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Review paper

Changing Views of Arctic Protists (Marine Microbial Eukaryotes) in a Changing Arctic

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Abstract. Advances in sequencing technology and the environmental genomic approaches have brought attention to the vastness of protist biodiversity. While over much of the world's oceans the species and phylotypes making up this diversity are assumed to be something previously hidden and now revealed, the recent rapid changes in the Arctic mean that such assumptions may be a simplification. Historical morphological species data can be used to validate new records provided that more of these species are identified using standard molecular markers. Environmental surveys can also go further by identifying species over regions, seasons and depths. High throughput sequencing and bioinformatics tools provide a means of monitoring and eventually predicting the consequences of change. We give an example of how microbial eukaryote communities differ over pan-arctic scales, emphasizing the need for additional sampling and the need for caution in extrapolating the results of one region to the entire Arctic.

Key words: Polar, marine microbial eukaryotes, protists, water masses, climate change, Subsurface Chlorophyll Maxima, High Throughput Sequencing.

Abbreviations: DNA – deoxyribonucleic acid, PCR – polymerase chain reaction.

BIOLOGICAL DIVERSITY

Up until recently the diversity and biogeography of marine microbial eukaryotes or protists (Caron *et al.* 2012) was largely based on microscopy, with bias toward larger species delimited by a suite of morphological characters. Relevant characters are usually based on

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close examination of isolates (Medinger *et al.* 2010). For groups difficult to maintain in culture, descriptions were based on drawings and reference imaging (Thessen *et al.* 2012) of preserved and sometimes freshly collected material. For many heterotrophic flagellates robust identification relied on accurately observing swimming behaviour (Vørs 1993, Ikavalko *et al.* 1996, Ikavalko and Gradinger 1997, Ikavalko and Thomsen 1997). However, species may exist as different morphotypes with different life stages, and can be misclassified (McManus and Katz 2009). Other species are identified by electron microscopy (Seoane *et al.* 2009), while oth-

ers are very small and missed altogether using classical microscopy techniques (Vaulot *et al.* 2008). The advent of molecular approaches, especially polymerase chain reaction (PCR) targeting the 18S rRNA gene followed by cloning and Sanger sequencing, resulted in the realization that the diversity of uncultivated marine protists was much greater than previously thought, with novelty at all taxonomic levels from divisions and phyla (Lopez-Garcia *et al.* 2001, Seenivasan *et al.* 2013) to ecotypes (Lovejoy *et al.* 2007). 18S rRNA gene phylogenies have also contributed to the resolution of higher level classifications of many previously enigmatic marine protists, including many reported from the Arctic (Vørs 1993), such as *Telonemia* (Shalchian-Tabrizi *et al.* 2007), *Leucocryptos* (Kathablepharida, Okamoto and Inouye 2005) and *Cryothecomonas* (Kuhn *et al.* 2000).

Sequences are deposited in public databases such as the National Center for Biotechnology Information (NCBI) and the Community Cyberinfrastructure for Advanced Microbial Ecology Research & Analysis (CAMERA). Consequently, researchers can easily compare sequences from different ecosystems, environments and geographic locations (Jungblut *et al.* 2012, Harding *et al.* 2011) independent of the availability of expert taxonomists. However, cloning and sequencing remain very time consuming and costly, and most studies are considered "snapshots" that describe a subset of common organisms in an environment. Since these snapshots do not capture whole communities (Pedrós-Alió 2006), comparisons at finer spatial and temporal scales are limited. Fortunately, breakthroughs in sequencing technology combined with multiplexing, where many samples are combined for high throughput sequencing (HTS) during a single run, coupled with improved bioinformatics approaches (Huse *et al.* 2008, Caporaso *et al.* 2010) are generating unprecedented information on protist diversity and distribution. HTS yields many thousands of reads and facilitates the identification of common and rare species in a single sample. Within the 18S rRNA gene are several short variable regions that are phylogenetically informative enabling identification to species if reference data bases exist (Luddington *et al.* 2012). For the Arctic Ocean, the V4 region of the 18S rRNA gene has been used the most frequently to identify taxa to the level of genera with confidence (c.f. Comeau *et al.* 2011, Monier *et al.* 2013). While sequencing errors from pyrosequencing, for example, can inflate overall diversity (Bachy *et al.* 2013) depending on the bioinformatics stringency, careful screening and mapping the short reads onto phylogenies constructed with nearly full length 18S rRNA gene trees can provide a high level of confidence for separating clades to species level units (Monier *et al.* 2013).

