BIOHYDROGRAPHY OF EUKARYOTIC MICROORGANISMS

IN A

COLD-OCEAN ECOSYSTEM

by

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Abstract

Picoeukaryotes (0.2 – 3 μm) dominate the planktonic biomass of the Arctic Ocean for most of the year, strongly influencing primary production and carbon and nutrient cycles. Despite their importance in this cold-ocean ecosystem, little is known about factors controlling picoeukaryote diversity and distribution. Picoeukaryote community composition and distribution in relation to the physical characteristics of the water column was investigated, and we introduce the term biohydrography to describe studies of this type. Samples were collected at 6 depths across 9 stations in the North Water Polynya (NOW), a large recurring polynya in northern Baffin Bay. The hydrography of the NOW was determined from 52 CTD casts, and several different water masses were identified by their distinct temperature and salinity characteristics. The circulation of water masses in the region was reported and evidence of interleaving and mixing was found along the frontal zone where water masses converged. Picoeukaryote community composition was determined from denaturing gradient gel electrophoresis (DGGE) profiles; samples showed 42 distinct band types or operational taxonomic units (OTUs) overall, with 8 to 22 OTUs per sample, and considerable variation in OTU composition among samples. Similarity analysis of DGGE profiles showed assemblages from different depths at the same station shared as little as 6% similarity, whereas assemblages from locations hundreds of kilometers apart shared as much as 90% similarity. Similarity among picoeukaryote communities was most closely related to the origin of the water mass sampled; for example, Arctic derived waters showed a unique and very different community than those of Atlantic origin. Separate community assemblages were also identified along the frontal zone, suggesting water masses maintain their signature community until further physical mixing disperses the organisms. Matching of excised DGGE band sequences identified organisms from taxonomic groups Acantharea, Cercozoa, Chrysophyceae, Bacillariophyceae, Dinophyceae, Prasinophyceae, and Prymnesiophyceae; however, many sequences matched uncultured organisms, whose function in the environment is unknown, highlighting the need for both culture and ecosystem-based studies. Canonical correspondence analysis (CCA) revealed that

latitude, depth, chlorophyll levels, and community size structure were important factors that partially explained 42.6% of the variability in assemblages, indicating contemporary environmental conditions influence picoeukaryote community structure. A detailed understanding of water mass distribution, circulation patterns, and physical mixing processes was required to further explain assemblage relatedness among sites, revealing the importance of investigating hydrographic processes in studies of picoeukaryote community dynamics.

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Dedication

For the northern lights that have seen strange sights…

Co-authorship Statement

I will be the primary author of the manuscript titled '*Biohydrography of picoeukaryotes in the North Water Polynya*' that results from Chapter 2. I devised the sampling scheme, collected and processed the samples and wrote the manuscript. Samples were collected from the Canadian research icebreaker *Amundsen* during August 2005. Processing of CTD data was performed by Yves Gratton's group at the Institut National de la Recherche Scientifique (INRS), and nutrient data was generously provided by the laboratory of Jean-Eric Tremblay at Université Laval. The second author on the manuscript is Connie Lovejoy, the primary investigator of the laboratory at Université Laval where I conducted my research. She provided the workplace, materials, training in molecular analysis, and helpful guidance. The final author of the manuscript is Grant Ingram. He is the principal investigator of the physical oceanographic group at University of British Columbia where I analyzed the data and wrote the manuscript. Connie Lovejoy and Grant Ingram provided impetus and funding for this work. Both co-authors edited the manuscript and they, along with the members of their respective laboratories, provided insightful discussion on the project.

I certify that the above statements about authorship are correct.

1. Introduction and Background

1.1 Microbial Biohydrography

Life on earth is overwhelmingly microbial, both in biomass and abundance (Nee 2004). Marine microbes are responsible for half of the Earth's primary productivity and are a fundamental component in global carbon and nutrient cycles (Arrigo 2005). Understanding what controls marine microbial distribution and diversity has been highlighted as one of the major challenges facing contemporary oceanographers (Arrigo 2005; Martiny et al. 2006). Growing evidence suggests that microbial composition affects ecosystem processes, and even under similar conditions, communities from different environments might function differently (Martiny et al. 2006). Understanding the diversity and distribution of marine microbes is therefore fundamental to the understanding of carbon and nutrient cycling, and autotrophic and heterotrophic productivity in the world's oceans.

Biogeography is the study of the distribution of biodiversity over space and time. Due to the very small size, extremely high abundance and potentially unrestricted dispersal of microorganisms, there is still some debate whether they exhibit any biogeographic patterns (Finlay 2002). If they do, which a large body of recent work supports, there is no conclusion whether they follow the same biogeographic patterns of macroorganisms (Martiny et al. 2006). Environmental microbiology has only recently moved beyond the stage of natural history characterization and into the application and development of ecological and evolutionary theory. Very basic hypotheses of microbial diversity and distribution are only now being tested, enabled to a large degree by advancement in community survey techniques. The basic hypotheses being tested ask fundamental questions and were summarized by Martiny et al. (2006). Are microbial communities non-randomly distributed, (i.e. do they exhibit biogeography)? If so, does distribution reflect the influence of contemporary environmental variation (i.e. the Baas Becking hypothesis (Baas Becking 1934) for microbial taxa which states that "everything is everywhere, but the environment selects")? Or is spatial variation due to historical events (i.e. dispersal limitation or past environmental conditions that led to genetic divergence)? Or finally, is distribution a reflection of both past events and contemporary environmental factors (i.e. similar biogeographic processes as macroorganisms)?

In the marine environment, biogeographic provinces have been distinguished for much of the open ocean (Longhurst 1998). The specification of ocean provinces by Longhurst was primarily based on the seasonal evolution of the surface chlorophyll field determined from remote sensing. While a major advancement in marine biogeography, Longhurst acknowledged the provinces were essentially a two-dimensional description of surface ecosystems, limited by the ability of satellite sensors to penetrate into the interior of the ocean. To move beyond the two-dimensional notion of biogeography, with terrestrial origins, and into the ocean realm, a three-dimensional approach is required. In the fluid ocean, where one oceanic province and its suite of biota may overlie an entirely different province, gradients across both horizontal and vertical planes are equally important. This is particularly apparent for marine microorganisms, whose dispersal is largely influenced by hydrography, the physical characteristics of the water (circulation, tides, mixing, pycnoclines, fronts, eddies, etc.). With this in mind, the study of the distribution of biodiversity in the dynamic marine environment is termed "biohydrography". Justification could also be made for using the term "hydrobiogeography", but for the purposes of this thesis the term biohydrography is preferred, and will be used throughout.

1.2 Analysis of picoeukaryote communities

As a group, marine microbes represent enormous biodiversity, dominating all three domains of life, the prokaryotic (lacking a nucleus) Archaea and Bacteria, and the Eukarya (having a membrane bound nucleus). Picoeukaryotes, between 0.2 and 3 μm in diameter (Diez et al. 2001a; Massana et al. 2004), both autotrophic and heterotrophic, are the most abundant eukaryotes on Earth. Despite being found in concentrations between 10^2 to 10^5 cells per ml in the world's oceans (Throndsen and Kristiansen 1991; Li 1994), and having significant roles in marine biogeochemical cycles their phylogenetic diversity and distribution have only recently been characterized (Lopez-Garcia 2001, Diez et al. 2004, Massana et al. 2004*a*; Lovejoy et al. 2006). The capacity of conventional microscopy to identify these small cells, many of which have relatively few morphologically distinct features, is limited. Characterizing the phylogenetic diversity and environmental distribution of marine picoeukaryotes has therefore been greatly facilitated by the development of molecular techniques.

Description of microbial biodiversity, including the definition of the three domains of life, is largely based on comparative analysis of ribosomal RNA (rRNA) sequences (Woese et al. 1990). Ribosomal RNA, found in all cellular life forms, is comprised of highly conserved sequence regions interspersed with more variable ones. Conservative portions have sequences that are maintained within a phylogenetically related group of organisms. Each domain of life, and even each species, has a unique rRNA signature. Sequence variation in rRNA has been exploited to determine the genetic diversity of microbial communities in environmental samples. As well, the development of sequence-specific primers allows the amplification and identification of targeted organisms. Cloning and sequencing of the eukaryotic-specific small-subunit rRNA (18S rRNA) genes have revealed the extraordinary diversity of picoeukaryote communities in the world's oceans (Lopez-Garcia 2001; Massana 2004*b*). However, to address the fundamental questions of picoeukaryote diversity and distribution implicit in biohydrography requires the analysis of more samples than is practical using a cloning and sequencing approach. These issues can be resolved using community fingerprinting methods, such as denaturing gradient gel electrophoresis (DGGE).

DGGE uses the specific melting points of double stranded DNA to separate different sequences. The melting point (Tm) of a DNA strand, the point at which 50 % of the two strands are denatured, is dependent on the sequence of its nucleotide base-pairs, particularly the proportion of guanine-cytosine (GC) triple H-bond pairs. For DNA fragments of a particular length, different sequences have different melting points. In general Tm in a solution containing a salt can be estimated as:

$$Tm = 81.5 + 16.6(\log M\ [Na+]) + 0.41(\%G+C)-0.63(\%\ f) - 600/L$$

Where % f is the percentage of formamide ($HCONH_2$) which is a denaturant and has the effect of lowering the Tm and L is the length in base pairs of the strand of interest.

The migration of a partially denatured DNA fragment is greatly reduced in a polyacrylamide gel compared to the helical form of the molecule. A gel with a linear gradient of increasing concentrations of denaturant (e.g. urea and formamide) will therefore separate sequences to different positions based on their melting point.

Prior to DGGE analysis, extracted DNA from the environment is amplified by polymerase chain reaction (PCR) using target-specific primers. In this study, primers targeting the domain Eukarya are based on two conserved regions of 18S rRNA genes. The primers are also designed to add a GC-rich clamp to the end of the DNA fragment, ensuring the helix does not fully denature in the DGGE gel. The products of PCR amplification are DNA fragments, all of the same length, representing each of the eukaryotic taxa present in the original environmental sample. These fragments are separated by DGGE, producing a signature band pattern, or community fingerprint, that is dependent upon the taxa present.

DGGE does have limitations. As with all PCR-based techniques, there may be biases in template-to-amplicon ratios so the intensity of a band may not reflect the abundance of the organism in the environment (Suzuki and Giovannoni 1996; Polz and Cavannaugh 1998; Suzuki et al. 1998). A heteroduplex DNA molecule can be formed from two different PCR products during re-annealing, resulting in multiple bands and an overestimation of community constituents (Myers et al. 1987). Differences in the extractability of DNA among cells (Polz et al. 1999) may cause some band types, called operational taxonomic units (OTUs), to be underrepresented in DGGE fingerprints. Depending on the resolution of the denaturant gradient, sequences with minor differences in base-pairs may migrate to the same position (Muyzer et al. 1992). Conversely, a single species may have slight sequence variants and appear as separate bands. Despite these limitations, DGGE has been used successfully to analyze the genetic diversity of complex microbial populations, able to identify constituents that represent only 1% of the total population (Muyzer et al. 1992). DGGE has been used to describe the diversity of prokaryotes in a variety of marine environments; the Arctic Ocean (Ferrari and Hollibaugh 1999); along estuarine gradients (Crump et al. 2004; Henriques et al. 2006); and at hydrothermal vents (Muyzer et al. 1995). The application of DGGE to describe marine picoeukaryotic diversity was first assessed by Diez et al. (2001*a*), where it was demonstrated to be as revealing as for prokaryotic communities. The utility of DGGE for evaluation of spatial distributions of picoeukaryote assemblages was further demonstrated by Diez et al. (2004) across hydrographic fronts in the Southern Ocean. That study determined that picoeukaryote assemblages grouped into distinct clusters that were generally consistent with the hydrography of the region. In this study, DGGE

fingerprinting is used to assess picoeukaryote assemblage distribution in another cold ocean ecosystem, one of the largest polynyas in the Arctic.

1.3 The North Water Polynya

The North Water Polynya (NOW) is an area of recurring open water or reduced sea-ice cover in northern Baffin Bay, between Greenland and Ellesmere Island. The occurrence of the polynya has been documented since 1616, when William Baffin navigated through heavy sea-ice along the west coast of Greenland until reaching a large area of open water (Dunbar and Dunbar 1972). Reduced ice coverage maintains conditions for enhanced primary production over longer periods during the year than surrounding areas. Productive phytoplankton communities support large herbivore populations, concentrating marine mammal and bird populations in a small region. Polynyas are regarded as "oases" in the ice and as local "hotspots" for biological production and biodiversity (Stirling 1997). The abundant and predictable populations of wildlife in the region have attracted Inuit hunters for hundreds, even thousands of years, and later brought commercial whalers, who called the area the "North Water". The NOW is thought to be one of the most biologically productive regions in the Arctic (Dunbar and Dunbar 1972).

Scientific interest in the region increased as the need for assessing environmental change in the Arctic became apparent. Research cumulated during the International North Water Polynya Study (1997-1999), a focused effort to understand the processes responsible for maintaining the polynya and contributing to conditions favorable for biological production. That project, along with a number of previous studies, provided an excellent overall understanding of the NOW and a strong basis for future work in the region. The research presented here is greatly enhanced by the findings of these earlier scientists.

