DIVERSITY AND COMMUNITY STRUCTURE OF EUKARYOTIC PHOTOTROPHS IN THE BERING AND CHUKCHI SEAS

By

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ABSTRACT

The phytoplankton of the Bering and Chukchi seas support highly productive ecosystems characterized by tight benthic-pelagic coupling. In this study, we focus on the northern Bering and Chukchi seas, considering them as one ecosystem. This community has historically been dominated by diatoms; however, climate change and accompanying warming ocean temperatures may alter primary producer communities. Using metabarcoding, we present the first synoptic, high-throughput molecular phylogenetic investigation of phytoplankton diversity in the Bering and Chukchi seas based on hundreds of samples collected from June to September in 2017. We identify the major and minor taxonomic groups of diatoms and picophytoplankton, relative abundances of genera, exact sequence variants (201 for diatoms and 227 for picophytoplankton), and describe their biogeography. These phylogenetic insights and environmental data are used to characterize preferred temperature ranges, offering insight into which specific phytoplankton (*Chaetoceros*, *Pseudo–nitzschia*, *Micromonas*, *Phaeocystis*) may be most affected as the region warms. Finally, we investigated the likelihood of using shipboard CTD data alone as predictive variables for which members of phytoplankton communities may be present. We found that the suite of environmental data collected from a shipboard CTD is a poor predictor of community composition, explaining only 12.6% of variability within diatom genera and 14.2% variability within picophytoplankton genera. Clustering these communities by similarity of samples did improve predictability (43.6% for diatoms and 32.5% for picophytoplankton). However, our analyses succeeded in identifying temperature as a key driver for certain taxa found commonly throughout the region, offering a key insight into which common phytoplankton community members may be affected first as the Alaskan Arctic continues to warm.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Primary producers in the ocean comprise a diverse group of microscopic organisms capable of using light energy to drive the fixation of inorganic carbon (in the form of CO_2) and releasing oxygen as a byproduct. Due to an increase in anthropogenic carbon emissions, the sub-Arctic and Arctic are warming at a faster pace compared to other regions (Held and Soden 2006; Zelinka and Hartmann 2011). As a result, sea ice forms later and melts earlier in the year, leading to increased areas of open water, more light, and changing patterns of productivity as the Arctic shifts from a light-limited to a nutrient-limited system (Henson et al. 2013).

Presently, diatoms are the paramount primary producers in the Bering and Chukchi seas, contributing as much as 470 g C m^{-2} $year^{-1}$ (Springer and McRoy 1993), playing an essential role in Arctic marine biological carbon pump and biogeochemical cycles. Diatom cells are relatively large, falling predominantly within the nanoplankton (2-20 μm) and microplankton (20-200 μm) size ranges, and are typically associated with food webs dominated by larger copepods and an increase in biological pump efficiency due to sedimentation of large cells and chains (Pomeroy 1974; Azam et al. 1983; Laws et al. 2000). The amount of carbon that reaches the seafloor is positively related to the aggregation of organic matter from phytoplankton, especially the diatoms that dominate communities in the nutrient-rich regions found throughout the Bering and Chukchi seas. In addition to their silicate frustules, which enhance sinking rates, diatoms produce extracellular polymeric substances (Passow 2002; Piontek et al. 2010) that cause cells and other detrital material to aggregate and sink (Alldredge 1993; Cowen and Holloway 1996; Holloway and Cowen 1997; Alldredge et al. 1998).

While large-celled diatoms are currently the most important primary producers in the Arctic, recent studies suggest that phytoplankton communities may shift towards mixotrophy (single cells capable of both photosyntheis and phagotrophy or osmotrophy) in response to climate change (Stoecker et al. 2017a; Stoecker and Lavrentyev 2018). Should these predictions prove correct, the decreased presence of diatoms could result in a reduced flux of carbon to the benthos and seafloor via the biological carbon pump. Other studies also speculate that regional productivity could increase on the Chukchi shelf (Arrigo et al. 2008; Grebmeier 2012), with the potential to offset some excess CO_2 emissions. While cell size is important for carbon flux, other factors are

also at play: grazing zooplankton contribute to the pool of POM by sloppy feeding and fecal pellet production (Azam et al. 1983; Willhelm and Suttle 1999; Stemman and Boss 2012). Heterotrophic bacteria and archaea also colonize particulates and utilize dissolved organic carbon in the water column, remineralizing it back to CO_2 (Pomeroy 1974; Azam, Smith and Hagström 1994; Burkhardt et al. 2014). The origin of this remineralized inorganic carbon plays an essential role in the marine carbon cycle, especially in regulating the export and sequestration of carbon to the seafloor.

For millennia, diatoms have been the dominant phytoplankton of the Bering and Chukchi seas, as evidenced by microscopic observations (Moran et al. 2012; Giesbrecht et al. 2019) and the presence of siliceous seafloor sediment originating from diatoms (Ran et al. 2013) dating back to the late Quaternary when the region became covered by ocean (Sancetta et al. 1984). In today's ocean, Pacific waters transported northwards into the Bering Sea from depth have relatively high nutrient concentrations (Harrison et al. 2004; Pisareva et al. 2015), spurring diatom productivity along the way (Walsh et al. 1989; Codispoti et al. 2005). Large-celled diatoms, particularly *Thalassiosira* and *Chaetoceros*, bloom at retreating sea ice edges in spring and early summer (Sukhanova et al. 2009) and tend to be prominent along the coast (Hill et al. 2005). In one study on the Bering Sea shelf, microplankton-sized diatoms made up about 80% of the carbon in biomass (Moran et al. 2012). High diversity within *Chaetoceros spp.* has been observed in the Chukchi and Beaufort seas (Balzano et al. 2017) with four distinct genetic clades reported within *C. neogracilis* using 18S and 28S rRNA sequencing. *Thalassiosira* and *Pseudo–nitzschia* were also prevalent throughout the study region, although *Chaetoceros* was the dominant genus. Phytoplankton blooms have also been observed under and around sea-ice, where *Chaetoceros*, *Thalassiosira*, and *Fragilariopsis* were the dominant diatoms, forming unique seawater assemblages (Arrigo et al. 2012).

Picoplankton, operationally defined as plankton between 0.2 and 2 μm, (Sieburth et al. 1978), were once thought to be exclusively bacterioplankton (Platt et al. 1983). Now, clades of mostly flagellated eukaryotic protists are recognized as important members of the picoplankton (Vaulot et al. 2008), and commonly referred to as picoeukaryotes. Picoeukaryotes play many roles in the marine ecosystem; for example, many taxa are phototrophs (picophytoplankton) or heterotrophs (Worden and Not 2008), with some documented mixotrophs (McKie-Krisberg and Sanders 2014). Picophytoplankton are important primary producers in our study region (McKie-

Krisberg and Sanders 2014), and can account for up to 90% of primary production in other marine environments (Worden et al. 2004; Jardillier et al. 2010).

Broadly, the prominent picophytoplankton taxa in the Arctic belong to flagellated groups including Chrysophyta, Cryptophyta, Haptophyta (Prymnesiophyta), and Chlorophyta (Stoecker and Lavrentyev 2018). Among chrysophytes, the genera *Ochromonas* and *Dinobryon* are common in the Arctic. Both are known to be mixotrophic (Estep et al. 1986; Andersson et al. 1989; Keller et al. 1994; McKenzie et al. 1995), a mode of metabolism that is thought to be important in the transfer of biomass up trophic levels due to photosynthesis compensating for respiratory loss (Ward and Follows 2016). *Dinobryon* forms colonies important to particle flux (Olli et al. 2002; Stoecker and Lavrentyev 2018). Cryptophytes have rarely been identified down to genera and species (Stoecker and Lavrentyev 2018), however some have been identified in the southeastern Bering Sea (Olson and Strom 2002). *Teleaulax amphioxeia* is one of the few species identified and was confirmed as mixotrophic (Yoo et al. 2017), highlighting the need for more research into this important group of picophytoplankton. The most common haptophyte in the Arctic is *Phaeocystis* (Stoecker and Lavrentyev 2018). These colonial picophytoplankton can form massive blooms, spurring seasonal production and affecting marine carbon cycling (Smith et al. 1991). Chlorophytes are becoming increasingly recognized as important in the Arctic, especially the genus *Micromonas*. Once thought to be comprised of a single species, the genus *Micromonas* is now known to contain greater diversity (Simon et al. 2017). Laboratory experiments have also found high rates of bacterivory by *Micromonas* under oligotrophic conditions similar to those found in polar seas in the summer (McKie-Krisberg and Sanders 2014).

This study aims to establish a robust baseline of phytoplankton community composition at the molecular level, in the midst of changing sub-Arctic and Arctic environments, with a focus on diatom and picophytoplankton communities. This study is the first molecular analysis to cover such a large area over the Bering and Chukchi seas and includes hundreds of samples collected over the course of three spring and summer months (June, August, and September). This unique dataset provides an opportunity to describe the seasonal variations in diversity and geographical distributions of phytoplankton communities in the Chukchi and Bering Seas using metabarcoding. To describe latitudinal changes within these communities over the Bering and Chukchi seas, we selected sites within the Distributed Biological Observatory (DBO), an established set of monitoring stations designed to study biodiversity and productivity shifts in response to global

climate change (Grebmeier et al. 2010; Grebmeier 2012). Phytoplankton size, biomass, and composition are core standardized ship-based sampling parameters of the DBO; our study adds genetic analyses for taxonomic identification of phytoplankton over a large sampling gradient and time period. We collected samples from transects DBO1, DBO2, DBO3, DBO4, and DBO5 in June, August, and September 2017, providing insights into seasonal community shifts of phytoplankton at the molecular level that are not captured by microscopy and pigment analyses. Additional time series studies in this region will be necessary to assess other predicted changes in sub-Arctic and Arctic phytoplankton communities.

Equally important to identifying the key taxonomic groups in the Bering and Chukchi seas is determining the environmental drivers that shape these communities. This knowledge enables better predictions of community response to climate change in the Bering and Chukchi seas, and for the Arctic at large. Over the Bering and Chukchi shelves, multiple water masses converging over a shallow shelf system create a dynamic environment that may influence these communities, providing opportunities to observe how environmental drivers shape them. Community structure analysis provides insights into the diversity and potential resilience of the community to climate change: higher taxonomic diversity in ecosystems tends to buffer communities from environmental changes (Needham et al. 2017). This magnifies the need to identify not only the dominant groups, but diversity at the genus level.

Our study is the first synoptic, high-throughput molecular phylogenetic investigation of phytoplankton diversity in the Bering and Chukchi seas based on hundreds of samples collected from June to September in 2017 (Figure 1). We use this unique opportunity to describe the seasonal diversity and geographic distributions of phytoplankton communities in the Chukchi and Bering Seas using metabarcoding. We focus primarily on diatoms and picophytoplankton, both prominent primary producer groups in this region. We also highlight the diversity within these groups and explore the environmental and biological drivers of phytoplankton community structure. These results will help in determining how sub-Arctic and Arctic microbial communities might respond to changes in their environment resulting from anthropogenic global warming.

MATERIALS AND METHODS

Sampling Sites

Seawater samples were collected during June, August, and September 2017. June sampling took place aboard *RV Sikuliaq* as part of the Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments (ASGARD) project. August sampling took place aboard *RV Norseman II* as part of the Arctic Marine Biodiversity Observing Network (AMBON). September sampling took place aboard *USCGC Healy* as part of the DBO-Northern Chukchi Integrated Study (DBO-NCIS). ASGARD covered transects in the northern Bering Sea, across the Bering Strait, and into the southern Chukchi Sea, AMBON covered study sites in the southern and northern Chukchi Sea, and DBO-NCIS covered sites in the Chukchi Sea (Figure 1). The DBO3 line was visited on each of the three cruises, the DBO4 line was visited by the DBO-NCIS and AMBON cruises, and lines DBO1 (DBO-NCIS), DBO2 (ASGARD), and DBO5 (DBO-NCIS) were visited on one cruise each.

Seawater was generally collected from a subset of standard sampling depths (e.g. surface, 10, 20, 30, 40, 50, 75, 100, 125, 150, 250, 500, 1000, 2000, 3000 m) and, if present, from oceanographic features (chlorophyll maxima, pycnocline, thermocline, halocline, etc.). A median of 3 depths were sampled per station, and the modal depths per station were 5 m, 20 m, and 40 m. Seawater was collected using Niskin bottles on a rosette with an attached Sea-Bird CTD (Sea-Bird Electronics Inc., Bellevue, WA, USA).

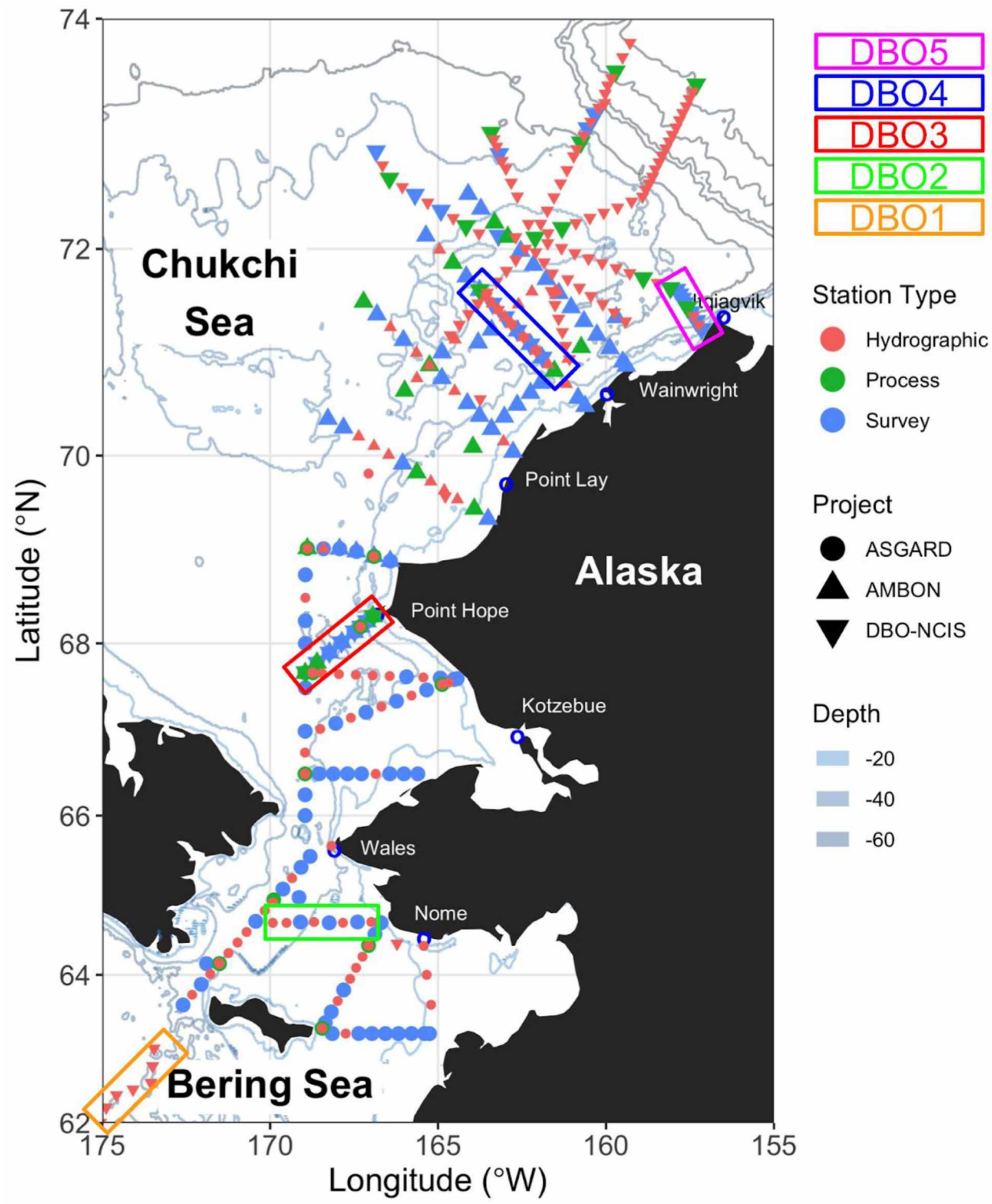


Figure 1: Map of sampling sites.

