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ORIGINAL PAPER

Protist community composition during spring in an Arctic flaw lead polynya

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Abstract The overwintering deployment of an icebreaker during the Canadian Flaw Lead study provided an opportunity to evaluate how protist communities (phytoplankton and other single-celled eukaryotes) respond to changing spring irradiance conditions in flaw lead polynyas, where open water persists between the central pack ice and land fast ice. We combined microscopic analysis of the protist communities (all cell sizes) with clone libraries of 18S rRNA genes and 18S rRNA (from RNA converted to cDNA) of size-fractionated seawater (0.2–3.0 μm) from 10 to 12 m depth in the surface mixed layer. The rRNA gene analysis provided information on the presence of organisms, while the rRNA analysis provided information on the most active members of the community. There was little overlap between the two types of clone libraries, and there were large community shifts over time. Heterotrophic dinoflagellates and ciliates were the most common sequences recovered. The relative proportion of photosynthetic protist sequences increased in March and April, and there was greater representation of Bacillariophyta, Prasinophyta, Haptophyta, and Cryptophyta in the rRNA compared to rRNA gene libraries. Microscopy indicated that large-celled diatoms dominated the community in May, when chlorophyll concentrations were greatest. However, the RNA sequencing showed that heterotrophic and putative parasitic protists were proportionately more active, and the concomitant decrease in nutrients suggested that the spring phytoplankton bloom had begun to decline by this time. These observations provide evidence of substantial changes in protist community structure and function during the spring transition.

Keywords Euphotic zone · Phytoplankton · Protist · Arctic Ocean · Beaufort Sea · Environmental clone libraries · Biodiversity · Microbial food webs

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Introduction

Arctic protist communities, comprising photosynthetic, mixotrophic, and heterotrophic species, experience a unique combination of environmental factors that constrain their biomass and productivity (Sakshaug et al. 2004; Greene and Pershing 2007). Phytoplankton and associated microbial communities in the Arctic Ocean have especially pronounced seasonal patterns because of the wide fluctuations in physical and chemical conditions over the year (Sherr et al. 2003; Carmack et al. 2004). For example, arctic primary production is regulated by the strong seasonality of incident irradiance, the extent and duration of sea ice, and the availability of inorganic nutrients (Smith Jr and Sakshaug 1990).

Spring is a critical time at high latitudes, with the transition from winter darkness to rapidly increasing day length. These improved irradiance conditions may be further amplified by the seasonal melting of ice and the transition to open water, thereby stimulating high photosynthetic activity (Smith Jr and Sakshaug 1990). In winter, Arctic marine waters are characterized by dominance of

heterotrophic organisms, and during spring, the relative abundance of phototrophs increases rapidly (Terrado et al. 2008). Although the transition is mostly driven by increased irradiance (Sherr et al. 2003; Terrado et al. 2008), nutrient supply can shape the final structure of Arctic protist communities (Lovejoy et al. 2004).

Protist succession has been studied by microscopy in coastal and offshore regions of the ocean (Larsen et al. 2004; Litchman et al. 2006) and by pigment signatures (Anderson et al. 2008). Environmental surveys using 18S rRNA gene clone libraries have been increasingly used to investigate seasonal changes in small eukaryotes (Romari and Vaulot 2004; Medlin et al. 2006; Worden 2006; Terrado et al. 2009). Molecular environmental surveys conducted in Arctic waters report the presence of diverse microbial communities, largely influenced by the different water masses (Lovejoy et al. 2006; Hamilton et al. 2008; Terrado et al. 2009; Lovejoy and Potvin 2010). These microbial communities contain cold-adapted ecotypes (Lovejoy et al. 2007), which could be vulnerable to shifts in global circulation patterns (Hakkinen and Rhines 2009).

Most molecular-based protist diversity studies have been carried out on genomic DNA by targeting the gene coding for small subunit ribosome RNA, the 18S rRNA gene. An additional approach is to directly target the 18S rRNA using RNA as the template, thus obtaining not only information on diversity but also on gene expression and the potential activity of cells in the community. Several studies have compared the rRNA and rRNA gene approaches and reported a relatively small and variable proportion of taxa retrieved by both methods (Stoeck et al. 2007b; Not et al. 2009). Although some of the differences in results of rRNA and the rRNA gene surveys may be due to incomplete sample coverage and inherent differences in gene copy number among organisms, the RNA template only targets the active community (defined as cells actively synthesizing ribosomes and proteins) under the environmental conditions at the time of sampling. In contrast, the resident community, defined as the total assemblage of all active plus non-active cells, reflects the source water masses and the history of environmental conditions prior to sampling. This difference between gene presence and expression should be particularly pronounced during the spring transition when the community is changing from a winter heterotrophic-dominated community to a spring photosynthetic community.

The Circumpolar Flaw Lead (CFL) program provided an opportunity to study the spring transition in the Arctic Ocean, specifically in a coastal flaw lead polynya ecosystem, where open water persists between central pack ice and land fast ice in Amundsen Gulf, Canadian Beaufort Sea. Our objective was to evaluate protist diversity and infer community structure during the spring transition by way of rRNA and rRNA gene cloning and sequencing. We targeted the smaller size class protists with our sample filtration approach, as they are abundant in the Arctic Ocean (Li et al. 2009) and difficult to identify by microscopy. As with all such environmental surveys, larger cells were also identified in our clone libraries (Vaulot et al. 2008) that along with our microscopic results provided a broader perspective on the protist communities.

Materials and methods

Sample collection and preparation

The CCGS Amundsen was kept mobile in Amundsen Gulf (Fig. 1) from December 2007 to June 2008 during the CFL study (Barber et al. 2010). Samples analyzed for this study were collected at ca. 3 week intervals from 17 March to 19 May (Fig. 1) from 10 to 12 m depth in the surface mixed layer using Niskin-type bottles (Ocean Test equipment Inc., Fort Lauderdale, USA) mounted on a rosette system equipped with a conductivity, temperature, and depth (CTD) profiler (Sea-Bird SBE-911 CTD, Bellevue, USA).

Seawater for environmental DNA and RNA was collected into clean carboys directly from the Niskin-type bottles. Six L for DNA and four L for RNA were sequentially pre-filtered through a 50-μm nylon mesh, a 47-mm diameter 3-μm polycarbonate filter, and either a 0.2-μm Sterivex unit (Millipore Canada Ltd, Mississauga, Canada) for DNA or a 47-mm diameter 0.2-μm polycarbonate filter for RNA. All clone libraries were constructed from material captured on the 0.2-μm filters, the nominal 0.2–3 μm size fraction. DNA samples were preserved by adding buffer (1.8 mL of 50 mM Tris–HCl, 0.75 M sucrose, 40 mM EDTA, pH = 8.3) to the Sterivex units, while the RNA filters were conserved in 2-mL cryovials following the addition of 600 μL of RLT buffer (Qiagen, Mississauga, Canada) and 1% beta-mercaptoethanol (Sigma–Aldrich, Oakville, ON, Canada). All samples were stored at −80°C until nucleic acid extraction.

