007; Priscu and Sullivan 1998; Rysgaard et al. 2008; Rysgaard and Glud 2004). In this study, we quantified the N cycling processes that underpin production within the ice at a western Arctic landfast site during spring and winter.

Biology

Ice algae biomass and production are concentrated on the underside and bottom few centimeters of sea ice (Horner and Schrader, 1982). During winter, flagellates make up the bulk of algal biomass in sea ice (Różańska et al. 2009) and are succeeded by diatoms, which generally dominate the phytoplankton community in the entire core in both first year and multiyear ice during spring (Eddie et al. 2010; Werner et al. 2007). In addition to adaptations to brine channel conditions (Krembs et al. 2002; Krembs et al. 2000), ice algae can also modify the physical structure of the ice by the release of extracellular polysaccharide substances, which provides protection from freezing, osmotic stress, and potential refuge from predation (Krembs et al. 2011). It is not clear how phytoplankton survive the dark winter months, but possible overwintering strategies include survival by tapping into stored lipids and carbohydrates, a switch to heterotrophy, or formation of resting spores (Lizotte 2003). In our study, Chl *a* in the middle and upper

ice, although at low concentrations, were still higher than values in the water column, so it may be that the sea-ice environment itself acts as a refuge.

Previous studies indicate that bacterial and archaeal communities are evenly distributed throughout the ice column (Collins et al. 2010; Mock and Thomas 2005) and that respiration can exceed primary production (Deming 2010; Rysgaard et al. 2008) even during winter when bacterial growth is extremely slow (Wells and Deming 2006). In our study, there was little difference in bacterial abundance and community structure with depth of ice during spring, which could be explained by presence of brine channel connections between the different depths, inclusion into freezing ice by the same pelagic seed population, or competitive exclusion of other groups. Prior studies found more than 10% of Arctic surface water prokaryotic community to be Crenarchaeota during the winter, which decreases during spring and summer (Alonso-Sáez et al. 2008; Kirchman et al. 2007). Crenarchaeota are also negatively correlated to sediment loads (Galand et al. 2008). In the central Arctic, Alpha- and Gamma-proteobacteria and cytophaga account for more than 80% of clades in first year ice (Junge et al. 2004; Junge et al. 2002), with archaea only showing up in winter (Collins et al. 2010). Relative abundance of bacteria have been shown to increase as temperature decreases within the ice (Junge et al. 2004). Our results generally agree with prior studies, although Betaproteobacteria were numerically more important in our samples than Gammaproteobacteria. It is interesting to note that of the five groups we probed for (crenarcheota, gamma-, alpha-, beta-proteobacteria and bacteroides), almost 99% of DAPI cells are represented by eubacteria (probe EUB338; Table 1) in the bottom ice, but only 78% in the upper two sections,

indicating that there is a slightly different community in the top two layers that didn't hybridize well with the probes we used.

Primary and secondary production

April Chl *a* and absolute uptake of HCO_3^- in the bottom ice was on the lower end of the spectrum of other bottom sea ice primary production measurements in our study region (Lee et al. 2008) and the Chukchi Sea generally (Gosselin et al. 1997; Gradinger 2009), highlighting the extreme seasonal productivity of the sea ice in the Arctic. Within the ice, there are relatively few measurements of primary production. In Barents Sea bottom ice, Mock and Gradinger (1999) measured primary production ranging from 0.25 - 7.11 mg C m^{-2} d^{-1}. Similar studies in the Chukchi Sea found a range from 0.24 – 55.2 mg C m^{-2} d^{-1} (Gradinger 2009) and 0.5 – 310 mg C m^{-2} d^{-1} (Gosselin et al. 1997). It is likely that our lower rates (Table 2) were due either to the patchiness or that we sampled prior to those other studies and prior to the full development of the spring bloom in this region, which culminated in late May (A. Juhl, pers. comm.).

The prokaryotic community was active throughout the water and ice columns during both seasons, as indicated by a cross-seasonal range of bacterial production of 0.1 – 0.22 μg C m^{-2} d^{-1}. In sea ice of the Amundsen Gulf (Canadian Arctic), Nguyen and Maranger (2011) found highly variable bacterial production, but with a mean (4.3 and 7.1 μg C L^{-1} d^{-1} during March and July respectively) higher than our study. Sea ice BP:PP in our study (0.03 – 0.13) was within range of ratios found in the summer (August) water column of the Chukchi Sea (0.04 - 0.10; Cota et al. 1996; Rich et al. 1997), but low

compared to spring water column BP:PP on the shelf of this region (0.05 - 0.96; Kirchman et al. 2009). In fact, the BP:PP in this study was an order of magnitude lower than the BP:PP found in winter Antarctic sea ice (Helmke and Weyland, 1995). These are the first measurements we know of for bacterial production in landfast sea ice of Alaska, and the lower numbers could simply reflect geographic or seasonal differences with prior studies.

Nutrients

The accepted paradigm of nutrition for sympagic organisms, at least in the bottom ice layer, is the physical exchange of nutrients with the water column (Thomas and Dieckman 2002; Thomas et al. 2010). However, inorganic nutrients and metals tend to be enriched in sea ice compared to the water column (Loose et al. 2011; Thomas et al. 1995; Tovar-Sánchez et al. 2010). The exception to this excess is NO_3^-, which is thought to have limited uptake due to low availability, high NH_4^+ concentrations inhibiting NO_3^- uptake, and poor cellular transport at low temperatures (Cochlan and Bronk 2001; Pineault et al. 2013; Priscu et al. 1989; Priscu and Sullivan 1998; Smith et al. 1997). Limitation of biological production by low NO_3^- concentrations, however, is equivocal, as it could be a function of geography, temperature, or light conditions (Harrison et al. 1990; Lee et al. 2010). In our study, we found no evidence of any nutrient limitation (as defined by concentrations of any macronutrient below 0.05 μM) in the ice, with the exception of possibly PO_4^{3-} during spring, which fell to near zero in the middle and upper ice (Figure 5).

Dissolved organic N sources have received relatively less attention than inorganic N in sea ice, and should not be overlooked as an alternative or supplement to inorganic N (Conover et al. 1999; Harrison et al. 1990). High extracellular DNA concentrations occur in Arctic sea ice (Collins and Deming 2011) and recent work shows amino acids are important source of nutrition to the coastal Arctic microbial community, especially during winter (Chapter 2; Alonso-Sáez et al. 2008; Nikrad et al. 2012). In this study, high urea and DPA concentrations, along with lower DOC:DON ratios, indicate relatively N-rich organic matter in the sea ice compared to the water column (Figure 4). Within the ice, concentrations of urea and DPA were both evenly distributed throughout the ice in January, but DPA was highly enriched in the bottom ice, and urea in the upper ice, during spring (Figure 5). With only one spring measurement of urea uptake in the bottom ice, which itself was very low, further study of urea and amino acid uptake is warranted.

Absolute and specific uptake of NH_4^+ *and* NO_3^-

Working in Resolute Bay, Canada during the summers of 1985 and 1986, Harrison et al. (1990) found a wide range of absolute uptake of NH_4^+ (2.64-36.15 μmol N L^{-1} h^{-1}), NO_3^- (0.87-80.04 μmol N L^{-1} h^{-1}) and urea (2.00-5.37 μmol N L^{-1} h^{-1}) and concluded that the bottom ice community was not N limited. On the other side of the Arctic basin, in the landfast ice of the White Sea (Russia), Krell et al. (2003) found high DOC:DON, low N:P ratios, and limitation by NO_3^-, but conditions there are highly influenced by riverine inputs and therefore may be localized. In the coastal seas near

Barrow, Alaska, where this study took place, Lee et al. (2008) concluded that light, not inorganic N, was the primary environmental variable controlling ice algal C uptake.

Among N substrates tested in sea ice, one study from the Baltic Sea shows that bacterial communities have higher dependence on NH_4^+, as opposed to NO_3^- (Kuparinen et al. 2011). In our study, absolute uptake of NH_4^+ was at least double absolute uptake of NO_3^- in the bottom ice during both April and January, but reliance on NH_4^+ decreased in the middle and upper ice. There are limited comparable ice data, but this is counter to Reay et al. (1999) who used chemostat experiments with the water column community of the Southern Ocean and found increasing dependence on NH_4^+ with decreasing temperature. Despite our experimental conditions being very different, this discrepancy highlights the need for field studies within sea ice, where there are a number of variables (e.g. community composition, light, temperature) that all interact to influence the uptake rates.

In contrast to absolute uptake, specific uptake of NH_4^+ did not change much going up the ice profile, with the highest value in the middle ice during April and statistically equal rates in all ice depths during January (Figure 6). All of the specific uptake rates for NH_4^+ or NO_3^- are outside of the range reported in the water and bottom ice of the Ross Sea during austral spring (Olson 1980). It is surprising that the lowest specific NO_3^- uptake in both cases is in the bottom ice where primary production, which has traditionally been thought to preferentially derive from NO_3^-, is highest. These results indicate a high capacity for N uptake in the brine channels, even in the upper horizons. Because of dilution with ASW and the melting of bulk ice during the experiment, even

these rates should be considered conservative, as ambient nutrient concentrations were diluted during the incubation set up. Because we corrected for isotope dilution, however, our rates reflect the most accurate representation within the experimental conditions. Isolation of NH_4^+ and NO_3^- is labor intensive and costly. These analyses are necessary to correct for isotope dilution, however, and necessary when calculating NH_4^+ uptake rates (Glibert et al. 1982). There is increasing recognition that NO_3^- uptake rates should be corrected as well (Lipschultz 2008; Preston et al. 1998), especially in regions where the N cycling processes are not well understood. Measurement of the isolated pools post-incubation can also be used to directly calculate regeneration and nitrification rates (Ward 2008).

