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ORIGINAL PAPER

Comparison of picoeukaryote community structures and their environmental relationships between summer and autumn in the southern Chukchi Sea

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Abstract

Picoeukaryotes constitute an important component of the living biomass of oceanic communities and play major roles in biogeochemical cycles. There are very few studies on picoeukaryotes found in the Chukchi Sea. This work shows the relationship between community distribution and composition of picoeukaryotes residing in water masses and physicochemical factors in the southern Chukchi Sea studied in both midsummer (July) and early autumn (September), 2012. Illumina 18S V4 rDNA metabarcoding were used as the main tool. In July, Mamiellophyceae, Dinophyceae, and Trebouxiophyceae were the main microbial classes, with *Micromonas*, *Prasinoderma*, *Telonema*, *Amoebophrya*, *Bathycoccus*, *Picomonas,* and *Bolidomonas* representing the main genera. In September, Trebouxiophyceae surpassed Dinophyceae and was the second main microbial class, with *Micromonas*, *Prasinoderma*, *Bathycoccus*, *Bolidomonas*, *Telonema*, *Choricystis,* and *Diaphanoeca* representing the main genera. Water mass was the primary factor determining the community composition and diversity of picoeukaryotes. Abundance of *Bathycoccus* was found to be highly correlated with Alaskan Coastal Water and that of *Prasinoderma*, *Bolidomonas,* and *Diaphanoeca* with Bering Seawater. Nitrate and phosphate content of water in midsummer and dissolved oxygen (DO) and temperature in early autumn were the main factors that shaped the abundance of the picoeukaryote community.

Keywords Picophytoplankton · Picozooplankton · Seasonal variations · Water mass indicators · Physicochemical correlation

Abbreviations

ACW	Alaskan Coastal Water
ANOVA	Analysis of variance
BSW	Summer Bering Sea Water
Chl *a*	Chlorophyll *a*
DCA	Detrended correspondence analysis
DO	Dissolved oxygen
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
RDA	Redundancy analysis

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Introduction

The Chukchi Sea is a shallow, wide marginal region of the Arctic Ocean. The average depth of the Chukchi Sea is 50 m, and its length is approximately 1000 km. The Chukchi Sea lies north of the Bering Sea and connects to it via the Bering Strait (Grebmeier et al. 2006). Water from the Pacific Ocean flows into the Arctic Ocean by means of the southern Chukchi Sea, delivering freshwater, nutrients, and Pacific biota into the Arctic Ocean (Woodgate and Aagaard 2005; Woodgate et al. 2005; Grebmeier et al. 2006; Pisareva et al. 2015; Linders et al. 2017). The following four main types of water masses are found in the Chukchi Sea: Alaskan Coastal Water (ACW), Summer Bering Sea Water (BSW), Siberian Coastal Water, and remnant Pacific Winter Water (Pisareva

et al. 2015). The southeastern Chukchi Sea is mainly influenced by BSW and ACW. The former is cold (3–6 °C) with high salinity (> 32), and the latter is warmer with low salinity (Coachman et al. 1975; Woodgate et al. 2005). The Chukchi Sea is one of the most productive oceanic areas in the world (Grebmeier 2012). It also acts as an important carbon sink, especially upon the formation of sea-ice-associated phytoplankton blooms (Lee et al. 2007; Arrigo et al. 2012; Lowry et al. 2014, 2015). The Chukchi Sea is also a hotspot for studying zooplankton (Springer et al. 1989; Questel et al. 2013; Sigler et al. 2017), benthic fauna, pelagic-benthic coupling (Feder et al. 2005; Grebmeier et al. 2006; Piepenburg et al. 2011; Blanchard and Feder 2014), and seabirds and marine mammals (Moore and Laidre 2006; Aerts et al. 2013; Clarke et al. 2013; Gall et al. 2013; Kuletz et al. 2015). Scientists have also examined the structure and biomass of microbial communities in both seawater (Zhang et al. 2012; Thaler 2014; Yun et al. 2014; Pedrós-Alió et al. 2015) and sea ice (Eddie et al. 2010; Poulin et al. 2011; Majaneva et al 2017; Belevich et al. 2017). However, to the best of our knowledge, very few studies (Zhang et al. 2012; Thaler 2014; Pedrós-Alió et al. 2015) have examined picoeukaryotes (< 3 μm) in the Chukchi Sea, and none of them have compared picoeukaryote community compositions in different water masses during different seasons.

Picoeukaryotes are vital to polar marine ecosystems because they are the most abundant photosynthetic plankton found for the greater part of the year (Lovejoy et al. 2007). They are estimated to thrive with increasing temperatures in the Arctic Ocean (Li et al. 2009). Autotrophic and heterotrophic organisms play important roles in the microbial loop, which is particularly important in polar oceans (Whitman et al. 1998). Along with other regions of the Arctic Ocean, the Chukchi Sea is undergoing increase in temperatures and freshwater and a reduction in volumes of sea ice (Steele et al. 2008; Polyakov et al. 2010). These changes drive shifts in the composition of marine species and carbon cycling and affect the structure of the marine ecosystem in the Chukchi Sea (Grebmeier et al. 2010; Grebmeier 2012; Häder et al. 2014). Picoeukaryotes are significantly associated with the circulation of global oceanic waters and are sensitive to changes in both physicochemical factors and water mass. Some species have been found only in certain water masses and can be used as bioindicators of water mass (Hamilton et al. 2008; Zhang et al. 2012, 2016). Hence, it is essential to record the composition of the picoeukaryote community and their diversity in the Chukchi Sea during different seasons. It is also important to understand how picoeukaryote communities are associated with water masses and physicochemical factors (Hamilton et al. 2008; Zhang et al. 2019). These are the main objectives of the present study, with data presented from midsummer and early autumn of 2012.

Materials and methods

Study area, sample collection and analysis of environmental factors

Samples were collected from seven stations in the southern Chukchi Sea (Fig. 1) aboard the R/V "Xuelong"during the fifth Chinese National Arctic Expedition in both the summer (18 and 19 July) and early autumn (8 September) of 2012. Three stations overlapped in both seasons. Five depths were selected at each station (Table 1), and seawater was

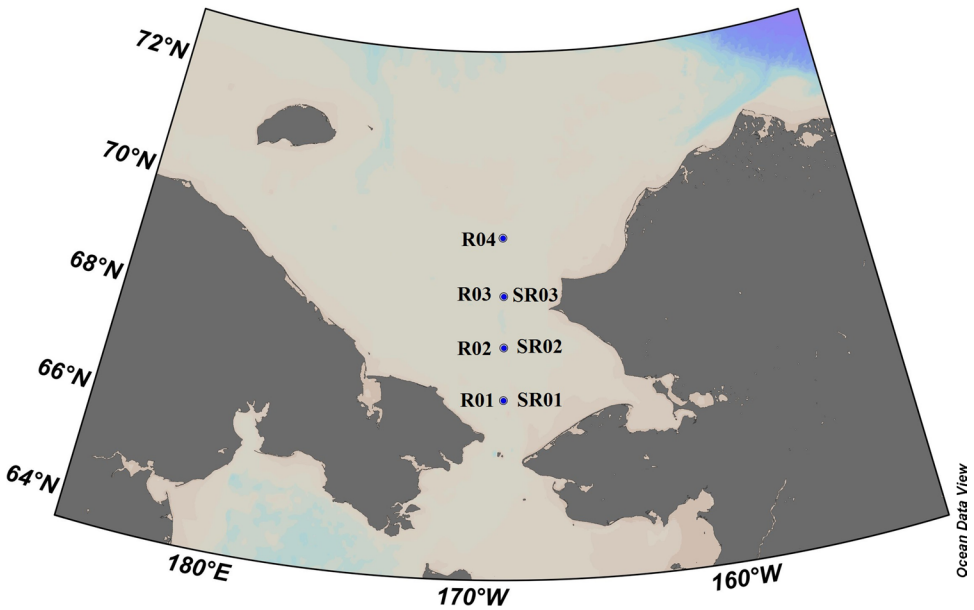

Fig. 1 Sampling sites in the southern Chukchi Sea of 2012: the sampling stations of R1 to R3 were in both July and September, and R4 was only July. Created by Ocean Data View (ODV 4.5, http://odv.awi.de), and modified by Painter Windows Operating System in-built Toolkit of image editor

