RESEARCH/REVIEW ARTICLE

Insight into protist diversity in Arctic sea ice and melt-pond aggregate obtained by pyrosequencing

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Abstract

Protists in the central Arctic Ocean are adapted to the harsh environmental conditions of its various habitats. During the *Polarstern* cruise ARK-XXVI/3 in 2011, at one sea-ice station, large aggregates accumulated at the bottom of the melt ponds. In this study, the protist assemblages of the bottom layer of the sea-ice and melt-pond aggregate were investigated using flow cytometry and 454-pyrosequencing. The objective is to provide a first molecular overview of protist biodiversity in these habitats and to consider the overlaps and/or differences in the community compositions. Results of flow cytometry pointed to a cell size distribution that was dominated by 3–10 μm nanoflagellates. The phylogenetic classification of all sequences was conducted at a high taxonomic level, while a selection of abundant ($\geq$1% of total reads) sequences was further classified at a lower level. At a high taxonomic level, both habitats showed very similar community structures, dominated by chrysophytes and chlorophytes. At a lower taxonomic level, dissimilarities in the diversity of both groups were encountered in the abundant biosphere. While sea-ice chlorophytes and chrysophytes were dominated by *Chlamydomonas/Chloromonas* spp. and *Ochromonas* spp., the melt-pond aggregate was dominated by *Carteria* sp., *Ochromonas* spp. and *Dinobryon faculiferum*. We suppose that the similarities in richness and community structure are a consequence of melt-pond freshwater seeping through porous sea ice in late summer. Differences in the abundant biosphere nevertheless indicate that environmental conditions in both habitats vary enough to select for different dominant species.

To access the supplementary material for this article, please see supplementary files under Article Tools online.

The Arctic Ocean is a harsh environment and subject to seasonal variations in temperature, sea-ice concentration and solar radiation. These strong variations have the potential to change protist community composition and to promote the occurrence of species that are especially adapted to the local constraints (Sakshaug & Slagstad 1991; Li et al. 2009; Tremblay et al. 2009). In particular, small cells with faster rates of nutrient uptake and little metabolic requirements are most able to adapt to changes in these abiotic conditions and hence dominate the Arctic Ocean (Grover 1991; Hein et al. 1995; Lovejoy et al. 2007). Protists in the Arctic Ocean are found in the deep sea as well as within melt ponds and sea ice. Abiotic environmental factors vary strongly between these habitats. Wide variations in chemical and physical parameters control the sympagic protist assemblages of brine channels in sea ice (Horner et al. 1992; Gradinger 1999; Schünemann & Werner 2005). Arrigo (2003) reported a strong relation between sea-ice thickness and protist community composition, with a greater biomass at the

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sea-ice–water interface. Sea-ice communities include micro- and nanoplankton species, such as diatoms, dinoflagellates and ciliates, but can also contain picoeukaryotes (Medlin & Priddle 1990; Thomas & Dieckmann 2002; Piwosz et al. 2013). In summer, when snow starts melting, melt ponds form and cover up to about 80% of the ice floe area (Luthje et al. 2006). So-called open melt ponds are connected with seawater and therefore show a higher salinity (ca. 29 PSU) than closed melt ponds that include freshwater (Gradinger 2002; Lee et al. 2011). Consequently, open ponds are characterized by marine protist species whereas closed ponds hold freshwater species. During an expedition of the RV *Polarstern* in summer 2011, we observed large numbers of melt-pond aggregates in the Atlantic sector of the Arctic Ocean. The number of observed aggregates declined towards the Pacific sector. Records of melt-pond aggregates are nothing new. Gran (1904) and Nansen (1906) first reported the occurrence of small algae flakes (aggregates) at the bottoms of melt ponds. In September 2008, Lee et al. (2012) found aggregates in melt ponds of the Chukchi Sea and Canadian Basin. Aggregates are chemical microenvironments that are enriched in nutrient concentration (Alldredge & Cohen 1987; Shanks & Trent 1979). They can be composed of either living or non-living material and can harbour dense attached microbial communities (MacIntyre et al. 1995; Silver et al. 1998). The formation of such aggregates is suggested to occur primarily through physical processes like collision and sticking of particles (Alldredge & Jackson 1995). In particular, calm water is supposed to favour the aggregation process (del Negro et al. 2005).