Nitrogen regeneration

In addition to uptake, nutrient concentrations in sea ice are generally considered to result from exchanges with the water column and heterotrophic regeneration (Arrigo and Thomas 2004; Gradinger and Ikävalko 1998; Thomas et al. 1995) and there has been some circumstantial evidence for remineralization through indirect measurements (Harrison et al. 1990; Riedel et al. 2007a; Riedel et al. 2007b). Here we were able to directly measure an internal regenerated N pool that is not dependent on outside sources. Heterotrophic bacteria constitute a large portion of sea ice biomass (Deming 2010) but because of the isolated nature of many of the brine pockets in the ice, the microbial loop is not able to fully develop, leading to accumulation of organic C (Lizotte 2003). If active, however, heterotrophic activity would remineralize both organic C and N

(Kirchman 2000). Direct quantification of NH_4^+ and NO_3^- regeneration in sea ice has heretofore not been tested polar sea-ice.

In this study, regeneration rates of NH_4^+ are generally higher than rates of NH_4^+ absolute uptake at each depth. The pattern of mean NH_4^+ regeneration closely aligns with NH_4^+ concentrations at every depth for both April and January. Curiously, NH_4^+ regeneration was below our detection limit in the middle ice in April and in the bottom ice during January. When not below the detection limit, NH_4^+ regeneration was 1.6 – 33 times greater than absolute uptake of NH_4^+. Measured by changes in NH_4^+ concentration over time, Riedel et al. (2007b) calculated an extremely high NH_4^+ regeneration rate of 200 nmol L^{-1} h^{-1} in new sea ice (<7 cm thick) during autumn, contributing 67% of the sea-ice NH_4^+ concentrations. In our study, such high NH_4^+ regeneration in April bottom ice might indicate very high turnover of NH_4^+, but without time course sampling during our 24 hour incubation period, we potentially underestimate NH_4^+ regeneration in other samples, as the NH_4^+ is recycled faster than our sampling regime could account for (Dickson and Wheeler 1995). Additionally, in temperate marine ecosystems when NO_3^- is replete, diatoms release NH_4^+ with rapid increases in irradiance and decreases in ambient temperature (Lomas et al. 2000), which potentially helps to explain high NH_4^+ turnover. In our study, we were careful to prevent light, temperature, and osmotic shock, and did not subject the communities to either of these conditions, but it remains a possibility that sea ice microorganisms are releasing NH_4^+ due to some stress response. What is clear is the NH_4^+ regeneration rates, when above our detection limit, indicates a highly regenerative system, with tight coupling between autotrophic and heterotrophic

production. Whether NH_4^+ regeneration in sea ice is highly variable or suffering from sparse sampling has yet to be determined.

During both April and January, NO_3^- regeneration was an order of magnitude higher than absolute uptake of NO_3^- at all three depths in the ice (Figure 6). In combination with the isolated brine pocket found in the January upper ice, elevated NO_3^- regeneration helps explain the high NO_3^- concentrations we saw in January upper ice (Figure 5), as it was 69 – 165 times the NO_3^- absolute uptake rate. Strangely though, nitrification rates only account for 24 ± 17% of the NO_3^- regeneration rates that we measured. It is unclear what process other than nitrification would increase NO_3^- concentrations during the incubations. Cells could potentially lose NO_3^- in response to stressors, such as grazing, virally-induced lysis, or elevated light (Bronk and Steinberg 2008; Lomas et al. 2000; Wells and Deming 2006). While we controlled light in our experimental setup, grazers were not excluded and viruses were not enumerated. More research is needed to understand if high NH_4^+ and NO_3^- regeneration rates are a universal feature of sea ice and what conditions might influence it.

In addition to regeneration, transformational processes between N compounds can have important implications for sympagic biology. Heterotrophic activity is clearly high during both spring and winter, and denitrification and annamox have been found in Arctic sea ice despite generally high oxygen availability (Rysgaard et al. 2008; Rysgaard and Glud 2004). On the other hand, nitrification could help explain high NO_3^- by oxidation of NH_4^+ into the NO_3^- pool. Nitrifiers are present in large numbers in central Arctic waters (Bano and Hollibaugh 2000), but their rates in sea ice have only been directly

measured in Antarctic landfast ice, where it was found that NH_4^+ oxidizers could account for a significant portion of heterotrophic C fixation and provide sufficient NO_3^- for uptake (Priscu et al. 1990). Nitrification rates in this study were highest in the bottom ice during spring, and were an order of magnitude lower in the upper section (Table 3), potentially indicating light limitation of the process (Ward et al. 1984). On the other hand, nitrification remained lower than the water column during the dark winter, which would point to some other environmental factor influencing this process. We have limited information on community composition in our ice samples, but Betaproteobacteria, of which prominent nitrifiers (*Nitrosomonas*) are a part, were a large percentage of our identified spring ice clades. In April upper ice, we also found low abundance of Crenarchaea, which are globally important nitrifiers (e.g. Francis et al. 2005; Wuchter et al. 2006). During January, when temperature decreased rapidly towards the surface, nitrification rates in all three sections were not statistically different from each other, which confirm the results from water column manipulation experiments.

Conclusions

Sympagic primary productivity is expected to increase in a warming climate until the N supply from the water column becomes limiting (Arrigo et al., 2008, Carmack and McLaughlin 2011; Gradinger 2009, Pabi et al. 2008). But organismal adaptations may be acting to retain more nutrients in the ice (Krembs et al. 2011), and our study shows that N regeneration within the ice can match or exceed absolute uptake rates. As ice extent and volume continues to be reduced (e.g. Maslowski et al. 2012; Stroeve et al. 2007; Wang

and Overland 2009), timing changes in the onset of the spring bloom will have impacts on food webs throughout the Arctic (Kahru et al. 2011; Tremblay et al. 2006), and one would expect remineralization in spring ice and the water column to increase as more organic material is available to the heterotrophic bacterial community, further reducing the capability of the Arctic Ocean to act as a CO_2 sink in the future (Cai et al. 2010; Else et al. 2013; Vaquer-Sunyer et al. 2010).

In this study, we were able to resolve sea ice N uptake and regeneration processes in landfast ice of the Chukchi Sea during winter and spring. The high concentrations of nutrients and diversity of organisms within the brine channels indicate that it is a thriving ecosystem, even within the constraints of variable light regimes, low temperatures and high salinities. While ice algae are widely considered to be an important driver of marine Arctic food webs, this study shows that the microbial N transformations throughout the ice help support their growth, and that biogeochemical cycling above the layer of ice algae is at least as important as in the water column and needs to be taken into account when attempting to understand nutrient dynamics in the Arctic, now and in the future.

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References

Alonso-Sáez L, Sánchez O, Gasol JM, Balagué V, Pedrós-Alio C (2008) Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. Environ Microbiol 10 (9):2444-2454.

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56 (6):1919-1925.

Arrigo KR, Mock T, Lizotte MP (2010) Primary producers and sea ice. In: Thomas DN, Dieckman GS (eds) Sea Ice. 2nd edn. Wiley-Blackwell, Oxford, UK, pp 283-325

Arrigo KR, Thomas DN (2004) Large scale importance of sea ice biology in the Southern Ocean. Antarct Sci 16 (4):471-486.

Arrigo KR, van Dijken G, Pabi S (2008) Impact of a shrinking Arctic ice cover on marine primary production. Geophys Res Lett 35:L19603, doi:19610.11029/12008GL035028.

Bano N, Hollibaugh JT (2000) Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the β subdivision of the class proteobacteria in the Arctic Ocean. Appl Environ Microbiol 66 (5):1960-1969.

Boé J, Hall A, Qu X (2009) September sea-ice cover in the Arctic Ocean projected to vanish by 2100. Nature Geoscience 2 (5):341-343.

Bronk DA, Lomas MW, Glibert PM, Schukert KJ, Sanderson MP (2000) Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. Mar Chem 69 (1-2):163-178.

Bronk DA, Steinberg DK (2008) Nitrogen regeneration. In: Bronk DA, Capone DG, Carpenter EJ, Mulholland MR (eds) Nitrogen in the Marine Environment. 2nd edn. Elsevier Inc., pp 385-467

Brown TA, Belt ST (2012a) Closely linked sea ice-pelagic coupling in the Amundsen Gulf revealed by the sea ice diatom biomarker IP25. J Plankton Res 34 (8):647-654.

Brown TA, Belt ST (2012b) Identification of the sea ice diatom biomarker IP_{25} in Arctic benthic macrofauna: direct evidence for a sea ice diatom diet in Arctic heterotrophs. Polar Biol 35:131-137.

Brown ZW, Arrigo KR (2013) Sea ice impacts on spring bloom dynamics and net primary production in the Eastern Bering Sea. J Geophys Res-Oceans 118:1–20, doi:10.1029/2012JC008034.

Brzezinski MA (1987) Colorimetric determination of nanomolar concentrations of ammonium in seawater using solvent extraction. Mar Chem 20 (3):277-288.

Cai W-J, Chen L, Chen B, Gao Z, Lee SH, Chen J, Pierrot D, Sullivan K, Wang Y, Hu X, Huang W-J, Zhang Y, Xu S, Murata A, Grebmeier JM, Jones EP, Zhang H (2010) Decrease in the CO_2 uptake capacity in an ice-free Arctic Ocean basin. Science 329 (5991):556-559.

Carmack E, McLaughlin F (2011) Towards recognition of physical and geochemical change in Subarctic and Arctic Seas. Prog Oceanogr 90 (1-4):90-104.