Table 1 Summary information for Miseq sequencing data from the 35 samples

Station-depth	Reads	OTUs	Shannon	Station-depth	Reads	OTUs	Shannon
R01-0m	15,486	158	1.40	SR01-0 m	46,888	177	2.12
R01-14m	10,071	188	2.29	SR01-10 m	52,355	241	2.53
R01-20m	5670	115	2.47	SR01-20 m	27,400	315	3.13
R01-30m	7110	170	2.99	SR01-30 m	39,226	303	2.48
R01-41m	3918	131	2.40	SR01-37 m	38,335	325	3.19
R02-10m	2333	140	3.88	SR02-0 m	42,719	193	1.41
R02-22m	5828	209	3.91	SR02-10 m	64,905	215	1.27
R02-30m	1239	113	3.77	SR02-20 m	17,853	283	3.02
R02-47m	6016	187	3.82	SR02-30 m	19,279	270	3.22
R03-0m	29,052	296	2.98	SR02-45 m	26,205	339	3.37
R03-10m	21,990	271	3.01	SR03-0 m	54,449	170	1.48
R03-20m	20,076	310	3.70	SR03-10 m	51,351	293	2.11
R03-30m	45,991	233	1.64	SR03-20 m	34,292	283	2.99
R03-50m	6718	263	3.80	SR03-30 m	12,816	293	3.91
R04-0m	32,989	242	2.04	SR03-45 m	6781	337	3.62
R04-10m	62,691	169	0.87				
R04-20m	17,752	173	1.79				
R04-30m	7839	252	3.71				
R04-48m	11,949	286	3.80				
ANOVA $F_{\text{J-St}}$	3.124	10.116	2.959	ANOVA $F_{\text{S-St}}$	0.204	0.064	0.153
$\text{Sig.}_{\text{J-St}}$	0.057	0.001	0.066	$\text{Sig.}_{\text{S-St}}$	0.819	0.938	0.860
$F_{\text{J-S}}$	14.21	8.46	0.468	$F_{\text{S-w}}$	27.139	23.602	33.095
$\text{Sig.}_{\text{J-S}}$	0.001	0.007	0.499	$\text{Sig.}_{\text{S-w}}$	0.0002	0.0004	0.00009
$F_{\text{JS-BBW}}$	2.794	19.720	1.136	$F^{w}_{\text{JS-BAW}}$	26.41	0.12	6.091
$\text{Sig.}_{\text{JS-BBW}}$	0.107	0.0002	0.297	$\text{Sig.}_{\text{JS-BAW}}$	0.00003	0.743	0.021

$F_{\text{J-St}}$, $\text{Sig.}_{\text{J-St}}$ and $F_{\text{S-St}}$, $\text{Sig.}_{\text{S-St}}$ stands for significance among difference stations in July and September, respectively. $F_{\text{S-W}}$, $\text{Sig.}_{\text{S-W}}$ stands for significance between different water masses in September. $F_{\text{JS-BBW}}$, $\text{Sig.}_{\text{JS-BBW}}$ and $F_{\text{JS-BAW}}$, $\text{Sig.}_{\text{JS-BAW}}$ stands for significance between BSW in July and September, and between BSW in July and ACW in September, respectively

collected from each depth using Niskin bottles attached to an SBE911 plus a CTD rosette system (Sea Bird Inc., USA). Water temperatures and salinities were recorded directly using the CTD; nutrients at each depth, including phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+) and silicate (Si), were immediately measured onboard the ship with a SKALAR SAN++ nutrient automatic analyzer (Netherlands). Dissolved oxygen (DO) was measured using the Winkler titration method (Grasshoff et al. 1983). Five-hundred-milliliter water samples were collected from each depth. Each sample was passed through 20-µm, 3-µm and Whatman GF/F glass filters (0.47-mm pore size, 47-mm diameter). Each filter was inserted into a clean glass tube for Chlorophyll *a* (Chl *a*) measurement. Chl *a* was extracted using 10 mL of 90% acetone for 24 h in a − 20 °C freezer and measured with a Turner Designs 10 fluorometer (Parsons et al. 1984).

Sampling and molecular detection of picoeukaryote community abundance and composition

One hundred milliliters of water were collected from each depth at the seven stations and prefiltered through a 50-µm-pore-size mesh to analyze the eukaryotic picophytoplankton. Three milliliters of the filtrate from each sample was directly used for measuring the abundance of picophytoplankton by a BD FACSCalibur Flow Cytometer. This analysis process is described in Zhang et al. (2016). Two-liter water samples were collected from each depth at each station. Next, each sample was passed through 20-µm, 3-µm and 0.2-µm filters. The pico-fractions (0.2–3 µm) were collected for analysis of the picoeukaryote biodiversity and community composition. The analysis methods, including DNA extraction and PCR amplification of rRNA genes, are described in Zhang et al. (2019). The V4 region of the

eukaryotic SSU rRNA gene was amplified using the universal forward primer 3NDf (5′-GGCAAGTCTGGTGCCAG-3′) and the reverse V4_euk_R2 primer (5′-ACGGTATCT RATCRTCTTCG-3′) (Bråte et al. 2010). These fused primers each included an Illumina adapter, the sequencing primer and an eight-nucleotide barcode inserted between the Illumina adapter and the sequencing primer.

Barcodes were used to sort multiple samples. First, samples were individually amplified for the eukaryotic SSU rRNA V4 region. PCRs were performed in a 20 μL reaction volume containing 2 μL DNA template, 250 μM dNTPs, 0.25 μM of each primer, 2 μL 10 × PCR buffer, and 2.5 U Pfu polymerase (MBI, Fermentas, USA). The PCR conditions consisted of denaturation at 95 °C for 2 min, 25 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension cycle at 72 °C for 10 min. Subsequently, using a limited-cycle PCR on 5 μL of each PCR gel-recycled product, Illumina sequencing adapters and dual-index barcodes were added to each amplicon. Aliquots of PCR products (3 μL) were checked on a 2% agarose gel, purified using a DNA gel extraction kit (Axygen, China), and quantified using a TBS-380 Mini-Fluorometer (Turner BioSystems). Following quantification, products from the different samples were mixed in equal molar ratios for sequencing on a MiSeq platform using a 2 × 300 cycle V3 kit following standard Illumina sequencing protocols.