Biodiversity studies of Arctic protist communities and particularly of those that inhabit sea ice and melt ponds are lacking (Gradinger 2002; del Negro et al. 2005; Precali et al. 2005; Werner et al. 2007; Lee et al. 2011; Piwosz et al. 2013). We used a molecular approach for diversity analysis of the small protists (e.g., picoplankton and nanoplankton), firstly because they are assumed to be major contributors to these communities and secondly because the high detrital load and the degraded condition of protist cells in melt-pond aggregates can hamper microscopy analysis. 454-Pyrosequencing allows assessment of microbial communities at high resolution given sufficiently deep taxon sampling (Margulies et al. 2005; Stoeck et al. 2010). The method suffers from a comparatively high intrinsic error rate that can be compensated for by a rigorous sequence processing step prior to analysis (Huse et al. 2007; Huse et al. 2010; Kunin et al. 2010). The use of ribosomal genes (rRNA genes), such as the 18S rDNA, is well suited for molecular diversity analysis. In this study, we used the hypervariable V4 region of the 18S rDNA (Nickrent & Sargent 1991; Ebenezer et al. 2012).

The objective of the study is to provide a first molecular insight into the diversity of melt-pond aggregate and a deeper one into the diversity of sea-ice bottom protist communities, including a survey of their cell size distribution. Furthermore, we want to assess potential connections between these communities, by focusing on species overlaps. To our knowledge, an investigation of the genetic diversity of the sea-ice bottom layer and melt-pond aggregate has not been accomplished so far.

Material and methods

Study area and sampling procedure

Samples were collected on 14 August 2011, during the ARK-XXVI/3 expedition of RV *Polarstern* to the central Arctic Ocean. The sampling site was located at 59°55.91′E, 85°48.19′N (Fig. 1). Melt-pond quantity, ice concentration and ice thickness were categorized to assess the condition of sea ice (data made publicly available by the Alfred Wegener Institute: doi: 10.1594/PANGEA.803312). Sea ice was collected with a Mark II 9 cm inner diameter ice corer (Kovacs Enterprise, Roseburg, OR, USA). The ice cores were sectioned into 10-cm vertical slices. For DNA analysis, the bottom section was diluted with 0.2 μm filtered seawater (200 ml for each cm of ice) and thereafter allowed to melt over the following 24 h at a constant 4°C temperature and under low light conditions. A volume of 250 ml was size fractionated at 200 mbar (0.4–3 μm, 3–10 μm and larger than 10 μm) on Millipore isopore membrane filters (Billerica, MA, USA) and immediately frozen at −80°C. Aggregates (Supplementary Fig. S1), from the bottom of the melt ponds, were collected in a glass vacuum flask with a hand-held vacuum pump. This way the samples were diluted with the ambient melt-pond water. The particulate organic matter and chlorophyll *a* of melt-pond water and aggregate slurry were compared to estimate the potential bias introduced by this sampling step. A volume of 10 ml was size fractionated from this slurry as described previously. Sequential filtration was conducted in order to obtain a best possible representation of all cell sizes in the molecular approach. The filtration facilitates separate amplification of the size fractions in the subsequent polymerase chain reaction step. This minimizes the danger of under-amplifying picoeukaryotes, due to the limited gene copy number (Zhu et al. 2005).

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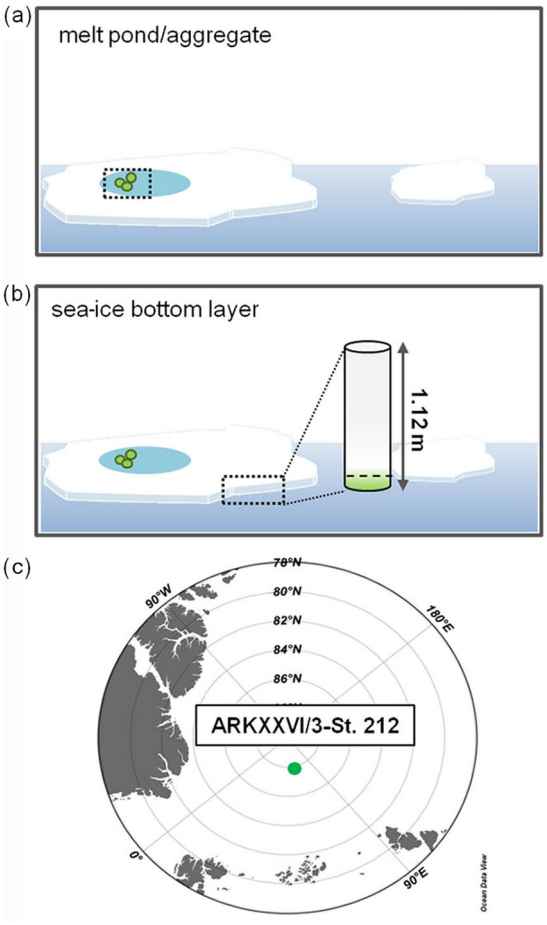

