

Key Arctic phototrophs are widespread in the polar night

Anna Vader · Miriam Marquardt ·
Archana R. Meshram · Tove M. Gabrielsen

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Abstract Small photosynthetic pico- and nanoeukaryotes contribute substantially to the biomass and primary production in the Arctic, often producing large blooms during spring and summer. During the civil polar night, which in the Svalbard Archipelago lasts from the middle of November to the end of February, no light is present, thus providing photosynthetic organisms with the challenge of how to survive several months of darkness. The small green alga *Micromonas pusilla* and the haptophyte *Phaeocystis pouchetii* are two key phototrophs in the Arctic, commonly blooming during the arctic spring and summer. Their occurrence in Arctic waters during the polar night period is, however, less well known. In the present study, we used a molecular approach to show that *M. pusilla* and *P. pouchetii* are widely distributed in Svalbard waters also at the height of the polar

night. Both species were detected in pelagic samples from both fjords and the open ocean, ice-covered and ice-free locations, shallow and deep water and from Atlantic, Arctic and coastal water masses. PCR screening was performed on both DNA and RNA samples, the latter allowing the detection of viable cells of both species in the mesopelagos. As far as we are aware this is the first systematic study on the persistence of these important photosynthetic organisms through the polar night.

Keywords Polar night · *Micromonas pusilla* · *Phaeocystis pouchetii* · Photosynthetic organisms · Svalbard Archipelago

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A. Vader (✉) · M. Marquardt · A. R. Meshram ·
T. M. Gabrielsen
The University Centre in Svalbard (UNIS), Longyearbyen,
Norway
e-mail: annav@unis.no

M. Marquardt
Department of Biology, CEES, University of Oslo, Oslo,
Norway

A. R. Meshram
Faculty of Biosciences, Fishery and Economics, University of
Tromsø, Tromsø, Norway

T. M. Gabrielsen
Department of Biology, University of Bergen, Bergen, Norway

Introduction

The Arctic is characterized by extreme seasonality in light conditions varying from 24-h sunlight in summer to complete darkness in winter, thus providing Arctic phototrophs the challenge of surviving long periods with no available light for photosynthesis. The classic paradigm of the Arctic winter that life comes to a standstill has recently been challenged by data showing that species of zooplankton were active and feeding even during the polar night period (Berge et al. 2009; Kraft et al. 2013). Indeed even photosynthetic organisms have been found to persist through the dark polar night, although at reduced abundances (i.e., Weslawski et al. 1988; Sherr et al. 2003).

Dominant arctic bloom-forming microalgae of Bacillariophyceae and Dinophyceae are well known to persist unfavorable conditions like the Arctic winter in resting stages such as spores or cysts (Garrison 1984; Smetacek 1985; Kremp and Anderson 2000). Such survival strategies are not known for the important photosynthetic Arctic

pico- and nanoflagellates who also contribute significantly to the diversity, biomass and primary production of Arctic waters (Booth and Horner 1997; Booth and Smith 1997; Gosselin et al. 1997; Sherr et al. 2003; Lovejoy et al. 2006). Typically, small cells (<10 µm) contribute 50 % of total carbon production in the Barents Sea, even though large cells (>10 µm) often dominate at the peak of the bloom (i.e., Hodal and Kristiansen 2008). In spite of their unknown winter survival strategies, photosynthetic pico- and nanoflagellates persist at reduced abundances in Arctic waters throughout the polar night (i.e., Sherr et al. 2003).

A key picoflagellate (1–3 µm) Arctic species is *Micromonas pusilla* (Butcher) Manton and Parke 1960 (Mamiellophyceae) which seems to have taken over as baseline phototroph in Arctic waters (Lovejoy et al. 2006), a role that *Synechococcus* and *Prochlorococcus* perform in temperate and tropical areas (Scanlan and West 2002). A distinct Arctic ecotype of *M. pusilla* adapted to cold temperatures and low-light conditions has been identified (Lovejoy et al. 2007). Densities of $2.4\text{--}2.8 \times 10^7$ cells L⁻¹ have been reported in the Barents Sea (Thronsdén and Kristiansen 1991) and the Arctic Ocean (Sherr et al. 2003). *Micromonas* persists at low population densities throughout the winter in northern Norway (Straumsbukta, 69° 33'N, Thronsdén and Heimdal 1976) and the Canadian Arctic (Franklin Bay, Beaufort Sea, 70°N, Terrado et al. 2008), whereas samples taken at latitudes experiencing civil polar night are rare. Sherr and coworkers (Sherr et al. 2003), however, identified a 2-µm micromonad believed to be *Micromonas* in the Arctic Ocean throughout winter (SHEBA/JOIS drift experiment, 75–80°N). On Svalbard, *Micromonas* was detected in clone libraries from Billefjorden in January 2009, although it was most common in the pre- and post-bloom periods (Sørensen et al. 2012).

