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ORIGINAL ARTICLE

Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples

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The composition of photosynthetic pico and nanoeukaryotes was investigated in the North East Pacific and the Arctic Ocean with special emphasis on the Beaufort Sea during the MALINA cruise in summer 2009. Photosynthetic populations were sorted using flow cytometry based on their size and pigment fluorescence. Diversity of the sorted photosynthetic eukaryotes was determined using terminal-restriction fragment length polymorphism analysis and cloning/sequencing of the 18S ribosomal RNA gene. Picoplankton was dominated by Mamiellophyceae, a class of small green algae previously included in the prasinophytes: in the North East Pacific, the contribution of an Arctic *Micromonas* ecotype increased steadily northward becoming the only taxon occurring at most stations throughout the Beaufort Sea. In contrast, nanoplankton was more diverse: North Pacific stations were dominated by *Pseudo-nitzschia* sp. whereas those in the Beaufort Sea were dominated by two distinct *Chaetoceros* species as well as by Chrysophyceae, Pelagophyceae and *Chrysochromulina* spp.. This study confirms the importance of Arctic *Micromonas* within picoplankton throughout the Beaufort Sea and demonstrates that the photosynthetic picoeukaryote community in the Arctic is much less diverse than at lower latitudes. Moreover, in contrast to what occurs in warmer waters, most of the key pico- and nanoplankton species found in the Beaufort Sea could be successfully established in culture.
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Introduction

Photosynthetic pico and nanoeukaryotes account for a significant proportion of marine primary production (Li, 1994). Assessing their composition is crucial for a better understanding of carbon fluxes in the ocean as some taxa account for higher CO_2 fixation rates than other (Jardillier *et al.*, 2010). Molecular-based approaches such as cloning/sequencing techniques have revealed a high diversity of small eukaryotes highlighting the presence of many uncultured lineages (Lopez-Garcia *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Diez *et al.*, 2001b). However, assessing the diversity of small photosynthetic eukaryotes is complicated by the prevalence in marine waters of sequences from heterotrophic eukaryotes (Vaulot *et al.*, 2002) including small predators (Massana *et al.*, 2004) and parasites (Guillou *et al.*, 2008). 18S ribosomal RNA (rRNA) gene primers biased toward known photosynthetic groups (Viprey *et al.*, 2008) or plastidial primers for the 16S rRNA (Fuller *et al.*, 2006; McDonald *et al.*, 2007; Treusch *et al.*, 2011) or psbA (Man-Aharonovich *et al.*, 2010) genes allow to target phototrophic groups. However, biased 18S rRNA primers do not recover all the photosynthetic taxa and plastidial-based approaches are limited by the lack of a sufficient number of reference sequences. Flow cytometry sorting of photosynthetic populations based on size and pigment composition followed by amplification and cloning of the 18S rRNA nuclear gene (Shi *et al.*, 2009; Yoshida *et al.*, 2009; Cuvelier *et al.*, 2010; Marie *et al.*, 2010) or of the 16S rRNA plastid gene (Jardillier *et al.*, 2010; Shi *et al.*, 2011) have confirmed the importance of uncultured microorganisms within photosynthetic pico and nanoplankton.

Small plankton in polar waters was previously investigated in the Southern Ocean (Diez *et al.*,

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2001b), North Atlantic (Not *et al.*, 2005; Luo *et al.*, 2009) and the Canadian Arctic (Lovejoy *et al.*, 2006). Seawater temperature rise and ice pack retreat (Comiso *et al.*, 2008) are highly affecting phytoplankton biomass, production and composition in the Arctic (Wassmann *et al.*, 2011) implying an increase in picoplankton and a decrease in nanoplankton abundances (Li *et al.*, 2009). Recent studies have demonstrated that a picoplanktonic Mamelliophyceae, forming an endemic lineage within the genus *Micromonas* (and referred as Arctic *Micromonas* throughout this paper) is widespread throughout the Arctic (Lovejoy *et al.*, 2007). Larger phytoplankton is more diverse and mainly dominated by diatoms (Lovejoy *et al.*, 2002; Sukhanova *et al.*, 2009) with late spring/early summer blooms of *Thalassiosira* species, *Chaetoceros socialis* and *Phaeocystis pouchetii* (Booth *et al.*, 2002; Wassmann *et al.*, 2005). However, most previous studies either provided information on a very limited number of sites or did not focus on the composition of small photosynthetic eukaryotes.

In the present work, flow cytometry was used to sort photosynthetic pico and nanoeukaryote populations in North Pacific and Arctic Oceans, with a special focus on the Beaufort Sea. The diversity of these populations was mapped by terminal-restriction fragment length polymorphism (T-RFLP) of the 18S rRNA gene, which allows the rapid analysis of a very large number of samples (Baldwin *et al.*, 2005; Vigil *et al.*, 2009). In a second step, cloning/sequencing was applied to two selected stations deemed to be representative of the Beaufort Sea based on the T-RFLP patterns.

Materials and methods

Sample collection and processing

The MALINA cruise took place on board the Canadian research vessel CCGS Amundsen during summer 2009 from Victoria (BC, Canada) to the Beaufort Sea (Leg 1b) and then throughout the Beaufort Sea (Leg 2b). Seawater samples were collected in surface during Leg 1b and at different depths during Leg 2b (Figure 1). Ancillary data of temperature, salinity, chlorophyll and nitrate concentration were kindly provided by JE Tremblay and J Gagnon (Table 1). Seawater was collected with a bucket (Leg 1b) or using Niskin bottles mounted on a CTD (conductivity temperature depth probe) frame (Leg 2b). Chlorophyll-*a* was measured by high pressure liquid chromatography after methanol extraction (Ras *et al.*, 2008). Samples for nitrates were poisoned by $HgCl_2$ and nitrates were analysed using an automated colorimetric procedure (Raimbault *et al.*, 1990).

Samples were analysed on-board by flow cytometry (Marie *et al.*, 1997) using a FACSAria (Becton Dickinson, San José, CA, USA) to determine the abundance of the photosynthetic pico and nanoeukaryotes (Table 1). These two groups were defined operationally on the basis of scatter vs chlorophyll fluorescence cytograms (Supplementary Figure S1) in a manner consistent with our previous work (Shi *et al.*, 2009; Marie *et al.*, 2010). The boundary between the two populations does not correspond exactly to the precise size threshold of 2 μm that formally separates pico from nanoplankton. Flow cytometry data are available at http://tinyurl.com/67wn5qc. Four litres were concentrated down to 25 ml by tangential flow filtration as described previously (Marie *et al.*, 2010). Concentration factors averaged 64- and 81-fold for pico and nanoplankton, respectively, with average recovery rates of 38% and 49%. In contrast with our previous work (Marie *et al.*, 2010), we performed during the MALINA cruise a two-step sorting procedure to minimise contamination (Supplementary Information). First, between 10 000 nano to 100 000 picoeukaryotic cells were sorted in enrichment mode, based on their scatter and chlorophyll fluorescence. Then, these sorted samples were stained by SYTO 13, a live stain for DNA (del Giorgio *et al.*, 1996) at a final concentration of 5 μM. Pico and nanoeukaryotes were discriminated as described previously (Marie *et al.*, 2010) and about 5000 and 50 000 cells of pico and nanoeukaryotes, respectively, were sorted in purity mode. Sorted populations were immediately frozen at −80 °C.

Cultures

Twenty phytoplankton strains (Supplementary Table S1) isolated during the MALINA cruise (Balzano *et al.* in preparation) and available from the Roscoff Culture Collection (http://www.sb-roscoff.fr/Phyto/RCC) were used to calibrate the T-RFLP patterns (see below). DNA was extracted from these strains using Qiagen Blood and Tissue kit (Qiagen, Courtaboeuf, France) as described in Supplementary Information.

Molecular and phylogenetic analysis

Molecular methods are described in greater details in Supplementary Information. For T-RFLP, PCR of the 18S rRNA gene was performed in triplicate, directly from lysed cells (95 °C, 5 min) of pico (59 samples) and nanoplankton (79 samples) using the primers 63f (6-FAM labelled) and 1818r (Lepère *et al.*, 2011). Amplification from lysed cells was found to be more reproducible than from extracted DNA. For 12 samples that could not be amplified directly, we performed first a Multiple Displacement Amplification of genomic DNA (Table 1).

Replicate amplicons were combined and incubated with Mung Bean Nuclease (New England Biolabs, Ipswich, MA, USA), purified with a UltraClean PCR kit (Mo-Bio Laboratories, Carlsbad, CA, USA), and digested with the restriction endonucleases *MnlI*, *HhaI* and *Hpy188I* (New England Biolabs) as described previously (Vigil *et al.*, 2009). *Hpy188I* was only used to discriminate among the different Mamiellophyceae.