The raw fastq files were demultiplexed based on the barcode. PE reads for all samples were run through Trimmomatic (version 0.35) to to remove low-quality base pairs using these parameters (SLIDINGWINDOW: 50:20 MINLEN: 50). Trimmed reads were then further merged using FLASH program (version 1.2.11) with default parameters. The low quality contigs were removed based on screen.seqs command using the following filtering parameters, maxambig = 0, minlength = 200, maxlength = 580, maxhomop = 8. The 18 s sequences were analyzed using a combination of software mothur (version 1.33.3), UPARSE (usearch version v8.1.1756, http://www.drive5.com/uparse/), and R (version 3.2.3). The demultiplexed reads were clustered at 98% sequence identity into operational taxonomic units (OTUs) using the UPARSE pipeline (http://www.drive5.com/usearch/manual/uparsecmds.html). The OTU representative sequences were an assignment for taxonomy against Silva 128 database with a confidence score ≥ 0.6 by the classify.seqs command in mothur. Then, the attributions of each sequence at different levels (from phylum to genus) were added according to the NCBI database. The OTUs with relative DNA abundance larger than 1% were blasted in the NCBI database to make sure the existence of the main species. All singletons and sequences belonging to Metazoa and other traditional non-picoeukaryotes, including most diatoms, dinoflagellate, ciliates and cercozoa, and some cryptophytes and chrysophytes, were removed, and R (version 3.4.1) was used to construct an alpha-diversity index (Shannon) from the left sequences. Variations in the alpha-diversity and the corresponding phylotypes between groups of samples were estimated by one-way ANOVA. Venn diagram was used to show the sharing of OTUs among different groups. Similarity of the beta-diversity among different samples were analyzed by Bray–curtis method and ploted by pheatmap. Both MRPP and Anosim was used to analyze the significant of the difference of OTUs between different groups. All the analyses above were done in R (version 3.4.1). The sequence data were submitted to the National Center for Biotechnology Information Sequence Read Archives (SRA) under BioProject ID PRJNA340039.

Statistical analysis of microbial and environmental factors

Two statistical approaches were used to analyze the relationships among microbial communities and environmental factors. The relationships between the biological group, including both the main classes and all the present OTUs, and their corresponding environmental group (environmental factors, including temperature, salinity, nutrients and chl *a*) were analysed (Canoco for Windows 4.5 software).

The relationships between both biological groups (picoeukaryotic community structure with all the present OTUs) at R and SR stations and their corresponding environmental groups (physicochemical factors, including temperature, salinity, nutrients, DO and chl *a*) were analyzed using redundant analysis (RDA) (Canoco for Windows 4.5 software). A Detrended correspondence analysis was used for the selection of RDAs of both relationships, as their relative largest axial lengths were 3.17 (R section) and 2.92 (SR section) (< 4, Leps and Smilauer 2003).

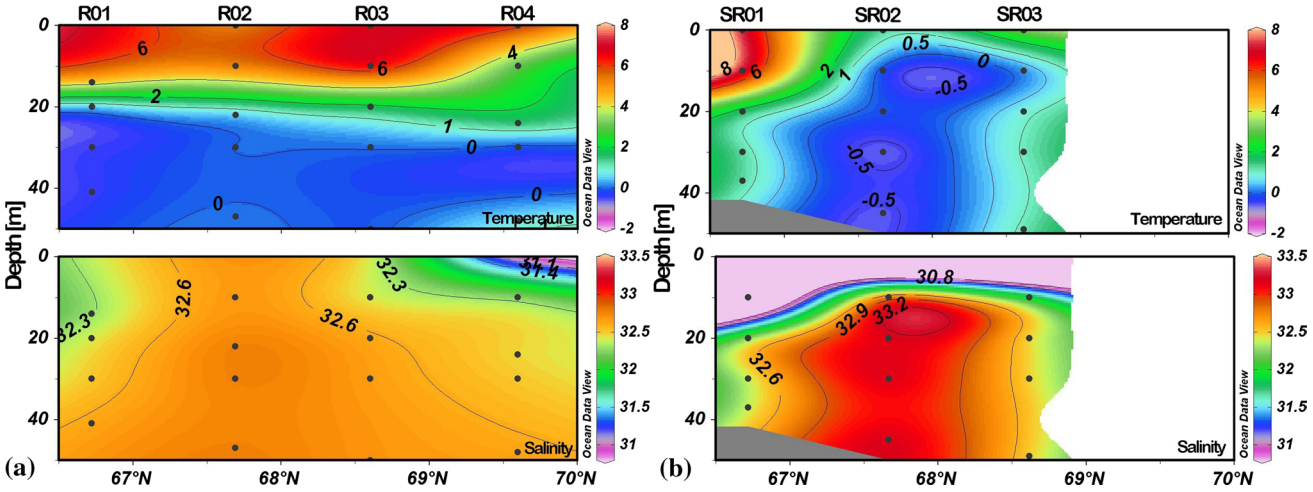

Fig. 2 Water temperature (°C) and salinity in the southern Chukchi Sea in July (**a**) and September (**b**) of 2012

Results

Hydrology and water masses in the southern Chukchi Sea

The temperature of water in July ranged from − 0.47 to 6.73 °C (Fig. 2a) and decreased with increasing depth of water. Salinity ranging from 31.05 to 32.77 (Fig. 2a). The profiles showed that the waters were mixed; and only weakly stratified at stion R04. The water mass mainly belonged to the BSW as the whole salinity was > 32, except that at R04-0m (Coachman et al. 1975; Woodgate et al. 2005), comprising Bering Shelf Water and Andre Water (Grebmeier et al. 2006). The temperature of water in September ranged from − 0.64 °C to 7.21 °C (Fig. 2b) and decreased with increasing depth of water. Salinity profiles also showed that the stratification of water ranged from 27.46 to 33.17 (Fig. 2b). They mainly belonged to ACW and BSW (Coachman et al. 1975; Woodgate et al. 2005). ACW, 0–10 m deep, consists of a mixture of the Alaskan Coastal Current and Bering Shelf Water (Grebmeier et al. 2006; Pisareva et al. 2015). ACW showed a lower silicate concentration (< 17 µM) than that seen in BSW (> 23 µM), which received nutrient supplements from Andre Water (Pisareva et al. 2015).

Nutrient-supplemented, upwelled water was detected at station R02 in the months of both July and September (Le et al. 2014). However, nutrient levels here (Supplementary material 1) were lower, and pH, DO, and Chl *a* were higher at depths of 10–30 m in July (R section) than those found in September (SR section) (average values in July: silicate = 0.12 µM, phosphate = 1.38 µM, ammonia = 1.76 µM, nitrite = 0.11 µM, nitrate = 8.63 µM, pH = 7.83, DO = 12.65 mg L^{-1}, Chl *a* = 11.86 µg L^{-1}; average values in September: silicate = 27.20 µM, phosphate = 1.81 µM, ammonia = 6.81 µM, nitrite = 0.30 µM, nitrate = 8.63 µM, pH = 7.62, DO = 9.49 mg L^{-1}, Chl *a* = 1.52 µg L^{-1}). These values suggest that a diatom bloom probably occurred in July, exhausting nutrients, especially the silicate content at station R02 (Perrette et al. 2011; Laney and Sosik 2014; Le et al. 2014). During the bloom, the pico-fraction only accounted for 14% of the total amounts of Chl *a*. Comparatively, the proportion of Chl *a* increased up to 56% in September. These findings are in accordance with the results of Le et al. (2014) and Danielson et al. (2017).