ARCTIC OCEAN AND CLIMATE

The recent rapid changes in Arctic Ocean summer ice cover (Laxon *et al.* 2013) has raised questions on how this will affect the annual carbon budget and primary productivity in the Arctic. One approach has been to use available satellite data to extrapolate to a future ice-free summer Arctic, with some authors suggesting higher future production (Arrigo and van Dijken 2011), while others suggest a decrease (Hill *et al.* 2013). Both analyses rely on interpretation of the timing and extent of surface chlorophyll and assumptions on how this acts as a proxy for net productivity. In fact, net productivity is strongly dependent on microbial food web structure and the depth distribution of photosynthetic activity and associated heterotrophic microbes, including heterotrophic protists. This is important because the Arctic is strongly salinity stratified (Carmack 2007), and while surface phytoplankton biomass is high in spring (Lalande *et al.* 2009, Forest *et al.* 2010) over the remaining summer and early fall, nutrients are depleted near the surface, and photosynthetic production is mostly within a sub-chlorophyll maximum layer (SCM) that is invisible to satellite remote sensing (Martin *et al.* 2010, Ardyna *et al.* 2013). Although the classic view is that diatoms dominate; for much of the year photosynthetic communities consist of small flagellates (Sukhanova *et al.* 2009, Martin *et al.* 2010, Lapoussiere *et al.* 2013), some of which may by mixotrophic, and are rarely identified to species level in microscopy based studies.

The Arctic is surrounded by land and can therefore be thought of as a double estuary (Carmack and Wassmann 2006, McClelland *et al.* 2012). The Arctic Ocean itself consists of a very large shelf region and two deep basins: the Canada Basin and Nansen Basin (Fig. 1). It is connected to the Pacific Ocean through the Bering Strait and to the Atlantic via Fram and Davis Straits. The West Spitsbergen Current brings Atlantic water into the Arctic via Fram Strait. This water circulates within Nansen Basin and eventually the East Greenland Current transports Arctic water into the North Atlantic. Most Western Arctic water exits through the Canadian Archipelago to Baffin Bay between Ellesmere Island and Greenland (Fig. 1) then to the North Atlantic from Davis Strait (Aagaard *et al.* 1981, Aagaard *et al.* 1985).
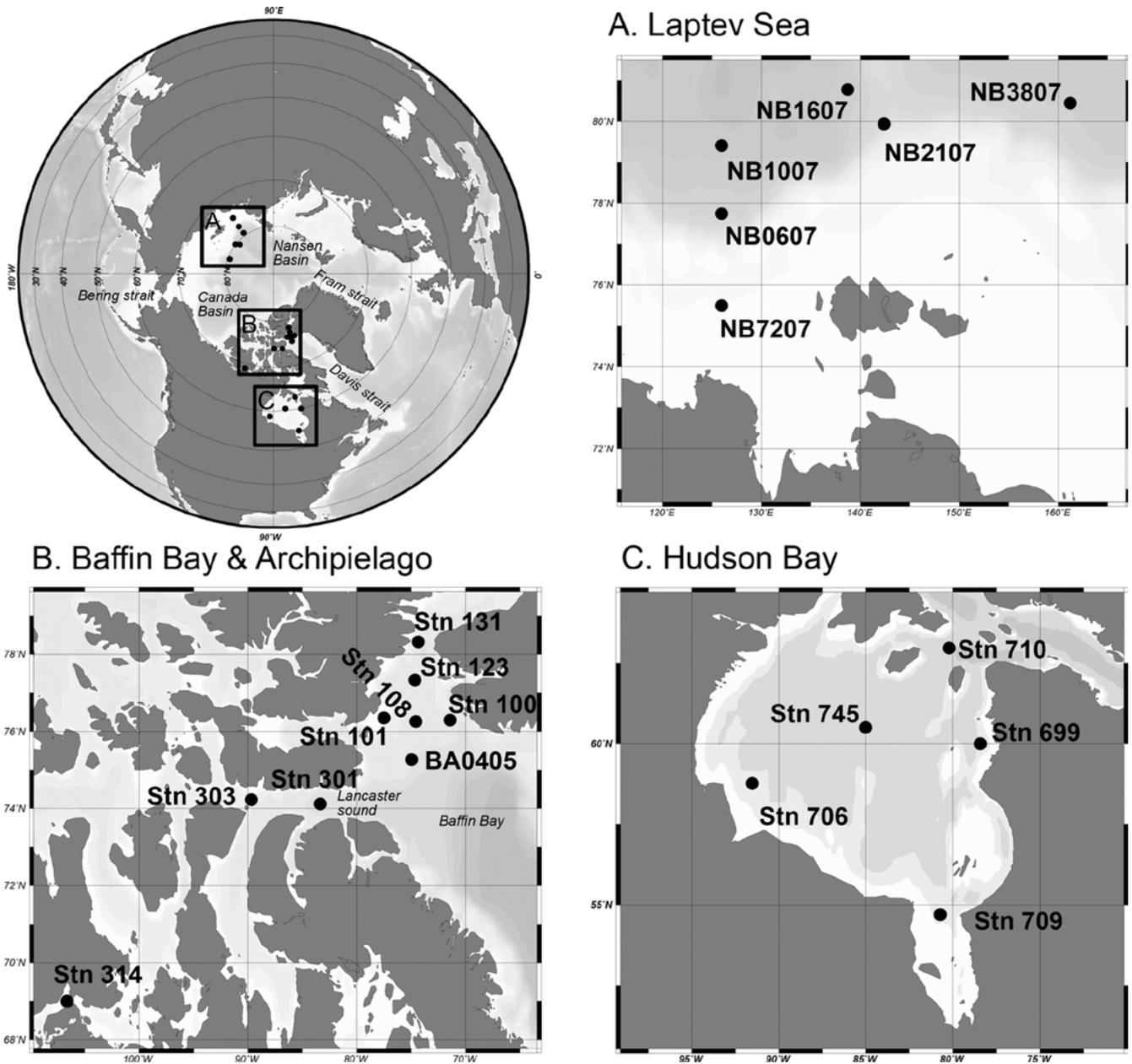
Fig. 1. Polar projections of Arctic Ocean, indicating the three regions outside of the Beaufort Sea, used as an example of community clustering in this review.