This project is part of ArcticNet, a Network of Centres of Excellence of Canada that studies the impacts of climate change in the coastal Canadian Arctic. The Earth's climate is warming and global climate models predict the effects will come earliest and most severely at Arctic latitudes (ACIA 2005). To predict how these changes will affect northern ecosystems, and the ability of Arctic biota to survive and adapt, depends on having knowledge of the current state of the environment. Picoeukaryotes have been

found to dominate the photosynthetic biomass of cold Arctic waters for most of the year (Lee and Whitledge 2002; Sherr et al. 2003; Lovejoy et al. in press) and small heterotrophic populations are both abundant and diverse in these perennially cold waters (Lovejoy et al. 2006). Despite their obvious importance in the Arctic marine ecosystem, very little is known about the distribution and diversity of eukaryotic microorganisms in this region.

1.4 Objectives and Hypotheses

The analysis presented here is the first report on picoeukaryote diversity and distribution in relation to hydrography in the North Water Polynya. The objectives of the research were: 1) to describe the current hydrographic conditions in the NOW, focusing on water masses, circulation, and regions of mixing; 2) to describe the diversity and distribution of picoeukaryotes in the NOW; and 3) to determine if picoeukaryote diversity and distribution were related to hydrography. Based upon previous work on picoeukaryote distribution in the Southern Ocean (Diez et al. 2004), and initial studies of general picoplankton distribution in the surface waters of the NOW (Mostajir et al. 2001), it was hypothesized that particular water masses would harbor distinct picoeukaryote communities.

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2. Biohydrography of picoeukaryotes in the North Water Polynya[1]

2.1 Introduction

Microscopic life, both eukaryotic and prokaryotic, is responsible for about half of global primary productivity and most of the nutrient cycling on the planet. The importance of microbial community structure on the ultimate fate of carbon and energy transfer in the ocean has been highlighted recently (Arrigo 2005; Le Quere et al. 2005). Over much of the world's oceans, picoplankton (0.2- 3 μm; Massana et al. 2004) dominate both photosynthetic and heterotrophic processes. Recently, environmental surveys probing for the eukaryotic 18S rRNA gene have revealed unanticipated diversity of both small phototrophic and heterotrophic eukaryotes, and that their depth distribution in marine systems is largely unknown (Lopez-Garcia et al. 2001; Massana et al. 2002, 2004*a*). Picoeukaryotes dominate the photosynthetic biomass of cold Arctic waters for most of the year (Lee and Whitledge 2002; Sherr et al. 2003; Lovejoy et al. in press) and small heterotrophic populations are both abundant and diverse in these perennially cold waters (Lovejoy et al. 2006). Molecular techniques and community fingerprinting methods, such as denaturing gradient gel electrophoresis (DGGE), have been valuable tools to describe the spatial and temporal variation of entire assemblages in other regions of the world's oceans (Diez et al. 2001*a*). The horizontal and vertical distribution of picoeukaryote communities in the Arctic, and the environmental factors responsible for their distribution, has not been previously investigated.

Picoeukaryotes are an ideal study group for investigating marine species patterns and their distribution in relation to the physical characteristics of the water column. Mobility and sinking rates are very low in picoplankton; the theoretical sinking rate of a 3μm cell is 1.7cm day^{-1} (as predicted by Stokes Law assuming 74 kg m^{-3} excess density over seawater at 0°C with a dynamic viscosity of 1.88 x 10^{-3} kg m^{-1} s^{-2}). Empirically picoplankton have been shown to be basically neutrally buoyant (Takahashi and Bienfang 1983), and the mesoscale spatial distribution of small cells is determined by lateral advection and vertical mixing. Community structure of picoeukaryote assemblages (both autotrophic and heterotrophic), while dependent upon biological factors such as resource availability, competition, grazing and viral lysis, will also be strongly influenced by

[1] A version of this chapter will be submitted for publication to *Limnology and Oceanography* as Hamilton, A. K., C. Lovejoy, and R. G. Ingram. Biohydrography of picoeukaryotes in the North Water Polynya.

hydrographic factors. These include water mass origin, circulation, and mixing, as well as temperature, salinity, irradiance, and density. With this in mind, we will refer to the description of the distribution of biological particles within dynamic oceanographic systems as biohydrography.

The North Water Polynya (NOW), a large region of perennially ice-free water between Ellesmere Island and Greenland, and one of the most productive marine ecosystems in the Arctic (Dunbar and Dunbar 1972), is well suited to investigate the biohydrography of picoeukaryotes in polar seas (Fig. 2.1). The hydrography of the region has been well described (Melling et al. 2001; Bacle et al. 2002; Ingram et al. 2002), characterized by opposing currents of cold Arctic water and warmer Atlantic water meeting over complex bathymetry, with evidence of a dynamic frontal zone showing strong interleaving and mixing features in the center of the polynya (Lobb et al. 2003). The extent and duration of primary productivity has been described (Klein et al. 2002; Mei et al. 2002; and Odate et al. 2002), with a spring bloom occurring as early as March and high productivity lasting as late as October. Lovejoy et al. (2002*a*) described vertically distinct micro- and nano- sized (2 – 20 μm and 20 – 200 μm diameter, respectively) protist communities associated with interleaving layers and intrusions along the frontal zone of the polynya. They proposed that the density differences at the base of these layers act as traps for particulate organic matter, and that the physical heterogeneity of the water column likely contributed to the diversity and community structure of the protist assemblages.

The objectives of the present study were to examine variability of picoeukaryotes in the NOW and determine factors that affect community diversity and distribution; whether community distribution could be explained by hydrographic patterns, and what environmental factors influence community structure. Here we describe the distribution of water masses, circulation patterns, and photosynthetic biomass to understand the physical environment of the NOW at the time of the study. We used denaturing gradient gel electrophoresis (DGGE) to characterize the community assemblages and relatedness among 57 sites. Multivariate statistics were used to determine factors that may be affecting assemblages – such as temperature, salinity, depth, irradiance, location, chlorophyll concentrations, and community size structure. We also sequenced DNA from extracted DGGE bands to identify the picoeukaryote taxa present in the NOW.

2.2 Material and Methods

Study Area – We sampled the NOW polynya between 16 and 22 August 2005. The NOW is a large recurring region of low ice cover in Northern Baffin Bay (between latitudes 76° and 78.5°N) bounded longitudinally by Ellesmere Island and Greenland (Fig. 2.1). The polynya is of critical importance to marine mammal, bird, and Inuit populations (Dunbar and Dunbar 1972). The region was intensively sampled during the International North Water Polynya (NOW) Study from 1997-1999, providing an excellent background to our present work.

Hydrography – Our physical oceanographic survey followed 6 E-W transects (Fig. 2.1). The general hydrography, inflowing water masses, and sites of strong physical interleaving in the frontal zone where these water masses mix were identified from 52 CTD casts using a Sea Bird SBE-911. CTD salinity (S) was calibrated with water samples analyzed by a Guildline Auto-Sal salinometer. Values of potential temperature (θ) and potential density were computed using algorithms from UNESCO (1983). Fluorescence (Seapoint), transmissivity (WetLabs C-Star Transmissometer), photosynthetically available radiation (PAR; Biospherical) and relative nitrate (Satlantic MBARI ISUS) were also recorded.

Sample collection and analysis - Sampling stations were chosen to maximize hydrographic coverage within logistical constraints of the overall project and to investigate sites of strong water-column interleaving. At nine stations, samples were collected at the following 6 depths, determined by both physical and biological characteristics: the surface (5 m), the subsurface chlorophyll max (10-50 m), the nitricline (40-80 m), the deep water (180 m), and two 'wildcard' temperature related depths (unique subsurface temperature excursions that indicating interleaved layers between 40-150 m). Sample depths at each station were determined by viewing the sensor profiles on the CTD downcast. Water samples were collected on the upcast in 12-liter PVC bottles (Ocean Test Equipment) mounted on a 24-bottle rosette sampler (General Oceanics). To ensure the reproducibility of the methods, six field replicates (six bottles collected at the same depth on the same cast) were also collected at the chlorophyll max in the Beaufort Sea (not shown on map) (Station CA04-05: 71°03.75 N, 133°36.07 W).

Nutrient tracer –To trace water-mass origin we collected samples for silicate and nitrate at most stations. Concentrations were determined using an ALPKEM autoanalyzer with routine colorimetric methods (Grasshoff 1976) and a detection limit of 0.05 μM. Tremblay et al. (2002) developed a quasi-conservative nutrient tracer (Si^{ex}) for the NOW based on the concentration of silicate in excess of nitrate. Si^{ex} was calculated as:

$$Si^{ex} = 100(Si - N - 1.06)/(12.75 - 1.06) \quad \text{(Eq. (8); Tremblay et al. 2002)}$$

where Si and N are the concentrations of silicate and nitrate, respectively. In the upper 200 m, relatively high Si^{ex} values indicate the presence of silicate-rich Arctic water (SRAW) and low values indicate Baffin Bay water (BBW) of mixed Atlantic and Arctic origin. At stations along the frontal zone observed by Lobb at al. (2003), where mixing and interleaving occurs (Lovejoy et al. 2002*a*), this index was useful in determining the water-mass origin of each sample.

Chlorophyll – Size fractionated samples for Chl *a* were filtered onto Whatman GF/F filters before (total Chl *a*) and after pre-filtration (pico Chl *a*) through 3 μm pore-size Nucleopore polycarbonate membranes and stored at -80°C until analysis. Pigments were extracted from the filters in 95% ethanol at 70°C for 5 min (Nusch 1980) and concentrations were determined by spectrofluorometry (Cary Eclipse) before and after acidification (Strickland and Parsons 1972).

DNA extraction - Samples for microbial DNA were obtained following the methods described in Diez et al. (2001a). Briefly, 6 liters of water was sequentially filtered through a 53 μm nylon mesh, a 3 μm polycarbonate filter, to remove zooplankton and macro- and nanoplankton, then a 0.2 μm sterivex unit (Millipore). The sterivex was emptied of seawater and 1.8 ml of buffer (40 mM EDTA; 50 mM Tris pH=8.3; 0.75 M Sucrose) was added to maintain the cells. Samples were stored at -80°C until extraction. DNA was extracted by adding lysozyme (final concentration 1 mg ml^{-1}) and incubated at 45°C with slight agitation. Proteinase K (final concentration 0.2 mg ml^{-1}) and sodium dodecyl sulphate (final concentration 1%) were added and incubated at 55°C for 1 h. The lysate was recuperated into a 15 ml falcon tube and the sterivex unit was rinsed with 1 ml of lysis buffer at 55°C for 15 min to recover any additional lysate. The lysate was extracted two times with an equal volume of phenol-chloroform-isoamyl alcohol

(25:24:1, pH=8). The aqueous phase was recuperated and extracted once with an equal volume of chloroform-isoamyl alcohol (24:1). The aqueous phase was recuperated and concentrated in an Amicon tube (Millipore) using a centrifuge (3500 rpm). The concentrate was rinsed and centrifuged 3 times with 2 ml of TE (or sterile water) to a final volume of 200 µl. To verify extraction success, 5 µl of DNA was run on agarose gel electrophoresis stained with ethidium bromide (final concentration 0.5 µg ml^{-1}) with a standard (High DNA Mass Ladder). The DNA extract was stored at -80°C until DGGE analysis.

PCR - Extracted environmental DNA was amplified by polymerase chain reaction (PCR) with eukaryotic 18S rRNA specific primers EukA (5'- AAC CTG GTT GAT CCT GCC AGT -3') and Euk516r-GC (5' - ACC AGA CTT GCC CTC C - 3' with a GC clamp) (Diez et al. 2001a). The PCR mixture (25 µL) contained 1 µl (~10 ng) of extracted DNA as template, each deoxynucleoside triphosphate at 200 µM, 1.5 mM of bovine serum albumin (BSA; Fermentas), each primer at a concentration of 0.3 µM, and 2.5 U of cTaq DNA polymerase (BioLabs) with buffer. The thermocycler program was optimized from Diez et al. (2001a) and included an initial denaturing cycle at 94°C for 120 s, 30 times amplification cycles of denaturing at 94°C for 30 s, annealing at 56°C for 45 s, and extension at 72°C for 120 s, with a final extension at 72°C for 6 min then cooled to 4°C. The PCR product was run on agarose gel electrophoresis stained with ethidium bromide to verify amplification of the 560 base-pair DNA fragment using a standard Low DNA Mass Ladder.

DGGE - The DGGE was run on a 0.75 mm thick 6% polyacrylamide gel with a linear denaturing gradient of 35-55% (100% denaturant is 7 M urea and 40% deionized formamide) on a Bio-Rad system at 100V for 16 hours submerged in 1X TAE buffer (40 mM Tris [pH 8.0], 20 mM acetic acid, 1 mM EDTA) at 60°C (Diez et al. 2001a). Each environmental sample lane was loaded with 12 µl (~500 ng) of PCR product and standard lanes were loaded with 5 µl (~100 ng) of in-lab culture standard. The in-lab standard consisted of extracted DNA PCR product from 6 picoeukaryote cultures grown in the lab, including CCMP 2298, 2436, 2296, 2097, 2098, 2099 from taxonomic groups Chrysophyceae, Haptophyceae, Polarella, Pelagophyceae, and Micromonas. Individual gels had 14 lanes, including 2 standards and 12 samples (6 samples each from 2 stations). Analytical replicates (repeated PCR of a single sample) for all samples were run on a

different gel alongside samples from a new station resulting in an overlapping pattern of stations on the gels (AB, BC, CD, etc.). This pattern permitted precise alignment of gels later during image analysis. Gels were stained with SYBRGold nucleic acid stain (Molecular Probes) for 1 h in the dark and rinsed with 500 ml of 1X TAE buffer before photographed. Exposure was set just below saturation level of bands. Gel images were acquired with the Bio-Rad Gel Doc imaging system using Quantity One (Bio-Rad v.4.6.0) software and exported as Tiff files. A composite image of all gels was created with Photoshop (Adobe v8.0). The multiple standard and analytical replicate lanes facilitated precise alignment of multiple gel images side-by-side on the same vertical scale. The final composite image was saved as a Tiff file for band analysis with Quantity One. We also attempted DGGE image analysis using Gel-Compar software (Applied Maths), which permits band matching between separate gels. We found that the automatic band matching performed by Gel-Compar introduced improper scaling of gels and band-matching errors that required additional manual quality control, with nothing gained compared to the Quantity One Tiff analysis.