Fig. 1 (a) Melt-pond aggregate and (b) sea-ice bottom layer (sea-ice–water interface; 10 cm) habitats. (c) The position of sampling station 212 in the Arctic Ocean (ARKXXVI/3 cruise).

Flow cytometry

Small algae (<50 μm) were directly counted on board with a BD Accuri C6 flow cytometer (San Jose, CA, USA). The algae were identified based on their orange and red fluorescence according to Marie et al. (2005) and were classified in three size groups of 0.6–3 μm, 3–10 μm and >10 μm.

DNA processing

DNA extraction and amplification of the V4 region (ca. 630 bp) were conducted according to Kilias et al. (2013). Purified polymerase chain reaction products were sent to GATC Biotech, Constance, Germany, where the final pyrosequencing was done with a Roche 454 Genome Sequencer FLX System (Penzberg, Germany).

Quality control and 454-pyrosequencing data processing

Raw sequences were processed to increase the quality and to decrease the effect of low-quality reads on diversity estimates. Sequences shorter than 300 bp and longer than the target fragment (ca. 670 bp) were excluded from the data set. Furthermore, sequences were screened by means of Perl scripts to remove those starting with incorrect/incomplete F-primer sequences, those with more than one ambiguous base (N) and those with homopolymers of seven or more successive bases (≥ 7 hps). Huse et al. (2007) showed that the removal of sequences with ambiguous bases can reduce the error rate from initial 0.5 % to 0.25 %, while homopolymers of 5 hps were found to contribute the highest proportion of induced errors in the Genome Sequencer FLX System (Behnke et al. 2011). Chimeras were detected and excluded using UCHIME 4.2.40 software (Edgar et al. 2011), which uses the SSU Ref 108 (SILVA) reference database. A random subsampling to the minimum quality sequence number (after quality processing) was carried out because sequencing depths at the two habitats differed by a factor of two. Final-processed sequences were clustered (furthest neighbour algorithm) into artificial operational taxonomic units (OTUs) with a threshold of 97%, using the software package Lasergene 10 Seqman Pro (DNAStar, Madison, WI, USA). All singletons, defined as OTUs composed of uniquely occurring sequences, were removed to evade possible errors induced by the assembly of the sequencing progress. We used consensus sequences of the OTUs to further reduce the number of sequencing errors in the diversity analysis. In order to estimate the potential bias by using consensus instead of original sequences, we compared their taxonomic information by performing BLAST searches and constructing a phylogenetic tree based on consensus sequences, original sequences and BLAST search sequences (Supplementary Table 1, Supplementary Fig. S2).

Consensus sequences were finally placed into a quality-trimmed reference tree built up from a selection of 1200 high-quality sequences from the SSU Ref 111 (SILVA) database, including representatives of all the main eukaryotic phyla, using the bioinformatics pipeline PhyloAssigner (Vergin et al. 2013). This procedure has the benefit of preserving as much phylogenetic signal as possible. Multiple phylotypes that clustered to the same genus but differed by at least 3% were numbered. Relative abundances of high quality reads were calculated and used for discrimination between abundant and rare OTUs that accounted for $\geq 1\%$ and $<1\%$ of the total read number.

The 454-pyrosequencing sequences were deposited at GenBank (SRP033264).

Results

Physical and chemical environment

Two habitats, sea ice (bottom layer) and melt pond (aggregate), were sampled at one station in the Arctic Ocean and analysed for protist biodiversity (Fig. 1). Sea-ice coverage was 100% with a thickness of approximately 0.7–1.2 m. An ice core of 1.12 m length was drilled, of which the last 10 cm (sea-ice–water interface) were used for analysis. The areal coverage of melt ponds reached 40%. This station was chosen since it represented the greatest accumulation of aggregates at the bottom of the melt ponds. Aggregate sampling was carried out in an almost-closed melt pond. Particulate organic matter and chlorophyll *a* of the melt-pond water contributed less than 1% of the aggregate, suggesting that the analysed biomass mainly came from the aggregate (data not shown).

