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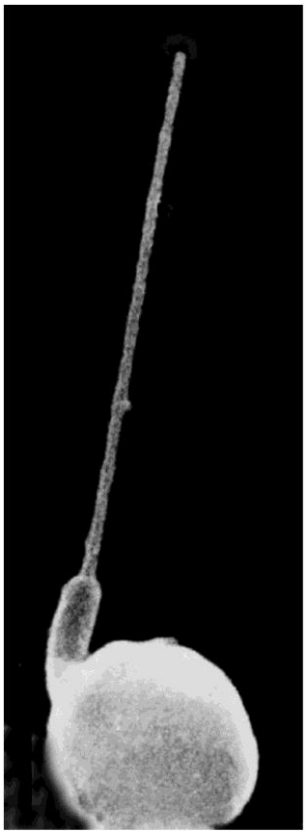
Temporal variation of arctic marine picophytoplankton focusing on *Micromonas pusilla* (Mamiellophyceae)



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Master's thesis

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Front page photos: *Micromonas pusilla* as seen in electron microscopy, picture provided by Wenche Eikrem and 17th of May celebration during fieldwork on the sea ice in Billefjorden 2011, taken by Lene Christensen.

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Abstract

Arctic ecosystems are strongly seasonal by nature, and photosynthetic organisms in particular so. In this study, the seasonality of one of the most commonly found marine photosynthetic picoeukaryotes, *Micromonas pusilla*, was investigated in Billefjorden, Svalbard by real-time quantitative polymerase chain reaction (qPCR). In addition, the relative abundance of pico- vs. larger-sized phytoplankton was studied throughout the spring and summer of 2011. The arctic sill fjord Billefjorden on the western coast of Spitsbergen is annually covered by fast ice and is dominated by cold, locally produced water throughout the year. Seawater samples from five depths was collected monthly from April to August, and the abundance of *M.pusilla* (via qPCR), the fractionated chl *a* biomass (cells < 3 µm vs. larger cells), and the amount of nutrients were analysed. In addition, CTD profiles and phytoplankton net hauls (20 µm mesh size) were collected at every sampling date. The Billefjorden pelagic system was in 2011 characterized by advection of warmer sea water from outside the sill in early May, and a *Phaeosystis*-dominated bloom that peaked in mid-May. The phytoplanktonic community had a high abundance of cells >3 µm during the peak of the spring bloom, and a picoplanktonic dominance of the chl *a* biomass in post bloom and late summer. *Micromonas pusilla* was found to be abundant below the chl *a* maximum in the post bloom situation in June and July, with cell numbers exceeding 6.5 x 10^5 cells/mL, but unidentified picoplankton dominated the phytoplanktonic community at the chl *a* maximum depth in June and August. During the *Phaeocystis*-dominated bloom in mid-May, the *M. pusilla* cell numbers were considerably lower and the species contributed minimally to the phytoplankton biomass.

Introduction

Arctic waters are characterized by annual and relatively short peaks in phytoplankton biomass during the spring (Sakshaug et al. 2009; Wassmann et al. 2006). In ice-covered waters, this spring bloom of the pelagic system is often preceeded by a sympagic bloom (Hegseth 1998). Diatoms (Bacillariophyceae) generally dominate both the sympagic and the pelagic arctic spring blooms (Sakshaug et al. 2009; Wassmann et al. 2006), although the haptophyte *Phaeocystis pouchetii* can form nearly monospecies blooms in pelagic waters (Schoemann et al. 2005). Outside the spring bloom period, arctic phytoplanktonic communities are often dominated by picoeukaryotes (cell size <2-3 µm) (Lovejoy et al. 2007; Sørensen et al. 2012). These small cells are important components of marine ecosystems worldwide and may contribute significantly to the total planktonic biomass (Stockner 1988; Worden et al. 2004; Zubkov et al. 1998). Their importance is further amplified in the arctic where picoprasinophytes seem to replace the key cyanobacterial primary producers *Synecococcus* and *Prochlorococcus* from temperate waters as the main picoplanktonic primary producers (Li 1998; Not et al. 2005; Vincent 2002). In a study carried out in the

Arctic Ocean during summer, picoeukaryotes were found to contribute 36% of total autotrophic biomass (Booth and Horner 1997). There is, however, a significant temporal variation in the relative contribution of picoplankton to autotrophic biomass, and small cells seem to be more important when chlorophyll *a* concentrations are low, thus outside the main bloom season (Brewin et al. 2010; Hodal and Kristiansen 2008). With millions of picoeukaryote cells per liter seawater and a doubling time of hours to days there is an extreme capacity of turnover of abundance patterns among the picoplankton community to be expected (Nolte et al. 2010). This trend may be even more pronounced in the Arctic where seasonal variation is especially great. Studies have shown that predicted climate conditions like less mixing, increased stratification and thus reduced nutrient concentrations in the surface layer may favor picoplankton growth compared to the growth of larger cells (Li et al. 2009; Worden et al. 2009). Picoeukaryote dynamics may thus be useful ecosystem indicators in arctic waters (Li et al. 2009; Worden et al. 2009).

The picoprasinophyte *Micromonas pusilla* was the most frequently recorded microbial autotrophic species in a recent study of environmental clone libraries from Isfjorden in 2009 (Sørensen et al. 2012). This morphologically uniform species has been discovered to comprise a cryptic species complex (Guillou et al. 2004). Phylogenetic analyses have also shown that while *M. pusilla* represents a single morphological species, this cryptic complex contains three (Guillou et al. 2004) or five phylogenetically distinct clades (Slapeta et al. 2006). The different clades can at many locations be found to co-exist year-round, but studies point to a niche partitioning between different clades within *M. pusilla*, indicating that it may represent different ecological species (Foulon et al. 2008). The finding of a unique pan-arctic, psychrophilic ecotype of *M. pusilla* supports this view (Lovejoy et al. 2007). The arctic *M. pusilla* differs from all other *M. pusilla* isolates, genetically and in terms of growth characteristics, as it is shade adapted and restricted to cold waters with an inability to grow at temperatures as high as 15° C (Lovejoy et al. 2007). Several studies have found the arctic ecotype of *M. pusilla* to be the only *Micromonas* sp. present in the arctic, and it may thus represent a true arctic species (Lovejoy et al. 2007; Sørensen et al. 2012). *Micromonas pusilla* has been detected in various habitats in the waters off Spitsbergen (e.g. fjords, open ocean) and throughout most of the year (Not et al. 2005; Sørensen et al. 2012). It has also been suggested to play a key role in the Arctic where it can dominate picoplanktonic communities for parts of the year (Not et al. 2005; Throndsen and Kristiansen 1991).

Due to their small size, morphological features as seen in light microscopy are usually not sufficient to distinguish between groups or species of picoplankton. Most picoeukaryote species can be distinguished by the use of electron microscopy, but this is costly and time consuming. Therefore recent advances in molecular biology hold great promise for the study of environmental samples of picoeukaryotes. Molecular methods are fast, relatively cheap and since species identification is based on sequence information they are often considered

more accurate than traditional microscopy methods. Real-time quantitative polymerase chain reaction (qPCR) involve amplification of a gene (in this case part of 18s nrDNA) by primers specific to a taxonomic unit and monitoring the formation of a product in real-time by fluorescence (Galluzzi and Penna 2010). Several studies have found that detection and quantification of target phytoplankton species in environmental samples by the use of qPCR are both fast and accurate compared to standard microscopy counting and FISH (Fitzpatrick et al. 2010; Galluzzi et al. 2004; Kamikawa et al. 2005; Tobe et al. 2010; Touzet et al. 2009; Zhu et al. 2005).

The relative abundance of *P. pouchetii* and diatoms in the blooms of northern waters show high inter-annual variability (Eriksen 2010), as well as a trend of succession with *P. pouchetii* dominating later in the bloom (Hodal et al. 2012; Rousseau et al. 2000; Wassmann et al. 1999). Zooplankton time their life cycle so that nauplii are released into the water-column in time to graze on the rich food source available during the spring bloom (Søreide et al. 2010). The timing of the bloom is therefore crucial for the whole arctic food-web, as an uncoupling between the phytoplankton bloom and the zooplankton will lead to an increase in phytoplankton sinking out, and cascading effects to higher trophic levels (Søreide et al. 2010). The nature of the bloom also affect the success of the zooplankton and thereby the amount of energy transferred to higher trophic levels, as not all species are ingested at the same rate, and different species may have different nutritional values (Brown et al. 1997). *Phaeocystis pouchetii* form gelatinous colonies when at high abundances, and the increased size combined with the tough outer skin of the colonies have been suggested to work as a defense against grazing (Hamm 2000). This is supported by studies documenting inefficient grazing by small copepods on *Phaeocystis* colonies, larger copepods (mainly *Calanus spp*.) show no such trend (Schoemann et al. 2005). However, selective grazing of diatoms in preference to *Phaeocystis* has been documented for krill, and particle size differences alone was not enough to explain the results given by the study (Haberman et al. 2003). Thus, the selective grazing identified in krill may be based on e.g. the nutritional value of diatoms vs. *Phaeocystis* or on toxins released by *Phaeocystis* (Haberman et al. 2003). Since the nature of the arctic spring bloom affects the whole marine ecosystem, understanding the arctic spring bloom development and its dominating species is important.