Christman GD, Cottrell MT, Popp BN, Gier E, Kirchman DL (2011) Abundance, diversity, and activity of ammonia-oxidizing prokaryotes in the coastal Arctic Ocean in summer and winter. Appl Environ Microbiol 77 (6):2026-2034.

Cochlan WP, Bronk DA (2001) Nitrogen uptake kinetics in the Ross Sea, Antarctica. Deep-Sea Res Pt II 48 (19–20):4127-4153.

Collins RE, Deming JW (2011) Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. Polar Biol 34:1819-1830.

Collins RE, Rocap G, Deming JW (2010) Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 12 (7):1828-1841.

Conover RJ, Mumm N, Bruecker P, MacKenzie S (1999) Sources of urea in arctic seas: seasonal fast ice? Mar Ecol Prog Ser 179:55-69.

Cota GF, Anning JL, Harris L, R., Harrison WG, Smith REH (1990) Impact of ice algae on inorganic nutrients in seawater and sea ice in Barrow Strait, NWT, Canada, during Spring. Can J Fish Aquat Sci 47 (7):1402-1415.

Cota GF, Pomeroy LR, Harrison WG, Jones EP, Peters F, Sheldon WM, Jr, Weingartner TR (1996) Nutrients, primary production and microbial heterotrophy in the southeastern Chukchi Sea: Arctic summer nutrient depletion and heterotrophy. Mar Ecol Prog Ser 135:247-258.

Cox GFN, Weeks WF (1983) Equations for determing the gas and brine volumes in sea-ice samples. Journal of Glaciology 29 (102):306-317.

Cozzi S (2008) High-resolution trends of nutrients, DOM and nitrogen uptake in the annual sea ice at Terra Nova Bay, Ross Sea. Antarct Sci 20 (5):441-454.

Deming JW (2010) Sea ice bacteria and viruses. In: Thomas DN, Dieckman GS (eds) Sea Ice. 2nd edn. Wiley-Blackwell, Oxford, UK, pp 247-282

Dickson M-L, Wheeler PA (1995) Ammonium uptake and regeneration rates in a coastal upwelling regime. Mar Ecol Prog Ser 121:239-248.

Dudek N, Brzezinski MA, Wheeler PA (1986) Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments. Mar Chem 18 (1):59-69.

Dugdale RC, Goering JJ (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol Oceanogr 12 (2):196-206.

Dugdale RC, Wilkerson FP (1986) The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. Limnol Oceanogr 31 (4):673-689.

Dupont F (2012) Impact of sea-ice biology on overall primary production in a biophysical model of the pan-Arctic Ocean. J Geophys Res 117:C00D17,doi:10.1029/2011jc006983.

Eddie B, Juhl A, Krembs C, Baysinger C, Neuer S (2010) Effect of environmental variables on eukaryotic microbial community structure of land-fast Arctic sea ice. Environ Microbiol 12 (3):797-809.

Eicken H (2003) From the microscopic, to the macroscopic, to the regional scale: growth, microstructure, and properties of sea ice. In: Thomas DN, Dieckman GS (eds) Sea Ice. Blackwell Science Ltd., Oxford, UK, pp 22-81

Else BGT, Galley RJ, Lansard B, Barber DG, Brown K, Miller LA, Mucci A, Papakyriakou TN, Tremblay J-É, Rysgaard S (2013) Further observations of a decreasing atmospheric CO_2 uptake capacity in the Canada Basin (Arctic Ocean) due to sea ice loss. Geophys Res Lett 40 (6):1132–1137, doi:1110.1002/grl.50268.

Forest A, Tremblay J-É, Gratton Y, Martin J, Gagnon J, Darnis G, Sampei M, Fortier L, Ardyna M, Gosselin M, Hattori H, Nguyen D, Maranger R, Vaqué D, Marrasé C, Pedrós-Alió C, Sallon A, Michel C, Kellogg C, Deming J, Shadwick E, Thomas H, Link H, Archambault P, Piepenburg D (2011) Biogenic carbon flows through the planktonic food web of the Amundsen Gulf (Arctic Ocean): A synthesis of field measurements and inverse modeling analyses. Prog Oceanogr 91 (4):410-436.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. P Natl Acad Sci USA 102 (41):14683-14688.

Frankenstein G, Garner R (1967) Equations for determining the brine volume of sea ice from -0.5°C to -22.9°C. Journal of Glaciology 6 (48):943-944.

Galand PE, Casamayor EO, Kirchman DL, Lovejoy C (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. P Natl Acad Sci USA 106 (52):22427-22432.

Galand PE, Lovejoy C, Pouliot J, Vincent WF (2008) Heterogeneous archaeal communities in the particle-rich environment of an arctic shelf ecosystem. J Mar Syst 74:774-782.

Garrison DL, Buck KR (1986) Organism losses during ice melting: A serious bias in sea ice community studies. Polar Biol 4:237-239.

Glibert PM, Lispschultz F, McCarthy JJ, Altabet MA (1982) Isotope dilution models of uptake ammonium by marine plankton. Limnol Oceanogr 27 (4):639-650.

Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl Environ Microbiol 65 (8):3721-3726.

Golden KM, Ackley SF, Lytle VI (1998) The percolation phase transition in sea ice. Science 282 (5397):2238-2241.

Gosselin M, Lavasseur M, Wheeler PA, Horner RA, Booth BC (1997) New measurements of phytoplankton and ice algal production in the Arctic Ocean. Deep-Sea Res Pt II 44 (8):1623-1644.

Gradinger R (1995) Climate change and biological oceanography of the Arctic Ocean. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences 352 (1699):277-286.

Gradinger R (2009) Sea-ice algae: Major contributors to primary production and algal biomass in the Chukchi and Beaufort Seas during May/June 2002. Deep-Sea Res Pt II 56 (17):1201-1212.

Gradinger R, Ikävalko J (1998) Organism incorporation into newly forming Arctic sea ice in the Greenland Sea. J Plankton Res 20 (5):871-886.

Grebmeier JM, Cooper LW, Feder HM, Sirenko BI (2006) Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. Prog Oceanogr 71 (2-4):331-361.

Hama T, Miyazaki T, Ogawa Y, Iwakuma T, Takahashi M, Otsuki A, Ichimura S (1983) Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. Mar Biol 73 (1):31-36.

Harrison WG, Cota GF, Smith REH (1990) Nitrogen utilization in ice algal communities of Barrow Strait, Northwest Territories, Canada. Mar Ecol Prog Ser 67:275-283.

Helmke E, Weyland H (1995) Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. Mar Ecol Prog Ser 117:269-287.

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9-18.

Horner R, Schrader GC (1982) Relative contributions of ice algae, phytoplankton, and benthic microalgae to primary production in nearshore regions of the Beaufort Sea. Arctic 35 (4):485-503.

Johnson KM, Sieburth JM, Williams PJl, Brändström L (1987) Coulometric total carbon dioxide analysis for marine studies: Automation and calibration. Mar Chem 21 (2):117-133.

Jones KA, Ingham M, Eicken H (2012) Modeling the anisotropic brine microstructure in first-year Arctic sea ice. J Geophys Res 117:C02005, doi:02010.01029/02011JC007607.

Junge K, Eicken H, Deming JW (2004) Bacterial activity at -2 to -20°C in Arctic wintertime sea ice. Appl Environ Microbiol 70 (1):550-557.

Junge K, Imhoff F, Staley T, Deming W (2002) Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol 43 (3):315-328.

Kaartokallio H (2001) Evidence for active microbial nitrogen transformations in sea ice (Gulf of Bothnia, Baltic Sea) in midwinter. Polar Biol 24 (1):21-28.

Kahru M, Brotas V, Manzano-Sarabia M, Mitchell BG (2011) Are phytoplankton blooms occurring earlier in the Arctic? Global Change Biol 17 (4):1733-1739.

Keil RG, Kirchman DL (1991) Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar Ecol Prog Ser 71:1-10.

Kirchman DL (2000) Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. In: Kirchman DL (ed) Microbial Ecology of the Oceans. Wiley-Liss, New York, pp 261-288

Kirchman DL, Elifantz H, Dittel AI, Malmstrom RR, Cottrell MT (2007) Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol Oceanogr 52 (2):495-507.

Kirchman DL, Hill V, Cottrell MT, Gradinger R, Malmstrom RR, Parker A (2009) Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. Deep-Sea Res Pt II 56:1237-1248.

Kirchman DL, T. CM, Lovejoy C (2010) The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. Environ Microbiol 12 (5):1132-1143.

Koroleff F (1983) Determination of nutrients. In: Grasshoff K, Ehrhardt M, Kremling K (eds) Methods of seawater analysis. Verlag Chemie, New York, pp 125-187

Krell A, Ummenhofer C, Kattner G, Naumov A, Evans D, Dieckman GS, Thomas DN (2003) The biology and chemistry of land fast ice in the White Sea, Russia—A comparison of winter and spring conditions. Polar Biol 26:707-719.

Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. P Natl Acad Sci USA 108 (9):3653-3658.

Krembs C, Eicken H, Junge K, Deming JW (2002) High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. Deep-Sea Res Pt I 49 (12):2163-2181.

Krembs C, Gradinger R, Spindler M (2000) Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. J Exp Mar Biol Ecol 243 (1):55-80.

Kuparinen J, Autio R, Kaartokallio H (2011) Sea ice bacterial growth rate, growth efficiency and preference for inorganic nitrogen sources in the Baltic Sea. Polar Biol 34:1361-1373.

Kuparinen J, Kuosa H, Andersson A, Autio R, Granskog MA, Ikävalko J, Kaartokallio H, Karell K, Leskinen E, Piiparinen J, Rintala J-M, Tuomainen J (2007) Role of sea-ice biota in nutrient and organic material cycles in the northern Baltic Sea. Ambio 36 (2):149-154.