The composite DGGE image (Appendix A) consisted of 107 lanes; 57 unique sample lanes, 17 standard lanes, and 33 replicate lanes. Background was subtracted from the densitometry scan of all lanes using the rolling disk (size 10) method. Bands contributing $\geq$1% of the total band intensity for each lane were automatically detected and scored as present, all others as absent. All 17 standard lanes were manually identified and bands matched. Given the narrow vertical range of the standard lane band patterns, the replicate lanes were also identified to facilitate automatic band matching. The software then compared all lanes and identified matching bands with identical vertical positions across the image. The banding profile of the composite DGGE image was converted into two matrices: 1) a binary presence-absence matrix and 2) an intensity matrix, where each band is scored with its intensity relative to the strongest band in its lane (0-100%). This focused the analysis on relative differences in band intensity within each sample, and eliminated the variability that may have been introduced by slight differences in the mass of DNA loaded.

Ecological Statistics - Similarity matrices were generated from the two banding matrices using Pearson product-moment correlation coefficients. The robustness of each

matrix was determined by comparing the average similarity of replicates for both matrices; the intensity matrix showed much higher similarity between field replicates and analytical replicates (0.98 ± 0.01 and 0.93 ± 0.05, respectively) than the presence-absence matrix (0.87 ± 0.03 and 0.73 ± 0.16, respectively). The error inherent in presence-absence method is due to the increased emphasis on faint bands, near the 1% intensity minimum, whose presence may differ between replicates. The more robust intensity matrix was therefore used for subsequent ecological analysis, unless otherwise noted. Richness was calculated from the number of different bands, called operational taxonomic units (OTUs). Where analytical replicates had a different number of OTUs the higher of the two was used. The Shannon diversity index (H'; Shannon and Weaver 1963) was calculated as:

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i)$$

where S is the total number of OTUs (richness) and p_i is the proportion of the ith OTU in the sample. The Simpson index of diversity (1 - D; Simpson 1949) was calculated as:

$$1 - D = 1 - \sum_{i=1}^{S} (p_i)^2$$

Symbols used are the same as those for the Shannon diversity index.

Relatedness of DGGE band patterns was visualized using dendrograms produced by Agglomerative Hierarchical Clustering (AHC) using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

Community composition was directly related to known environmental gradients using the ordination technique Canonical Correspondence Analysis (CCA) (ter Braak 1986, 1995) calculated with XLStat (Addinsoft). CCA seeks to explain the variance in community composition under the constraint that gradients are a linear combination of physical variables. After checking for collinearity among environmental variables, density, salinity, nitrate and fluorescence were eliminated from the analysis. The physical variables included in the CCA were pressure, total chlorophyll (total Chl *a*), the percentage of the pico-sized fraction in total Chl *a* (% pico Chl *a*), latitude, temperature, transmissivity, and PAR. The normality of all variables except temperature was improved by a log (X+ 1) transformation (Legendre and Legendre 1998). All variables were

standardized as z-scores by subtracting the mean and dividing by the standard deviation (Legendre and Legendre 1998). One additional qualitative category variable (ter Braak 1995) was added to classify different water masses (see Results). Each sample point was overlain on a θ-S diagram and scored if present or absent in a particular water mass using binary notation. The classes of water masses were not mutually exclusive; if it was not readily apparent which water mass the sample was from then more than one class was scored as present. This classification was instructive in that it separated samples from obviously different water masses and yet allowed for samples from mixing regions.

Sequencing - The most prominent DGGE bands in each sample were extracted with a sterile blade, and dissolved in 1X TE at 4°C overnight. A 5 µl aliquot of the dissolute was amplified by PCR as above using Euk1F and Euk516r (no GC clamp). The 25 µl PCR product was sequenced using the forward primer at the Plateforme de Séquençage et de Génotypage des Genomes du Centre de Recherche du CHUL/CHUQ Québec, Canada using an Applied Biosystems ABI 3730xl. Nucleotide sequences (~560 bp) were manually edited using FinchTV software (Geospiza v.1.4) and noisy or incomplete sequences were discarded. The closest match to each sequence was found from NCBI Blast (Altschul et al. 1990) search against the GenBank online database.

2.3 Results

Hydrography – Potential temperature sections show the 3-D variability in the upper water column and the presence of different water masses (Fig. 2.2). The northern stations (MB02, MB07, MB09, MB11, and MB21), represented by the section along Line 3, show relatively cold water between the freezing point and -1°C throughout most of the upper 250 m, with some atmospheric warming > 0°C of the top 25 m. The southern stations (BA01, BA02, BA04, and L03), represented by the section along Line 6, show a warm surface layer (> 1°C) to 50 m overlying the wintertime convection water (WCW), which extends down to 150-200 m. Below the cold WCW is a warmer (> 1°C) water mass of Atlantic origin that extends to the bottom. The north-south section (Line 7; Fig. 2.2.) shows the strong frontal zone between the cold Arctic derived waters in the north and the warm surface waters of the south, with temperature interleaving layers between 50 and 100 m at station MB21. We noted the presence of warm Atlantic waters below 150 m at the southern stations (BA02, BA04, L03).

The θ-S diagram of all casts showed distinct water masses were present in the NOW (Fig. 2.3). Referencing previous descriptions of θ-S characteristics (Melling et al. 2001; Bacle et al. 2002), we identified the following five water masses: Arctic surface water ($S < 32.5$ and $\theta < -0.5°C$), Arctic water ($S > 32.5$ and $\theta < 0°C$), Baffin Bay surface water ($S < 32.5$ and $\theta > 0.5°C$), the wintertime convection water ($33.5 > S < 33.6$ and $\theta < -1°C$), and Atlantic deep water ($S > 33.6$ and $\theta > 1°C$). These are the same water mass categories used in the CCA. Interleaving layers are detected as strong temperature excursions between salinities of 31 and 33.2.

The Si^{ex} tracer calculations distinguished samples with relatively high proportions of SRAW (Fig. 2.4). Si^{ex} is usually expressed as a percentage of SRAW present, but due to changes in absolute values between years, here it is expressed in relative non-dimensional units (Tremblay, J. E., pers. comm.). The low Si^{ex} values and low salinities of the surface samples from stations MB02 and MB07 (A030, A041, and A042) are associated with ice melt. Other samples show varying proportions, with less SRAW in more southerly and deeper samples. There was significant variability over depth at different stations: MB07 (A037-42) varied from mostly BBW water at depth, to mostly SRAW in the mid-depths, to melt water at the surface, while MB21 (A043-48) had relative constant proportions at all depths.

Chlorophyll – At most stations, the near-surface (5 m) Chl *a* concentration, estimated from relative fluorescence, was low (<1 μg l^{-1}), but increased to a subsurface chlorophyll maximum between 15 and 50 m (Fig. 2.5). Surface values were lowest at MB07 (0.2 μg l^{-1}), and highest at MB09 (3.1 μg l^{-1}). The highest overall Chl *a* concentrations were observed at kilometer 25 along Line 6 (Fig. 2.5), where values peaked at 40.2 μg l^{-1} at 30 m. At the more northern stations, Chl *a* concentrations were highest at stations MB09 and MB07, with values of 4.8 and 4.7 μg l^{-1} at 27 m and 45 m, respectively. Overall, concentrations decreased below the subsurface maximum to <2 μg l^{-1}. Chlorophyll concentrations were generally higher in the west and north, consistent with the seasonal northwest migration of the phytoplankton bloom shown for this region by Lewis et al. (1996) and Booth et al. (2002).

Station profiles (Fig. 2.6) show large variability in temperature, salinity and chlorophyll concentrations among stations. Interleaving layers were detected between 40 and 60 m at MB11, MB21 and BA01. These layers were also associated with small secondary chlorophyll peaks, 0.5 to 1 $\mu g\ l^{-1}$ above the background level. MB02 and MB07 show a more diffuse interleaving layer between 100 and 150 m and have a relatively fresh surface layer. BA02, BA04, and L03 show a warm mixed surface layer and the presence of the warm Atlantic layer at depth.

DGGE fingerprints – The DGGE fingerprints showed 42 unique OTUs overall, with individual samples containing between 8 and 22 OTUs (Table 2.1). Maximum richness was found at MB11, minimum at MB02 and L03. A number of stations (BA02, BA04, MB07, MB11, and MB21) showed decreasing richness from the surface to a minimum at about 30m, then an increase with depth, and subsequent variability with increasing depth. Others stations differed; BA01 showed minor variability in richness from surface to depth, whereas MB02 showed a minimum at the surface, increasing with depth to a maximum at 160m and then decreasing with depth. Surface values were lowest at MB02 and highest at MB07, having 8 and 20 OTUs, respectively. Richness values in the chlorophyll maxima were lowest at BA02, with 10 OTUs, and highest at MB09 and MB21, both with 18 OTUs. The average richness per station was lowest at MB02 and highest at MB11, with values of 12.4 and 18.8 OTUs, respectively. Maxima and minima for both diversity indices corresponded to the samples having maximum and minimum richness values.

Histograms of the relative intensity of OTUs in each sample show the large variability in picoeukaryote community composition between stations and over depth (Fig. 2.7). MB02 shows the two surface samples are quite different than the deeper samples, largely influenced by the intensity of OTU 7. A similar pattern is observed in MB07. MB11 and MB21 are not as strongly influenced by changes in one OTU; instead these stations show minor changes in the intensity and composition of all OTUs. We found a strong change in community structure at L03, where OTU 27 was more intense in the deeper samples. The most ubiquitous OTUs were 28 and 30, found in 49 different samples. The rarest, OTU 41, was only found in sample A048, a surface sample from MB21. Analysis of the OTUs present indicated that 5 OTUs had cosmopolitan

distributions (defined as being present in > 75% of all samples). Conversely, 12 OTUs had a metropolitan distribution (defined as being present in < 25% of all samples).

DGGE fingerprint similarity –We were able to define 9 major clusters from the similarity dendrogram (Fig. 2.8). The samples generally separated on the basis of water mass origin, as indicated (see Discussion). Samples from one station were found in several different clusters (e.g. station BA04; samples A049-A054), sharing as little as 15% similarity overall. Surface samples were also found in multiple clusters, sharing as little as 25% similarity overall. Within each cluster samples shared at least 50% similarity, usually more, yet could be from stations hundreds of kilometers apart, and from depths ranging from 15 to 180 m. Some samples shared greater than 85% similarity but were from distant sites (e.g. A004 and A038 from BA01 and MB07, respectively).

CCA – Ordination analysis shows that 42.6% of the total variation in assemblages can be accounted for by two factors that are a linear combination of the 7 quantitative physical variables selected (Fig. 2.9). F1 alone accounted for 27.2% of the variation and was correlated with latitude and total Chl *a*. F2 accounted for 15.8% of the variation and was correlated with PAR and pressure. The diagram shows the approximate "centers" of each sample, and the water mass categories, along the environmental gradients. Projecting each sample orthogonally onto the variable vector permits ranking of samples along the physical gradient. For example, A049 (Fig. 2.9a) falls near the Atlantic water mass category, has a relatively high % pico chl *a*, is from relatively deep water (high pressure), and a low latitude (extrapolating the latitude vector through the origin). In contrast, A042 (Fig. 2.9a) is distinguished as a surface sample with very high PAR values. We note that southern samples from the Atlantic water mass group together with high proportions of pico chl *a*, while Arctic surface samples in the north tend to higher total chl *a* values. The CCA for environmental variables and OTUs (Fig. 2.9b) is interpreted similarily and shows that certain OTUs are correlated with particular physical conditions and water masses. For example, OTU 27 is closely associated with Atlantic water while OTU 7 is associated with Arctic surface water. OTUs 4, 9, 16, 20, 41 are present in samples with high total Chl *a* levels, while OTUs 11, 18, 25, 26, 29, 35, 38 are associated with a high proportion of pico-sized Chl *a*.

Sequence Identification – We detected 26 different taxa from the extracted bands (Table 2.2). Many sequences had closest matches to uncultured marine eukaryotes, but most were able to be classified within major taxonomic groups including, Acantharea, Cercozoa, Chrysophyceae, Bacillariophyceae (diatoms), Dinophyceae, Prasinophyceae, Prymnesiophyceae, and uncultured alveolate groups I and II, novel marine stramenopiles (MAST), as well as a recently discovered putative algal phyla the picobiliphytes (Not et al. in press). We also found two sequences from Metazoans (Hydrozoa and Appendicularia), organisms obviously larger than our 3μm mesh (see Discussion).