Nutrients and Chlorophyll

For each cruise, nutrients were collected immediately after recovering the CTD with 60 mL syringes, filtered through 0.45 μm Nuclepore filters, and kept frozen below −20 °C until processed colorimetrically by autoanalyser post-cruise (Gordon, Jennings and Krest 1993). ASGARD nutrient samples were collected June 9–28, 2017, with concentrations of nitrate, nitrite, phosphate, and silicate provided (S. Danielson, University of Alaska Fairbanks, unpublished data). AMBON nutrient samples were collected August 7–22, 2017 (L. Cooper, University of Maryland, unpublished data). DBO-NCIS nutrient samples were collected from August 28 to September 13, 2017 (C. Mordy, University of Washington, unpublished data).

Microbe and Particle Filtration

Seawater was drained from Niskin bottles into 20 L Cubitainers and stored at 4 °C until filtration using a peristaltic pump within six hours of collection. No pre-filter was used to exclude macroscopic plankton. A single 1–5 L seawater sample per depth was filtered directly onto 0.2 μm-pore size Sterivex cartridge filters to collect microbes at "Survey" stations. Seawater from selected "Process" stations was sequentially filtered based on size, first through a 47 mm-diameter 20 μm-pore size nylon net filter, then a 47 mm-diameter 3 μm-pore size membrane filter, and finally through a 0.2 μm-pore size Sterivex cartridge filter (all filters from Millipore Sigma, Burlington, MA, USA). The 20 μm and 3 μm filters were folded cells-in using forceps cleaned with ethanol and then placed in 2 mL microcentrifuge tubes with approximately 1 mL of RNAlater (Life Technologies Corporation, Carlsbad, CA, USA). Approximately 1 mL of RNAlater was injected directly into Sterivex filters prior to sealing. Filters were stored in freezers (below −20 °C) until lab processing and sequencing.

CHN and SPM Analyses

Precombusted 25 mm-diameter Whatman GF/F filters were used to collect particulate matter for carbon, hydrogen, nitrogen (CHN) analysis and suspended particulate matter (SPM), following established methods (Knap et al. 1996; Neukermans et al. 2016). For both CHN and SPM samples, 500 to 1000 mL of seawater was filtered from each target depth. After filtration, CHN and SPM filters were rinsed with fresh Milli-Q water to remove salts, dried at 60 °C for 12 hours, and stored in petri dishes until analysis. CHN filters were acidified with 10% hydrochloric

acid for 6 hours to remove inorganic carbon and then exposed to a standard high temperature combustion technique to determine levels of carbon, hydrogen, and nitrogen in each sample at the Alaska Stable Isotope Facility at University of Alaska Fairbanks' (UAF) Water and Environmental Research Center. SPM filters were massed and the original weight of the filter was subtracted and divided by the volume of seawater filtered to obtain in situ concentrations.

DNA Sequencing

DNA was extracted from filters using the DNeasy PowerWater kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, with the exception that prior to extraction, RNALater was expelled from the thawed Sterivex filter cartridge and the filter was rinsed with 1 mL ultrapure water. PCR-amplification of 18S rRNA genes was used for analysis of phytoplankton communities. The KAPA HiFi HotStart PCR Kit (Kapa Biosystems, Wilmington, MA, USA) was used for PCR-amplification of 18S rRNA genes. Thermocycling parameters were: one cycle at 98 °C for 1-min, 26 cycles at 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and one cycle at 72 °C for five minutes. Primers used in PCR to target the eukaryotic V4 hypervariable region were TAReuk454FWD1 5'-CCAGCASCYGCGGTAATTCC-3' and TAReukREV3_modified 5'-ACTTTCGTTCTTGATYRATGA-3' (Stoeck et al. 2010). Amplified DNA was dual-indexed using unique adapters (Glenn et al. 2016) before TruSeq library preparation and sequencing on an Illumina MiSeq in the UAF DNA Core Lab.

Table 1: Sample sizes and sequencing depth across each project.

		ASGARD	**AMBON**	**DBO-NCIS**
Cruise Dates (2017)		**June 9–28**	**Aug. 7–22**	**Aug. 28–Sept. 13**
Stations Sampled		74	69	46
Serial filtration samples	**20 µm**	34	63	96
	3 µm	33	68	96
	0.2 µm	34	24	66
Direct filtration samples	**0.2 µm**	206	203	129
Quality-filtered samples		240	338	117
Quality-filtered reads		13,425,698	18,161,396	6,379,108
Mean quality-filtered reads per sample		55,940	53,732	54,522

Data Analysis

Oceanographic data was visualized in Ocean Data View (Schlitzer 2016) and in R (R Core Team 2013). Open source scripts used for sequencing and statistical analyses are available at https://github.com/lekanor/thesis. Water masses definitions (Pisareva et al. 2015) were used with the addition of an extra water mass extending from 6 °C to 14 °C, denoted "WACW" here for "Warm Alaska Coastal Water". For some analyses and visualizations, samples were grouped by depth, with all samples taken between 0 and 7 m considered 'surface', all samples taken closest to the seafloor (within 10 m) considered 'bottom', and samples from depths in between denoted 'midwater'.

Bioinformatics and statistical analyses were carried out in R. After sequencing, samples were demultiplexed and primers were removed using cutadapt v2.8 (Martin 2011). Exact sequence variants (ESVs) were called using DADA2 (divisive amplicon denoising algorithm 2), an open source R package (Callahan et al. 2016) that performs quality control, error correction, merging, chimera checks, and taxonomic classification using SILVA database v132 (Quast et al. 2012). Samples with less than 3000 quality-controlled reads were omitted, resulting in the removal of about 12% of samples. For taxonomic group analyses, "diatoms" were defined as all ESVs that were classified to the class Diatomea, and "picophytoplankton" were operationally defined as all ESVs that were classified to the groups Chlorophyta, Haptophyta, or Chrysophyceae. Scripts implementing sequence analysis and visualization are freely available at https://github.com/rec3141/microscape. Scripts to generate the plots and tables found in this manuscript are also available at https://github.com/rec3141/rml_thesis.

Diatom and picophytoplankton relative abundance tables were subjected to fourth-root transformation before clustering (using 'ggplot2', 'gplots', and 'heatmap.plus' packages for R). A table of Bray-Curtis dissimilarities was calculated from the transformed relative abundance matrix, and samples were hierarchically clustered using Ward's minimum variance method.

Canonical Correspondence Analysis (CCA) finds response variables that are maximally related to linear combinations of the explanatory variables provided. In this study we used taxa relative abundances as response variables and the following metadata and environmental parameters as explanatory variables: day of year, depth, bottom depth, distance to shore, latitude, longitude, temperature, salinity, fluorescence, and dissolved oxygen. These parameters were chosen because they can be obtained in situ via shipboard data streams and during a CTD cast.

Using the R package 'vegan' (Oksanen et al. 2019), CCA was performed across taxonomic subsets of the relative abundance tables. In addition to ESVs, diatom and picophytoplankton communities were analyzed after aggregation to the taxonomic levels of genus and family to explore patterns of community composition at higher taxonomic levels.

RESULTS

Environmental Conditions

During the ASGARD expedition in June 2017, sampled surface water temperatures ranged from 1.1 °C to 10.9 °C, with the warmest water consistently above 6 °C appearing south of Nome (Figure 2). Beneath the surface, sampled water temperatures ranged from −1.4 °C to 7.3 °C. Water masses present at the time of sampling included Alaskan Coastal Water (ACW), Bering Shelf Water (BSW), and Remnant Winter Water (RWW; Figure 3). Concentrations of chlorophyll a were highest during June, with an average across sampled stations of 2.8 mg m^{-3}; surface waters averaged 2.8 mg m^{-3}, midwater depths 3.8 mg m^{-3}, and bottom depths 1.7 mg m^{-3}. The highest concentration observed was 26.2 mg m^{-3} at 3 m depth at station DBO3.6.

During the AMBON cruise in August, sampled surface seawater temperatures ranged from 3.8 °C to 10.1 °C, with subsurface temperatures ranging from −0.2 °C to 9.9 °C (Figure 2). Water masses encountered were the ACW, BSW, and RWW (Figure 3). Concentrations of chlorophyll a were lower during August sampling, averaging 1.4 mg m^{-3} across all samples and depths, 1.1 mg m^{-3} at the surface, 1.6 mg m^{-3} at midwater depths, and 1.2 mg m^{-3} at bottom depths. The highest concentration observed was 7.9 mg m^{-3} at 32 m depth at station DBO4.6.

During the DBO-NCIS cruise in late August–early September, sampled surface seawater temperatures ranged from 1.8 °C to 7.5 °C, with subsurface temperatures ranging from −1.7 °C to 8.6 °C (Figure 2). Water masses encountered included ACW, Atlantic Water (AW), ACW, BSW, Melt water/river water (MWR), RWW, and Winter water (WW; Figure 3). Across the Chukchi shelf, strong winds from the east drove upwelling through Barrow Canyon and even reversed the ACW, detectable at lines DBO3, DBO4, and DBO5. Concentrations of chlorophyll a were the lowest of the three cruises, averaging 0.8 mg m^{-3} across all samples and depths; mean values of 1.1 mg m^{-3} were observed at the surface, 0.7 mg m^{-3} at midwater depths, and 0.9 mg m^{-3} at bottom depths. The highest concentration observed was 5.8 mg m^{-3} at 33 m depth at station W-4.

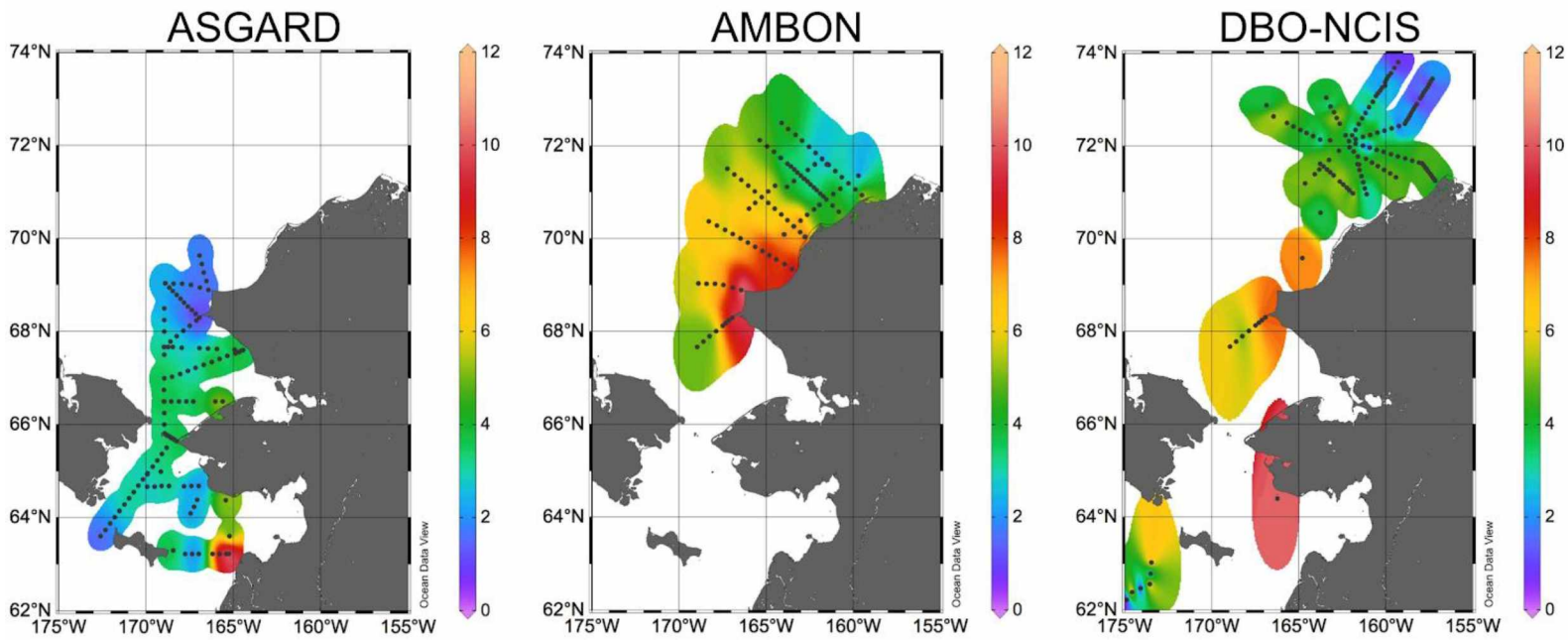


Figure 2: Sea surface temperature (°C) at each sampling site for each cruise in the northern Bering and Chukchi seas during the spring and summer of 2017. Values were gridded using weighted-average gridding in ODV. From left to right: ASGARD (June 9–29), AMBON (August 7–22), and DBO-NCIS (August 28 to September 13).

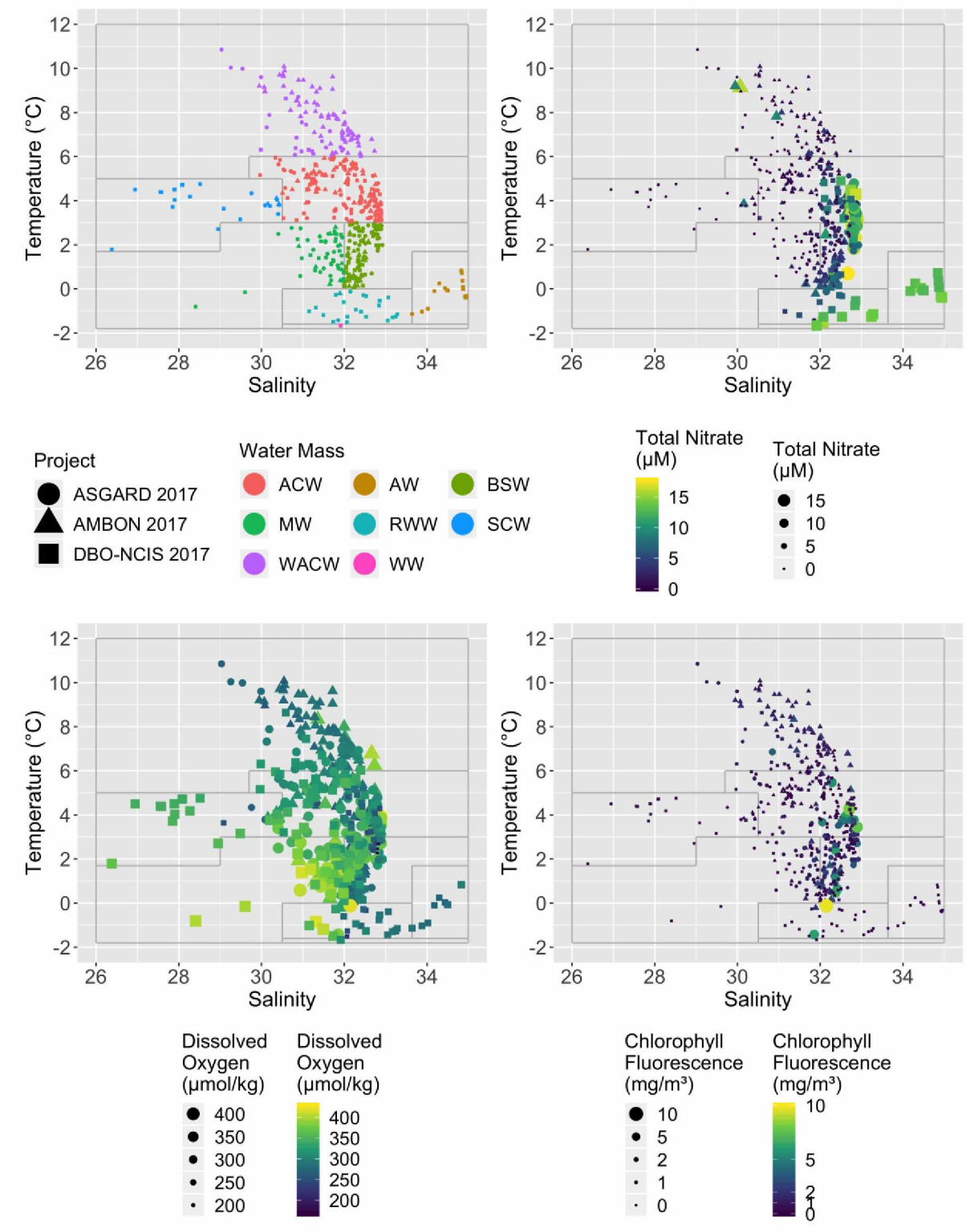


Figure 3: Temperature-Salinity plots of water sampled in the northern Bering and Chukchi seas during the 2017 cruises overlaid with environmental metadata: (A: upper left) water mass designations; (B: upper right) total nitrate (µM; NO_3^{2-} + NO_2^-); (C: lower left) dissolved oxygen (µmol/kg); (D: lower right) chlorophyll fluorescence (mg m^{-3}; from the CTD fluorometer).