Canadian Arctic Archipelago water contains almost no signal from waters of Atlantic origin (Jones *et al.* 2003) and is composed of Pacific water from the Bering Sea with some influence from the Mackenzie River. The upper waters are, as elsewhere in the Arctic, influenced by ice melt to form a Polar Mixed Layer (PML). Hudson Bay (Fig. 1) is also considered an Arctic Sea and is strongly influenced by freshwater discharge (Kuzyk *et al.* 2010) but can be impacted by deep brine enriched waters from the Foxe Basin polynya in the Canadian Archipelago (Defossez *et al.* 2010) increasing stratification and maintaining fresher surface waters. With such linkages between neighbouring oceans, the biodiversity of Arctic microbial eukaryotes could represent important tracers of water masses and connectivity and enable comparisons of composition and biogeography.

However, data are currently sparse and there is an urgent need to document the community composition and species diversity of Arctic regions. A first step is to verify whether there are differences in eukaryotic marine microbial communities in the surface and SCM waters of different regions. In this review, I firstly briefly review the major species groups of smaller marine microbial eukaryotes in Arctic Seas and secondly report on recent HTS results that document community level differences over space and time.

ARCTIC PROTISTS AND ECOLOGY

Protists are morphologically, phylogenetically and functionally diverse; the term protist is employed for convenience and is not used as a valid taxonomic classification; it should also be noted that terminology and evolutionary relationships at the highest taxonomic ranks remain in flux (Adl *et al.* 2012). While many larger protists such as ciliates, foraminifera and dinoflagellates have well-defined taxonomies based on morphology, the diversity of smaller flagellates is not well understood, and the lack of qualified taxonomists working on specific groups is problematic. High throughput sequencing technology will facilitate comparisons among sites and seasons (Comeau *et al.* 2011). However, matching historical records to the genetic signatures generated from sequences remains a major challenge (Lovejoy 2013).