Lee SH, Jin M, Whitledge TE (2010) Comparison of bottom sea-ice algal characteristics from coastal and offshore regions in the Arctic Ocean. Polar Biol 33 (10):1331-1337.

Lee SH, Whitledge TE, Kang S-H (2008) Carbon uptake rates of sea ice algae and phytoplankton under different light intensities in a landfast sea ice zone, Barrow, Alaska. Arctic 61 (3):281-291.

Lipschultz F (2008) Isotope tracer methods for studies of the marine nitrogen cycle. In: Bronk DA, Capone DG, Carpenter EJ, Mulholland MR (eds) Nitrogen in the Marine Environment. Elsevier Inc., pp 1345-1384

Lizotte MP (2003) The microbiology of sea ice. In: Thomas DN, Dieckman GS (eds) Sea Ice. Blackwell Science Ltd, Oxford, UK, pp 184-210

Lomas MW, Rumbley CJ, Glibert PM (2000) Ammonium release by nitrogen sufficient diatoms in response to rapid increases in irradiance J Plankton Res 22 (12):2351-2366.

Loose B, Miller LA, Elliott S, Papakyriakou T (2011) Sea ice biogeochemistry and material transport across the frozen interface. Oceanography 24 (3):202-218.

Manz W, Amann R, Ludwig W, Wagner M, Schleifer K-H (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*: problems and solutions. Syst Appl Microbiol 15 (4):593-600.

Maslowski W, Kinney JC, Higgins M, Roberts A (2012) The future of Arctic sea ice. Annu Rev Earth Pl Sc 40:625-654.

Massana R, Murray AE, Preston CM, DeLong EF (1997) Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl Environ Microbiol 63 (1):50-56.

Mock T, Gradinger R (1999) Determination of Arctic ice algal production with a new *in situ* incubation technique. Mar Ecol Prog Ser 177:15-26.

Mock T, Thomas DN (2005) Recent advances in sea-ice microbiology. Environ Microbiol 7 (5):605-619.

Moore SE, Huntington HP (2008) Arctic marine mammals and climate change: impacts and resilience. Ecol Appl 18 (sp2):S157-S165.

Nguyen D, Maranger R (2011) Respiration and bacterial carbon dynamics in Arctic sea ice. Polar Biol 34:1843-1855.

Olson RJ (1980) Nitrate and ammonium uptake in Antarctic waters. Limnol Oceanogr 25 (6):1064-1074.

Pabi S, van Dijken GL, Arrigo KR (2008) Primary production in the Arctic Ocean, 1998-2006. J Geophys Res 113 (C8):C08005, doi:08010.01029/02007jc004578.

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon, Oxford

Pernthaler A, Preston CM, Pernthaler J, DeLong EF, Amann R (2002) Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine bacteria and archaea. Appl Environ Microbiol 68 (2):661-667.

Perrette M, Yool A, Quartly GD, Popova EE (2011) Near-ubiquity of ice-edge blooms in the Arctic. Biogeosciences 8 (2):515-524.

Pineault S, Tremblay J-É, Gosselin M, Thomas H, Shadwick E (2013) The isotopic signature of particulate organic C and N in bottom ice: Key influencing factors and applications for tracing the fate of ice-algae in the Arctic Ocean. J Geophys Res-Oceans 118:1–14, doi:10.1029/2012JC008331.

Preston T, Zainal K, Anderson S, Bury SJ, Slater C (1998) Isotope dilution analysis of combined nitrogen in natural waters. III. Nitrate and nitrite. Rapid Commun Mass Sp 12 (8):423-428.

Price NM, Harrison PJ (1987) Comparison of methods for the analysis of dissolved urea in seawater. Mar Biol 94:307-317.

Priscu JC, Downes MT, Priscu LR, Palmisano AC, Sullivan CW (1990) Dynamics of ammonium oxidizer activity and nitrous oxide (N_2O) within and beneath Antarctic sea ice. Mar Ecol Prog Ser 62:37-46.

Priscu JC, Palmisano AC, Priscu LR, Sullivan CW (1989) Temperature dependence of inorganic nitrogen uptake and assimilation in Antarctic sea-ice microalgae. Polar Biol 9 (7):443-446.

Priscu JC, Sullivan CW (1998) Nitrogen metabolism in Antarctic fast-ice microalgal assemblages. In: Lizotte MP, Arrigo KR (eds) Antarctic Sea Ice: Biological Processes, Interactions and Variability, vol 73. Antarct. Res. Ser. AGU, Washington, DC, pp 147-160. doi:10.1029/AR073p0147

Reay DS, Nedwell DB, Priddle J, Ellis-Evans JC (1999) Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl Environ Microbiol 65 (6):2577-2584.

Redfield AC (1934) On the proportions of organic derivatives in sea water and their relation to the composition of plankton. In: Daniel RJ (ed) James Johnstone Memorial Volumes. University Press of Liverpool, pp 177-192

Renaud PE, Riedel A, Michel C, Morata N, Gosselin M, Juul-Pedersen T, Chiuchiolo A (2007) Seasonal variation in benthic community oxygen demand: A response to an ice algal bloom in the Beaufort Sea, Canadian Arctic? J Mar Syst 67 (1–2):1-12.

Rich J, Gosselin M, Sherr E, Sherr B, Kirchman DL (1997) High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. Deep-Sea Res Pt II 44 (8):1645-1663.

Riedel A, Michel C, Gosselin M (2007a) Grazing of large-sized bacteria by sea-ice heterotrophic protists on the Mackenzie Shelf during the winter–spring transition. Aquat Microb Ecol 50:25-38.

Riedel A, Michel C, Gosselin M, LeBlanc B (2007b) Enrichment of nutrients, exopolymeric substances and microorganisms in newly formed sea ice on the Mackenzie shelf. Mar Ecol Prog Ser 342:55-67.

Różańska M, Gosselin M, Poulin M, Wiktor JM, Michel C (2009) Influence of environmental factors on the development of bottom ice protist communities during the winter–spring transition. Mar Ecol Prog Ser 386:43-59.

Rysgaard S, Glud R, Sejr M, Blicher M, Stahl H (2008) Denitrification activity and oxygen dynamics in Arctic sea ice. Polar Biol 31 (5):527-537.

Rysgaard S, Glud RN (2004) Anaerobic N_2 Production in Arctic sea ice. Limnol Oceanogr 49 (1):86-94.

Sala M, Arrieta J, Boras J, Duarte C, Vaqué D (2010) The impact of ice melting on bacterioplankton in the Arctic Ocean. Polar Biol 33 (12):1683-1694.

Serreze MC, Francis JA (2006) The Arctic amplification debate. Clim Change 76 (3-4):241-264.

Sharp JH, Carlson CA, Peltzer ET, Castle-Ward DM, Savidge KB, Rinker KR (2002) Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. Mar Chem 77 (4):239-253.

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73 (17):4145-4153.

Smith REH, Gosselin M, Taguchi S (1997) The influence of major inorganic nutrients on the growth and physiology of high arctic ice algae. J Mar Syst 11:63-70.

Stoecker DK, Gustafson DE, Baier CT, Black MMD (2000) Primary production in the upper sea ice. Aquat Microb Ecol 21 (3):275-287.

Stroeve J, Holland MM, Meier W, Scambos T, Serreze M (2007) Arctic sea ice decline: Faster than forecast. Geophys Res Lett 34 (9):L09501, doi:09510.01029/02007gl029703.

Stroeve JC, Serreze MC, Holland MM, Kay JE, Malanik J, Barrett AP (2012) The Arctic's rapidly shrinking sea ice cover: a research synthesis. Clim Change 110 (3-4):1005-1027.

Thomas DN, Dieckman GS (2002) Antarctic sea ice -- a habitat for extremophiles. Science 295:641-644.

Thomas DN, Lara RJ, Eicken H, Kattner G, Skoog A (1995) Dissolved organic matter in Arctic multi-year sea ice during winter: major components and relationship to ice characteristics. Polar Biol 15 (7):477-483.

Thomas DN, Papadimitriou S, Michel C (2010) Biogeochemistry of sea ice. In: Thomas DN, Dieckman GS (eds) Sea Ice. 2nd edn. Wiley-Blackwell, Oxford, UK, pp 425-467

Tovar-Sánchez A, Duarte CM, Alonso JC, Lacorte S, Tauler R, Galbán-Malagón C (2010) Impacts of metals and nutrients released from melting multiyear Arctic sea ice. J Geophys Res 115 (C7):C07003, 07010.01029/02009jc005685.

Tremblay C, Runge JA, Legendre L (1989) Grazing and sedimentation of ice algae during and immediately after a bloom at the ice-water interface. Mar Ecol Prog Ser 56:291-300.

Tremblay J-É, Michel C, Hobson KA, Gosselin M, Price NM (2006) Bloom dynamics in early opening waters of the Arctic Ocean. Limnol Oceanogr 51 (2):900-912.

Vaquer-Sunyer R, Duarte C, Santiago R, Wassmann P, Reigstad M (2010) Experimental evaluation of planktonic respiration response to warming in the European Arctic Sector. Polar Biol 33 (12):1661-1671.

Wallner G, Amann R, Beisker W (1993) Optimizing fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry 14 (2):136-143.

Wang M, Overland JE (2009) A sea ice free summer Arctic within 30 years? Geophys Res Lett 36:L07502, doi:07510.01029/02009GL037820.

Ward BB (2008) Nitrification in marine systems. In: Bronk DA, Capone DG, Carpenter EJ, Mulholland MR (eds) Nitrogen in the Marine Environment. 2nd edn. Elsevier Inc., pp 199-262

Ward BB, Talbot MC, Perry MJ (1984) Contributions of phytoplankton and nitrifying bacteria to ammonium and nitrite dynamics in coastal waters. Cont Shelf Res 3 (4):383-398.

