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To cite this article: Anique Stecher, Stefan Neuhaus, Benjamin Lange, Stephan Frickenhaus, Bábk Beszteri, Peter G. Kroth & Klaus Valentin (2015): rRNA and rDNA based assessment of sea ice protist biodiversity from the central Arctic Ocean, European Journal of Phycology, DOI: 10.1080/09670262.2015.1077395

To link to this article: <http://dx.doi.org/10.1080/09670262.2015.1077395>



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rRNA and rDNA based assessment of sea ice protist biodiversity from the central Arctic Ocean

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(Received 10 November 2014; revised 7 May 2015; accepted 1 June 2015)

Sea ice is a large and diverse ecosystem contributing significantly to primary production in ice-covered regions. In the Arctic Ocean, sea ice consists of mixed multi-year ice (MYI), often several metres thick, and thinner first-year ice (FYI). Current global warming is most severe in Arctic regions; as a consequence, summer sea ice cover is decreasing and MYI is disappearing at an alarming rate. Despite its apparent hostility, sea ice is inhabited by a diverse microbial community of bacteria and protists, many of which are photosynthetic. Here we present an assessment of eukaryotic biodiversity in MYI and FYI from the central Arctic Ocean using high-throughput 454 sequencing of 18S rRNA and rDNA amplicons. We compared the rDNA-based ‘total’ biodiversity with the ‘active’ biodiversity from rRNA amplicons and found differences between them including an over-representation of Ciliophora, Bicosoecida and Bacillariophyceae operational taxonomic units (OTUs) in the active part of the community. Differences between the two libraries are more pronounced at the lower taxonomic level: certain genera, such as *Melosira*, are more abundant in the rRNA library, indicating activity of these genera. Furthermore, we found that one FYI station showed a higher activity of potential grazers which was probably due to the advanced stage of melt evident by higher ice temperatures and highly porous ice compared with the other stations.

Key words: 18S rDNA, 18S rRNA, 454 sequencing, alveolates, Arctic Ocean, diatoms, sea ice

INTRODUCTION

Sea ice appears to be a hostile habitat with respect to its abiotic properties. Nevertheless, a diverse sympagic (i.e. ice associated) community thrives within the sea ice matrix, contributing significantly to primary production, to net carbon flux of the Arctic Ocean (Comeau *et al.*, 2013), and representing an important energy source for higher trophic levels. Gosselin *et al.* (1997) found that sea ice algae from first-year ice (FYI) and multi-year ice (MYI) in the central Arctic Ocean, on average, contributed 57% of the total primary production. However, the annual primary production of sea ice algae (5 to 10 g C m⁻² year⁻¹) is low compared with Arctic pelagic phytoplankton (12 to 50 g C m⁻² year⁻¹) (Legendre *et al.*, 1992; Gosselin *et al.*, 1997). Ice-related primary production can have large spatial and temporal variability and may be more important in areas with dense ice coverage. The

on-going shift from older, thicker MYI towards younger, thinner FYI (Kwok & Rothrock, 2009; Maslanik *et al.*, 2011) observed in the Arctic Ocean will have a pronounced impact on sea ice-related primary production. MYI has different physical characteristics than FYI, but more importantly MYI comprises two different ice types: (1) older ice corresponding to the upper portion, which has survived at least one previous melt season and which is representative of the multi-year component; and (2) younger ice corresponding to the bottom portion, which has formed since the previous year’s melt season and therefore is similar to first year ice (Perovich, 2011). Different bacterial communities have been found to reside within these different components of MYI (Hatam *et al.*, 2014). Therefore, it is likely that there are also differences in the protist communities within MYI and between MYI and FYI.

It remains difficult to describe a ‘typical’ sea ice community as it can be impacted by several environmental variables (e.g. abiotic, grazing pressure,

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seasonal changes) or by the species composition present within the water column during ice formation. During this process, microorganisms can be passively trapped within or at the surface of these ice crystals (Garrison *et al.*, 1983; Eicken, 1992) and later they are enclosed within the sea ice matrix (Arrigo *et al.*, 2010). During winter, the abundance of photosynthetic species, and thus primary production, decreases drastically (Bachy *et al.*, 2011), resulting in a community dominated by non-photosynthetic organisms (Bachy *et al.*, 2011; Majaneva *et al.*, 2012). However, as soon as light levels begin to increase in springtime, phototrophic species thrive and may form bloom communities by late-spring to early-summer (see Arrigo, 2014). The decline of this bloom is accompanied by increased grazing pressure of heterotrophs, indicating a post-bloom situation (Wassmann & Reigstad, 2011).

In addition to seasonal changes, the sea ice physical environment itself is a highly variable system on all spatial scales which has an influence on the distribution of sea ice communities (Vancoppenolle *et al.*, 2013; Arrigo, 2014). Snow cover, and thus light transmission, significantly influences the distribution of phototrophic sea ice inhabitants, especially during spring and early summer (Perovich, 1990; Rysgaard *et al.*, 2001; Granskog *et al.*, 2005). Other physical properties can also play an important role in structuring sea ice communities, such as ice thickness (Granskog *et al.*, 2005) and variations in ice structure associated with ice rafting (Babko *et al.*, 2002).

In the past, many studies investigated the diversity of sea ice assemblages by light microscopic surveys and cell counts (Booth & Horner, 1997; Gradinger, 1999; Melnikov *et al.*, 2002; Mundy *et al.*, 2011; Niemi *et al.*, 2011) and/or flow cytometry (Mundy *et al.*, 2011). However, some species might be difficult to identify due to their small size, paucity of morphological traits and sometimes absence of plastids (not detectable by methods based on photosynthetic pigments, e.g. HPLC), resulting in an incomplete view of the biodiversity. More recently, high-throughput sequencing techniques have enabled the acquisition of more detailed information about the structure of communities (Bik *et al.*, 2012). Until present, there are only a few studies investigating the protist communities in sea ice by molecular methods (Eddie *et al.*, 2010; Bachy *et al.*, 2011; Poulin *et al.*, 2011; Majaneva *et al.*, 2012; Comeau *et al.*, 2013; Piwosz *et al.*, 2013).

In this study, we investigated sympagic protist communities in sea ice cores from four different regions of the central Arctic Ocean using 454 sequencing of the 18S rDNA and the 18S rRNA, and compared the biodiversity of FYI and MYI. We were also interested in comparing the total (as assessed by rDNA sequences) vs. active (rRNA based) part of the protist communities, as these approaches for other habitats

have been shown to reveal more detailed information on the biological activity of individual phyla (Stoeck *et al.*, 2007; Logares *et al.*, 2014). Clustered operational taxonomic units (OTUs) were taxonomically classified using both a method based on sequence similarity characteristics and a method based on phylogenetic relationships.

MATERIALS AND METHODS

Sampling

Sea ice algal community samples were taken at four different ice stations (FYI: 237, 255, 277, MYI: 360, Fig. 1) during the expedition ARK-XXVII/3 (PS80/3) of *RV Polarstern* to the central Arctic Ocean from 2 August to 10 October 2012. We have used the original cruise station numbers as assigned on board according to *RV Polarstern* conventions for the sake of traceability; additional station information has been deposited at <http://doi.pangaea.de/10.1594/PANGAEA.792734>. Ice cores with a diameter of 9 cm were extracted with a Mark II Coring System (Kovacs Enterprises, Roseburg, Oregon, USA). At each station, three ice cores were sampled and processed for salinity, temperature, texture and density. Ice temperature measurements were conducted on texture cores immediately after extraction. Texture and density cores were stored at -20°C for later analyses. Immediately after extraction, the salinity cores were melted on the ship and analysed for salinity using a conductivity meter (WTW 3300i, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Within 1 m of the above cores, two cores were drilled adjacent to each other for RNA and DNA extraction. Both were cut into 0.1 m sections and immediately transported to the ship for further processing. The RNA cores were sampled last and processed first to avoid degradation of the RNA and