2.4 Discussion

Analysis of picoeukaryote assemblages - The diversity of picoeukaryotes in the world's oceans has been revealed in recent studies (Diez et al. 2001b; Lopez-Garcia et al. 2001; Moon-van der Staay et al. 2001). The spatial distribution of picoeukaryote assemblages has only very recently been examined in the Antarctic Ocean (Diez et al. 2004). We were interested in examining the distribution of picoeukaryote assemblages in relation to the hydrographic environment in the Arctic.

We used DGGE community fingerprinting to describe picoeukaryote distribution, a technique that has been applied previously (Diez et al. 2004). The presence of a band in a DGGE gel indicates the presence of a corresponding organism in the environment. Due to biases inherent in PCR based studies however, band intensity is not a direct indication of organism abundance in the environment, but band intensity changes within a sample set do show changes in the relative community structure in nature (Casamayor et al. 2002; Diez et al. 2004). Muylaert et al. (2005) found that errors introduced by faint bands in presence-absence datasets were most likely to obscure relationships between species and the environment, indicating intensity datasets are preferred in studies of this type. Acknowledging the limitations of the technique, we used the intensity dataset as the more robust for revealing changes in community composition, which was the aim of this study.

Analysis of distribution patterns across a large region required many samples, which necessitated comparison of DGGE fingerprints among gels. We ran multiple

replicates on different gels to ensure community comparisons were valid. The reliance of DGGE results on a specific and repeatable protocol, which often varies slightly among labs, and the lack of a universal standard limited our ability to compare fingerprint patterns among studies. Currently, the only practical method for comparing results among studies is to sequence extracted DGGE bands.

To improve confidence in relating sequences to bands, we extracted multiple replicates for each band from each gel. There was however, sequence variability among replicates and we were unable to confidently match a given band with a taxonomic group. Following the same protocol, other studies have reported sequences related to particular bands (Diez et al. 2004; Gast et al. 2004), so we were not expecting such variability between replicates. It is possible that contaminating DNA from adjacent bands may have out-competed our target during the re-amplification PCR, resulting in variable sequences. Similarly, due to the nature of DGGE and the high diversity of picoeukaryotes in this region (Lovejoy et al. 2006), sequence variants could have migrated to the same position in the gel, confusing the results. The method would likely have been improved by re-running the extracted band PCR product alongside the original sample to confirm a match with the original band (Schafer and Muyzer 2001; Crump et al. 2004). Thus, we report all organisms identified from the sequences, but do not match taxonomic names to particular bands or OTUs (Table 2.2).

Hydrography - Our aim was to relate the community distribution pattern of picoeukaryotes to their environment, which requires a detailed understanding of the polynya. The creation and persistence of the NOW polynya is strongly influenced by the circulation in the region (Fig. 2.1). The polynya occurs when an ice bridge forms across the northern end of Smith Sound, preventing Arctic pack ice from flowing south into Northern Baffin Bay (Melling et al. 2001; Ingram et al. 2002). The ice-bridge had broken-up prior to our sampling, bringing scattered ice flows into northern Smith Sound. Land fast and pack ice were present along the coast of Ellesmere Island whereas the remainder of the NOW was relatively ice-free. The strong southward flow (10-15 cm s^{-1}) of cold and relatively fresh Arctic water is likely of Pacific origin, as deeper Atlantic water is

restricted by the 230 m sill in Nares Strait (Melling et al. 2001). This water is characterized by relatively high silicate content (Tremblay et al. 2002), likely supporting diatom blooms. The excess silicate enabled us to track the Arctic water as it entered Smith Sound (Tremblay et al. 2002). The Arctic water continues south along the east coast of Ellesmere and Devon Island (Melling et al. 2001). Surface freshening, observed at MB02 and MB07 (Fig. 2.2 and 2.6), is likely associated with melting of sea-ice and runoff from the surrounding ice-sheets. Modified Arctic surface water flowing eastward through the Canadian Archipelago enters the NOW through both Lancaster and Jones Sound, near station L03 (Fig 2.1). The combined water forms the Baffin Current flowing along the coast of Baffin Island. On the east side of Baffin Bay, the West Greenland Current (WGC) flows north along the coast of Greenland into the NOW. The WGC is evident as downward sloping isopcynals to the east in Line 6 at BA01 (Fig. 2.2). The WGC brings warmer and more saline water originating in the North Atlantic into the NOW at depth. The majority of the Atlantic water is bathymetrically steered west toward Devon Island in the vicinity of the 500 m isobath, and is observed at BA04 and L03 (Line 7; Fig. 2.2). A trough to the south of the Carey Islands (Fig. 2.1) allows some northward excursions of warm deep Atlantic water further north, possibly as far as MB02 in Smith Sound (Melling et al. 2001). Surface waters of the WGC flow parallel to the Greenland coast until veering west in the vicinity of the Carey Islands and encountering the southward flowing Arctic water (Bacle et al. 2002; Ingram et al. 2002). This can be seen as the sloping isopycnals at MB21 (Line 7, Fig. 2.2), indicating westward circulating water. The lateral mixing of water masses in the frontal zone is readily observed as density-compensating thermohaline intrusions at MB11 and MB21 (Fig. 2.6). Given the circulation pattern, it is likely that the source of these temperature intrusions is the surface waters of BA01, which is located in the WGC. There also appears to be an anti-cyclonic eddy centered between MB09 and MB21 (Line 7, Fig. 2.2), further evidence of lateral current shear in the region. All water is eventually re-circulated southward and exits the NOW by the Baffin Current. The general southward flow across most of the width of the NOW can be observed by the downward sloping isopycnals to the west in Line 6 (Fig. 2.2).

Picoeukaryote distribution - Patterns in picoeukaryote community assemblages were strongly related to the hydrography described above. Assemblages located closely in space, often only a few meters apart, differed substantially, yet others located large distances away revealed very similar communities. The clustering of assemblages (Fig. 2.8) was best understood in relation to the circulation of water masses. Cluster 1 was composed of the two surface samples from BA01, representing the warm surface WGC assemblages. The samples in Cluster 2 share the presence of rare OTUs (see corresponding samples in Fig. 2.7), but did not have any obvious hydrographic relationship. Cluster 3 was composed of Baffin Bay surface samples that were related by the presence of the relatively intense OTU 28 (see corresponding samples in Fig. 2.7). Cluster 4 contained samples from the WCW of BA04 and L03, as well as replicates A103-A106 collected at 51 m in the chlorophyll max of Station CA04-05, in the Beaufort Sea, 2000 km upstream (through the Canadian Archipelago). The similarity between these distant assemblages could be the result of circulation of Beaufort Sea waters through the Canadian Archipelago into the NOW via Lancaster and Jones Sounds. Cluster 5 was comprised of samples from the northern stations and representative of Arctic water assemblages. The Si^{ex} tracer showed further evidence of this division, many of the samples from Cluster 5 (Fig. 2.8) had a high proportion of SRAW water (Fig. 2.4). Cluster 6 was also comprised of samples from the same northern stations as Cluster 5, but in addition Cluster 6 contained samples from southern BA01 and BA02. It is likely that Cluster 6 represented assemblages that originated in WGC waters and were circulated throughout the NOW. Clusters 5 and 6 both shared about 30% overall similarity with Cluster 1 (WGC surface assemblage) on the basis of OTU intensity fingerprints (Fig. 2.8). However, on the basis of the OTU presence-absence dataset, we found that 73% of the OTUs present in Cluster 1 were also present in Cluster 6, whereas only 57% were present in Cluster 3 (data not shown). The higher degree of identical OTUs between Cluster 1 and Cluster 6 supported the idea that these were WGC derived assemblages. Cluster 7 contained surface samples from northern stations near the pack ice along Ellesmere Island (Fig. 2.1) that had low salinity, indicating assemblages likely associated with meltwater. Cluster 7 was strongly defined by the intensity of OTU 7 (Fig. 2.7).

Atlantic deep-water assemblages defined Cluster 8, which had only 20% similarity to any other cluster. This cluster was strongly influenced by the presence of OTU 27, which was rarely found in other clusters (Fig. 2.7). Cluster 9, which had the least similarity (6%) to any other cluster, contained samples from the warm halocline between the WCW and deep Atlantic water. Perhaps this can be explained by considering that ecotones, transition zones between two ecological communities that are associated with a strong environmental gradient, may be the habitat of specialized "edge-effect" species (Odum 1971). Cluster 9 may represent an ecotone community along the environmental gradient between these two water masses, different from either of the communities associated with the separated water masses.

The frontal zone between Arctic water and WGC water is a region of water mass mixing, and is indicated by the presence of thermohaline intrusions, or interleaving layers, at MB11 and MB21 (Fig. 2.6). Lovejoy et al. (2002) sampled the NOW in 1998 and observed that stations with interleaving layers had larger changes in nano- and microplankton community structure through the water column than stations without interleaving. They hypothesized that these layers acted as processing traps for particulate organic matter, contributing to microbial diversity. At the same stations in 2005, we found remarkably similar interleaving layers, suggesting the processes responsible for these features are consistent between years. In this study, stations with interleaving layers (e.g. MB21) showed consistently high diversity (Table 2.1), but relatively constant picoeukaryote communities across all layers (Fig. 2.7). Lovejoy et al. (2002) focused on nano-and micro-plankton using abundance data as a measure of community shifts. This study focused only on picoplankton, using community fingerprint patterns as indicators of community shifts. A possible explanation for this difference between size fractions is the mobility of the biological particles involved. Picoplankton, being neutrally buoyant, are confined to their parent water mass. Larger plankton however, will potentially sink out of a water mass until reaching a pycnocline. Minor density gradients, like those associated with interleaving layers, may be sufficient to act as accumulation sites for these larger plankton, and particulate organic matter, while picoplankton will simply track along with their associated water mass as it circulates. Samples collected specifically from

interleaving layers did not show distinct communities compared to samples collected from the surrounding water column. However, samples from these stations, and below the fresher surface layer did separate into two different clusters (Cluster 5 and 6, Fig. 2.8), so there are two distinct picoeukaryote communities present at the interleaving stations. Where two distinct water masses mix, we find two distinct picoeukaryote communities.

Environmental influences on community composition - To understand how environmental variables influence community composition we used CCA. As an ordination technique, CCA is similar to the more commonly used principle component analysis (PCA) and multi-dimensional scaling (MDS). It differs however, in that it calculates ordination coefficients from both the original species and variable datasets and forces the environmental variables to fit the species ordination. Unlike PCA, which assumes a linear species distribution along an environmental gradient, CCA assumes a more realistic unimodal species distribution, where species have a particular niche.

From the ordination plot of environmental variables and samples (Fig. 2.9a), samples can be differentiated by these environmental gradients. Samples were roughly separated by latitude and depth, spatial parameters that separate samples according to water mass. There is also a notable separation of samples by size-fractionated chlorophyll. Assemblages from northern stations within or above the Chl *a* max group together, while corresponding assemblages from southern stations are loosely associated with an increase in the proportion of pico-sized Chl *a*. This separation may be due to a strong diatom bloom in the north and west, while southern stations may be in a post-bloom state. Generally, picoeukaryote communities within and above the chl max are distinct from communities below

Ordination of environmental variables and OTUs (Fig. 2.9b) indicated those taxonomic units that are associated with particular environmental conditions or water masses. For example, OTU 27 was closely associated with Atlantic water, which can be confirmed by the histogram of deep samples at L03 (Fig. 2.7). OTU 37 was associated with northern samples and primarily found in samples belonging to Cluster 5 (Arctic

water). Attaching taxonomic identification to these OTUs in the future will provide the next level of understanding of the ecosystem.

Sequence Identification - The phylogenetic diversity of picoeukaryotes has been documented recently (Massana et al. 2004*a*; Lovejoy et al. 2006; Not et al. in press). Many of the taxonomic groups identified in this study (Table 2.2) were described in these previous studies. Sequences from novel marine stramenopiles (MAST) were identified; these are heterotrophic organisms thought to have a cosmopolitan distribution and graze on bacteria (Massana et al. 2004*b*). Other phototrophic stramenopiles found here, such as the diatom *Fragilariopsis cylindricus*, were originally isolated from the Arctic Ocean (Lovejoy et al. 2006), perhaps linked to the high silicic acid concentrations of the Pacific layer in the upper ocean which promotes diatom growth. The alveolates, Group 1 and 2, are likely parasites of dinoflagellates and zooplankton with picoplankton life stages (Lovejoy et al. 2006). Organisms such as the small green algae prasinophytes, the novel picobiliphytes (Not et al. in press), and the prymnesiophyte *Phaeocystis jahnii* were present. We also found sequences belonging to metazoans and dinoflagellates, organisms generally larger than our 3 μm filter pore size. This occurrence has been reported in previous studies (Massana et al. 2004; Lovejoy et al. 2006), and could be due to cell breakage during filtering or sloppy feeding by zooplankton, larger flexible cells that can be forced through the filter pores, or dissolved free DNA adhering to small particles (Lovejoy et al. 2006). Despite identifying numerous sequences belonging to picoeukaryotes, our understanding of the functioning of these organisms in the environment is limited because many of them remain uncultivated. Massana et al. (2004*a*) expressed optimism however, as previously "uncultivable" prokaryotes have now been successfully cultured by new techniques (Rappé et al. 2002), and we expect similar developments for difficult eukaryotes.