Phytoplankton fix the carbon and energy required by all other trophic levels, and unlike temperate and tropical oceans, planktonic cyanobacteria are virtually absent from the Arctic marine environment, where marine microbial eukaryotes are always the primary photosynthetic functional group (Li *et al.* 2009). Some photosynthetic groups are also mixotrophs that supplement energy or nutrient requirements by phagotrophy. Among mixotrophs are the phylogenetically diverse flagellates, dinoflagellates and a few ciliates that are kleptoplastidic (enslavers of chloroplasts from ingested prey). Among potentially mixotrophic flagellates are chrysophytes that are reported from sea ice and Arctic marine waters (Lovejoy *et al.* 2002b, Rozanska *et al.* 2008). These include small free living *Ochromonas* spp., large tree shaped colonies of *Dinobryon balticum* and other colonial species. Other stramenopile groups that contain mixotrophic taxa are the Dictyophyceae, Pelagophyceae and Raphidophyceae (Poulin *et al.*

2011). Several other phyla level groups also include mixotrophs, among these are the Euglenozoa, Cryptophyceae, Haptophyceae and many small non-thecate dinoflagellates (Lovejoy *et al.* 2002b, Poulin *et al.* 2011). Mixotrophic ciliates including *Mesodinium rubrum* and *Loboea stobila* (Fig. 2) have been reported from both microscopy-based and 18S rRNA gene surveys (Lovejoy *et al.* 2002b, 2006; Comeau *et al.* 2011). Chloroarchniophytes, which are Cercozoa with chlorophyll *b* (derived from a green algal secondary endosymbiosis), have been recovered from most surface Arctic marine 18S rRNA gene surveys (Lovejoy *et al.* 2006, Lovejoy and Potvin 2011).

Primarily, heterotrophic protists that depend on bacteria and other protists for energy are reported from microscopy-based (Vørs 1993, Lovejoy *et al.* 2002b, Ikavalko and Gradinger 1997) and molecular 18S rRNA gene surveys (Terrado *et al.* 2009, Monier *et al.* 2013). Most frequent among the small marine heterotrophic flagellates are the phylogenetically diverse marine stramenopiles (MASTS, Massana *et al.* 2006), Picozoa, *Telonemia*, cerozoans, choanoflagellates and Katablepharidia. A brief summary of the known ecological difference among these major groups follows.

MASTs, belonging to clades 1, 2, 3, 7 and 8 (Massana and Pedrós-Alió 2008), are common in upper Arctic waters (Lovejoy *et al.* 2006, Comeau *et al.* 2011) and Sea Ice (Comeau *et al.* 2013). MAST-4 is rarely reported compared to other clades (Monier *et al.* 2013). Three subclades within MAST-1 occur in the Arctic (MAST 1a, 1b and 1c), the different subclades appear to be associated with different environments, such as the sea ice edge (Thaler and Lovejoy 2014). MAST 7 and MAST 8 are more often found in and below subsurface chlorophyll maximum (SCM) layer compared to near surface waters (Monier *et al.* 2013) and are rarely found at depth (Thaler and Lovejoy 2014).

Picozoa, previously known as "picobiliphytes" (Seenivasan *et al.* 2013), are nearly always retrieved from Arctic Ocean 18S rRNA gene surveys (Comeau *et al.* 2011, Monier *et al.* 2013). Picozoa were first discovered from samples that had been pre-filtered through a 3 μm filter (picoplanktonic size). Alignments of full sequences put them as a sister of the Cryptophyta, prompting Not *et al.* (2007) to use flow cytometry to sort cells with fluorescence similar to cryptophyte plastids, "picobiliphyte" specific 18S rRNA fluorescent in situ hybridization (FISH) probes hybridized to some of these cells. Yoon and colleagues (Yoon *et al.* 2011) had
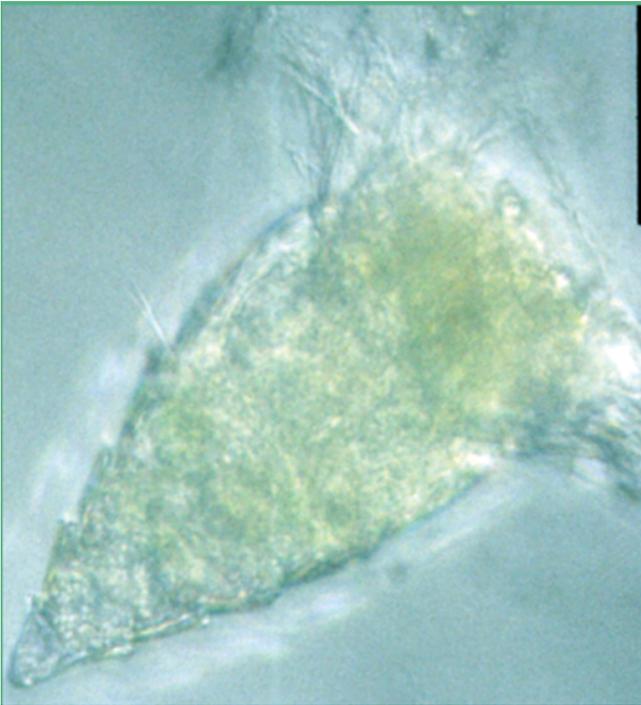
Fig. 2. *Loboea*, collected from Northern Baffin Bay. Scale bar: 8 μm.

