The ISME Journal (2013) 7, 1461–1471

www.nature.com/ismej

ORIGINAL ARTICLE

Mesoscale distribution and functional diversity of picoeukaryotes in the first-year sea ice of the Canadian Arctic

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Sea ice, a characteristic feature of polar waters, is home to diverse microbial communities. Sea-ice picoeukaryotes (unicellular eukaryotes with cell size $<3\,\mu m$) have received little attention compared with diatoms that dominate the spring bloom in Arctic first-year sea ice. Here, we investigated the abundance of all picoeukaryotes, and of 11 groups (chlorophytes, cryptophytes, bolidophytes, haptophytes, Pavlovaphyceae, *Phaeocystis* spp., pedinellales, stramenopiles groups MAST-1, MAST-2 and MAST-6 and Syndiniales Group II) at 13 first-year sea-ice stations localized in Barrow Strait and in the vicinity of Cornwallis Island, Canadian Arctic Archipelago. We applied Catalyzed Reporter Deposition–Fluorescence *In Situ* Hybridization to identify selected groups at a single cell level. Pavlovaphyceae and stramenopiles from groups MAST-2 and MAST-6 were for the first time reported from sea ice. Total numbers of picoeukaryotes were significantly higher in the vicinity of Cornwallis Island than in Barrow Strait. Similar trend was observed for all the groups except for haptophytes. Chlorophytes and cryptophytes were the dominant plastidic, and MAST-2 most numerous aplastidic of all the groups investigated. Numbers of total picoeukaryotes, chlorophytes and MAST-2 stramenopiles were positively correlated with the thickness of snow cover. All studied algal and MAST groups fed on bacteria. Presence of picoeukaryotes from various trophic groups (mixotrophs, phagotrophic and parasitic heterotrophs) indicates the diverse ecological roles picoeukaryotes have in sea ice. Yet, $>50\%$ of total sea-ice picoeukaryote cells remained unidentified, highlighting the need for further study of functional and phylogenetic sea-ice diversity, to elucidate the risks posed by ongoing Arctic changes.

The ISME Journal (2013) **7,** 1461–1471; doi:10.1038/ismej.2013.39; published online 21 March 2013
Subject Category: Microbial population and community ecology
Keywords: first-year sea ice; picoeukaryotes; chlorophytes; cryptophytes; stramenopiles; Syndiniales Group II

Introduction

Sea ice is the key feature that strongly influences the abiotic and biotic components of Arctic marine ecosystems. Presence of the sea-ice cover and overlying snow drastically reduces transmission of photosynthetic active radiance to the water column (Perovich *et al.*, 1993; Mundy *et al.*, 2007), which at times limits phytoplankton production (Fortier *et al.*, 2002; Glud *et al.*, 2007). On the other hand, sea ice provides a unique habitat for diverse and highly productive microbial communities (Gradinger, 2009; Deming, 2010). Ice algae form the base of the polar food web before the melting of sea ice (for example, (Conover *et al.*, 1986; Werner, 1997), as ice-algal fatty acids are essential for the development of key Arctic zooplankton species (Soreide *et al.*, 2010).

The rapid and dramatic changes taking place in the Arctic cryosphere include a decline in multi-year ice extent, with an estimated pan-Arctic monthly averaged ice volume loss of about 70% in September 2012 (Polar Science Center). Moreover, the ice-covered season is shortening due to delayed ice formation and advanced melt (Polyakov *et al.*, 2012). A recent ice–ocean regional model predicts spring ice breakup to advance by as much as 2–5 weeks in the Canadian Arctic Archipelago (Sou and Flato, 2009). This may pose a particular threat to the Arctic ecosystem, as the Archipelago is the region where the highest ice-algal biomasses are reported in the Arctic, associated with strong mixing and replenishment of nutrients at the ice-water interface (Cota *et al.*, 1987; Michel *et al.*, 2006). An exhaustive

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Received 1 October 2012; revised 10 January 2013; accepted 4 February 2013; published online 21 March 2013

review of dominant physical and biological processes in the Canadian Arctic Archipelago and recent changes in this area can be found in Michel *et al.* (2006).

In the Arctic, sea-ice microbial biomass is concentrated in the bottom section of first-year sea ice (Laurion *et al.*, 1995). Pennate diatoms are major component of the spring ice-algal bloom. Other unicellular eukaryotes contribute substantially to protists' biomass in bottom-ice communities in winter, but they typically constitute only a minor fraction of the biomass during the spring bloom (Riedel *et al.*, 2008; Niemi *et al.*, 2011). In result, they have received substantially less attention than sea-ice diatoms, and their composition and role are largely understudied. The phylogenetic diversity of pico- (cell size $<3\,\mu m$) and nano- (cells size 3–20 μm) eukaryotes in the sea ice has been studied only recently (Eddie *et al.*, 2010; Bachy *et al.*, 2011; Majaneva *et al.*, 2012; Comeau *et al.*, 2013), but reliable identification and enumeration is still lacking. Knowledge on the distribution and abundance of sea-ice protists is essential to understand and predict possible changes in microbial communities that will have profound impacts on Arctic marine food webs (Davidson *et al.*, 2010). Although genomic sequencing provides insights into processes, it is also essential to gain information on the abundance and community structure of picoeukaryotes. Hybridization with fluorescently labeled specific oligonucleotide probes is an invaluable tool for reliable detection and enumeration of morphologically undistinguishable microbial phylotypes in environmental samples (Amann and Fuchs, 2008).

The aim of this study was to determine the community structure of picoeukaryotes in first-year sea ice in the Canadian Arctic Archipelago during the spring period, and to elucidate environmental factors affecting their distribution. We applied Catalyzed Reporter Deposition–Fluorescence *In Situ* Hybridization (CARD–FISH) technique to detect and enumerate 12 groups of flagellates, including groups that so far have not been reported from the sea ice.

Materials and methods

Samples collection

The sampling was carried out from 9–17 May 2010 in Canadian Arctic Archipelago. A total of 13 first-year sea-ice stations (three stations in Barrow Strait and 10 in the vicinity of Cornwallis Island: Resolute Passage and McDougall Sound) were visited by snowmobile or helicopter, and sampled for biochemical measurements in bottom ice (Figure 1). Routine physical measurements included snow and ice thickness measurements at the coring sites. Because of technical obstacles, we could not routinely measured under-ice photosynthetic active radiance radiation.