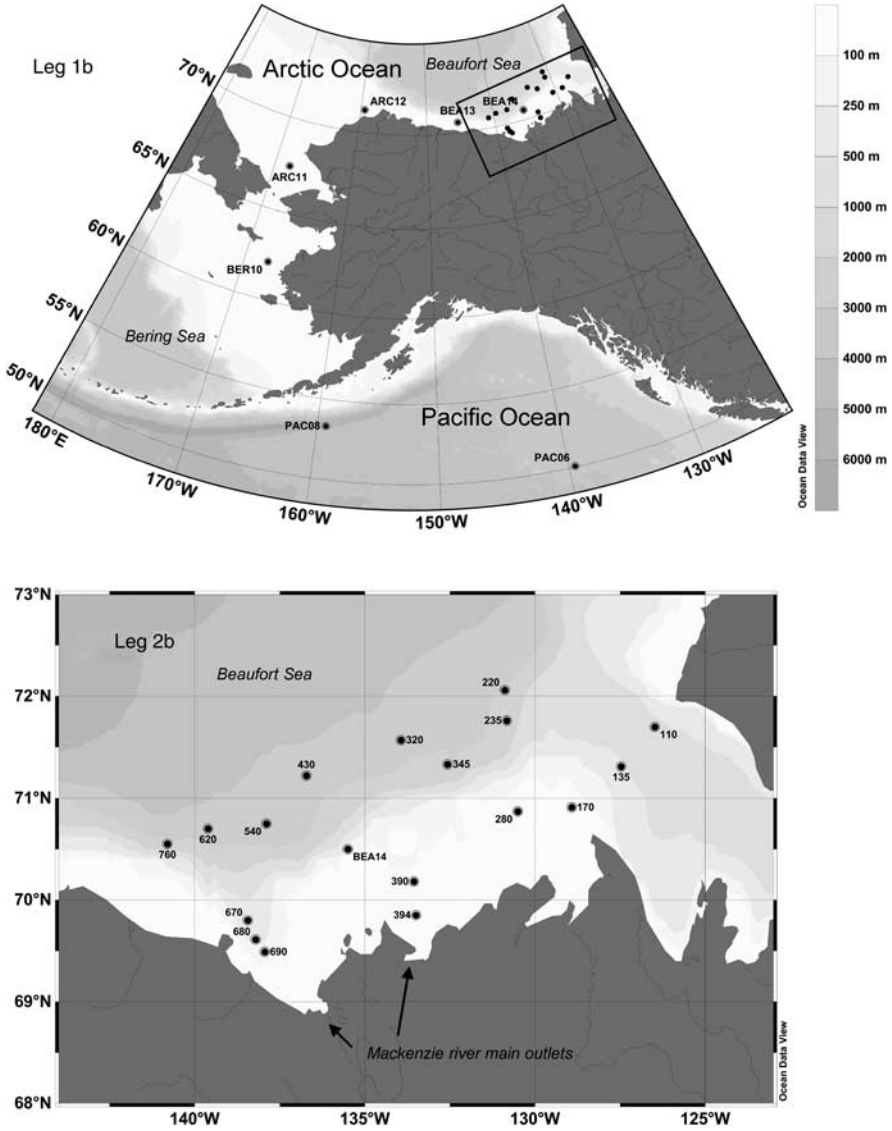

Figure 1 MALINA station locations for Legs 1b and 2b. Grey shades correspond to bottom depths.

The T-RFLP digests were then diluted in HiDi Formamide (Applied Biosystems, Foster City, CA, USA) and terminal-restriction fragments (T-RFs) were separated in a 3130 *xl* Genetic Analyzer (Applied Biosystems). Data were analysed using the PeakScanner software (Applied Biosystems). Peaks with T-RFs comprised between 100 and 500 bp were binned at 0.4-bp resolution, the relative peak area was exported, and the total peak area of each sample was normalised to one.

We define a ribotype by a unique set of T-RFs for the enzymes used (2–4, Table 2). T-RFs obtained experimentally from our clone libraries (see below) and phytoplankton cultures were compared with T-RFs obtained from environmental samples for ribotype identification. Other ribotypes were tentatively identified using an *in silico* T-RF database (Supplementary Information).

For cloning and sequencing purposes, the 18S rRNA gene was amplified from four samples of nanoeukaryotes and four samples of picoeukaryotes, sorted from the surface and the DCM of the stations 320 and 390 (Figure 1). PCR was performed in triplicate as described above, but an unlabelled rather than labelled 63f primer was used. Replicate amplicons were combined and purified using a UltraClean PCR kit (Mo-Bio Laboratories). Purified PCR products were cloned into vector PCR4-TOPO (Invitrogen, Carlsbad, CA, USA) and transformed into *Escherichia coli* competent cells following the manufacturer instruction. Clone inserts were then amplified using the same (unlabelled) primers as above and purified using Exosap (USB products, Santa Clara, CA, USA). Partial sequences were determined by using Big Dye Terminator V3.1 (Applied Biosystems) and the internal primer Euk528f (Zhu *et al*., 2005) or a slightly modified Euk528f primer (5′-CCGCGGTAATTCCA GCT-3′) for *C. socialis*, which has a mismatch to Euk528f. DNA was sequenced using an ABI prism 3100 sequencer (Applied Biosystems).

Table 1 MALINA sample locations, main physico-chemical characteristics[a], abundance of bacteria and photosynthetic pico and nanoeukaryotes

Station	*CTD*	*Sampling date*	*Latitude (°N)*	*Longitude (°W)*	*Depth (m)*	*Temperature (°C)*[a]	*Salinity (psu)*[a]	*Chl-a (µg l⁻¹)*[a]	*Nitrate (µM)*[a]	*Picoeukaryotes (cell ml⁻¹)*	*Nanoeukaryotes (cell ml⁻¹)*	*Picoeukaryote sample*	*Picoeukaryote cells sorted*	*Nanoeukaryote sample*	*Nanoeukaryote cells sorted*
PAC060709		06/07/2009	50.06	139.53	0	12.1	32.5		10	6900	7200			ES060709 N	5000
PAC080709		08/07/2009	53.36	159.29	0	11.8	32.7		11.6	10 000	2900	ES080709 P	50 000	ES080709 N	5000
BER100709		10/07/2009	62.14	167.54	0	6.6	30.5		1.19	5100	3300	ES100709 P	50 000	ES100709 N	5000
ARC110709		11/07/2009	67.49	168.12	0	6.8	31.7		0.98	5300	2200	ES110709 P	50 000	ES110709 N	5000
ARC120709		12/07/2009	71.19	159.42	0	2.0	30.5		0.15	21 000	6100	ES120709 P	50 000	ES120709 N	10 000
BEA130709		13/07/2009	70.56	145.40	0	8.8	17.6		0.27	1800	720	ES130709 P	30 000		
BEA140709		14/07/2009	70.50	135.50	0	3.3	25.6		0.18	1200	530	ES140709 P	40 000	ES140709 N	10 000
690	31	01/08/2009	69.49	137.94	3	7.39	19.0	0.17	0.03	5400	450	ES022[b]	50 000	ES024	10 000
		01/08/2009										ES023[b]	50 000		
690	31	01/08/2009	69.49	137.94	29	−1.30	31.2	0.93	4.9	220	960	ES025	50 000	ES026	10 000
680	35	02/08/2009	69.61	138.21	3	8.33	14.7	0.18	0.03	3100	800	ES030[b]	4000	ES028[b]	1100
		02/08/2009										ES031[b]	4000	ES032[b]	2000
		02/08/2009												ES035	10 000
680	35	02/08/2009	69.61	138.21	40	−1.17	31.3	0.87	3.30	930	2300	ES034	25 000	ES033[b]	10 000
		02/08/2009												ES035	10 000
670	89	10/08/2008	69.80	138.44	3	3.81	23.4	0.07	0.01	1900	400	ES075[b]	15 000	ES077[b]	3600
		10/08/2008										ES076[b]	15 000	ES078[b]	3000
670	89	10/08/2008	69.80	138.44	70	−1.16	31.9	0.10	6.2	1000	430	ES071[b]	30 000	ES073	1600
		10/08/2008										ES072[b]	13 000		
394	38	03/08/2009	69.85	133.50	3	7.00	25.1	0.47	0.00	1300	2000	ES038	50 000	ES039	10 000
390	27	31/07/2009	70.18	133.56	3	4.74	28.0	0.17	0.01	990	1200	ES020	50 000	ES021	10 000
390	27	31/07/2009	70.18	133.56	30	−0.72	31.5	1.0	2.9	220	3100	ES018	50 000	ES019	5000
760	106	12/08/2008	70.55	140.80	3	0.59	22.3	0.09	0.01	4300	720	ES091[b]	60 000	ES093[b]	10 000
		12/08/2008										ES092[b]	20 000	ES094[b]	5000
760	106	12/08/2008	70.55	140.80	70	−1.10	31.5	0.33	6.2	4400	1200	ES085[b]	50 000	ES088[b]	5000
		12/08/2008										ES086[b]	50 000	ES089[b]	5000
		12/08/2008												ES090[b,c]	2000
620	99	11/08/2008	70.70	139.61	3	1.56	22.1	0.093	0.00	4000	520	ES083	50 000	ES084	5000
620	99	11/08/2008	70.70	139.61	65	−1.13	30.7	0.090	2.0	110	150	ES079	25 000	ES080[b]	5000
		11/08/2008												ES081[b]	2500
		11/08/2008												ES082[b,c]	1000
540	134	17/08/2009	70.75	137.89	3	−0.39	25.8	0.063	0.01	2900	490	ES113[b]	30 000	ES115[b]	3000
		17/08/2009										ES114[b]	30 000	ES116[b]	3000
		17/08/2009												ES117	5000
540	134	17/08/2009	70.75	137.89	70	−1.14	31.7	0.43	6.1	2100	920	ES106[b]	60 000	ES108[b]	5000
		17/08/2009										ES107[b]	20 000	ES110[b]	250
		17/08/2009												ES112[b]	10 000
280	42	04/08/2009	70.87	130.51	3	4.69	27.7	0.13	0.02	1800	2100	ES042	50 000	ES043	10 000
280	42	04/08/2009	70.87	130.51	30	−0.74	32.2	2.6	6.5		6700			ES041[c]	10 000
170	65	07/08/2009	70.91	128.92	5	3.39	29.3	0.94	0.04	2400	2600	ES060	50 000	ES061	5000
		07/08/2009												ES062[b]	4000
		07/08/2009												ES063[b]	1200
170	65	07/08/2009	70.91	128.92	20	−1.22	31.8	0.41	3.6	2500	220	ES055	50 000	ES056	5000
		07/08/2009												ES057[b,c]	250
		07/08/2009												ES058[b,c]	150
		07/08/2009												ES059[b,c]	150
430	138	18/08/2009	71.22	136.72	3	−0.78	25.9	0.009	0.01	5200	430	ES126	50 000	ES130	5000
		18/08/2009										ES127[b]	20 000	ES132[b]	1000
		18/08/2009										ES128[b]	12 000	ES133[b]	1000
		18/08/2009										ES129[b]	2500		

Table 1 (Continued)