The key Arctic nanoflagellate *Phaeocystis pouchetii* (Hariot) Lagerheim 1893 (Prymnesiophyceae) can produce massive nearly monospecific blooms in its colony-forming stage (Schoemann et al. 2005). Its complex life cycle is only partly resolved, but consists of one or two solitary flagellated stages (3–8 µm) and one colonial form (up to 2 mm) where non-motile cells are encapsulated in a mucilaginous matrix (Rousseau et al. 2007). *Phaeocystis* sp. plays a prominent role in biogeochemical fluxes (Smith et al. 1991) and is especially important for sulfur cycling (Stefels and vanBoekel 1993). Bloom densities of 1.2×10^7 cells L⁻¹ have been reported from Kongsfjorden, Svalbard (Eilertsen et al. 1989) where viable solitary cells were observed during the civil polar night (December 2006; Rokkan Iversen and Seuthe 2011). *Phaeocystis* cells were detected but only rarely during winter in the Arctic Ocean (Sherr et al. 2003).

Our objective was to test the occurrence of the two phototrophs *M. pusilla* and *P. pouchetii* during the civil

polar night, a period of the year that is rarely sampled due to logistic constraints. Our extensive material includes pelagic samples from fjords and the open ocean, from shallow and deep water and from Atlantic, Arctic and coastal water masses. Molecular methods were used to overcome the difficulties associated with microscope detection and identification of minute flagellates in low-biomass winter samples. PCR screening was performed on sample DNA and RNA, the latter in order to test for the presence of viable cells.

Materials and methods

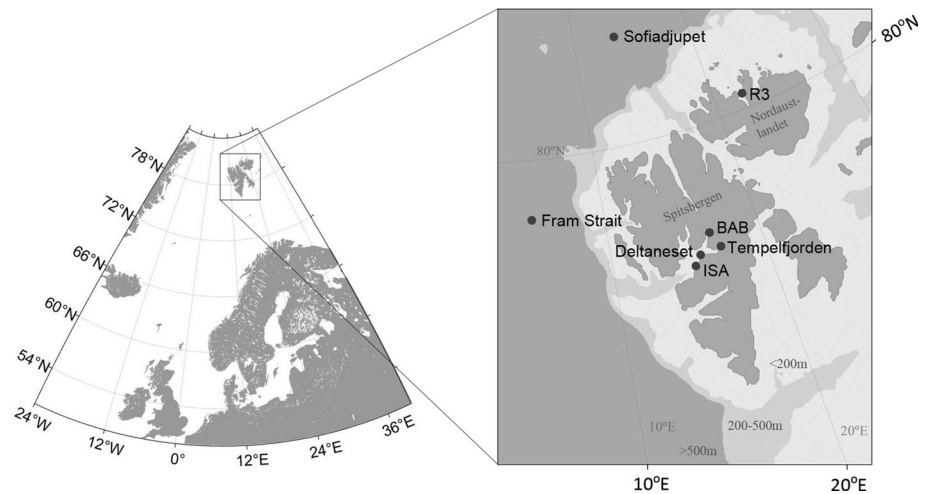
Sample collection

Water samples were collected from sites in the waters of Svalbard during several research cruises with “KV Svalbard,” “RV Helmer Hanssen” and “RV Viking Explorer,” between December 2008 and January 2013 (Fig. 1). Open ocean deep water stations include Sofiadjupet north of Spitsbergen and the Fram Strait station northwest of Kongsfjorden. Samples from fjords were taken in the north-facing Rippfjorden (station R3) at Nordaustlandet, and from stations within Isfjorden, including Billefjorden (station BAB), Deltanaset, Tempelfjorden and Adventfjorden (station ISA; see Fig. 1). The 2008 and 2009 samples from Tempelfjorden and Billefjorden (BAB), as well as the samples from Sofiadjupet, were collected through holes in the sea ice. All samples were collected using a 10-L Niskin bottle and kept cold and dark until further processing. At each sampling station, a CTD profile was obtained using either a SAIV SD204 CTD equipped with a Seapoint fluorescence sensor or the ship CTD (Sealogger CTD, SBE Seabird Electronics equipped with a fluorometer from Seapoint Sensors Inc).