The development of the phytoplankton spring bloom in the high-Arctic sill fjord Billefjorden was studied in 2011 by genetic tools, fractionated chlorophyll *a* biomass, phytoplankton net hauls and nutrient analyses. The temporal variability identified was related to abiotic factors and compared to earlier studies from Billefjorden(Kuckero 2009; Sørensen et al. 2012). The main focus of this study was on the relative importance of picoplankton vs. larger phytoplankton to the autotrophic biomass throughout the spring bloom and post bloom period, and in particular on the temporal variability in the abundance of the picoprasinophyte *M. pusilla* in this high-Arctic fjord. The quantitative PCR technique was established at UNIS during the project period according to the description of Zhu et al. (2005), and utilized to quantify the abundance of *M. pusilla* in the time period from April to

August 2011. Several investigations have aimed at describing the molecular diversity of picoeukaryotes in the Arctic (Lovejoy et al. 2006; Luo et al. 2009; Piquet et al. 2010; Sørensen et al. 2012). This study is to my knowledge the first seeking to quantify the temporal variation of a picoplanktonic species in arctic waters.

Materials and Methods

Study site and sampling

The sampling location chosen for this project was Adolfbukta in the inner part of Billefjorden (78°39 N, 16°44 E) of the Isfjorden system, on the western coast of Spitsbergen (Fig. 1). Two sills restrict the inflow of warm and saline Atlantic-influenced water often dominating in the outer part of the Isfjorden system (Nilsen et al. 2008) into Billefjorden below c. 50 m. Thus, Billefjorden is generally dominated by locally produced and winter cooled water, and a fast-ice cover forms annually in the inner parts (Nilsen et al. 2008). Because of the limited influence from the outer part of Isfjorden, Billefjorden is usually considered particularly useful for temporal studies of arctic marine communities (Arnkværn et al. 2005).

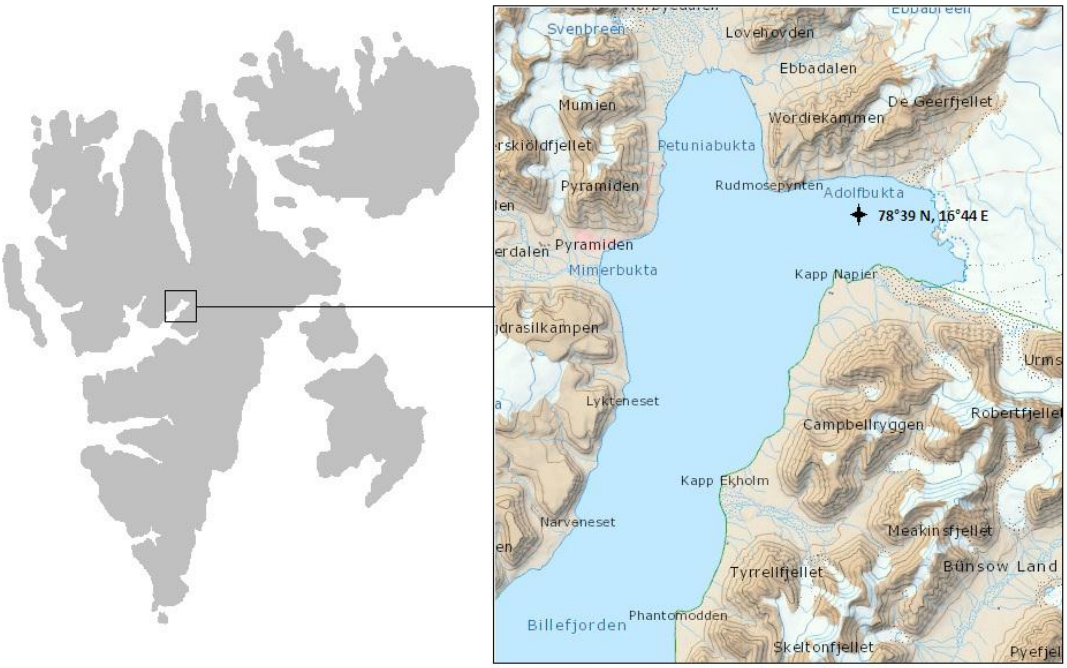

Figure 1. The study site in Billefjorden, in the innermost part of the Isfjorden system. The sampling location is marked by a star.

A mooring was deployed in Billefjorden as a collaboration between UNIS and SAMS (Scottish Association for Marine Sciences) in the time period October 2010 to August 2011. Temperature, salinity, photosynthetically active radiation (PAR) and fluorescence was measured at different depths every 10 minutes throughout the period, and these data were made available to the present study (Jørgen Berge unpublished data).

Seawater samples were collected once or twice a month from April to August 2011 using a 10 L Niskin bottle and a 10 L water sampler (both from KC Denmark, Denmark). Water was collected from 5 m, 15 m, 35 m, 75 m and 150 m depth and kept in an insulated box in the dark until further processing. CTD profiles were taken at every sampling date from the surface down to the fjord bottom with a STD/CTD model SD204 (SAIV A/S, Norway). Ice cores were sampled in April and May at an undisturbed site on the ice by a Kovacs ice corer either with a 9 cm or a 15 cm barrel. Ice thickness, snow depth and freeboard were measured for each ice core. The bottom 0-3 cm segment of the ice core was sawed off into a container and 90-400 mL sterile filtered seawater added to avoid osmotic shock to the organisms in the core while thawing. The cores were kept in the dark and melted slowly.

Meteorological data

Data on the sea ice cover in Billefjorden was collected from satellite images (NASA 2012), ice charts (PolarView 2012) and meteorological data was obtained from the observatory at Longyearbyen airport (Meterologisk-institutt 2012).

Nutrients

100 mL seawater was sampled from 5 m, 15 m, 35 m, 75 m and 150 m depth at each of the sampling dates in acid washed plastic bottles and stored at -20 °C in the dark until processing. Chemical analysis of nitrate and nitrite (NO_3/NO_2), phosphate (PO_4) and silicic acid ($Si(OH)_4$) was done at the University of Tromsø using a Flow Solution IV analyzer (O.I. Analytical, college station, USA). The Analyzer was calibrated with reference seawater from Ocean Scientific International Ltd (England). Each sample was divided in three subsamples that were measured individually.

Phytoplankton net haul

Phytoplankton samples were collected from 35 m and up using a 20 µm phytoplankton net (KC Denmark, Denmark). The samples were preserved in 1-2 % hexamine-buffered

formaldehyde dependent on the sample biomass, and stored at room temperature in the dark until processing. Four mL of each sample was investigated under Leica inverted microscope at 200 times magnification by Miriam Marquardt (Marquardt et al. unpublished data).

Chlorophyll *a* biomass

Seawater sampled for chl *a* biomass estimation was collected in 10 L plastic bottles that were rinsed in distilled water between each sampling. The samples were processed within 36 hours. Filtrations were done in 3 replicates for each depth and type of filter. Between 0.5 and 1 L of seawater was run through a 3 µm isopore membrane polycarbonate filter (Millipore, USA) for non picoplanktonic autotrophic biomass, and the same volume was filtered through a glass microfiber GF/F filter (0.7 µm; Whatman, England) for total autotrophic biomass by a KNF lab vacuum pump (KNF lab, Germany). The filters were packed individually in aluminum foil, snap frozen in liquid nitrogen and stored at -80 °C until processing. The filtration units were cleaned in distilled water between each filtering. Chl *a* extractions were done by adding 10 mL 100% methanol to the filters and incubating them in the dark at +5 °C for 20-24 h. After 24 h the solution was mixed, 7-8 mL was filtered through a 0.22 µm cellulose acetate filter (Whatman, England) into a 13 mm round cuvette and measured immediately in a 10-AU-005-CE Flourometer (Turner designs, USA). The same filter was used for the three replicates from the same depth and type of filter. Syringes and cuvettes were cleaned in 100% methanol between the samples. Samples with an expected high chl *a* concentrations were diluted 1:10 with 100% methanol before measuring. The readout from the flourometer was used to calculate the chl *a* concentration in the samples (µg/L) according to the following formula:

$$read\ out\ fluorometer\ \left[\frac{\mu g}{L}\right] \times \left(\frac{(\ volume\ of\ methanol\ [mL])}{(\text{dilution filtered volume of sample}[mL])}\right) \times \text{factor}$$

The factor was only used for diluted samples.

DNA extractions

Seawater sampled for DNA extractions were prefiltered through a 38 µm nylon net (KC Danmark, Denmark) and collected in 2 L and 4 L plastic bottles that were rinsed in distilled water between samplings. The samples were processed within 36 hours. Between 200 mL and 2 L of the sampled water were filtered using a Heidolph pumpdrive 5001 peristaltic pump at 50 rpm (Heidolph, Germany) or a KNF lab vacuum pump (KNF lab, Germany) through a 3 µm isopore membrane polycarbonate filter (Millipore, USA) to remove the

bigger size fraction and picoplankton was collected on a 0.22 µm durapore membrane filter (Millipore, USA). The 0.22 µm filters were cut in two, and all filters placed in 1 mL 2% CTAB solution, snap frozen in liquid nitrogen and then stored at -80 °C until extraction.