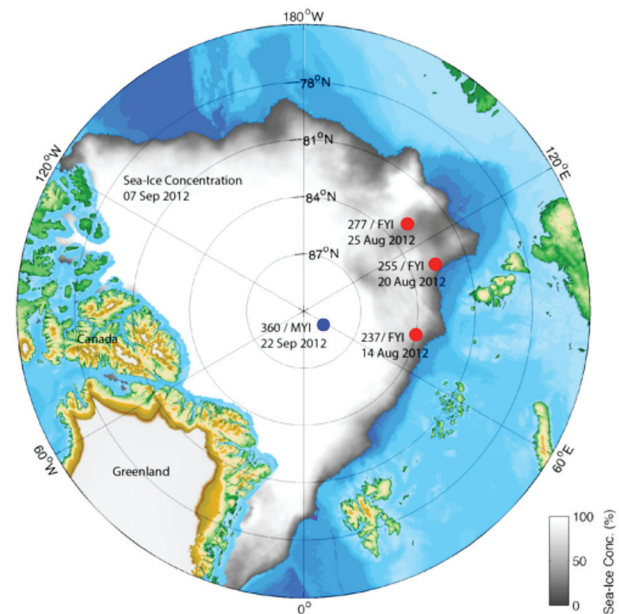


Fig. 1. Locations and dates of the four ice stations sampled in the central Arctic Ocean between 2 August and 10 October 2012. Red dots indicate first-year ice (FYI) stations 237, 255 and 277. Blue dot indicates the multi-year ice (MYI) station 360.

changes in the expression rates. Ice sections were crushed manually, filled with 0.1 l (per 0.1 m of ice core) seawater with a salinity of 70 PSU to avoid osmotic stress of the algae, pooled and melted at room temperature with occasional shaking. After melting, the complete ice core was filtered on 1.2–2.0 μm IsoporeTM polycarbonate membrane filters (1.2 μm RTTP, 2.0 μm TTTP, Merck Millipore, Schwalbach, Germany) to collect the protist community. Replicate filters were taken from each DNA and RNA ice core. RNA filters were stored in liquid nitrogen until further analyses. The DNA cores were processed in the same way except that they were melted at 4°C for 1–2 days.

Abiotic properties

Density measurements were done by first calculating the volume V of each piece ($V = \pi r^2 h$) and afterwards the density ρ ($\rho = \frac{m}{V}$). In some instances, calculated density values were greater than the expected theoretical ice density value of 917 kg m^{-3} (Yen *et al.*, 1991). These values were replaced with the theoretical density value in further calculations. This was likely due to refreezing of seawater or brine along the outside of the core resulting in the overestimation of mass and thus density. Brine volume V_b estimates were calculated from the measured temperature and salinity values using equations in Cox & Weeks (1983) and Yen *et al.* (1991). The relative air volume V_a/V , hereafter referred to as porosity, was calculated following equations in Cox & Weeks (1983).

Satellite-derived sea ice concentration data were acquired from the University of Bremen for each corresponding sampling date (<http://www.iup.physik.uni-bremen.de/>; Spreen *et al.*, 2008, updated). Sea ice concentrations are reported as the average calculated over a 9 grid cell square (~380 km^2), with the ice station at the centre cell. Distance to the ice edge was calculated for each ice station by measuring the distance from the ice station to the closest point/grid cell where large open water areas were present (e.g. grid cells < 15%).

RNA extraction

Total RNA was extracted from pooled replicate filters of the same ice core. Filters were washed with 1 ml of 65°C pre-warmed TRI Reagent[®] (Sigma-Aldrich, Munich, Germany) before adding glass beads to disrupt cells mechanically for 30 s with a beadbeater. Samples were incubated 5 min at 65°C, treated a second time for 30 s in the beadbeater and then centrifuged at 13 000 rpm for 5 min. Purification of RNA from the extracts was carried out following the manufacturer's protocol of the Direct-Zol RNA MiniPrep kit (Zymo Research, Freiburg, Germany) with an additional DNase I digestion (Zymo Research, Freiburg, Germany) step for 20 min at 35°C. Concentration and purity of the extracted RNA was quantified spectrophotometrically with a NanoDrop ND-1000 spectrometer (Thermo Fisher Scientific, Wilmington, Delaware, USA). When required, the RNA was further purified with the RNeasy MinElute Cleanup kit (Qiagen, Hilden, Germany). The quality of the extracted RNA was verified by a RNA Nano Chip Assay with the 2100 Bioanalyzer device (Agilent Technologies, Böblingen, Germany). Only non-degraded RNA samples of high concentration and without contaminations were used for further analyses.

DNA extraction

Total DNA was extracted using DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with an additional mechanical 10 s cell disruption step using glass beads and an additional proteinase K (AmpliChem, Darmstadt, Germany) digestion step during the RNase A (Qiagen, Hilden, Germany) digestion. The isolated genomic DNA was analysed and visualized by gel electrophoresis in 1% TAE agarose. All samples that showed large (> 10 000 bp) non-degraded fragments were used for further analyses.

Reverse transcription and full-length 18S amplification

Single-stranded cDNA was produced using a SuperScript III Reverse Transcriptase kit (Invitrogen, Darmstadt, Germany) with an initial concentration of approximately 100 ng of template RNA. The manufacturer's protocol was modified as follows: incubations were conducted at 37°C and 42°C, each for 45 min, followed by the addition of 1 μl of fresh SuperScript III RT, an additional incubation at 50°C and 55°C, both for 30 min, and a final incubation step at 37°C for 20 min with 1 μl of RNase H (Invitrogen, Darmstadt, Germany). Immediately after cDNA synthesis, samples were purified using a MinElute PCR purification kit (Qiagen, Hilden, Germany). The concentration of each sample was again quantified spectrophotometrically (NanoDrop ND-1000). The 18S gene was then amplified by PCR in 50 μl total volume, with both cDNA and DNA as templates, as follows: 10 mmol/dNTP dNTP mix (Qiagen, Hilden, Germany), 10 $\mu\text{mol/l}$ F and R primer (Eurofins MWG Operon, Ebersberg, Germany), 10 \times Taq polymerase buffer, 5 U μl^{-1} Hot Master Taq Polymerase (5' Prime, Hamburg, Germany), and 1 μl template DNA (10 ng μl^{-1}). Cycling conditions were: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min, elongation at 72°C for 2 min, and a final elongation at 72°C for 10 min. The primers 1F (5'-AACCTGGTTGATCCTGCCAGT-3') and 1528R (5'-TGATCCTTCTGCAGGTTACCTAC-3') (Medlin *et al.*, 1988) were chosen to amplify the complete gene. The amplified gene was visualized by gel electrophoresis and products were excised and purified from agarose residuals using a PeqGold Gel Extraction kit s-line (Peqlab, Erlangen, Germany).

18S PCR with specific adaptor-barcode-primer

From each of the excised 18S full-length amplicons, a smaller amplicon with a size of approximately 400–600 bp was amplified. During this amplification, adaptors and specific Multiplex Identifiers (MIDs) to allow multiplexing of multiple samples in the Pico Titer Plate (PTP) of the 454 sequencer were incorporated into the amplicons. PCR biases (unequal amplification/primer efficiencies) or PCR errors (sequences artefacts due to chimeras or nucleotide errors incorporated by the Taq polymerase) may artificially increase diversity estimates (Acinas *et al.*, 2005). To minimize these potential biases, each PCR was performed in triplicates which were pooled prior to the sequencing procedure (Bik *et al.*, 2012). PCR reaction mix and cycling conditions were used as described above except that we used primers 528F (5'-

GCGGTAATTCAGCTCCAA-3') (Edgcomb *et al.*, 2002) and 1055R (5'-ACGGCCATGACCACCACCCAT-3') (Elwood *et al.*, 1985) with incorporated MIDTs and adjusted annealing temperature to 75°C. The PCR products were analysed by agarose gel electrophoresis. Products with the correct size were excised and purified using a PeqGold Gel Extraction kit s-line.

454 sequencing

The barcoded and purified amplicons were purified a second time by Agencourt® AMPure® XP PCR purification (Beckman Coulter, Krefeld, Germany) following the manufacturer's protocol to completely remove small fragments. PCR products were quantified spectrophotometrically by a NanoDrop ND-1000, and the triplicates were pooled in an equimolar ratio. The quality of the DNA was again verified using a High Sensitivity DNA Assay and the 2100 Bioanalyzer device (Agilent Technologies, Böblingen, Germany). To quantify the amount of template DNA for sequencing, the concentration of double-stranded DNA was determined using the ultrasensitive fluorescent Quant-iT™ PicoGreen® (Life Technologies GmbH, Darmstadt, Germany) according to the manufacturer's protocol. The emulsion PCR was performed with 0.7 molecules per bead following the Lib-L manufactures protocol (Roche, Branford, Germany). Sequencing was carried out using a GS Junior Titanium Series sequencer (Roche, Mannheim, Germany). rRNA and rDNA samples were sequenced separately in two runs (i.e. four samples on each run).