Station	*CTD*	*Sampling date*	*Latitude (°N)*	*Longitude (°W)*	*Depth (m)*	*Temperature (°C)*[a]	*Salinity (psu)*[a]	*Chl-a (μg l⁻¹)*[a]	*Nitrate (μM)*[a]	*Picoeukaryotes (cell ml⁻¹)*	*Nanoeukaryotes (cell ml⁻¹)*	*Picoeukaryote sample*	*Picoeukaryote cells sorted*	*Nanoeukaryote sample*	*Nanoeukaryote cells sorted*
430	138	18/08/2009	71.22	136.72	65	−1.06	31.7	0.47	6.7	13 000	830	ES118[b]	20 000	ES121	10 000
		18/08/2009										ES119[b]	50 000	ES122[b]	2500
		18/08/2009										ES120[b]	30 000	ES123[b]	1000
		18/08/2009												ES125[b,c]	400
135	161	21/08/2009	71.31	127.47	3	2.39	28.1	0.056	0.01	4200	370	ES139	90 000	ES140	7500
		21/08/2009										ES141[b]	50 000	ES143[b]	160 000
		21/08/2009										ES142[b]	80 000	ES144[b]	220 000
135	161	21/08/2009	71.31	127.47	60	−1.22	31.6	0.19	3.8	2700	490	ES134	90 000	ES135[b]	10 000
		21/08/2009												ES136[b]	2500
		21/08/2009												ES137[b]	7000
		21/08/2009												ES138	10 000
345	125	15/08/2008	71.33	132.57	3	1.98	27.8	0.061	0.06	3400	410	ES101[b]	50 000	ES103[b]	7300
		15/08/2008										ES102[b]	5000	ES104[b]	3500
		15/08/2008												ES105	7000
345	125	15/08/2008	71.33	132.57	70	−1.13	31.8	0.23	—	1200	370	ES096	20 000	ES097[b,c]	1000
		15/08/2008												ES098[b,c]	1000
		15/08/2008												ES099[b,c]	500
320	82	09/08/2008	71.57	133.94	3	−0.82	27.0	0.04	0.01	2900	500	ES068	50 000	ES069	10 000
320	82	09/08/2008	71.57	133.94	70	−1.17	26.8	0.16	3.4	3100	650	ES064	50 000	ES065	10 000
		09/08/2008												ES067[b,c]	1000
110	56	06/08/2009	71.70	126.48	3	4.41	28.7	0.071	0.00	6600	870	ES050	50 000	ES051	10 000
		06/08/2009										ES052[b]	20 000		
		06/08/2009										ES053[b]	5000		
110	56	06/08/2009	71.70	126.48	60	−1.20	31.6	0.24	0.33	2100	1100	ES048	50 000	ES049	10 000
235	191	24/08/2009	71.76	130.83	3	0.03	27.3	0.077	0.02	5100	490	ES160[b]	96 000	ES162[b]	6500
		24/08/2009										ES161[b]	64 000	ES163[b]	3600
		24/08/2009												ES164[b]	3000
235	191	24/08/2009	71.76	130.83	25	1.63	29.9	0.056	0.00	2400	600	ES155[b]	50 000	ES157[b]	3000
235	191	24/08/2009	71.76	130.83	45	−0.45	31.2	0.12	0.00	2400	280	ES152[c]	50 000	ES153	4200
		24/08/2009												ES158[b]	3300
		24/08/2009												ES159[b]	2000
235	191	24/08/2009	71.76	130.83	55	−0.96	31.4		0.30	2400	170	ES150	50 000		
235	191	24/08/2009	71.76	130.83	65	−1.17	31.7	0.14	2.9	1500	220	ES146	50 000	ES147[b]	1200
		24/08/2009												ES149[b]	2500
220	50	05/08/2009	72.06	130.89	3	0.64	27.9	0.061	0.01	6000	740	ES046	50 000	ES047	10 000
220	50	05/08/2009	72.06	130.89	70	−1.37	31.6	0.18	1.9	3500	460			ES045	10 000

[a]Temperature and salinity data were obtained from D Doxaran whereas Chl-*a* and nitrate data were obtained from JE Tremblay and J Gagnon. The detection limit for the NO_3^- is 3 nM.
[b]These samples correspond to specific pico and nanoplankton subpopulation sorted from the same seawater sample, see Supplementary Tables S2 and S3 for details.
[c]These samples have been analysed after Multiple Displacement Amplification (MDA) of their genomic DNA (Supplementary Information).

Table 2 List of the 18S rRNA ribotypes found in this study for T-RFLP chromatograms, clone libraries and cultures

Ribotype number[a]	*T-RF size (bp)*				*Number of samples where ribotype found*		*Phylogenetic classification*	*Ribotype putative identification*[b]	*MALINA OTU with same ribotype*	*No. of clones found for this OTU*	*MALINA culture with same ribotype*	*MALINA culture identification*	*Closest species*[c]
	Restriction endonuclease used				*Pico*	*Nano*							
	MnlI	*HhaI*	*HhaI*[d]	*Hpy188I*[e]									
1	107	400			1	2	Bacillariophyceae	*Skeletonema* sp.	ES018P1G2	8			*Skeletonema costatum*
2	127	368					Rhizaria	Uncultured Rhizaria	ES020P1C10	7			*Protaspis obliqua*
									ES020P1A10	1			*Cercomonas rotunda*
3	141	254					Prymnesiophyceae	*Haptolina* sp.			RCC2300	*Haptolina* sp.	*Haptolina hirta*
									ES021G6	1			*Haptolina fragaria*
4	152	390				1	Bacillariophyceae	*Cylindrotheca closterium*	ES021E9	1	RCC1985	*Cylindrotheca closterium*	*Cylindrotheca closterium*
									ES069B6	1			*Cylindrotheca closterium*
5	156	399				7	Prymnesiophyceae	*Chrysochromulina* sp. I	ES069C7	4			*Chrysochromulina scutellum*
6	163	201				1	Cercozoa	*Cryothecomonas* sp.	ES020P2D11	2			*Cryothecomonas* sp.
7	163	410	411				Cercozoa	Uncultured Cercozoa	ES020P1H8	1			*Protaspis grandis*
									ES020P1E10	1			*Protaspis* sp.
									ES021H9	1			*Ebria tripartita*
8	195	393					Bacillariophyceae	Undescribed Fragilariaceae	ES069E5	2	RCC2043	Undescribed Fragilariaceae	*Synedra minuscula*
9[f]	198	403				1	Bacillariophyceae	*Shionodiscus bioculatus*			RCC1991	*Shionodiscus bioculatus*	*Shionodiscus oestrupii*
10	199	402				13	Bacillariophyceae	*Attheya septentrionalis*			RCC1986	*Attheya septentrionalis*	*Attheya septentrionalis*
11	201	389	390	109	9		Mamiellophyceae	*Bathycoccus prasinos*			S664[g]	*Bathycoccus prasinos*	*Bathycoccus prasinos*
12	201	388	390	156	1		Mamiellophyceae	Undescribed Mamiellaceae	ES069D8	1	RCC2285	Undescribed Mamiellaceae	*Mantoniella squamata*
13	201	388	390	457	55	10	Mamiellophyceae	Arctic *Micromonas*	ES020P1D7	42	RCC2306	Arctic *Micromonas*	*Micromonas pusilla*
14	202	389	391	157	7	1	Mamiellophyceae	*Mantoniella squamata*	ES069D5	5			*Mantoniella squamata*
15	203	144					Cryptophyta	*Hemiselmis* sp.	ES020P2C9	2			*Hemiselmis cryptochromatica*
16	206	145				16	Prasinophyceae	*Pyramimonas* sp. I			RCC2009	*Pyramimonas* sp.	*Pyramimonas australis*
17	247	389			1	5	Bacillariophyceae	*Pseudo-nitzschia* sp.	ES020P1B10	19	RCC2004	*Pseudo-nitzschia* sp.	*Pseudo-nitzschia* sp.
									ES021A10	2			*Pseudo-nitzschia* sp.
18	264	205	207				Dictyochophyceae	*Florenciella* sp. I	ES065A8	2			*Florenciella parvula*
19	278	380	382			2	Cryptophyta	*Rhodomonas* sp.			RCC2020	*Rhodomonas* sp.	*Rhodomonas abbreviata*
20	287	400					Bacillariophyceae	*Chaetoceros decipiens*			RCC1997	*Chaetoceros decipiens*	*Chaetoceros muelleri*
21	296	399				38	Prymnesiophyceae	*Chrysochromulina* sp. II	ES065G2	1			*Chrysochromulina campanulifera*
									ES069D4	1			*Chrysochromulina cymbium*
									ES020P2G9	5			*Chrysochromulina simplex*
22	297	390				6	Bacillariophyceae	Uncultured Naviculales	ES069B5	1			*Cymbella minor*
									ES069F7	2			*Cymbella minor*
23	298	146				12	Prasinophyceae	*Pyramimonas* sp. II	ES065F2	2	RCC2015	*Pyramimonas* sp.	*Pyramimonas gelidicola*
24	300	403				1	Prymnesiophyceae	*Phaeocystis* sp.	ES065D7	2			*Phaeocystis pouchetii*
25	324	397			2	30	Bacillariophyceae	*Chaetoceros* cf. *neogracile*	ES020P2H11	26	RCC2016	*Chaetoceros* cf. *neogracile*	*Chaetoceros neogracile*
									ES018P1G4	3			*Chaetoceros neogracile*
									ES018P2D4	2			*Chaetoceros neogracile*
									ES021E7	1			*Chaetoceros neogracile*
									ES069A6	1			*Chaetoceros neogracile*
26	325	398	400		5	33	Bacillariophyceae	*Chaetoceros socialis*	ES018P1B1	95	RCC1992	*Chaetoceros socialis*	*Chaetoceros socialis*
									ES020P1H10	5			*Chaetoceros socialis*
									ES021H11	1			*Chaetoceros socialis*
									ES020P2F12	1			*Chaetoceros socialis*
27[h]	329	402				2	Bacillariophyceae	*Eucampia* sp.					*Eucampia antarctica*
28	340	389			1	24	Bacillariophyceae	*Fragilariopsis*/*Cylindrotheca*	ES020P1H7	26			*Fragilariopsis cylindrus*
									ES018P1G1	5			*Cylindrotheca closterium*
29[h]	341	391				2	Bacillariophyceae	*Nitzschia* sp.			RCC2276	*Nitzschia* sp.	*Nitzschia linearis*
30[h]	342	392			2	3	Chrysophyceae	Uncultured Chrysophyceae					Uncultured Chrysophyceae