Total chlorophyll *a* (chl *a*) samples were collected by filtering 500 mL of whole seawater (not prefiltered) onto Whatman GF/F filters (Whatman, Sanford, USA). Pigment extraction in acetone was done onboard the ship, and the extracts were then analyzed by spectrofluorometry (Parsons et al. 1984). Samples for dissolved nutrients were collected directly from the Niskin-type bottles and analyzed onboard the ship with an Autoanalyzer 3 (Bran + Luebe, Nordestedt, Germany) using colorimetric methods (Grasshoff 1999).

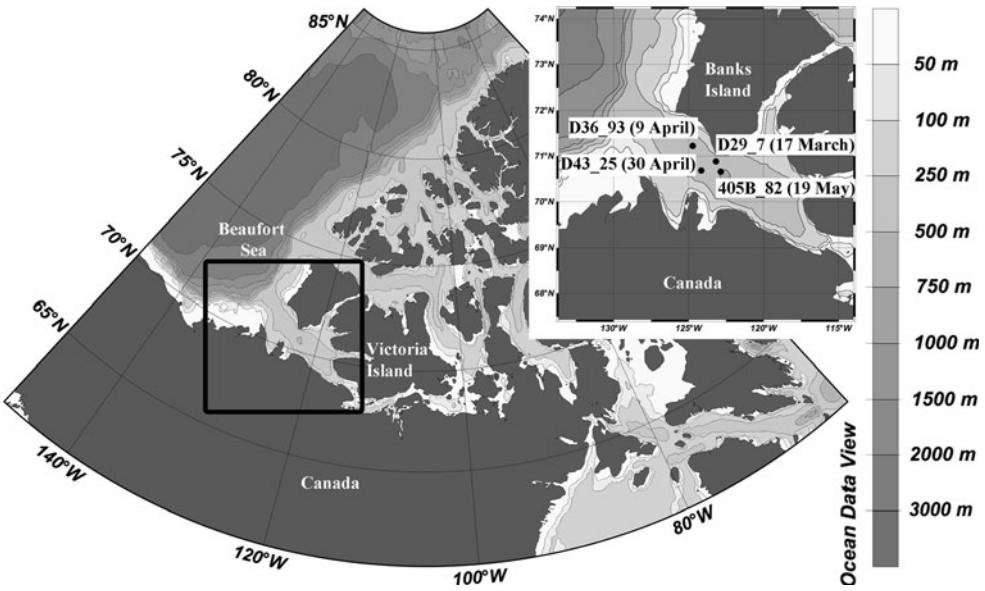

Fig. 1 Map of the Amundsen Gulf region indicating the location and the date of the stations sampled. *Gray scale* indicates bottom depth

Microscopy

Whole seawater samples (not prefiltered) for analysis by epifluorescence microscopy were fixed with glutaraldehyde (Electron Microscope Sciences, Fort Washington, PA, USA) at a final concentration of 1% (v/v). Fixation took place in the dark at 4°C for 1 h. After fixation, 45 ml of the 50 ml samples were filtered onto 0.8-μm black 25-mm diameter polycarbonate filters (Poretics Corp., Livermore, CA). After filtering to 5 ml, the last 5 ml was added, filtration stopped, and 4′6-diamidino-2-phenylindole (DAPI, 5 μg mL^{-1} final concentration, Sigma–Aldrich, Oakville, Canada), added and left for at least 5 min (Porter and Feig 1980). After completing the filtration, the filters were mounted onto slides with non-fluorescent mounting oil (Immersol 518 M) and left at 4°C for 4–8 h then stored at −20°C. Cells were enumerated at 1,000× magnification using an Olympus BX40 fluorescence microscope equipped with a mercury light source within 6 months of collection. Excitation/emission filters were 330/400 nm for DAPI and 450–490/515 nm for chlorophyll. Cells were grouped into categories based on size (<2, 2–5, 5–10 and >10 μm) and the presence or absence of fluorescence from chloroplasts. Diatoms, dinoflagellates, and cryptophytes were counted as separate groups. Cell counts were converted to biomass carbon using conversion factors for diatoms and flagellates given in Menden-Deuer and Lessard (2000) based on estimated cell volumes.

Nucleic acid extraction

DNA was extracted from the filters based on a salt extraction method (Aljanabi and Martinez 1997). Briefly, lysozyme (final concentration of 1 mg mL^{-1}) was added to the Sterivex units and incubated for 45 min at 37°C. Proteinase K (final concentration of 0.2 mg mL^{-1}) and SDS (final concentration of 1%) were then added and incubated at 55°C for 1 h. The contents were then removed from the Sterivex unit and transferred to a 15-mL centrifuge tube with 1 mL of lysis buffer used to rinse the Sterivex unit and incubated for another 15 min at 55°C. Concentrated NaCl (6 M) was then added to the tubes (final concentration of 2.3 M), which were vortexed for 1 min followed by centrifugation at 6,000 rpm for 10 min. The supernatant was transferred into a new 15-mL tube, and an equal volume of cold 70% ethanol was added into each sample, mixed, and left at −20°C for 2 h. For each sample, 1.8 mL of the total volume was transferred into a 2-mL microcentrifuge tube and centrifuged at 14,000 rpm for 10 min at 4°C and the supernatant discarded. This step was repeated until the entire volume of the sample had been centrifuged. The remaining pellet was washed with 200 μL of 70% ethanol. Once the pellet was dry, it was resuspended in 100 μL of 1X TE buffer and stored at −80°C.

RNA was extracted as in Church et al. (2005) using a bead beater (Disruptor GenieTM, Scientific Industries, Inc., Bohemia, NY, USA) followed by the extraction with the RNeasy Kit (Qiagen, Mississauga, ON, CAN) following the manufacturer's instructions, including the optional on-column digestion with the RNase-Free DNase kit (Qiagen, Mississauga, ON, CAN). RNA was eluted with 30 μL of RNAse-free water and immediately transformed into cDNA using a High Capacity Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommendations. Briefly, RT–PCR mixtures consisted of 30 μL of eluted RNA, 6 μL of RT buffer,

2.4 μL of dNTP, 6 μL of random primers, 3 μL of Multiscribe reverse transcriptase, and 12.6 μL of water. Samples were reverse transcribed using the thermal cycling conditions: 10 min at 25°C, 120 min at 37°C, and 5 min at 85°C. The cDNA was then stored at −80°C.