Bioinformatics

The raw reads (stored in Standard Flowgram Format, sff) were first demultiplexed by using Roche gsSeqTools and then analysed by an in-house pipeline, which wraps and connects QIIME (Quantitative Insights Into Microbial Ecology) scripts (version 1.6) (Caporaso *et al.*, 2010) to form a full analysis workflow. The demultiplexed raw files were first converted into sequence fasta files and corresponding quality files. Afterwards, the sequences were pre-processed to improve quality: the barcodes and forward primers were removed and the sequences trimmed at the first occurrence of an ambiguous base or a homopolymer exceeding a length of six bases. The sequence was cut/trimmed at first position of the reverse primer. Any sequence with a length less than 200 bp or an average phred quality value less than 25 was discarded. Denoising was not performed, due to the fact that this process might erroneously alter sequences to adapt them to a denoising cluster representing sequence, which can potentially lead to the loss of rare sequence variants (Gaspar & Thomas, 2013). After quality control, the mean read length was approximately 520 bp and approximately 60% of the sequences remained. OTU clustering was done by USEARCH (version 5.2.236) (Edgar, 2010) at a sequence similarity level of 98%. All sequencing reads from both rDNA and rRNA samples were clustered together in order to ensure traceability of OTUs across all samples. Checking for and exclusion of chimeras was done with the UCHIME algorithm (Edgar *et al.*, 2011), included in the USEARCH package by using *de novo* and

reference-based chimera detection (reference dataset built by Wolf *et al.*, 2014). The intersection of the chimera checked sequence sets obtained by both methods were kept for further processing. A minimum of four sequences per cluster was required to keep it as OTU. The longest sequence of each cluster was selected as the OTU representative sequence for assigning taxonomy. The taxonomic identification was based on the Ribosomal Database Project (RDP) Classifier (version 2.2) (Wang *et al.*, 2007) with a confidence threshold of 0.8. We used the SILVA (Pruesse *et al.*, 2007; Quast *et al.*, 2013; Yilmaz *et al.*, 2014) SSURefNR (www.arb-silva.de, version 111) sequence set as taxonomic reference, which was pruned to only contain eukaryotic sequences and clustered at a similarity level of 97% to reduce computational costs (ftp.microbio.me/pub/QIIME_nonstandard_referencedb/Silva_111.tgz). The Shannon biodiversity index and the rarefaction curve were generated by a QIIME workflow script for alpha diversity. The script computes alpha diversity (in this case Shannon index) for each sample and collates the results to generate alpha diversity rarefaction plots (in this case based on observed species). Trophic affiliation of OTUs as phototroph, heterotroph, mixotroph, parasitic and unknown was done based on a literature search and is included into the OTU table (see section data availability).

Due to the expected higher accuracy of phylogeny-based methods over similarity-based methods, we decided to use the similarity-based RDP program solely for a preliminary classification. For accurate classification of selected sequences the phylogeny-based PhyloAssigner pipeline (version 6.166) (Vergin *et al.*, 2013) was applied. This pipeline aligns sequences to a reference multiple sequence alignment and involves pplacer (version 1.1) (Matsen *et al.*, 2010) to place them upon a fixed rooted phylogenetic backbone tree using a maximum likelihood phylogenetic placement algorithm. Following this concept, all OTU sequences predicted by RDP to be Bacillariophyceae or Alveolata were extracted and analysed in more detail by PhyloAssigner. The required reference set, which consists of a tree and a corresponding alignment, was achieved by selecting sequences from the SILVA alignment and filtering the SILVA guide tree using ARB (version 5.5) (Ludwig *et al.*, 2004). The reference set for Bacillariophyceae was built using 1390 diatom sequences and 196 other Stramenopile sequences as outgroup. The Alveolata reference set was built based on 2504 sequences with 28 Chloroplastida sequences as outgroup. To ensure a conservative taxonomic assignment of a certain OTU sequence, PhyloAssigner uses the last common ancestor (LCA) node of the most likely sequence placement positions up to a cumulative likelihood weight cut-off of 0.9 for taxonomic placement. To assign taxonomy to an OTU sequence placed at its respective LCA, which might be an inner node without its own taxonomic information, the full taxonomic strings of all tree leaves below the LCA node were extracted and the common prefix string was used as a taxonomic assignment.

For OTUs classified by QIIME (similarity-based) as Bacillariophyceae and Alveolata, a heatmap of OTU abundance ratios in the rRNA vs. rDNA samples was plotted with the R software (version 3.0.2, package: gplots, default parameters). For this, all OTU counts for both groups were first normalized against total read counts per sample (see Table 1 for number of reads per sample). Normalized rDNA

Table 1. QIIME output for the different stations and sample type.

	237		255		277		360		total
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	
(a)									
raw reads	48658	39328	51489	39912	46804	56990	84325	67322	434828
reads after quality clipping	31522	17725	34876	22358	28411	33997	54825	38252	261966
reads after OTU clustering	22255	12030	28879	16029	23884	26097	40310	28878	198362
OTUs	427	438	402	442	298	412	447	465	714
diatom reads per sample	14709	8376	20595	12597	4628	10627	37284	10491	119307
alveolate reads per sample	4416	2717	2741	1017	5780	8290	1481	365	26807
(b)									
Shannon index	6.6	7.1	5.9	6.8	5.4	6.1	6.0	6.2	

(a) Summary of recovered reads before and after processing.

(b) Shannon biodiversity index.

abundances of OTUs with a count of 0 in rDNA samples were set to the smallest value of rDNA samples found. The highest ratios ranked by the sum of rRNA/rDNA ratios across each sample were then plotted in a heatmap with an attached dendrogram from complete linkage hierarchical clustering of euclidean distances to visualize the most striking differences between rRNA and rDNA OTU abundances. R-scripts, OTU-count data and further QIIME analysis results, including configuration details, are provided to the public; see data availability section.

RESULTS

Abiotic properties

The FYI stations 237, 255 and 277 had sea ice surface ‘scattering’ layers, which consisted of deteriorated sea ice that was characterized by an unconsolidated granular structure and could closely resemble wet snow (Table 2). MYI station 360 had

no obvious scattering layer, showed the thickest sea ice and was the only station with snow accumulation (Table 2). FYI stations 255 and 277 had comparable core lengths, which both corresponded to the thinnest sea ice sampled, and station 237 was the thickest FYI sampled (Table 2). FYI station 277 showed the lowest core-averaged salinity, brine volume (although comparable to MYI station 360), density and porosity values in addition to the warmest core-averaged temperature values (Table 2 and Fig. 2). This pattern was even more pronounced when comparing only the bottom parts of each core (Table 2 and Fig. 2). MYI station 360 had the lowest averaged core-temperatures and was the only station with a bottom temperature near the freezing point (Table 2) of -1.86°C for seawater with a salinity of 34 PSU (Petrich & Eicken, 2010). MYI station 360 was also sampled latest in the season and from the highest latitude (Table 2). All stations had comparable sur-

Table 2. Abiotic properties of the three first-year sea ice (FYI) and one multi-year sea ice (MYI) stations.

	237 FYI	255 FYI	277 FYI	360 MYI
Date (M/D/Y)	08/14/12	08/20/12	08/25/12	09/22/12
Latitude (N)	83° 59.19'	82° 40.24'	82° 52.95'	88° 49.66'
Longitude (E)	78° 6.20'	109° 35.37'	130° 7.77'	58° 51.81'
Sea ice concentration (%)	96	97	97	100
Distance to ice edge (km)	214	156	38	515
Freeboard (m) ^a	0.20 ± 0.03	0.13 ± 0.01	0.18 ± 0.01	0.26 ± 0.02
Snow cover (m) ^a	0	0	0	0.02 ± 0.0
Scattering layer (m) ^a	0.06 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0
Core length (m) ^a	1.47 ± 0.04	0.88 ± 0.02	0.85 ± 0.05	1.97 ± 0.06
Salinity (PSU) ^a	1.89 ± 1.12	1.66 ± 0.75	1.23 ± 0.72	2.39 ± 0.96
Bottom	3.1	2.5	1.1	3.2
Temperature (°C) ^a	-0.82 ± 0.42	-0.71 ± 0.43	-0.67 ± 0.32	-1.65 ± 1.05
Bottom	-1.6	-1.6	-1.2	-1.8
Brine volume (ppt) ^a	117 ± 62	194 ± 218	83 ± 36	83 ± 27
Bottom	93	70	53	89
Density (kg m ⁻³) ^a	872 ± 54	808 ± 98	757 ± 58	860 ± 37
Bottom	917 ^a	917 ^a	805	888
Porosity (V _a /V) ^a	0.07 ± 0.06	0.15 ± 0.13	0.17 ± 0.06	0.06 ± 0.04
Bottom	0.01 ^b	0.01 ^b	0.13	0.04

Freeboard, snow cover, and core length are the mean (arithmetic) and standard deviation (SD, one sigma) values from the five cores taken at each station. Salinity, temperature, brine volume, density, and porosity values are reported for each individual corresponding core and ‘bottom’ refers to the observation closest to the bottom (ice-water interface) of the core. ^a mean ± SD, ^b value determined or replaced by theoretical density due to overestimation.