At each station five to seven cores were collected using a manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprises, Lebanon, NH, USA). The bottom 3 cm of each core were immediately cut off with a stainless steel saw. Three of the cores collected at a station were processed for biological analyses: They were melted together in a dark isothermal container upon the addition of 0.22 μm-filtered surface seawater collected at the time of sampling (dilution factor: 3.7–6 times), to reduce osmotic shock of sea-ice microorganisms (Bates and Cota, 1986). The remaining two to four cores were pooled in sterile Whirlpack bags, slowly (24–36 h) melted without addition of seawater, and processed for nutrient analysis.

Chlorophyll a, nutrients and bacterial numbers

Duplicate subsamples (20–50 ml) were filtered onto Whatman GF/F (GE Healthcare, Little Chalfont, UK) glass–fiber filters (nominal pore size 0.7 μm; referred to as total Chl *a*) or onto 5 μm Nuclepore polycarbonate membrane filters (referred to as chl $a>5\,\mu m$; used to approximate biomass of sea-ice algae), extracted during 24 h in 90% acetone at 5 °C in the dark. Chl *a* concentrations were determined on a 10-AU Turner Designs (Sunnyvale, CA, USA) fluorometer calibrated with chl *a* extract from *Anacystic nidulans* (Sigma, Oakville, ON, Canada) (Parsons *et al.*, 1984). Chl $a>5\,\mu m$ fraction excluded all picoeukaryotes (cell size $<3\,\mu m$), and was, therefore, used for statistical analysis.

Samples for nutrients analysis (NO_3+NO_2, NO_2, PO_4 and $SiOH_4$) were filtered through precombusted (450 °C during 24 h) Whatman GF/F filters and immediately frozen in liquid nitrogen. They were analyzed on a Seal's Autoanalyzer 3 (NO_3+NO_2, NO_2, PO_4; Mequon, WI, USA) and on a Technicon II Autoanalyzer ($SiOH_4$; Sydney, NSW, Australia).

Samples for bacterial numbers were prefiltered through 40 μm plankton net, preserved with glutaraldehyde (final concentration 0.1%) and frozen in liquid nitrogen. They were thawed in a 30 °C water bath and stained with SYBR-Green I in tris-EDTA buffer (10 mMTris, 1 mMEDTA; Sigma) (Belzile *et al.*, 2008, Lebaron *et al.*, 1998). After cooling to ambient temperature, subsamples were analyzed with a FACSort flow cytometer (Becton and Dickinson, San Jose, CA, USA) equipped with an air-cooled argon laser (15 mW). The excitation wavelength was set at 488 nm to provide optimum excitation wavelength for SYBR-Green I 495 nm (Marie *et al.*, 1997).

Picoeukaryotes community composition

Aliquotes of 50–100 ml were prefiltered through 20 μm plankton net, and fixed with alkaline Lugol's solution, formalin (final concentration 2%) and 3% sodium thiosulphate (Sherr *et al.*, 1989). Samples were filtered onto polycarbonate membrane filters (pore size 0.8 μm, diameter 25 mm, Millipore, Billerica, MA, USA), washed with sterile deionized water, air-dried and stored at −20 °C. CARD–FISH

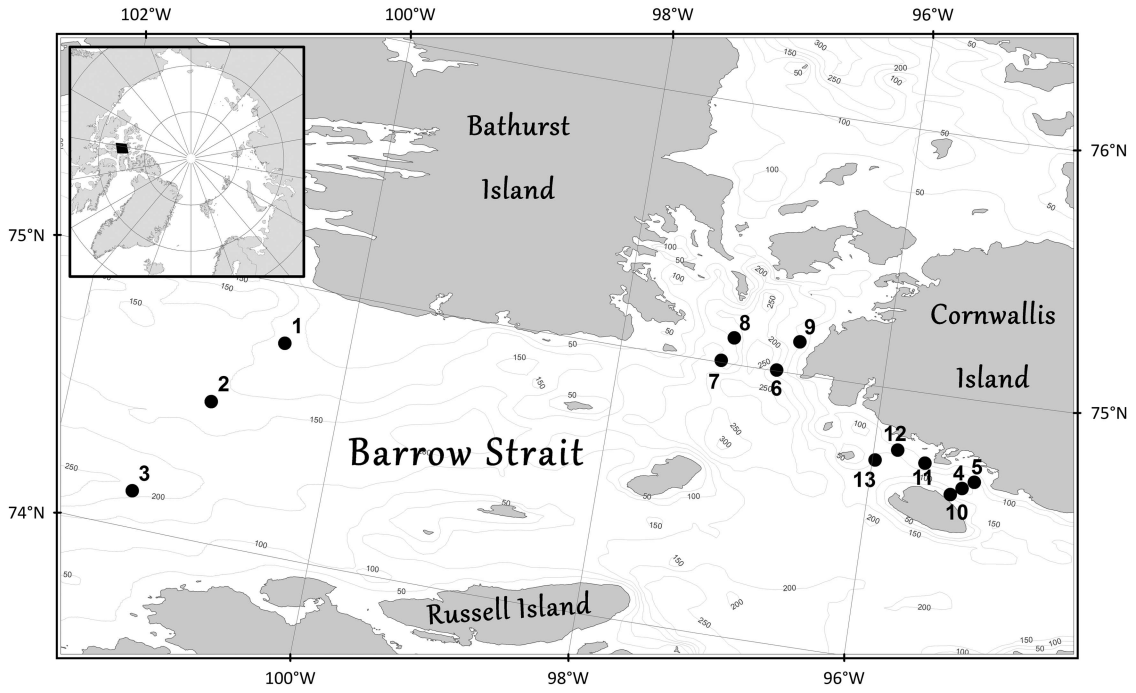

Figure 1 Location of the sampling stations in Barrow Strait (stations 1–3), and in the vicinity of Cornwallis Island (stations 4–13), Canadian Arctic Archipelago in May 2010. The inset shows the location of the sampling area in the Canadian High Arctic (black rectangle). Isobaths (in meters) are shown.