Clone libraries and phylogenetic analysis

Two different primer pairs were used to construct the DNA clone libraries; 336f-EUK R and NSF 4/18-EUK R (Sogin and Gunderson 1987; Medlin et al. 1988; Hendriks et al. 1989). Because there was little difference between libraries from the same sample constructed from the different primer pairs (Bray-Curtis similarity matrix >80% similarity, not shown), as has been reported elsewhere (Potvin and Lovejoy 2009); only the first primer pair (336f-EUK R) was used to construct the RNA clone libraries. Amplification steps and cloning were as in Potvin and Lovejoy (2009). Positive clones were reamplified using the vector M13 primers. Resulting PCR amplicons of all the clones were digested with *Hae*III and run on a 2.5% low melting point agarose gel for screening by Restriction Fragment Length Polymorphism (RFLP) analysis (Diez et al. 2001) At least one representative of each pattern in each library was sequenced using the 528f primer (Medlin et al. 1988) or the 336f primer at Service de séquençage et génotypage du Centre Hospitalier de l'Université Laval with an ABI 3730xl system. Sequences were manually checked using the 4Peaks software v.1.7.2 (A. Griekspoor and Tom Groothuis, mekentosj.com), and closest identity to other sequences was checked using NCBI BLAST. Sequences with BLAST results with less than 95% similarity to other sequences were tested using the Check Chimera program at the Ribosomal Data Project II (Michigan State University; http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). Separate short segments of suspected chimeras were subjected to additional BLAST searches; if the separate segments matched very different groups, they were discarded. The software KeyDNAtools was used to obtain preliminary taxonomic affiliations (Guillou et al. 2008). Sequences that passed chimeric screening were aligned using Clustal X v.1.83 (Thompson et al. 1997). Alignments were further checked using CLC sequence viewer v. 6.3 (CLC Bio A/S, http://www.clcbio.com). Neighbor-joining trees were constructed with PHYLIP (Felsenstein 2005) to identify major clades and similar sequences among the libraries (not shown). Operational taxonomic units (OTUs, defined as $< 2\%$ difference between sequences) were calculated with MOTHUR (Schloss et al. 2009). Clones with the same RFLP pattern in each clone library was considered the same sequence for relative abundance estimates. Sequences from this study have been deposited in GenBank (accession numbers HM560982 to HM561278).

Results

Site characteristics

The average salinity of the surface mixed layer from which the samples were collected ranged from 31.84 to 31.96; a slight freshening was observed in May (Table 1). Similarly, temperatures were relatively constant at −1.72 to −1.70°C until May when a slight warming to −1.32°C was recorded (Table 1). Intermittent ice cover was present throughout March and April, whereas in May the region of sampling was mostly ice-free. Concentrations of nitrate plus nitrite (hereafter called nitrate, as nitrite values were low) and of silica were highest in March. Nitrate values (at over 5 μM in March) dropped by 40% in April and by 80% in May (to ca. 1 μM), whereas silica declined steadily over the sampling period by ca. 12% (Table 1). Total chl *a* was extremely low (0.01 μg L^{-1}) in March, remained below 1 μg L^{-1} in April, and then increased to over 10 μg L^{-1} in May (Table 1). Microscope counts showed a general increase in total cells for both heterotrophic and autotrophic (chloroplastic) organisms from March to May (Fig. 2). Cryptophytes were observed in all samples at concentrations ranging 11–23 cells mL^{-1}. Dinoflagellates were also observed in all samples, at densities of 8–20 cells mL^{-1}. The proportional contribution of heterotrophs (protist cells with no discernable pigment fluorescence) to total protist biomass was highest in the March sample, with no diatoms observed at that time (Fig. 2). Autotrophic biomass dominated in April and May, with the smallest cells peaking in late April and diatoms making the largest contribution in the May sample (Fig. 2).

Comparison of rRNA gene and rRNA libraries

Overall, 805 cloned fragments of the targeted size were retrieved and screened using restriction fragment length polymorphism (RLFP). Of those, we identified 7 chimeric and 38 metazoan sequences, which were excluded from further analysis. In total, 452 DNA-derived and 308 RNA-derived clones were retained for further analysis.

There were differences in the relative proportions of the phylogenetic groups in the rRNA gene and rRNA libraries with over or under representation among several groups (Fig. 3). Cercozoa, unclassified marine alveolates (Marine Alveolates), *Telonemia*, fungi (Chytridiales), uncultured marine stramenopiles (MAST), dinoflagellates, and Cryptophyta nuclear sequences were more frequently detected in rRNA gene libraries compared to rRNA libraries. In contrast, Haptophyta, Bacillariophyta, Prasinophyceae, and Ciliophora were more often retrieved in rRNA libraries. Bolidophyceae, Dictyochophyceae, "picobiliphytes", and Choanoflagellida sequences were only recovered from

Table 1 Environmental properties of the stations used for the construction of clones libraries

	Station			
	D29_7	D36_93	D43_25	405B_82
Date	17/03/2008	09/04/2008	30/04/2008	19/05/2008
Latitude (°N)	70.91	71.31	70.66	70.66
Longitude (°W)	123.48	124.57	123.77	122.88
Depth (m)	10	10	10	12
Temperature (°C)	−1.74	−1.72	−1.74	−1.32
Salinity	31.89	31.89	31.96	31.84
Chl *a* (μg L^{-1})	0.01	0.60	0.76	10.55
Nitrate (μM)	5.13	3.18	3.08	0.95
Nitrite (μM)	0.28	0.27	0.10	0.12
Silica (μM)	10.02	9.32	8.99	8.77

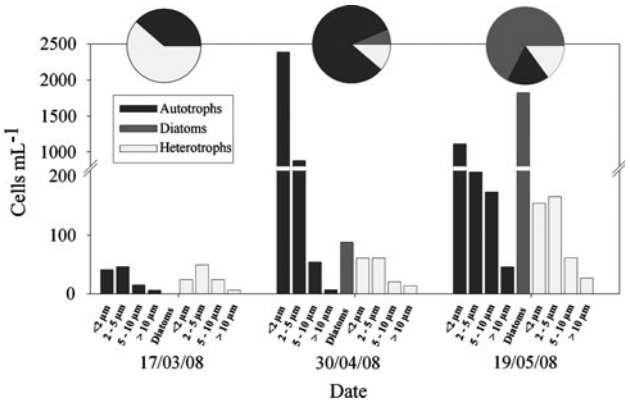

Fig. 2 Abundance of heterotrophic and autotrophic protists by sampling date of this study expressed as cells mL^{-1}. No data were available for sample date 09/04/2008. Values are given for 4 different size classes, with diatoms classified as a separate group. *Pie charts* show the proportional contribution to biomass carbon derived from microscope counts for diatoms, other autotrophs, and heterotrophs, by sampling date. Cell counts were converted to carbon biomass using the conversion factors of Menden-Deuer and Lessard (2000)

rRNA gene libraries. Labyrinthulida, Radiolaria, and cryptophyte nucleomorph sequences were only recovered from rRNA libraries. Nucleomoph rRNA operons are located in subtelomeric regions of their chromosomes that have a high GC content (Archibald 2007). This may have contributed to the lack of nucleomorph sequences in the rRNA gene libraries compared to the rRNA libraries, as these regions are more difficult to amplify by standard PCR. Alternatively, this absence may reflect the lower gene copy numbers in the nucleomorph.