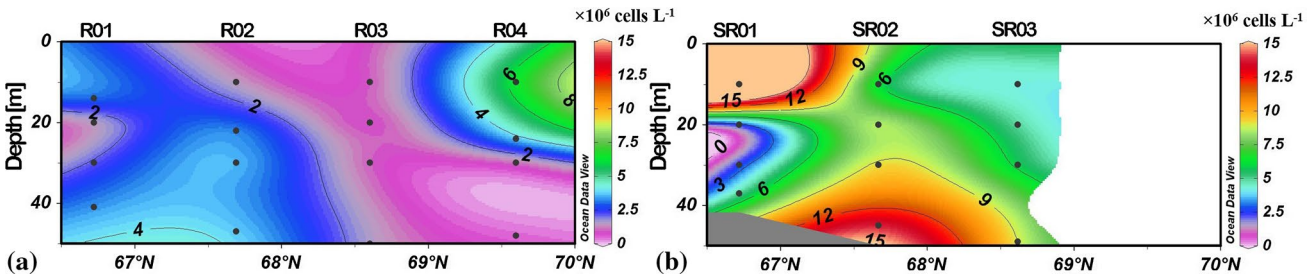

Fig. 3 Community abundance of eukaryotic picophytoplankton in the southern Chukchi Sea in July (**a**) and September (**b**) of 2012

Community abundance, diversity, and composition of picoeukaryotes in the southern Chukchi Sea

The community abundance of eukaryotic picophytoplankton in the southern Chukchi Sea (Fig. 3) was found to be 0.47–6.42 × 10^6 cells L^{-1} in July, which was approximately one-fourth of their abundance (2.31–23.70 × 10^6 cells L^{-1}) recorded in September. Average abundance increased fourfold, from 2.09 to 8.76 × 10^6 cells L^{-1}. Distribution of data for community abundance showed some peaks indicating regional abundance for both surface and bottom water in July as well as September. Comparatively, the distribution of data showed two tongues for the R02/SR02 station corresponding to upwelling.

A total of 849,572 sequences (reads) and 7940 OTUs (at 98% similarity) were identified in our study. The sequence number of each sample ranged from 1239 to 64,905, of which 113–339 OTUs were recognized with 98% similarity (Table 1). The Good's coverage estimator of the OTUs for all samples was higher than 99%, except for sample R02-30m, which had relatively low values (1239 reads, 113 OTUs and coverage of 97.82%). There were significant differences in both sequence numbers ($F = 14.21$, $p = 0.001$) and OTUs ($F = 8.46$, $p = 0.007$) between July and September; however, no discernible differences in the Shannon index were found (Table 1). Although specimens belonged to the same water mass, they showed significant differences in the OTUs ($F = 10.116$, $p = 0.001$) among different stations in July; however, no clear differences were seen in the sequence numbers and in the Shannon index ($p > 0.5$). Comparatively, no discernible differences were found between all the three parameters among different stations in September ($p > 0.5$).

There were significant differences in sequence numbers/reads ($p = 0.0003$), OTUs ($p = 0.00021$), and Shannon index values ($p = 0.00462$) among the three water masses, i.e., ACW-S (ACW in September), BSW-S (BSW in September), and BSW-J (BSW in July). Significant differences were also found between ACW-S and BSW-S ($F_{reads} = 27.139$, $p_{reads} = 0.0002$, $F_{OTUs} = 23.602$, $p_{OTUs} = 0.0004$, $F_{Shannon} = 33.095$, $p_{Shannon} = 0.00009$) (Table 1). Significant differences were found only between OTUs of BSW-J and BSW-S ($F = 19.720$, $p = 0.0002$), sequence numbers ($F = 26.41$, $p = 0.00003$), and Shannon index values ($F = 6.091$, $p = 0.021$) of ACW-S and BSW-S. Generally, ACW-S had the fewest OTUs (821) and BSW-S had the highest number of OTUs (956). The three water masses shared 373 OTUs: BSW-J and BSW-S shared 117 OTUs; BSW-S and ACW-S shared 42 OTUs; and BSW-J and ACW-S shared 25 OTUs (Fig. 4). Figure 5 shows that the picoeukaryote community had distinct regional distributions. The picoeukaryotes community belonged to similar depths of water and closely situated latitudes, and the same water masses probably had similar structures. The

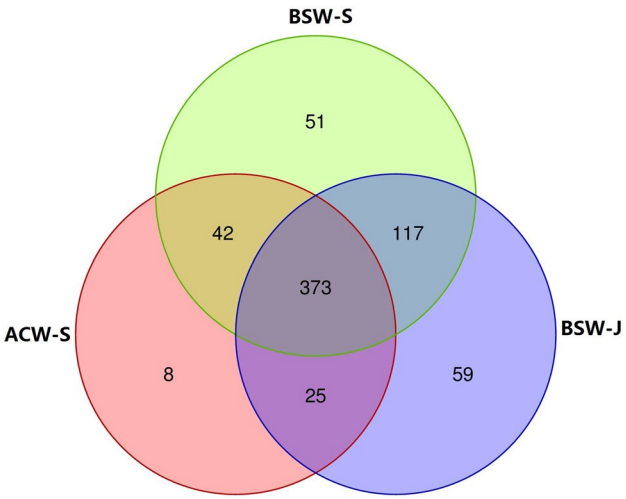

Fig. 4 Venn diagram for OTUs among different water masses and in different seasons

community structure at closer depths and latitudes may be more similar than the structure of communities belonging to the same water masses but located at distant sites. The community structure found in September was similar to that seen at higher latitudes and/or deeper waters in July, i.e. SR03-10m had a similar community structure to that of R03-30m and SR04-0m.

The picoeukaryotes identified both in the months of July and September mainly belonged to nine divisions, twelve classes, seven genera, and one species (Table 2). Picoeukaryotes found in the month of July were classified into ten orders and seven families with a proportion (relative 18S rDNA read abundance) greater than 0.5% among all reads, whereas those found in the month of September were classified into eight orders and six families. Phytoplankton, all of which were mixotrophs, were found to have the highest contribution in sequencing libraries of the picoeukaryotic community in the months of July and September, respectively, accounting for 70.7% and 83.8% of the total number of reads. Comparatively, heterotrophs including Choanozoa, Picozoa, Telonemia, and Ciliophora acounted for 5.4% and 7%, respectively, in July and September. Chlorophyta was found to be the most common division, accounting for 56.8% and 69.9% of the total number of reads in the months of July and September, respectively, with Mamiellophyceae, Trebouxiophyceae, and Dinophyceae identified as the first three-domain classes. The contribution of Chlorophyta, Dinoflagellata, Choanozoa, and Chrysophyta to the total picoeukaryotic sequence library increased in September, whereas that of Ochrophyta, Picozoa, Telonemia, and Ciliophora was found to be decreased. Syndiniales was found to be the main order identified in Dinoflagellata, and its increased contribution to the picoeukaryotic library was