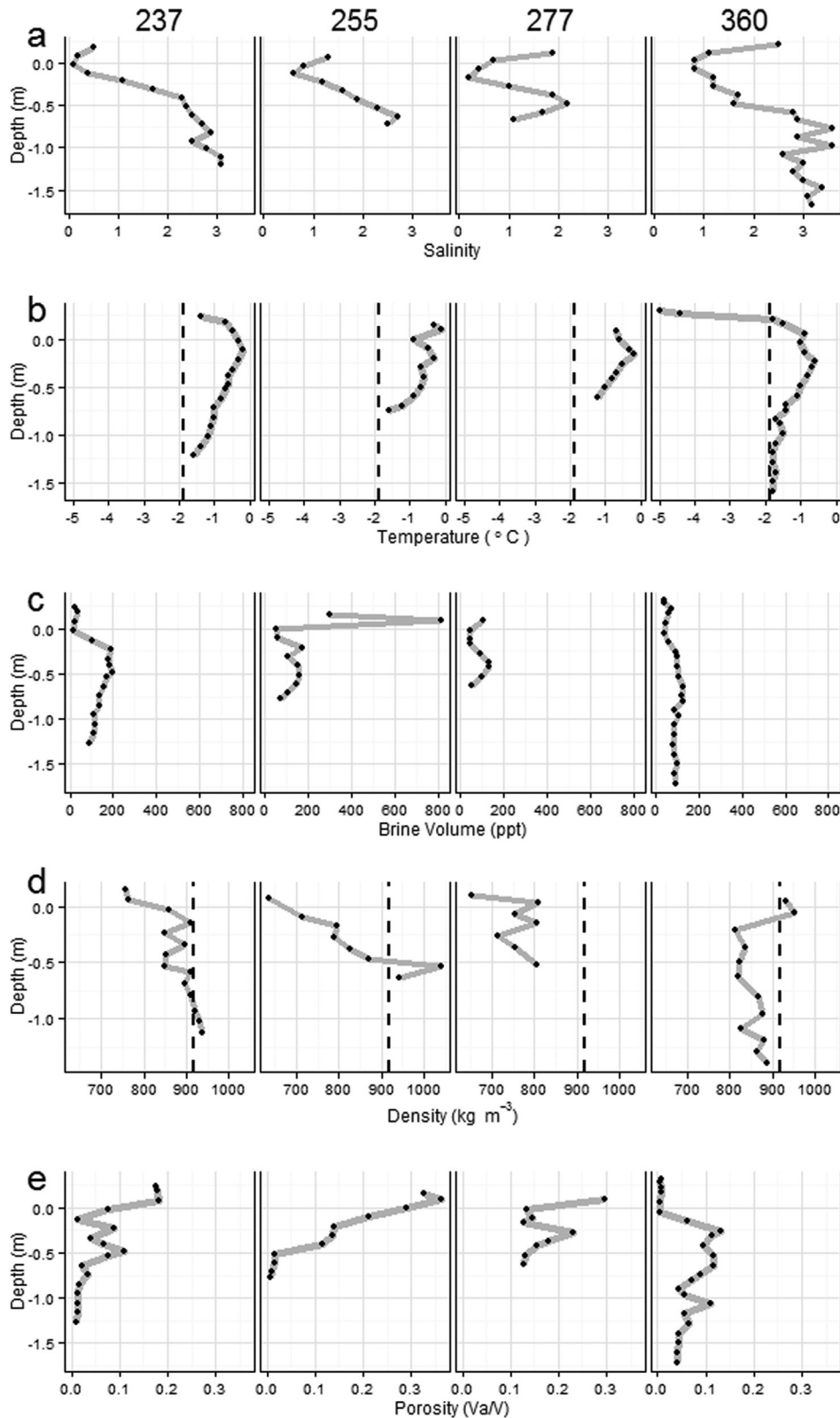


Fig. 2. Vertical sea ice core profiles, for each station, of: (a) salinity; (b) temperature, dashed vertical lines at -1.86°C correspond to freezing point of seawater with salinity of 34; (c) brine volume; (d) density, dashed vertical lines at 917 kg m^{-3} correspond to theoretical density of ice (shown are raw values without correction for overestimated values > 917); and (e) porosity (calculated using corrected density values).

rounding sea ice concentrations, with values greater than 95%; however, FYI station 277 was considerably closer to the ice edge than all other stations (Table 2).

Sequence analyses

A total of approximately 430 000 raw reads (across all rRNA and rDNA samples) with assignable barcodes were generated (Table 1). After quality clipping and

discarding reads < 200 bps approximately 260 000 reads were left and of these, 198 000 reads were clustered to OTUs, corresponding to 45.6% of the total raw reads. In total, 11 222 potential chimeras were removed. An average of 24 750 reads per sample were clustered into OTUs (range: 12 030 to 40 310). We found a total of 714 different OTUs for all stations. Rarefaction curves (Fig. S1) showed no saturation in any sample, meaning that the diversity of sea ice protists was not fully recovered.

OTU diversity of Arctic sea ice protists

Taxonomic classification of OTUs was performed by two different methods: the QIIME and the PhyloAssigner pipeline. The whole dataset was pre-processed and clustered using QIIME, including a similarity-based taxonomic assignment by RDP. Additionally, for OTUs that were classified by QIIME to belong to the two most abundant and diverse groups, Bacillariophyceae and Alveolata, phylogenetic placement was performed using PhyloAssigner. In the

following, we describe the phylogenetic composition based on the number of different OTUs found (as a measure of diversity) and not on sequence abundance of the described OTUs across all samples and both rRNA and rDNA libraries.

Based on QIIME, > 95% of the 714 OTUs were classified into one of the three super-groups: Stramenopiles (64% OTUs), Alveolata (17% OTUs) and Rhizaria (13% OTUs) (Fig. 3, Table S1). The remaining OTUs (< 5%) belonged to the super-groups Chlorophyta, Cryptophyceae, Haptophyta, Holozoa and Telonema. The largest amount of Stramenopile OTUs (79%) clustered as Bacillariophyceae (diatoms) (Fig. 3, Table S2), with Bacillariophycidae (raphid pennate diatoms, 83.8%) as the most abundant subclass based on PhyloAssigner (Table S3). Five per cent of the Stramenopile OTUs were clustered as Bicosoecida (Fig. 3, Table S2). Within Alveolata, PhyloAssigner classified 27.9% of the OTUs as Dinoflagellata and 71.3% of Alveolata OTUs as Ciliophora (Table S4). A large fraction of the Rhizaria OTUs (79.9%) was classified into Thecofilosea, with *Cryothecomonas*