Table 1 List of probes used in this study

Probe name	*Target group*	*Formamide in hybridization buffer (%)*	*Reference*
Euk516	All eukaryotes	20	Amann *et al.* (1990)
Chlo02	Chlorophyta	40	Simon *et al.* (2000)
CryptB	Cryptophyceae	50	Metfies and Medlin (2007)
Bolido02	Bolidophyceae	40	Guillou *et al.* (1999b)
Prym02	Haptophyta	40	Simon *et al.* (2000)
Pavlova01	Pavlovaphyceae	40	Eller *et al.* (2007)
Phaeo02	*Phaeocystis* spp.	50	Zingone *et al.* (1999)
Ped675	Pedinellales	45	Piwosz and Pernthaler (2010)
NS1A, B and C	MAST-1 stramenopiles	45	Massana *et al.* (2006)
NS2	MAST-2 stramenopiles	45	Massana *et al.* (2006)
MAST-6	MAST-6 stramenopiles	35	Piwosz and Pernthaler (2010)
Alv01	Syndiniales Group II	50	Chambouvet *et al.* (2008)

Optimal hybridization conditions were maintained by varying the concentration of formamide in hybridization buffer at the constant temperature (37 °C). Formamide concentration was increased for all probes, except for Ped675 and MAST-6, compared with the original methodology due to lower hybridization temperature applied in this study (+20% formamide for every 10 °C decrease in hybridization temperature).

followed the procedure by Pernthaler *et al.* (2004), modified as described in Piwosz and Pernthaler (2010). Permeabilization step by enzymatic digestion was omitted. Filters were embedded in 0.1% agarose, and incubated 20 min in 0.01 M HCl. Filters were cut into 12 sections, which were hybridized at 35 °C for 3 h, and washed at 37 °C for 30 min. The list of 12 applied oligonucleotide probes and hybridization conditions are given in Table 1, and probe sequences and coverage in Supplementary Table S1. The numbers of MAST-1 stramenopiles were estimated with all three probes (NS1A, NS1B and NS1C) mixed together. Amplification with tyramides (Sigma) labeled with carboxyfluorescein (Molecular Probes, Invitrogen, Eugene, OR, USA) was performed at 37 °C for 30 min. The hybridized samples were mounted in glycerol medium containing 4′,6-diamidino-2-phenylindole dihydrochloride (1 μg ml^{-1}) to counterstain the cells. Preparations were stored at −20 °C. They were examined under ×1000 magnification by epifluorescence microscopy (Olympus BX 50) under green/blue excitation. At least 400 hybridized cells were counted for the general eukaryotic probe Euk 516, and 100 cells were counted with other probes. Whenever these numbers could not be reached due to the low abundance of hybridized cells, the complete filter section was counted. Cells size was constantly

monitored with an eyepiece New Porton measuring graticule NG12, and only cells $<3\,\mu m$ were counted. Coverage with specific probes is given in relation to the cell numbers obtained with the Euk516 probe.

The food vacuole content was examined for the most numerous groups and stations: total eukaryotes (all stations), chlorophytes (all stations), cryptophytes (all stations), bolidophytes (stations 8, 9), haptophytes (stations 1–3), Pavlovaphyceae (station 1), stramenopiles MAST-2 (stations 4–7, 10 and 11) and MAST-6 (station 5). 25–50 hybridized cells were examined by epifluorescence microscopy (AxioImager.M2, Carl Zeiss, Oberkochen, Germany). The presence of bacteria in food vacuoles was assessed based on their 4′,6-diamidino-2-phenylindole dihydrochloride signal at ultraviolet excitation. Prey items were counted only if they were in the focal plane of a flagellate cell to ensure that they had not settled onto the surface of the examined cell or did not shine through from underneath (Piwosz and Pernthaler, 2010).

All variables measured in ice cores were corrected for dilution when applicable. Numbers of bacterial and picoeukarote cells are presented in areal (cells m^{-2}) and volume (cells m^{-3}) concentrations to facilitate comparison with available literature.

Statistical procedures

Mann–Whitney *U*-test was used to compare measured variables between stations from Barrow Strait and in the vicinity of Cornwallis Island. Correlations between the groups of picoeukaryotes and environmental variables were calculated with Spearman rank correlation test.

Similarity analysis of the samples was based on the Bray–Curtis index (Bray and Curtis, 1957), calculated from (i) the untransformed abundances, (ii) percentage contribution to the total picoeukaryote numbers estimated with probe Euk516, of ten studied populations (with exclusion of total picoeukaryotes, and of Pavlovaphyceae and *Phaeocystis* spp. that were enumerated only at the stations in Barrow Strait). Calculations were performed using the PRIMER6 software (PRIMER-E Ltd, Plymouth, UK).

Results

Environmental variables

Physico–chemical conditions differed significantly between the stations in Barrow Strait and the vicinity of Cornwallis Island (Mann–Whitney *U*-test, $P<0.05$, Figure 2). Drift pack ice present in Barrow Strait was significantly thinner (77–132 cm) than land-fast ice in the vicinity of Cornwallis Island (138–189 cm; Figure 2a, Supplementary Table S2). Concentrations of inorganic nutrients in sea ice were significantly lower in Barrow Strait (NO_3: 0.3–2.0 mmol m^{-3}; PO_4: 0.5–1.0 mmol m^{-3}; $SiOH_4$: 1.5–2.7 mmol m^{-3}) than in the vicinity of Cornwallis Island (NO_3: 3.8–9.4 mmol m^{-3}; PO_4: 2.6–15.2 mmol m^{-3}; $SiOH_4$: 2.4–21.0 mmol m^{-3}; Figures 2b–d), except for NO_2 (0.07–0.08 mmol m^{-3} in Barrow Strait and 0.03–0.09 mmol m^{-3} in the vicinity of Cornwallis Island). Similar trend was observed for concentration of nutrients in water column under the sea ice (Supplementary Table S2). Thickness of snow cover ranged from 1.5 to 25.0 cm (Figure 3a), but the differences between Barrow Strait and the vicinity of Cornwallis Island were not significant.

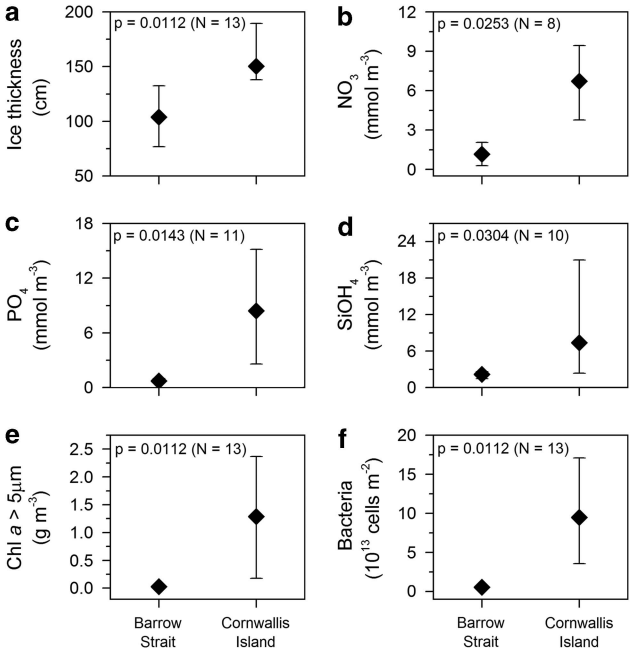

Figure 2 Comparison of (**a**) ice thickness, (**b**) NO_3 concentration, (**c**) orthophosphate concentration, (**d**) silicate concentration, (**e**) chl a (fraction $>5\,\mu m$) concentration and (**f**) bacterial numbers measured in bottom sea ice at stations in Barrow Strait and in the vicinity of Cornwallis Island. Significance level (*P*) from Mann–Whitney *U*-test, and number of observations (N) are indicated.