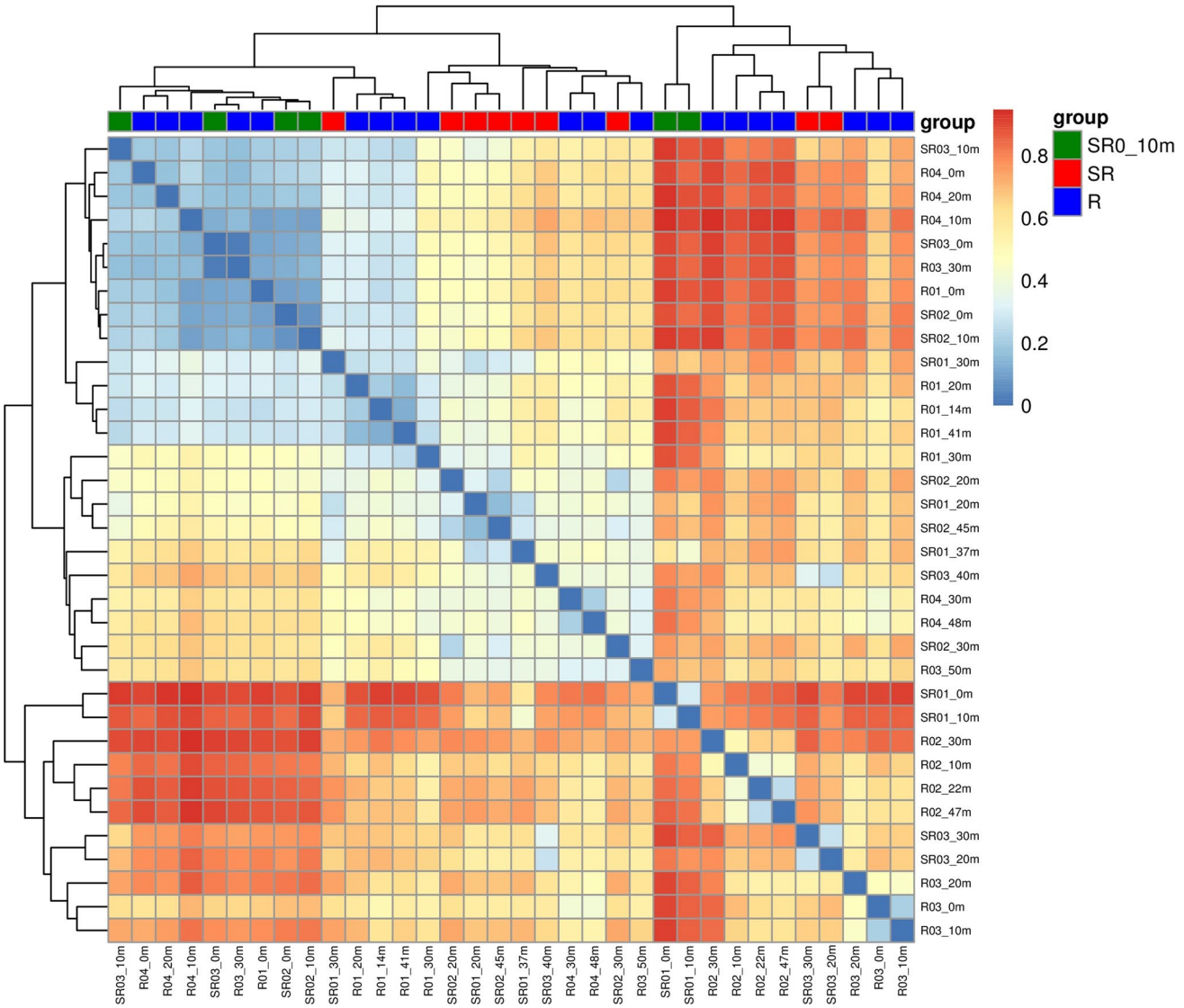

Fig. 5 Cluster analysis of picoeukaryote community at different sampling sites

accompanied by a predominant transfer (from Syndiniales Group II to Syndiniales Group I).

We found eight and seven classes with proportions larger than 1%, respectively, in the months of July and September (Table 1). These classes were distributed differentially at various stations, depths of water, in varying seasons and different water masses (Fig. 6). Mamiellophyceae was found to contribute markedly in all samples, with proportions of 7.9–98.0% and 17.3–89.8% in the months of July and September respectively. The contribution of Mamiellophyceae did not always decrease or increase along with increasing depths of water in both July and September (Fig. 6a, b). This class showed a relatively higher contribution in the ACW than in the BSW (Fig. 6c). Tragin and Vaulot (2019) show that the genus *Micromonas* is divided into 9 clades corresponding to four species: *M. commoda*, *M. bravo*, *M. polaris*, *M. pusilla*) and some clades/candidate species. *M. pusilla* was found mostly in temperate locations while *M. polaris* and *Micromonas* clade B3 dominate in arctic and subarctic waters, respectively. In our study, *M. pusilla* only accounted for 3.1% of the *Micromonas* and most species cannot be identified.

In September, Trebouxiophyceae surpassed Dinophyceae were found to be the second most predominant class (Table 2). Prasinophyceae and Choanoflagellatea were two other classes with relatively higher contribution to picoeukaryotic libraries in September than in July. Among the main classes, only Dictyochophyceae ($p = 0.0049$), Spirotrichea ($p = 0.009$), and Bolidophyceae ($p = 0.0399$) showed significant differences in contribution between July and September. Like Mamiellophyceae, Trebouxiophyceae also showed relatively higher contribution in ACW than in BSW (Fig. 6c).

Table 2 Composition of picoeukaryotic community at different levels (contributions > 0.5%) obtained by MiSeq platform and bioinformatics: a. July, b. September

a	Compositions with their reads contributions	Unclassified proportions (%)
Phylum	Chlorophyta (56.8%), Dinoflagellata (5.4%), Ochrophyta (3.3%), Chrysophyta (3.1%), Picozoa (2.1%), Ciliophora (1.7%), Telonemia (1.1%), Cryptophyta (0.7%), Choanozoa (0.5%)	22.9
Class	Mamiellophyceae (48.3%), Dinophyceae (5.4%), Trebouxiophyceae (4.6%), Chrysophyceae (3.1%), Dictyochophyceae (3.1%), Prasinophyceae (2.9%), Spirotrichea (1.6%), Telonemea (1.1%), Cryptophyceae (0.7%), Picomonadea (0.7%), Bolidophyceae (0.6%), Choanoflagellatea (0.5%)	24.9
Order	Mamiellales (45.6%), Syndiniales (5.4%, Group_I, 2.9%, Group_II, 2.0%), Prasinococcales (2.6%), Pedinellales (2.2%), Telonemida (1.1%), Chromulinales (0.8%), Picomonadida (0.7%), Cryptomonadales (0.7%), Bolidomonadales (0.6%), Choanoflagellida (0.5%)	37.1
Family	Mamiellaceae (44.8%), Prasinococcaceae (2.6%), Telonemidae (1.1%), Bathycoccaceae (0.7%), Picomonadidae (0.6%), Amoebophryaceae (0.6%), Bolidomonadaceae (0.6%)	46.0
Genus	*Micromonas* (44.8%), *Prasinoderma* (2.6%), *Telonema* (1.1%), *Amoebophrya* (1.1%), *Bathycoccus* (0.7%), *Picomonas* (0.6%), *Bolidomonas* (0.6%)	44.1
Species	*Micromonas pusilla* (1.4%)	49.5
b	Compositions with their contributions	Unclassified proportions (%)
Phylum	Chlorophyta (69.9%), Dinoflagellata (6.5%), Choanozoa (4.3%), Chrysophyta (4.1%), Ochrophyta (1.9%), Picozoa (1.3%), Telonemia (0.7%), Ciliophora (0.7%), Cryptophyta (0.6%)	9.7
Class	Mamiellophyceae (52.5%), Trebouxiophyceae (11.2%), Dinophyceae (6.5%), Prasinophyceae (5.2%), Choanoflagellatea (4.3%), Chrysophyceae (4.1%), Bolidophyceae (1.3%), Telonemea (0.7%), Dictyochophyceae (0.7%), Cryptophyceae (0.6%), Spirotrichea (0.5%), Picomonadea (0.5%)	11.1
Order	Mamiellales (51.8%), Syndiniales (6.5%, Group_I 5.3%, Group_II 0.9%, Group_III 0.1%), Prasinococcales (5.1%), Choanoflagellida (4.2%), Bolidomonadales (1.3%), Telonemida (0.7%), Cryptomonadales (0.6%), Picomonadida (0.5%)	27.7
Family	Mamiellaceae (47.9%), Prasinococcaceae (5.1%), Stephanoecidae (4.1%), Bathycoccaceae (3.9%), Bolidomonadaceae (1.3%), Telonemidae (0.7%)	35.4
Genus	*Micromonas* (47.9%), *Prasinoderma* (5.1%), *Bathycoccus* (3.7%), *Bolidomonas* (1.3%), *Telonema* (0.7%), *Choricystis* (0.6%), *Diaphanoeca* (0.5%)	37.6
Species	*Micromonas pusilla* (4.4%)	24.9