a different strategy, where they used flow cytometry to select non-chlorophyll containing cells, targeting strict heterotrophs for single cell genome sequencing. Among their single-cell genomes were several with 18S rRNA genes within the "picobiliphytes". However, they failed to detect any plastid-related genes. Recently, Seenivasan *et al.* (2013) using both genetics and electron microscopy showed that an isolate described as *Picomonas judraskeda* belonging to the lineage lacks plastids. The description of this isolate provided sufficient information to erect a new eukaryotic phylum; Picozoa that includes at least 3 subclades with Arctic representatives. The association with chloroplasts in some samples remains unresolved, but could represent mutualistic (Thompson *et al.* 2012) or other inter-species interactions. The ultra-structure of *P. judraskeda*, described by Seenivasan *et al.* (2013) suggests that Picozoa take up colloidal substrates, which are often abundant in Arctic waters as transparent exopolymers (Riedel *et al.* 2006).

The genus *Telonema* has been reported in most Arctic surface water 18S rRNA gene surveys (Monier *et al.* 2013). Taxonomic affinities to other eukaryotes remains unresolved, but the genus has been placed in its own phyla, Telonemia, by Shalchian-Tabrizi *et al.* (2006, 2007). Those authors initially suggested that there may

be at least one Arctic restricted clade of Telonemia, but that is now thought to have been an artefact (Brate *et al.* 2010). Monier *et al.* (2013) suggested that the uniformity of very closely related phylotypes occurring sporadically over different geographic regions was consistent with Telonemia being able to survive long distance transport and then 'bloom' when conditions were favourable.

Other heterotrophic flagellates include the Cercozoa, Choanoflagellidia and Katablepharidia. Cryothecomonads are the most common heterotrophic cercozoan group found in Arctic waters (Thaler and Lovejoy 2012) and occur both in the water column and in sea ice (Comeau *et al.* 2013). Choanoflagellates are efficient bacterivoures, and both historic records and recent molecular markers indicate that they are very diverse (del Campo and Massana 2011). While nearly always retrieved at low frequency in the Arctic, there has been little work specifically targeting Choanoflagellates, but they may well be very diverse over pan-Arctic scales (Lovejoy and Thaler unpublished data). Katablepharids, especially *Leucocryptos* spp., have been reported from microscopy-based studies (Vørs 1993) but only occasionally reported from Arctic 18S clone libraries and never in high proportions (Monier *et al.* 2013).

In addition to the small heterotrophic flagellates, larger mostly heterotrophic protists are reported from microscopy-based and 18S rRNA gene surveys. Polycystinea and Acantharia are frequently recovered from Arctic 18S rRNA gene libraries; often the sequences are most similar to sequences from the deep ocean (Lovejoy and Potvin 2011). Their frequencies in environmental gene surveys suggest they may be important phytoplankton consumers in polar waters. As in other oceanic regions, alveolates, which include dinoflagellates, ciliates and uncultivated groups, are common and often dominate 18S rRNA gene surveys; in part this may be due to high gene copy numbers (Zhu *et al.* 2005). Records from microscopy-based studies tend to report larger thecate dinoflagellates (Okolodkov and Dodge 1996, Okolodkov 1999), whereas difficlult to indentify *Gymnodinium* and *Gyrondinium* species complexes dominate 18s rRNA gene surveys (Comeau *et al.* 2011). Ciliates are also diverse in Arctic waters, but gene surveys tend to indicate the predominance of two clades (Lovejoy and Potvin 2011) with nearest matches to *Strombidium* SNB99.2 (*Strombidium* sp.2 in Struder-Kypke and Lynn 2003). There are substantial microscopical records of tinntinids (Dolan *et al.* 2013) that can be used to infer ecological changes. However, these