Following the RLFP analysis, a total of 760 clones were matched with a sequence from its RLFP pattern. Sequences from the same pattern were always >99% similar to each

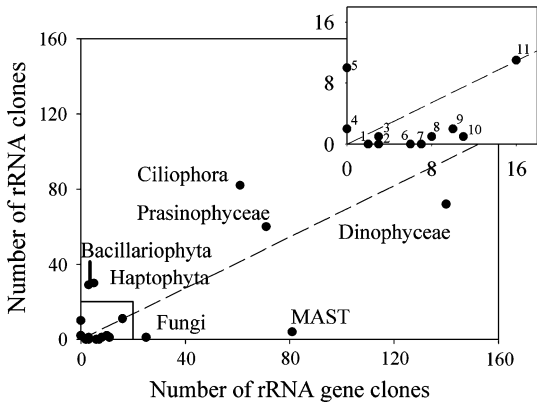

Fig. 3 Number of clones recovered for each phylogenetic group from rRNA clone libraries versus rRNA gene clone libraries. The *dashed line* represents the sampling effort (number of clones) for rRNA clone libraries compared to rRNA gene clone libraries. Groups with fewer than 20 clones are represented by numbers in the *upper right insert*. *1* Dictiophyta. *2* Bolidophyceae. *3* Marine Alveolates Group I + V. *4* Labyrinthulida. *5* Cryptophyta nucleomorph. *6* Choanoflagellida. *7* Picobiliphytes. *8* Cryptophyta nuclear. *9* Telonemia. *10* Cercozoa. *11* Marine alveolate groups II + III + IV

other as reported elsewhere (Lovejoy et al. 2006). Sequences >98% similar were classified as operational taxonomic units (OTU), yielding 114 OTUs recovered from rRNA gene libraries and 65 from rRNA libraries (Fig. 4). The fact that we had two libraries per sample from DNA-extracted template explains the higher number of rRNA gene OTUs, but the ratios of pooled OTUs to pooled number of clones were similar (0.25 for rRNA gene and 0.21 for rRNA). Just over 10% of the OTUs were common to both the rRNA gene and rRNA libraries. Dinoflagellates and ciliates had the greatest number of overlapping sequences recovered from both types of libraries with 14% overlap for ciliates and 19% for dinoflagellates (Fig. 4).

Community composition

Overall, the most commonly recovered sequences from both rRNA gene and rRNA clone libraries for all samples were alveolate taxa that included representatives from a diversity of the known marine groups (Adl et al. 2005; Vaulot et al. 2008). Dinoflagellates were present in both rRNA gene and rRNA clone libraries in all samples except for the rRNA clone library from early April (Table 2). Generally, dinoflagellate sequences were closest to other environmental sequences sometimes within clades defined by their closest cultured relative. For example, rRNA gene clone libraries all had sequences within the cluster associated with the heterotrophic *Gyrodinium rubrum* (GenBank accession number AB120002). Other sequences were related to dinoflagellates with chloroplasts including a morphospecies of *Gymnodinium aureolum* (GenBank

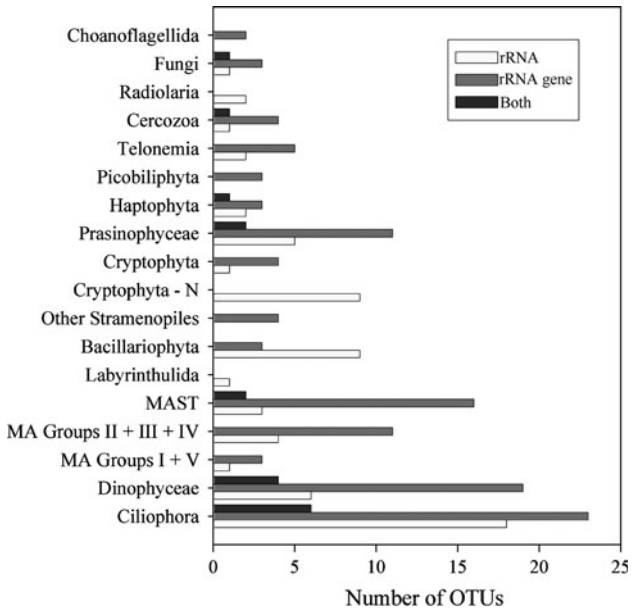

Fig. 4 Number of operational taxonomic units (OTUs) for each phylogenetic group present in rRNA clone libraries, rRNA gene clone libraries, or in both types of clone libraries. Marine stramenopiles (MAST): the groupings are as in Massana et al. (2006) and Marine alveolates (MA): the groupings (I–V) are as in Guillou et al. (2008)

accession number AJ415517) and an isolate from Antarctic sea ice designated Dinophyceae W-5 (GenBank accession number AY434687; Table 2).

Ciliate sequences were recovered in all libraries (Fig. 3, Table 2). Environmental sequences falling into the Spirotrichea were recovered from all clone libraries and were especially common in both rRNA gene and rRNA libraries from May. Sequences falling within a large environmental cluster, with the closest match to *Strombidium* SNB99, (*Strombidium* sp 2 in Strüder-Kypke and Lynn 2003) (GenBank accession number AY143564), were recovered from all rRNA gene clone libraries, representing 3–10% of the total clones. However, it was only in May that the sequences from this cluster were recovered in the rRNA libraries when they accounted for 27% of all sequences. Environmental sequences with closest affinities within the Phyllopharyngea were recovered only from the rRNA libraries from March to early April, where they accounted for 6 and 26% of the total clones, respectively (Table 2).