The average bottom ice total chl *a* concentration was >30-fold higher in the vicinity of Cornwallis Island (10.3–59.5 mg m^{-2}; 364.0–2438.8 mg m^{-3}) than in Barrow Strait (0.6–1.5 mg m^{-2}; 28.9–64.9 mg m^{-3}, Supplementary Table S2). Chl $a>5\,\mu m$ made up >90% of total chl *a*, except for stations two (79%) and five (<20%), and also was significantly higher in the vicinity of Cornwallis Island (3.7–53.5 mg m^{-2}; 178.0–2367.2 mg m^{-3}) than in Barrow Strait (0.5–0.7 mg m^{-2}; 22.9–35.8 mg m^{-3} (Figure 2e). Numbers of bacteria were more than two orders of magnitude higher in the vicinity of Cornwallis Island ($3.6–17.1\times10^{13}$ cells m^{-2}; $13.6–77.6\times10^{14}$ cells m^{-3}) than in Barrow Strait ($5.0–6.5\times10^{12}$ cells m^{-2}; $2.1–3.2\times10^{14}$ cells m^{-3}, Figure 2f, Supplementary Table S2).

Picoeukaryotes in sea ice

The total numbers of sea-ice picoeukaryotes ranged over one order of magnitude, from 0.2 to $2.0\pm1.3\times10^{9}$ cells m^{-2} ($0.9–8.7\pm1.3\times10^{10}$ cells m^{-3}, Figure 3b). A total of 32–48% (average 39.8%) of cells contained at least one bacterium inside food vacuoles (Table 2).

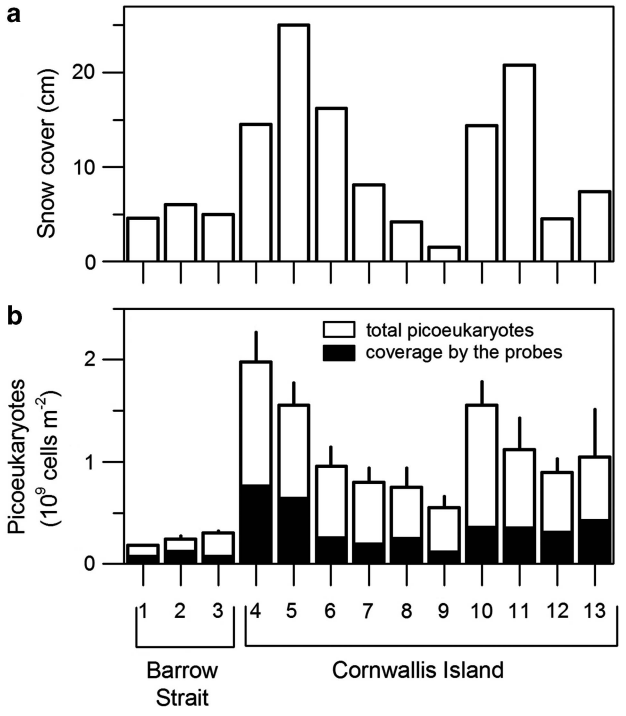

Figure 3 (**a**) Thickness of snow cover at 13 first-year sea-ice stations, Canadian Arctic Archipelago (**b**) Numbers of picoeukaryotes detected with probe Euk516. Shaded area depicts the proportions of cells hybridized with specific probes (see Figure 4). Error bars are s.d. of triplicate determinations.

Significantly higher numbers of picoeukaryotes were observed in the vicinity of Cornwallis Island than in Barrow Strait ($P<0.05$). 20–50% of total picoeukaryote numbers could be detected with the specific probes used (Figure 3b). The observed cells, hybridized with Euk516 and group specific probes, had similar morphology (rounded or slightly elongated cells, 1.5–3 μm in diameter).

The most numerous picoeukaryote groups at all the stations visited were chlorophytes and cryptophytes (Figure 4). Cell numbers for these two groups ranged from 2.5 ± 0.6 to $35 \pm 3 \times 10^7$ cells m^{-2} (1.0 ± 0.2 to $12 \pm 1 \times 10^9$ cells m^{-3}). The contribution to total picoeukaryote numbers was 5–30% for chlorophytes and 9–30% for cryptophytes. Less than 15% (average 7.7%) of chlorophytes contained bacteria in food vacuoles (Table 2). Percentage of cryptophytes that actively grazed bacteria ranged from 2.5 to 35.0% (average 15.4%, Table 2).

Bolidophytes were detected at all the stations visited. Their numbers varied from 0.6 to 18×10^6 cells m^{-2} (3.9 to 67×10^7 cells m^{-3}, Figure 4), and their contribution to picoeukaryote numbers was <1–2.5%. About 17.5% of the investigated bolidophytes cells contained bacteria in food vacuoles (Table 2).

Pedinellids were present only at stations five and 12 at very low numbers ($<8.2 \times 10^5$ cells m^{-2}; 3.9×10^7 cells m^{-3}; Figure 4). Their contribution to total picoeukaryote numbers did not exceed 1%.

Table 2 Food vacuole content of the studied populations of picoeukaryotes

Group	*Cells with at least one bacterium in food vacuoles (%)*	*Number of investigated cells*	*Number of investigated samples*
All picoeukaryotes	39.8	650	13
Chlorophyta	7.7	520	13
Cryptophyceae	15.4	480	12
Bolidophyceae	17.5	80	2
Haptophyta	30.8	91	3
Pavlovaphyceae	44.0	25	1
MAST-2 stramenopiles	29.4	211	6
MAST-6 stramenopiles	20.0	25	1