Weller R, Glöckner FO, Amann R (2000) 16S rRNA-targeted oligonucleotide probes for the *in situ* detection of members of the phylum *Cytophaga-Flavobacterium-Bacteroides*. Syst Appl Microbiol 23 (1):107-114.

Wells LE, Deming JW (2006) Modelled and measured dynamics of viruses in Arctic winter sea-ice brines. Environ Microbiol 8 (6):1115-1121.

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol Oceanogr 39 (8):1985-1992.

Werner I, Ikävalko J, Schünemann H (2007) Sea-ice algae in Arctic pack ice during late winter. Polar Biol 30 (11):1493-1504.

Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl GJ, Middleburg JJ, Schouten S, Damsté JSS (2006) Archaeal nitrification in the ocean. P Natl Acad Sci USA 103 (33):12317-12322.

Yentsch CS (1965) Distribution of chlorophyll and phaeophytin in the open ocean. Deep Sea Research and Oceanographic Abstracts 12 (5):653-666.

Tables

Table 1. Sequences of oligonucleotides (16S rRNA) used as FISH probes.

Probe name	Target group	Sequence (5'-3')	Reference
EUB338	Bacteria	GCTGCCTCCCGTAGGAGT	Amann et al. (1990)
CREN554	Crenarchaeota	TTAGGCCCAATAATCMTCCT	Massana et al. (1997)
GAM42a	Gammproteobacteria	GCCTTCCCACATCGTTT	Manz et al. (1992)
ALF968	Alphaproteobacteria	GGTAAGGTTCTGCGCGTT	Glöckner et al. (1999)
BET42a	Betaproteobacteria	GCCTTCCCACTTCGTTT	Manz et al. (1992)
CF319a	Bacteriodes	TGGTCCGTGTCTCAGTAC	Weller et al. (2000)
NON338	Control[1]	ACTCCTACGGGAGGCAGC	Wallner et al. (1993)

[1] complimentary to EUB338.

Table 2. Specific and absolute C uptake rates. Rates and standard deviations for HCO_3^- and ^{3}H-leu uptake. Chl *a* and bacterial abundance for the sea ice depths are brine-corrected concentrations. BDL is below detection limit. BP:PP values could not be calculated for January 2012 since no HCO_3^- uptake was detected in the winter incubations. Negative values denote water column depths.

Month	Depth (cm)	Chl a (μg L^{-1})	Bacterial abundance (10^8 cells L^{-1})	Particulate C (μmol C L^{-1})	HCO_3^- specific uptake (h^{-1}) x 1000	HCO_3^- absolute uptake (nmol C L^{-1} h^{-1})	HCO_3^- absolute uptake (mg C m^{-2} d^{-1})	^{3}H-leu uptake (pmol leu L^{-1} h^{-1})	^{3}H-leu uptake (μg C m^{-2} d^{-1})	BP:PP
April	35	6.0 ± 2.1	62 ± 0.2	4.81 ± 0.31	1.3 ± 0.2	6.32 ± 1.11	0.18 ± 0.03	2.38 ± 0.19	0.01 ± 0.00	0.047
	20	3.3 ± 0.4	72 ± 2.2	4.51 ± 0.24	1.4 ± 0.3	6.19 ± 1.14	0.12 ± 0.07	6.19 ± 0.34	0.02 ± 0.00	0.125
	5	415 ± 17	148 ± 2.1	23.4 ± 1.72	6.5 ± 0.8	153 ± 17.9	4.41 ± 0.51	37.9 ± 2.16	0.14 ± 0.01	0.031
	-400	0.1 ± 0.0	1.8 ± 0.0	2.07 ± 0.18	0.49 ± 0.13	0.15 ± 0.01	0.004 ± 0.002	8.23 ± 0.14	0.03 ± 0.00	7.0
January	35	0.7 ± 0.0	149 ± 0.6	15.5 ± 3.09	BDL	BDL	BDL	0.99 ± 0.24	0.07 ± 0.01	-
	20	0.6 ± 0.0	227 ± 1.7	10.4 ± 0.45	BDL	BDL	BDL	1.18 ± 0.09	0.04 ± 0.00	-
	5	16 ± 0.0	165 ± 4.0	10.2 ± 2.18	BDL	BDL	BDL	6.07 ± 0.20	0.22 ± 0.01	-
	-100	0.0 ± 0.0	1.7 ± 0.0	23.4 ± 1.72	BDL	BDL	BDL	1.75 ± 0.32	0.06 ± 0.01	-

Table 3. Nitrification rates. Measured nitrification rates and standard deviations in the sea ice and water column (denoted by negative values) for spring and winter sampling.

Depth (cm)	Nitrification rate ($nmol\ N\ L^{-1}\ h^{-1}$)	
	April	January
35	0.60 ± 0.06	3.86 ± 1.34
20	2.21 ± 2.09	3.77 ± 1.08
5	5.40 ± 0.28	2.26 ± 0.05
-100		21.6 ± 8.16
-400	24.9 ± 16.6	

Figures

Figure 1. Physical conditions of the sea ice and brine channels. Temperature (open triangles), brine salinity (open squares), and brine channel volume percent (filled circles) profiles for cores during spring (left panel) and winter (right panel). The profiles are for the full depth of the ice cores, which was 130 cm in spring, but 60 cm in winter. The vertical dashed lines are the 5% brine channel volume percent, which corresponds to the lower limit of permeability in the ice core (Golden et al. 1998). Note the different scales for salinity and temperature. A depth of zero corresponds to the ice-water interface.

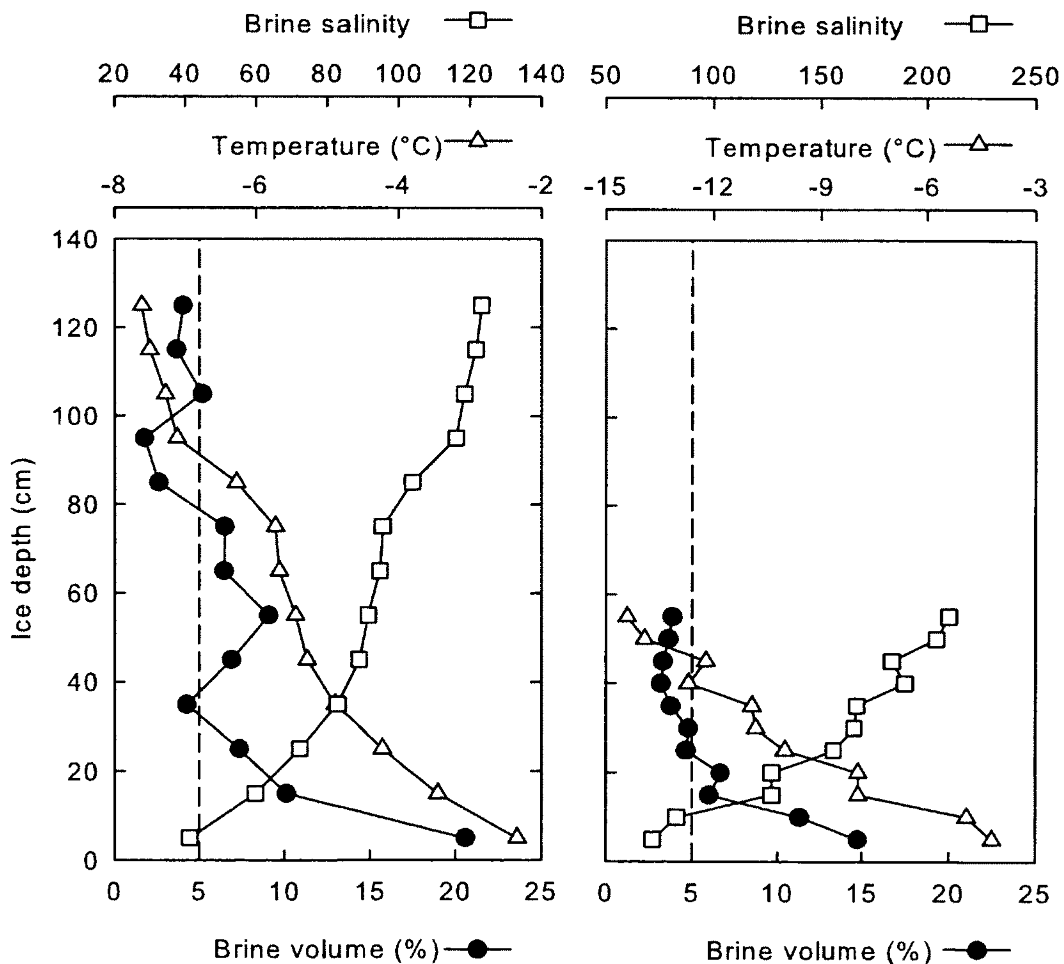


Figure 2. Chlorophyll a and bacterial abundance in sea-ice and water column. Chl *a* and bacterial abundance for spring (a) and winter (b). Error bars indicate standard deviation; when no error bars are seen, they were smaller than the symbol. The dashed horizontal line indicates the ice-water interface; the sea ice values are above and water column values are below that line. The inset boxes are brine-normalized values for the Chl *a* and bacterial abundance of just the sea ice communities; note the different scales from the larger plots, and between the seasons.