Marine alveolate groups I and V (as defined by Guillou et al. 2008) were relatively rare, representing <2% of total sequences. Both groups were recovered from rRNA gene libraries in March; Group I was later recovered in the rRNA library from late April. Marine alveolate group II sequences were recovered from rRNA gene libraries in March and April. In contrast, the closely related Groups III and IV (from Guillou et al. 2008) were only recovered from rRNA libraries, where they were relatively common (up to 11%) in March and early April (Table 2).

Stramenopile (Heterokonta) sequences were recovered from all clone libraries. Non-cultivated heterotrophic marine stramenopiles (MAST, Massana et al. 2004) and a clone 96% similar to known Labyrinthulida were more frequent in rRNA gene libraries compared to rRNA libraries (Fig. 3, Table 2). The MAST-1A clade represented 9–23% of clones from rRNA gene libraries, but was never recovered from RNA libraries (Table 2). Similarly, MAST-1C and MAST-2 were only recovered in the rRNA gene libraries. MAST-3, MAST-7, MAST-8, and a single sequence closely related to the Labyrinthulida were recovered from rRNA libraries (Table 2). Diatom sequences did not appear in the libraries until April and were frequent (1–9%) in the rRNA libraries in late April and May (Table 2). Small flagellated photosynthetic stramenopiles in the Bolidophyceae and Dictyochophyceae were rare and only found in the rRNA gene libraries.

Among prasinophytes, *Micromonas* sequences >99% similar to the Arctic isolate CCMP 2099 (GenBank accession number DQ025733, Lovejoy et al. 2007) were recovered from all sites in both rRNA gene and rRNA clone libraries. *Bathycoccus* sequences were common in the March rRNA library, and sequences with nearest matches to the genus *Pyramimonas* were recovered in samples from March (rRNA) and April (both rRNA gene and rRNA) (Table 2).

Haptophyte sequences were recovered from rRNA gene and rRNA libraries, but were most common in the rRNA library from early April (Table 2). Cryptophyte sequences were recovered beginning in early April. Cryptophyte nucleomorph sequences were all from the rRNA libraries and were quite diverse with closest matches to *Falcomonas* sp., *Hemiselmis* sp., *Teleaulax* sp., and *Geminigera cryophila* (Table 2). The majority of cryptophyte sequences for the nuclear 18S rRNA gene were from rRNA gene libraries in April and May (Table 2), except for one sequence from the May rRNA library, with 99% similarity to an Arctic Isolate (CCMP2293) in the *Falcomonas* group. The majority of other cryptophyte sequences were closest to *Geminigera cryophila* but there were also matches to *Teleaulax* (Table 2).

Other groups recovered included two Radiolarian sequences belonging to an environmental Acantharia clade related to *Acanthometra*. Cercozoan sequences had closest matches to the clade containing *Cryothecomonas* and *Protapsis*. The choanoflagellate sequences were most closely related to other environmental sequences previously recovered from the Arctic, with affinities to *Diaphanoeca grandis*. Other sequences aligned with the heterotrophic flagellate *Telonemia*, the as yet uncultured picobiliphytes, and the fungal group Chytridiales (Table 2).

Table 2 Proportion of clones for rRNA gene and rRNA clone libraries for the different phylogenetic groups retrieved

	Group	Subgroup	D29_7		D36_93		D43_25		405B_82	
			17 March		9 April		30 April		19 May	
			rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene	rRNA
Alveolate	Dinophyceae	*Gyrodinium rubrum*	37	3.0	27	0.0	27	0.0	5.8	33
		Gyrodinium aureolum (p)	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0
		Dinophyceae W5 (p)	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0
		Other Dinophyceae	5.1	24	8.2	0.0	4.2	29	11	0.0
	Ciliophora	*Strombidium* SNB99-2	10	0.0	3.1	0.0	4.2	0.0	6.5	27
		Other Spirotrichea	7.2	6.1	2.1	5.5	5.0	8.1	11	25
		Phyllopharyngea	0.0	6.1	0.0	26	0.0	0.	0.0	0.0
		Oligohymenophorea	0.0	0.0	0.0	1.4	0.0	0.0	0.7	0.0
		Prostomatea	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
		Litostomatea	1.0	0.0	0.0	0.0	0.8	0.0	0.7	1.2
	Marine Alveolates	Group I	2.1	0.0	0.0	0.0	0.0	0.8	0.0	0.0
		Group II	4.1	0.0	8.2	0.0	3.3	0.0	0.0	0.0
		Group III	0.0	3.0	0.0	11	0.0	0.0	0.0	0.0
		Group IV	0.0	6.1	0.0	0.0	0.0	0.0	0.0	0.0
		Group V	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stramenopiles	MAST	MAST 1A	23	0.0	9.3	0.0	9.2	0.0	16	0.0
		MAST 1C	2.1	0.0	1.0	0.0	0.8	0.0	1.4	0.0
		MAST 2	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0
		MAST 3	1.0	3.0	0.0	0.0	0.0	0.0	0.7	0.0
		MAST 7	0.0	3.0	0.0	0.0	0.0	0.0	2.2	0.0
		MAST 8	0.0	0.0	0.0	1.4	0.0	0.8	0.7	0.0
	Labyrinthulida		0.0	6.1	0.0	0.0	0.0	0.0	0.0	0.0
	Bacillariophyta	*Chaetoceros* (p)	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0
		Porosira (p)	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0
		Navicula (p)	0.0	0.0	0.0	0.0	0.0	8.9	0.0	0.0
		Fragilaria (p)	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
		Pseudo-nitzschia (p)	0.0	0.0	0.0	0.0	0.8	0.0	0.7	6.2
		Thalassiosira (p)	0.0	0.0	1.0	0.0	0.0	4.1	0.0	2.5
	Bolidophyceae (p)		0.0	0.0	2.1	0.0	0.0	0.0	0.7	0.0
	Dichtyochophyceae (p)		0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0
Chlorophyta	Prasinophyceae	*Pyramimonas* (p)	0.0	0.0	3.1	0.0	1.7	8.9	0.0	0.0
		Bathycoccus (p)	0.0	24	1.0	0.0	0.8	0.8	0.0	0.0
		Micromonas (p)	1.0	3.0	14	1.4	33	29	6.5	2.5
Haptophyta	Prymnesiophyceae	*Chrysochromulina* (p)	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
		Phaeocystis (p)	0.0	0.0	3.1	40	0.0	0.0	1.4	0.0
Cryptophyta	Nucleomorph	*Falcomonas* (p)	0.0	0.0	0.0	2.7	0.0	0.8	0.0	0.0
		Hemiselmis (p)	0.0	0.0	0.0	1.4	0.0	1.6	0.0	0.0
		Teleaulax (p)	0.0	0.0	0.0	4.1	0.0	0.0	0.0	0.0
		Geminigera (p)	0.0	0.0	0.0	1.4	0.0	1.6	0.0	0.0
	Nuclear	*Cryptophyceae* (p)	0.0	0.0	0.0	0.0	0.8	0.0	0.0	1.2
		Teleaulax (p)	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
		Geminigera (p)	0.0	0.0	3.1	0.0	1.7	0.0	0.7	0.0
Rhizaria	Radiolaria		0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
	Cercozoa		0.0	3.0	0.0	0.0	0.0	0.0	8.0	0.0