Chl *a* measurements

Seawater samples for chlorophyll *a* (Chl *a*) measurements were collected from the Niskin bottle into 10-L plastic buckets that were rinsed with distilled water between sampling. In order to measure the total Chl *a*, 0.4–1.0 L seawater was filtered in triplicate (or in duplicate for the Sofiadjupet samples) onto GF/F glass microfiber filters (Whatman, England) using a vacuum pump. In order to determine the Chl *a* contribution of larger cells, an equal volume of seawater was filtered in triplicate onto either 3-µm (for 2008–2010 samples) or 10-µm (for 2011–2013 samples) Isopore membrane polycarbonate filters (Millipore, USA). For Sofiadjupet, duplicate samples were drained through a 10-µm mesh, prior to collecting the <10-µm cells on GF/F filters. All filters were stored at –80 °C

Fig. 1 Map of Northern Europe and Svalbard indicating the location of the sampling stations



until processing. Chl *a* was extracted in 10 ml methanol for 20–24 h at +4 °C in the dark (Holm-Hansen and Riemann 1978) and fluorescence determined using a 10-AU-005-CE Fluorometer (Turner, USA). After measuring total Chl *a* from each filter, non-degraded Chl *a* was degraded by the addition of 5 % HCl, and fluorescence measurements were repeated. Both uncorrected and acid-corrected Chl *a* values were calculated.

DNA/RNA collection and extraction

Prior to 2011, water for DNA and RNA filtration was collected in clean 4-L plastic buckets, and 1–2 L of seawater was filtered through two in-line 47-mm filter holders using a peristaltic pump at 40 rpm (Heidolph, Germany). The first filter holder contained a 3- μ m Isopore membrane polycarbonate filter and the second filter holder contained a 0.22- μ m Durapore membrane hydrophilic PVDF filter (both Millipore, USA). After filtration, samples were immediately frozen at -80 °C or on dry ice (Deltaneset samples). In 2011, the water filtration procedure, as well as the methods for DNA and RNA isolation, was changed. After this, approximately 4 L of water was poured through a funnel with 10- μ m mesh (KC Denmark) immediately after collection, before organisms were collected on a 0.45- μ m polycarbonate filter (Millipore, USA) using vacuum. Immediately after filtration both the 10- μ m mesh and the 0.45- μ m filters were snap-frozen in liquid nitrogen and stored at -80 °C until processing. DNA from the 2008 and 2009 samples was extracted by CTAB (see Sørensen et al. 2012), while all other DNA was extracted using the DNeasy Plant Mini kit (Qiagen, USA) following the manufacturers' protocol, except that a bead-beating step was included to ensure efficient lysis. Briefly, each filter was cut into two halves which were individually extracted. During the lysis step, beads were added (200- μ m zirconium

beads, molecular biology grade, OPS Diagnostics, USA) and the tubes were beaten 2 times for 1 min at frequency 1/22 s using a Retsch MM400 bead beater. At the end of the protocol, DNA from each filter half was eluted two consecutive times using 75 μ l elution buffer and combined for further analysis. RNA from Deltaneset was isolated using Trizol (Invitrogen) according to standard protocol, except that a bead-beating step was added. Prior to freezing, 1 ml Trizol was added to the filter. After thawing, beads were added and the samples beaten (see above). The Trizol was subsequently transferred to a fresh tube, and the filter re-extracted with another 1 ml of Trizol, thereby yielding 2 ml of Trizol-extracted sample from each filter. These were subsequently pooled. For each sample 10 μ g glycogen (RNase free, Fermentas) was added as a carrier during precipitation. Finally, the precipitated RNA was dissolved in 27 μ l of nuclease-free water. All other RNA samples were extracted using the RNAqueous kit (Ambion) according to the standard protocol except that each filter was extracted twice and a bead-beating step was added (see above). Prior to freezing, 600 μ l of LB buffer from the kit was added. After filter-binding and washing, the RNA was eluted into 60 μ l of elution buffer. All RNA samples were DNase-treated using the Turbo DNase-kit (Ambion) according to the manufacturers' protocol and stored at -80 °C until further processing.