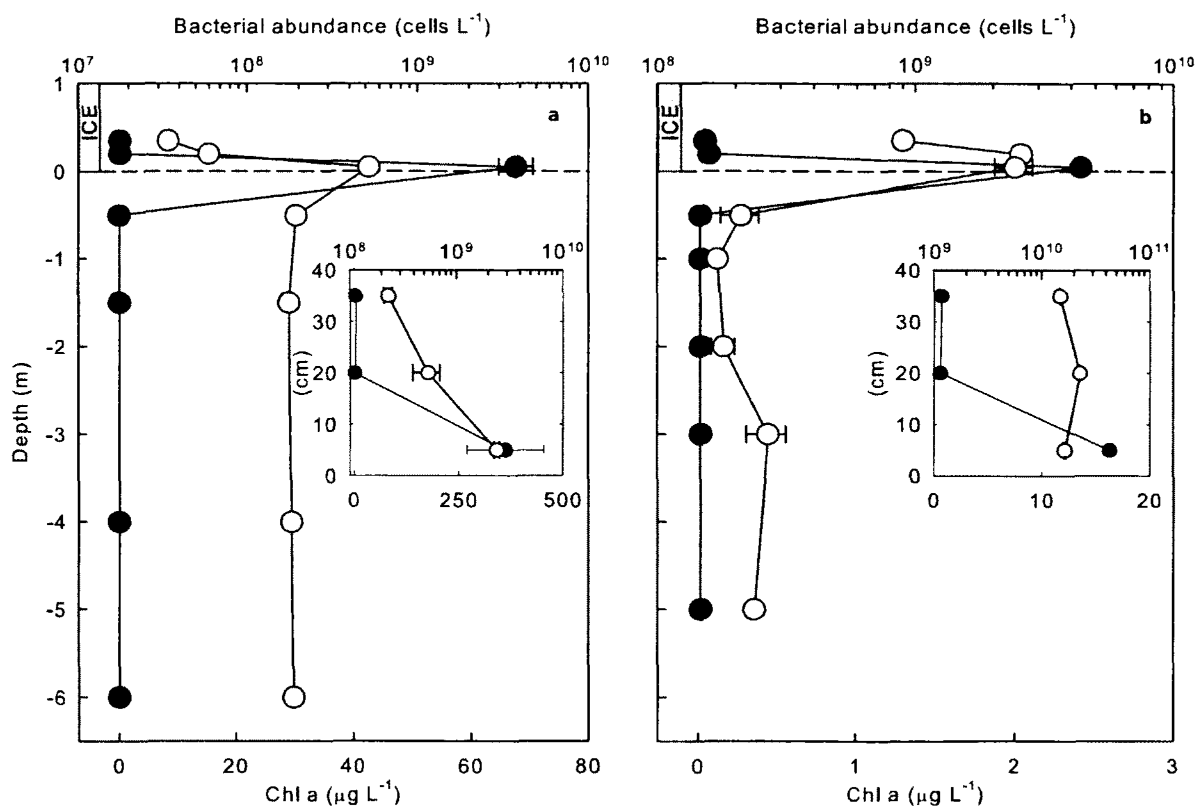


Figure 3. Community composition of sea ice communities. Means of CARD-FISH results as a function of overall percentage of the sea ice community for spring. Probes used for alpha- (ALF), beta- (BET), and gamma- (GAM) proteobacteria, bacteriodes (CF), and crenarchaeota (CREN). Positive (eubacteria) control discussed in Results and negative (antisense) control not shown.

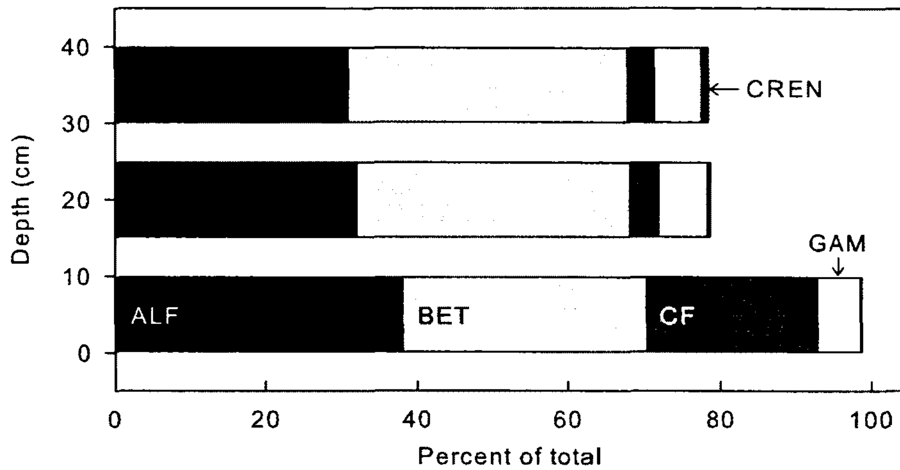


Figure 4. Bulk nutrients in the ice and water column. Ambient nutrient concentrations in the water column and bulk ice for spring (upper panel) and winter (lower panel). The dashed line indicates the ice-water interface; the sea ice values are above and water column values are below that line. Symbols, as noted in the bottom panel, indicate NH_4^+ (filled circles) and NO_3^- (open circles), urea (filled triangles) and DPA (open triangles), DOC (filled squares) and DON (open squares), PO_4^{3-} (filled diamonds) and Si (open diamonds). Error bars indicate standard deviation; some error bars are smaller than the symbol.

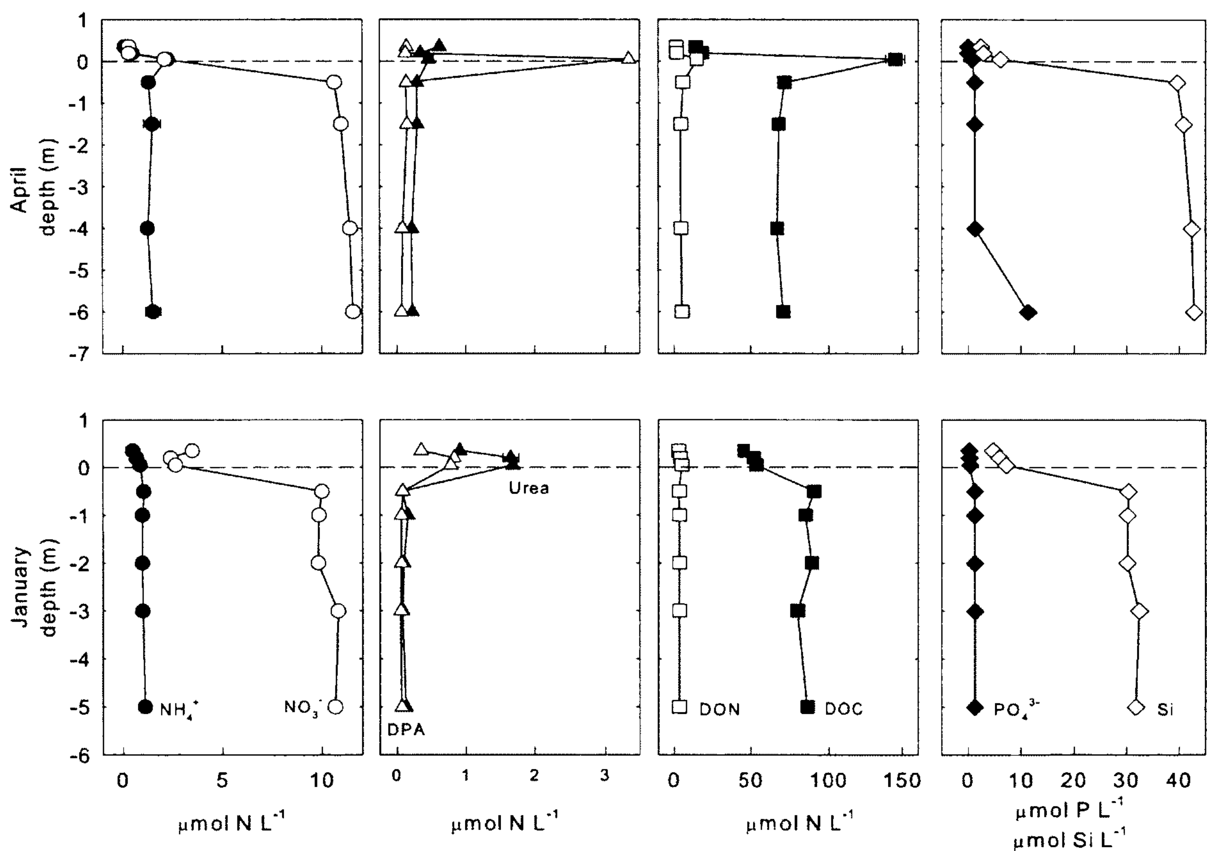


Figure 5. Brine normalized nutrient concentrations in the sea ice. Concentrations of ambient nutrients in the sea ice brine channels for spring (upper panel) and winter (lower panel). Symbols, as noted in the bottom panel, indicate NH_4^+ (filled circles) and NO_3^- (open circles), urea (filled triangles) and DPA (open triangles), DOC (filled squares) and DON (open squares), PO_4^{3-} (filled diamonds) and Si (open diamonds). Error bars indicate standard deviation; some error bars are smaller than the symbol. The DOC:DON axis does not include a break.