are not often indentified from gene surveys, perhaps in part due to their under representation in sequence databases. Other ciliates such as *Euplotes* spp. have been isolated from both the Arctic and Antarctic (Di Giuseppe *et al*. 2013) but also appear less often in 18S rRNA gene surveys.

Uncultivated alveolates (Lopez-Garcia *et al.* 2001, Guillou *et al.* 2008) that have nearest NCBI Blast matches to parasitic taxa, mostly fall into two major clades in the Arctic: Group I and Group II (Lovejoy *et al.* 2006, Terrado *et al.* 2009). The most commonly recovered clades in the Arctic fall into Group II, which contains the dinoflagellate parasitoid *Amoebophyra*. Other sequences are related to parasites of fish (Skovgaard *et al.* 2010).

The bipolar distribution of protist taxa has been used to argue for lack of endemism among microbes; e.g. the cyst-forming dinoflagellate *Polarella glacialis* is found associated with ice in both the Arctic and Antarctic (Montresor *et al.* 2003). As more sequences from widely dispersed regions become available, biogeographic studies are becoming more common, and a level of similarity among taxa from different regions can be defined at levels from genera to strain, depending on the resolution of the marker (Gomez 2013). Cloning and sequencing studies of the 18S rRNA gene suggest a certain level of, if not endemicity then at least, restricted distributions of several marine Arctic protists. A recent survey of over 2,500 microbial algal sequences originating from Arctic 18S rRNA gene clone libraries indentified several Arctic specific taxa (Terrado *et al.* 2012); among these were phylotypes of *Chrysochomulina* and *Ochromonas*, which are mixotrophic genera (Jones *et al*. 1993, Keller *et al.* 1994). Other protists with possible restricted distributions include several ciliates, dinoflagellates and Acantharia (Lovejoy and Potvin 2011). Eukaryote 18S rRNA gene sequences with best matches to sequences previously reported from marine Arctic sites have been recovered from Arctic snow and air, suggesting the importance of local transport reinforcing species distribution patterns (Harding *et al.* 2011).

HIGH THROUGHPUT SEQUENCING

We have collected microbial DNA from the Canadian Beaufort Sea and Amundsen Gulf since 2005, and much more is known about both the physical oceanography and microbial communities of the area compared to elsewhere in the Arctic. The Beaufort Sea is density stratified because of characteristic temperature and salinity properties of waters that flow along the shelf. The surface waters are locally formed and relatively fresh, this upper Polar Mixed Layer (PML) lies atop Bering Sea Summer Water (BSSW) that originates in the Pacific, and just below BSSW is colder Bering Sea Winter Water (BSWW). Finally warmer saltier Atlantic Water can be detected at depth (Carmack and McLaughlin 2011). The BBSW is rich in nutrients and supports production and biomass buildup in the SCM. In the southern Beaufort Sea the communities from the surface PML and SCM in the Pacific origin water are distinct from each other, and SCM heterotrophic protist communities are similar over hundreds of km (Monier *et al.* 2013). Exploiting this community stability, Comeau *et al*. (2011) investigated the summer and late summer Amundsen Gulf SCM before and after the 2007 record sea ice minimum and found significant changes in the relative frequency of different microbial eukaryotic clades. In particular MASTs were less common, and small phototrophs including an Arctic ecotype of *Micromonas* were more common, suggesting local (Arctic) selection for certain biological species or traits.