PCR screening

Extracted DNase-free RNA was first reverse transcribed into cDNA using M-MLV reverse transcriptase according to standard protocol. Briefly, 4 μ l of RNA was combined with 2 μ l 50 μ M random decamer primers and 6 μ l of nuclease-free water (both Ambion), denatured by heating at 80 °C for 3 min and immediately transferred to ice. Subsequently, 2 μ l 10 \times first-strand synthesis buffer, 4 μ l

2.5 mM dNTP mix, 1 μl 40 U/ μl RNase inhibitor and 1 μl 100 U/ μl M-MLV reverse transcriptase (all Ambion) were added. The RT reaction mix was incubated at 44 °C for 1 h, prior to denaturing the enzyme at 92 °C for 10 min. Extracted DNA or cDNA was amplified in 25- μl reactions containing 1 \times Dream Taq green mix (Fermentas), 0.4 μM of each primer and 1 μl DNA/3 μl cDNA template. Cycling conditions were as follows: An initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The primer-pairs PhaeoF-489/PhaeoR-683 (Nejstgaard et al. 2008) and Euk528F (Elwood et al. 1985)/Micro04R (Zhu et al. 2005) amplify 209 and 134 bp regions of the nuclear 18S small subunit rDNA of *Phaeocystis* sp and *Micromonas pusilla*, respectively. Both primer sets were originally designed to amplify their target species from environmental samples, and their specificities have been tested (Zhu et al. 2005; Nejstgaard et al. 2008). For all RNA preparations, PCR reactions were also run using 1 μl DNase-treated RNA as a template, to make sure amplification products were not due to residual DNA. No PCR products were generated from these controls, or from mock PCR reactions (containing water instead of DNA/cDNA template). PCR products were visualized on a 1.5 % 1 \times TAE agarose gel. The identities of the amplified products were determined by Sanger sequencing of eight random PCR products produced with each primer pair. The PCR products were cleaned using the EZNA CyclePure Kit (Omega Bio-Tek, USA) prior to bidirectional sequencing at the CEES DNA lab, University of Oslo, on a ABI 3730 DNA analyzer (Applied Biosystems, USA). The resulting sequences were manually inspected and trimmed for primer sequences before performing a megablast search (Altschul et al. 1990) against the NCBI-NT database (updated 22.03.2013) using default parameters. In all cases, the top hits of the amplified sequences were to *M. pusilla* or *Phaeocystis* sp, thus confirming the specificity of the PCR reactions (data not shown). While the amplified *Phaeocystis* rDNA was different from *P. globosa* (3–4 nucleotide differences within the amplified segment), it is not possible to distinguish *P. pouchetii* from *P. antarctica* based on the amplified nucleotide sequence (identical sequences). However, only *P. pouchetii* is expected to be found in the Arctic (Schoemann et al. 2005).

Results and discussion

Hydrography

Samples were collected from a wide range of oceanographic environments and corresponding water masses

(Fig. 1; Table 1). At Sofiadjupet very cold and less saline Arctic Water (-1.9 °C, 34.0 psu at 5–35 m depth) was overlying warmer more saline water of Atlantic origin (2.5 °C, 34.9 psu at 150 m depth) from the West Spitsbergen Current (WSC). At the bottom, colder water was present (-0.8 °C), most likely representing Eurasian Basin Deep Water. At the Fram Strait station water of Atlantic origin was at the surface (3.1–2.9 °C, 35.1 psu at 15–150 m depth) overlying colder and relatively saline water (-0.8 °C, 34.9 psu at 1,000 m depth). The mesopelagic water probably represents Arctic Intermediate Water that originates from the Greenland Sea and is commonly found below the WSC (Saloranta and Svendsen 2001). Various intermediate water types typical of coastal environments that are influenced by local fresh-water additions from rivers and glaciers were identified in the sampled fjords on the western and northern coasts of Svalbard. The conditions at the ISA station varied greatly between the different sampling dates. On November 29, 2012, relatively warm and salty water was found, especially at the bottom (2.6 °C, 34.6 psu). This was due to Atlantic water from the WSC that flooded into Isfjorden at several occasions during 2012 (our own unpublished data). Some of the samples were taken from ice-covered waters (BAB 03.12.2008 and 14.01.2009, Tempelfjorden and Sofiadjupet). These sampling dates were characterized by cold “surface” water (-1.9 to -0.8 °C at 5–15 m depth).