Biohydrography - Martiny et al. (2006) discussed the different hypotheses concerning microbial biogeography, whether distribution of very small organisms is due to contemporary environmental influence (i.e. the Baas-Becking hypothesis - "everything is everywhere, but the environment selects"; Baas Becking 1934), or lingering historical

effects, or a combination of past events and environmental conditions. This study has shown that picoeukaryotes are not randomly distributed (to the detection limit of PCR-DGGE methods) in the ocean; there are indeed distinct marine provinces with unique microbial assemblages. On the scale of 10s to 100s of kilometers, the provinces appear to be the result of historical events; Arctic water has evolved a separate eukaryotic assemblage than Atlantic water. We were able to distinguish separate communities even in regions of mixing; indicating water masses keep their picoeukaryotic signature. But these findings could also be interpreted as the result of contemporary environmental influence, as Arctic and Atlantic water had significantly different physical and chemical properties that would have selected for divergent communities. We observed potential evidence of a novel assemblage along an ecotone between Atlantic water and WGC derived water that had very little similarity to either of the neighboring water masses, further evidence supporting the hypothesis of contemporary environmental influence.

We can be certain that picoeukaryote community distribution is strongly related to water mass distribution. It is apparent that the "distance effect" of variation in microbial assemblages needs to be accounted for in marine biogeographic, or biohydrographic studies. Due to circulation in the marine environment, the "landscape" is itself moving, so the notion of a fixed station, as on land, is somewhat of a misnomer. To truly account for distance effects we need to standardize the sample locations in reference to circulation. We need to track water masses. Two sample locations may be very closely situated, but if they lie in different provinces with opposing flow, then they are effectively very distant from one another. Therefore, the Lagrangian approach to oceanography, utilizing neutrally buoyant floats to follow a given water mass in its trajectory and track changes over time, would be more appropriate than the Eulerian approach described here, which monitors changes at distinct geographic locations.

The results of this study provide evidence that distinct water masses have an associated unique picoeukaryote community. The large- and small-scale distribution of a picoeukaryote community, comprised of small neutrally buoyant cells lacking significant mobility, is determined by the circulation of the associated water mass. In regions where

water masses meet and interleave, the community associated with each individual water mass seems to retain its unique structure and composition, as long as the water mass itself maintains its original physical characteristics. However, where water mass mixing results in a gradient of physical conditions between two very different water masses, the new conditions along the gradient may promote the success of an entirely different community, distinct from those associated with the two separate water masses. Ecological studies of microbial eukaryotes in the Arctic are few. This study demonstrates that physical factors strongly influence the community structure of picoeukaryotes, which will influence understanding of primary productivity and nutrient cycling in the world ocean. With a rapidly changing Arctic it is imperative that we have a solid understanding of the structure and diversity of these key players in the ecosystem.

Table 2.1 Picoeukaryote diversity indices and total Chl*a* concentrations of samples from the NOW. Indices are derived from DGGE intensity band patterns. Maximum index values are indicated in bold, minimum in bold italic. Note: Station CA04-05 is in the Beaufort Sea (see text).

Station	Sample	Depth (m)	Richness (# of OTUs)	Shannon (H) Index	Simpson (1-D) Index	Total Chl*a* ($\mu g\ l^{-1}$)
MB02	A030	5	***8***	***1.20***	***0.52***	0.08
	A029	40	11	1.84	0.76	1.22
	A028	75	15	2.37	0.87	0.49
	A027	99	16	2.36	0.87	0.21
	A026	140	17	2.51	0.89	0.16
	A025	180	9	1.60	0.68	0.08
MB07	A042	5	20	2.53	0.88	0.05
	A041	25	11	1.91	0.79	0.15
	A040	45	16	2.15	0.82	1.91
	A039	80	17	2.55	0.91	0.20
	A038	140	17	2.36	0.85	0.04
	A037	180	17	2.45	0.88	0.02
MB09	A023	27	18	2.61	0.91	1.87
	A021	66	19	2.61	0.90	0.17
	A019	180	19	2.67	0.91	0.07
MB11	A036	5	17	2.47	0.89	0.90
	A035	23	15	2.41	0.89	0.84
	A034	45	19	2.55	0.89	0.55
	A033	62	20	2.68	0.91	0.13
	A032	85	**22**	**2.81**	**0.92**	0.09
	A031	180	21	2.58	0.90	0.03
MB21	A048	5	18	2.55	0.90	1.55
	A047	15	18	2.55	0.90	1.55
	A046	32	15	2.30	0.85	0.63
	A045	55	17	2.44	0.86	0.78
	A044	90	16	2.33	0.85	0.09
	A043	180	14	2.17	0.83	0.01
BA01	A006	5	16	2.55	0.91	0.41
	A005	22	17	2.46	0.88	0.57
	A004	48	14	2.19	0.83	0.40
	A003	55	15	2.22	0.85	0.27
	A002	80	17	2.28	0.83	0.01
	A001	150	15	2.20	0.84	0.01
BA02	A012	5	15	2.48	0.90	0.22
	A011	23	10	1.78	0.73	1.58
	A010	40	16	2.36	0.87	0.03
	A009	65	17	2.48	0.88	0.00
	A008	125	17	2.36	0.86	0.02
	A007	180	19	2.64	0.91	0.01
BA04	A054	5	15	2.28	0.86	0.21
	A053	12	13	2.23	0.86	0.21
	A052	23	13	2.11	0.83	0.79
	A051	55	16	2.40	0.88	0.18
	A050	110	14	2.08	0.78	0.01
	A049	200	20	2.64	0.90	0.02
L03	A060	20	14	2.06	0.83	0.55
	A059	80	18	2.62	0.91	0.04
	A058	200	15	2.50	0.91	0.01
	A057	240	15	2.35	0.87	0.01
	A056	270	17	2.40	0.87	0.01
	A055	400	***8***	1.38	0.60	0.01
CA04-05	A106	51	19	2.60	0.91	0.25

Table 2.2 Sequence matches of excised DGGE bands

Closest Match	BLAST Accession ID	Sequence Similarity %, (bit score)	Taxonomic Group
SCM15C12	AY665098.1	99.4 (961)	Acantharea
SCM28C151	AY665054.1	97.6 (907)	alveolate Group 1
OLI11511	AJ402343.1	99.8 (969)	alveolate Group 1
SCM27C18	AY665021.1	98.4 (911)	alveolate Group 1
Amoebophrya sp. ex Dinophysis norvegica	AY260469.1	89.3 (462)	alveolate Group 2
OLI11023	AJ402335.1	95.8 (821)	alveolate Group 2
BB01_105	AY885033.1	89.8 (559)	alveolate Group 2
NOR46.14	DQ314811.1	100.0 (1025)	Cercozoa
Spumella sp. GOT220	EF027354.1	100.4 (916)	Chrysophyceae
Fragilariopsis cylindrus strain CCMP1102	AY485467.1	100.0 (908)	Bacillariophyceae
Pseudo-nitzschia pungens	U18240.1	100.0 (969)	Bacillariophyceae
Thalassiosira aestivalis strain CCMP 975	DQ093369.1	99.2 (950)	Bacillariophyceae
Gymnodinium sp	AF274260	100.0 (989)	Dinophyceae
Gyrodinium spirale	AB120001.1	99.8 (967)	Dinophyceae
SCM37C61	AY664880.1	98.0 (916)	Dinophyceae
SCM38C58	AY664956.1	99.0 (954)	Dinophyceae
SCM27C56	AY664944.1	99.6 (987)	Dinophyceae
Phaeocystis jahnii	AF163148	97.8 (910)	Prymnesiophyceae
Stephanomia amphytridis	AY937322.1	99.0 (955)	Hydrozoa
ME1-21	AF363190.2	99.6 (973)	MAST
HE000427.21	AY381157.1	99.0 (955)	MAST
UEPACLp5	AY129067.1	98.0 (914)	MAST
Oikopleura sp. cf. dioica-TTGS-2	AY116613.1	95.9 (821)	Appendicularia
NOR50.52	DQ060527.1	98.2 (914)	Metazoa
NW617.02	DQ060525.1	96.0 (809)	picobiliphytes
CCMP2099	DQ025753.1	100.0 (967)	Prasinophyceae

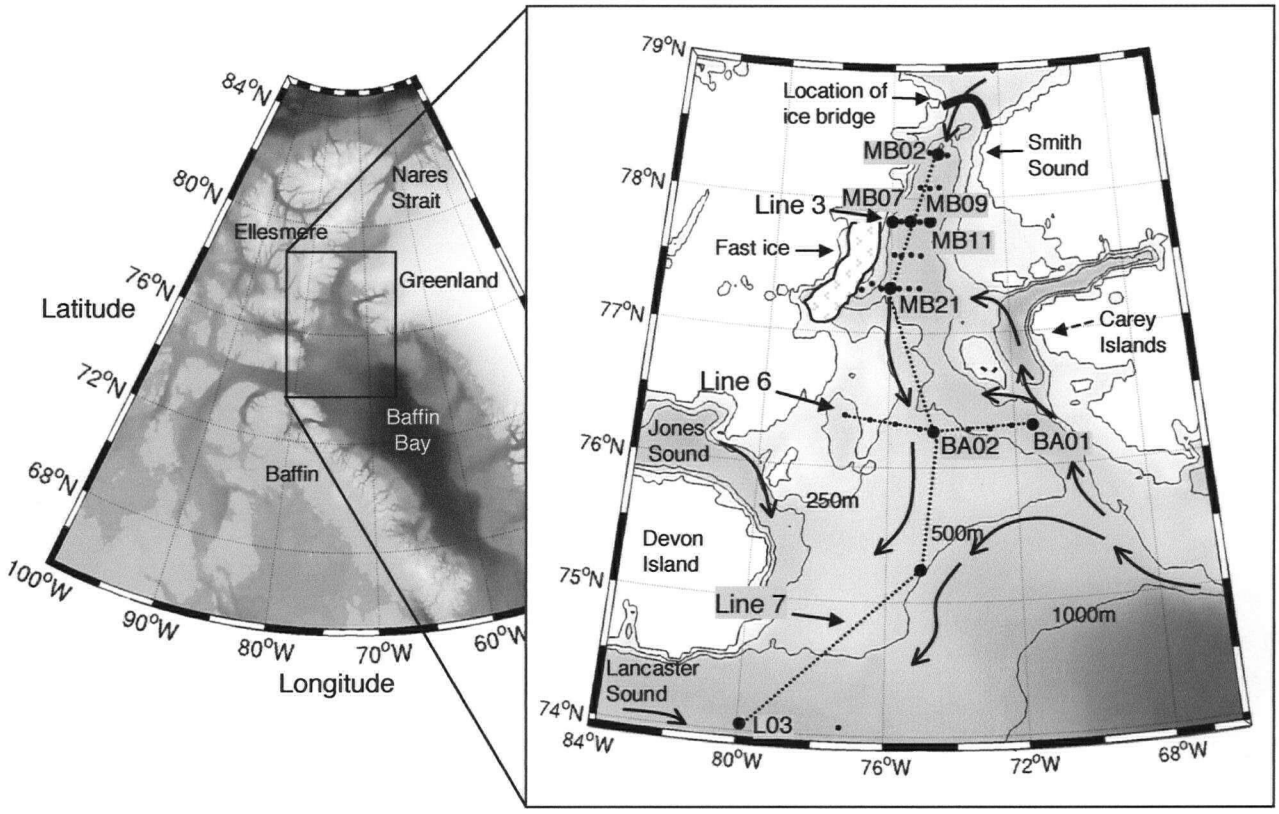

Figure 2.1 Shaded bathymetric map of the North Water Polynya showing locations of CTD casts, sample stations, and the average surface circulation (adapted from Melling et al. 2001). Lines 3 (West to East), 6 (West to East), and 7 (North to South) indicate section transects shown in Fig. 2.2 and Fig. 2.5.