The PML is formed locally by mixing of melting ice and river runoff, and PML are found throughout the Arctic. SCM also form throughout the Arctic at the base most of these PML (Ardyna *et al.* 2013, Martin *et al.* 2010). An interesting question is whether other SCM that form in the absence of Pacific Water are as biologically different from surface waters as SCM and surface waters in the Beaufort Sea, where evidence of an SCM persists even over the winter (Lovejoy and Medrinal unpublished data, Terrado *et al.* 2011). The answer to this question has implications for predicting the strength of environmental selection and community adaptability in the face of rapid changes. Accurate information is needed for the parameterization of coupled biological physical models used to predict future Arctic productivity.

Taking advantage of microbial DNA samples collected by my laboratory from different Arctic Regions, we used HTS to compare near surface and SCM samples where Pacific Summer Water was not evident at the base of the PML. Samples had been collected as part of other missions since 2004 (Comeau *et al.* 2011, Terrado *et al.* 2012), and details of methodology can be found in those publications; a more in depth analysis of new data will be published elsewhere. Included were samples from Baffin Bay, Hudson Bay
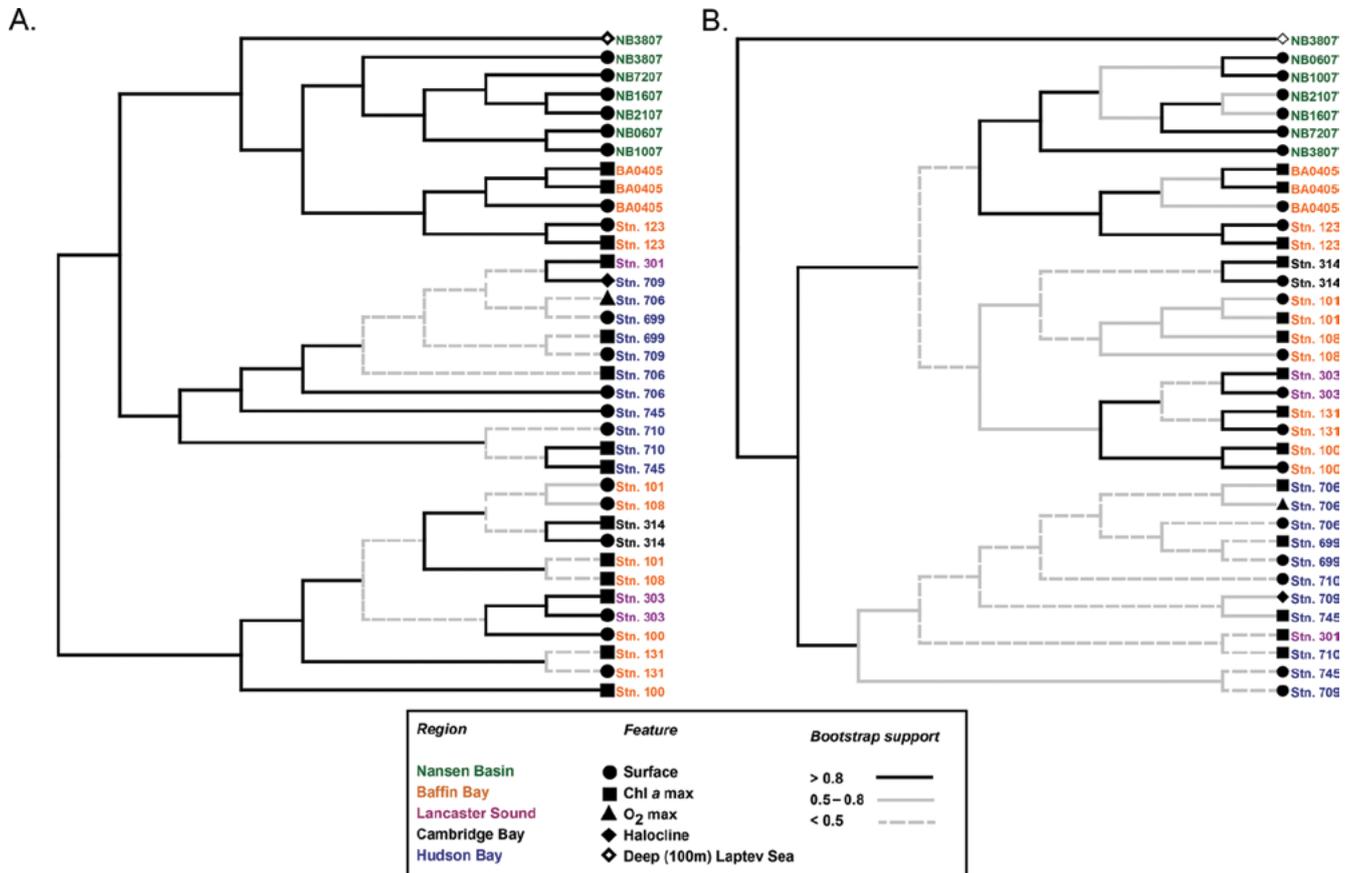
A.