However, Dinophyceae, Prasinophyceae, Choanoflagellatea ($p = 0.0016$), Chrysophyceae ($p = 0.0213$), and Bolidophyceae ($p = 0.0113$) showed higher contribution in BSW-S. Telonemea, Dictyochophyceae ($p = 0.0301$), and Spirotrichea showed higher contribution in BSW-J. As to the main genera with relative DNA contribution greater than 0.5% (Table 1), *Amoebophrya*, *Cryptocaryon*, *Parauronema,* and *Picomonas* were not found in either of the water masses in September. Comparatively, *Diaphanoeca* was only found in BSW-S, while *Choricystis* was found in both ASW-S and BSW-S. Usually, *Cryptocaryon* (WoRMS, http://www.marinespecies.org/) and *Parauronema* (Soldo et al. 1978; Pan et al. 2011) were thought as nonpico eukaryotes. However, we did find some OTUs belonging to both genera were identified as picoeukaryotes or picoplankton. Consequently, new candidate species of these genera that are possibly belonging to pico sized protists.

Compared with other stations, station R02 showed the presence of distinct picoeukaryote communities, with relatively high biodiversity (Table 1) however, a low contribution from the eight predominant classes, especially Mamiellophyceae (Fig. 6a). This is in accordance with the algal bloom, during which the blooming species, probably a type of diatom, inhibited the growth of picoeukaryotes.

Environmental correlations of the microbial community

Relationships between water masses, environmental factors and picoeukaryote assemblages were different between July and September, as revealed by RDA (Fig. 7). In July, canonical eigenvalues explain 70.2% of the total relationships, and the sum of the first two axes explains 66.0%. The contribution of environmental factors (C) to microbial distribution, from highest to lowest was as follows: nitrogen ($C = 15.73\%$, $p = 0.389$) > phosphate ($C = 13.33\%$, $p = 0.156$) > Chl *a* ($C = 10.94\%$, $p = 0.847$) > salinity ($C = 12.33\%$, $p = 0.001$) > pH ($C = 9.33\%$, $p = 0.005$) > DO ($C = 6.76\%$,

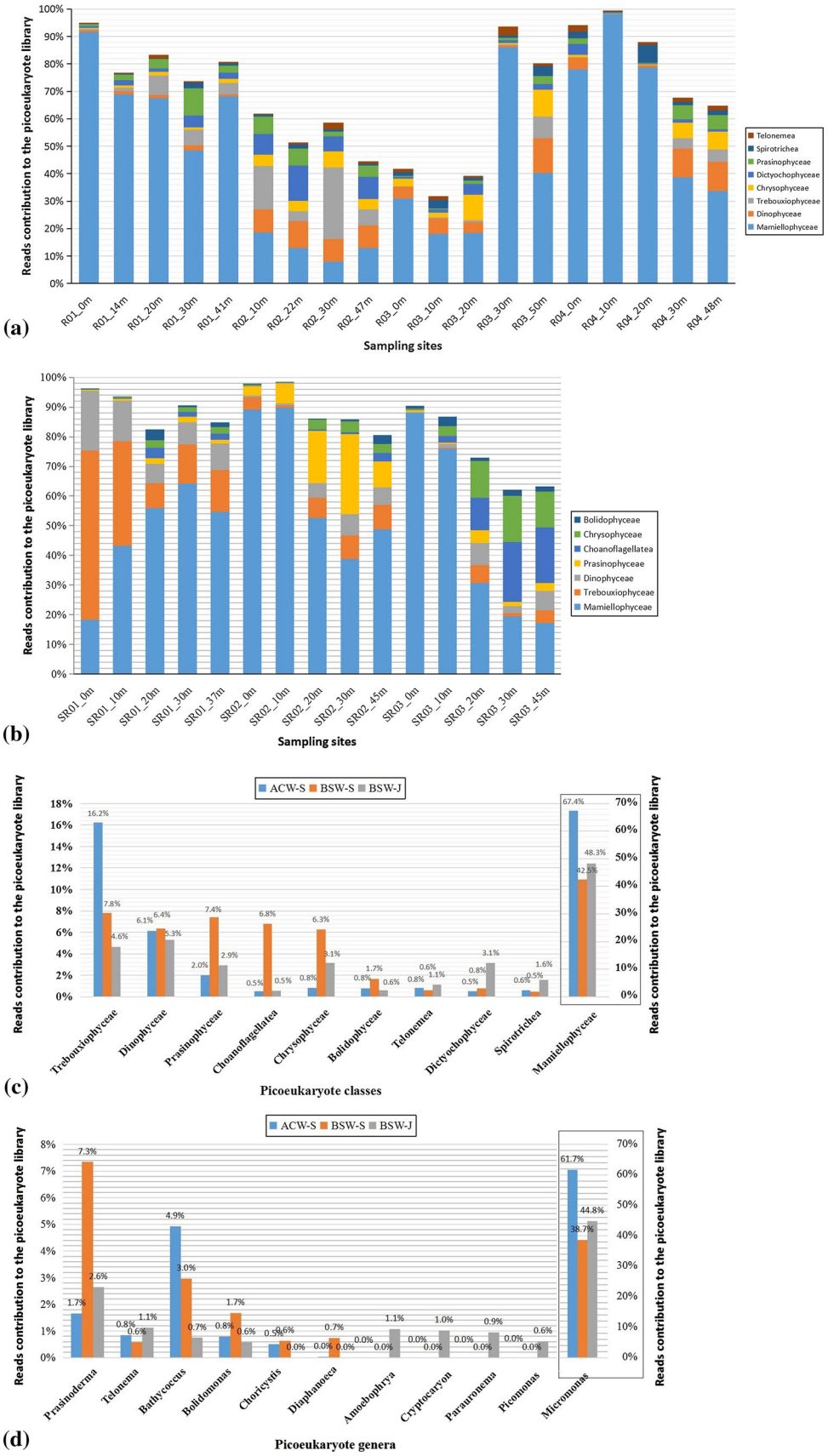

Fig. 6 Proportions of the identified picoeukaryotes at class level in July (**a**), September (**b**) and different water masses (**c**), and at genus level in different water masses (**d**) of the southern Chukchi Sea in 2012: assemblages with average proportions (relative 18S rDNA read abundances) of larger than 0.5% are shown