Table 2 continued

	Group	Subgroup	D29_7		D36_93		D43_25		405B_82	
			17 March		9 April		30 April		19 May	
			rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene	rRNA	rRNA gene	rRNA
Opisthokonta	Choanoflagellida		1.0	0.0	5.2	0.0	0.0	0.0	0.0	0.0
	Fungi	Chytridiales	0.0	0.0	0.0	0.0	0.0	0.0	18	1.2
Incertae sedis	Picobiliphytes (p)		4.1	0.0	1.0	0.0	0.8	0.0	0.7	0.0
	Telonemia		0.0	3.0	5.2	0.0	3.3	0.8	0.7	0.0

Subgroups were defined by closest BLAST match and after inspection of phylogenetic trees (not shown). Marine Stramenopiles (MAST) sensu Massana et al. (2006). Note that *Gyrodinium aureolum* is a polyphyletic morphospecies, and our sequences were similar to the sequence noted in the text. Photosynthetic organisms are marked with (p). Picobiliphytes are uncultured, and their status as photosynthetic is doubtful

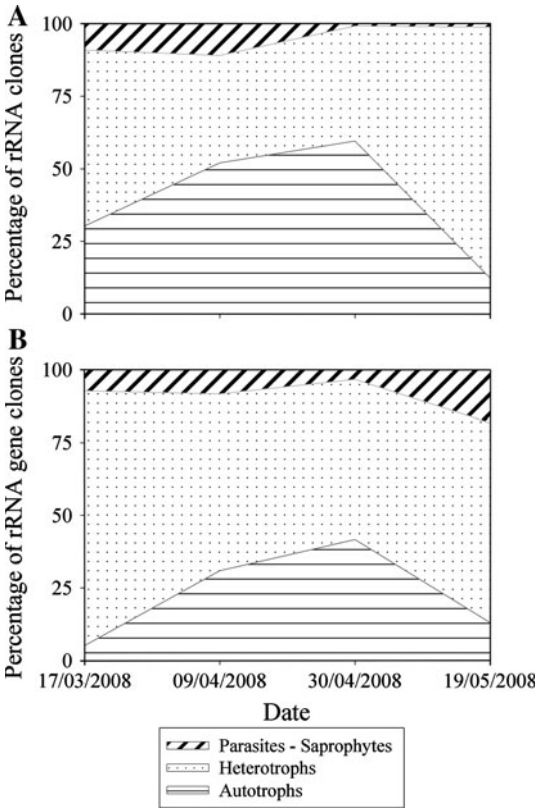

Fig. 5 Percentage of autotrophic-, heterotrophic-, or parasite/saprophyte-related clones recovered for **a** rRNA clone libraries and **b** rRNA gene clone libraries by sample date

The MOTHUR-generated OTUs defined at 98% similarity level, were assigned to phototrophic, heterotrophic, or parasitic–saprophytic trophic levels based on what is known of their nearest cultured match (Table 2); OTUs related to mixotrophic organisms were considered phototrophs. Whether originally from the rRNA gene or the rRNA clone libraries, the functional groups in the size fraction we collected followed similar trends from March to May. Sequences from heterotrophs accounted for a higher proportion of total sequences at the beginning of the study and phototrophs made up higher proportions in April, decreasing in May. The proportion of sequences belonging to heterotrophic groups in the rRNA gene clone libraries was always greater than in the rRNA clone libraries (Fig. 5). The parasitic–saprophytic groups remained below ca. 10% of the total clones recovered from the rRNA gene and rRNA libraries until May when these groups accounted for ca. 20% of the rRNA gene clones, all of which were related to the Chytridiales (Table 2, Fig. 5).

Discussion

The sampling strategy for the Canadian Flaw Lead study (CFL) was to remain mobile within the flaw lead throughout the entire sampling period; because of shifting ice and currents, there was drift and displacement between sampling dates. However, temperature and salinity data indicated that all sampling was carried out within the Arctic surface winter layer (SWL) (Carmack et al. 1989). Because Arctic water masses are relatively stable entities with distinctive microbial communities (Hamilton et al. 2008; Galand et al. 2009; Lovejoy and Potvin 2010), it is likely that the protist community changes observed in this study were due to the increasing irradiance and nutrient drawdown in the salinity-stratified upper water column, which are the characteristic of spring. During the Canadian Arctic Shelf Exchange Study (CASES), an earlier (2003–2004) overwintering expedition in Franklin Bay (Amundsen Gulf, Beaufort Sea), chl *a*, and cell concentrations began to increase soon after the spring solstice (Terrado et al. 2008; Tremblay et al. 2008). However, chl *a* values were fourfold greater in the CFL study region during 2008, reaching over 10 μg chl *a* L^{-1} in May, and were comparable to the values recorded in Northern Baffin Bay (15 μg chl *a* L^{-1}) during the International North Water (NOW) Polynya Study (Mei et al. 2002).

Environmental conditions in the CFL study region during March were similar to those in early April 1998 reported for Smith Sound and the Outer Murchison Region in the northern part of the North Water Polynya during the NOW study (Ingram et al. 2002). Microscopic analysis showed that protist biomass in this NOW study region was dominated by ciliates and some dinoflagellates in April but changed to a more mixed community the next month when chl *a* concentrations were greater (Lovejoy et al. 2002). Analogous to the microscopy data from the NOW study and consistent with the dominance of heterotrophs observed microscopically in the present study (Fig. 2), we found that the majority of 18S rDNA sequences from both rRNA gene and rRNA libraries belonged to ciliates and other heterotrophs in March. The community identified from 18S rRNA in the present study shifted to a more mixed community in early April and to a greater proportion of phototrophs at the end of April (Fig. 5). In May, chl *a* concentrations and autotrophic biomass derived from microscope counts were at their maximum (Table 1, Fig. 2); however, the proportion of sequences associated with photosynthetic groups recovered in the PCR-based libraries fell as the proportion of heterotrophic groups increased. Concurrent with the high chl *a* concentrations, nitrate values decreased substantially, similar to the inverse relationship observed between chl *a* values and nitrate concentrations over April and May in the North Water Polynya study (Mei et al. 2002). However, although nitrate concentrations had diminished, silica concentrations remained relatively high, consistent with the mixed community indicated from the clone libraries, where cryptophyte, *Micromonas*, and haptophyte sequences were recovered as well as diatoms. Although the bloom was ongoing (especially of diatoms, undersampled by our filtration approach), the relative importance of heterotrophic protists in our May libraries, in particular in the rRNA library, suggests that a complex microbial food web was well developed with active protist grazing, as reported from the Western Arctic Ocean (Weisse et al. 1990; Sherr and Sherr 2002; Sherr et al. 2009).