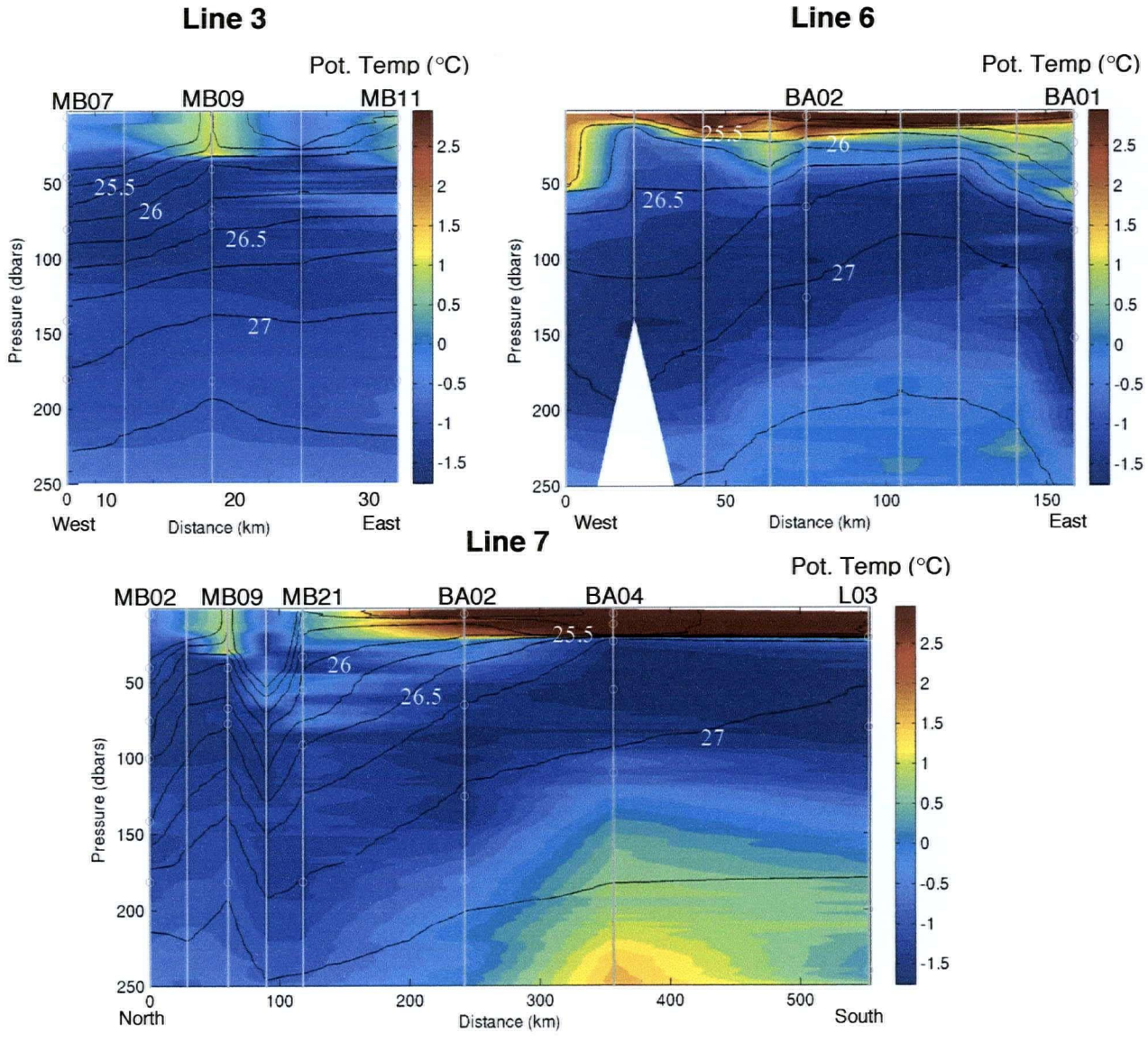

Figure 2.2 Transects of potential temperature and potential density along lines 3, 6, and 7 (see Fig. 2.1 for line locations). Stations are labeled and cast locations indicated.

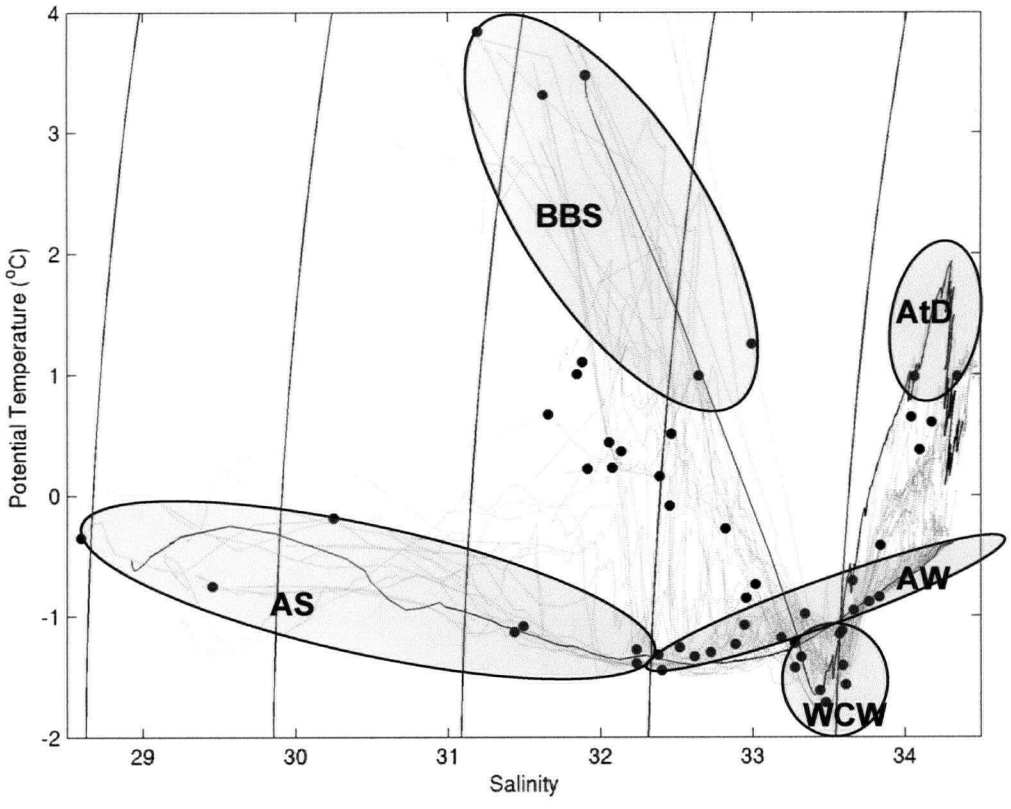

Figure 2.3 θ-S diagram indicating water masses present in the North Water Polynya; Arctic surface water (AS), Arctic water (AW), Baffin Bay surface water (BBS), wintertime convection water (WCW), and Atlantic deep water (AtD). Black dots mark θ-S characteristics of water bottle samples. Black lines are two end member casts that show all water masses present with little mixing. Dotted grey lines show θ-S profiles from all casts.

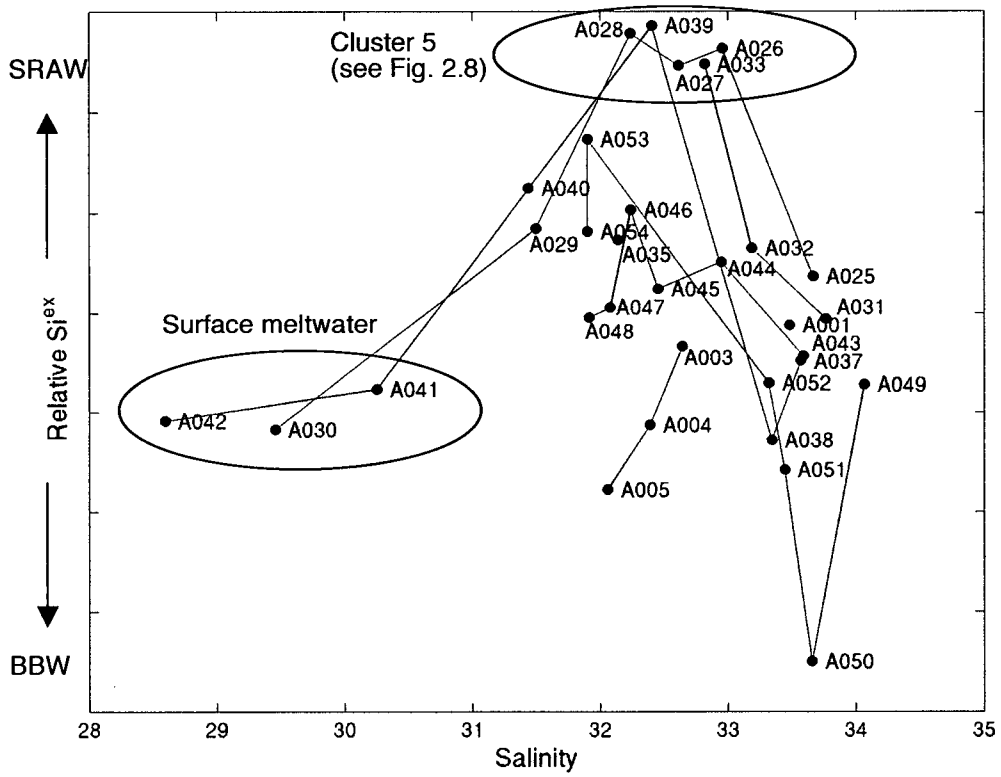

Figure 2.4 Relative Si^{ex} versus salinity diagram for station profiles from the North Water Polynya (NOW). Si^{ex} is calculated as per Eq. (8) in Tremblay et al. (2002). High Si^{ex} values indicate silicate-rich Arctic water (SRAW); low values indicate Baffin Bay water (BBW).

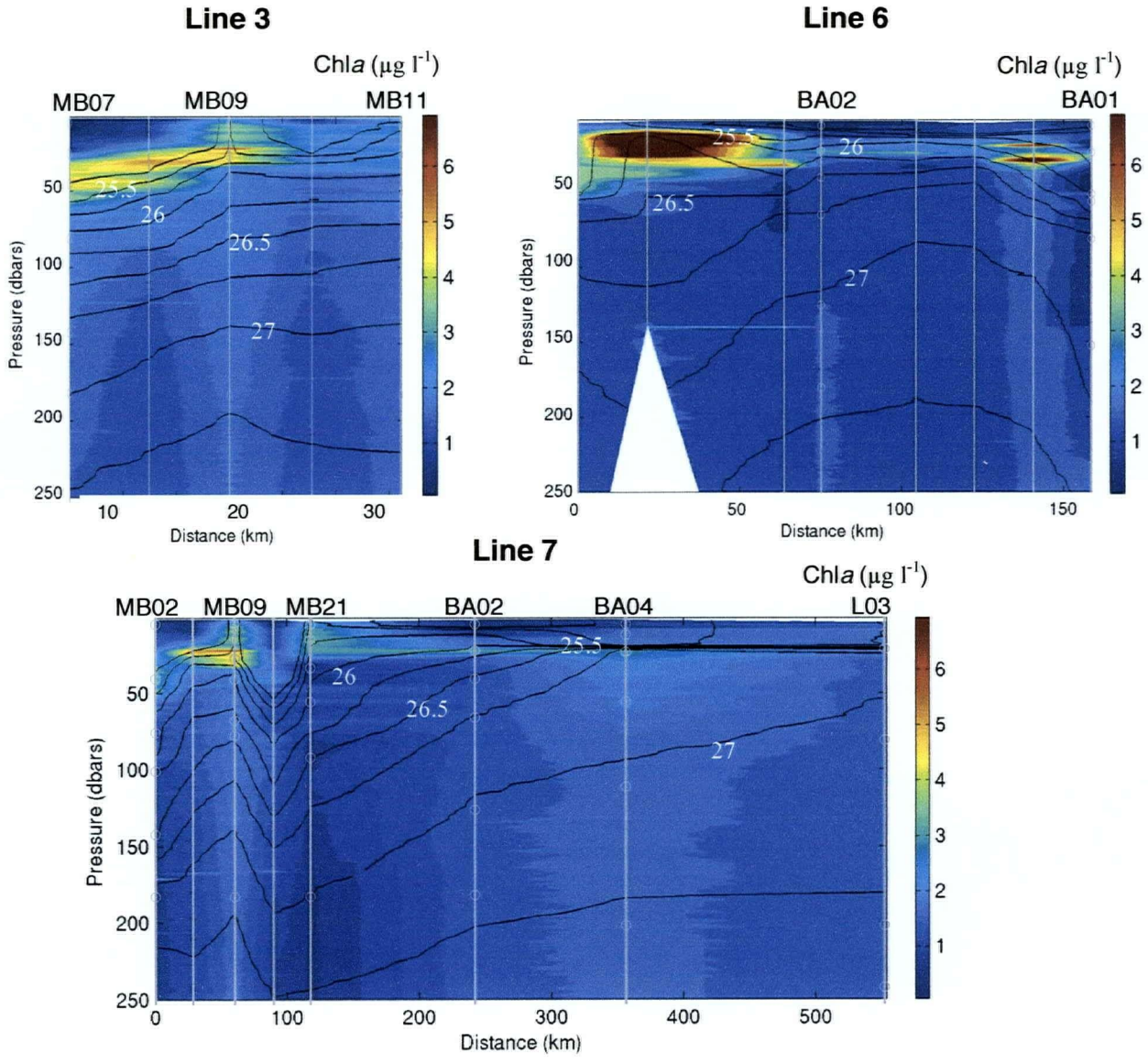

Figure 2.5 Transects of chlorophyll fluorescence and potential density along lines 3, 6, and 7 (see Fig. 2.1 for line locations). Stations are labeled and casts indicated.