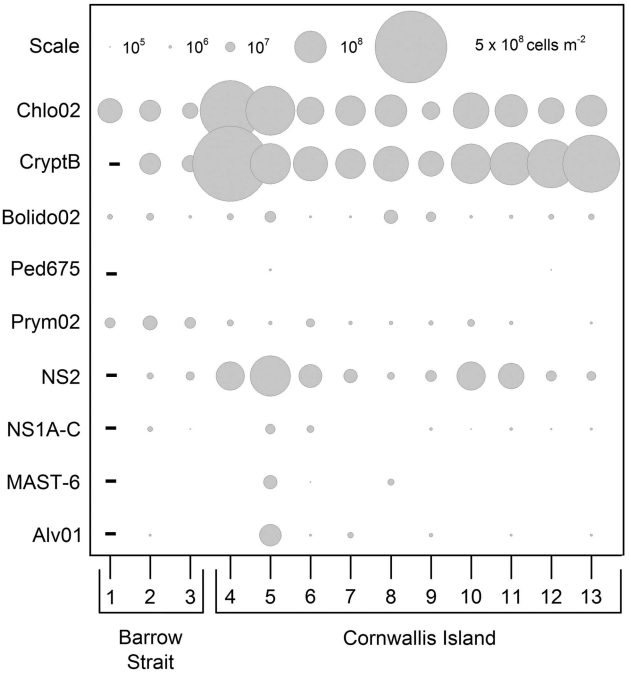

Figure 4 Distribution of the investigated groups of picoeukaryotes at 13 first-year sea-ice stations, Canadian Arctic Archipelago. Chlo02—Chlorophyta; CryptB—Cryptophyceae; Bolido02—Bolidophyceae; Ped675—Pedinellales, Prym02—Haptophyta; NS2—marine stramenopiles MAST-2; NS1A-C—marine stramenopiles MAST-1A, B & C; MAST-6—marine stramenopiles MAST-6; Alv01—Syndiniales Group II. '–' indicates no data, lack of data point indicates samples that were analyzed, but no cells were detected.

Numbers of haptophytes ranged from 0.8 to $20 \pm 13 \times 10^6$ cells m^{-2} ($3.2–91 \pm 59 \times 10^7$ cells m^{-3}, Figure 4). In contrast to other investigated groups, haptophytes were more numerous in Barrow Strait (stations one–three), where their contribution to total picoeukaryotes numbers was up to 8%. At these stations about 31% of haptophyte cells contained bacteria in food vacuoles (Table 2). The Barrow Strait stations were further investigated for the presence of class Pavlovaphyceae and genus *Phaeocystis*. Pavlovaphyceae were relatively numerous at station one (6.8×10^6 cells m^{-2},

3.1×10^7 cells m^{-3}), where they contributed ca. 4% of total picoeukaryote numbers, and 63% of haptophyte numbers. About 44% of cells had at least single bacterium ingested (Table 2). At stations two and three numbers of Pavlovaphyceae were 1.2×10^6 cells m^{-2} (5.5×10^7 cells m^{-3}) and 9.9×10^5 cells m^{-2} (4.2×10^7 cells m^{-3}), respectively. *Phaeocystis* spp. was only detected at station one, in very low numbers (8.8×10^5 cells m^{-2}, 4.3×10^7 cells m^{-3}).

Heterotrophic stramenopiles from the uncultured lineage MAST-2 were found at all the stations visited (Figure 4), with numbers ranging from $4.2 \pm 2.3 \times 10^6$ to $1.6 \pm 1.5 \times 10^8$ cells m^{-2} (0.2 ± 0.1–$7.6 \pm 7.1 \times 10^9$ cells m^{-3}). They were most numerous in the vicinity of Cornwallis Island, where their contribution to total picoeukaryote numbers was 3.9–10.3%. About 30% of the investigated cells actively grazed bacteria (Table 2). Stramenopiles from MAST-1 lineage were at least one order of magnitude less numerous than MAST-2 (0.2–9.3×10^6 cells m^{-2}; or 8.1×10^6–4.4×10^8 cells m^{-3}), and were undetectable at stations four, 7 and 8 (Figure 4). MAST-6 stramenopiles were found only at stations five, six and eight. Their numbers were very low ($<1.8 \times 10^7$ cells m^{-2}; 9.0×10^8 cells m^{-3}), and their contribution to total picoeukaryote numbers did not exceed 1% (Figure 4). About 20% of population contain bacteria in food vacuoles (Table 2).

Dinospores (that is, invasive single-cell stage) of parasitic Syndiniales Group II were generally absent or present in low numbers, except for station five ($4.6 \pm 0.8 \times 10^7$ cells m^{-2}; $2.2 \pm 0.3 \times 10^9$ cells m^{-3}), where they contributed ca. 3% to the total numbers of picoeukaryotes (Figure 4). Vermiforms (that is multicellular stage released from a host's cell) were not observed.

The observed differences between Barrow Strait and the vicinity of Cornwallis Island with respect to the numbers of the picoeukaryotic groups studied were also established by non-metric multidimensional scaling (Figure 5a). However, station nine also grouped with stations two and three from the Barrow Strait at 60% of similarity. The remaining stations grouped at 40% of similarity, but at 60% stations four and five were separated. Non-metric multidimensional scaling analysis performed on percentage contributions of the investigated groups to total numbers of picoeukaryotes resulted in different grouping (Figure 5b). All stations could be grouped at similarity level 60%. At 80% of similarity level, three cluster could be defined: C1 (stations 6, 7 and 9–11), C2 (stations 12, 13) and C3 (stations 2, 4 and 8). Stations 3 and 5 did not fell into any of clusters. Cluster C1 could be characterized by moderate contribution of chlorophytes (5.5–10.5%), cryptophytes (10–16%) and of MAST-2 stramenopiles (2–5%), cluster C1 was formed by stations with very high contribution of cryptophytes ($>25\%$), and cluster C3 was characterized by high contribution of chlorophytes ($>12\%$), cryptophytes