B.

Region		Feature		Bootstrap support	
Nansen Basin		● Surface		> 0.8	▬▬
Baffin Bay		■ Chl *a* max		0.5 – 0.8	▬▬
Lancaster Sound		▲ O₂ max		< 0.5	- - -
Cambridge Bay		◆ Halocline			
Hudson Bay		◇ Deep (100m) Laptev Sea			

Fi**g. 3. A** – Whole eukaryotic microbial community bootstrap-supported UPGMA hierarchical clustering tree based on Bray-Curtis ß-diversity metrics. Metazoa were excluded of this analysis; OTU definition as 98% similarity. Analysis were carried out in Qiime as in Kuczynski *et al.* 2002; samples were subsampled 100 times selecting 2533 sequences (75% of the smallest subsample). **B** – Only rare eukaryotic community bootstrap-supported UPGMA hierarchical clustering tree based on Bray-Curtis ß-diversity metrics. Metazoa and abundant OTUs (> 0.1%) were excluded of this analysis; OTU definition as 98% similarity. Analysis were carried out in Qiime as in Kuczynski *et al.* 2002; samples were subsampled 100 times selecting 125 sequences (75% of the smallest subsample).

and the Laptev Sea (referred to here as Nansen Basin), collected during a Nansen-Amundsen Basin Observational System (NABOS, http://www.arcus.org/search/catalog/259) mission. We also included samples from the entry (Cambridge Bay) and exit (Lancaster Sound) of the Canadian Archipelago (Fig. 1). Over the wider Arctic upper mixed layer microbial eukaryotic communities tended to separate by region (Fig. 3) with both surface and SCM samples in most samples grouping by station. Similar patterns were detected in the total community and in the rare community; although with poorer bootstrap support. Hudson Bay communities clustered apart from Canadian Archipelago and Baffin Bay communities, as did the Nansen Basin (Laptev Sea) samples. Interestingly, Baffin Bay Stations did

not cluster together, but formed several sub-clusters. Baffin Bay itself is hydrologically complex (Lovejoy *et al.* 2002c), and both predominant water masses and ice conditions vary substantially over short distances. Recent ice conditions may have had the strongest influence on surface communities (Thaler and Lovejoy 2012), and the dissimilarity of communities from the separate regions likely reflects local selection.

TRENDS

Microbial communities are expected to be influenced by environmental changes that are now underway. Recent changes in ice cover are already associated with

microbial community structure changes (Li *et al.* 2009, Comeau *et al.* 2011), likely caused by changing light availability and increased water column stratification. Both phototrophic and heterotrophic species are vulnerable to change, as the seasonally open water increases over time and space. The timing and extent of the spring surface bloom will change, along with the timing and geographical extent of ice edge blooms, with consequences for zooplankton and higher food webs (Soreide *et al.* 2010). Day length can influence food quality as well as species composition (Leu *et al.* 2010). Ongoing loss of ice and earlier blooms could result in a rearrangement of food webs in the Arctic, since annual light availability at high latitudes remains fixed and there is an increased probability of decoupling production patterns from annual animal cycles resulting in major ecosystem shifts (Grebmeier *et al.* 2006, Post *et al.* 2013). In addition, microbial community interactions and dominant species largely determine the efficacy of the biological carbon pump, where CO_2 is drawn down from the atmosphere and sequestered in the deep ocean. This absorption of CO_2 by the global ocean is now affecting the pH of oceanic waters and the biological availability of carbonate and aragonite minerals. There are already signs of such changes in the Arctic Ocean (Carmack and McLaughlin 2011), and these pH effects will add to the selection pressures on microbial communities. There is an urgency to understand community assemblages and functions to predict impacts on marine food webs and global processes, and careful application of HTS studies will be a useful tool in this quest.

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