Table 2 (Continued)

Ribotype number[a]	T-RF size (bp)				Number of samples where ribotype found		Phylogenetic classification	Ribotype putative identification[b]	MALINA OTU with same ribotype	No. of clones found for this OTU	MALINA culture with same ribotype	MALINA culture identification	Closest species[c]
	Restriction endonuclease used				Pico	Nano							
	MnlI	*HhaI*	*HhaI*[d]	*Hpy188I*[e]									
31[h]	343	394				9	Chrysophyceae	*Chromulina* sp.					*Chromulina* sp.
32	344	394	396			4	Alveolata	Uncultured Alveolata	ES069B8	1			Uncultured Alveolate Group II
33	346	395				26	Chrysophyceae	*Dinobryon* spp.	ES069E8	3			*Ochromonas* sp.
											RCC2290	*Dinobryon faculiferum*	*Ochromonas* sp.
34	347	397	398	641			Telonemia	Uncultured Telonemia	ES065G7	1			*Telonema antarcticum*
35	347	251		113	1	10	Dictyochophyceae	Undescribed Pedinellales	ES069F4	1	RCC2289	Undescribed Pedinellales	*Helicopedinella tricostata*
36	347	398		628		19	Bacillariophyceae	*Chaetoceros* sp. I	ES021G7	5			*Chaetoceros gracilis*
37	350	400				6	Bacillariophyceae	*Chaetoceros* sp. II	ES20P1H10	5			*Chaetoceros socialis*
38[h]	353	200				5	Dinophyceae	Uncultured Dinophyceae					Uncultured Dinophyceae
39	354	146				1	Dinophyceae	*Gymnodinium* sp.	ES065H7	2			*Gymnodinium* sp.
40	354	404				1	Bacillariophyceae	*Thalassiosira nordenskioeldii*			RCC2000	*Thalassiosira nordenskioeldii*	*Thalassiosira aestivalis*
41	355	405					Bolidophyceae	*Triparma* sp.	ES065D3	2			*Triparma* sp.
42	357	204				7	Dictyochophyceae	*Florenciella sp.* II	ES069A5	4			*Florenciella parvula*
43	360	411			1	25	Pelagophyceae	Pelagophyceae			RCC2040	Undescribed Pelagophyceae	*Ankylochrysis lutea*
									ES065B3	2			*Aureococcus anophagefferens*
									ES069C5	1			Pelagophyceae sp.

Abbreviations: OTU, operational taxonomic unit; rRNA, ribosomal RNA; T-RFLP, terminal-restriction fragment length polymorphism.
[a]Ribotypes that were observed by T-RFLP in sorted samples are in bold.
[b]Putative identification of T-RFs was based on the digestion of 18S rRNA sequences representative of distinct 48 OTUs obtained from 39 clones, and 20 cultures used in a parallel study (Balzano *et al.* unpublished) using two or three endonucleases. Moreover for five ribotypes, the T-RFLP profile was identified based on the *in silico* digestion of a large 18S rRNA database (≈20 000 sequences) with the same enzymes as above.
[c]Closest species in the Genbank for OTU or, if no OTU, on culture and if no culture and no OTU, from *in silico* analysis.
[d]Digestion with this enzyme occasionally produced two rather than one T-RFs. The second T-RFs is indicated where it occurred.
[e]Endonuclease *Hpy188I* was used to discriminate between the ribotypes producing T-RFs of identical sizes with both *HhaI* and *MnlI* (for example, Arctic *Micromonas* and *Mantoniella squamata*). The T-RF size is therefore indicated only for those ribotypes where *Hpy188I* was used.
[f]This ribotype when digested with *MnlI* has a second T-RF at 208 bp.
[g]This strain has been lost.
[h]For these ribotypes, identification is based on the *in silico* restriction map of the 18S database.

Partial sequences were grouped into 48 operational taxonomic units (OTUs, 99.5% similarity) and the full 18S rRNA gene was sequenced from at least one sequence per OTU as well as from 20 phytoplankton cultures using the primers 63f, 528f and 1818r. Full-length 18S rRNA gene sequences were analysed using Bioedit software (Hall, 1999) then aligned using clustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2). A neighbour-joining (Saitou and Nei, 1987) phylogenetic tree was constructed using Geneious software (www.geneious.com, Supplementary Information).

Sequences have been deposited to GenBank under the accession numbers JF698738 to JF699043 for the MALINA samples and JF794039 to JF794059 for the MALINA cultures.

Statistical analyses

Spearman rank correlation coefficients (ρ) and Pearson's product-moment correlation between nanoeukaryote ribotypes and environmental conditions (Supplementary Information) were computed with the Vegan package (Legendre and Legendre, 1998) of the R software (http://www.r-project.org). As both methods provided similar results, only ρ-values are shown here.

Results

Oceanographic context

During Leg 1b of the MALINA cruise (Figure 1), temperature, salinity and nitrates decreased more or less regularly going northward through the Pacific and Arctic Oceans (Table 1). During Leg 2b in the Beaufort Sea, the salinity was generally lower at the western stations whereas the temperature was generally higher at coastal stations. Both temperature and salinity varied very little at the deep chlorophyll maximum (DCM, −0.7 to −1.4 °C and 26.8 to 31.9 psu).

Chlorophyll-*a* concentration was higher at the DCM compared with the surface for all stations

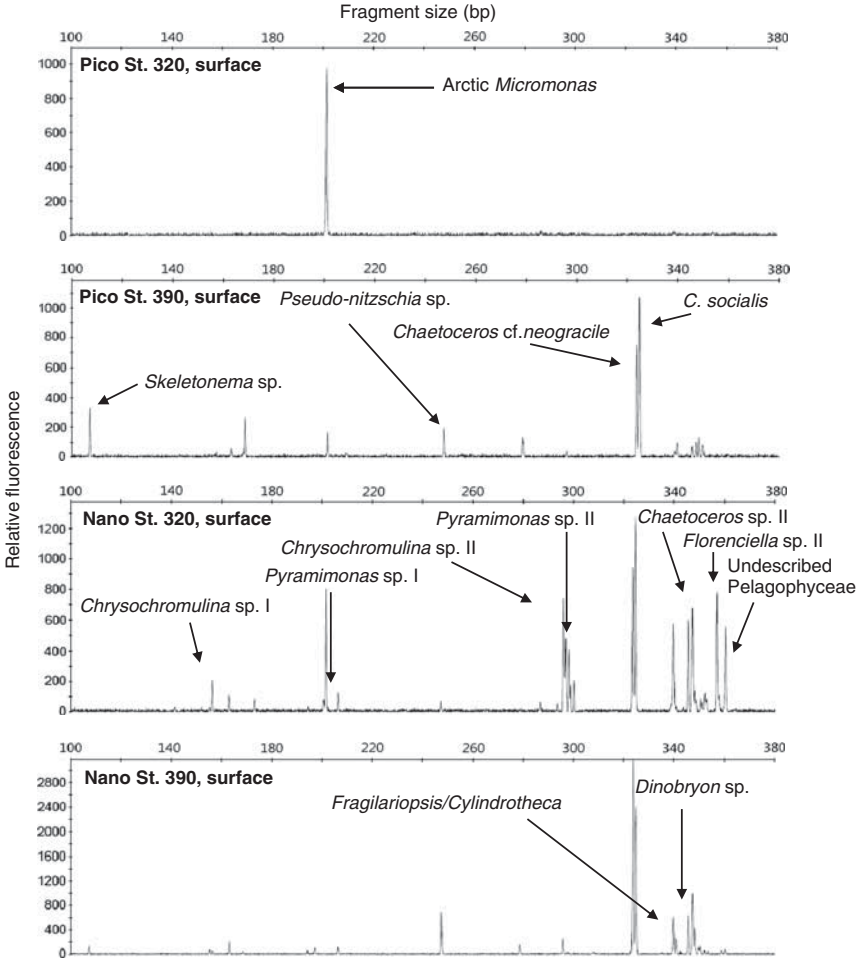

Figure 2 Diversity of flow cytometry sorted photosynthetic picoeukaryotes and nanoeukaryotes from the surface at stations 320 and 390 assessed by T-RFLP chromatograms of *MnlI* digests of 18S rDNA. Please note that the identification shown here has been confirmed by T-RFLP chromatograms of *HhaI* digests. The enzyme *Hpy188I,* which allows discriminating among the different *Micromonas* clades (Supplementary Table S7), was also used to validate the identification of the Arctic *Micromonas* ecotype. The full list of ribotypes identified is shown on Table 2. *C.*, *Chaetoceros.*

except Stn 170. Surface waters were depleted in nitrates (0.01–0.04 μM) whereas much higher levels (1.88–6.93 μM) were found at the DCM for all the stations except Stn 110 (0.33 μM, Table 1).

Cyanobacteria were present in the North Pacific, found in very low concentrations in the Bering Sea, and not detected at all the other stations of both Leg 1b and Leg 2b. During Leg 1b, photosynthetic pico and nanoeukaryotes were more abundant in the Pacific Ocean and the Bering and Arctic Seas compared with the Beaufort Sea. During Leg 2b, photosynthetic picoeukaryotes ranged two orders of magnitude (110–13 000 cell ml^{-1}) and were generally more abundant in surface compared with the DCM (Table 1) whereas photosynthetic nanoeukaryotes at the DCM often exceeded those measured at the surface and ranged from 170 to 7200 cell ml^{-1}.

T-RFLP of the 18S rRNA gene

In order to assess the diversity of photosynthetic pico and nanoeukaryotes, we amplified the 18S rRNA gene from populations sorted by flow cytometry on the basis of their size and chlorophyll fluorescence. The diversity of the amplified sequences was analysed by T-RFLP following enzyme digestion, which allowed obtaining a semi-quantitative image of the major taxa present (Figure 2, Table 2). Environmental ribotypes were identified up to the species level by comparison with ribotypes obtained from clones and strains or Genbank sequences.