Taxonomic Diversity

Overall Structure

Using high-throughput DNA sequencing, 201 unique diatom ESVs (taxa) and 227 unique picophytoplankton taxa were identified in the study region. Diatoms and the phytoflagellates that fell within the picophytoplankton classification used here are known to be important primary producers in the sub-Arctic and Arctic, so the current analysis will focus on these groups. Other photosynthetic taxa and all heterotrophic taxa were thus excluded from the current analysis, but future studies should consider these taxa in their own right, and as proxies for top-down processes like grazing, to better understand the factors that control community diversity.

Eukaryotic microbial communities in the Bering Strait and Chukchi Seas during the open water season of 2017 were assigned (bootstrap support > 60%) to 35 Phyla and 58 Classes. The 7 most abundant Phyla (as classified using SILVA) made up 93% of the relative sequence abundance across all samples: Ochrophyta (39.8%, primarily Diatoms), Dinoflagellata (24.6%, mostly mixotrophic taxa), Ciliophora (8.0%), Protalveolata (6.4%, primarily Syndiniales), Chlorophyta (5.2%, primarily *Mamiella*les), Chytridiomycota (4.8%), and Prymnesiophyceae (4.4%). Overall, the groups we defined as "picophytoplankton" made up 10.0% of the relative sequence abundance across all samples.

Despite an opportunistic sampling scheme that resulted in spatiotemporal heterogeneity of sampling sites and little direct overlap among cruises, the dominance of diatoms was particularly consistent over the course of the season, with mean relative abundances of 36.0 ± 0.1% across cruises. Chlorophyta also remained fairly consistent over the summer at 4.8 ± 0.7% across cruises. Prymnesiophyceae (i.e. Haptophytes) reached maximal relative abundances in June (8.2%) but were nearly absent in September (0.5%). The dominant mixotrophs and heterotrophs (Dinoflagellata and Ciliophora, respectively) reached maximal relative abundances in August (27.6% and 11.3%, respectively). Among parasites, *Sydiniales* represented 1.0% of the mean relative abundance in June and 9.6% in September (with high spatial variability), while the Chytridiomycota exhibited the opposite pattern with maximal relative abundances in June (6.5%) and minimum in September (1.1%).

Size Structure

Picophytoplankton, generally defined as single-celled eukaryotes that are less than 3 μm in diameter, were operationally defined here as all taxa that were classified to the taxonomic groups Chlorophyta (green algae), Haptophyta, or Chrysophyceae (golden algae). While size does not necessarily correlate with taxonomy, we found that these flagellated cells generally fell into the expected size range, with the prominent exception of *Micromonas* ESV 20, which was found frequently on 20 μm filters (Figure 4). This abundant organism may have fallen prey to larger protists that were captured on the large filter. Some picophytoplankton taxa are known to be able to form colonies (e.g. *Phaeocystis*), but these taxa were quite rare on 20 μm filters, suggesting they were free-living (or easily disaggregated) in the study region. The remainder of this manuscript will focus only on results from the "Survey" stations, which were not size-fractionated prior to filtration onto 0.2 μm filters.

Diversity within Genera

A significant positive correlation was observed between genus relative abundance and ESV richness for diatoms ($N=25$, $p<<0.001$, Spearman correlation) but not for picophytoplankton ($N=16$, $p>0.05$, Spearman correlation). In diatoms, the genera with the highest relative abundances (*Chaetoceros*, *Thalassiosira*) also had the highest diversity at the ESV level (43 and 44 ESVs, respectively).

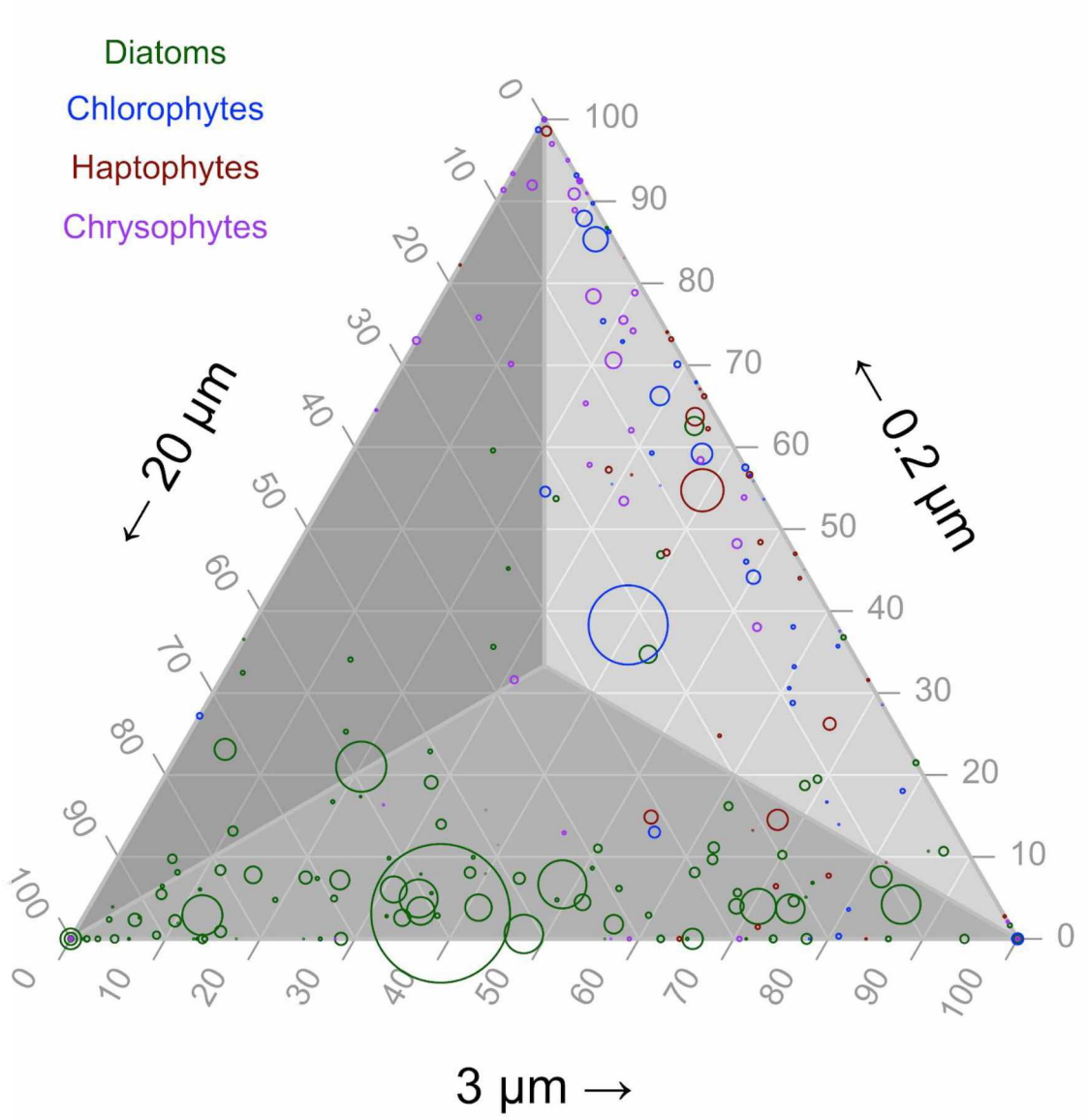


Figure 4: The proportion of each exact sequence variant (ESV) within each phytoplankton size fraction for the northern Bering and Chukchi seas during 2017. The ESVs in the lightest grey triangle were found most commonly on 0.2 µm and 3 µm filters, the ESVs in the lower triangle were found most commonly on 3 µm and 20 µm filters, and the ESVs in the darker triangle were found most commonly on 0.2 µm and 20 µm filters. Point area is scaled to the mean relative abundance of each ESV.

Diatoms

Diatom taxa were classified to 5 families, 25 genera, and 201 unique ESVs, which were used as molecular proxies for species (or lower) level taxonomy (Table 2). The most prominent family was Mediophyceae (88% diatom relative abundance) with the other families contributing from <1% to 6% of the diatom relative abundance. Multiple genera represented the families Mediophyceae (9), Bacillariophyceae (9), and *Fragilaria*les (3), while only 2 genera each were found within the Melosirids and Rhizosolenids (Table 2). The distribution of diatom proportions was highly skewed, with only 14 ESVs making up 80%, 26 ESVs making up 90%, and 99 ESVs making up 99% of the cumulative relative abundance (Figure 5).

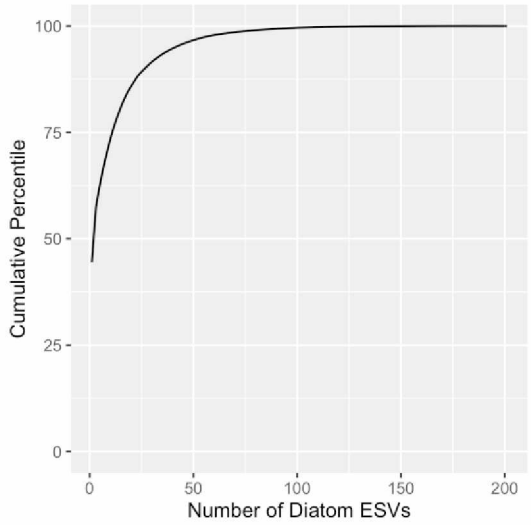


Figure 5: Cumulative percentile of diatom ESVs across the northern Bering and Chukchi seas cruises during 2017.

Among diatoms, 90% of the relative abundance was contributed by the most abundant 26 ESVs, including genera that are well known from the Pacific Arctic like *Chaetoceros*, *Fragilariopsis*, *Navicula*, *Nitzschia*, *Pseudo–nitzschia*, and *Thalassiosira* (Sakshaug 2004; von Quillfeldt 2005).

Chaetoceros was the most common diatom genus across all depth bins (48–71%) followed by *Thalassiosira* (15–26%). Other genera ranged from <1–6% at all depths. Some prominent diatom ESVs were putatively identified to species using best BLAST hits, including ESV 2 (*Chaetoceros socialis* complex) and ESV 19 (*Chaetoceros diadema*).

Table 2: Diatom taxonomy list for the northern Bering and Chukchi seas cruises during 2017 using a taxonomic bootstrap cutoff of 60%.

Family	Genus	Relative Abundance (%)	ESVs	Mean bootstrap support
Mediophyceae	*Chaetoceros*	61.7	43	96
	Thalassiosira	18.6	44	98
	Unidentified	2.8	12	28
	Lauderia	2.1	1	92
	Arcocellulus	1.9	3	91
	Skeletonema	1.0	4	100
	Attheya	0.3	2	100
	Brockmanniella	0.1	2	100
	Cyclotella	<0.1	1	100
	Eucampia	<0.1	1	100
Bacillariophyceae	Unidentified	1.6	21	46
	Pseudo−nitzschia	1.5	6	94
	Fragilariopsis	1.3	2	88
	Cylindrotheca	0.5	7	93
	Nitzschia	0.3	6	82
	Navicula	0.2	4	96
	Pleurosigma	0.1	3	75
	Entomoneis	0.1	1	95
	Asterionellopsis	<0.1	2	100
	Amphora	<0.1	1	100
Rhizosolenids	*Rhizosolenia*	0.3	2	92
	Guinardia	0.2	3	95
	Unidentified	0.1	2	45
Fragilariales	*Thalassionema*	<0.1	2	100
	Fragilaria	<0.1	1	60
	Synedropsis	<0.1	1	73
Melosirids	*Melosira*	<0.1	1	100
	Stephanopyxis	<0.1	1	100
Unidentified Diatoms	Unidentified	5.4	22	15

Picophytoplankton

Picophytoplankton taxa were classified to 3 kingdoms and 16 genera, with 227 unique ESVs (Table 3). Multiple genera represented the kingdoms Chloroplastida (8) and Haptophyta (7), while only 1 was identified in the Stramenopiles. The most prominent ESVs (Table 3) were within the kingdom Chloroplastida (52% picophytoplankton relative abundance), followed by Haptophyta (42%), and Stramenopiles (6%). The distribution of picophytoplankton proportions was highly skewed, with only 9 ESVs making up 80% of the total, 23 ESVs making up 90%, and 99 ESVs making up 99% of the cumulative relative abundance (Figure 6).

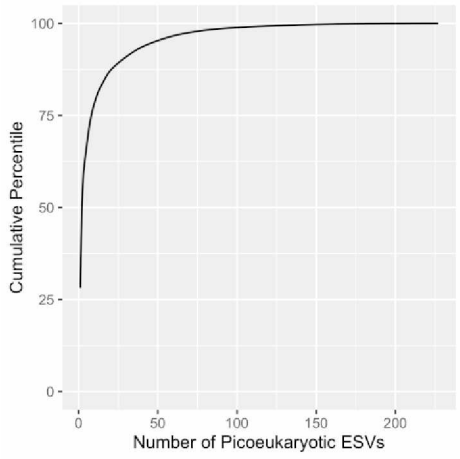


Figure 6: Cumulative percentile of picophytoplankton ESVs across the northern Bering and Chukchi seas cruises during 2017.

Among the picophytoplankton, 90% of the total relative abundance was contributed by the most abundant 23 ESVs, including well-known genera like *Bathycoccus*, *Micromonas*, *Mamiella*, *Chrysochromulina*, *Phaeocystis*, and Nannochloris (Lovejoy et al. 2006; McKie-Krisberg and Sanders 2014).

Micromonas was the most common genus in the picophytoplankton assemblage, comprising 36% of the picophytoplankton sequences at the surface, 36% in midwater depths, and 24% at the bottom. *Phaeocystis* was the second most common genus with 20% relative abundance at the surface, 19% at midwater depths, and 38% at the bottom, followed by *Chrysochromulina* which comprised 11%, 10%, and 8% of picophytoplankton sequences in the surface, midwater, and bottom, respectively. All other identified genera varied from <1 to 3% across all depths.

Prominent picophytoplankton ESVs that were putatively identified to species level using best BLAST hits included ESV 20 (*Micromonas pusilla*), ESV 31 (*Phaeocystis pouchetii*), and ESV 188 (*Bathycoccus prasinos*).

While many of these top ESVs for diatoms and picoeukaryotes alike were easily identified, numerous others did not have good matches to sequences in the database used for taxonomic identification. Future studies should work to identify these sequences using cultured representatives that might also be identified in older studies based on morphological data.

Table 3: Diatom taxonomy list for the northern Bering and Chukchi seas cruises during 2017 using a taxonomic bootstrap cutoff of 60%.