Flow cytometry

Flow cytometer measurements revealed three size classes of algae in the aggregates and ice cores (Fig. 2). Cells larger than 50 µm cannot be identified with the flow cytometer due to the set-up of the instrument. In any case, most of the cells in the largest size class were still less than 25 µm. Nano-algae (3–10 µm) dominated both biomes with 74 and 84% in the aggregate and in the bottom sea ice. Pico-algae in the melt-pond aggregate had a higher proportion, at almost 23%, than in the ice core.

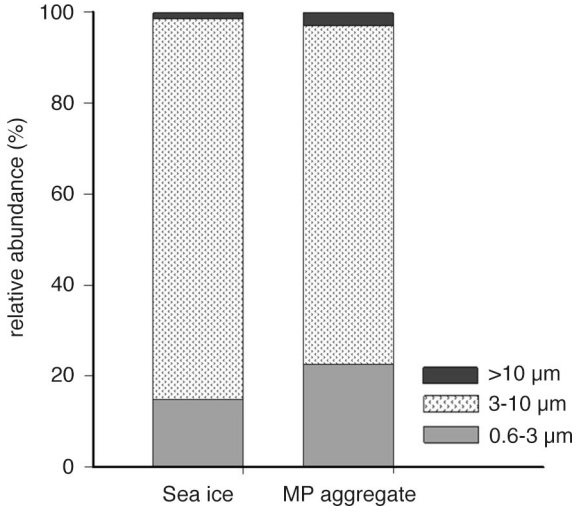

Fig. 2 Histogram of the protist cell size distribution in the sea-ice bottom layer and melt-pond aggregate obtained by flow cytometry.

454-Pyrosequencing

The sequencing depth differed by a factor of two between the two habitats. While the sea-ice sample recorded 27 289 raw reads, the melt-pond aggregate yielded 43 826 sequences (Table 1). Quality filtering removed 33% of the initial sea ice reads and 35% of the melt-pond aggregate reads. Subsampling to the minimum sequence number (sea ice) reduced melt-pond aggregate sequences to 17 193 reads. The clustering of quality-screened subsampled sequences at a 97% identity threshold, presented 358 OTUs for the sea-ice sample and 440 OTUs for the melt-pond aggregate.

The Venn diagram in Fig. 3 presents the overlap of 140 OTUs between the habitats. Numbers of unique OTUs were greatest for the melt-pond aggregate (330) and least for the sea-ice bottom layer (218).

The relative abundance of the major taxonomic group distribution, including all phylotypes, is presented in Fig. 4, while a more detailed classification of abundant phylotypes ($\geq$1% relative abundance) is presented in Table 2. Chrysophytes and chlorophytes dominated the community structures in both habitats with 96% (chrysophyte: 69%; chlorophyte: 27%) in the sea ice and 74% (chrysophyte: 47%; chlorophyte: 28%) in the aggregate sample. Stramenopiles, as bacillariophytes, were weakly represented and ranged between 0.3 and 0.8% in both habitats. Pelagophytes accounted for greater percentages (2.6%) in the melt-pond aggregate than were recovered in the sea-ice bottom layer ($<$1%). Alveolates in the sea ice showed a small contribution of about 1% and were mostly represented by dinoflagellates. In the aggregate, alveolates were of greater abundance, contributing 15.9% dinoflagellates and 3.2% ciliates.

The distribution of species within major taxonomic groups was characterized by a few phylotypes defined by many sequences, and many phylotypes of low sequence

Table 1 Summary of 454-pyrosequencing data processing, showing the quality filtering and the number of operational taxonomic units (OTUs). Quality check includes size trimming ($\geq$300 bp), denoising and removal of chimeras. OTUs were generated with a 97% threshold for read identity.