Chlorophyll *a*

The levels of chlorophyll *a* were consistently low but detectable, with total Chl *a* values in the 0.01–0.05 $\mu\text{g L}^{-1}$ range (acid-corrected Chl *a* values, Table 1, see Supplementary Table 1 for uncorrected Chl *a* values). Small cells were the major photosynthetic component, contributing 50–100 % of total Chl *a* biomass in nearly all samples. One exception was the 60-m sample from the ISA station in November 2012, where total chlorophyll *a* levels were 0.1 $\mu\text{g L}^{-1}$ and the large cells' contribution was 65 %. This sample probably represents a recent influx of Atlantic water from the WSC.

The fact that chlorophyll *a* levels remained detectable suggests that at least some of the phototrophs retained their pigments throughout the winter. Although the contribution of the small cells was substantial (35–100 %), the numbers cannot be attributed to specific species (e.g., the species under study). It has previously been reported that *Micromonas* retained its pigments throughout the winter in Franklin Bay and was able to start exponential growth as soon as enough light was available in February (Lovejoy et al. 2007; Terrado et al. 2008).

Table 1 Locations, physiochemical parameters, chlorophyll *a* concentrations (acid corrected), type of samples used for PCR screening and results

Date	Station ^a	Latitude	Longitude	Depth (m)	T (°C)	Salinity (psu)	Chl <i>a</i>		Sample type ^d	Presence	
							Total (µg L ⁻¹)	% small ^b		% large ^c	<i>M. pusilla</i>
03.12.2008	BAB-ICE	78.6600	16.6900	15	-0.8	34.2	ND	ND	DNA	Y	Y
14.01.2009	BAB-ICE	78.6600	16.6900	15	-1.7	34.2	0.016 ± 0.000	ND	DNA	Y	Y
				150	-1.5	34.6	ND	ND	DNA	Y	(Y)
22.01.2009	Tempelfjorden-ICE	78.3810	16.9324	15	-1.4	34.5	0.000	ND	DNA	Y	Y
26.11.2010	Deltaneset	78.3467	15.6961	5	ND	ND	0.042 ± 0.002	71	DNA/RNA	Y/Y	Y/Y
				15	ND	ND	0.038 ± 0.000	68	DNA/RNA	Y/Y	Y/Y
				35	ND	ND	0.042 ± 0.004	74	DNA/RNA	Y/Y	Y/Y
				75	ND	ND	0.037 ± 0.003	62	DNA/RNA	Y/Y	Y/Y
				150	ND	ND	0.034 ± 0.005	71	DNA/RNA	Y/Y	Y/Y
13.12.2011	BAB	78.6600	16.6900	5	-0.1	34.1	0.018 ± 0.002	89	DNA	Y	Y
				15	-0.1	34.1	0.016 ± 0.005	81	DNA	Y	Y
				35	-0.1	34.1	0.020 ± 0.001	80	DNA	Y	Y
				60	0.9	34.3	0.019 ± 0.000	16	DNA	Y	Y
				75	0.2	34.5	0.014 ± 0.001	79	DNA	Y	Y
				150	-1.6	34.6	0.006 ± 0.001	83	DNA	Y	(Y)
14.12.2011	ISA	78.2610	15.5350	5	0.9	34.3	0.026 ± 0.003	88	DNA	Y	Y
				15	0.9	34.3	0.024 ± 0.000	79	DNA	Y	Y
				25	0.9	34.3	0.024 ± 0.004	88	DNA/RNA	Y/Y	Y/Y
				60	1.3	34.4	0.025 ± 0.001	80	DNA	Y	Y
12.01.2012	R3	80.3036	22.2542	5	-1.1	34.2	0.014 ± 0.003	100	DNA	Y	Y
				15	-1.0	34.2	0.013 ± 0.001	100	DNA/RNA	Y/Y	Y/Y
				150	1.4	34.6	0.016 ± 0.004	81	DNA	Y	Y
				240	0.6	34.6	0.015 ± 0.001	73	DNA/RNA	Y/Y	(Y)/Y
15.01.2012	Sofiadjupet-ICE	81.6861	14.2881	5	-1.9	34.0	0.034 ± 0.005	75	DNA	Y	Y
				15	-1.9	34.0	0.038 ± 0.000	68	DNA/RNA	Y/Y	Y/Y
				35	-1.9	34.0	0.028 ± 0.001	100	DNA	Y	Y
				60	-0.1	34.4	0.037 ± 0.005	59	DNA	Y	Y
				150	2.5	34.9	0.022 ± 0.001	95	DNA	Y	Y
				500	1.6	35.0	ND	ND	DNA	Y	Y
				2,290	-0.8	34.9	ND	ND	DNA	(Y)	N
17.01.2012	ISA	78.2610	15.5350	5	-1.3	34.2	0.035 ± 0.001	83	DNA	Y	Y
				15	-1.3	34.2	0.038 ± 0.001	79	DNA	Y	Y
				25	-1.3	34.2	0.040 ± 0.001	75	DNA/RNA	Y/Y	Y/Y
				60	-1.1	34.2	0.041 ± 0.001	71	DNA	Y	Y