When extracted, half of the 0.22 µm Durapore filters were thawed, the rest were kept as reserves. DNA was extracted using the following CTAB protocol:

One % mercaptoethanol was added to the thawed filter tubes containing CTAB. Two hundred µm molecular biology grade zirconium beads (New Jersey, USA) was added and bead beating was performed twice with 1 min at 22 /s in a Retsch MM301 bead beating machine (Retch, Germany). The extracts were transferred to new eppendorf tubes, and 1 mL of 2% CTAB with 1% mercaptoethanol was added to the filters and the bed-beating step was repeated. The samples were put on the heating block at 65 °C for 30 min, vortexed each 15 min and left at -80 °C overnight. The next day the samples were put back at the heating block at 65 °C for 45 min, and vortexed every 15 min. Extracts from the second Bead-beating step were transferred to new eppendorf tubes. Five hundred µL chloroform mix (96:4 chloroform: isoamylalcohol) was added and the samples vortexed twice and shaken regularly for 10 min, then centrifuged at 12 000 rpm for 5 min in an Eppendorf Centrifuge 5424 (Eppendorf AG, Germany). The aqueous phase was recovered and transferred to new tubes, 500 µL chloroform mix added and the procedure repeated. The water phase was transferred to new tubes and two third of the sample volume was added with 100% isopropanol holding -20 °C, flipped gently to mix and kept at -20 °C for a minimum of 30 min and a maximum of 12 h. The samples were centrifuged at 13 000 rpm for 10 min, the supernatant discarded and 1 mL of 70% ethanol added. The tubes were then flipped gently for mixing and centrifuged at 12 000 rpm for 2 min. The supernatant was discarded, 1 mL of 70% ethanol added, centrifuged at 12 000 rpm for 2 min and the supernatant discarded again. The tubes were placed upside-down on lab tissue and put a few min on the heating block at 65 °C to make residual ethanol evaporate. Fifty µL of 1x TE buffer was added directly to the bottom of the tubes, the tubes tapped to mix and stored at -20 °C until further processing.

To purify the DNA, parts of the protocol for purification of total DNA from plant tissue (Qiagen, Germany) was used. 1.5 volumes of buffer AP3/E was added to 20 µL of extracted DNA in a tube and mixed by pipetting. The mix was then added to DNeasy mini spin columns placed in 2mL collection tubes and spun in an Eppendorf Centrifuge 5417 R (Eppendorf AG, Germany) at 8000 rpm for 1 min. The flow through was discarded, 500 µL of buffer AW added and the tubes spun again at 8000 rpm for 1 min. The flow through was again discarded, 500 µL of buffer AW added and centrifuged at 14 000 rpm for 2 min to dry the membrane for ethanol. Again the flow through was discarded, and the mini column was moved to a new eppendorf tube. Twenty-two µL of buffer AE was pipetted directly onto the DNeasy membrane and incubated for 5 min at room temperature before it was centrifuged

for 1 min at 8000 rpm. The flow through was then added to the column again and spun through one more time for 1 min at 8000 rpm to get residual DNA in the membrane through.

DNA from a monoculture of *Micromonas pusilla* (UIO004) for construction of a plasmid standard, was extracted by spinning down the cultured cells in four 2 mL eppendorf tubes in recurrent rounds of 6 rcf for 5 min in an Eppendorf Centrifuge 5424 (Eppendorf AG, Germany). At the end of every centrifugation, the upper 1.5 mL was pipette out carefully, and new 1.5 mL added from the culture. When the whole culture was added, the 4 tubes were spun down at 6 rcf for 5 min and the upper 1.5 mL of water carefully removed. The rest was resuspended using a pipette and transferred to one tube which was spun one last time at 10 rcf for 5 min to collect a pellet. The water was poured off carefully and the DNA extracted from the cells by the CTAB extraction protocol as described. The cleaning step was not used for the culture extract.

All extracted samples were tested with a NanoDrop2000 spectrophotometer (Thermo scientific, USA) to quantify the amount of DNA in the sample and its purity.

Polymerase chain reaction

Polymerase chain reactions were carried out on an Eppendorf Mastercycler Ep Gradient S PCR cycler (Eppendorf AG, Germany). The PCR was run in a final volume of 25 µL and the mix used was as follows: 200 µM dNTP, 0.2 µM forward and reverse primer, 1x supplied buffer, 1 U Dream Taq DNA polymerase (Fermentas, Germany) and 2 µL template diluted 1:10 to avoid inhibitors possibly co-extracted with the DNA, -per reaction. For testing the results of the DNA extractions the following thermal cycling program was used: initial denaturation at 94 °C for 3 min, 30 cycles of (94 °C denaturation for 45 sec, 54 °C annealing for 60 sec, 72 °C elongation for 120 sec) and a final extension step at 72 °C for 10 min. To test primer specificity and optimality the following thermal cycling program was used: initial denaturation at 94 °C for 3 min, 30 cycles of (94 °C denaturation for 30 sec, 54-64 °C annealing for 30 sec, 72 °C elongation for 30 sec) and a final extension step at 72 °C for 10 min. The tests were run in several different PCR runs, and annealing temperature gradually increased to attain maximum specificity. When optimal conditions of the primers were found, different dilutions of the environmental samples were tested (1:5, 1:10, 1:20 and 1:100), 1:10 dilution gave good results for all samples and future analyzes were run at this concentration. The forward primer used is a universal eukaryote primer and species specificity was therefore attained by the reverse primer. Primers used in the different tests can be seen in Tables 1, 2. The PCR result was displayed on a 0.75 % agarose gel (for test of extracted DNA) or a 2 % agarose gel (for test of primer specificity and optimality) after a run at 90 V for 1 h.

Table 1. Primers used for PCR, QPCR and sequencing.

Name	Sequence (5' → 3')	Tm (°C)	Reference
Euk528f	CCGCGGTAATTCAGCTC	49	Elwood et al. (1985)
EukB	TGATCCTTCTGCAGGTTCACCTAC	64	Medlin et al. (1988)
Chlo02r	CTTCGAGCCCCCAACTTTC	58	Simon et al. (2000)
Micro04r	CGCGTCCTCTACAGGAAGTTG	54	Not et al. (2004)

Table 2. Primer sets used in this study.

Aim	Target group	Forward primer	Reverse primer
Test of DNA extraction	Eucaryotes	Euk528f	eukB
Amlification of culture for cloning	Chlorophyta	Euk528f	chlo02r
Running of qPCR samples	*Micromonas pusilla*	Euk528f	micro04r

QPCR assays

The SYBR Green dye was chosen to quantify the amount of product at the end of each PCR - cycle since it binds to double stranded DNA as it is formed. The number of cycles required to cross a certain fluorescence threshold relative to a known plasmid standard (Cycle threshold Ct) was then used to deduce the number of gene copies in the original sample. The initial runs with primer concentration 200 nM had a high frequency of amplification in the negative controls, a known problem with qPCR (Suzuki et al. 2000). The primer concentration in the reaction setup was thus reduced to 100 nM, which significantly reduced the frequency of amplification in the negative controls. When amplifications in the negative were still present, the Ct was always above 35 (which was selected as the cut-off).

In this project real time qPCR was carried out according to Zhu et al. (2005) with minor deviations. Reactions were run in a final volume of 20 µL using 1 X SYBR Green master mix (Applied Biosystems, USA), 0.1 µM of the forward primer Euk528f and the reverse primer Micro04r, 2 µL of template in 1:10 dilution and nuclease free water (Fermentas, Germany). The master mix, primers and H2O were mixed and added to a MicroAmp 48-well optical reaction plate (Applied Biosystems, USA) and 1:10 diluted template added directly to each well. The plate was sealed with MicroAmp 48-well optical adhesive film (Applied Biosystems, USA). All samples were run in triplicates in a StepOne real time PCR system from Applied Biosystems (USA), and some samples were run multiple times to check the reproducibility of the analysis.

The following thermal cycling program was used, an initial denaturation step at 95 °C for 3 min followed by forty cycles of denaturation at 95 °C for 15 sec, annealing-extension at 60 °C for 1 min and 25 sec of data collection at 77 °C. This was followed by a melt curve stage with 95 °C for 15 sec, 60 °C for 1 min and 95 °C for 15 sec with data collection at the change from 60 to 95 degrees and at 95 °C. All data were analyzed using StepOne software v2.1 (Applied Biosystems, USA). Cutoff was set at CT 35, and all samples with CT values above this limit considered negative.