Kingdom	**Genus**	**Relative Abundance (%)**	**ESVs**	**Mean bootstrap support**
Stramenopiles	Unidentified	6.5	54	59
	Paraphysomonas	0.8	9	100
Chloroplastida	*Micromonas*	33.5	6	99
	Unidentified	14.4	34	64
	Prasinoderma	1.2	6	97
	Pyramimonas	0.8	2	94
	Mamiella	0.4	7	85
	Pterosperma	0.3	12	82
	Dolichomastix	0.2	10	87
	Cymbomonas	0.2	1	73
	Nephroselmis	0.1	1	100
Haptophyta	*Phaeocystis*	23.8	8	99
	Chrysochromulina	9.9	26	89
	Unidentified	6.4	32	45
	Prymnesium	0.6	6	87
	Braarudosphaera	0.4	2	100
	Haptolina	0.3	5	95
	Imantonia	0.1	1	100
	OLI16029	<0.1	5	76

Community Clustering

Diatom Assemblages

Samples were classified into seven clusters (D1–D7) based on hierarchical clustering of diatom community composition, with 32–140 samples per cluster (Table 4). The sample assemblages separated into two major clusters, defined primarily by the relative abundance of *Chaetoceros* ESV 2, which was singularly dominant in clusters D1 and D2, while of varying importance in D3–D7 (Figure 7). Of the clusters with abundant *Chaetoceros* ESV 2, its relative abundance increased from D4 (16%) to D6 (23%) to D7 (31%) to D2 (58%) to D1 (96%). Cluster D3 was highly diverse, with no single dominant ESV. Compared to other clusters, D3 had relatively high proportions of ESV 56 (8%) and *Skeletonema* ESV 336 (4%).

Cluster D6 was the only cluster to have a majority of *Thalassiosira*, dominated by ESV 85 (27%), ESV 453 (12%), and ESV 389 (8%). Cluster D2 had a higher proportion of *Thalassiosira* ESV 104 (16%) compared to any other cluster. Cluster D4 was distinguished by higher concentrations of ESV 323 (12%; tentatively assigned to *Nitzschia*) and *Chaetoceros* ESV 27 (12%) than any other cluster. Cluster D5 was characterized by high proportions of *Chaetoceros* ESV 19 (27%), which was common throughout the study region, and ESV 246 (20%; tentatively assigned to *Helicotheca*), and ESV 56 (5%; tentatively assigned to *Guinardia*), which were not.

Table 4: Hierarchical clustering of samples based on diatom community composition (D1-D7) from the northern Bering and Chukchi seas cruises during 2017. Numbers of samples per cluster are shown for each cruise and depth bin. Within each cruise and depth bin, the most frequent cluster is shown in bold.

Project	Depth Bin	D1	D2	D3	D4	D5	D6	D7
ASGARD	surface	2	2	3	12	0	7	**32**
ASGARD	midwater	3	3	1	10	0	16	**32**
ASGARD	bottom	0	1	3	10	0	9	**34**
AMBON	surface	1	1	**33**	1	14	0	1
AMBON	midwater	3	4	**66**	6	18	0	17
AMBON	bottom	1	5	**22**	0	1	0	13
DBO-NCIS	surface	**11**	10	1	0	0	0	1
DBO-NCIS	midwater	47	**55**	6	2	0	0	5
DBO-NCIS	bottom	**22**	1	1	0	0	0	5

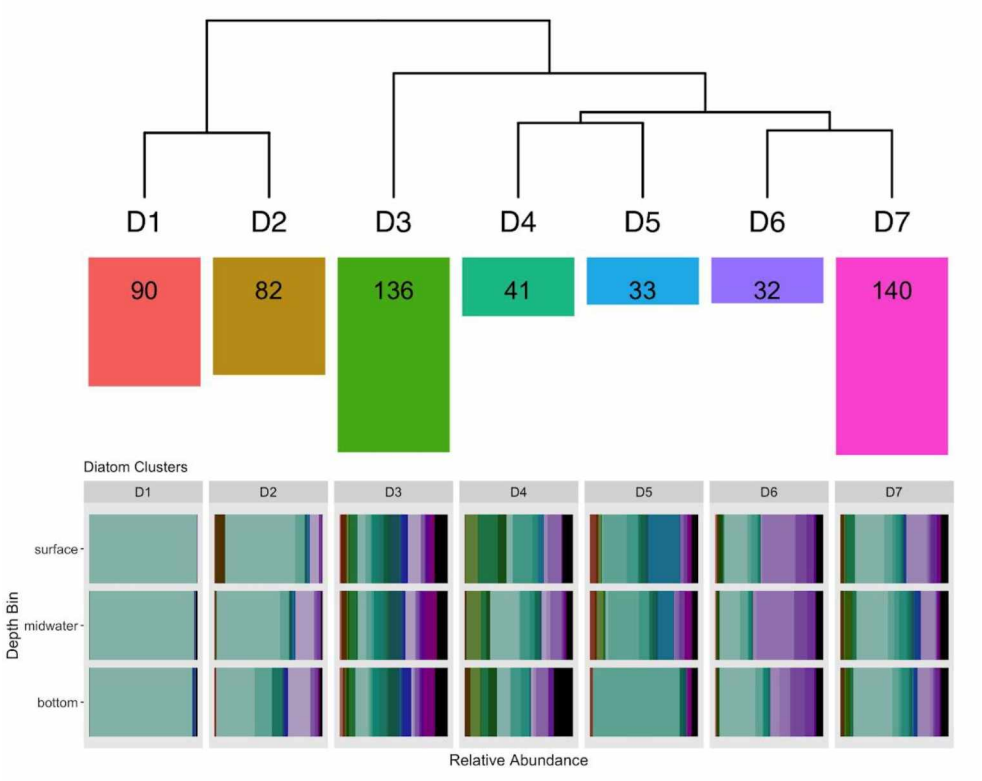
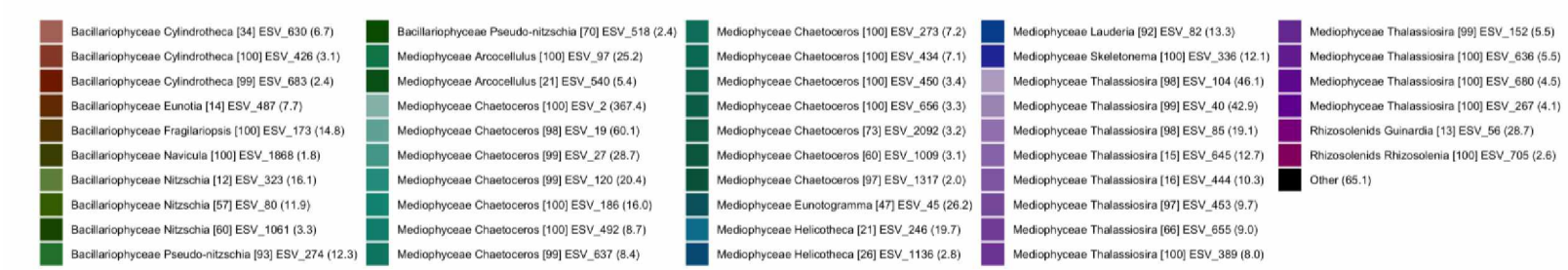


Figure 7: Hierarchical clustering of samples based on diatom community composition from the northern Bering and Chukchi seas cruises during 2017. (a) The dendrogram shows the relationships among the clusters (D1—D7) and number of samples classified to each cluster. (b) The barplots show the relative abundance of the top 90% most abundant ESVs in each cluster. (c) The color legend shows the taxonomic identification of each ESV, with numbers in [brackets] indicating taxonomic bootstrap support for the genus label, and numbers in (parentheses) indicating the mean relative abundance of that ESV across all samples in parts per thousand.



Each cluster was composed of samples originating from a range of environmental conditions, with D3 standing out as having a particularly high median temperature (7 °C), D1 having relatively high nutrient concentrations, and D4 and D5 having relatively low nutrient concentrations (Figure 8). Cluster D3 was found in the largest range of environmental conditions, from –1 to 11 °C and from salinities of 25 to 35. Cluster D6 had the lowest temperature range, from 2 °C to 5 °C.

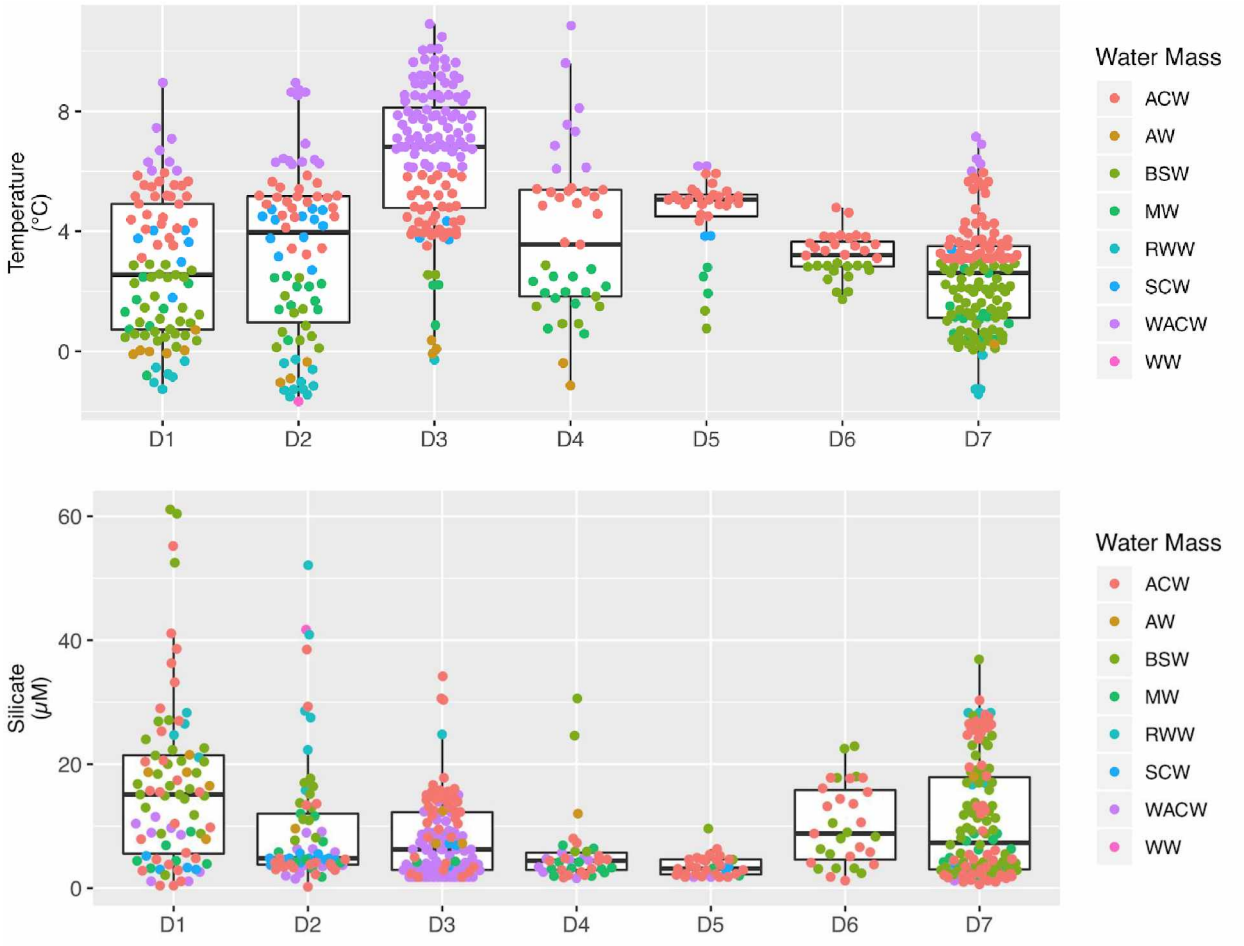


Figure 8: Distribution of temperature (above) and silicate concentrations (below) from samples in each diatom assemblage cluster (D1–D7), colored by water mass for the northern Bering and Chukchi seas cruises during 2017.

Diatom assemblage spatiotemporal distributions

June

D7 was the most frequently observed cluster during the ASGARD cruise (54% of samples; Table 4; Figure 9), followed by D4 and D6 (18% each). D7 was prominent throughout the Bering Strait region, with the exception of the DBO3 line off the coast of Point Hope, AK, where *Thalassiosira*-rich cluster D6 was common. A surface bloom was apparent at the western-most station of that transect, indicated by elevated chlorophyll fluorescence (>12 mg m^{-3}). D7 was again common along the northernmost transect sampled during June (Figure 9), where fluorescence values were lower (<3 mg m^{-3}).

D4 and D6 occurred overwhelmingly in June (87% of all occurrences) and were mostly confined to the Bering Strait region (Figure 9), where chlorophyll fluorescence values were relatively high; they appeared proportionally across surface, midwater, and bottom depths (Figure 9). D4 appeared in coastal zones both north and south of the Bering Strait, whereas D6 appeared only in a patch in the central channel offshore of Point Hope (Table 4; Figure 7; Figure 9).

August

During the AMBON cruise in August, D3 was the most common cluster, occurring 58% of the time (Table 4), followed by D5 (16%), and D7 (15%). Cluster D5 was observed exclusively during the AMBON cruise, primarily in surface waters of an offshore transect crossing Hanna Shoal (Figure 9). Cluster D7 was still common, but found almost exclusively at midwater and bottom depths, potentially indicating a sinking *Chaetoceros* bloom (Table 4; Figure 7; Figure 9).

August-September

During the DBO-NCIS cruise in late August and September, clusters D1 (48%) and D2 (40%) were most prominent. Both the overall diversity and chlorophyll fluorescence values were lower during this cruise, with most samples dominated by *Chaetoceros* ESV 2 (Table 4; Figure 7; Figure 9).

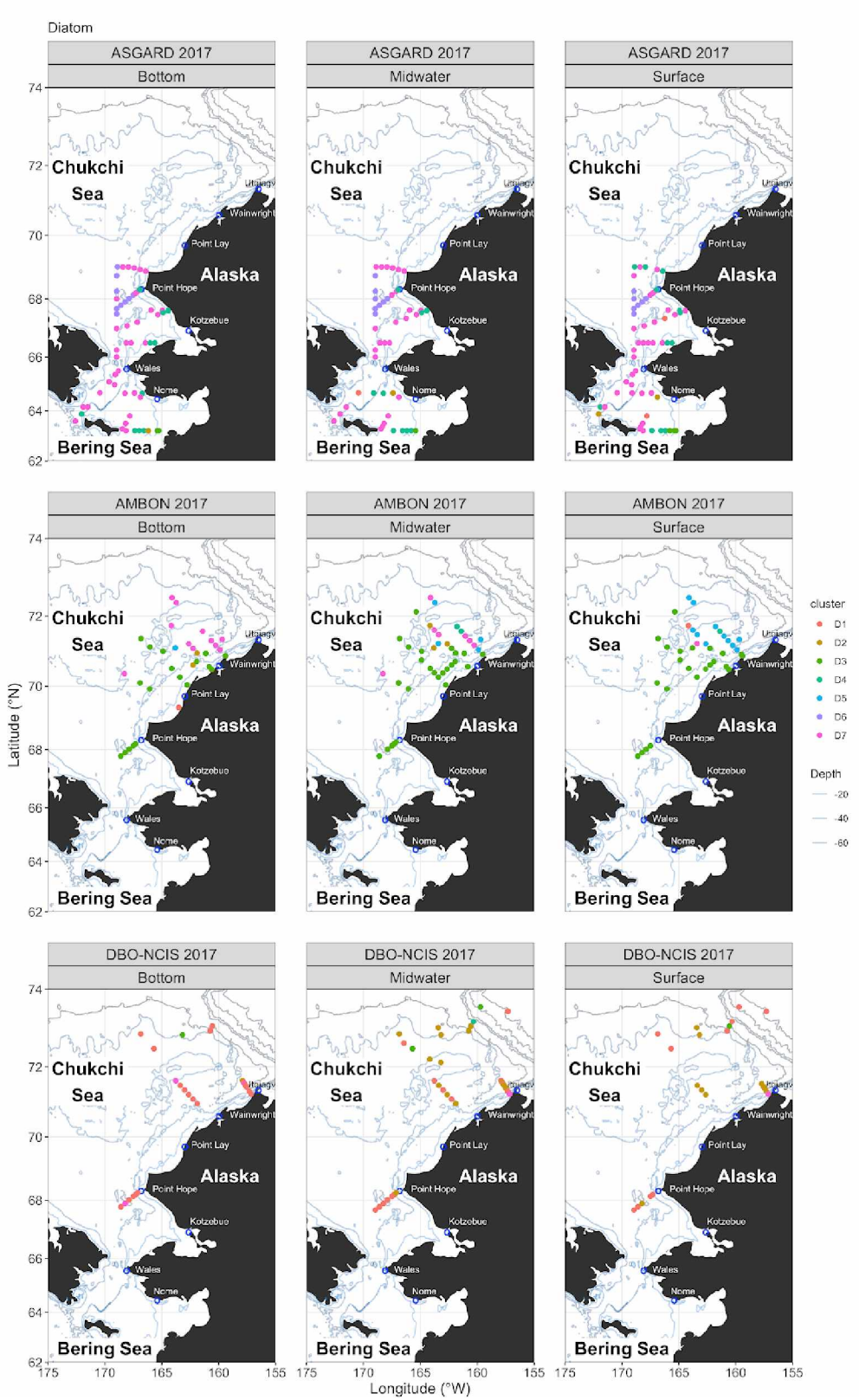


Figure 9: Spatiotemporal trends in diatom community clusters from the northern Bering and Chukchi seas cruises during 2017.