Table 1 continued

Date	Station ^a	Latitude	Longitude	Depth (m)	T (°C)	Salinity (psu)	Chl <i>a</i>		Sample type ^d		Presence	
							Total ($\mu\text{g L}^{-1}$)	% large ^c	% small ^b	% large ^c	<i>M. pusilla</i>	<i>P. pouchetii</i>
28.01.2012	ISA	78.2610	15.5350	5	-0.8	34.2	0.028 ± 0.001	68	32	DNA	Y	Y
				15	-0.8	34.2	0.039 ± 0.004	54	46	DNA	Y	Y
				25	-0.8	34.2	0.036 ± 0.005	56	44	DNA/RNA	Y/Y	Y/Y
29.11.2012	ISA	78.2610	15.5350	60	-0.8	34.2	0.025 ± 0.003	64	36	DNA	Y	Y
				5	1.7	34.3	0.043 ± 0.006	79	21	DNA	Y	Y
				15	1.8	34.4	0.041 ± 0.001	80	20	DNA	Y	Y
14.01.2013	Fram strait	79.3407	7.2647	25	1.8	34.4	0.047 ± 0.002	81	19	DNA/RNA	Y/Y	Y/Y
				60	2.6	34.6	0.103 ± 0.008	35	65	DNA	Y	Y
				15	3.1	35.1	0.011 ± 0.001	64	36	DNA/RNA	Y/Y	Y/Y
				150	2.9	35.1	0.012 ± 0.004	67	33	DNA/RNA	Y/Y	Y/Y
				500	-0.2	34.9	0.004 ± 0.000	86	14	DNA/RNA	Y/Y	Y/Y
				1000	-0.8	34.9	0.004 ± 0.000	75	25	DNA/RNA	(Y)/Y	N/N

Y and N denote whether *Micromonas pusilla* and *Phaeocystis pouchetii* were detected (Yes) or not (No), (Y) indicates a weak PCR signal and ND indicates No Data. Latitude and longitude are indicated in decimal degrees (WGS84). Total Chl *a* values are given as mean and standard deviation of triplicate filters, except from Sofiadjupet which was only sampled in duplicate

^a ISA = Isfjorden Adventfjorden, BAB = Billefjorden Adolfbukta, R3 = Rijpfjorden station3

^b Small is either <3 μm (2008–2010 samples) or <10 μm (2011–2013 samples)

^c Large is either >3 μm (2008–2010 samples) or >10 μm (2011–2013 samples)

^d DNA/RNA was isolated from the 3- to 0.22- μm (2008–2010 samples) or 10- to 0.45- μm (2011–2013 samples) size fraction

Micromonas and *Phaeocystis* are found “everywhere”