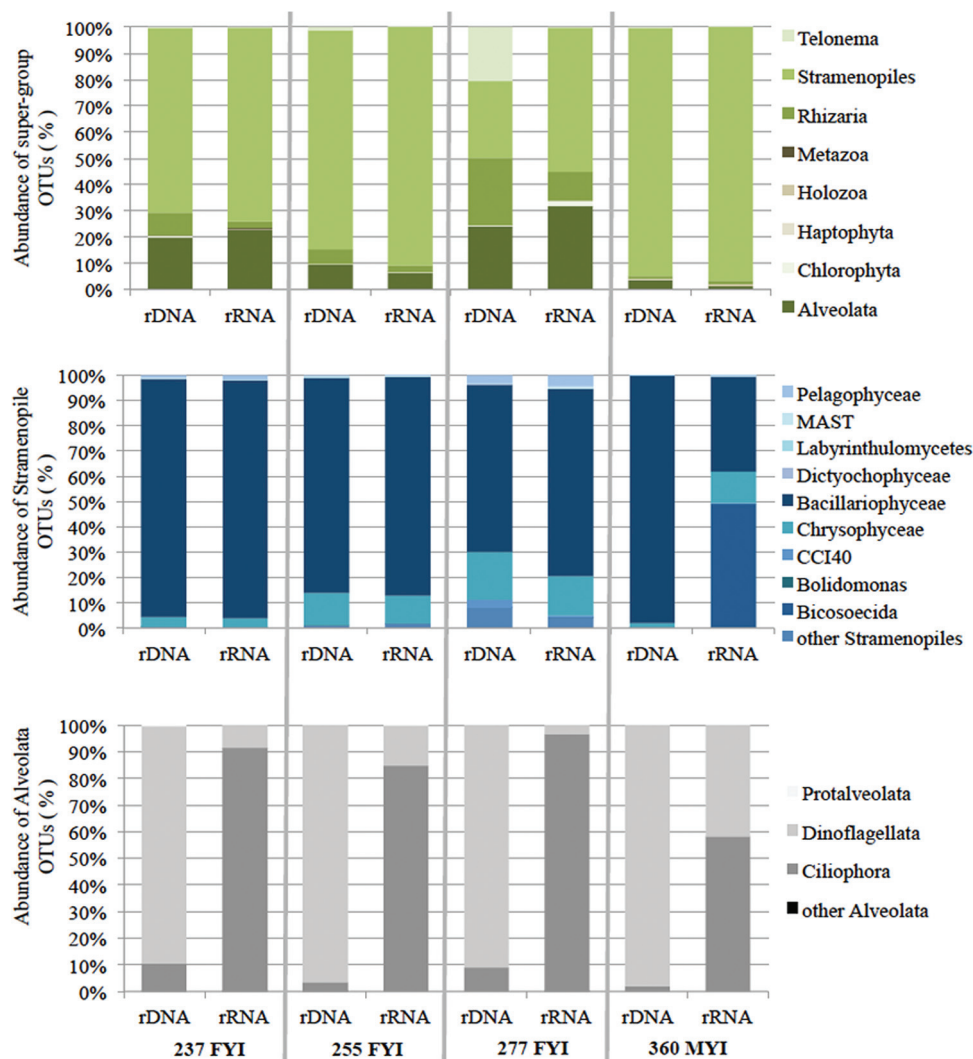


Fig. 3. Relative OTU abundance (%) of the super-groups (top, greenish colours), Stramenopiles (middle, bluish colours), and Alveolata (bottom, greyish colours) found in the rDNA and rRNA libraries at each sampling location based on QIIME.

Thomsen Buck, Bolt & Garrison (incertae sedis) as the most abundant genus (data not shown).

Due to the complexity of the dataset, we here focus on the representatives of the three most abundant protist groups: Stramenopiles, Alveolata and Rhizaria. Furthermore, we present the results for the two different sample types (FYI and MYI) in the context of both rDNA and rRNA libraries.

Relative protist OTU abundances in Arctic FYI

We characterized relative abundance patterns of taxa among the samples based on counts of sequences assigned to OTUs. The three FYI stations differed only slightly with respect to their biodiversity on the super-group level. rRNA libraries had a higher Shannon diversity index than their corresponding rDNA counterparts irrespective of the location (Table 1).

Apart from these similarities, FYI station 277 differed markedly from the other two FYI stations. The lowest relative abundance of Stramenopiles of all stations was found in FYI station 277, together with the highest relative abundance of Rhizaria and Alveolata OTUs of all stations. Looking at this station in more detail showed that the rDNA library of this station had a lower relative abundance of OTUs classified as Stramenopiles (rDNA: 29%, rRNA: 55%) and Alveolata (rDNA: 24%, rRNA: 32%), but had a higher relative abundance of Rhizaria OTUs (rDNA: 26%, rRNA: 11%) than the corresponding rRNA library (Fig. 3, Table S1). The trophic affiliation of this station also shows differences to the other FYI stations. At FYI station 277, we observed a lower amount of

phototrophs (rDNA: 26%, rRNA: 54%), compared with FYI station 237 and 255 (rDNA: 70% and rRNA: 74% at station 237; rDNA: 82% and rRNA: 89% at station 255) (Fig. 4). The differences of FYI station 277 from the other FYI stations are even more pronounced between both libraries on a lower taxonomic level analysed by PhyloAssigner: we found the highest relative amount of pennate diatoms (Bacillariophycidae) at FYI station 277. The pennate diatoms *Bacillaria* J.F. Gmelin, a phylotype most closely related to the naviculoid strain CCMP2297, and *Melosira* C. Agardh were most abundant at FYI station 277 compared with the other FYI stations. Whereas *Bacillaria* was most abundant in the rDNA library, CCMP2297 and *Melosira* showed higher abundances in the rRNA library, with *Melosira* being absent from the rDNA library (Table S3). This is also reflected in the high rRNA/rDNA ratios of *Melosira* OTUs, whereas no other Bacillariophyceae OTU showed an rRNA/rDNA ratio signal at station 277 (Fig. 5).

We also found an alternating pattern between Alveolata sequence abundances in rDNA and rRNA libraries, irrespective of the sampling location. In every FYI rDNA sample, there were more Dinoflagellata OTUs compared with the corresponding rRNA library and in every rRNA library, we found a greater relative abundance of Ciliophora OTUs than in the rDNA library (Fig. 3, Table S5). This pattern is also reflected by the trophic affiliation of the community: each rDNA library had more mixotrophic genera, whereas the rRNA library had more heterotrophic genera (Fig. 4). Again, FYI station 277 showed a different pattern from the other FYI stations, by having the greatest amount of heterotrophs of all FYI

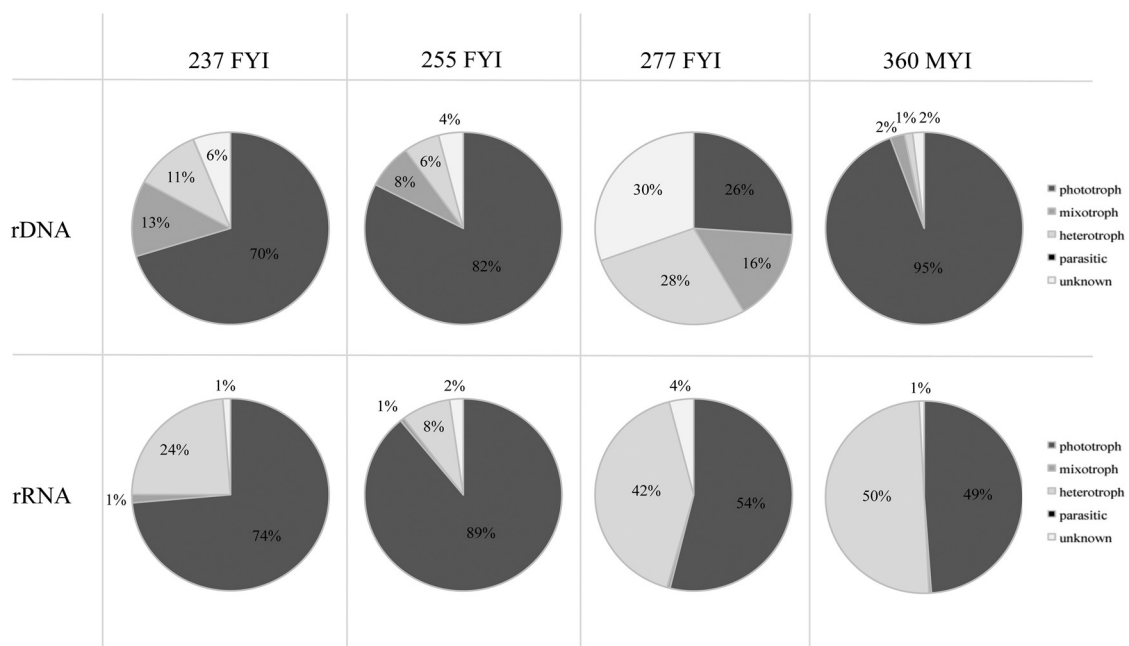


Fig. 4. Classified OTUs divided into photo-, hetero- and mixotrophs (trophic affiliation) for both rRNA and rDNA libraries for all stations.