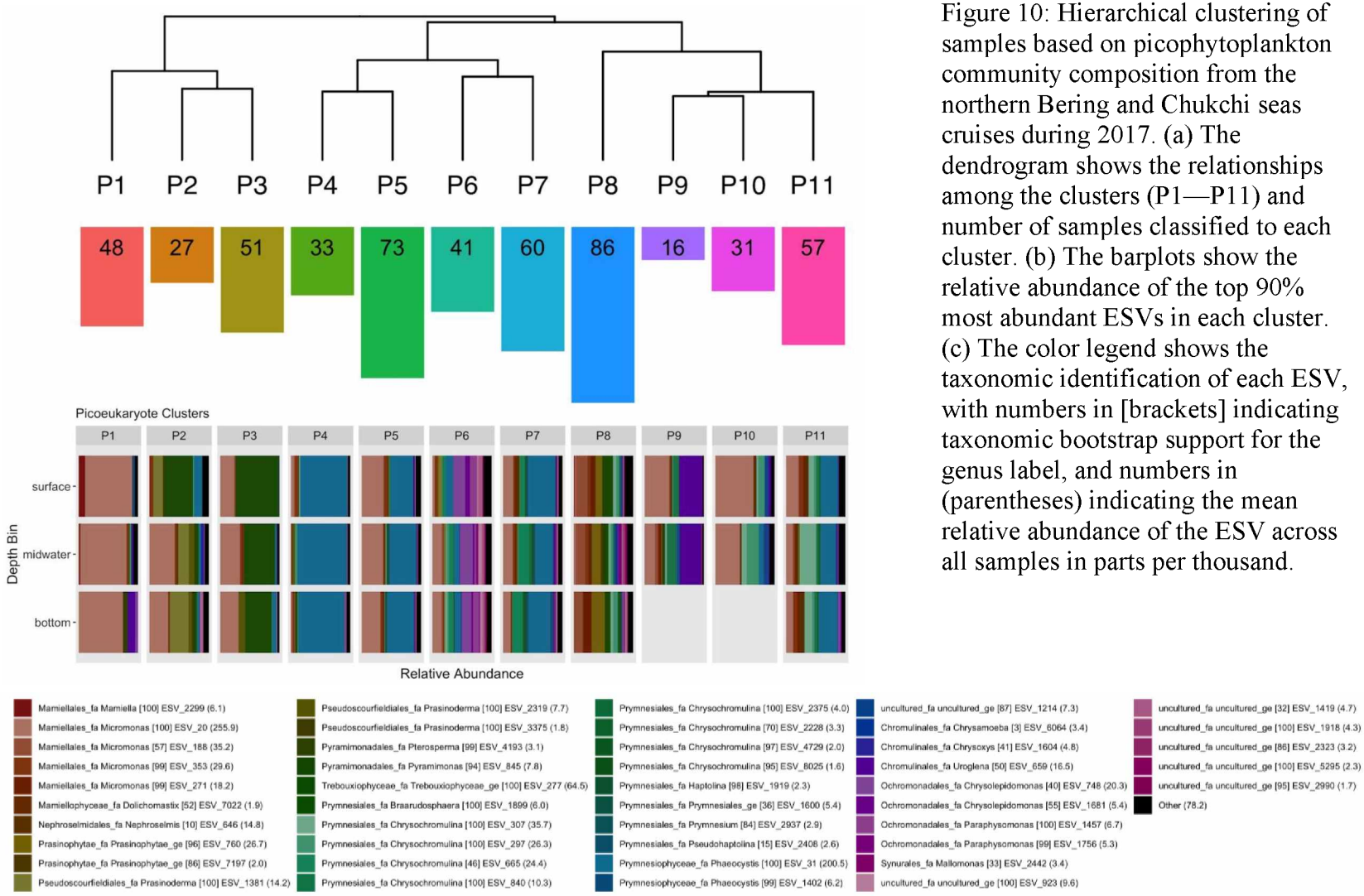


Figure 10: Hierarchical clustering of samples based on picophytoplankton community composition from the northern Bering and Chukchi seas cruises during 2017. (a) The dendrogram shows the relationships among the clusters (P1—P11) and number of samples classified to each cluster. (b) The barplots show the relative abundance of the top 90% most abundant ESVs in each cluster. (c) The color legend shows the taxonomic identification of each ESV, with numbers in [brackets] indicating taxonomic bootstrap support for the genus label, and numbers in (parentheses) indicating the mean relative abundance of the ESV across all samples in parts per thousand.

Picophytoplankton Assemblages

Samples were classified into eleven clusters (P1–P11) based on hierarchical clustering of picophytoplankton community composition, with 16–86 samples per cluster (Table 5, Fig 10). The sample clusters split first between P1–P3 and P4–P11, followed by a second major split between P4–P7 and P8–P11; these divisions roughly reflect the seasonality/cruise schedule.

Clusters P1 and P4 were both characterized by very low diversity (Figure 10), dominated by *Micromonas* ESV 20 (78%) and *Phaeocystis* ESV 31 (81%), respectively. *Micromonas* ESV 20 also made up major portions of the picophytoplankton diversity in P2 (36%), P3 (32%), P5 (35%), P6 (15%), P7 (15%), P9 (29%), P10 (49%), and P11 (14%). *Phaeocystis* ESV 31 was also an important contributor to P5 (43%), P7 (37%), P10 (6%), and P11 (28%).

Other taxa that contributed to distinguishing clusters include *Bathycoccus* ESV 188 (P8: 17%), *Nannochloris* ESV 277 (P3: 54%), *Chrysochromulina* ESV 297 (P10: 17%), *Chrysochromulina* ESV 307 (P11: 18%), ESV 659 (P9: 37%; possibly *Uroglena*), ESV 665 (P7: 15%; possibly *Chrysochromulina*), ESV 748 (P6: 19%; possibly *Chrysolepidomonas*), ESV 760 (P8: 15%; unidentified), *Chrysochromulina* ESV 840 (P9: 12%), and *Prasinoderma* ESV 1381 (P2: 23%).

Table 5: Hierarchical clustering of samples based on picophytoplankton community composition (P1-P11) for the northern Bering and Chukchi seas cruises during 2017. Numbers of samples per cluster are shown for each cruise and depth bin. Within each cruise and depth bin, the most frequent cluster is shown in bold.

Project	Depth Bin	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
ASGARD	surface	0	0	0	4	16	0	**17**	5	0	0	**17**
ASGARD	midwater	1	0	0	11	**23**	0	17	0	0	0	12
ASGARD	bottom	0	0	0	6	**18**	1	17	3	0	0	13
AMBON	surface	2	0	0	0	0	2	0	**29**	7	10	0
AMBON	midwater	3	2	1	2	2	18	5	**36**	7	20	11
AMBON	bottom	1	0	2	1	2	**19**	3	10	0	0	3
DBO-NCIS	surface	6	1	**7**	2	2	0	0	0	1	0	0
DBO-NCIS	midwater	34	13	**37**	5	7	0	1	3	1	1	1
DBO-NCIS	bottom	1	**11**	4	2	3	1	0	0	0	0	0

Each cluster was composed of samples originating from a range of environmental conditions, with P8 standing out as having a particularly high median temperature, P2 and P6 for having relatively high nutrient concentrations, and P8 and P9 for having relatively low nutrient concentrations.

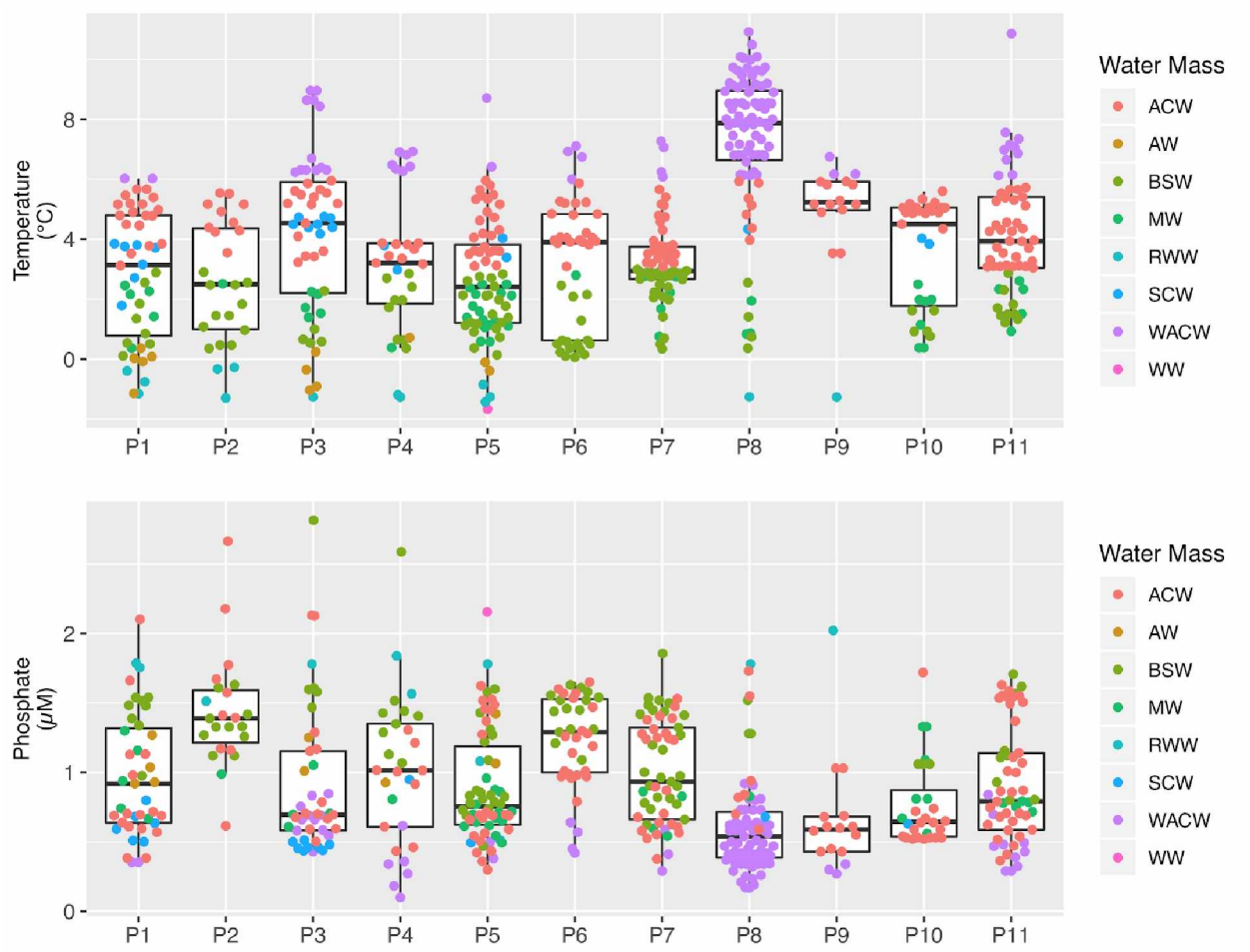


Figure 11: Distribution of temperature (*above*) and phosphate concentrations (*below*) from samples in each picophytoplankton assemblage cluster (P1–P11), colored by water mass, from the northern Bering and Chukchi seas cruises during 2017.

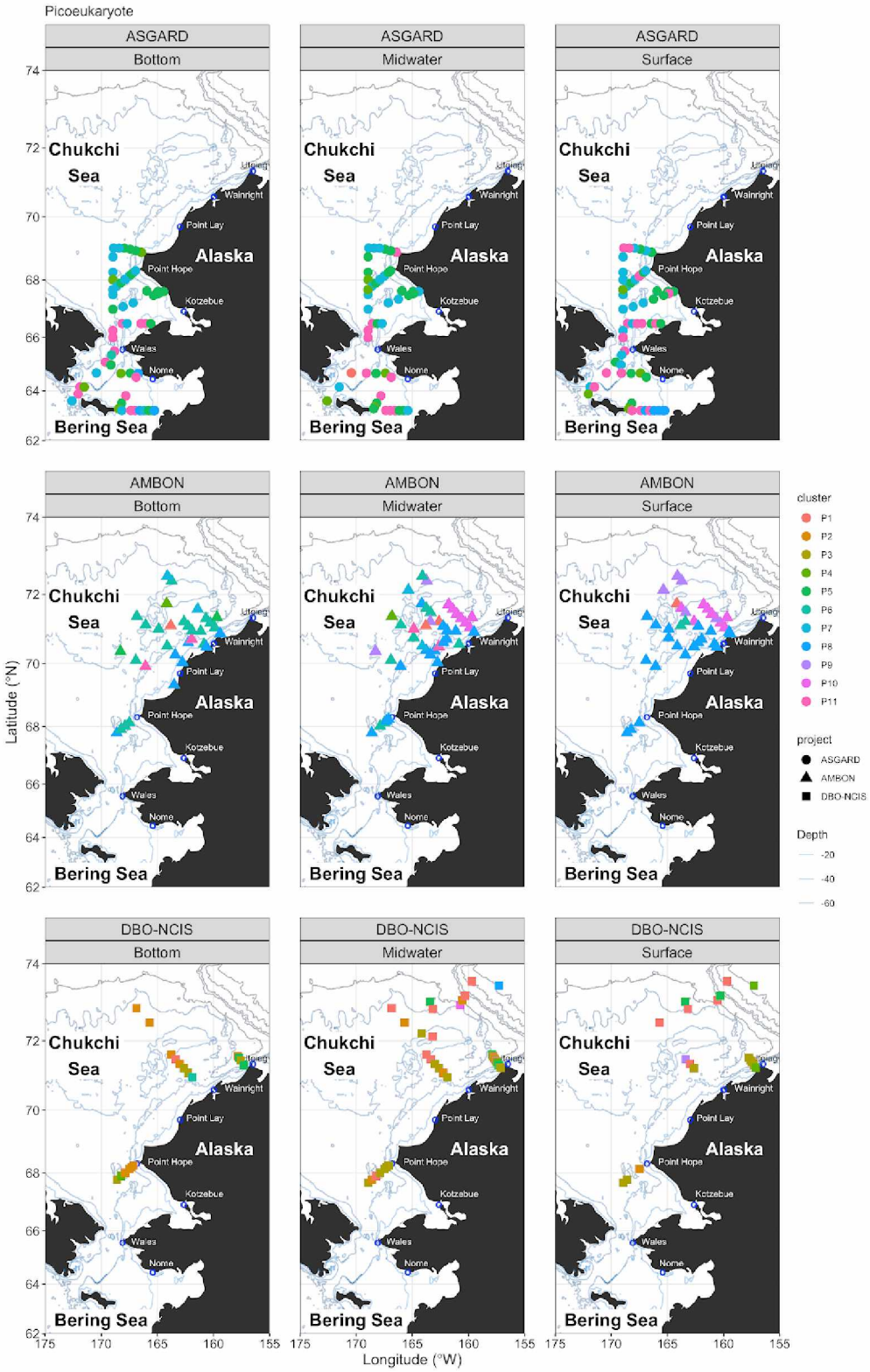


Figure 12: Spatiotemporal trends in picophytoplankton community clusters for the northern Bering and Chukchi seas cruises during 2017.