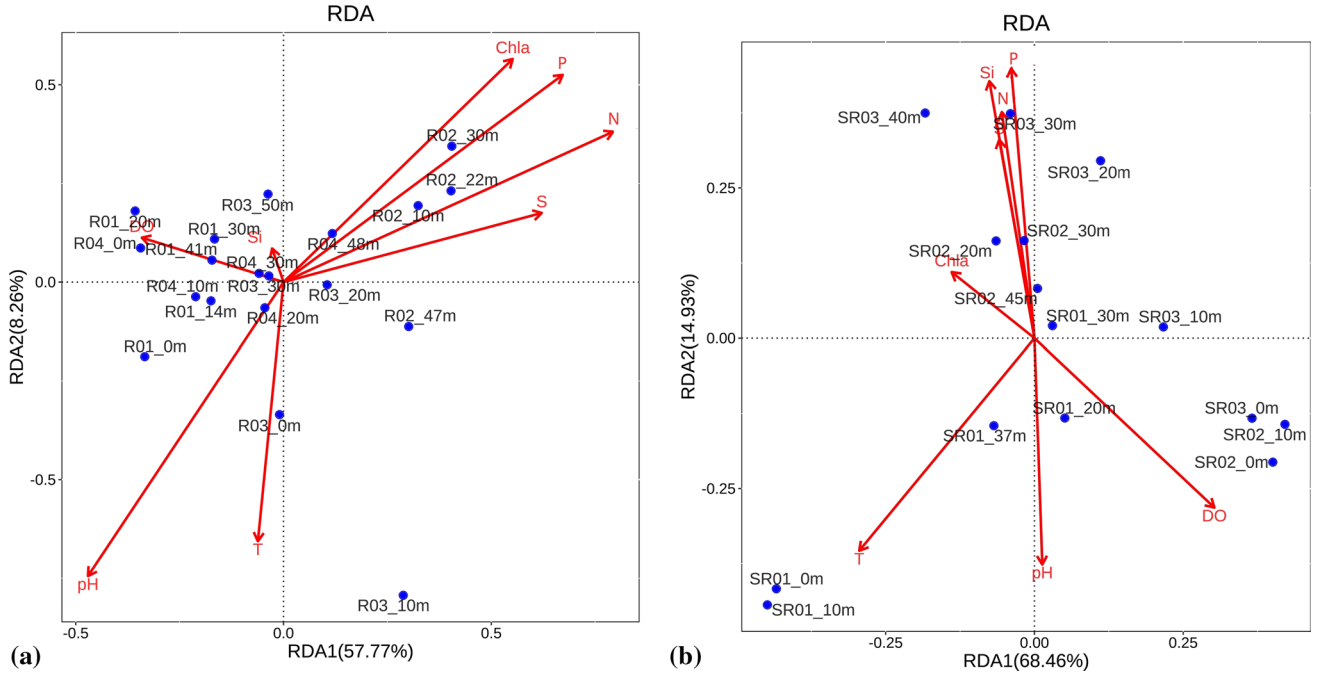

Fig. 7 Relationships of picoeukaryote community structure at different sampling sites with physiochemical factors in an ordination diagram with the first two axes of the RDA in July (**a**) and September (**b**). Red arrows with different lengths denote relative correlations of different independent variables with the biological factors. *T* temperature, *S* salinity, *N* $NO_3+NO_2+NH_4^+$, *DO* dissolved oxygen, *Chl a* Chlorophyll *a*

$p=0.329$) > temperature ($C=1.22\%$, $p=0.284$) > silicate ($C=0.54\%$, $p=0.637$). In September, canonical eigenvalues explain 89.5% of the total relationships, and the sum of the first two axes explains 83.4%. The influence of environmental factors on community composition is given here. The contribution of environmental factors (C) to microbial distribution, from highest to lowest was as follows: DO ($C=27.67\%$, $p=0.030$) > temperature ($C=26.94\%$, $p=0.001$) > Chl *a* ($C=12.12\%$, $p=0.109$) > silicate ($C=6.93\%$, $p=0.637$) > salinity ($C=5.41\%$, $p=0.002$) > nitrogen ($C=5.02\%$, $p=0.275$) > phosphate ($C=3.57\%$, $p=0.366$) > pH ($C=1.21\%$, $p=0.253$). Their interactions had different correlation with community structure at different sampling sites. Nitrogen and phosphate were the primary environmental factors influencing the community at BSW-J. Comparatively, silicate and salinity were the primary environmental factors influencing the community at BSW-S. However, DO and temperature were the primary environmental factors influencing the community at ACW-S. DO and temperature were important environmental factors in surface waters in both months. The increase in members of Mamiellophyceae and Trebouxiophyceae was seen mainly in ACW-S with lower nutrients and more fresh water than in BSW-S. However, the numbers of Prasinophyceae, Choanoflagellatea, Chrysophyceae, and Bolidophyceae mainly increased in BSW-S with nutrient supplements (Fig. 6c).

Discussion

As an important part of the Pacific Arctic Gateway, the Chukchi Sea has a strong influence on the Arctic Ocean through the transport of freshwater, heat, nutrients, and plankton from the Subarctic to the Arctic (Roach et al. 1995). This region is characterized by varying gradients in species composition, diversity, and abundance of fish and invertebrates (Stevenson and Lauth 2012; Mueter et al. 2013). Types of water masses with different physicochemical factors were found to change from midsummer to early autumn in the southern Chukchi Sea (Danielson et al. 2017). Water stratification was seen in both seasons. Both temperature and salinity were higher during midsummer. All macronutrients were supplemented during early autumn, especially silicates, which were exhausted during the diatom bloom (Springer and McRoy 1989; Sakshaug 2004; Laney and Sosik 2014; Le et al. 2014). The nutrient supplement was most discernible at the R02 (SR02) station, which was a typical bloom area, with extremely low picophytoplankton abundance (Fig. 3) and low DNA contribution to picoeukaryotes in July (Fig. 6a). We found that there was a competition between large taxa and smaller ones (Zhang et al. 2016), the predominant inhibited the growth of others (Zhang et al. 2019). However, the diversity of the picoeukaryote community was not affected by the bloom (Shannon Index > 3.80).

We know that the fractionated filtration does not ensure a complete separation of pico-sized forms from nano- and microorganisms (Vaulot et al. 2002; Nielsen et al. 2007; Charvet et al. 2012; Belevich et al. 2017). The picoeukaryotes in our study still contain some OTUs identified as real picoplankton by the SILVA database, although they may belong to classes and domains that are traditionally thought as non-pico ones. Some species of diatoms can be < 3 µm in one dimension and hence are capable of passing through a 3 µm filter. Vaulot et al. (2008) also reported central diatoms as potentially "true"picoplankton. Both Lovejoy et al. (2006) and Kilias et al. (2014) reported the presence of the arctic diatoms Fragilariopsis in picoplankton libraries. Of course, sloppy feeding or cell breakage also bought some non-pico fractions (Vaulot et al. 2002; Nielsen et al. 2007; Charvet et al. 2012; Belevich et al. 2017). We have tried our best to wipe these sequences. The proportion of the increase in the contribution of picophytoplankton to that of total sequencing libraries of the picoeukaryotic community was (70.7%: 83.8%), that of increase in the pico-fraction to the total Chl *a* was found to be (38%: 53%). The abundance of picophytoplankton (2.09: 8.76 cells L^{-1}), along with a decrease in the levels of total Chl *a* (3.06:1.08 µg L^{-1}) and the levels of nutrient supplements in water masses in September, do not substantiate the classical assumption that larger phytoplankton would be associated with higher nutrient levels and higher biomass (Springer and McRoy 1989; Danielson et al. 2017).