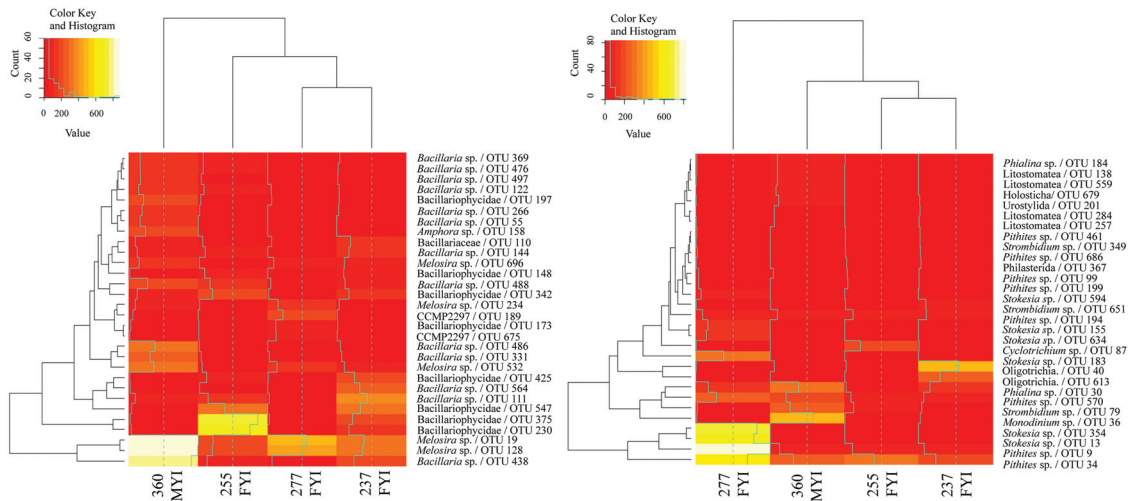


Fig. 5. Heatmap of the top 30 rRNA/rDNA ratios of single Bacillariophyceae OTUs (left) and Alveolata OTUs (right) from four different stations. White and yellow colours indicate higher RNA than DNA ratios, orange and red colours indicate low or no rRNA/rDNA ratios.

stations in both, rDNA and rRNA libraries (Fig. 4). A similar pattern was observed for the rRNA/rDNA ratios: FYI station 277 had the highest rRNA/rDNA ratios with signals only for the most abundant Ciliophora genera *Stokesia* Wenrich and *Pithites* Deroux & Dragesco (Fig. 5. Table S4).

OTU abundances of an Arctic MYI floe

Analysis of the relative abundance of specific OTUs within the MYI community yielded similarities and differences compared with the FYI communities. As for the FYI stations, the rRNA library of the MYI station had a higher Shannon index compared with their corresponding rDNA library (Table 1) and a greater proportion of heterotrophs (50%, compared with 1% in the rDNA library) (Fig. 4). Of all stations, MYI station 360 showed the highest relative abundance of Stramenopile OTUs in both the rDNA (95%) and rRNA (97%) library and also the smallest amount of Alveolata (rDNA 4%, rRNA 1%) and Rhizaria (1%, each) OTUs of all stations (Fig. 3, Table S1). In this respect, rRNA and rDNA libraries from the MYI station appeared to be similar to the FYI stations 237 and 255. On the other hand, at a lower taxonomic level, the MYI station was different than the FYI stations: almost half (49%) of the Stramenopile OTUs in the rRNA library were classified as Bicosoecida, which were exclusively found at the MYI station, and only 38% of the Stramenopile OTUs were classified as Bacillariophyceae. The rDNA library, however, had a relative Bacillariophyceae OTU abundance of 98% and a relative Bicosoecida OTU abundance of less than 1% (Fig. 3, Table S2). This taxonomic difference also has a strong effect upon the trophic composition. The MYI rRNA library appears to harbour the highest amount of heterotrophs and its rDNA library shows the highest representation

of phototrophs (95%) across all libraries (Fig. 4). Although the abundance of Bacillariophyceae OTUs in the rRNA library was lower compared with the other stations, based on PhyloAssigner *Bacillaria*, *Melosira* and CCMP2297 were the most abundant Bacillariophyceae genera or groups at this station in the rRNA library (Table S3). Overall, MYI station 360 had the highest observed rRNA/rDNA ratios for Bacillariophyceae OTUs, with *Melosira* and *Bacillaria*, showing the highest rRNA/rDNA ratios (Fig. 5).

Similar to the FYI samples, the Alveolata groups from the MYI station also showed an alternate pattern between the rDNA and the corresponding rRNA libraries, although less pronounced than at the FYI stations (Fig. 3, Table S5). We found a greater relative abundance of Dinoflagellata OTUs in the rDNA library compared with the rRNA library and a higher amount of Ciliophora OTUs in the rRNA library compared with the rDNA library, which can also be seen in the trophic affiliation (Fig. 4). Apart from these similarities, the rDNA library of MYI station 360 had the highest relative abundance of the Dinoflagellata genera *Gymnodinium* Stein and *Karlodinium* J. Larsen and the lowest relative abundance of the otherwise abundant ciliate genus *Pithites* of all stations (Table S4). Although we also observed other Ciliophora genera, like *Phialina*, *Strombidium*, *Monodinium* and *Pithites* at the MYI station, rRNA/rDNA ratios of Ciliophora species are similar to FYI stations 237 and 255 and were lower than at FYI station 277 (Fig. 5).

DISCUSSION

Abiotic properties

The sea ice environment of the four stations exhibited three different stages of melt which can help to describe the variability of the sea ice communities.

The stage of melt can be an important factor for structuring sea ice communities due to flushing of important nutrients and organic material (Riedel *et al.*, 2008), increased losses of bottom ice algal biomass associated with higher melt rates (Pogson *et al.*, 2011) and increased grazing pressure owing to increased pore space and access for grazers (see also reviews by Vancoppenolle *et al.*, 2013 and Arrigo, 2014). The physical state of the bottom ice and the loss of biomass from the bottom ice are of particular interest in terms of community composition since this is where the majority of the biomass is located (Arrigo, 2014).

FYI station 277 was particularly different from all other stations because, at the time of sampling, it was in an advanced stage of melt. This was evident by the observation that FYI station 277 had the lowest core-averaged and bottom salinity, brine volume, density, and porosity values in addition to the warmest core-averaged and bottom temperature values. Typically, higher brine volume occurs during melt and at the ice-bottom; however, due to the advanced state of melt, it is likely that much of the internal brine was already flushed from the ice (Vancoppenolle *et al.*, 2013), which was evident from the high porosity and low salinity at the bottom ice of this station. The advanced stage of melt at FYI station 277 was likely due to its proximity to the ice edge where melt rates are typically higher for proximity of open water and increased absorption of solar radiation. Although the ice floe at FYI station 237 was considerably thicker than the ice at FYI station 255, the other physical properties were very similar. Based on the date of sampling and physical properties, FYI stations 237 and 255 could also be classified as being in a state of melt, but not as advanced as FYI station 277.

MYI station 360 was sampled latest in the season during the transition from end of melt to freeze-up, which was evident from the lowest bottom and surface ice temperatures. In addition, MYI station 360 was sampled at the highest latitude, and from a region dominated by thicker MYI. Due to a thicker surrounding sea ice pack and higher latitude, MYI station 360 likely experienced a lower melt rate than the other sites, which could have limited biomass loss (Pogson *et al.*, 2011) and increased the potential to retain bottom-ice communities. The presence of bottom-ice biomass at the end of the melt season has been observed in previous studies (e.g. Meiners *et al.*, 2003) and is also suggested from internal biomass layers within MYI (e.g. Thomas *et al.*, 1995; Gradinger, 1999), that likely corresponded to previous seasons/years bottom ice.

Based on the analyses of the physical sea ice environment, we have classified the ice stations into three different melt stages: FYI stations 237 and 255 corresponded to the early- to mid-melt season; FYI station 277 corresponded to advanced melt; and MYI station

360 corresponded to the end of melt before the start of freeze-up.

Diversity of sea ice protists

High throughput sequencing of 18S rDNA and 18S rRNA of eukaryotic sea ice communities collected from sea ice cores conducted in this study yielded a detailed view on the sympagic community of the ice cores. Most sequences were assigned as Stramenopile OTUs, followed by Alveolata and Rhizaria OTUs in both rRNA and rDNA libraries. Other super-groups that were detected in both libraries were also found in other studies, such as: Chlorophyta (Eddie *et al.*, 2010; Majaneva *et al.*, 2012; Comeau *et al.*, 2013; Piwosz *et al.*, 2013), Cryptophyceae (Majaneva *et al.*, 2012; Comeau *et al.*, 2013; Piwosz *et al.*, 2013), Haptophyta (Comeau *et al.*, 2013; Piwosz *et al.*, 2013), Holozoa and Telonema (Bachy *et al.*, 2011; Mundy *et al.*, 2011; Majaneva *et al.*, 2012). Overall, there was only a slight difference in the number of different OTUs on the super-group level found between rDNA and rRNA libraries. This indicates that, with regard to larger taxonomic groups like Stramenopiles or Rhizaria, the observed OTU diversity of both libraries were comparable, which is in accordance with other studies (Stoeck *et al.*, 2007; Baldrian *et al.*, 2012; Logares *et al.*, 2014). However, we found a higher biodiversity index in the rRNA libraries compared with the rDNA libraries, which might be due to a different representation of taxa (e.g. OTUs that were more abundant in the rRNA library or absent in the rDNA library) at lower taxonomic levels in rRNA and rDNA libraries.