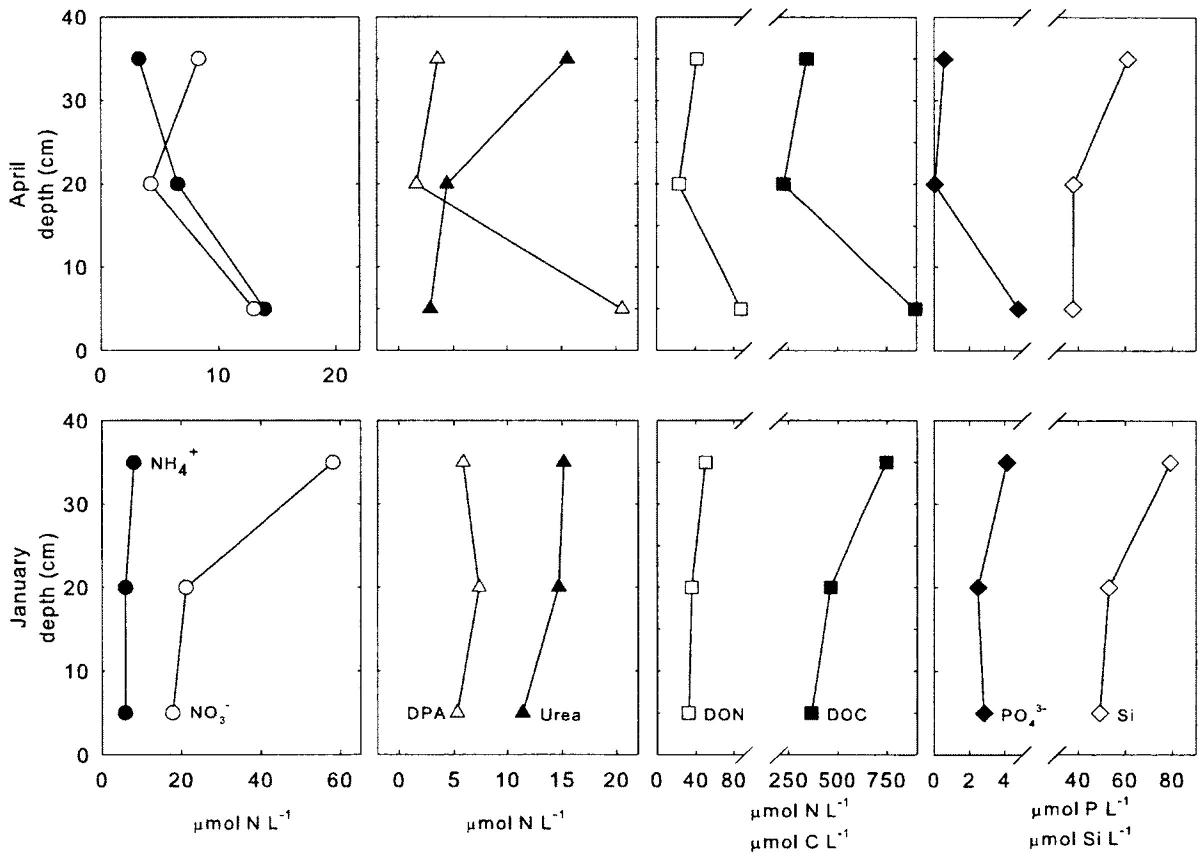
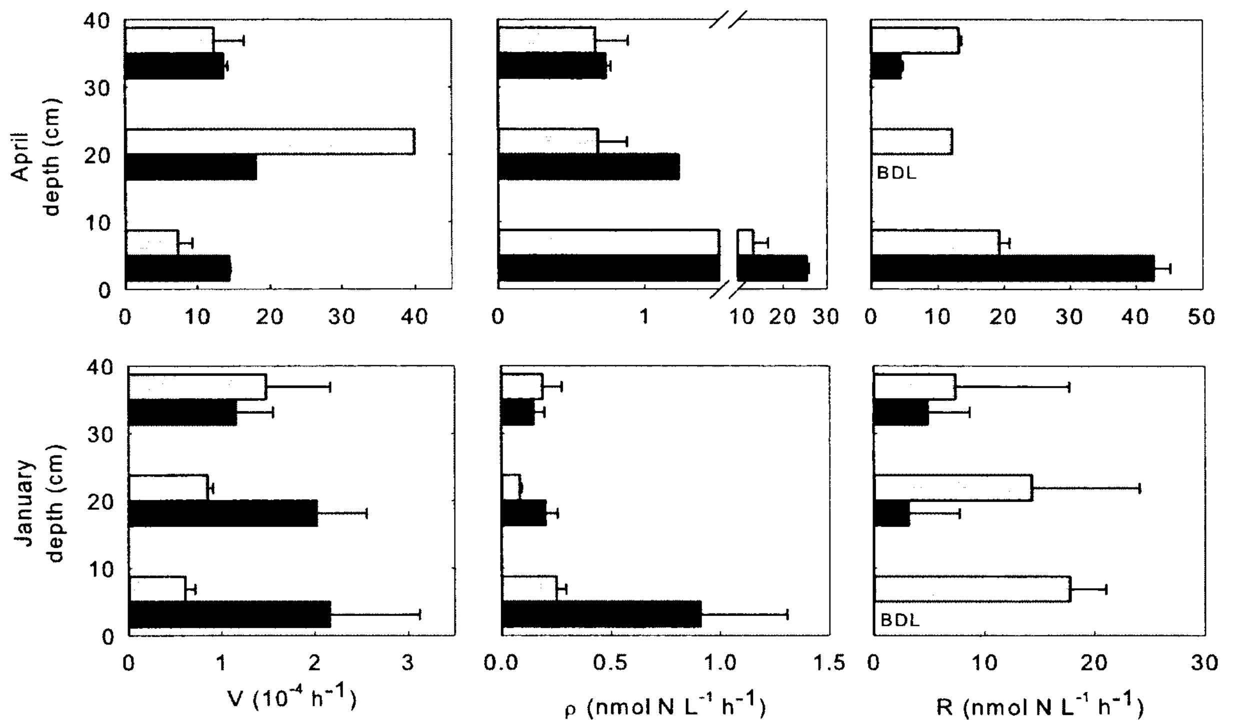


Figure 6. Uptake and regeneration rates of NH_4^+ and NO_3^-. For each sampling depth (centered on 5, 20, and 35 cm from the ice-water interface), values of specific uptake (V), absolute uptake (ρ) and regeneration (R) of NH_4^+ (black) and NO_3^- (gray) are given for spring (top row) and winter (bottom row). BDL is below the detection limit. Note different x-axis scales for different seasons (upper and lower panels), especially for V, which is an order of magnitude lower during January.



Chapter 6 – Conclusion

This chapter summarizes the principal findings and theorizes about potential impacts on our understanding of the coastal Chukchi Sea ecosystem as well as the potential for future biogeochemical changes in the ecosystem. The research was full of both scientific and physical adventures, which helped to add to our knowledge of nitrogen (N) cycling, but also sparked new ideas for how to extend the scope of the research questions. In that regard, limitations, lessons learned and future directions of the research are also presented.

The essential issue that was addressed was the limited data on N uptake and regeneration across the Arctic, despite the hypothesized importance of these processes in sea ice and during winter. Furthermore, there is very little data from anywhere in the Arctic on N uptake and regeneration rates within the sea ice matrix (i.e. not just the bottom ice) and in the water column beneath sea ice (i.e. winter and spring). This lack of knowledge is especially striking in the shallow regions away from large rivers such as the Chukchi Sea. Even though it is known that the Arctic is warming faster than anywhere else on Earth (e.g. Serreze and Francis 2006), there is very little empirical information on how increased temperatures will impact N cycling.

Chapter 2 addresses the shortage of data sets on N uptake and regeneration in the western coastal Arctic. The sampling regime consisted of experiments to quantify N uptake rates in the water column and sea ice during January, April, and August. Reported herein is evidence of the importance of both NH_4^+ and amino acids compared NO_3^- in this system. Chapter 3 presents data that show this preference holds for both phytoplankton fractions and the bacterial LNA and HNA populations. Chapter 5 extends this

generalization further to the landfast sea ice community. This preference for NH_4^+ is also different from N uptake measured in other Arctic regions, where NO_3^- uptake tends to be more important. While it appears that NH_4^+ is preferentially used in the Chukchi Sea generally, it may be a feature of coastal regions across the Arctic.

Polar amplification of climate change is rapidly changing the system. Temperature increases could potentially cause changes in biogeochemical rates and is already triggering declines in multiyear sea ice. The results from Chapter 4 illustrate the magnitude of changes to NH_4^+ uptake and nitrification rates that may be expected to happen along with changes in temperature. Rates of NH_4^+ uptake increased with temperature and were positively correlated to ambient NH_4^+ concentrations, but were likely to be inhibited by light. If sea ice recedes earlier in the year, it is likely to precipitously reduce nitrification rates and therefore NO_3^- concentrations. This could potentially reduce the timing and magnitude of the spring phytoplankton bloom, which would have drastic impacts on the Arctic food web.

The results from Chapter 5 showed that N uptake rates within the sea ice are typically greater than the water column during January and April. The landfast first year ice could be a harbinger of sea ice biogeochemistry in the future. Although there is little comparable data from multiyear ice, the light regime within the thinner sea ice is likely to promote changes in the timing and magnitude of the spring phytoplankton bloom, thereby increasing biomass and N uptake rates throughout the ice. With greater biomass, the microbial community would be expected to respond with greater N regeneration rates and

a larger fresh organic matter pulse to the water column, further enhancing primary production throughout the Arctic (Arrigo et al. 2008).

Research limitations

Our research plan focused on a wide variety of N substrates and sampling across different seasons and was heavily influenced by the physical conditions present in Barrow, including the location of impassable pressure ridges, stability of the sea ice, and avoidance of the annual bowhead whale hunt of the local community. During the summer, high seas and low visibility made it difficult at times to procure water for sampling. Some of these difficulties were anticipated in the sampling plan and strategies were devised to overcome any obstacles, within the confines of physical safety for all involved.

It could be argued that to truly understand seasonal dynamics and the intense pulses of activity known to occur in the region extended continuous time sampling the region would be warranted. This was done for the CASES project in the southeastern Beaufort Sea, which overwintered aboard the CCGS *Amundsen* (details first presented in Tremblay et al. 2008). Our study was funded to visit at discrete time periods and we performed as much sampling as we could within the limitations of what was funded. While we attempted to be at our sampling site as close to the time of the historical phytoplankton bloom period, we were early by a few weeks each year (A. Juhl, pers. comm.)