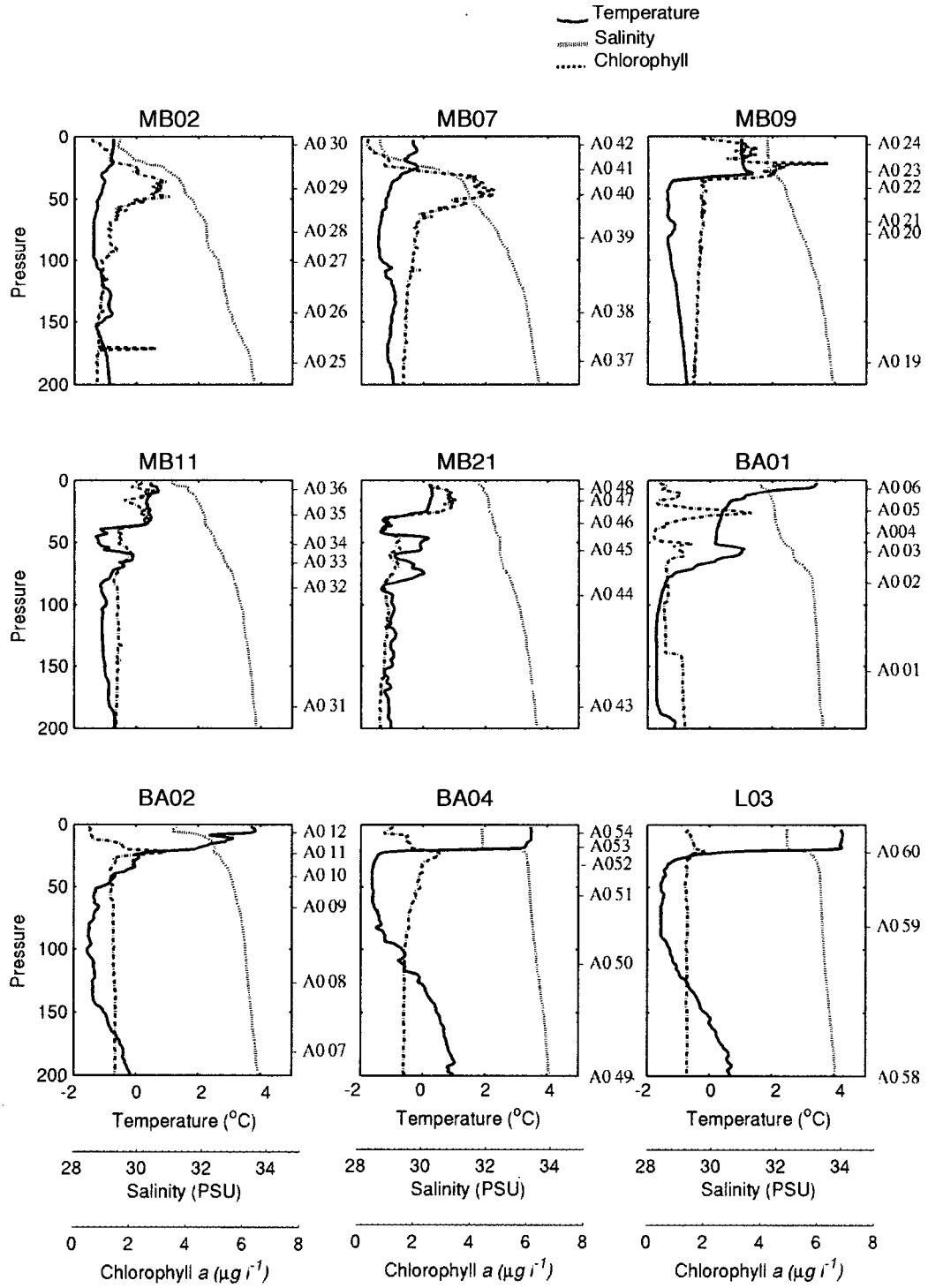

Figure 2.6 Station profiles showing temperature, salinity, and chlorophyll *a* concentration (estimated from fluorescence). Sample names are indicated at their corresponding depth.

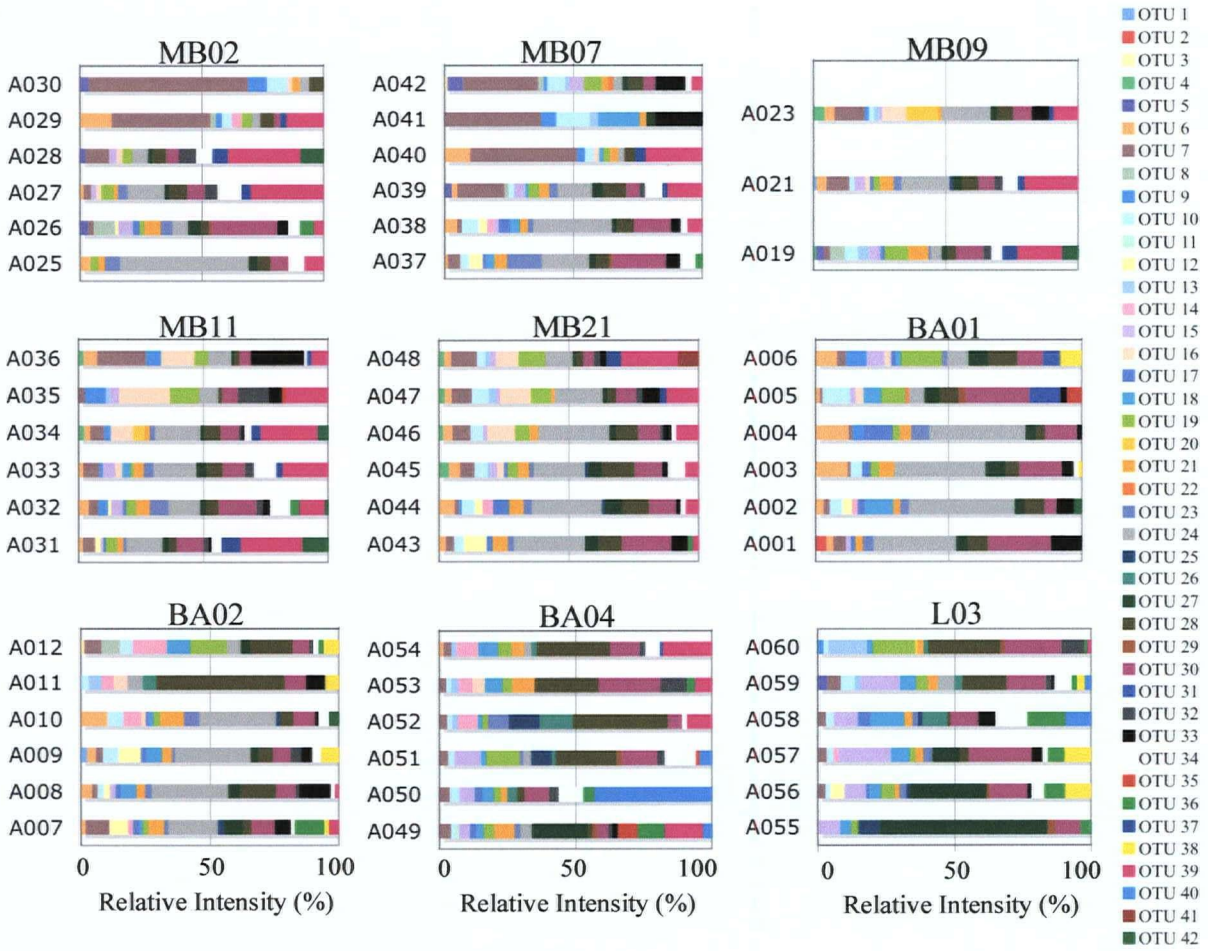

Figure 2.7 Histograms showing changes in picoeukaryote community structure at each station. The intensity of each DGGE band type, or operational taxonomic unit (OTU), relative to the total band intensity in each sample is shown. Samples are in order down the water column at each station. Refer to Figure 2.5 for corresponding depth.

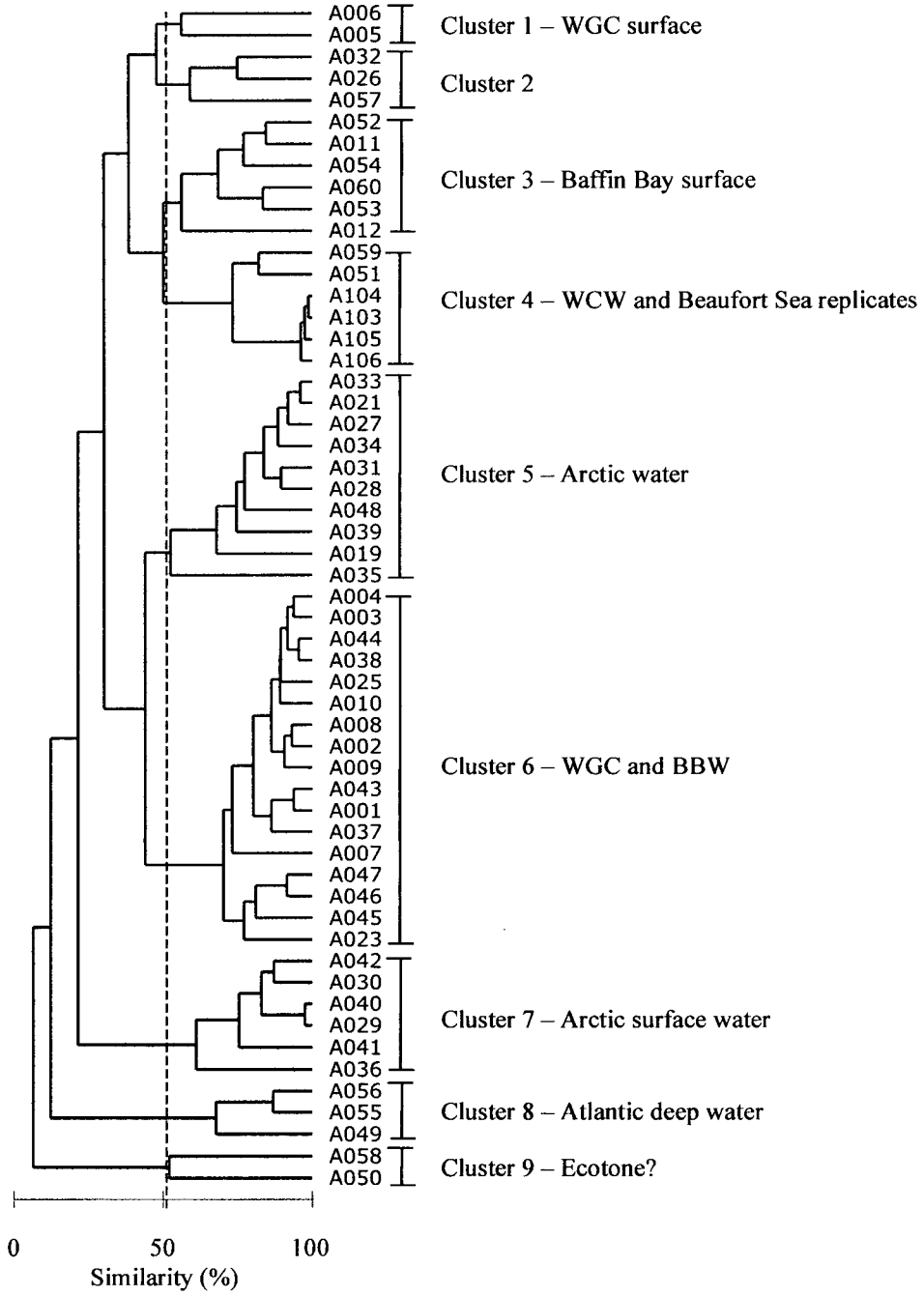

Figure 2.8 Dendrogram showing similarity of picoeukaryote assemblages from the North Water polynya. Dendrogram was derived from DGGE intensity band patterns by Agglomerative Hierarchical Clustering (AHC) using a Pearson Correlation Coefficient with Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Dotted line indicates cluster branch cutoff similarity of 50%. WCW – wintertime convection water; WGC – West Greenland Current; BBW – Baffin Bay Water.