Both *Micromonas* and *Phaeocystis* rDNA was detected at every tested locality, demonstrating that both species were present at many depths, in different water masses, in ice-covered and ice-free locations, in open ocean as well as enclosed fjord systems and in different years (Table 1). While *Micromonas* rDNA was detected in all samples, *Phaeocystis* was not detected in the bathypelagic sample from Sofiadjupet (2,290 m) and the deep mesopelagic sample from the Fram Strait station (1,000 m). Weak signals [denoted as (Y) in Table 1] were observed when deep samples (below 150 m) were screened for *Phaeocystis*. Also *Micromonas* produced weak signals in the very deep samples from Sofiadjupet and the Fram Strait. It has been speculated that *Phaeocystis* may have a bottom stage and possibly overwinter in the sediment (Hegseth and Tverberg 2013), thus representing an inoculum for the spring bloom similar to what is known for diatoms (Smetacek 1985). A proposed zygote resting stage has recently been reported for *P. antarctica* (Gaebler-Swarz et al. 2010), although no such stages have so far been detected for *P. pouchetii*. In the current study, *Phaeocystis* cells were detected in water samples at all localities. These results suggest that the species overwinter in the pelagos, although at present a yearly cycle that includes a bottom stage cannot be excluded.

Viable cells exist in the deep

Extracellular DNA (excreted or from dead organisms) may be amplified by the sensitive PCR technique (Paul et al. 1990). To test whether the detected *Micromonas* and *Phaeocystis* 18S rDNA originated from living organisms, RNA was included in the screening. RNA is a much less stable molecule than DNA, thus it is unlikely to be detected once an organism is dead. In all cases where RNA was tested, the results of cDNA amplification were identical to those obtained from amplification of the corresponding DNA. This suggests that the PCR assay detected living cells.

In the present study, viable cells of *Micromonas* and *Phaeocystis* were detected in the mesopelagic zone down to 1,000 and 500 m depths, respectively. *Micromonas* DNA was also detected in a bathypelagic sample from Sofiadjupet (2,290 m), but since only DNA was tested from this depth we cannot rule out the possibility that this weak signal was due to the amplification of extracellular DNA (i.e., Paul et al. 1990). Finding viable photosynthetic organisms in the mesopelagos is surprising, and we are not aware of any reports on the presence of *Phaeocystis* at such depths. However, viable *Micromonas* cells have earlier been found at 500 m in the Bay of Biscay (Manton and

Parke 1960) and at 600 m in the Kuroshio area off the coast of Japan (Thronsen 1983). While the Arctic subclade of *Micromonas* is shade adapted (Lovejoy et al. 2007), these are depths where the irradiance is too low for photosynthesis to cover basic cellular metabolic requirements even at the height of summer. Thus, the presence of these phototrophs in the mesopelagos strongly suggests the existence of alternative life strategies: either the presence of resting stages or alternative trophic modes, such as phagotrophy. Indeed, *Micromonas* has been suggested to be mixotrophic (Gonzalez et al. 1993; Sanders and Gast 2011). Both species are too small to sink in their solitary life stage, and *Phaeocystis* colonies are reported to be buoyant (Skreslett 1988), although the contribution of *Phaeocystis* to the benthos remains a point of controversy (Wassmann 1994; DiTullio et al. 2000). Fast sinking of *P. pouchetii* colonies has been reported in the Barents Sea, where viable cells were prevalent in sediment traps at 100 m depth (Wassmann et al. 1990). In the current study, both *M. pusilla* and *P. pouchetii* RNA was detected in deep samples, showing that they were alive in the mesopelagos. Currently, we do not know whether the presence of *Micromonas* and *Phaeocystis* in the deep represents a dead end or an alternative ecological niche. The clear stratification of the water column at the two open ocean stations (Sofiadjupet and Fram Strait) makes it unlikely that the presence of the living cells in the mesopelagos was due to a recent mixing event.

Conclusion

The two key arctic phototrophs *M. pusilla* and *P. pouchetii* that may dominate the entire photosynthetic community during spring and summer (Thronsen and Kristiansen 1991; Booth and Horner 1997; Not et al. 2005; Schoemann et al. 2005; Hegseth and Tverberg 2013) were found to be widely distributed in the Svalbard waters also at the height of the polar night. This shows that these tiny phototrophs are able to survive long periods without light. Currently, it is not known which mechanisms are involved.

Further studies will include the quantification of *M. pusilla* and *P. pouchetii* cells through the polar night and into the spring. Keeping low but persistent stocks of viable cells through the winter may give these important phototrophs a competitive advantage allowing immediate response when conditions become favorable for growth in the spring.

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