Analysis of melt curves

In an initial test run a dissociation curve was run from 60 to 95 °C at the end of the program as described in the software manual. The resulting melting temperature (T_m) of the plasmid standard was 80 °C, the environmental sample 81 °C and the primer-dimers 65 °C. Since SYBR Green binds unspecific to all double stranded DNA, primer-dimers would have led to an overestimation of the sample; therefore the temperature of the detection step was set at 77 °C, above the melting temperature of the primer-dimers, but still well below the T_m of the specific qPCR product.

Cloning and preparation of plasmids for calibration

Linearized plasmids from cloned 18S nrDNA of *M. pusilla* was used as a standard for the qPCR analysis. Part of the 18S nrDNA was amplified from DNA extract of a monoculture of *M. pusilla* obtained from the culture collection at UIO (UIO004) using the primer set Euk528f + Chloro02r, and cloning was carried out following the sticky end protocol of the CloneJET PCR cloning kit (Fermentas, Germany). Transformation was carried out using TransformAid Bacterial Transformation Kit (Fermentas, Germany) together with JM107 cells (Fermentas, Germany) using ampicillin as selective media. The colonies of the cloned cells were grown overnight at 37 °C in 3 mL liquid LB-ampicillin medium and plasmids isolated by E.Z.N.A^{tm} Plasmid Miniprep Kit l (Omega Bio-Tek, USA) using the spin protocol. The isolated plasmids were sequenced at the NSC at the University of Oslo using Euk528f and Micro04r as sequencing primers. The clones were confirmed to be identical to the arctic strain of *M. pusilla* based on comparisons of their sequences to existing GenBank sequences. The plasmids were linearized by cutting with the restriction enzyme Hindlll (Fermentas, Canada) at 37 °C for 3 h followed by a thermal inactivation step at 80 °C for 20 min.

A NanoDrop2000 spectrophotometer (Thermo scientific, USA) was used to quantify the amount of DNA in the isolated plasmids and the plasmid copy number for the standard curve was calculated using the following formula:

$$\text{Molecules } \mu L^{-1} = (A \times 6.022 \times 10^{23}) / (660 \times B)$$

Where A is plasmid concentration (g/µL) as measured from the NanoDrop, 6.022×10^{23} is Avogadro's number, 660 is the average molecular weight for one base pair and B is the length of the plasmid including the cloned sequence.

Standard curves were constructed for each run of the qPCR by serial dilutions with 5 different plasmid concentrations in the range 2.9×10^{7} to 5.9×10^{3} copies/µL.

Calculations of cell numbers in the environmental samples from qPCR results

The standard curve was used to calculate the number of target DNA copies in the sample by the following formula:

$$N = [(A/B) \times d] \times (V/C)$$

Where N is the total number of cells in the initial sample, A the number of target DNA copies per PCR tube as calculated by the instrument software. B is the number of µL of cell lysate in the PCR tube and d the lysate dilution factor. V is the initial lysate volume expressed in µL and C is the target DNA copies per cell which was found to be 4.09±0.11 for *M. pusilla* by Zhu et al. (2005). The total number of cells in the initial sample was divided by the volume of seawater (mL) from which the DNA was extracted to get cells/mL in the environmental sample.

One positive qPCR product from each of the sampling dates was sequenced at the NCS at the University of Oslo using Euk528f as sequencing primer. For samples with Ct values below the cutoff, product from a regular PCR was used. The obtained sequences were aligned and checked manually before blasting (Altschul et al. 1990) to confirm the identity of the *M. pusilla* in the samples.

Results

Oceanographic measurements

Temperature

The temperature at 183 m stayed stable below -1° C throughout the year, while in the higher water masses it varied between -1.7° C and 7° C (Fig. 2). The temperature in the surface layers decreased steadily from October until December when it reached about -1.7° C. The temperature of the water masses then stayed stable throughout the winter months and was well mixed with temperatures below -1.5° C throughout the water column for both sampling dates in April (Fig. 3). At the start of May there was a sudden increase in the temperature of the upper 40 m of the water column, and the temperature kept rising until the start of august with the exception of one sudden decrease of about 4° C in July. As the summer preceded the heated surface layer reached deeper into the water column to about 40 meters in August, below 60 meters the temperature stayed below -1.5° C throughout the summer. At the start of August the temperature in the upper water layer reached a peak at 7° C, and then started to decline rapidly, but by mid-August a new period of warming occurred.

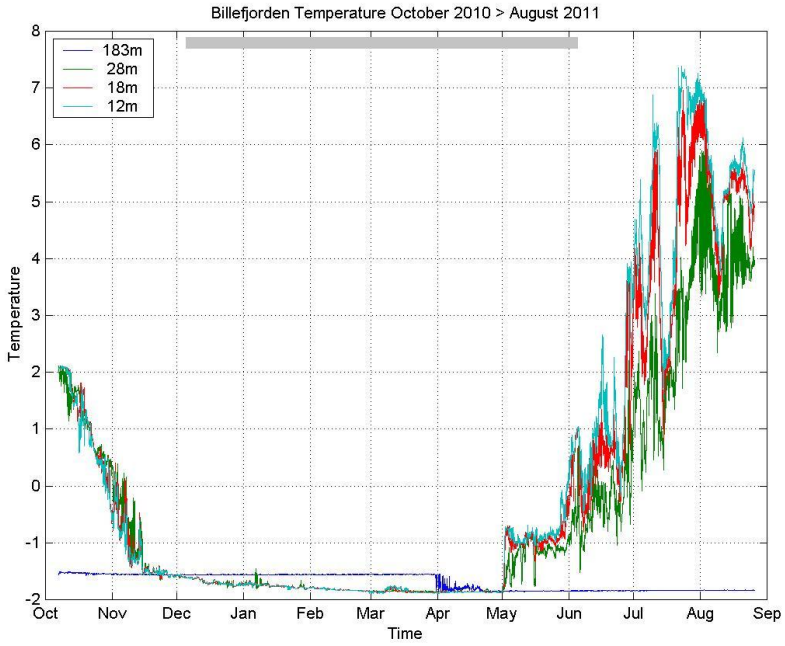

Figure 2. Temperature (°C) of the water column in Billefjorden from October 2010 to September 2011 detected by temperature loggers at 4 different depths in the moored observatory. The grey bar indicates the period with sea ice cover.

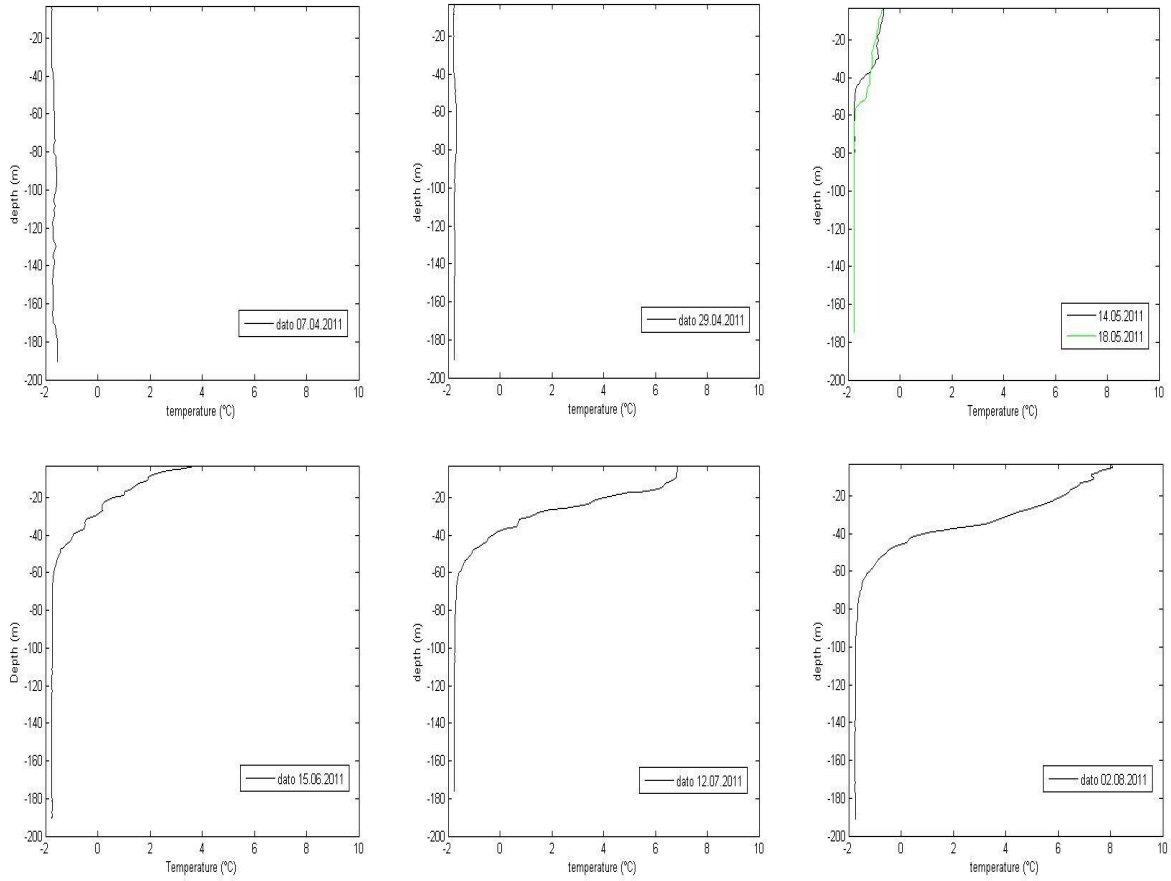

Figure 3. Temperature (°C) of the water column in Billefjorden recorded by vertical CTD profiles collected at the sampling dates.