The Chukchi Sea is one of the most N-limiting area amongst global oceans and is severely N-limited during the season of phytoplankton growth (Brown et al. 2015). During midsummer, nitrogen and phosphate levels were the primary factors affecting the community structure of picoeukaryotes. Diatom blooming exhausted silicates (Supplement Material 1), whereas, relatively high nitrogen and phosphate levels were still detected. These nutrients could nevertheless support the growth of other picophytoplankton except that of Mamiellophyceae, i.e., Trebouxiophyceae and Chrysophyceae. Diatom blooming inhibited the growth of Mamiellophyceae, which would be more abundant in post-bloom conditions, i.e., in the whole water column at station R01 and at a depth of 0–10 m at R03, where the silicate had been exhausted. Interestingly, the bloom was mainly found in the 10–30 m region, with insufficient light (Martini et al. 2016). NO_3^- reduction and O_2 supersaturation in surface waters indicate the growth of phytoplankton. Comparatively, DO and temperature became the primary factors affecting picoeukaryote growth in September when phytoplankton of the picoeukaryote community increased in abundance. Although levels of nutrient supplements did not stimulate primary productivity and biomass of larger phytoplankton, the contribution of autotrophs was higher and some heterotrophs of the picoeukaryote community appeared to have perished. Water stratification with lower nutrient levels, especially N-limiting (N/P = 9) is responsible for such phenomena. Picophytoplanton are predicted to thrive in a warmer more stratified Arctic Ocean (Li et al. 2009; Zhang et al. 2016), because small cells are more effective in acquiring nutrients and less susceptible to gravitational settling (Ardyna et al. 2011). In accordance with Danielson et al. (2017) the Chukchi Sea shows a predominance of smaller phytoflagellates which suggests the possibility of a more important microbial loop in early autumn. Lower levels of DO and reduced pH indicated considerable respiratory activity of heterotrophs in the upwelling, where some mixotrophs and heterotrophs dominated the picoeukaryote community (Fig. 6). The mixotrophs can use light and nutrients to synthesize carbon and can also swallow other microbes to obtain carbon. Most phytoflagellates were mixotrophs, including members of Mamiellophyceae, Chrysophyceae, and Dictyochophyceae (Lovejoy et al. 2002; Rozanska et al. 2008; Lovejoy 2013). They are commonly known to be predominant in the arctic seas (Lovejoy et al. 2006; Terrado et al. 2009; Lovejoy and Potvin 2011). *Micromonas* and *Bathycoccus* are abundant in marine coastal waters (Kilias et al. 2014). *Amoebophyra* is a most commonly recovered clade of Syndiniales Group II, which has been reported to be found at all depths and in all seasons in the Arctic (Terrado 2011). The Syndiniales are either parasitoids, parasitic, or commensally dependent on a host and have complex stages in their life cycles. Diversity of these protists suggests that they evolve rapidly and many varieties may be restricted to a single host (Guillou et al. 2008). Consequently, seasonal changes in composition Syndiniales indicated variations in their hosts in the southern Chukchi Sea. Generally, the composition of the picoeukaryote community in the southern Chukchi Sea is different from that in both, the Central Arctic Ocean and in the European polar Seas (Lovejoy et al. 2006; Zhang et al. 2015). *Micromonas* are pan-Arctic-dominant (Lovejoy et al. 2007; Zhang et al. 2015). *M. polaris* and *Micromonas clade B3* dominate in arctic and subarctic waters respectively (Tragin and Vaulot 2019). *Micromonas* with many species or clades were also found to be predominant in the southern Chukchi Sea. *M. pusilla*, which was found mostly in temperate locations only account for a very small fraction (1.4% of the total reads and 3.1% of the *Micromonas* reads). This may indicate a complex water environment (mixed of water masses). The abundance of picophytoplankton in the southern Chukchi Sea in 2012 was slightly higher than that in 2008 (July: 1.00×10^6 cells L^{-1}, Zhang et al. 2012) and was comparable to that in the Northern Bering Sea in 2008 (July: 3.48×10^6 cells L^{-1}) and to that in the central Arctic Ocean in 2010 (August: 4.97×10^6 cells L^{-1}, Zhang et al. 2015, 2016).

As in other oceanic waters, the picoeukaryotic community has a distinct composition and diversity in different

water masses (Hamilton et al. 2008; Winter et al. 2008; Lovejoy et al. 2011; Zhang et al. 2019), as their movements are primarily determined by passive lateral advection and vertical mixing in the water column (Hamilton et al. 2008; Zhang et al. 2012, 2019; Sigler et al. 2017). Each water mass can be considered as a habitat with its own protists; even rare organisms have a biogeography that is best explained by their water mass of origin (Galand et al. 2009). Water mass was also the primary factor determining community composition and diversity of picoeukaryotes in the southern Chukchi Sea. Variations found in some picoeukaryote communities reflected variations in water masses. Some species of Trebouxiophyceae and *Bathycoccus* (Mamiellophyceae) were probably carried by the ACW, especially from the Alaskan Coastal Current (Grebmeier et al. 2006; Pisareva et al. 2015). *Prasinoderma* (Prasinophyceae), *Bolidomonas* (Bolidophyceae), *Diaphanoeca* (Choanoflagellatea) and some species of Chrysophyceae, Dictyochophyceae, and Spirotrichea were brought by the BSW because they showed relatively high abundance in waters deeper than 10 m at station R2 with upwelling.

Seasonal changes, varying depths, and varying stations (latitudes) at the same water mass also had a significant influence on the picoeukaryotic community. Some phylotypes are ubiquitous in surface waters (Kirchman et al. 2010) and others are predominantly found in deeper waters (Galand et al. 2010). We found clear changes such as replacement of species at different regions as well in different seasons, which presents changes in community functions and indicates a great effect on the whole system (Lovejoy et al. 2011). In different seasons, the assemblages in the same water mass may be less similar to those in different water masses which are located close by. This observation is different from the results of Hamilton et al. (2008).

As temperatures of the Arctic Ocean are increasing with climate change, its physiochemical environment is changing. These changes will continue to have an impact on the microbial community in the Chukchi Sea. The Chukchi Sea may become a more flagellate-based system, especially a picophytoplankton-based system, favored by warm temperatures and strong vertical stratification of the upper water column (Li et al. 2009; Lovejoy et al. 2011), as the phytoflagellates were primarily supported by regenerated nutrients (Carmack 2007; Tremblay et al. 2009). The success of macrozooplankton is tied to higher trophic levels (Hatun et al. 2009); these organisms are dependent on phytoplankton and are sensitive to species composition (Vargas et al. 2006). Consequently, the changes in microbial communities have a great impact on trophic levels, which might change along with the changing climate.

Conclusion

The community distribution and composition of picoeukaryotes had distinct seasonal features. Contribution of picophytoplankton, especially chlorophytes increased in early autumn compared with their contribution in midsummer (July). Water mass was the primary factor determining the community composition and diversity of picoeukaryotes. Seasonal changes, varying depths, and varying stations (latitudes) at the same water mass also had a significant influence on the picoeukaryotic community. The Chukchi Sea will become a smaller phytoflagellate-based system along with Arctic warming. This will change the whole pelagic ecosystem in this region. Long-term monitoring of biodiversity and community composition of picoeukaryotes is necessary to evaluate the effects of warming of waters. Such studies will also provide essential primary data to study changes in the whole pelagic ecosystem of the Arctic Ocean.

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