	Sea ice	Melt pond
Raw reads	27 289	43 826
Removal of reads:		
$<$300 bp	167	406
Homopolymers and ambiguous N	8719	11 601
Chimeras and metazoan	1210	3363
Final read number:	17 193	28 456
Subsampling	17 193	17 193
After removal of singletons	14 699	14 072
OTU (97%)	358	440

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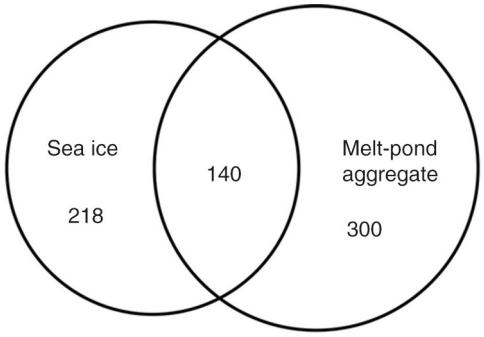

Fig. 3 Venn diagram of the operational taxonomic unit (OTU) overlap between the sea-ice bottom layer and melt-pond aggregate habitats. OTUs were generated with a threshold of 97% identity.

number. Concerning the whole biosphere (all reads), the abundant biosphere (≥1% of total reads) was dominated by chlorophyte and chrysophyte species. However, species composition differed between the sea-ice bottom layer and melt-pond aggregate. While *Carteria* sp. was particular abundant in the melt-pond aggregate (20%), phylotypes, affiliating to Chlamydomonadaceae were more abundant in the sea ice. A precise genus-level-specific characterization of Chlamydomonadaceae phylotypes was not possible because sequence annotations resulted in two genera of similar probability (99%), *Chlamydomonas* and *Chloromonas*. Chlamydomonadaceae 1 accounted for the greatest percentage of ca. 16% in the sea-ice sample and was also the only recorded phylotype in the abundant biosphere of the melt-pond aggregate. Chrysophytes in the melt-pond aggregate were more diverse, with six species, than in the sea-ice bottom layer, with only two species. In this regard, one species, *Ochromonas* sp. 1 (58%), strongly dominated the sea-ice sample while species in the melt-pond sample were more evenly

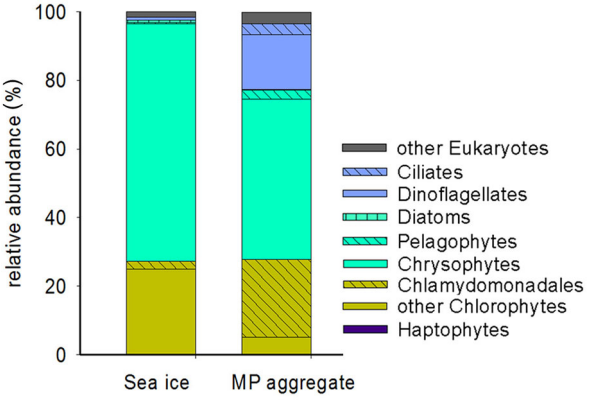

Fig. 4 Histogram showing the relative abundances (%) of major taxonomic groups, obtained by 454-pyrosequencing read distribution in the sea-ice bottom layer and melt-pond aggregate.

Table 2 Classification of abundant phylotypes (≥1% of total reads) in the sea-ice bottom layer and melt-pond aggregate. Values are given in percent (relative abundance). *r* refers to the occurrence of the phylotype in the rare biosphere (<1% of total reads). Phylotypes with a similar taxonomic classification were numbered.

Taxonomic classification	Sea-ice bottom 10 cm	Melt-pond aggregate
Alveolate		
Unc.[a] Gymnodiniales	*r*	12.9
Peridinium sp.	*r*	1.8
Strombidium basimorphum	—	1.4
Chlorophyte		
Carteria sp.	1.2	19.8
Chlamydomonadaceae 1	15.9	2.9
Chlamydomonadaceae 2	1.6	*r*
Chlamydomonadaceae 3	3.45	*r*
Chlamydomonadaceae 4	1.4	*r*
Stramenopile		
Dinobryon faculiferum 1	*r*	8.9
Dinobryon faculiferum 2	*r*	2.6
Ochromonas tuberculata	*r*	2.7
Ochromonas sp. 1	58.1	8.7
Ochromonas sp. 2	2.0	13.3
Ochromonas sp. 3	*r*	1.3
Pelagomonas sp.	*r*	2.6

[a]Uncultured.

distributed with *Ochromonas* sp. 2 (13%), *Ochromonas* sp. 1 (9%) and *Dinobryon faculiferum* 1 (9%) accounting for the highest proportions. Alveolates were not abundant in the sea-ice bottom layer but represented by three species in the melt-pond aggregate, an unclassified Gymnodiniales species (13%), *Peridinium* sp. (2%) and *Strombidium basimorphum* (1%).