The filtration strategy and the choice of the 0.2–3 μm fraction in this study were selected to better detect small eukaryotic cells, as in previous molecular surveys (Diez et al. 2001; Lovejoy et al. 2006). However, larger protists and even metazoan sequences are also recovered from these small size fraction clone libraries (Lovejoy et al. 2006; Vaulot et al. 2008), indicating imperfect separation of cells by filtration. The sequences associated with large protists recovered in our clone libraries (ciliates and dinoflagellates) would be due to factors such as cell breakage and deformation of flexible-walled cells that then pass through the prefilters. These factors together with the variable 18S rRNA gene copy number in different protists (Zhu et al. 2005) means that PCR-based eukaryotic rRNA gene surveys cannot be used to infer cell numbers or biomass (von Wintzingerode et al. 1997; Potvin and Lovejoy 2009). The cloning and sequencing of the small size fraction are useful for identifying important and often major components in a community, and even though the relative abundance of sequences in the clone libraries is not a representation of protist biomass, it does indicate differences in community composition (Potvin and Lovejoy 2009; Terrado et al. 2009; Medinger et al. 2010).

Clone libraries prepared from cDNA following reverse transcription of RNA are likely to target cells with abundant 18S ribosomal RNA (Buckley and Szmant 2004; Stoeck et al. 2007b). The percentage of OTUs shared between the rRNA gene and rRNA libraries was ca. 10.5% (Fig. 4), which is five times greater than the overlap reported by Not et al. (2009) from the Mediterranean Sea, but less than the 27% overlap reported from a deep anoxic basin (Stoeck et al. 2007b). Not et al. (2009) examined a sample pooled from different depths (surface to 140 m) and found only two OTUs in common. Stoeck et al. (2007b) working in a deep anoxic basin suggested that only cells surviving or recently entering anoxic waters were recovered from rRNA libraries, whereas rRNA gene libraries recovered DNA mostly from cells that were dead or dying. Since all eukaryotes have the 18S rRNA gene and DNA represents the total pool of organisms, including those that are still present but less active, the total number of taxa ought to be greater in the rRNA gene libraries. The differences in recovery of common OTUs between the two sorts of libraries most likely reflect the incomplete coverage of total diversity using the cloning and sequencing techniques (Amaral-Zettler et al. 2009). Here, as in similar studies elsewhere, diversity was under-sampled and rare organisms would not be represented in either library (Sogin et al. 2006). However, we found that both the rRNA gene and rRNA libraries showed a similar temporal pattern when grouped by functional trophic level. This pattern was usually more pronounced in the rRNA-based libraries compared to the rRNA gene-based libraries, which is consistent with RNA being more sensitive to current environmental conditions, while DNA is more indicative of organism gene copy number (Zhu et al. 2005) or water mass history (Hamilton et al. 2008; Lovejoy and Potvin 2010).

Alveolates often dominate rRNA gene clone libraries (Massana and Pedrós-Alió 2008) and have been reported as more rare in rRNA libraries (Stoeck et al. 2007b; Not et al. 2009). We also found that alveolates were well represented in the rRNA gene libraries, accounting for 47 to 68% of all clones, but they also contributed a high proportion of sequences in the rRNA libraries representing 39–88% of those clones (Table 2, Fig. 3). Different groups of

alveolates were found in different libraries and samples, suggesting a complex ecology among the different functional groups of heterotrophic, mixotrophic, and parasitic alveolate taxa. Generalizations that alveolates never dominate the active communities or that they only dominate the DNA-based libraries because they have more 18S rRNA gene copies (Cavalier-Smith 2005) should be treated with caution.

Sequences clearly within the Alveolates but not belonging to well-known major groups were first identified from uncultured 18S rRNA gene surveys and fell into two distinct clusters termed Marine Group I and Marine Group II, (MG I and MGII) (López-García et al. 2001). Group II was later found to include dinoflagellate parasites in the genus *Amoebophyra* and a copepod parasite *Syndinium* (Skovgaard et al. 2005), placing Group II in the Syndinales. Because of these associations, members of Group II were considered to be either parasitoids or parasites. The sequences of several tintinnid parasites morphologically described as *Duboscquella* clustered with the original López-García et al. (2001) MG I sequences, indicating that MG I was also likely to be parasitic (Harada et al. 2007). Subsequently, Guillou et al. (2008) reanalyzed environmental sequences from a wide range of marine habitats and separated a small cluster from Group I, which was designated Group V and contained only environmental sequences. They further separated the original Group II into three groups designated Group II encompassing *Amoebophyra* and environmental sequences, Group III consisting only of environmental sequences, and Group IV which included all known *Syndinium* spp. (López-García et al. 2001; Vaulot et al. 2008). Marine Groups I and II are nearly always recovered in clone libraries from the Arctic (Lovejoy et al. 2006; Terrado et al. 2009). Interestingly, the sequences falling within the Guillou et al. (2008) groups III and IV were only in rRNA libraries in March and early April. Since Group IV includes the zooplankton parasites *Syndinium* and *Hematodinium* (Skovgaard et al. 2005; Stentiford and Shields 2005), their presence in the rRNA libraries could indicate that zooplankton were infected, coincident with the onset of active behavior of Arctic copepods at that time of the year (Seuthe et al. 2007). However, at this time, the vast majority of all of these Marine Alveolates taxa are known solely from their 18S rRNA sequences and whether they all have parasite life stages has yet to be determined.