At the North Pacific station PAC08 (Leg1b, Figure 1) photosynthetic picoplankton was dominated by an undescribed Mamiellophyceae. Its relative abundance decreased northward and the Arctic *Micromonas* ecotype became increasingly dominant (Figure 3). During Leg 2b through the Beaufort Sea, the only ribotype found in 36 out of 54 sorted picoeukaryote samples and dominating 12 other samples corresponded to Arctic *Micromonas* (Supplementary Table S2). It was the only photosynthetic picoeukaryote species present at most stations, especially in offshore waters (Figure 4). Ribotypes associated with other Mamiellophyceae (*Bathycoccus prasinos* and *Mantoniella squamata*), diatoms (*Chaetoceros socialis* and *Chaetoceros* cf. *neogracile*) and Pelagophyceae were occasionally present. Only 4 samples from three coastal stations (680, 690 and 390) did not contain, or contained in very low proportions, T-RFs specific of Arctic *Micromonas* (Figure 4). In these samples, ribotypes of *C. socialis* were in general dominating, but the total abundance of photosynthetic picoplankton was very low compared with that measured for the other stations (Table 1). A more detailed vertical profile was analysed at station 235 (eastern Beaufort Sea), revealing that Arctic *Micromonas* was the unique taxon throughout the water column, except in the very surface layer (Figure 5).

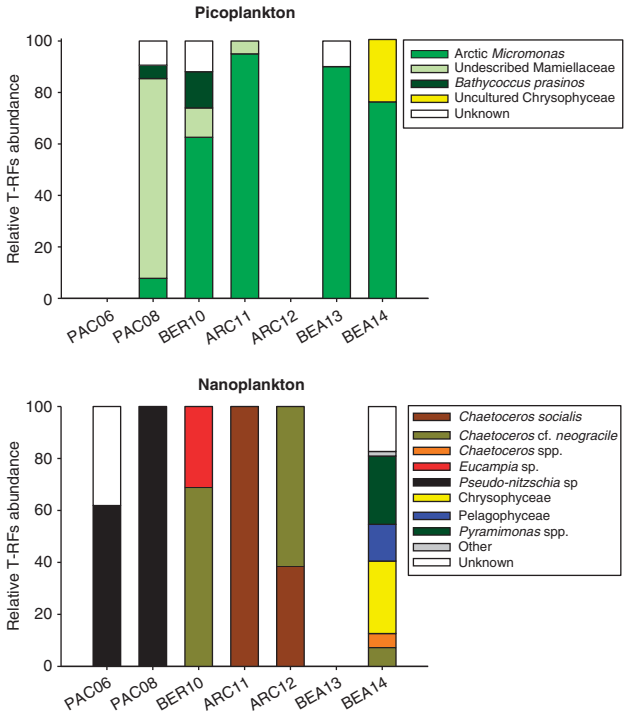

Figure 3 Taxonomic composition of photosynthetic pico and nanoeukaryotes based on T-RFLP on 18S rRNA gene sequences obtained from sorted photosynthetic populations at the different surface stations across the Leg 1b. Please note that while for picoplankton only one Chrysophyceae ribotype has been found (uncultured Chrysophyceae, Table 2), several have been found for nanoplankton. See Figure 1 for station locations.

During Leg 1b, photosynthetic nanoplankton was dominated by *Pseudo-nitzschia* sp. in the North Pacific and by *C.* cf. *neogracile* and *C. socialis* in the Bering and Arctic Seas (Figure 3). Station BEA14 in the Beaufort Sea was more diverse than the others and dominated by *Pyramimonas* spp., Pelagophyceae, and Chrysophyceae. During Leg 2b in the Beaufort Sea, nanoplankton communities were more diverse at the surface than at the DCM and in offshore compared with coastal waters (Figure 6). Surface samples were dominated by *Chaetoceros* species (*C.* cf. *neogracile*, *C. socialis* and, to a minor extent, two additional *Chaetoceros* spp., Supplementary Table S3) as well as *Chrysochromulina* spp., Chrysophyceae and Pelagophyceae. Within surface samples, the contribution from *Chaetoceros* species tended to be higher in coastal compared with offshore waters. At the DCM, ribotypes from *C. socialis* dominated at 10 out of 15 stations. Pelagophyceae, Arctic *Micromonas* and *Chrysochromulina* spp. occasionally dominated offshore stations. The detailed profile obtained at station 235 demonstrated sharp community changes with depth as well as a decrease in diversity (Figure 5). In surface waters, *C.* cf. *neogracile*, Chrysophyceae and *Pyramimonas* sp. I dominated, whereas *Chrysochromulina* spp., mainly occurred in colder deeper layers.

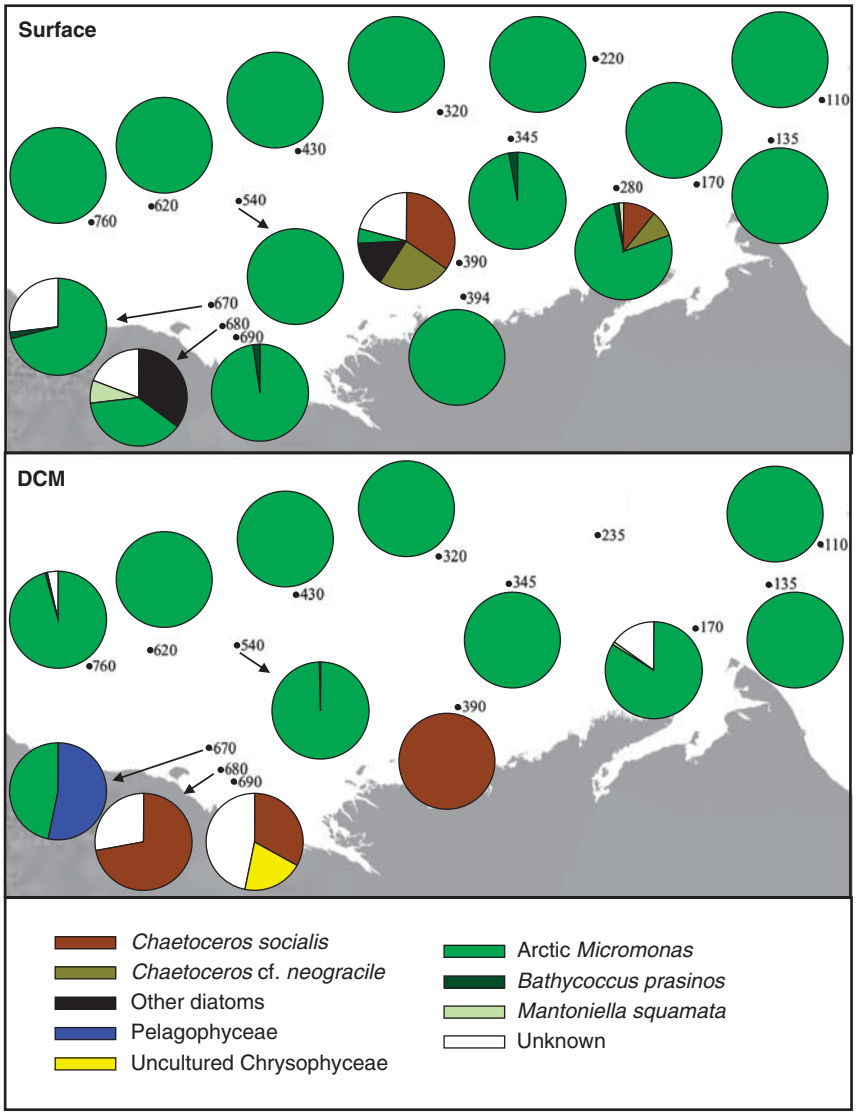

Figure 4 Taxonomic composition of photosynthetic picoeukaryotes based on T-RFLP on 18S rRNA gene sequences obtained from photosynthetic populations sorted from the surface and the DCM throughout the Beaufort Sea.

Cloning/sequencing

Genetic libraries of the 18S rRNA were constructed for pico and nanoeukaryotes samples sorted from the surface and the DCM at one coastal (390) and one offshore (320) station. These stations were selected because they are located on the same transect and showed remarkably different microbial compositions (Figures 4 and 6). Overall, we obtained 303 partial 18S rRNA gene sequences: 289 belonged to putative photosynthetic groups (Supplementary Table S4), and the others belonged to groups containing mainly heterotrophic micro-organisms (mostly Cercozoa, Supplementary Information).

At the coastal station 390, the composition of the pico and nanoplankton communities were quite similar (Table 3). Communities were more diverse in surface compared with the DCM. In surface, picoplankton was dominated by *C. socialis*, *C.* cf. *neogracile*, and uncultured Cercozoa, whereas nanoplankton was dominated by *C.* cf. *neogracile* and *Pseudo-nitzschia* sp. At the DCM, only diatoms (mostly *C. socialis*) were recovered in both fractions.

In contrast, at the offshore station 320, the picoplankton communities were monospecific (Arctic *Micromonas*) at both depths and different from nanoplankton communities (Table 3), which were rather diverse and dominated by diatoms: the most abundant sequences retrieved from the surface layer belonged to *C.* cf. *neogracile*, *M. squamata*, *Chrysochromulina* sp., *Florenciella parvula*, *Fragilariopsis cylindrus*, uncultured Naviculales, whereas *C. socialis* and *F. cylindrus* dominated the DCM nanoplankton communities.

The sequences belonging to the Arctic *Micromonas* clade were highly similar (>99.5% identity) whereas those affiliated to the genera *Chaetoceros* and *Chrysochromulina* were more divergent because we obtained 11 and 4 OTUs for these two genera, respectively (Figure 7).