Picophytoplankton assemblage spatiotemporal distributions

The three primary splits in the picophytoplankton assemblage clustering roughly reflected the seasonality of the cruise that the samples were collected on (Table 5), with P1–P3 composed primarily of samples from DBO-NCIS (90%), P4–P7 mostly from ASGARD (63%), and P8–P11 mostly from AMBON. However, P6 was an exception to this trend (95% collected on AMBON), as was P11 (74% collected on ASGARD).

June

During the ASGARD cruise in June, *Phaeocystis*-rich clusters P4, P5, P7, and P11 occurred 94% of the time (Table 5). Cluster P11 was prominent throughout the Bering Strait, while P4 and P7 appeared more frequently at the edges of P11 along the central channel, and P5 appeared more often closer to the Alaskan coast (Figure 12).

August

During the AMBON cruise in August, picophytoplankton communities were *Phaeocystis*-poor (Table 5), with diverse clusters P8 (38%) and P6 (20%) becoming common (Figure 11), along with *Micromonas*-rich P10 (15%). Clusters P8 and P10 were widely distributed across the Chukchi Shelf, and about 10 times as common at surface and midwater depths than at the bottom (Table 5; Figure 11; Figure 12). Cluster P10 was particularly prevalent in the channel between Wainwright and Hanna Shoal, and was not found at the warm, fresh coastal sites, which were primarily affiliated to P8. While cluster P6 was common across the central Chukchi Shelf, it was never observed near shore, and was found at midwater and bottom depths 94% of the time (Table 5; Figure 11; Figure 12).

August-September

During the DBO-NCIS cruise in late August and September, the *Phaeocystis*-rich clusters were again absent, as were the diverse clusters P8 and P10 (Table 5). In their place were clusters P1 (28%, rich with *Micromonas* ESV 20), P3 (33%, rich with *Nannochloris* ESV 277), and P2 (17%), a mixture of P1 and P2 with the addition of *Prasinoderma* ESV 1381 (Table 5; Figure 11; Figure 12).

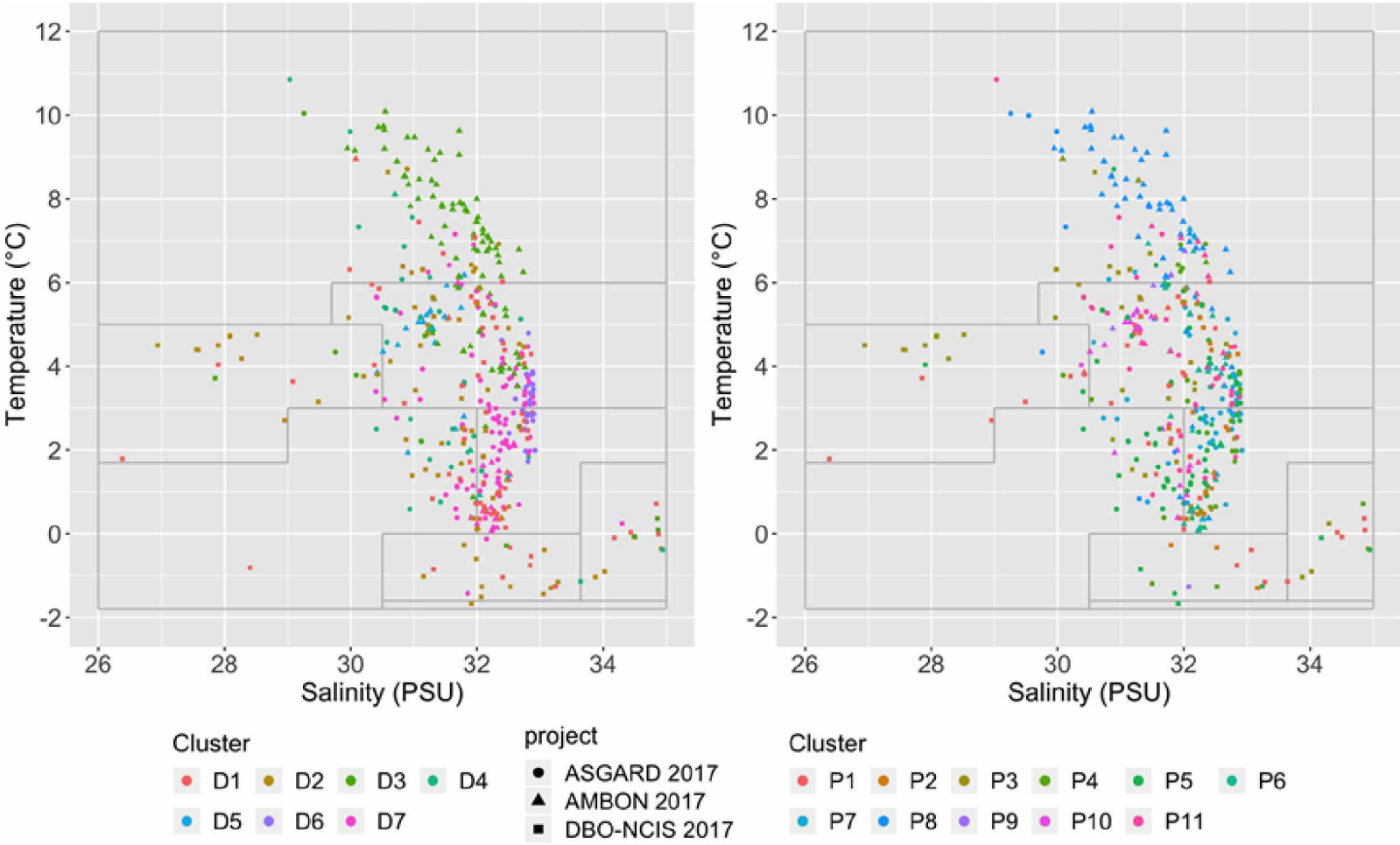


Figure 13: Diatom (*left*) and picophytoplankton (*right*) sample clusters overlaid on temperature-salinity plots for the northern Bering and Chukchi seas cruises during 2017.

Environmental Drivers of Community Variability

One of the future goals of this research is to predict microbial communities in Arctic seawater in real time while aboard the ship. To test our current ability to make these predictions, we restricted the analysis of environmental drivers to those parameters that are readily available at sea, i.e. those available during a CTD cast: day of year, depth, bottom depth, distance to shore, latitude, longitude, temperature, salinity, fluorescence, and dissolved oxygen.

Aggregation by Taxonomy

In general, diatom and picophytoplankton relative abundances were not easily predictable based on metadata or environmental variables available at the time of sampling (Figure 13). After aggregating at the ESV, Genus, and Family levels, no linear combination of environmental parameters explained more than 12.6% of variability within diatom communities or 19.7% in picophytoplankton (Table 6).

Table 6: Proportion of constrained variability captured by selected environmental variables for the northern Bering and Chukchi seas cruises during 2017.

	Diatoms	**Picophytoplankton**
Family	11.6%	19.7%
Genus	12.6%	14.2%
ESV	12.5%	7.4%
Cluster	43.6%	32.5%

For diatom ESVs, the first two CCA axes contributed 47% of the inertia (Figure 14), driven primarily by day of year and salinity, respectively. At the genus level, the first two axes contributed 61% of the inertia, driven primarily by temperature and day of year, respectively. At the family level, the first two axes contributed 99% of the inertia, driven primarily by temperature and distance to shore, respectively.

For picophytoplankton ESVs, the first two CCA axes contributed 44% of the inertia (Figure 14), driven primarily by day of year and temperature, respectively. At the genus level, the first two axes contributed 59% of the inertia, driven primarily by day of year and dissolved oxygen, respectively. At the family level, the first two axes contributed 68% of the inertia, driven primarily by day of year and distance to shore, respectively.

Analysis of variance (ANOVA) was used to determine the significance of each environmental parameter in explaining the variability in relative abundance of taxa (Figure 14). For diatoms, the number of significant ($p < 0.05$) parameters decreased from ESV (9/10) to genus (8/10) to family (5/10). For picophytoplankton, 9/10 parameters were significant at each taxonomic level.

Aggregation by Cluster

CCAs were also performed on communities that were aggregated by cluster classifications. To aggregate by sample cluster (D1–D7 in diatoms, P1–P11 in picophytoplankton), the relative abundance of each taxon in each sample was replaced by the mean relative abundance of that taxon in the samples within the sample cluster prior to CCA analysis.

Significant improvements in explaining community variability were detected after aggregating by cluster: 43.6% in diatoms and 32.5% in picophytoplankton (Table 6). For diatom clusters, the first two CCA axes contributed 83% of the inertia, driven primarily by day of year and temperature, respectively. For picophytoplankton clusters, the first two axes contributed 73% of the inertia, also driven primarily by day of year and temperature, respectively. Cluster aggregation resulted in 9/10 significant parameters for both diatoms and picophytoplankton.

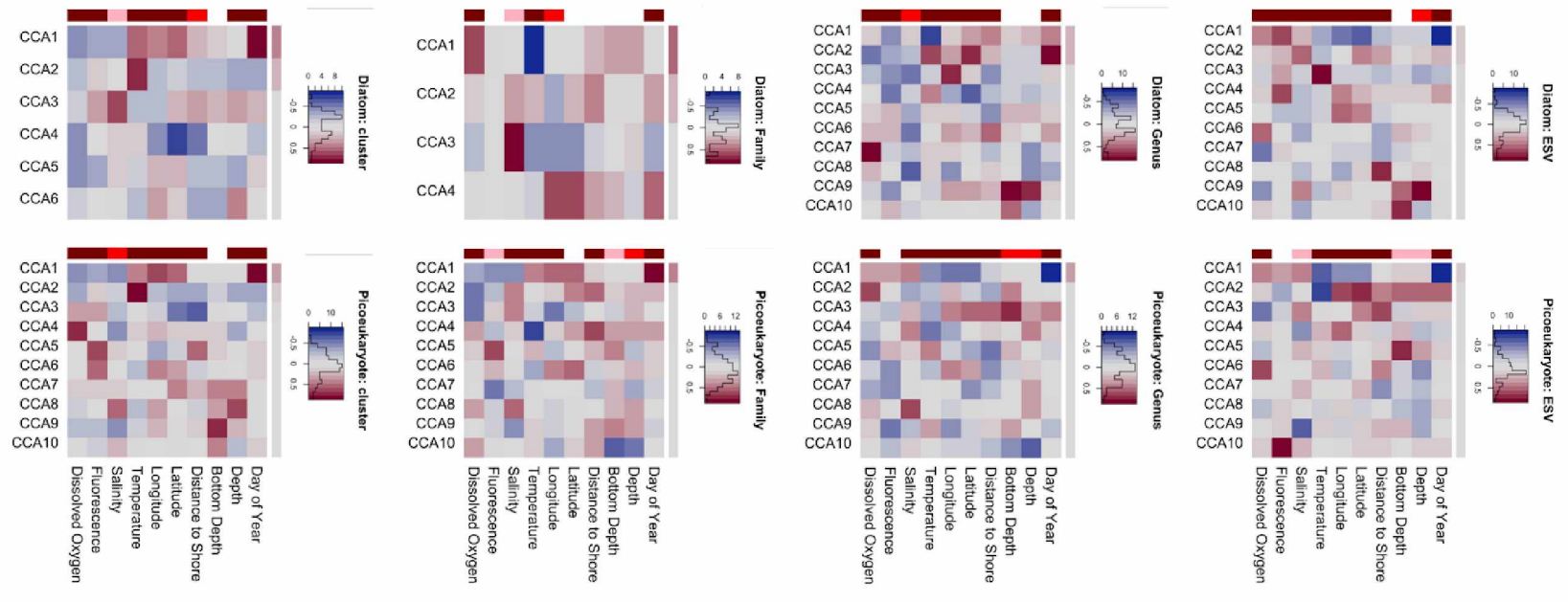


Figure 14: Canonical coefficients of each environmental parameter for each diatom and picophytoplankton constrained canonical axis (CCA) at each aggregation level for the northern Bering and Chukchi seas cruises during 2017. In the top row, the ANOVA p-value is shown, where white is $p > 0.05$, pink is $p < 0.05$, red is $p < 0.01$, and dark red is $p < 0.001$. In the right-most, the proportion of the constrained variability explained by each CCA axis is shown using the color scale in the key.

Temperature Effects on Community Structure

Using CCA, temperature was repeatedly identified as an important factor structuring the microbial communities in our study region. The clustering analyses (Figure 8; Figure 11; Figure 13) also suggested that a shift in community structure may have occurred around 5–7 °C, so we used Student's t-tests to identify breakpoints in ESV relative abundance as a function of temperature (Table 7).

Table 7: Breakpoints in preferred water temperature identified for some abundant ESVs for the northern Bering and Chukchi seas cruises during 2017. The t-tests tested the hypothesis that the relative abundance of an ESV was significantly different (two-sided, alpha = 0.05, Bonferroni-corrected) in samples collected in warm water (> breakpoint) compared to cold water (< breakpoint). For each ESV, the test statistics were computed for every unique temperature in the dataset and breakpoints were identified as local minima of the test p-values, all of which were <<0.0001.

	ESV	**Breakpoint (°C)**	**Prefers warm/cold**
Diatoms	*Chaetoceros* ESV 2	6.7	cold
	Chaetoceros ESV 19	7.0	cold
	Chaetoceros ESV 27	7.0	cold
	Skeletonema ESV 336	5.2	warm
	Pseudo–nitzschia ESV 274	4.5	warm
	Pseudo–nitzschia ESV 518	4.5	cold
	Thalassiosira ESV 104	4.2	warm
Picophytoplankton	*Micromonas* ESV 20	6.2	cold
	Phaeocystis ESV 31	6.9	cold
	Bathycoccus ESV 188	5.9	warm
	Nannochloris ESV 277	3.4	warm

The three most abundant diatoms (all *Chaetoceros*) showed a clear preference for waters colder than 7 °C (Table 7; Figure 15), as did the two most abundant picophytoplankton: *Micromonas* ESV 20 and *Phaeocystis* ESV 31 (Table 7; Figure 16). Among diatoms, about 70% of ESVs had a maximal relative abundance in waters colder than 7 °C (Figure 15); among picophytoplankton, it was over 80% (Figure 16). Some taxa preferred warmer waters, however,

including *Skeletonema* ESV 336 and *Pseudo–nitzschia* ESV 274, as well as *Bathycoccus* ESV 188 and *Nannochloris* ESV 277.