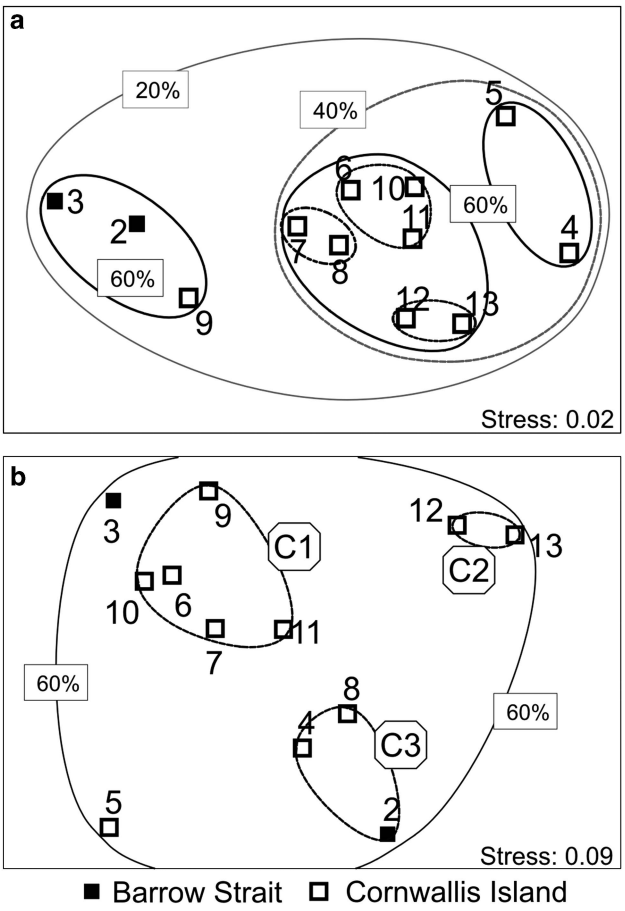

Figure 5 Configuration plots of non-metric multidimensional scaling based on Bray-Curtis similarity. (**a**) untransformed numbers (**b**) percentage contribution to total picoeukaryote numbers. Lines encircle stations at percentage similarity level given in rectangles, unlabeled lines corresponds to 80% of similarity. Labels in hexagons in panel (**b**) are names for three clusters defined at 80% similarity level (see text).

($>15\%$) and for station two and eight also bolidophytes ($>2\%$). Station three was quite similar to stations grouped in cluster C1, but had higher percentage contribution of haptophytes ($>4\%$). Station five, on the other hand, was different from the other stations, as it have the highest percentage contribution of MAST-2 ($>10\%$), MAST-6 ($>1\%$) stramenopiles, and of Syndiniales Group II (3%).

Correlations with abiotic and biotic variables

The total numbers of picoeukaryotes were positively and significantly correlated with thickness of snow cover (Spearman rank correlation test, Table 3). However, among eleven of the investigated picoeukaryote groups, only chlorophytes and MAST-2 stramenopiles were also correlated with this variable. Sea-ice chl $a > 5\,\mu m$ was positively correlated with sea-ice concentrations of $NO_2^- + NO_3^-$ and PO_4^{3+} (Table 3). Chl $a > 5\,\mu m$ was further positively correlated with numbers of bacteria, picoeukaryotes, chlorophytes and MAST-2 stramenopiles (Table 3). Inorganic nutrients

Table 3 Correlation matrix for selected environmental variables and picoeucaryote groups

Parameter	*Picoeukaryotes (Euk516)*	*Chlorophyta (Chlo02)*	*Cryptophyta (CryptB)*	*Bolidophyceae (Bolido02)*	*Haptophyta (Prym02)*	*MAST-1 (NS1 A-C)*	*MAST-2 (NS2)*	*Chlorophyll-a (>5 μm)*
Ice thickness	0.10	0.21	0.28	0.45	−0.49	−0.32	0.02	0.31
Snow cover	**0.72****	**0.66***	0.38	−0.27	0.04	0.39	**0.76****	0.41
Chlorophyll-*a* (>5 μm)	**0.64***	**0.57***	0.39	−0.33	−0.15	−0.27	**0.59***	—
Bacteria	0.30	0.37	0.03	0.08	−0.07	−0.05	0.41	**0.72****
$NO_3 + NO_2$	0.37	0.50	0.46	0.57	−0.39	−0.27	0.28	**0.78***
PO_4	0.28	0.50	0.15	0.21	−0.21	−0.36	0.39	**0.75****
$SiOH_4$	0.15	0.56	0.30	0.36	−0.41	−0.49	0.11	0.56
Chlorophyta (Chlo02)	**0.88****	—						
Cryptophyta (CryptB)	**0.80****	**0.72****	—					
Bolidophyceae (Bolido02)	−0.05	0.21	0.17	—				
Haptophyta (Prym02)	−0.26	−0.24	−**0.62***	−0.07	—			
MAST-1 (NS1 A–C)	0.01	−0.10	0.08	0.15	0.15	—		
MAST-2 (NS2)	**0.85****	**0.70***	0.44	−0.16	−0.08	0.19	—	

Spearman rank correlation coefficients are given. Significance level of bold entries is marked by asterisks: $*P<0.05$, $**P<0.01$.

and bacterial numbers were not correlated with any of the studied picoeukaryote groups.

Three most abundant groups: chlorophytes, cryptophytes and MAST-2 stramenopiles were significantly correlated with total picoeukaryote numbers (Table 3). Numbers of chlorophytes were also positively correlated with numbers of cryptophytes and MAST-2 stramenopiles. Finally, numbers of cryptophytes and haptophytes were negatively correlated (Table 3).

Discussion

Arctic sea ice is an ecosystem threatened by ongoing global warming. Positive feedbacks, including decrease in surface albedo due to ice- and snow-melting, accelerate the climate change in this region. In result, the Arctic temperature is rising two to three times faster than the global temperature (Trenberth *et al.*, 2007). First-year sea ice is replacing rapidly vanishing multi-year ice, becoming dominant form of ice in the Arctic (Polyakov *et al.*, 2012). These changes affect the whole Arctic ecosystem, as it is first-year sea ice that supports most active algae communities: an important source of nutrition for both, pelagic and benthic organisms. Larger area of the first-year sea ice cover is predicted to increase energy available for pelagic organisms, but to lower quality of organic matter exported to the bottom (Wassmann and Reigstad, 2011). These scenarios, however, do not account for possible changes in microbial processes within sea ice, which are still inadequately described. Picoeukaryotes are among the least known protistan groups in the sea ice. The first studies on the diversity of sea-ice protists (not only picoeukaryotes) have shown sea-ice communities to consist of various phylotypes (Eddie *et al.*, 2010; Bachy *et al.*, 2011; Majaneva *et al.*, 2012; Comeau *et al.*, 2013). Our study complements these recent results with the first report on the quantitative distribution of particular groups of picoeukaryotes in first-year sea ice, including percentage of cells actively feeding by phagocytosis. However, as every method, CARD–FISH also suffers from limitations. The most important are: group coverage by a probe (percentage of sequences within a group that are targeted by the probe), and hits outside a group (Amann and Fuchs, 2008). All but two probes used in our study has coverage >80% (Supplementary Table S1), and all but one (Bolido02) matches all sequences retrieved from sea ice in the previous studies (Majaneva *et al.*, 2012). This indicates that use of CARD–FISH technique enabled reliable estimation of numbers of most of the investigated groups, providing baseline data on the distribution and relative importance of these groups in the sea ice.