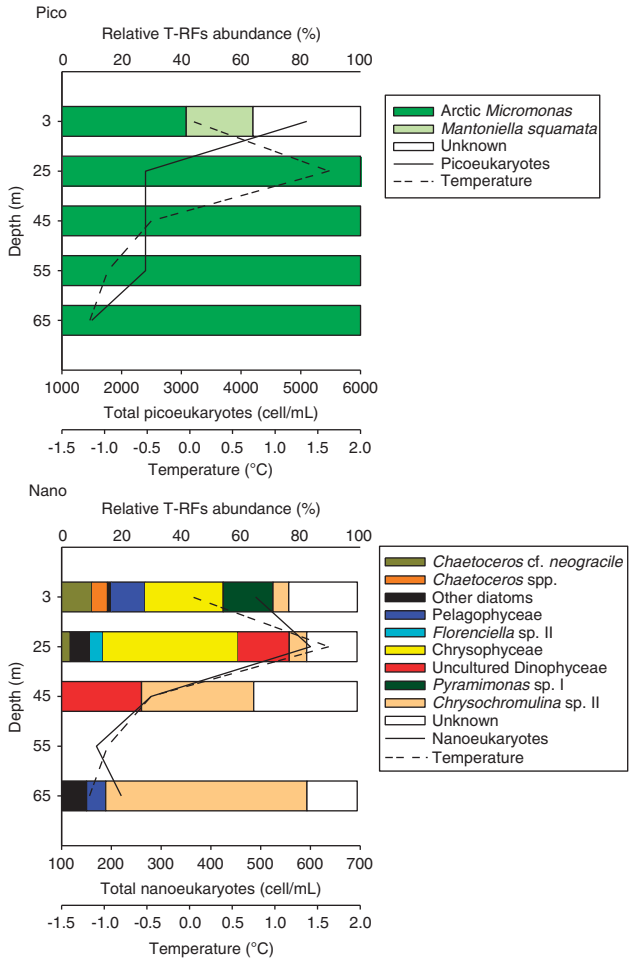

Figure 5 Temperature profile, absolute abundance and taxonomic composition of photosynthetic pico and nanoeukaryotes sorted from different depths at station 235.

Comparison of cloning/sequencing vs T-RFLP

The comparison of the cloning and the T-RFLP data revealed that the two approaches provided very similar images of the communities in particular for the major taxonomic groups (Figure 8). OTU richness generally exceeded the number of T-RFs detected for each enzyme (Supplementary Table S5). For example, the different OTUs found within the genera *Chaetoceros* (11) and *Chrysochromulina* (4) grouped into 5 and 2 ribotypes, respectively. Overall from 43 ribotypes occurred within our T-RFLP chromatograms, 31 were associated to OTUs sequenced from clones (Table 2). Discrepancies occurred (Supplementary Figure S2) but rather in terms of relative abundance of the different ribotypes. Only at station 390 in surface, pico and nanoplankton sequences affiliated to Rhizaria and at station 320, *M. squamata* sequences were recovered by cloning but not by T-RFLP.

Statistical analysis

The Spearman rank correlation coefficient showed in general a poor (<0.5) correlation between ribotypes and environmental conditions (Table 4). *Chaetoceros socialis* appeared related (0.59) with Chl-*a*, whereas *Chaetoceros* spp., *Pyramimonas* spp. and Chrysophyceae displayed significant negative correlations (<-0.5) with salinity, nitrate concentration and Chl-*a*.

Discussion

In this study, eukaryotes were sorted by flow cytometry to allow focusing on photosynthetic communities and to remove heterotrophic eukaryotes, which often dominate 18S rRNA gene sequences obtained from filtered samples (Marie *et al*., 2010). Sorted populations were analysed by T-RFLP. We chose this approach because it is rapid, cost-effective, and highly reproducible. T-RFLP was successfully applied to investigate microbial eukaryotes in aquatic systems from filtered samples (Diez *et al*., 2001a; Countway *et al*., 2005; Lepère *et al*., 2006). In this study, a total of 59 picoplankton and 79 nanoplankton samples were analysed (Supplementary Tables S2 and S3). Treating such a large number of samples with the classical cloning/sequencing approach would have been expensive and time consuming.

The combination of flow cytometry sorting and T-RFLP is particularly interesting because the complexity of the community is reduced compared with filtered samples, making T-RFs identification much easier. The use of two (or three) restriction enzymes allowed identifying most of the T-RFs found in the environmental samples by comparing them with those determined from our clones and cultures (Supplementary Information) or alternatively, for T-RFs not represented in clones and cultures, by an *in silico* analysis of the large 18S rRNA gene database. Overall, we identified 43 ribotypes (Table 2) by comparison with the experimental database from clones (48 OTUs) and strains (20 OTUs) or with an *in silico* database (5 OTUs). Several T-RFs, especially occurring at the DCM of Stn 110 (Figure 6) could not be identified and were likely associated with unknown eukaryotes. Overall unidentified peaks did not seriously affect our ribotype identification (Supplementary Information). The validity of our assignments is confirmed by the good agreement ($\rho>0.5$) of community structures estimated from T-RFLP vs cloning/sequencing for seven out of eight samples for which both approaches were used (Figure 8, Supplementary Figure S2).

Picoplankton community composition in the Arctic

In opposition to other oceanic waters, picocyanobacteria (*Synechococcus* and *Prochlorococcus*) were completely lacking in Arctic waters as observed previously (Li, 1998). This contrasts with the fact that cyanobacteria are an important component of Arctic freshwater systems including Mackenzie

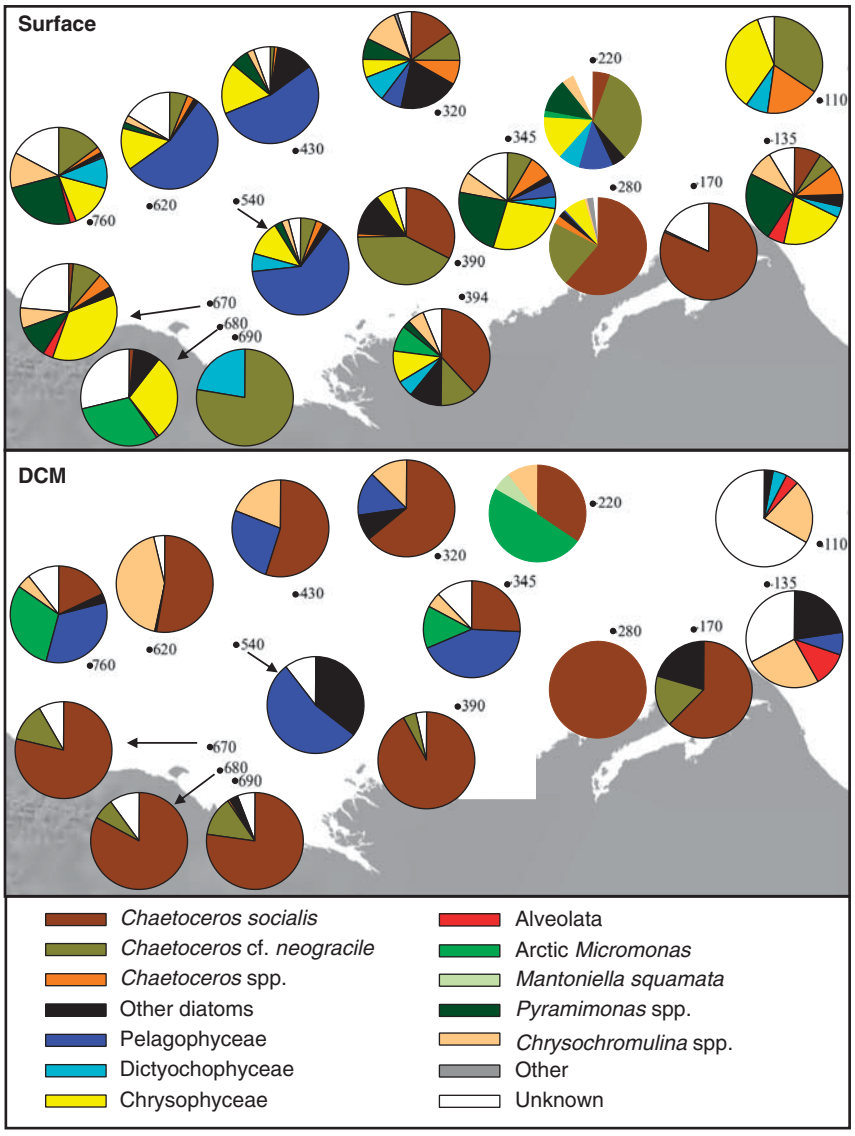

Figure 6 Taxonomic composition of photosynthetic nanoeukaryotes based on T-RFLP of 18S rRNA gene sequences obtained from photosynthetic populations sorted from the surface and the DCM throughout the Beaufort Sea.

River, but their abundance decreases sharply with increasing salinities (Vallieres *et al.*, 2008). Therefore, only eukaryotes account for marine primary production in the Arctic.

The most dramatic observation from our data set, which covers with unprecedented resolution the Beaufort Sea during mid-summer, is that Arctic *Micromonas* was the unique photosynthetic picoeukaryote occurring at many stations, confirming its importance within Arctic picoplankton (Not *et al.*, 2005; Lovejoy *et al.*, 2007). The other Mamiellophyceae, *B. prasinos*, only had a very marginal role (Figure 4), in contrast with observations in the Beaufort Sea in late summer (Lovejoy *et al.*, 2007) and in the Barents Sea in mid-summer (Not *et al.*, 2005). The genus *Micromonas* has been clustered into three to six distinct clades depending on the investigators (Guillou *et al.*, 2004; Slapeta *et al.*, 2006; Worden, 2006; Lovejoy *et al.*, 2007). Almost all the *Micromonas* sequences recovered from Arctic waters in the present (Figure 7) and previous studies (Lovejoy *et al.*, 2007; Luo *et al.*, 2009) are highly homogeneous and belong to a distinct lineage within clade B *sensu* Guillou *et al.* (2004). *Hpy188I* digests of the 18S rRNA gene from our picoplankton samples, which allows the different *Micromonas* clades to be distinguished, have confirmed that only clade B occurred during the MALINA cruise (Supplementary Table S7). In contrast, clade A occurred in the Barents Sea, dominating surface waters, probably because of the influence of Atlantic water (Foulon *et al.*, 2008) whereas a single study detected sequences from clade C in the Beaufort Sea, although in very low number compared with those of the Arctic ecotype (Lovejoy and Potvin, 2011).