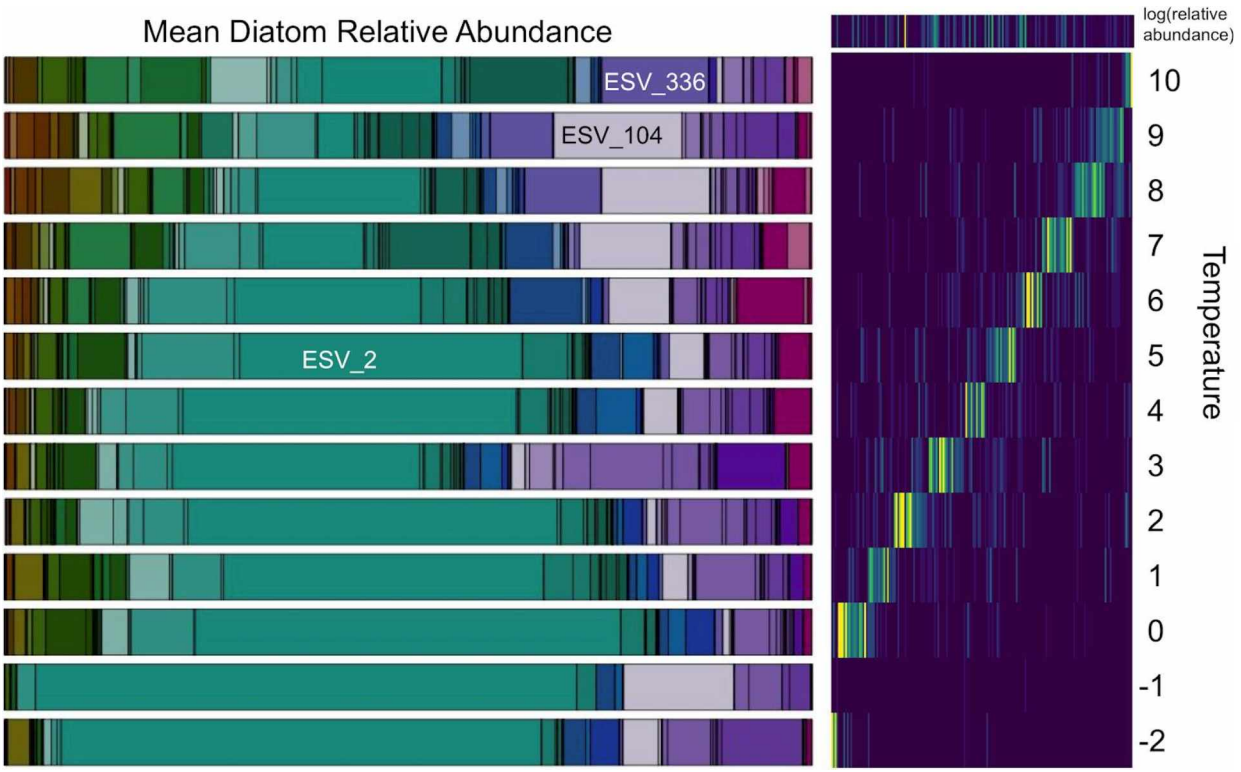


Figure 15: (*left*) Mean diatom ESV relative abundance for the northern Bering and Chukchi seas cruises during 2017 after binning by temperature; ESVs are colored by genus. (*right*) Scaled diatom ESV relative abundances sorted by temperature at maximum relative abundance (each column is an ESV).

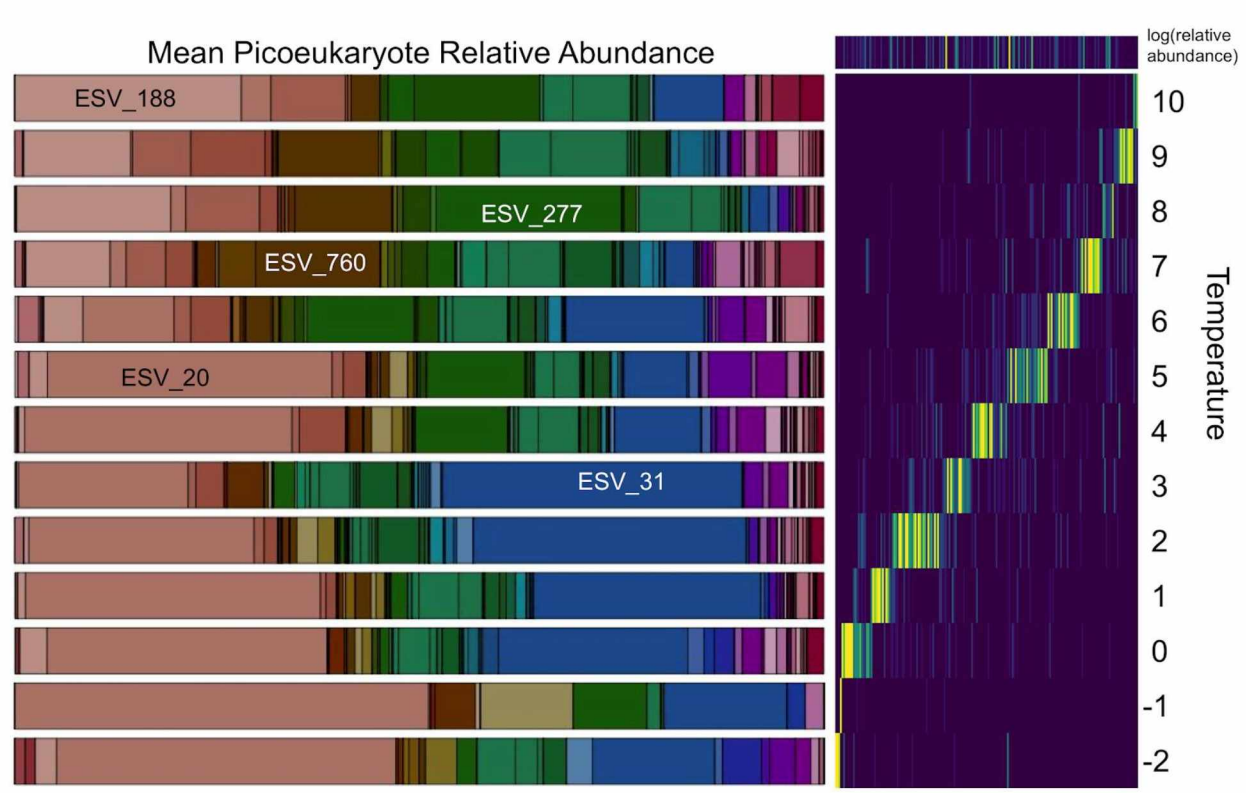


Figure 16: (left) Mean picophytoplankton ESV relative abundance for the northern Bering and Chukchi seas cruises during 2017 after binning by temperature; ESVs are colored by genus. (right) Scaled picophytoplankton ESV relative abundance sorted by temperature at maximum relative abundance (each column is an ESV).

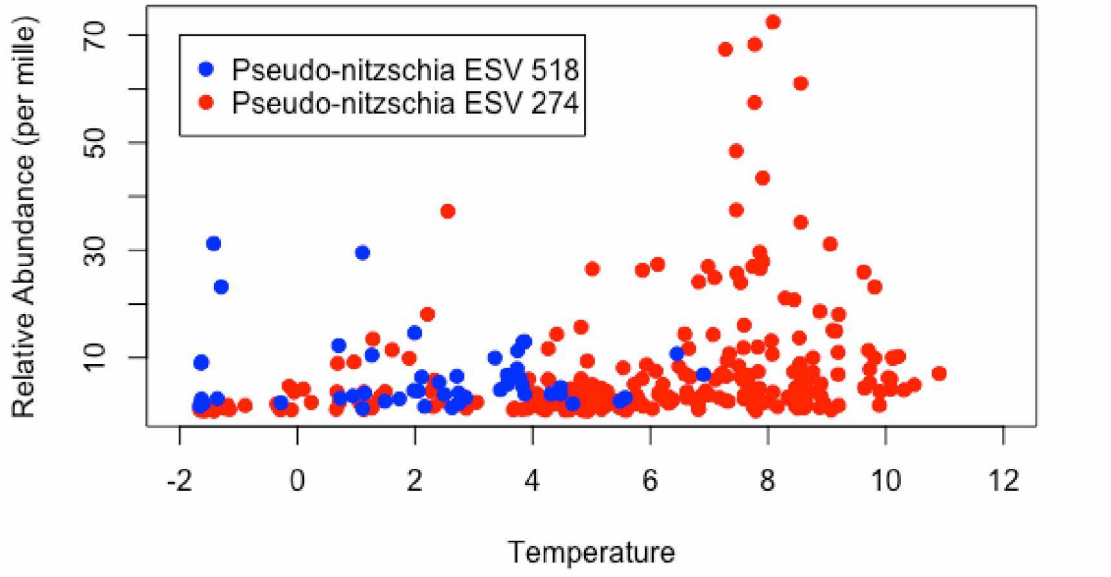


Figure 17: Relative abundance of two diatom ESVs from the HAB-forming genus *Pseudo–nitzschia*, as a function of temperature, for the northern Bering and Chukchi cruises during 2017.

Statistical analyses suggested that overall, environmental parameters measured at the time of sample collection (e.g. temperature, salinity, chlorophyll, dissolved oxygen) were not the primary drivers of phytoplankton community structure at broad spatial and temporal scales. While the composition of individual samples across all cruises were not well characterized using these measured variables, the amount of variability explained (i.e. the quality of predictions) increased somewhat when samples were clustered by community assemblage. Environmental parameters accounted for only 12.5% of variability for diatoms at the ESV level and 7.4% for picophytoplankton, but accounted for 43.6% and 32.5% of the variability across clustered sample assemblages for diatoms and picophytoplankton, respectively (Table 6). This suggests clustering samples based on similarity improves the ability to anticipate community composition from environmental data.

Clustering the samples by community assemblage revealed cases where sample clusters dominated by chain-forming diatoms (e.g. *Chaetoceros* and *Thalassiosira* in clusters D1, D2, and D7) were more prevalent in midwater and bottom depth bins, suggestive of a bloom in the process of sinking as also indicated by low chlorophyll concentrations. Alternatively, these diatoms could be indicative of sustained production due to higher nutrient concentrations found deeper in the water column since the vast majority of sampling depths fell within the euphotic zone.

A similar phenomenon was observed for *Phaeocystis*, another important group in the carbon cycle. After blooming, *Phaeocystis* can aggregate, sink, and carry large quantities of organic carbon to the seafloor, supporting the benthos and potentially sequestering carbon. Two *Phaeocystis*-rich clusters, P4 and P5, were more prevalent at midwater and bottom depth bins, again suggesting a sinking bloom or sustained production at the bottom due to nutrients and suitable conditions at depth.

Similarly, clusters were also useful in identifying temperature preferences for certain taxa, allowing the prediction of certain ESVs that may be more sensitive to a warming Alaskan Arctic. Each of these putatively sinking communities (clusters D1, D2, P4, and P5) was substantially more prevalent in waters colder than 6 °C compared to waters warmer than 6 °C, suggesting that these sinking communities may indeed be negatively affected by warmer waters in the future Arctic.

We found several *Micromonas* ESVs to be prominent members of the picophytoplankton community, though the most abundant, *Micromonas* ESV 20, was most commonly found at temperatures below 6.2 °C, replaced by *Bathycoccus* ESV 188 in warmer coastal waters. Future

studies could investigate the different physiologies of *Micromonas* and *Bathycoccus* to predict potential changes in biogeochemical cycling or primary or secondary productivity in the case that *Bathycoccus* expanded further into the *Micromonas* niche. While this pattern may hold at the large scale, in more localized settings, factors like currents, wind direction, advection of water from off shelf, or upwelling of nutrients may provide stronger indications of community composition due to mixing water masses, or the growth of opportunistic taxa when certain conditions are met, e.g. increased nutrient loads.

DISCUSSION

Drivers of Community Structure

During the ice-free summer of 2017, our results indicate that environmental parameters measured at the time of sampling exerted little influence over phytoplankton community structure. Many studies have debated which parameters are most important to driving community structure (Krug et al. 2013; Sunagawa et al. 2015; Neeley et al. 2018), with a lack of clear consensus. However, our findings consider these communities at the ESV-level (genus, species, and, when possible, subspecies taxonomic resolution), while prior studies characterized to genus and species. Our higher taxonomic resolution allowed us to determine temperature as having more of a measurable effect on certain taxonomic groups, and especially those ESVs that are most prevalent (Table 7). Water mass has previously been attributed to shaping Arctic phytoplankton communities with some water masses observed to hold distinct communities—including diatoms, chlorophytes, and haptophytes—specifically within Pacific Halocline Water (originating through the Arctic Ocean) and deep Atlantic Water (Fehling et al. 2012; Kalenitchenko et al. 2019). The mixing of these distinct water masses, separated by global thermohaline circulation, may explain the high degree of community dissimilarity observed there, whereas in our study many communities originated from closely related water masses flowing northward from the Pacific Ocean and into the Bering and Chukchi seas. However, some mixing of water masses appeared to have an impact on our samples. At midwater and bottom depths at AMBON's furthest northeast transect, an inflow of water through Barrow Canyon, suggested by ADCP and nutrient profiles from the area, may have introduced deep Arctic water onto the shelf, with its own unique community, consistent with other observations of distinct communities between on- and offshore environments (Siemering et al. 2016). Other instances of community introduction via advection of water have been documented with significant differences attributed to hydrography (Hamilton et al. 2008; Kalenitchenko et al. 2019).

While our study did not identify any diatom and picophytoplankton taxa found exclusively in temperate waters, our results confirm temperature shifts are likely to affect the distribution of taxa. Changes in temperature have driven poleward shifts of numerous temperate taxa, including phytoplankton, across the globe (Poloczanska et al. 2013). On the Atlantic side of the Arctic, the fronts of Atlantic water masses have moved further north due to sea temperature warming, bringing

with it phytoplankton communities characteristic of warmer Atlantic waters (Neukermans et al. 2018). While our study did not find strong correlations with water mass, the local trends observed in combination with previous studies indicate water movement is important to communities on a regional level. Highly different water masses in conjunction with increased water masses have a potential to bring in their own distinct communities, creating conditions more suitable for taxa not traditionally found in the Arctic. A clear understanding of the physical environment is needed to understand the context of changes within communities across all oceans, and future studies could usefully incorporate backtracing of water parcels using models of current flow to better predict the origins of sampled water.

Diversity, Community Resilience, and Potential Impacts of a Warmer Arctic

Paleoecological reconstruction of marine microbial community structure during past periods of climatic change may provide insight into the future of our oceans. At the species level, communities have proven resilient to large changes in environmental conditions (Moritz and Agudo 2013), showing that communities of today may be more resilient to climate change than previously thought. During a mass extinction event of the Late Cretaceous, the fossilized planktonic coccolithophorids, radiolaria, and foraminifera decreased in relative abundance by 73–92% (Thierstein 1982) while diatoms were only reduced by 23%, a success rate attributed to the life-history of diatoms that includes resting spores (Kitchell et al. 1986). At the time our studies were conducted, the Bering and Chukchi seas experienced the warmest temperatures on record. While numerous studies have characterized phytoplankton bloom timing (Kahru et al. 2011; Sigler et al. 2014), there is a lack of taxonomic studies that broadly characterize and identify the diversity of the Bering and Chukchi seas phytoplankton communities. However, the warming in the Arctic is not expected to decline, highlighting the need for continued monitoring. In addition to life-histories, and applicable to more than just diatom assemblages, intra-species diversity is thought to be one of the mechanisms of resilience to changes in environmental conditions in modern biological communities (Tesson et al. 2014; Sjöqvist and Kremp 2016).

Diatom and picophytoplankton assemblages each consisted of over 200 ESVs, all of which contribute to much of the production on the Bering and Chukchi sea shelves using upwelled nutrients to generate new productivity (Ardyna et al. 2011). Our study confirms the higher relative abundance of diatoms in the region (36.0 ± 0.1% mean relative abundance across all sampling

seasons), however with the onset of the 'new Arctic' (Overpeck et al. 2005; Carmack et al. 2015), an increase in temperatures and stratification could limit nutrient turnover in the surface waters, causing a shift to picophytoplankton (Li et al. 2009) and regenerated production can be expected (Ardyna et al. 2011). Additionally, diatoms are better adapted to lower light availability, provided nutrients are sufficient (Siemering et al. 2016), another indicator that diatoms as a whole may be negatively affected with less ice coverage throughout the year and with stratification limiting nutrient turnover. For example, particular ESVs of diatoms and picophytoplankton were sensitive to a temperature transition zone observed at around 5–7 °C (Table 7). One of these, *Chaetoceros* ESV 2, was cosmopolitan, found in nearly every sample in the study, across all seasons, temperatures, and salinities. However, this taxon reached its highest relative abundances in waters colder than 6.7 °C, and declined precipitously above that temperature. *Chaetoceros* ESV 2 was most closely related to *Chaetoceros socialis*, a species complex already known to have high intraspecific diversity (Degerlund et al. 2012; Gaonkar et al. 2017). *Chaetoceros* ESV 2 along with *Skeletonema* ESV 336, found almost exclusively in waters warmer than 5.2 °C, consistent with literature descriptions as a genus of temperate, coastal diatoms (Thornton and Thake 1998; Kooistra et al. 2008), and *Chaetoceros* ESV 186 and *Thalassiosira* ESV 104 all stand to be negatively affected by climate change due to increased temperatures. Among the picophytoplankton, *Bathycoccus prasinos* ESV 188 appeared almost exclusively in waters warmer than 5.9 °C, while *Phaeocystis pouchetii* ESV 31 and *Micromonas pusilla* ESV 20 appeared predominantly in waters colder than 6.9 °C and 6.2 °C, respectively. The clear temperature preferences for some of the most relatively abundant taxa in this region presage changes for these communities as the Arctic and sub-Arctic continues to warm.