Discussion

We investigated the coupling of protist biodiversity between the sea-ice bottom layer and melt-pond aggregate, using 454-pyrosequencing of the 18S rDNA (V4 region). Since studies on melt-pond and sea-ice protist diversity are scarce and in most parts based on non-molecular approaches (Gradinger 2002; del Negro et al. 2005; Precali et al. 2005; Werner et al. 2007; Lee et al. 2011), ours is a first molecular overview, including small cell size protists. In addition, we analysed cell size distributions in both habitats by applying flow cytometry.

Cell size distributions between the sea-ice bottom layer and melt-pond aggregate were similar and showed a dominance of cells ranging between 3 and 10 µm. Cells larger than 10 µm were weakly represented. Although flow cytometer analysis missed cell sizes >50 µm, our data suggest a stronger representation of nanoplankton overall in both habitats, compared to the pico- and

microplankton. For the sea-ice sample, this size distribution is surprising in view of numerous reports that diatoms in the size range 10–100 μm dominate sea-ice communities (Riaux-Gobin et al. 2003; Werner et al. 2007; Winder et al. 2009). On the other hand, Piwosz et al. (2013) reported large numbers of picoplanktonic cells (0.9–$8.7 \pm 1.3 \times 10^{10}$ cells m^{-3}) in first-year sea ice. Due to the lack of data for melt-pond aggregates no comparison with our data is possible. Lee et al. (2012) studied the species composition in an open melt pond and observed a dominance of nano- and pico-sized flagellates, including *Chlamydomonas nivalis*, *Dinobryon belgica* and *Pyramimonas* sp. Melt-pond aggregates might be expected to show a similar species composition if formed by physical aggregation processes. Another scenario might be that species in melt-pond aggregates originate from sea ice, released during sea-ice surface melt in summer (Fernández-Méndez et al. 2014). However, no sea-ice algae like *Pseudonitzschia* sp., *Nitzschia* sp. or *Navicula* sp. (Syvertsen 1991) were recovered in our data set.

Sequence data presented an OTU overlap of ca. 30–40% between the sea-ice bottom layer and melt-pond aggregate, suggesting a similar richness in both habitats. The similarity is further illustrated by the dissimilarity with the corresponding chlorophyll maximum water sample (data not shown; Kilias et al. 2014). While the resemblance on a high taxonomic level was mirrored by a dominance of chrysophytes and chlorophytes, seawater was dominated by dinoflagellates (Syndiniales) and chlorophytes (*Micromonas pusilla*). Chrysophyte and chlorophyte species distribution in the abundant biosphere, however, differed between both habitats.

In our study, abundant chlorophytes affiliated to different Chlamydomonadaceae phylotypes and *Carteria* sp. Chlamydomonadaceae phylotypes accounted for greater percentages in the sea ice than in the melt-pond aggregate. In contrast, *Carteria* sp. was more abundant in the aggregate. Both genera are composed of different freshwater species (Buchheim & Chapman 1992; del Campo & Massana 2011; Pentecost 2011). Chlamydomonadaceae, such as *Chlamydomonas* sp. and *Chloromonas* sp., are widely distributed in the polar regions and have been recorded in sea ice, snow and melt ponds (Gradinger & Nürnberg 1996; Müller et al. 1998; Weissenberger 1998; Gradinger 2002; Sandgren 2009; Harding et al. 2011; Piwosz et al. 2013). Phylotypes in this study grouped in the genera *Chlamydomonas* and *Chloromonas* but could not be separated in more detail. This finding is similar to those of former studies that reported polyphyly for both genera (Buchheim et al. 1990; Buchheim et al. 1996; Pröschold et al. 2001). The life cycles of both genera include a cyst formation stage, enabling high photoprotective efficiency and promoting survival in hostile conditions (Bidigare 1993; Müller et al. 1998).