Table 3 Summary of phylogenetic assignments for sequences obtained from the stations 320 and 390 for sorted photosynthetic pico and nanoeukaryotes

Division	*Station*	*390*	*390*	*320*	*320*	*390*	*390*	*320*	*320*
	Clone library	*ES018*	*ES020*	*ES064*	*ES068*	*ES019*	*ES021*	*ES065*	*ES069*
	Fraction	*Picoeukaryotes*				*Nanoeukaryotes*			
	Depth	30	3	70	3	30	3	70	3
	Class								
Haptophyta			1				1	4	9
Telonemia								1	
Alveolata	Dinophyceae							1	1
Alveolata	Unknown								1
Rhizaria	Cercozoa		4				1		
Rhizaria	Unknown		8						
Cryptophyta			2						
Chlorophyta	Prasinophyceae							1	1
Chlorophyta	Mamiellophyceae		1	17	22			1	7
Heterokontophyta	Chrysophyceae						1		2
Heterokontophyta	Dictyochophyceae							2	5
Heterokontophyta	Pelagophyceae							2	1
Heterokontophyta	Bolidophyceae							2	
Heterokontophyta	Bacillariophyceae	51	30			25	40	36	22
Number of clones sequenced		51	46	17	22	25	43	50	49

More details are shown on Supplementary Table S4.

As a consequence, the abundance of picoeukaryotes measured by flow cytometry during the MALINA cruise in the Beaufort Sea (Table 1) corresponds, for most stations, to that of Arctic *Micromonas*. The nitrate limitation detected in surface waters, as well as the wide ranges observed in temperatures (−1.1 to 7 °C) and salinities (19–31 psu), did not seem to affect the abundance of the Arctic ecotype ($\rho = -0.21$, *P*-value = 0.22). In contrast in the Barents Sea, clade B was outnumbered by clade A in waters where temperature was in the same range than at the coastal stations in the Beaufort Sea (≈7 °C), in August/September 2002 (Foulon *et al.*, 2008). However, it should be noted that the areas in the Beaufort Sea where the water temperature was higher are surrounded by low temperature areas. Therefore, the other *Micromonas* clades may not be able to recolonise these areas after the Arctic winter. In temperate areas, *Micromonas* abundance was high in the nutrient rich English Channel (Not *et al.*, 2004) but low in oligotrophic environments such as the Mediterranean Sea (Marie *et al.*, 2006) and the Indian Ocean Gyre (Not *et al.*, 2008) where only clade C occurred (Foulon *et al.*, 2008). In contrast, in the Beaufort Sea, the Arctic *Micromonas* ecotype was found under both nitrate deplete and nitrate replete conditions (Table 1).

Besides Arctic *Micromonas*, a few other species, mostly diatoms, were observed to contribute to the photosynthetic picoeukaryote community (Figure 4). Their presence was limited to samples with low picoeukaryote abundances, mostly in coastal waters (Table 1). Although most diatoms are >2 μm, diatom sequences are often found in picoplankton clone libraries (Vaulot *et al.*, 2008). These sequences may derive from male gametes or early stage auxospores, which could fit within the size range of picoplankton as shown for *Chaetoceros* (Jensen *et al.*, 2003; Assmy *et al.*, 2008) and *Pseudo-nitzschia* (Sarno *et al.*, 2010). Individual *Skeletonema* cells may also be occasionally ⩽2 μm in size (Sarno *et al.*, 2005; Balzano *et al.*, 2011).

The diversity found in this study for sorted photosynthetic picoeukaryotes is very low compared with that previously estimated for small (<3 μm) filtered plankton in the Beaufort Sea (Lovejoy and Potvin, 2011) and other Arctic systems (Lovejoy *et al.*, 2006). This is likely due to the removal of heterotrophic groups through sorting (Marie *et al.*, 2010). Tyramide signal amplification—fluorescent *in situ* hybridisation in the Norvegian and Barents Sea revealed that besides Mamiellophyceae, other Chlorophyta as well as Haptophyta occurred within the small (<3 μm) photosynthetic plankton (Not *et al.*, 2005). Overall, the diversity of photosynthetic picoeukaryotes in the Arctic is far lower than that found in the South East Pacific (Shi *et al.*, 2009, 2011), the Sargasso Sea (Not *et al.*, 2007; Cuvelier *et al.*, 2010), the North East Atlantic Ocean (Jardillier *et al.*, 2010) and the English Channel (Marie *et al.*, 2010). *Micromonas* has also been observed in winter in the Canadian Arctic (Sherr *et al.*, 2003) and is likely to be the only organism in this size range that can adapt to both the very low temperatures and the long period of darkness encountered in these waters.

Nanoplankton diversity in the Arctic

Photosynthetic nanoeukaryotes investigated here constitute a more diverse community compared

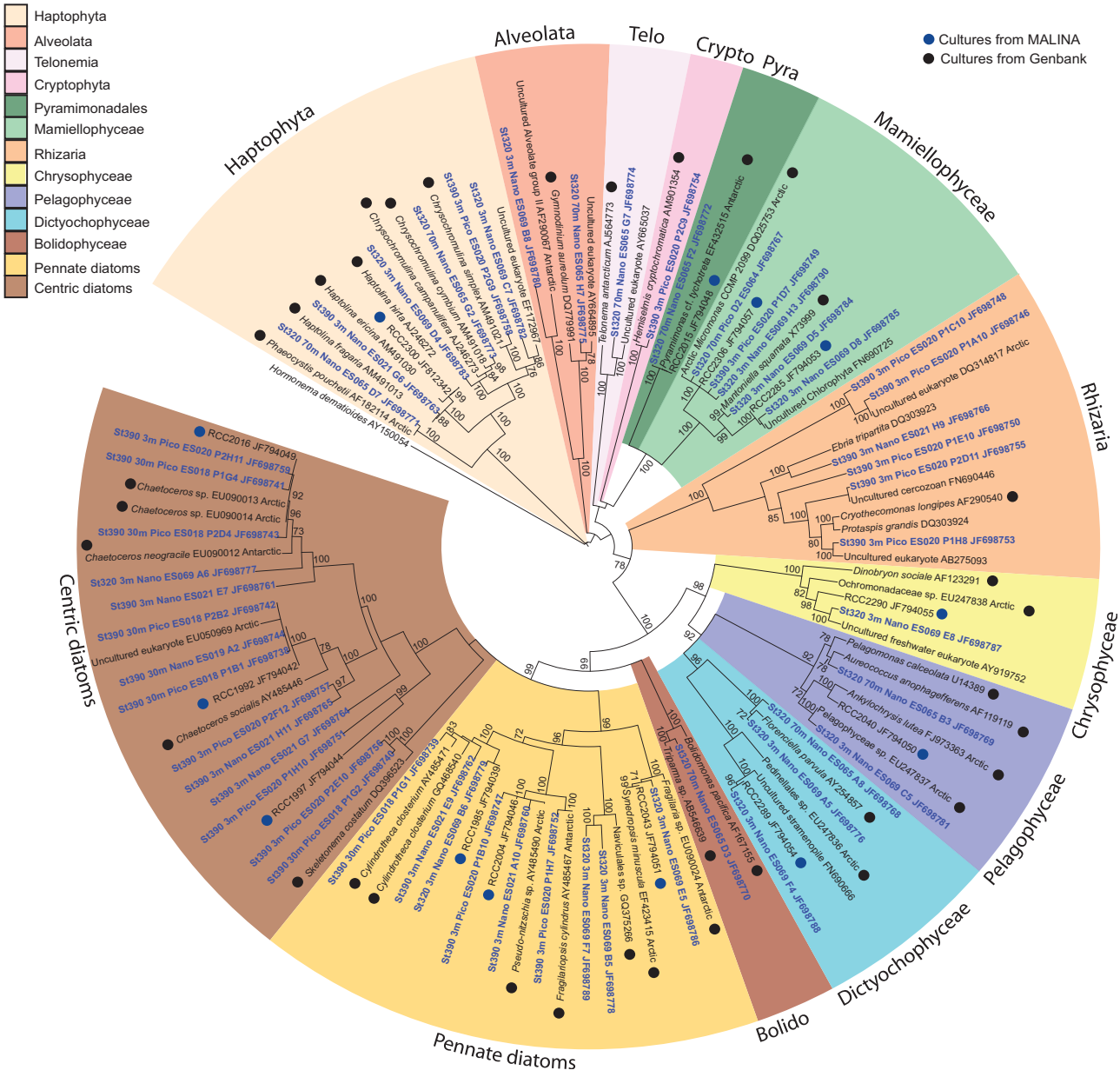

Figure 7 Neighbour joining (NJ) phylogeny of almost full-length 18S rRNA genes from photosynthetic pico and nanoeukaryotes sorted from the stations 320 and 390. A fungal sequence (*Hormonema dematioides*) was used as outgroup. Sequences corresponding to cultures are indicated by a dot (blue for cultures isolated during MALINA and black for others) whereas environmental sequences are in blue. Details on phylogenetic analyses are given in the Materials and methods Section. 1556 unambiguously aligned positions were considered from an alignment of 115 nucleotide sequences. The percentage of NJ bootstrap (based on 1000 replicates) is shown next to the branches for values $\geqslant$70%.

with picoeukaryotes. Only 7 out of 38 OTUs recovered from the nanoplankton are closely related ($\geqslant$99.5% similarity) to existing Arctic sequences (Supplementary Table S4), whereas the others either match sequences from elsewhere (8 sequences, mostly from the Baltic Sea) or belong to novel OTUs. This suggests that some of the OTUs found in this study have a global oceanic distribution and can be detected in similar (cold and salinity-changing) environments (Nolte *et al.*, 2010) whereas other OTUs might be restricted to the Beaufort Sea, which seems to constitute a microbial province favouring endemism (Lovejoy *et al.*, 2007).