Salinity

The salinity of the water masses at 183 m depth kept stable above 34.5 psu from October to mid-August where there was a small decrease (Figs 4, 5). In the surface layers the salinity ranged from 34.5 in the end of April to 30.5 in mid-August. In the higher water masses the salinity increased steadily from October to March. At the 7th of April the salinity was 34.5 psu throughout the water column, but at the 28th of April the surface layer had started to freshen and there was a steady increase in salinity with depth from 32.5 psu at 0 m to 34.5 psu at 60 m depth. In the beginning of May the salinity had started to decrease and a pycnocline had formed in the water masses. In mid-July there was a sudden decrease in salinity in the upper water column and the salinity continued to decrease until the start of August. Below 60 m depth the salinity kept at 34.5 psu until the start of August, when a decrease in salinity at 183 m depth coincided with an increase in salinity of the upper water masses.

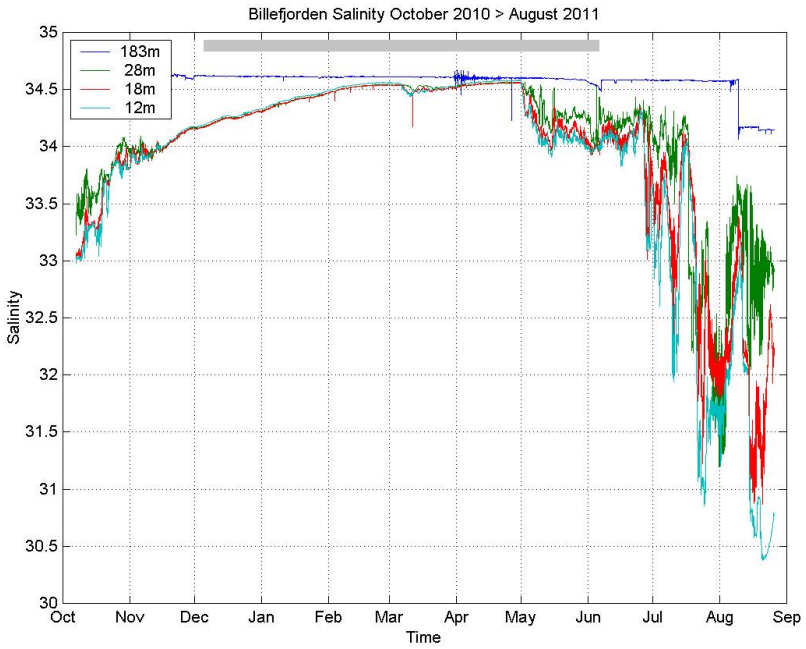

Figure 4. Salinity (psu) of the water column in Billefjorden measured from October 2010 to September 2011 detected by the moored observatory. The grey bar indicates the period with sea ice cover.

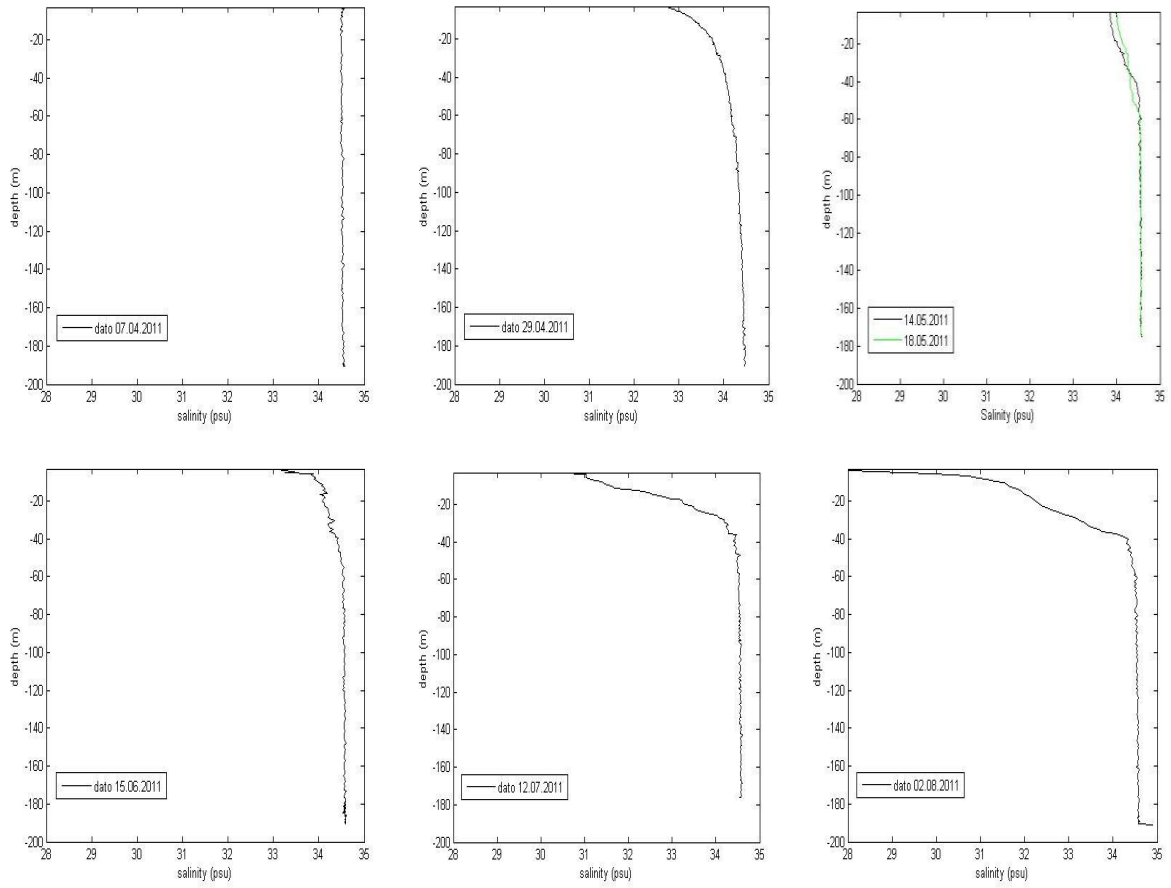

Figure 5. Salinity (psu) of the water column in Billefjorden measured by vertical CTD profiles collected at the sampling dates.

Fluorescence

The fluorescence measurements recorded by the mooring varied between 0 mV at 28 meters in March to over 3 mV in the start of May (Fig. 6). In October-February there was a record of periodically high measurements of fluorescence in the water column, but for both the April measurements based on the fluorometer of the veritical CTD profiler there was virtually no fluorescence detected (Fig. 7). In the beginning of May there were high levels of fluorescence at 14 m, followed by high fluorescence levels at 28 m depth a few days later (Fig. 7). CTD's taken at the 14th of May revealed a peak at 35m, whereas on May 18th there was a less clear peak but high fluorescence levels from 20-50 meter depth. During the summer there was little fluorescence at 28 m, but higher levels detected for short periods at 14 m depth.

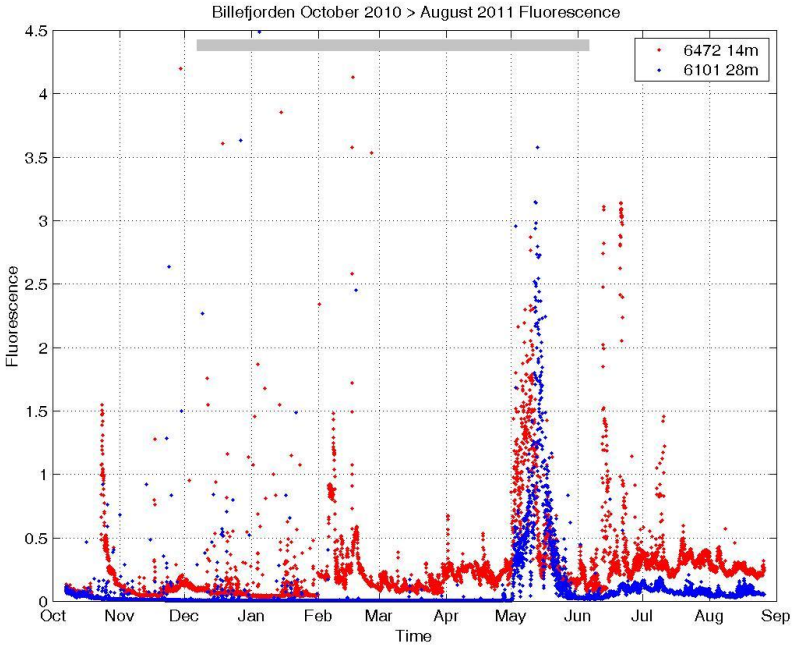

Figure 6. Fluorescence (measured in mV, mV x10 = µg/L) of the upper water column in Billefjorden from October 2010 to September 2011. The grey bar indicates the period with sea ice cover.