Importance of picoeukaryotes in sea ice

The total numbers of picoeukaryotes in sea ice, estimated with the Euk516 probe (Figure 3b), were at least an order of magnitude higher than numbers of small protists (<5 μm) determined either by flow cytometry ($0.4–3.1 \times 10^9$ cells m^{-3}, Różańska *et al.*, 2008) or epifluorescent microscopy ($0–6.6 \times 10^9$ cells m^{-3}, (Riedel *et al.*, 2007; Bachy *et al.*, 2011). Flow cytometry was applied to enumerate only plastidic protists, which explains the observed discrepancy. Microscopic discrimination between 4′,6-diamidino-2-phenylindole dihydrochloride-stained picoeukaryotes and large bacteria is challenging. Application of CARD–FISH and Euk516 probe significantly increased detection of picoeukaryotes in the North Sea (Beardsley *et al.*, 2005), and likely accounted for the higher numbers in our study.

Plastidic eukaryotes other than diatoms are minor component of sea-ice communities during the spring

algal bloom (Riedel *et al*., 2007). Chlorophytes and cryptophytes were the most numerous groups (Figure 4). Sequences of chlorophytes were also retrieved from multi-year sea ice close to the North Pole (Bachy *et al*., 2011), and first-year sea ice in the Beaufort Sea (Eddie *et al*., 2010; Comeau *et al*., 2013) and the Baltic Sea (Majaneva *et al*., 2012). These phylotypes were related to *Chlamydomonas*, *Mantoniella*, *Bathycoccus*, *Pyramimonas* and *Microsomonas*. The cold-adapted picoplanktonic *Micromonas* strain CCMP2099, which is the most frequent chlorophycean phylotype in Arctic waters (Terrado *et al*., 2011), grows at low light regimes (Lovejoy *et al*., 2007). The observed positive correlation between numbers of chlorophytes and snow cover thickness (Table 2), may indicate that also sea-ice chlorophytes are adapted to low light levels, and could be photoinhibited under low snow cover.

Cryptophytes were the second most numerous group (Figure 4). Cryptophyte phylotypes related to *Teleaulax* and *Hemiselmis* were found in first-year sea ice in the Canadian Beaufort Sea (Comeau *et al*., 2013) and the Baltic Sea (Majaneva *et al*., 2012), but not in multiyear ice close to the North Pole (Bachy *et al*., 2011). Sequences of cryptophytes have been recovered from size-fractionated (0.2–3 μm) Arctic waters (Terrado *et al*., 2011). Our results show that picocryptophytes are very numerous also in sea ice.

The presence of bolidophytes in our samples is particularly interesting. This recently discovered picoeukaryotes (Guillou *et al*., 1999a) form one phylogenetic clade with Parmalean algae *Triparma* (Ichinomiya *et al*., 2011), which is also targeted by probe Bolido02. Phylotypes related to bolidophytes were previously reported from pancake sea ice (Majaneva *et al*., 2012). This young form of sea ice contains many pelagic protists entrapped during freezing (Różańska *et al*., 2008). We confirmed that bolidophytes may survive winter in sea ice until spring. Considering that probe Bolido02 has mismatches to bolidophycean sequences of Arctic origin, the actual numbers of these organisms in sea ice could be higher than reported here.

HPLC-based studies of sea-ice algae showed photosynthetic prymnesiophytes to be relatively abundant during spring bloom in sea ice in the Canadian Beaufort Sea (Alou-Font *et al*., 2013). In contrast, our results showed haptophytes to be minor component of picoeukaryotic communities in sea ice, especially in the vicinity of the Cornwallis Island (Figure 4). Haptophycean sequences were also relatively rare in diversity studies, and these affiliated with *Chrysochromulina* sp., which is not a picoeukaryotic genus (cell size >6 μm, (Throndsen *et al*., 2007)). There is no report on *Phaeocystis* spp. and Pavlovaphyceae in sea ice (Eddie *et al*., 2010; Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). The latter was relatively abundant at station one in the Barrow Strait, where they contributed in $>60\%$ to numbers of picohaptophytes.

Pedinellids were present at two station only, and in low numbers (Figure 4). Most pedinellids are nano-sized organisms (4–15 μm, (Throndsen *et al*., 2007; Piwosz and Pernthaler, 2010)), and sequences of pedinellids were rare in sea-ice samples (Eddie *et al*., 2010; Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). This indicates these flagellates may be uncommon in sea ice.

Heterotrophic nanoeukaryotes generally make up a major part of non-diatom sea-ice communities in spring (Riedel *et al*., 2007). Majority of sequences of heterotrophic eukaryotes retrieved from sea ice affiliated with ciliates, dinoflagellates, Syndiniales groups I and II and cercozoans (Eddie *et al*., 2010; Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). Strains of *Cryothecomonas* have been frequently isolated from polar waters and sea ice (Thaler and Lovejoy, 2012), and related sequences form substantial part of 18S RNA gene libraries from sea ice (Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). *Cryothecomonas* probes were unavailable at the time of this study (Thaler and Lovejoy, 2012), thus we could not enumerate this group. *Cryothecomonas*-related protists are considered to grazed on other protists, and their cell size is usually above picoeukaryotic range (2.5–29 μm, Thaler and Lovejoy, 2012) Therefore, it could be possible that they had been not very numerous in the picoeukaryotic fraction.

We found two groups of bacterivorous picoeukaryotes: MAST-2 and MAST-1 (Massana *et al*., 2006). MAST-1 flagellates appear to be cosmopolitan with preference towards polar waters (Lovejoy *et al*., 2006), and MAST-1C phylotypes were also reported from sea ice in the Baltic Sea (Majaneva *et al*., 2012). However, MAST-1 flagellates were not very prominent in sea ice in the Canadian Arctic Archipelago (Figure 4). Because of these low numbers, we did not increased the resolution of our study by separately applying probes specific for every clade (NS1A, NS1B and NS1C Table 1 and Supplementary Table S1). In contrast, MAST-2 flagellates were relatively numerous in first-year sea ice (Figure 4). They have been reported to be present in the water column worldwide in very low numbers ($<10^6$ cells m^{-3}), including the Arctic and Antarctic regions (Massana *et al*., 2006), but not in sea ice (Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). We also found MAST-6 stramenopiles, but in very low numbers. These flagellates have been reported to have two morphotypes, both outside the picoekuoryotic range (Piwosz and Pernthaler, 2010), and were not reported from sea ice (Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). The MAST-6 probe coverage has decreased to $<50\%$ since its publication (Supplementary Table S1), which negatively affected our estimates for this group.

Finally, we also confirmed the presence of parasitic Syndiniales Group II, which often substantially contribute to 18S rRNA gene libraries from sea ice (Bachy *et al*., 2011; Majaneva *et al*., 2012; Comeau *et al*., 2013). These flagellates were generally present in low numbers except for station five, where they were abundant (Figure 4). The coverage of the probe

Alv01 (Chambouvet *et al.*, 2008) decreased since its publication (Supplementary Table S1), hence the actual numbers were likely higher than reported here. Presence of vermiforms would have confirmed whether this group is indeed parasitic for sea-ice algae. However, the absence of vermiforms may have resulted from distortion during samples processing. Sea ice could be also a source of parasites to water column upon melting.