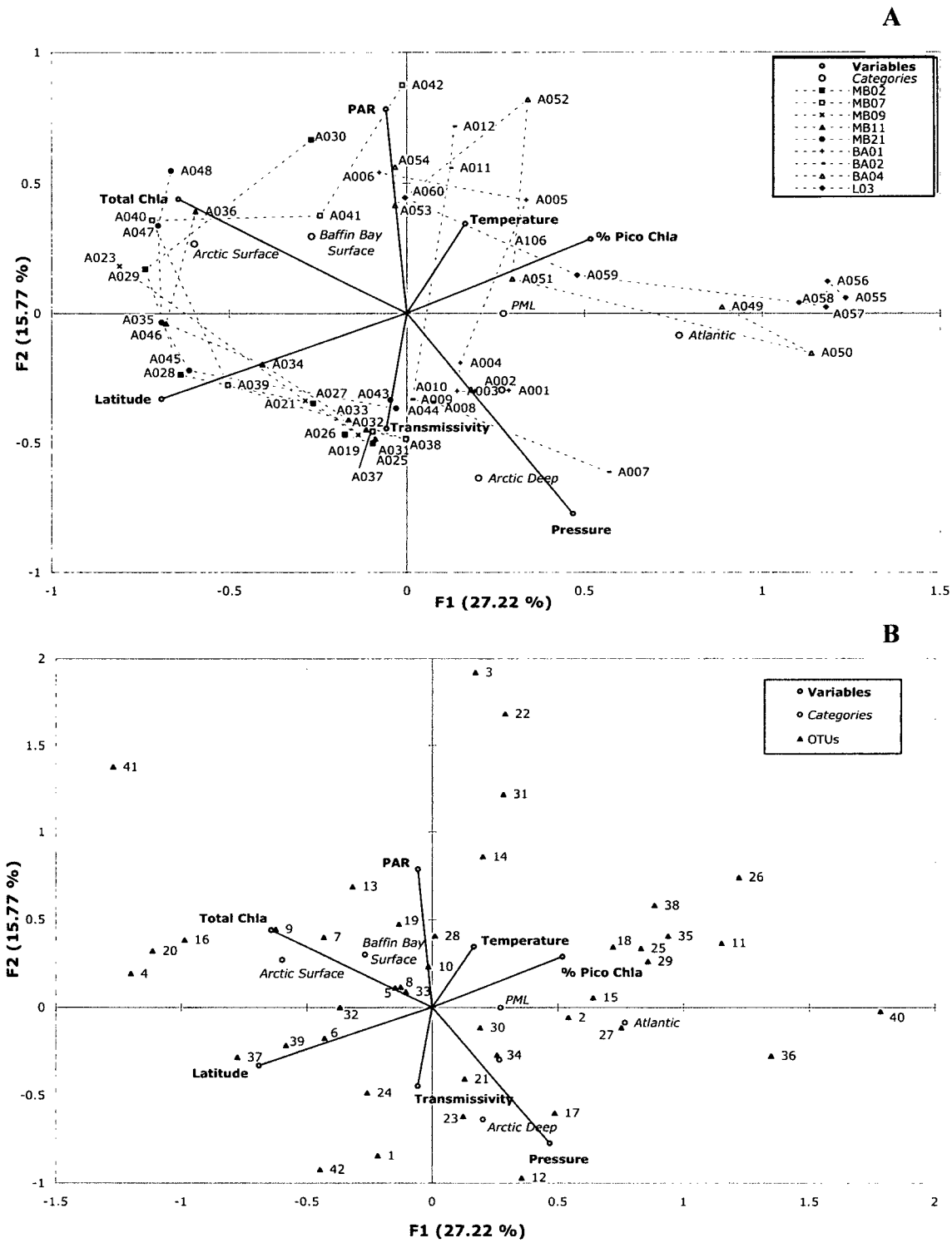

Figure 2.9 (A) Canonical Correspondence Analysis (CCA) ordination diagram relating environmental variables to picoeukaryote assemblages. Each sample represents a community assemblage derived from denaturing gradient gel electrophoresis (DGGE) band intensity patterns. (B) CCA ordination with operational taxonomic units (OTUs). Both CCAs display 18.3% of the inertia (weighted variance) in OTU intensity and 42.6% of the variance in the weighted averages with respect to the environmental variables. For both diagrams the eigenvalues of axis F1 and F2 are 0.272 and 0.158, respectively; the eigenvalue of F3 (not displayed) is 0.138.

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3. Conclusion

3.1 Picoeukaryote biohydrography

The importance of picoeukaryotes in the global ecosystem has become apparent in recent studies. While the significance of prokaryotic picoplankton species, such as Synechococcus and Prochlorococcus, in global marine productivity and biomass has been recognized, and substantial advancements have been made in the understanding of their spatial distribution and ecosystem functioning (Delong et al. 2006), investigation of picoeukaryotes is just beginning. Particularly in the Arctic, the dominant ecosystem role and genetic diversity of picoeukaryotes is only now being appreciated (Lovejoy et al. 2006). As global warming accelerates, drastic environmental change in the Arctic, including increased water temperatures, reduced ice-cover, and altered circulation patterns, will change the physical habitat for picoeukaryotes. How these changes will affect their community structure and diversity, and their ultimate functioning in the ecosystem, is currently unknown. This study provides one of the first analyses of the distribution of picoeukaryotes in the Arctic in relation to the physical environment.

We studied the biohydrography of picoeukaryotes, their distribution and diversity in relation to the physical characteristics of the water, in the North Water Polynya (NOW), one of the largest and most productive polynyas in the Arctic. The NOW is a region where distinct water masses intersect, Arctic water flowing south through Nares Strait meets Atlantic water flowing north through Baffin Bay. The distinct physical properties of these two water masses, and the frontal zone at their intersection, provided an excellent study region for biohydrographic investigation. Using molecular techniques to determine community assemblages, we were able to investigate basic hypotheses of microbial distribution. As it is virtually impossible to disprove the presence of a particular microbial species at one location, we used relative changes in DGGE community fingerprint intensity to reflect changes in community structure, a generally accepted method. Contrary to the conclusions of Finlay (2002), we found that picoeukaryotes are not randomly distributed; they do exhibit biogeographic (referred to as biohydrographic in this study) distribution patterns. Further, we were able to associate community assemblages with particular water masses. Arctic and Atlantic water masses showed different assemblages, as did surface waters with different physical

characteristics and different origins; Arctic meltwater, West Greenland Current, and Baffin Bay surface waters all showed distinct assemblages. At sites of water mass mixing, assemblages seemed to maintain the composition of their original water mass. Statistical analysis revealed the relative influence of certain physical variables on variation in communities. We found that spatially related variables, such as depth and latitude, were correlated with picoeukaryote community structure. We also found that variables related to phytoplankton biomass and overall community size structures were important influences. Samples associated with high total phytoplankton biomass, likely from diatom blooms, had a different picoeukaryote assemblage than samples with low phytoplankton biomass, those from depth or non-blooming sites. Using genetic sequencing of extracted DGGE bands, we were also able to identify many of the taxa present in these waters. This study was successful in providing insight into the factors structuring picoeukaryote community diversity in Arctic waters.

Although we were able to accomplish many of the original goals of the project, the study could have been improved by a number of factors. We were unable to conclusively distinguish between the roles of historical events versus contemporary environmental influence on the biohydrographic variation in communities. We did find that distinct waters masses had an associated distinct assemblage, and the similarities between assemblages carried over relatively large distances (100s of kilometers), even in regions of mixing, indicating that there were multiple biohydrographic "provinces" differentiated by past events. These provinces however, generally had different physical conditions (temperature, salinity, nutrients), so we cannot rule out the influence of contemporary environmental factors on community structure. We also found indications of ecotone communities, unique assemblages in the strong physical gradient between separate ecosystems, and an influence of phytoplankton size structure, evidence that picoeukaryotes are influenced by contemporary conditions. Martiny et al. (2006) outlined some of the factors that need to be accounted for to differentiate between past history and the contemporary environment. One of the most important is the "distance effect"; are closer samples more similar than distant ones? This is a non-trivial question for planktonic organisms in the marine environment where the habitat itself is fluid, moving and mixing. To truly account for the effect of distance we need to follow water mass trajectories. Depending on the flow of water, sites closely situated in space may, due to

opposing currents or dispersal boundaries such as pycnoclines, be effectively very distant from one another. To do so requires the use of conservative tracers, which was beyond the scope of this study, but could be utilized in future studies. As well, other non-independent statistical analyses, such as bootstrapped regression and the partial Mantel test (Borcard 1992; Mantel 1967), may be more instructive in disentangling between the effects of geographic distance versus the environment on assemblage composition. In the future, the utilization of neutrally buoyant drifters that could track changes in a water mass over time would be more appropriate for microbial oceanography, however this requires new technology. Real-time automated molecular sampling probes that could potentially sample the microbial realm at the same resolution as physical data are in development (Scholin et al. in press), and this technology would vastly expand our understanding of the microbial world. Also, increasing the depth resolution of sampling, even by traditional methods, at stations showing interleaving layers or strong physical gradients would provide greater insight into vertical variability of communities. Despite these approaches that would improve the current study, we were able to discern patterns with the available data, likely indicating that both historical events and contemporary environmental factors contributed to the structure of picoeukaryote communities.

Further areas for improvement center around the data collected and the methods used in the study. Substantial information would be gained if the taxonomic identification obtained by sequencing extracted bands could be confidently matched to a particular OTU. This would enable description of the spatial distribution of particular species, and investigation of biohydrographic patterns. Running the re-amplified extracted DGGE band beside the original sample would permit confident matching (Crump et al. 2003). Alternatively, analyzing samples on gels with a narrower range of denaturant could separate potentially overlapping bands that confused sequencing results. Neither of these methods is ideal however, as they both require substantial additional investment in time and resources, conflicting with the very characteristics of DGGE that make it appropriate for community analysis. Other technologies, such as culture cloning, DNA micro-arrays, and shotgun sequencing would provide the desired taxonomic information, but as with DGGE, each has its strengths and weaknesses. The DGGE technique itself would be improved by development of an in-lab standard with greater gel separation, permitting

more accurate standardization between gels, and potentially allow comparison among future projects utilizing the same protocol.

The biohydrographic approach used in this study acknowledged the influence of the physical environment on microbial assemblages, and the investigation was structured accordingly. We attempted to understand the hydrographic environment of the NOW in as much detail as we could using previous work and our observations. The interdisciplinary nature of the project, investigating both physics and biology, is important to understand processes in the North Water. Utilization of novel molecular genetic techniques, multivariate statistics, and traditional oceanographic tools, places this project at a unique juncture in marine research, where exciting advancements in our knowledge of the world's oceans will undoubtedly occur in the near future.

This project is an initial contribution to our understanding of picoeukaryote ecology in the Arctic. The results described here will provide a framework for designing on-going research in the NOW, and throughout the Arctic Ocean. Additional information on picoeukaryote communities is currently being obtained by our laboratory through continued sampling and an ever-increasing array of analytical tools, including cloning and culturing, flow cytometry, real-time polymerase chain reaction (RT-PCR), and fluorescent in-situ hybridization (FISH), all of which will enhance our understanding of picoeukaryote abundance, diversity, physiology, and ecosystem functioning. Research is concurrently expanding to include marine Bacteria and Archaea, for a more holistic understanding of microbial processes in the rapidly changing Arctic. In collaboration with researchers from other disciplines, from physical oceanographers and climate modelers to chemists and fisheries scientists, this work brings us one step closer to understanding this unique cold ocean ecosystem. The importance of which cannot be underestimated, as a changing Arctic may have pronounced affects on the state of the global ecosystem.

3.2 References

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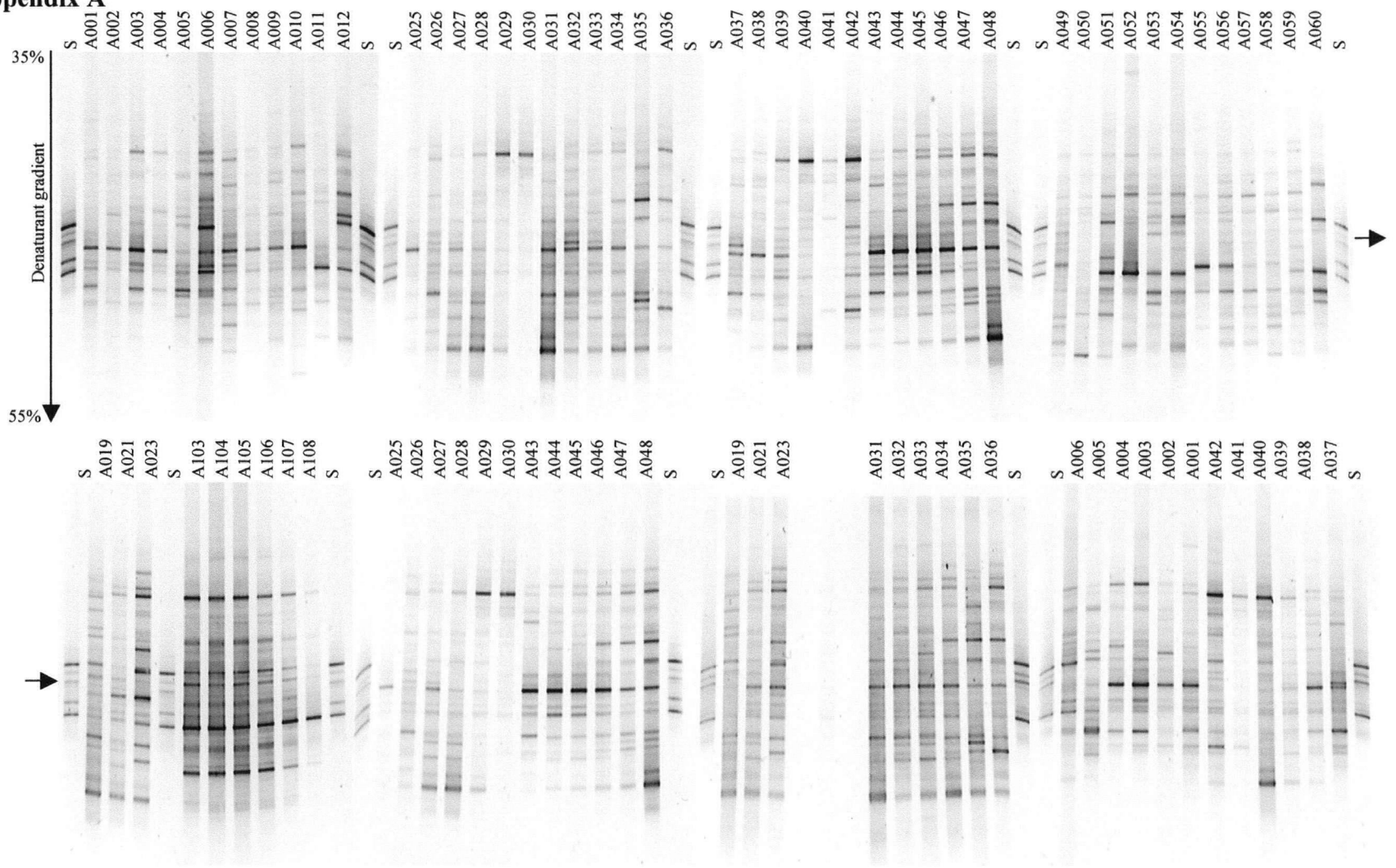
Appendix A



Appendix A Composite denaturing gradient gel electrophoresis image. S – standard lane.