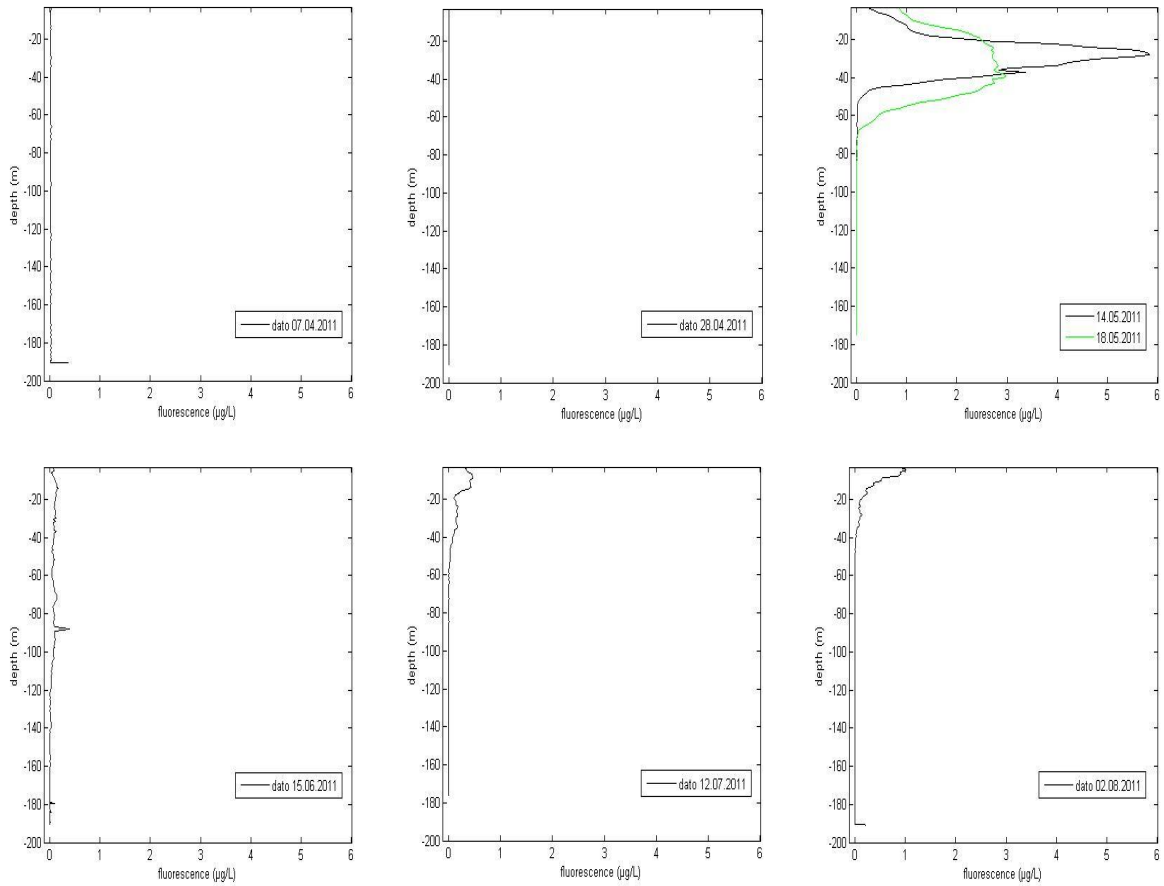

Figure 7. Fluorescence (µg/L) in the water column measured by the fluorometer of the CTD in Billefjorden at each sampling date.

Photosynthetically Active Radiation (PAR)

Light measurements ranged from no light measured during the winter darkness to the highest levels at the start of June (Fig. 8). From mid October the irradiation decreased until no light was detected at the start of November. The measurements then kept low until the end of February. During March the radiation increased, first slowly then more rapidly from the start of May reaching a peak in the photosynthetically active radiation (PAR) in the first half of June. From mid June the light measurements decreased steadily until the end of July, with a small increase in mid August.

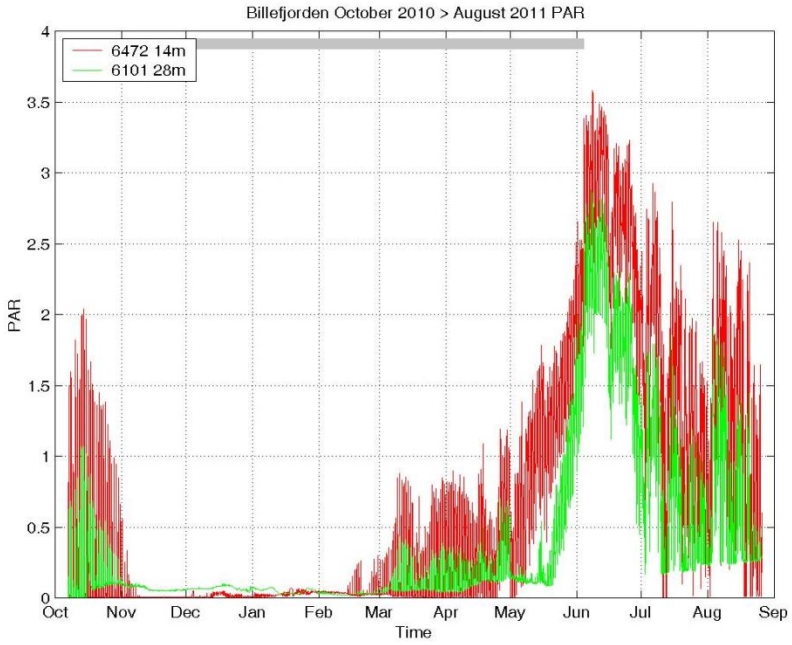

Figure 8. Photosynthetically active radiation (PAR) (µmol photon $m^{-2}s^{-1}$) trends of the upper water column (at 14 m and 28 m depth) in Billefjorden recorded from October 2010 to September 2011 by the moored observatory. The PAR values are relative numbers. The grey bar indicates the period of sea ice cover.

Sea ice measurements

Mean sea ice thickness increased from the start of April to mid May from 80.4 cm to 97.4 cm (Fig. 9). Snow cover increased slightly from the 5th to the 29th of April and then decreased to about half in mid May. In the start of May the ice cover in Billefjorden started to break up (NASA 2012), but the sampling site in Adolfbukta stayed covered by ice. The underside of the ice was, however, completely melted out. At the 15th of June the ice cover had broken up completely and was only left as thin ice floes.

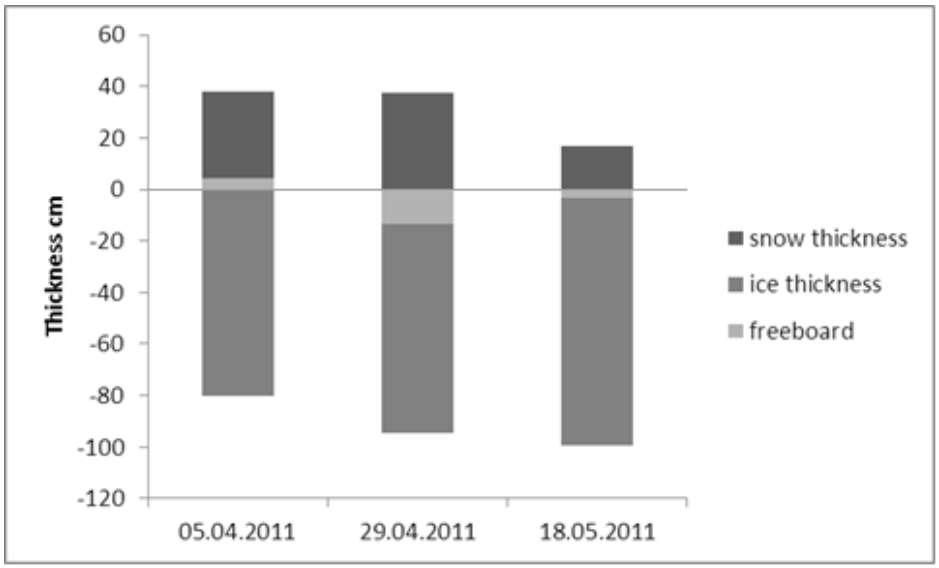

Figure 9. Sea ice thickness, freeboard (ice above water) and snow depth (given as means) at the Billefjorden sampling location in April and May.