In addition to a decline in some common diatoms, the Alaskan Arctic could see an increase in taxa related to known harmful algal bloom-forming species (HABs). *Pseudo–nitzschia* is a genus containing HAB-forming diatoms in which about 50% of species are known to produce domoic acid, a neurotoxin that has recently been identified in Alaskan waters (Lefebvre et al. 2016; Huntington et al. 2020). We found two prominent ESVs classified to *Pseudo–nitzschia* in our dataset, with ESV 518 predominant at lower temperatures, and ESV 274 predominant at higher temperatures (Figure 17), though there was no consensus on which species the two ESVs matched. Regardless, presence of a genus known to play a role in HABs is now confirmed, and expected to persist and increase in abundance with warming temperatures (Hallegraeff 2010). The

picophytoplankton *Phaeocystis pouchetii* ESV 31, detected prominently throughout our study region, is also a known toxin producer (Eilertsen and Raa 1995), however it's prominence in the Alaskan Arctic may decrease as temperatures rise (Table 7). Nonetheless, changing conditions in the Arctic are still expected to lead to increases in certain HAB-forming taxonomic groups that stand to affect higher trophic levels (Walsh et al. 2011).

These strong temperature preferences are exhibited by taxonomic groups that, combined, contribute a high proportion of the relative abundance. Climate change has occurred cyclically throughout Earth's history (Sarmiento and Bender 1994), though the current pace of anthropogenically driven climate change is unprecedented (Jeffries et al. 2014). Despite the evidence of diatoms persisting in past climatic events, a rapidly warming Arctic today means change for numerous prevalent ESVs. While other studies have debated between salinity (Lozupone and Knight 2007), nutrients, and temperature having a greater influence on community structure, our study demonstrates that in an ice-free season temperature plays a greater role than other environmental factors. Our study is in agreement with a comprehensive TARA Oceans metagenomic study (Sunagawa et al. 2015) examining prokaryotes and picoeukaryotes across temperate, tropical, and polar latitudes, which also concluded that temperature is one of the primary drivers of marine microbial community structure.

Our identification of key groups with strongly preferred temperatures allows us to confidently state that primary producer communities will change in the face of climate change, with ramifications felt throughout the Bering and Chukchi Sea ecosystem. Specifically, we expect to see a loss of *Chaetoceros*, especially ESV 2 and *Phaeocystis* ESV 31 (Fig. 15, Fig 16). Diatoms with a preference to warmer conditions could still be outcompeted if temperature driven stratification suppresses the upwelling of nutrients to the surface (Cermeño et al. 2012), which diatoms rely on to outcompete other phytoplankton (Litchman 2007). *Chaetoceros* and *Thalassiosira* rely on high nutrient concentrations to bloom, and form large chains that contribute significantly to the carbon cycle. The combination of high nutrient requirements and strong temperature preferences of some taxa (Figure 15) could lead to a decrease in their abundance in the future, and a subsequent increase from other taxa. If the replacement taxa are smaller, solitary diatoms, or picophytoplankton like *Micromonas*, this could have significant effects on the carbon cycle, leading to a reduced flow of particulate carbon to the benthos, that in the present ecosystem

drives the tight benthic-pelagic coupling observed over the Bering and Chukchi shelves (Grebmeier and Barry 1991; Dunton et al. 2005).

In general, we observed a spectrum of generalist to specialist diatom ESVs: some taxa were found to thrive in a range of environmental conditions while some were found only in specific and narrow environmental conditions. The CCA analysis captured only a rough summary of this diversity, making it difficult to distinguish specialist from generalist taxonomic groups, and illustrating that this type of analysis may be more meaningful for picking informative variables to investigate than in finding the root causes of community variability (Thaler and Lovejoy 2014). To better understand how these communities change with environmental conditions, collecting samples from the same area over a long period of time may be more useful than collecting samples at sites just one time during the year. Because ship-based observations are limited in temporal scope, long-term mooring deployments like the Chukchi Ecosystem Observatory (Hauri et al. 2018) will be critical for understanding the seasonal changes in planktonic community structure, and future deployments of the Chukchi Ecosystem Observatory are expected to include sampling of microbial communities for this purpose.

Top-Down and Bottom-Up Controls on Community Structure

Our study focused primarily on environmental variables that are bottom-up controls on primary producers. Top-down controls were not explored in this study, however grazing by heterotrophic protists has been demonstrated to impact phytoplankton community structure. Changes in phytoplankton bloom development has been shown to impact food web structure and top-down and bottom-up control of marine ecosystems in polar waters (Arrigo and van Dijken 2004). The PCR primers used in this study also amplify heterotrophic protist DNA, but their analysis was outside of the scope of this work at this time. Future studies should include them as potential drivers of phytoplankton community composition in this region.

The Arctic is responding to the effects of climate change faster than other regions of the ocean (Zhang 2005), so if environmental parameters are not driving change in these communities, changes in top-down controls like grazer diversity, abundance, and grazing rates are reasonable hypotheses. Besides single celled grazers, metazoans like copepods might also be important top-down drivers of phytoplankton community structure. Copepod diversity and abundance are known to be influenced by water mass and depth (Cooney and Coyle 1982; Kosobokova and Hopcroft

2010; Ashjian et al. 2017), and may be expected to change in the future as a result of global climate change. For example, copepods may experience stress as phytoplankton blooms occur earlier or later in the season in conjunction with early sea-ice retreat and melt. This mismatch in bloom and copepod timing has led to copepods decreasing in size in years with early ice retreat and warm waters (Hunt et al. 2011). Through top-down control, copepods could shape phytoplankton communities by consuming primary producers at different rates: oceanic copepods living off-shelf in the Bering Sea are capable of consuming 20 to 30% of primary production (Cooney and Coyle 1982), and on-shelf have been estimated to consume 26% of primary production per day (Campbell et al. 2016). Oceanic copepods are also advected onto the shelf with currents (Springer et al. 1989), possibly increasing grazing rates. Predation of phytoplankton off-shelf in the Bering Sea may shape what communities are eventually carried onto and established over the Bering shelf as well, considering the limited influence of environmental parameters over diatom and picophytoplankton assemblage structure. Copepods have been observed to increase grazing rates in warmer waters (Coyle et al. 2007). This could lead to a decrease in larger diatoms and be another way in which the carbon pump is weakened in the Arctic, and the benthic community could be negatively impacted.

Top-down and bottom-up controls have long been debated in the field. Results from this study suggest that bottom-up controls may influence diversity in terms of ESVs, but do not appear to drastically reshape communities at the family level and above. We suggest that top-down controls are more likely to drive community structure in terms of broad shifts in diversity. Our results also suggest that top-down and bottom-up controls may oscillate as environmental conditions shift over time and space, a trend demonstrated in another coastal sea (Mozetič et al. 2012). If shifts in community structure are more likely, climate change may not have the detrimental effects predicted, i.e., resilience in diatoms means the efficiency of the biological carbon pump to the seafloor will be maintained. As seawater temperatures continue to rise in the Arctic, some studies have suggested that a shift to more mixotrophic plankton could also occur, altering biogeochemical cycling (Ward and Follows 2016). Common throughout temperate oceans (Hartmann et al. 2012; Flynn et al. 2013), mixotrophs are multifunctional protists that photosynthesize when nutrient concentrations are high, and assume an osmotrophic or phagotrophic lifestyle in nutrient deplete conditions (Ward and Follows 2016). Diatoms, in general, are relatively large and heavy, meaning they sink quickly, raising the efficiency of transfer

of carbon to the seafloor. In contrast, mixotrophic picophytoplankton are much smaller, and expected to increase in abundance in a warmer Arctic due to the advantages that mixotrophy brings in conditions of high resource variability (Mitra et al. 2016; Stoecker et al. 2017b).

Hypothesized changes as a result of increased mixotrophy in the Arctic include increased carbon fixation but decreased vertical carbon flux (Stoecker and Lavrentyev 2018), which would be expected to increase trophic transfer, possibly raising planktonic production at higher trophic levels (Mitra et al. 2014; Ward and Follows 2016). An increase in mixotroph abundance could reduce carbon flux to the benthos, which could have long-lasting repercussions all the way up the food web to humans. Our study observed a relatively high abundance of *Micromonas* ESV 20, which could not be identified to species. *Micromonas* is a prominent mixotrophic picophytoplankton genus also found throughout the world ocean. Recent studies (Lovejoy et al. 2007; McKie-Krisberg and Sanders 2014) have demonstrated that this genus has dispersed widely throughout the Arctic Ocean, and has been observed to be particularly sensitive to temperature (Demory et al. 2018), however the strain found in the Arctic differs from the clades found in other oceans by notably thriving at 6–8 °C due to its adaptations to cold and low-light conditions (Lovejoy et al. 2007). Our study found the peak abundance of *Micromonas* ESV 20 occurred at 6.2 °C and preferred colder waters (Table 7), confirming a cold-adapted ESV. As a mixotroph, *Micromonas* could impact production in the Arctic if it were to displace phytoplankton that rely solely on photosynthesis, or the cold-adapted *Micromonas pusilla* were displaced by the more temperate clade. Combined with its strong temperature preferences and numerous studies from across the global ocean, *Micromonas* is a key genus to watch and monitor in the face of a changing Alaskan Arctic.

Taxonomic Resolution and Environmental Influence

Taxonomic resolution of communities influenced ability to constrain variability. Picophytoplankton families were better constrained by environmental variables, but that variability declined when reanalyzing picophytoplankton genera and then ESVs (Table 6). Diatom variability was consistent across family, genus, and ESV (Table 6). This suggests that communities become less predictable with higher taxonomic resolution, or that high taxonomic resolution is not necessary to capture general trends of communities. The ANOVAs also indicated that certain parameters were more important than others, further indicating that with limited data, community

trends can still be characterized if high resolution taxonomic data is lacking. Other studies have also explored taxonomic resolution, and how much is needed to sufficiently understand communities and their interactions with the environment, with no clear answer emerging as yet (Carneiro et al. 2010; Machado et al. 2015).

Our results suggest that over broad scales (multiple sampling seasons and regional sites), taxonomic genera provide a suitable level to understand general impacts of environmental variables on community structure. However, higher taxonomic resolution is needed to observe more subtle shifts not detectable at higher taxonomic levels (e.g. *Chaetoceros* ESV 2 and different temperature preferences of *Micromonas* within species). Not only is high taxonomic resolution the most useful for studying community changes, it also adds to our understanding of diversity in a rapidly changing ocean environment. When possible, it is best to identify organisms to the highest taxonomic resolution possible, underscored by numerous studies demonstrating the importance of microdiversity (Allison and Martiny 2008; Needham et al. 2017; García-García et al. 2019).

In our study we used primers targeting the V4 hypervariable region of the 18S ribosomal RNA gene, which is often specific enough to identify taxa to species level but often not specific enough to identify intraspecific diversity. Because many cultured representatives of Arctic phytoplankton remain missing from sequence databases, and we did not collect morphological data, we deferred from making strong claims to species identifications and focus instead on ESVs as indicators of taxonomic diversity. Future studies should consider the use of more sensitive primers in order to quantify microdiversity within these communities. Even with these limitations, it was still possible to identify several specific ESVs that stand to be the most affected in the face of a warming Arctic, as well as build a comprehensive taxonomic dataset in conjunction with environmental data across the late spring and summer in the Bering and Chukchi seas. We have clearly demonstrated specific groups that stand to be most affected in a new and warming Alaskan Arctic.

CONCLUSIONS AND FUTURE WORK

Objectives of this study were to explore the spatial and temporal distributions of eukaryotic phytoplankton communities in the Pacific Ocean inflow to the Arctic, covering the Chukchi Sea and Bering Strait regions. This project aimed to determine whether reproducible patterns of occurrence were present within community assemblages of diatoms and picophytoplankton, and the role of environmental conditions in structuring these communities. The sampling coverage attained in this study in both the spatial and temporal domains was much larger than typical studies of its kind, allowing for unique insights into the structuring the microorganisms at the base of the Alaskan Arctic food web.

Overall, the low predictability of community composition based on measured environmental variables suggests that more explanatory variables exist that were not considered in this study. However, our most notable finding is identifying temperature as a driver for certain taxa, especially ones that make up a high proportion of the primary producers (e.g. *Chaetoceros* ESV 2). Other integrative, bottom-up forcing factors that could contribute to phytoplankton community structure, such as historical light availability, cloud cover, weather patterns, stratification levels, mixing, ice extent, and freshwater input (e.g., ice melt, precipitation, rivers, runoff), were beyond the scope of this study, so we cannot comment on their utility in predicting microbial community structure at this time. Future studies should incorporate remote sensing observations or seascape predictions to include these additional parameters as explanatory variables.

Our study also captured the diversity of these communities, all the more important considering diversity is a key buffer in rapid environmental changes (especially as the Arctic warms in response to climate change). Our study identified key genera and ESVs of diatoms and picophytoplankton. While we did identify hundreds of ESVs, more work is needed to determine both abiotic and biotic drivers of community assembly, and more specific genetic studies are needed to delineate microdiversity within the primary producer communities of the Alaskan Arctic. Our work does begin to fill in the gaps, allowing us to contribute more genetic information to existing databases. Monitoring of changes in zooplankton grazer abundance and distribution (e.g. copepods, ciliates, dinoflagellates) will also be important to understand how top-down controls could change and affect the phytoplankton. However, we did reaffirm the importance of temperature in structuring many key members of the diatom and picoeukaryote communities.

Connecting certain taxa with temperature allowed for some insights into the future primary producer community of the Bering and Chukchi seas.

Our study highlights the diversity of primary producers and demonstrates that these communities are driven by a variety of environmental and biological parameters that are difficult to fully quantify. We observed a weaker influence of nutrients, water mass, water depth, and geography on diatom and picophytoplankton communities as a whole compared to other phytoplankton community studies (Sunagawa et al. 2015; Neeley et al. 2018; Kalenitchenko et al. 2019). However, we have demonstrated some key taxonomic groups are sensitive to temperature and these same taxonomic groups influence the structure of the communities. The taxonomic groups' responses to temperature are consistent with predictions of shifts to smaller celled mixotrophs and away from larger celled phytoplankton (Ward and Follows 2016). Since the biological carbon pump in the Arctic is currently driven primarily by the sinking of large (>10 μm) diatom cells and chains, we can expect to see declines in benthic-pelagic coupling and seafloor productivity and higher trophic levels. However, this is also dependent on the resilience provided by biodiversity, especially microdiversity within primary producers, an area which is in need of more study. A shift to small-celled phytoplankton in a freshening Alaskan Arctic could have disruptive implications for primary productivity that supports the seafloor (Li et al. 2009).

Our analyses, conducted on almost a thousand samples collected across four months and three cruises, has established a baseline of microbial communities in the Bering and Chukchi seas and identified prominent taxa that are the most vulnerable to climate change. Already we can see the impacts of climate change on the lowest trophic levels that support the entire Alaskan Arctic ecosystem. Long-term monitoring along our study sites, especially the DBO and Chukchi Environmental Observatory, will be imperative to continue increasing our understanding of photoautotrophs and other microbes in the warming Bering and Chukchi seas.

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