As described in Chapters 2 and 3, we used size fractionation to discriminate uptake by phytoplankton from that by bacteria. The biomass in our sample region is low, necessitating large volumes of water to filter in order to get enough mass for a reliable reading on our mass spectrometer. We attempted to capture bacterial cells passing through the GF/F filters by terminating the incubations over 0.2 μm silver filters but found the filtration times were greater than 1 hour each, and would have compromised our ability to perform multiple experiments with a variety of N forms. Additionally, such long filtering times risk exposing the microorganisms to artificial conditions (i.e. light, temperature) of the laboratory and making the end point of the incubations unclear, factors that we had worked hard to eliminate.

We attempted to mitigate size fractionation issues with parallel incubations intended for flow cytometric sorting, using 0.2 μm Supor filters. We hoped to identify phytoplankton and bacterial groups to measure their respective uptake rates unambiguously. Unfortunately, cell fluorescence was so low such that there was no clear separation of the phytoplankton and bacterial populations, unlike what we have observed in the other estuarine, coastal and ocean systems we have sampled. It is currently unclear whether the lack of separation has to do with the region we sampled or because of a problem with our sampling methodology. Now that the Bronk lab has a high-speed sorter in house, we intend to perform method tests to perfect our sample preparation processes. In an effort to make lemonade out of lemons, the sort procedure was adjusted to interrogate low nucleic acid and high nucleic acid populations, as described in Chapter 3. While not the original goal of the sampling procedure, the results of our LNA and HNA

sorting should result in compelling evidence of the ecological significance of the bacterial subpopulations.

Future directions

The research presented in this dissertation has provided information on N cycling processes in the coastal western Arctic. In addition to adding to the body of knowledge on polar biogeochemistry, it has raised some fundamental questions about how the coastal Chukchi Sea ecosystem functions at the microbial scale. Given the opportunity, future experiments should expand on the knowledge gained by connecting the discrete sampling of this study with connections to the wider ecosystem temporally and spatially along with connections to other elemental cycles and food web dynamics. As mentioned above, greater continuous coverage of N uptake, especially during the spring phytoplankton and bloom periods, could provide a better mechanistic understanding of the seasonal dynamics of the Chukchi Sea. There is great potential to recruit the local community, in collaboration with a qualified lab, to perform basic monitoring of nutrients and physical conditions. This kind of long-term and detailed monitoring would be invaluable for efforts to evaluate changes and provide context for more targeted uptake studies. In addition to more temporal coverage, wider spatial sampling could provide better understanding of variability in the region. Our sampling site was within a few miles of the flaw lead. How do ice conditions and uptake change even in that short distance? Barrow is located just west of the convergence of the Chukchi and Beaufort

Seas, which have vastly different conditions. Future work could focus on the gradients between these two seas along with onshore to offshore gradients.

Midway through this project, Christman et al. (2011) published the first report of winter nitrification rates in polar waters. Chapter 3 corroborated the vast seasonal difference in rates and furthermore explicitly tested the impact of warming temperatures on NH_4^+ uptake and nitrification. Additional work is warranted on warming impacts of NO_3^-, urea, and amino acid uptake. Climate change impacts will also influence light, pH and stratification. Incorporating these elements into future studies of N uptake would provide some predictive power to climate change modeling in the Arctic and beyond.

The N uptake in this study focused on the water column and sea ice. Sampling took place in a shallow system, but we did not account for any benthic processes. Future work should attempt to directly link biogeochemical cycling in all three (presumably) closely linked systems: the benthos, water column, and sea ice. Additionally, riverine inputs to the Arctic have great influence on the freshwater budget and nutrient regimes of the basin (Aagaard and Carmack 1989; Holmes et al. 2012). Barrow, however, is located far from these influences. While it is likely that the Yukon and Mackenzie Rivers have some impact on this region, it is currently unclear what role overland or submarine flow have on nutrient inputs there.

Flow cytometric sorting provides an opportunity to connect specific populations of microorganisms with their uptake rates (Lomas et al. 2011), which is otherwise methodologically difficult. An alternate avenue would be to incorporate genomic studies with uptake. While not part of this dissertation, samples were taken for stable isotope

probing to clearly indicate which microorganisms actively incorporate the ^{15}N label. Molecular data could provide more detail on the organisms present and their physiological capabilities. Combining genomic work with uptake studies in the Arctic has already provided new information on the link between urea and nitrification (Alonso-Sáez et al. 2012) and N_2 fixation (Blais et al. 2012). Nitrification measurements have been reported extensively herein, but there is limited information on N_2 fixation. The Bronk lab recently made N_2 fixation measurements and found that it is low but present in the summer and that this may be a relatively new phenomenon (Bronk et al. in prep.). Could this be an indication that the system has become more N-limited during the latter half of the twentieth century? To what extent, under what conditions, and what organisms are responsible for N_2 fixation in the Arctic?

As presented in Chapter 5, there was a discrepancy between measured NO_3^- regeneration and nitrification rates. There are few NO_3^- regeneration rates reported throughout the literature, making a comparison difficult. Additionally, many reported rates of uptake for NH_4^+ and NO_3^- are not corrected for isotope dilution, and instead account for the former with the equations of Kanda et al. (1987) and the latter not at all. The limited information on isotope dilution correction across a variety of oceanographic areas cries out for an analysis of the difference between measured and calculated dilution effects.

Synopsis

The physical changes happening in the Arctic are having a profound effect on biogeochemical cycling. With this dissertation research, empirical data has been

presented on N uptake rates and regeneration in the water column and sea ice of the western coastal Arctic. It has added to the field of Arctic biogeochemistry, expanding understanding of N cycling processes in the western coastal Arctic and provided a baseline for future studies. The research has undertaken the task of describing N cycling in the water column, including size fractionation of phytoplankton, detailed N uptake rates of bacterial subpopulations, and potential future changes to these processes with respect to irradiance and nutrient concentration. The research has also expanded our knowledge of N cycling in landfast sea ice and provided the first direct measurements of NH_4^+ and NO_3^- regeneration in Arctic sea ice. While the work presented here can stand on it's own, there are many future avenues of research for comparison and further expansion of our knowledge of the rapidly changing Arctic ecosystem.

References

Aagaard K, Carmack EC (1989) The role of sea ice and other fresh water in the Arctic circulation. J Geophys Res 94 (C10):14485-14498.

Alonso-Sáez L, Waller AS, Mende DR, Bakker K, Farnelid H, Yager PL, Lovejoy C, Tremblay J-É, Potvin M, Heinrich F, Estrada M, Riemann L, Bork P, Pedrós-Alió C, Bertilsson S (2012) Role for urea in nitrification by polar marine Archaea. P Natl Acad Sci USA 109 (44):17989-17994.

Arrigo KR, van Dijken G, Pabi S (2008) Impact of a shrinking Arctic ice cover on marine primary production. Geophys Res Lett 35:L19603, doi:19610.11029/12008GL035028.

Blais M, Tremblay J-É, Jungblut AD, Gagnon J, Martin J, Thaler M, Lovejoy C (2012) Nitrogen fixation and identification of potential diazotrophs in the Canadian Arctic. Global Biogeochem Cy 26 (3):GB3022,doi:3010.1029/2011gb004096.

Bronk DA, Sipler RE, Killberg-Thoreson LM, Dugdale R, Sanderson MP, Baer SE, Roberts QN (in prep.) Arctic coastal nitrogen fixation.

Christman GD, Cottrell MT, Popp BN, Gier E, Kirchman DL (2011) Abundance, diversity, and activity of ammonia-oxidizing prokaryotes in the coastal Arctic Ocean in summer and winter. Appl Environ Microbiol 77 (6):2026-2034.

Holmes R, McClelland J, Peterson B, Tank S, Bulygina E, Eglinton T, Gordeev V, Gurtovaya T, Raymond P, Repeta D, Staples R, Striegl R, Zhulidov A, Zimov S (2012) Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic Ocean and surrounding seas. Estuaries Coasts 35 (2):369-382.

Kanda J, Laws EA, Saino T, Hattori A (1987) An evaluation of isotope dilution effects from conventional data set of ^{15}N uptake experiments. J Plankton Res 9 (1):79-90.

Lomas MW, Bronk DA, van den Engh G (2011) Use of flow cytometry to measure biogeochemical rates and processes in the ocean. Annu Rev Mar Sci 3:537-566.

Serreze MC, Francis JA (2006) The Arctic amplification debate. Clim Change 76 (3-4):241-264.

Tremblay J-É, Simpson K, Martin J, Miller L, Gratton Y, Barber D, Price NM (2008) Vertical stability and the annual dynamics of nutrients and chlorophyll fluorescence in the coastal, southeast Beaufort Sea. J Geophys Res 113:C07S90, doi:10.1029/2007JC004547.

Vita

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Born in Boston, Massachusetts on April 18, 1973. Graduated from Framingham North High School in 1991. Received a bachelor's degree in Social Thought and Political Economy from the University of Massachusetts, Amherst. Worked for a variety of technology companies with responsibilities including personnel management, business relations, marketing, and project management. Returned to undergraduate work in biology at the University of Massachusetts, Boston. Worked as an undergraduate assistant in the zooplankton ecology lab of Juanita Urban-Rich. Entered graduate school in the Physical Sciences department at the Virginia Institute of Marine Science, working in the nitrogen biogeochemistry lab of Deborah Bronk. Taught an undergraduate seminar course on Arctic Oceanography at the College of William and Mary. Defended doctoral dissertation in July 2013.