Nutrients

Nitrate/nitrite (NO_3/NO_2) had concentrations above 6 mmol m^{-3} throughout the water column for both the April samplings (Fig. 10). At the 7th of April the lowest values were found at 5 m, and nitrate/nitrite increased with dept. At the 29th of April the nitrate/nitrite concentration had increased slightly in the upper 15 m, and decreased at 150 m depth. During the spring bloom in May nitrate/nitrite was depleted in the surface layers and stayed below 1 mmol m^{-3} down to 15 m throughout the summer. At 75 m the nitrate/nitrite concentrations were still above 7.5 mmol m^{-3} in May, but as the summer preceded concentrations declined throughout the water column to 4.5 mmol m^{-3} at the same depth in August. Phosphate (PO_4) concentrations at 5 m and 15 m decreased from 0.6 mmol m^{-3} in April to 0.1 mmol m^{-3} in May and then stayed below 0.2 mmol m^{-3} at these depths throughout the summer. At 35 m phosphate values were slightly higher, but showed the same trend as for the surface layers. Phosphate concentrations at 75 m and 150 m depth remained above 0.5 mmol m^{-3} throughout the sampling period with only a slight decrease in concentration in May and stable values throughout the summer. Silicic acid ($Si(OH)_4$) concentrations did not get depleted during this summer, but the concentration decreased from 4 mmol m^{-3} at 5 m depth in April to 2 mmol m^{-3} in May-June and 1 mmol m^{-3} in July and August. Below 35 meters the silicic acid concentration increased with depth down to 75 or 150 m but the maximum concentration in the water column decreased from 6.5 mmol m^{-3} at 150 m at the 7th of April to less than 3 mmol m^{-3} at 150 m depth in August.

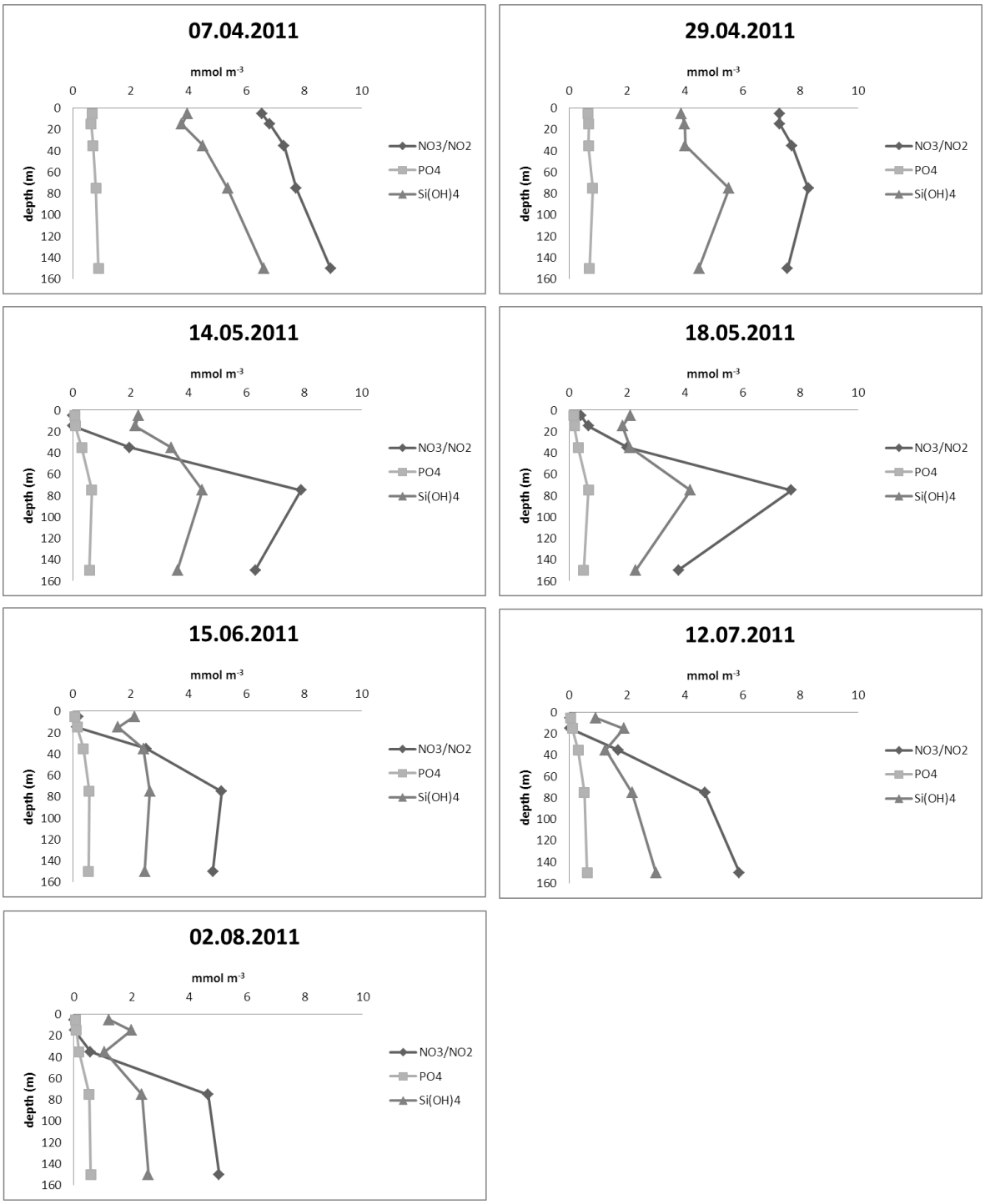

Figure 10. Nutrient concentrations (mmol m^{-3}) (given as means) at 5 m, 15 m, 35 m, 75 m and 150 m depth in Billefjorden from the beginning of April to August 2011.

Phytoplankton net haul

The phytoplankton bloom in Billefjorden in May 2011 was dominated by *Phaeocystis pouchetii* (Appendix 1 and personal observation). Several diatoms traditionally found in the arctic spring bloom were also recorded in the May samples, but in low numbers (Appendix 1). In June, the composition of the phytoplankton sample had few species of dinoflagellates, all in relatively low numbers. In July the phytoplankton sample also consisted of few species, and few individuals, the two most commonly identified groups being dinoflaggelates and tinttinids. In August the sample was dominated by cilliates, especially tintinnids.

Chlorophyll *a* biomass

Water samples

The photosynthetic biomass measured as the concentration of chl *a* in the water column increased from values below 0.2 µg/L in April to form a peak at the 14th of May with chl *a* values reaching 8 µg/L at 35 m depth under the sea ice (Fig. 11, The figures from April were not included here). The chl a biomass at the other sampling depths remained low, and cells in the >3 µm fraction made up 70% of the total photosynthetic biomass at this date (Fig. 12). The peak at 35 m was still pronounced at the 18th of May, though it was considerably lower with chl *a* concentration less than 5 µg/L. The chl *a* biomass at 15 m depth had increased slightly, while the other sampling depths remained low. Picoplanktonic cells now made up the bulk of the community accounting for almost 70% of the total chl *a* biomass. Chl *a* concentration remained above 2 µg/L at 5 m in June and August with a notable decrease to about 1 µg/L at 5 m depth in July. This decline was accompanied by a shift in the photosynthetic community from picoplanktonic dominance in June and August both with the picoplanktonic fraction making up 88 % of the total chl *a* biomass, to dominance by larger cells in July (76% of total chl a contributed by cells > 3 µm).