The appearance and disappearance of many major groups recovered from the rRNA libraries over the season likely reflected their relative activity within the system. Among the ciliates, for example, the Phyllopharyngea is a large family with some members classified as zooplankton parasites while others are free-living predators of other ciliates (Lynn 2008). Sequences from this group have not been reported previously from the Arctic but were common in March and early April at the same time that other ciliates were common in the rRNA gene libraries, suggesting that the Phyllopharyngea environmental sequences belonged to the free-living predator group. In contrast to the rarely recovered Phyllopharyngea, oligotrichous ciliates in the Spirotrichea (Oligochitrea, see Agatha and Strüder-Kypke 2007) are the most common class of ciliates recovered from Arctic rRNA gene clone libraries (Lovejoy et al. 2006). The Strom A and Strom B clades that cluster near *Strombidium* SNB99 (Lovejoy et al. 2006) were present in all rRNA gene libraries from March to April, but were most common in the rRNA libraries when chl *a* levels were highest in May. This pattern is consistent with Strombidiidae being algivorous, feeding on phytoplankton cells. Similarly, among dinoflagellates, sequences similar to *Gyrodinium rubrum,* which feeds on phytoplankton and ciliates (Hansen and Calado 1999; Jeong 1999), were found in all rRNA gene libraries but were only common in the May rRNA library (Table 2). Lovejoy et al. (2002) observed that ciliates and dinoflagellates were most abundant during the terminal stages of the phytoplankton bloom in the North Water Polynya. The high frequency of these groups in the rRNA library, particularly the clusters related to *Strombidium* SNB99 and *G. rubrum,* at the time of highest chl *a* biomass, suggests a coupling between phytoplankton blooms and these heterotrophs in the Arctic Ocean.

Not et al. (2009) reported that the most commonly recovered sequences in rRNA libraries in the Mediterranean Sea belonged to MAST groups. These small flagellates have been identified as marine bacterial grazers (Massana et al. 2006), and they are ubiquitous in Arctic marine rRNA gene libraries (Lovejoy et al. 2006; Terrado et al. 2009). Interestingly, MAST-1A and MAST-1C were absent from the rRNA libraries, despite representing a high percentage of clones in all of the rRNA gene libraries (Table 2). These small cells are likely to have few 18S rRNA gene copy numbers (Rodríguez-Martínez et al. 2009) yet were still recovered in the rRNA gene libraries; their underrepresentation in the rRNA libraries could indicate an ability to persist in the water column, with only slow growth rates. Vaqué et al. (2008) experimentally determined grazing rates on bacteria by small flagellates in Franklin Bay during 2004. These rates were low throughout winter and only increased slightly in spring, remaining well below rates estimated elsewhere. A similar trend was also noted in their concurrent study during the 2008 CFL project (D. Vaqué, personal communication). Bacterial concentrations are typically low in late winter/early spring (Garneau et al. 2008), perhaps because of their increased organic substrate requirements for activity at cold temperatures (Pomeroy et al. 1991; Middelboe and Lundsgaard 2003), reducing prey for bacterivorous MAST groups. In

contrast, ciliates were recovered in all of the rRNA libraries, reflecting a different range of prey items, notably pico- and nanophytoplankton (Vaqué et al. 2008) and their relatively high growth rates at lower temperatures (Rose et al. 2009).

In March, sequences most closely related to the Prasinophyte *Pyramimonas* were recovered from both rRNA gene and rRNA libraries. This genus is common in cold waters (Rodriguez et al. 2002) and is frequently reported in ice algal assemblages (Różańska et al. 2008). This taxon did not persist beyond March, in contrast to the Prasinophyte *Micromonas*. Sequences of the latter closely matching the *Micromonas* isolate from the Arctic Ocean (CCMP 2099) were recovered from all rRNA gene and rRNA libraries, consistent with its role as a key component of the Arctic marine food webs (Lovejoy et al. 2007). Our rRNA results show that not only was it widespread and persistent, but that the *Micromonas* cells were also always active.

As spring progressed, the increasingly favorable conditions for photosynthesis were reflected in the higher percentages of other photosynthetic groups recovered from the rRNA libraries. *Chaetoceros* cf. *socialis* and *Phaeocystis* are often reported ensemble in the Arctic (Booth and Horner 1997). We found sequences of *Chaetoceros* and *Phaeocystis* in early April rRNA clone libraries, with *Phaeocystis* persisting in the rRNA gene libraries through April. In late April and May, the diatom assemblage changed and we recovered chain and colonial diatom groups, including sequences with closest matches to *Thalassiosira* spp., which are routinely reported from Arctic waters (Booth and Horner 1997; Lovejoy et al. 2002; Różańska et al. 2008). Compared to microscopy, the molecular results provided incomplete information about colonial and larger diatoms since the filtration protocol was designed to focus on the smallest protists, and diatom breakage may be less important than for ciliates and non-thecate dinoflagellates. This incompleteness was especially reflected in the May clone libraries when diatoms dominated cell numbers and biomass but represented a low percentage of the clones recovered (Fig. 2, Table 2). The complementary cell count data, however, indicated a clear increase in diatoms over the study period.

In May, all putative parasite sequences recovered were related to the Chytridiales, a group of parasitic/saprophytic fungi that includes taxa that attack phytoplankton (Kagami et al. 2007), including diatoms (Hanic et al. 2009). These sequences were present in both rRNA and rRNA gene libraries, representing 18% of all clones from the rRNA gene library (Table 2). The growth and infection of this parasite is enhanced when algal growth is constrained, i.e., under conditions of low nutrient levels and high host density (Kagami et al. 2007). These same conditions prevailed in the flaw lead in May. Although such infections are not often reported in marine waters, diatoms with chytrid-like infections are often noted in microscopic examinations when calcofluor has been added to sedimented samples (Lovejoy et al. 2002). *Chytridium*-related sequences have been recovered from the Arctic in sediments of Disko Bay (Stoeck et al. 2007a) and from the mesopelagic zone of Franklin Bay (Terrado et al. 2009), suggesting an association with the sedimentation of moribund diatoms to the benthos. The role of chytrids in bloom dynamics in the Arctic warrants further investigation.

Conclusions

The Amundsen Gulf flaw lead system harbors a diverse protist community during spring. The transition in March to April was characterized by an increasing recovery rate from both rRNA gene and rRNA clone libraries of sequences falling into clusters belonging to phototrophic groups. The photosynthetic taxa that likely dominated springtime productivity included prasinophytes, diatoms, haptophytes, and cryptophytes. Despite maximum chl *a* concentrations in May attributable to larger diatoms not captured by our size-fraction approach, heterotrophic taxa and notably sequences with best matches to the dinoflagellate *Gyrodinium rubrum* and the ciliate *Strombidium* were common. Chytrid sequences were recovered from both rRNA gene and rRNA libraries in May, suggesting active parasitic infection during the phytoplankton bloom. RNA-based clone libraries, in combination with the more commonly used rRNA gene, DNA-based clone libraries, provided complementary information, which added to our microscopic observations on protist community changes over the Arctic spring transition.

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Conflict of interest The authors declare that they have no conflict of interest.

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