Our studies on numbers and distribution of picoeukaryotic groups in sea ice enabled deduction on functional roles of picoeukaryotes in sea ice. We confirmed the presence of bacterivorous phylotypes from groups MAST-1 and MAST-2 (Massana *et al.*, 2006). Bacterivory is well-documented in sea ice (Laurion *et al.*, 1995; Riedel *et al.*, 2007). MAST-1 and MAST-2 numbers were not correlated with numbers of bacteria, even though almost 30% of MAST-2 cells contained bacterial cells in food vacuoles (Table 2). Nevertheless, numbers of MAST-2 were significantly correlated with numbers of chlorophytes (Table 3). This would indicate that other picoeukaryotes may be more important food sources than bacteria, and agrees with the view that bacteria in sea ice are rather bottom-up than top-down controlled (Vezina *et al.*, 1997; Riedel *et al.*, 2007).

Mixotrophic algae also may contribute substantially to overall bacterivory (Zubkov and Tarran, 2008). Mixotrophic phylotypes made up 16% of 18S rRNA libraries (Comeau *et al.*, 2013), but the contribution of mixotrophic algae to total bacterivory in sea ice remains unknown. All investigated groups of algae were found to contain bacteria in food vacuoles (Table 2). Their widespread presence in sea ice and their apparent independence from light conditions (deduced from the lack of correlation with snow cover thickness, Table 3), suggested potential mixotrophic nutrition in sea ice. Such strategy may be advantageous due to low nutrients' concentrations, but surplus of bacteria in sea ice in spring (Vezina *et al.*, 1997; Riedel *et al.*, 2007). On the other hand, percentage contribution of algal cells containing bacteria inside food vacuoles was generally $<20\%$, except for less numerous haptophytes (Table 2). Moreover, none of these groups correlated with bacterial numbers (Table 3). Thus, importance of mixotrophy by picoeukaryotes still remained to be confirmed.

The observed co-occurrence of phylogenetically and functionally diverse (that is, autotrophs, mixotrophic and heterotrophic phagotrophs and parasites) groups reflects the varied roles picoeukaryotes have in sea-ice communities. However, the specific probes used in this study identified only 20–50% of total picoeukaryotes enumerated with the probe Euk516. Therefore, a large part of the picoeukaryote communities still remains to be characterized and likely comprise cells affiliated with Fungi, Amoebozoa and Rhizaria (for example, *Cryothecomans* (Eddie *et al.*, 2010; Bachy *et al.*, 2011; Majaneva *et al.*, 2012; Comeau *et al.*, 2013)). This emphasizes the need for more research on diversity and roles of picoeukaryotes in sea-ice food webs.

Impact of environmental factors on numbers and distribution of picoeukaryotes in sea ice

Numbers of picoeukaryotes significantly differed between the two investigated regions (Figure 3b). The reason could have been (i) much higher nutrients' concentrations in the vicinity of Cornwallis Island than in Barrow Strait (Figure 2, Supplementary Table S2), (ii) difference in ice type (land-fast ice vs drift pack ice, Supplementary Table S2). These factors likely caused the observed difference in biomass of sea-ice algae (Figure 2). Drift pack ice and land-fast ice have been shown to bear distinct protistan communities (Comeau *et al.*, 2013). Higher nutrients concentrations resulted in higher values of chl $a>5\,\mu m$ (Table 3). As sea-ice algae are fueling an active microbial food web by providing carbon sources (Vezina *et al.*, 1997; Riedel *et al.*, 2007), higher biomasses of sea-ice algae were associated with higher numbers of bacteria, total numbers of picoeukaryotes, chlorophytes and MAST-2 stramenopiles (Table 3). However, concentrations of nutrients were not directly correlated with total numbers of picoeukaryotes or with numbers of any of the investigated groups. The only variable significantly correlated with numbers of picoeukaryotes (and of chlorophytes and MAST-2 stramenopiles, Table 3) was thickness of snow cover. Snow cover limits light available for sea-ice organisms, which likely was the factor that directly influenced picoeukaryote communities. Complex relationships, shifting from positive to negative correlations at lower ($<20\,cm$) and higher ($>20\,cm$) snow cover depths respectively, have been reported in the same region (Mundy *et al.*, 2005).

Non-metric multidimensional scaling analysis did not resemble spatial distribution of stations (Figure 5), neither could be matched to any of the investigated environmental factors. Although one of the reasons could be low taxonomic resolution of our data, it may also points to possible effect of interspecies interaction on distribution of the investigated groups and picoeukaryote community composition. This view was also supported by the observed correlations between different groups of picoeukaryotes (Table 2).

Conclusion

This study showed that picoeukaryotes are important members of sea-ice communities, with diverse functional roles that support complex microbial food webs within the ice. We found auto- and mixo-trophic picoalgae (chlorophytes, cryptophytes, haptophytes and bolidophytes), heterotrophic flagellates (stramenopiles MAST-1 and MAST-2) and parasites (Syndiniales Group II) to be potentially numerous component of microbial communities in Arctic first-year sea ice in spring. The distribution of some, but not all, groups of picoeukaryotes in bottom

sea ice was related to snow cover thickness, highlighting the complexity and dynamic nature of the sea-ice physical and biological environment. Our data on the distribution and functional roles of picoeukaryotes in first-year sea ice provide a baseline for future investigations of changes in picoeukaryotic communities. Presently, the absence of comparable data sets on sea-ice Arctic picoeukaryote communities limits our understanding of Arctic ecosystem.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by IGS (International Governance Strategy, Fisheries and Oceans Canada) and the National Science Engineering Research Council (NSERC) Discovery Grants to CM. Logistical support was provided by Polar Continental Shelf Program (PCSP, Natural Resources Canada). Our sincere thanks go to the PCSP manager and staff for their invaluable help during the field program. We also thank Kelly Hille for help in the field, Line McLaughlin for nutrient analyses, Claude Belzile for flow cytometry analyses and Lena Szymanek for preparing the map with location of the sampling stations (Figure 1). This study was also supported by a grant from the Polish Ministry of Science and Higher Education, grant no 695/N-ARCTICNET/2010/0. This is a contribution of the Freshwater Institute, Fisheries and Oceans Canada, the National Marine Fisheries Research Institute (Poland), the Polish Academy of Sciences and the Arctic in Rapid Transition (ART) Initiative.

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