Phototrophic community of Arctic FYI and MYI

Within the Stramenopiles, the Bacillariophycidae (pennate raphid diatoms) were most abundant at each of the ice floes, irrespective of the library, which is typical for sea ice during the summer season (Eddie *et al.*, 2010; Poulin *et al.*, 2011; Comeau *et al.*, 2013; Kiliyas *et al.*, 2013). Although both libraries represent similar communities on a larger taxonomic scale, it is known that both library types might give different views of the community on smaller scales (Stoeck *et al.*, 2007; Majaneva *et al.*, 2012), which is in accordance with our results. Furthermore, we have not only observed differences in the communities between the two library types, but also pronounced differences between sample locations and ice types. At FYI station 277, *Bacillaria* was more abundant in the rDNA library, which can be an indicator for inactivity or dormancy of this genus (Jones & Lennon, 2010). In contrast, CCMP2297 and *Melosira* OTUs were more abundant within the rRNA library, which can be an indicator for a potential metabolic activity (Blazewicz *et al.*, 2013). It should be noted that DNA can be present in active, dormant and

dead cells (Hansen *et al.*, 2007) and can also persist as free or extracellular DNA (Nielsen *et al.*, 2007; Charvet *et al.*, 2012) and thus will be seen as an indicator of inactivity as well as of the total biodiversity. On the other hand, the presence of rRNA does not necessarily indicate current activity, but more likely the potential protein synthesis due to a higher amount of ribosomes of specific taxa (Blazewicz *et al.*, 2013). Thus, whenever a conclusion is made regarding 'activity', this indicates the potential for a cell to be active and not the actual *in situ* activity. Furthermore, it remains difficult to directly compare rRNA versus rDNA libraries due to, for example, varying rDNA copy numbers within protist taxa (Prokopowich *et al.*, 2003) and variable cell sizes (Zhu *et al.*, 2005; Godhe *et al.*, 2008). Thus, the results of the sequencing should be interpreted in the light of these restrictions. However, due to the fact that the naviculoid pennate diatom CCMP2297 (Potvin & Lovejoy, 2009) showed high relative abundances in both libraries, we cannot infer activity based on the rRNA/rDNA ratios. In general, CCMP2297 was a very abundant group, especially at the highly porous and advanced melt FYI station 277. This suggests that this taxon might contribute significantly to the community composition, particularly at the end of the summer season during advanced melt and high porosity of the ice floe. Based on the rRNA/rDNA ratios, the diatom *Melosira* appeared to be the only highly active genus at the FYI station 277, which might also be related to the high porosity of the ice floe and therefore accompanied higher grazing impact on other diatom species, due to higher prey accessibility (Vancoppenolle *et al.*, 2013; Arrigo, 2014). It has been shown that *Melosira arctica*, which grows loosely attached to the sub-ice environment (Syvertsen, 1991; Gutt, 1995; Ambrose *et al.*, 2005), escapes grazing by an unknown mechanism in its sub-ice habitat with no grazers present in its surrounding environment (Gutt, 1995; Fernández-Méndez *et al.*, 2014).

With regard to phototrophs, the MYI station 360 did not differ much from the FYI stations based on the rDNA library. Even though this site is located in the vicinity of the North Pole and was thicker and colder than the other FYI samples, its biodiversity or phototrophic genus composition was comparable to the FYI floes. Until now, detailed information about the species composition of sea ice protists from MYI from the central Arctic Ocean is limited (Werner *et al.*, 2007; Bachy *et al.*, 2011). When it comes to potential metabolic activity (rRNA/rDNA ratio), the MYI station showed especially high relative abundances and potential metabolic activity of *Bacillaria* and *Melosira*. In general, *Melosira* showed a notable pattern: we exclusively found *Melosira* OTUs in the rRNA but none in the rDNA libraries, irrespective of the station and ice type. This might be due to a combination of the following factors: (1) DNA concentrations below detection levels (Deangelis & Firestone,

2012), (2) high activity of species with low relative abundances (Baldrian *et al.*, 2012; Angel *et al.*, 2013), (3) fewer gene copy numbers in rDNA libraries and an accompanied underestimation in rDNA libraries due to 'dilution' of the species by high copy number taxa (Not *et al.*, 2009; Koid *et al.*, 2012), or (4) methodological biases (Angel *et al.*, 2013) such as the nucleic acid extraction (Kermarrec *et al.*, 2013). *Melosira* accounts for a high amount of sub-ice biomass (Syvertsen, 1991; Gutt, 1995; Boetius *et al.*, 2013; Fernández-Méndez *et al.*, 2014) and net primary production (Fernández-Méndez *et al.*, 2014), although its occurrence can be very patchy (Gosselin *et al.*, 1997). As we were investigating ice cores and not the sub-ice habitat, *Melosira* was not the most abundant diatom genus, but it was the most abundant centric diatom, which is in agreement with other studies conducted in the Arctic (Eddie *et al.*, 2010; Comeau *et al.*, 2013). This result together with the lowest relative abundance of phototrophs in the rRNA library of the MYI station 360 and the accompanied highest potential metabolic activity of *Melosira* at this station, point out that low abundance taxa can be highly active and thus important (Baldrian *et al.*, 2012). A similar activity pattern was observed for the genus *Bacillaria*, which has rarely been reported from sea ice samples in non-molecular studies (Majaneva *et al.*, 2012; Comeau *et al.*, 2013). *Bacillaria* is the most abundant diatom genus in our dataset, occurring in every sample and station. Majaneva *et al.* (2012), also identified a *Bacillaria* sequence (FN690573) in environmental samples from the Baltic Sea during winter (Pniewski *et al.*, 2010) and Comeau *et al.* (2013) also observed high abundances of a similar sequence in their sea ice 18S rDNA dataset. *Bacillaria* normally forms colonies in its typically pelagic habitat, but also occurs individually in sea ice. Therefore, our observations provide further evidence that this taxon is an important, previously overlooked member of sea ice communities, especially due to its high activity. Based on the present results, genera like *Bacillaria* and *Melosira* may become more endangered by the on-going loss of Arctic MYI (Maslanik *et al.*, 2011; Stroeve *et al.*, 2011; IPCC, 2013).

Hetero- and mixotrophic communities of Arctic FYI and MYI

Molecular studies have shown that heterotrophic species are common in sea ice assemblages (Bachy *et al.*, 2011; Majaneva *et al.*, 2012; Comeau *et al.*, 2013). We found a higher relative abundance of Ciliophora species (ciliates) and heterotrophic OTUs in every rRNA versus rDNA library, irrespective of the location, which suggests higher potential metabolic activity of ciliates and high potential grazing pressure on phototrophic organism. Majaneva *et al.* (2012), who investigated the sympagic community of the Baltic Sea

during winter based on rRNA, found that ciliates were more abundant and active parts of the sympagic community than previously thought. In all rDNA libraries, irrespective of their origin, we found higher relative amounts of Dinoflagellata (dinoflagellates) and mixotrophs than in rRNA libraries, with high relative abundances of *Gymnodinium*, *Karlodinium* and *Gyrodinium*, known to be common in sea ice (Comeau *et al.*, 2013; Kiliyas *et al.*, 2013). Again, this might indicate either a lower degree of metabolic activity or a high degree of dormancy of these taxa (Jones & Lennon, 2010). During immediate microscopic investigations, we found many dinoflagellate cysts (data not shown), which are known to be present in Arctic waters (Harland *et al.*, 1980; Mudie & Rochon, 2001; Potvin *et al.*, 2013) at unfavourable conditions. Other studies also found a very high amount of Alveolata (alveolates) in their DNA libraries (Massana & Pedros-Alio, 2008; Not *et al.*, 2009), although they were not investigating the rRNA library. Ciliates and dinoflagellates are known to possess high 18S gene copy numbers in their genomes, leading to an overestimation of these taxa and an underestimation of metabolically active and smaller taxa with a lower rDNA gene copy number in DNA-based libraries (Not *et al.*, 2009; Baldrian *et al.*, 2012; Gong *et al.*, 2013; Kim *et al.*, 2013). Furthermore, it is known that 18S rDNA libraries cannot only be biased by high gene copy numbers of certain taxa (Prokopowich *et al.*, 2003; Godhe *et al.*, 2008; Gong *et al.*, 2013), but also by cell sizes and biovolumes of marine protists (Zhu *et al.*, 2005; Godhe *et al.*, 2008), free and extracellular DNA in the environment (Nielsen *et al.*, 2007), slow decay rates of nucleic acids in cold environments (Willerslev *et al.*, 2004), and dormant or dead cells (Hansen *et al.*, 2007). All these factors have implications on the interpretation of diversity studies, because specific taxa might contribute to the structure of the community but not to its function (Hansen *et al.*, 2007). Taken together, this emphasizes the need for a combined approach when investigating sympagic communities via both rDNA and rRNA studies.