Strains representative of 28 out of 47 OTUs have been successfully brought in culture previously or during the MALINA cruise (Figure 7). The 11 T-RFLP ribotypes found more frequently (>10 samples) include OTUs from strains cultured during the MALINA cruise (8) or previously (3, Table 2) suggesting that the majority of phytoplankters from the Beaufort Sea have cultured representatives. This clearly contrasts with small phytoplankton from

oligotrophic areas such as the Mediterranean Sea (Viprey *et al*., 2008), the North East Atlantic (Jardillier *et al*., 2010), the Sargasso Sea (Not *et al*., 2007) or the South East Pacific (Shi *et al*., 2009), which are dominated by microorganisms that cannot be cultured despite extensive isolation efforts (Le Gall *et al*., 2008). Such waters may contain slow-growing, low-nutrient adapted microorganisms that cannot adapt to the media used for micro-algae or that are outcompeted by rarer but faster growing species (for example, *Pelagomonas calceolata*, a species often isolated from oligotrophic waters, Le Gall *et al*., 2008). In contrast, the seasonal variability in temperature, salinity and nutrients typical of the Beaufort Sea (Carmack and MacDonald, 2002; McLaughlin *et al*., 2004) may select resilient genotypes that can adapt to a broad range of conditions and therefore can be easily brought into culture.

The diversity and abundance of *Chaetoceros* species is confirmed by phytoplankton counts (S Lessard, personal communication) and has been previously documented in Arctic waters (Booth and Horner, 1997; Lovejoy *et al*., 2002) with *C. socialis* often forming late spring blooms (Booth *et al*., 2002; Degerlund and Eilertsen, 2010). The ribotypes found here are likely associated with single cells either from occasionally non-colonial species (*C.* cf. *neogracile*) or detached from colonies in the water column or during the tangential flow filtration (*C. socialis*). Resting spores, which have been observed previously for *C. socialis* (Booth *et al*., 2002) as well as in sorted samples from the MALINA cruise (M Kawachi, personal communication), probably contributed also to these sequences. The contribution of *C. socialis* was usually higher at the DCM compared with the surface (Figure 6). In contrast, the other *Chaetoceros* species were found more frequently in surface waters. The vertical profile at station 235 (Figure 5) displays a drastic change in the microbial community between 25 and 45 m associated with decreases in temperature and total abundance of photosynthetic nanoeukaryotes. This may suggest a transition between ribotypes adapted to surface waters (*C.* cf. *neogracile*, *Chaetoceros* spp., Chrysophyceae, *Pyramimonas* sp. I) and *Chrysochromulina* spp., which occur mainly at the DCM. This is consistent with the negative correlation between surface ribotypes and salinity, nitrate concentration and to a lesser extent with the positive correlation with temperature (Table 4).

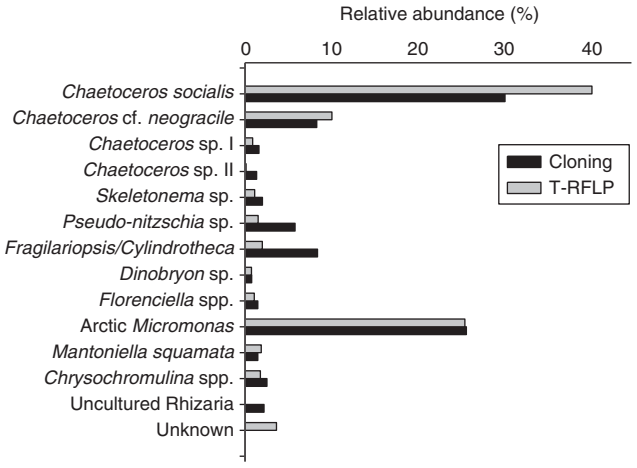

Figure 8 Overall comparison of composition of photosynthetic pico and nanoeukaryotes assessed by T-RFLP and cloning/sequencing of the 18S rRNA gene. Only ribotypes from which at least three sequences were recovered by cloning/sequencing are represented.

Chrysophyceae, mainly represented by *Dinobryon* spp. were restricted to surface waters (Figure 6). A number of *Dinobryon* species were previously reported in marine (Lovejoy *et al*., 2002) and freshwater environments (Brutemark *et al*., 2006) of the Arctic but they were never characterised genetically and we do not know whether they correspond to the ribotypes found here. The occurrence of Pelagophyceae in the Beaufort Sea is consistent with a previous study (Suzuki *et al*., 2002) indicating the prevalence of Pelagophyceae-specific pigments (19′-Butanoyloxyfucoxanthin) in the Bering Sea. Three OTUs undistinguishable

Table 4 Spearman rank correlation coefficients and *P*-values between nanoplankton groups or taxa and environmental variables for Leg 2b

	Temperature	*Salinity*	*Nitrate*	*Chlorophyll-a*	*Pico*	*Nano*
C. socialis	−0.16 (0.35)	**0.36 (0.03)**	**0.47 (<0.01)**	**0.59 (<0.01)**	**−0.49 (<0.01)**	**0.40 (0.02)**
C. cf. *neogracile*	**0.36 (0.04)**	**−0.40 (0.02)**	**−0.43 (0.01)**	−0.31 (0.07)	0.10 (0.58)	0.05 (0.78)
Chaetoceros spp.	0.33 (0.05)	**−0.51 (<0.01)**	**−0.50 (<0.01)**	**−0.62 (<0.01)**	0.28 (0.10)	−0.12 (0.48)
Other diatoms	−0.14 (0.41)	−0.20 (0.25)	−0.11 (0.53)	−0.22 (0.21)	0.02 (0.92)	−0.10 (0.58)
Pelagophyceae	−0.16 (0.36)	−0.11 (0.53)	0.14 (0.43)	−0.27 (0.12)	**0.41 (0.02)**	−0.11 (0.52)
Dictyochophyceae	**0.38 (0.02)**	**−0.43 (0.01)**	**−0.55 (<0.01)**	**−0.44 (0.01)**	**0.39 (0.02)**	<0.01 (0.98)
Chrysophyceae	**0.62 (<0.01)**	**−0.67 (<0.01)**	**−0.71 (<0.01)**	**−0.70 (<0.01)**	**0.39 (0.02)**	−0.09 (0.59)
Alveolata	0.11 (0.52)	−0.10 (0.55)	−0.33 (0.05)	−0.20 (0.24)	0.05 (0.76)	−0.20 (0.24)
Arctic *Micromonas*	0.04 (0.81)	−0.01 (0.94)	0.04 (0.83)	0.16 (0.36)	0.13 (0.46)	0.07 (0.68)
Mantoniella squamata	−0.29 (0.09)	0.15 (0.38)	0.05 (0.77)	0.06 (0.73)	0.12 (0.49)	−0.10 (0.56)
Pyramimonas spp.	**0.35 (0.04)**	**−0.58 (<0.01)**	**−0.50 (<0.01)**	**−0.64 (<0.01)**	**0.40 (0.02)**	−0.24 (0.16)
Chrysochromulina spp.	−0.25 (0.14)	<0.01 (0.99)	−0.12 (0.50)	−0.32 (0.06)	0.14 (0.41)	**−0.46 (0.01)**

Significant coefficients are indicated in bold.

by T-RFLP (Table 2) appear to constitute novel Pelagophyceae lineages (Figure 7).

Among Haptophyta, the high occurrence (Figure 6) and the wide diversity (Figure 7) of *Chrysochromulina* ribotypes found here is consistent with previous findings in North waters (Lovejoy *et al.*, 2002). Although *Phaeocystis pouchetii* forms blooms in the Barents (Wassmann *et al.*, 2005) and Greenland Seas (Cota *et al.*, 1994), it occurs rarely in the Beaufort Sea (Campbell *et al.*, 2009) and its contribution to Beaufort Sea nanoplankton in this study was very low (Table 2) as confirmed by phytoplankton counts (S Lessard, personal communication). Surprisingly, uncultured Haptophyta that typically dominate the 3–4 μm fraction in many marine waters (Cuvelier *et al.*, 2010; Jardillier *et al.*, 2010) were not detected in our samples.

Pyramimonas spp. were found only in surface waters (Figures 5 and 6). A number of *Pyramimonas* species have been isolated from Arctic (Daugbjerg and Moestrup, 1993) and Antarctic (Daugbjerg, 2000) environments. Previous reports from blooms under the ice (Gradinger, 1996) and growth in the laboratory across a broad (15–35 psu) salinity range (Daugbjerg, 2000) indicate that some *Pyramimonas* species are adapted to salinity-changing environments as encountered in surface waters of the Beaufort Sea.

The contribution of dinoflagellates to our samples was very low (Figures 5 and 6). Although a number of dinoflagellate species have been reported for the Arctic (Okolodkov, 1999), their presence in the Beaufort Sea remains very scarce (Okolodkov and Dodge, 1996), especially in mid-summer when pigments specific of diatoms, green algae, and Haptophyta mainly occur (Hill *et al.*, 2005). Dinoflagellates become more abundant in autumn (Brugel *et al.*, 2009).

The nanoplankton community was less diverse at the DCM compared with the surface (Supplementary Figure S3). This could be due to the narrower variability of both temperature and salinities encountered there (Supplementary Figure S4).

Conclusions

Although surface waters in the Beaufort Sea were quite oligotrophic in summer with nearly undetectable nitrate levels during the MALINA cruise (Table 1), small phytoplankton communities here were very different from those observed in warmer oligotrophic waters such as the South East Pacific gyre (Shi *et al.*, 2009) or the Mediterranean Sea (Man-Aharonovich *et al.*, 2010). First, photosynthetic picoeukaryotes were dominated by a single ecotype of the Mamiellophyceae genus *Micromonas* and we did not find any other species at most of the stations analysed, whereas temperate and tropical oligotrophic waters contain much more diverse communities. Second, nanoeukaryotes were dominated by diatoms and other stramenopiles groups, which representatives, at least for the taxa most frequently found, can be easily isolated and cultivated. This contrasts with temperate and tropical small phytoplankton communities, which contain many uncultivable taxa. These differences may be explained by the fact that only few resilient ecotypes can adapt to the sub-freezing temperatures and variable salinities observed in the Arctic.

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