Abundant chrysophytes affiliated to several *Ochromonas* phylotypes and to two of *Dinobryon faculiferum*. *Ochromonas* spp. were strongly represented in the sea-ice bottom layer and melt-pond aggregate while *D. faculiferum* was only abundant in the melt-pond aggregate. Chrysophytes are found across diverse aquatic habitats; *Ochromonas* has been found in brackish, marine and above all freshwater environments (Fenchel 1982; Doddema & Van Der Veer 1983; Anderson et al. 1985; Anderson et al. 1989). One explanation for the wide distribution of chrysophytes is their ability to form cysts during periods of unfavourable conditions (Nicholls 2009). Cyst formation is also common in *Ochromonas* (Hibberd 1977). The ability of *Ochromonas* and *Chlamydomonas* or *Chloromonas* to form cysts is beyond any doubt advantageous for their dispersal. The greater tolerance of resting stages to unfavourable abiotic conditions may have enabled their distribution via seawater or drift ice circulating outwards from the Laptev Sea (Rudels et al. 2012). Wind may also have acted as a long distance dispersal vehicle for cysts (Munoz et al. 2004). Mixotrophy may be another advantageous strategy for surviving extreme conditions, particularly in the polar regions (Moorthi et al. 2009). Bachy et al. (2011) observed a variety of likely mixotrophic taxa, including *Gyrodinium*, *Gymnodinium*, *Woloszynskia*, *Prorocentrum* or *Strombidium*, surviving the polar night in the water below the sea ice and in the sea ice itself. Charvet et al. (2012) studied protist communities in High-Arctic meromictic lakes. Their data showed high numbers of dinoflagellates, ciliates and chrysophytes (e.g., *Gymnodinium*, *Strombidium* and *Ochromonas*) in the under sea-ice community in summer 2008 and 2009. In our study, *Gymnodinium* and *Strombidium* were only abundant in the melt-pond aggregate, while sea ice was strongly dominated by the mixotroph *Ochromonas* sp. (Andersson et al. 1989; Hiltunen et al. 2012). These observations show that mixotrophic taxa not only constitute important contributors during the polar night but can also be dominant under the midnight sun or sea ice.

The dominance of freshwater chlorophytes in both samples points to a freshwater environment in the melt pond and a strong freshwater influence on sea ice during the summer. This study's abundant chrysophytes, such as *Ochromonas* sp. and *D. faculiferum*, have often been reported in freshwater environments (Sanders 1991; Caron et al. 1993). The presence and overlap of freshwater species in both the sea-ice and melt-pond aggregate OTUs poses the questions of whether (i) the sea-ice protist community constitutes the source of the melt-pond aggregate, released during melting or (ii) the melt-pond

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protist community dropped through the ice to accumulate in the bottom layer during summer melting. However, differences in the abundant biospheres of the two habitats suggested that environmental variables were distinct enough in each to select for different dominant community members.

Our sampling was carried out in late summer, when sea ice at the bottoms of ponds can become porous (Kramer & Kiko 2011). Current information about sea-ice community diversity, including cercozoans, dinoflagellates and diatoms, strengthens the second suggestion (Comeau et al. 2013). In general, diatoms are thought to dominate the lower part of sea ice and to contribute to aggregates, by macroaggregate formation (Brown et al. 2011; Assmy et al. 2013). Autotrophic and heterotrophic flagellates, in contrast, were found to be more common in the upper layers of sea ice (Grossi & Sullivan 1985; Gradinger 1999). In mid-August, diatoms were not found in abundance in the surface seawater (Kilias et al. 2014), the sea-ice bottom layer, or in the melt-pond aggregate. Environmental DNA sequencing is unlikely to have failed to detect this quantitatively important phylogenetic group (del Campo & Massana 2011) because of the high throughput of next-generation sequencing. Nevertheless, we have to keep in mind that genetic diversity studies can only provide indications of relative abundance. Seasonal variations of single protist groups might be one explanation for observed differences in community structures (Horner 1985). Microscopy of the aggregate protist assemblage presented a large proportion of empty diatom frustules, which, combined with a high C/N ratio may point to advanced degradation of the algae aggregates (Fernández-Méndez et al. 2014). Hence, it seems that large aggregates serve as a micro niche for nanoflagellates during summer.

In summary, our data confirm previous investigations in which melt ponds can provide a distinct freshwater habitat, including for specific protists (Gradinger & Nürnberg 1996; Brinkmeyer et al. 2004). The large aggregates found on the bottom of the melt ponds serve mainly as a habitat for nanoplanktonic algae, while microplankton were already too degraded to be detected with our methods. We cannot conclude to what extent the sea-ice protist community may serve as a seeding reservoir for melt-pond communities because of the possible seeping of melt-pond freshwater through the porous sea ice. However, our data suggest that the two habitats selected for different abundant species in spite of the potential for seepage to influence diversity estimates for the sea-ice bottom layer. Based on the findings of this short overview and the currently observed increase of Arctic sea-ice melt ponds, which has been attributed to on-going climate change, future studies of interactions between these habitats are urgently required. We recommend that these studies should cover a broad regional and seasonal range for a better understanding of the factors that govern species diversity in these central Arctic Ocean habitats.

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