FYI station 277 and MYI station 360 showed different hetero- and mixotrophic communities compared with the other stations. Ciliate genera like *Pithites*, and *Stokesia* were most active at FYI station 277. Due to the advanced stage of melt at FYI station 277 with its highly porous bottom ice, grazers would have had easier access into the ice and their prey. This can also be seen in our dataset by the highly abundant Rhizaria genus *Cryothecomonas*. It appears that *Cryothecomonas* species may act as important indicators for sea ice-melt in the Arctic (Thaler & Lovejoy, 2012). Furthermore, this genus, which was previously found in sea ice communities in several molecular studies (Lovejoy *et al.*, 2006; Bachy *et al.*, 2011; Majaneva *et al.*, 2012; Thaler &

Lovejoy, 2012; Comeau *et al.*, 2013), is indicative for advantageous grazing situations and/or good access to prey (e.g. diatoms) (Thomsen *et al.*, 1991; Thaler & Lovejoy, 2012; Comeau *et al.*, 2013). Majaneva *et al.* (2012) showed that Cerozoa species were most abundant in their 18S rRNA library, thus appear to be active grazers within the sea ice. Although the highest relative abundance of *Cryothecomonas* was found in the rDNA library, some *Cryothecomonas* OTUs had higher relative abundances in the rRNA library, indicating a certain degree of activity. Together with the high activity of ciliates at FYI station 277, heterotrophs are likely to have reduced the phototrophic community, in this case diatom members, by grazing. This is indicative of a post-bloom situation (Poulsen & Reuss, 2002; Kiliyas *et al.*, 2014) whereby a phototrophs-dominated sea ice community shifts towards a winter community dominated by hetero- and mixotrophs (Bachy *et al.*, 2011; Majaneva *et al.*, 2012). The only diatom genus that was active within FYI station 277 was *Melosira* that grows in a sub-ice habitat (Syvertsen, 1991). The presence of *Melosira* during advanced melt aligns well with previous findings that these algae by so far unknown means can prevent from grazing (Gutt, 1995).

The rRNA library of the MYI station had the highest proportion of heterotrophic protists of all stations with almost 50% of sequences assigned as Bicosoecida in the MYI rRNA library. Bicosoecida species are known to be halophilic, certain genera have been observed to grow within salinities between 60 to 150‰ (Park & Simpson, 2010). These two genera were the most abundant Bicosoecida genera in our dataset. Although the upper, older portions of MYI generally consist of fresher water, typically having a lower bulk salinity than FYI, MYI might include specific areas where the brine solution is highly concentrated due to potentially colder temperatures (Eicken *et al.*, 1995; Petrich & Eicken, 2010). The high relative abundance of Bicosoecida OTUs could be explained by the presence of high salinity patches within the MYI. This might also cause the high activity of Bicosoecida at this station: if the salinity is more favourable, the species might thrive better within the ice. This demonstrates that Bicosoecida might also be important active grazers within sea ice, in particular when temperatures are below freezing, in comparison to ciliates, which were mostly found at FYI station 277 with high porosity and advanced melt. Park & Simpson (2010) found that Bicosoecida species are able to form cysts, although we did not find Bicosoecida in our rDNA libraries (FYI and MYI). This indicates that this group might have been overlooked in the past and could possibly play an important role in the active microbial loop within sea ice. Furthermore, in cases where certain genera, such as Bicosoecida, are found mostly in MYI, they are

especially endangered by the loss of MYI and a shift towards a younger (Maslanik *et al.*, 2011) and thinner (Kwok & Rothrock, 2009) Arctic sea ice. Predicting the future state of the sea ice system remains a difficult task, which cannot fully be tackled with analyses of a limited number of ice cores, therefore more MYI samples over different seasons and from different regions are needed to better address the question how a rapidly changing sea ice cover may impact future trends in biodiversity.

Conclusion

We have shown that the taxonomic composition and trophic affiliation of sea ice protist assemblages varied both between and within ice types (FYI vs. MYI) especially at lower taxonomic levels. Comparing 18S rDNA and 18S rRNA libraries may reveal activity patterns of specific groups, but also identify species that have been overlooked and underestimated in the past. Our rDNA libraries from different ice types (FYI vs. MYI) showed that groups such as dinoflagellates and Rhizaria were the most important heterotrophic or mixotrophic grazers in the sea ice. However, their lower representation in our rRNA libraries indicates that most of the dinoflagellates and Rhizaria genera were probably less active, a conclusion that is in line with our microscopic observations of dinoflagellates, which we mainly observed in our samples in the form of cysts. On the other hand, ciliates and some other inhabitants such as *Melosira* and Bicosoecida species were underestimated when investigating only the rDNA. A higher potential metabolic activity of grazers at the FYI station 277 might be due to the advanced melt state of the ice matrix accompanied by a higher porosity and thus an easier accessibility to prey. Additionally, the higher representation of ciliates in the rRNA library indicates that they are more important members of the sympagic community than previously thought. Thus, we strongly recommend that future studies include both approaches to characterize sympagic communities, especially in the light of a changing Arctic Ocean.

ACKNOWLEDGEMENTS

This work was supported by the DFG [VA 105/18-1]. We would like to thank the captain and the crew of the expedition IceArc (ARK XXVII/3) of the *RV Polarstern* as well as our colleagues for their great support during the expedition. We are grateful to Marcel Nicolaus, Mar Fernández-Méndez, Judith Piontek, Christiane Uhlig, Luisa Galgani and Eva Kirschenmann for their excellent help during the fieldwork. We thank Marcel Nicolaus for assistance in preparation of the station map, Sylke Wohlrab for helping optimizing RNA extraction and cDNA synthesis, and Sandra Heinrich and Gerhard Dieckmann for fruitful discussions.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

SUPPLEMENTARY INFORMATION AND DATA AVAILABILITY

The 454-sequencing raw reads were deposited at the European Nucleotide Archive (ENA) under the accession number PRJEB7577. R-scripts, OTU-count data and further QIIME and PhyloAssigner analysis were deposited at <ftp://ftp.awi.de/pub/EDV/Supplements/TEJP-2014-0106>.

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at <http://dx.doi.org/10.1080/09670262.2015.1077395>.

Supplementary Table S1. Super-groups and their relative OTUs abundance (%) and relative number of different OTUs (%) found in rDNA and rRNA samples of each station based on the QIIME pipeline. Total relative amounts of OTU abundance and number refer to all OTUs found.

Supplementary Table S2. Stramenopile groups and their relative OTUs abundance (%) and relative number of different OTUs (%) found in rDNA and rRNA samples of each stations based on the QIIME pipeline. Total relative amounts of OTU abundance and number refer to Stramenopile OTUs found.

Supplementary Table S3. Assigned Bacillariophyceae OTUs. (a) Subclasses (bold) and genera in total number of OTUs and relative percentage of Bacillariophyceae (358) and total OTUs (714) based on PhyloAssigner. (b) Percental Bacillariophyceae OTU abundances of subclasses (bold) and genera for each of the four stations and sample type (rDNA and rRNA) based on QIIME.

Supplementary Table S4. Assigned Alveolata OTUs. (a) Orders (bold) and genera in total number of OTUs and relative percentage of Alveolata (122) and total OTUs (714) based on PhyloAssigner. (b) Percental Alveolata OTU abundances of orders (bold) and genera for each of the four stations and sample type (rDNA and rRNA) based on QIIME.

Supplementary Table S5. Alveolate groups and their relative OTUs abundance (%) and relative number of different OTUs (%) found in rDNA and rRNA samples of each stations based on the QIIME pipeline. Total relative amounts of OTU abundance and number refer to Alveolata OTUs found.

Supplementary Fig. S1. Rarefaction curves of 454 sequencing reads after quality clipping as number OTUs as a function of number of reads.

AUTHOR CONTRIBUTIONS

A. Stecher: sampling, RNA/DNA isolation and sequencing, data analysis and interpretation, drafting, writing and editing manuscript; S. Neuhaus: bioinformatic data analysis, writing and editing manuscript; B. Lange: sampling, abiotic data analysis, writing and editing manuscript; S. Frickenhaus: bioinformatics data analysis, editing manuscript; B. Beszteri: bioinformatic data analysis, data interpretation, drafting and

editing manuscript; P.G. Kroth: original concept, editing manuscript; K. Valentin: original concept, data interpretation, editing manuscript.

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