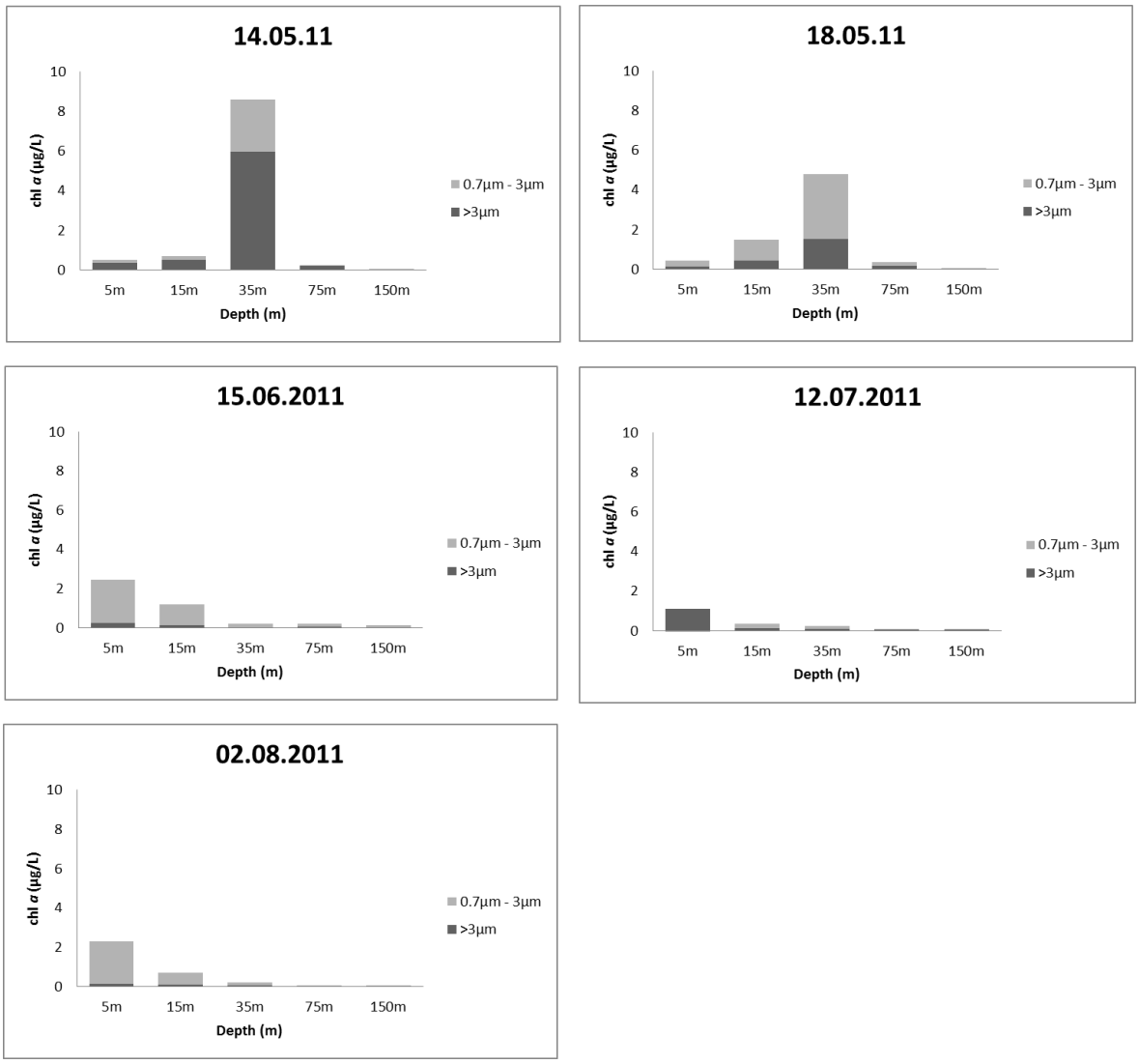

Figure 11. Chl *a* concentrations in µg/L (given as mean) at 5 m, 15 m, 35 m, 75 m and 150 m depth in Billefjorden from April to August 2011. The samples were fractionated into picoplankton (0.7-3 µm) and non- picoplankton (>3 µm).

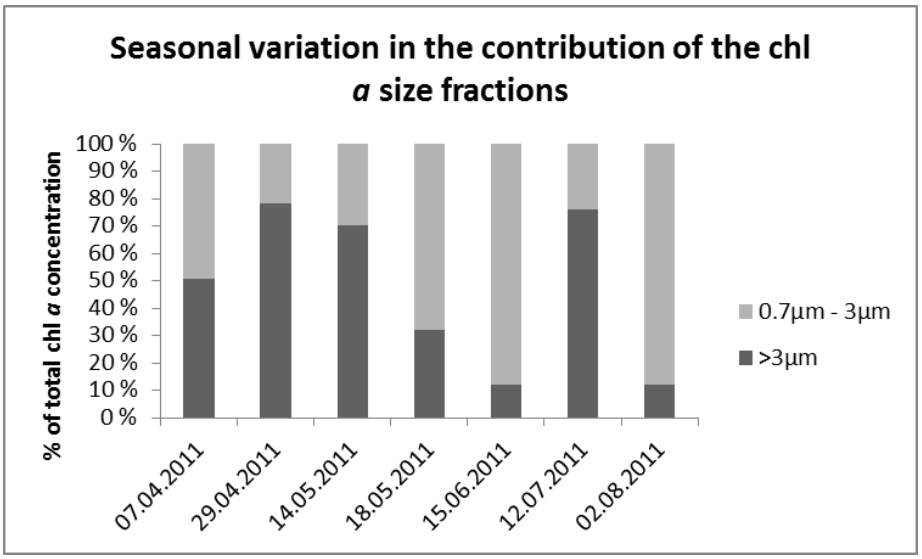

Figure 12. Percentage of the two cell size fractions of the total chl *a* biomass in the water column summed for all depths, at the different sampling dates in Billefjorden.

Ice cores

The chl *a* concentration in the ice cores increased from 0.01 µg/L at the 7th of April to 4 µg/L at the 15th of May (Fig. 13, 7th of April not included). There was a variation in size fraction dominance in the ice cores at the 15th of May. There was no ice algae bloom at any of the sampling dates.

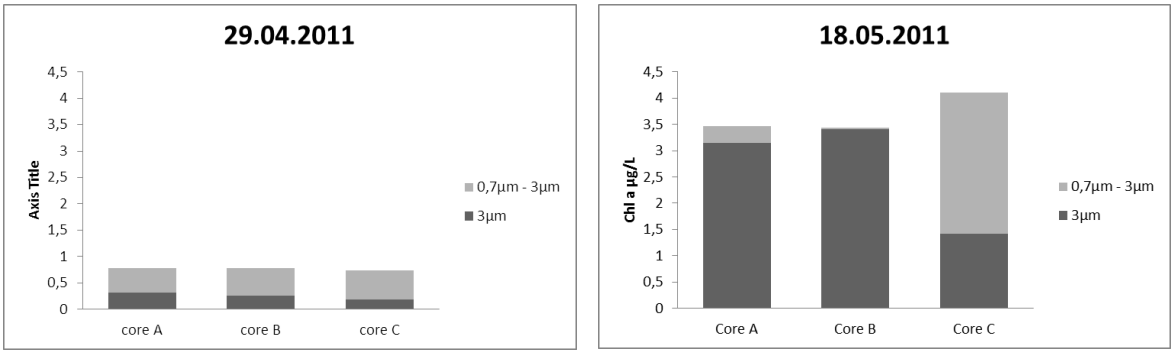

Figure 13. Chl *a* concentrations (µg/L) in the bottom 0-3 cm segment of the sea ice in Billefjorden in April and May. The concentrations were fractionated in picophytoplankton (0.7-3 µm) and non – picophytoplankton (>3 µm).

QPCR assays

Nine of the total ten qPCR and PCR products (one from each sample) were confirmed by sequencing to represent the arctic ecotype of *M. pusilla* (CCMP2099) as described in Lovejoy et al. (2007). The sequence profile to the sample collected at the 29th of April contained noise and could therefore not be identified.

Water samples

The cell counts for *M. pusilla* ranged from values too low to be quantified reliably by qPCR in April (not included here) to a peak in abundance with cell numbers exceeding 6.5 x 10^5 cells/mL at 15 m depth at the 15th of June (Fig. 15). The abundance of *M. pusilla* increased from April to May with 4 x 10^4 cells/mL at 5 m depth at the 14th of May, and cell numbers decreasing with increasing depth down to 35 m. At the 18th of May the *M. pusilla* cell numbers at 5 m had decreased to less than ¼ of the abundance detected at this depth 4 days earlier, there had also been a slight decrease in cell number at 15 m and an increased at 35 m depth. At the 15th of June there was a peak in *M. pusilla* abundance with cell numbers reaching 6.5 x 10^5 cells/mL at 15 m and exceeding 2.5 x 10^5 cells/mL at 5 m depth. The cell counts declined from June to July but were still high at the 12th of July with 1.2 x 10^5 cells/mL at 35 m, while the cell numbers had declined at 15 and 5 m depth. At the 2th of August the *M. pusilla* cell counts was in the range reported for May with 3 x 10^4 cells/mL at 35 m depth.

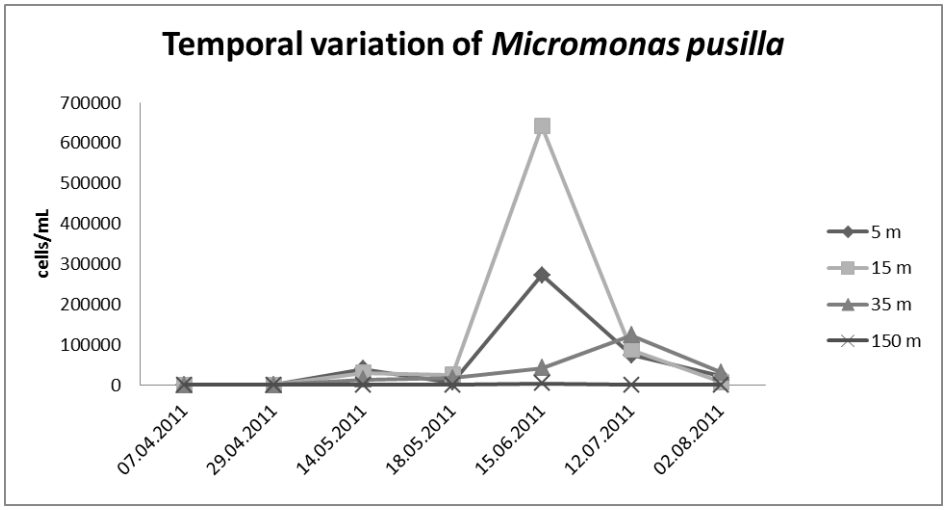

Figure 14. QPCR cell counts of *Micromonas pusilla* (cells/mL) in the environmental samples from 5 m, 15 m, 35 m and 150 m depth in Billefjorden from April to August 2011.

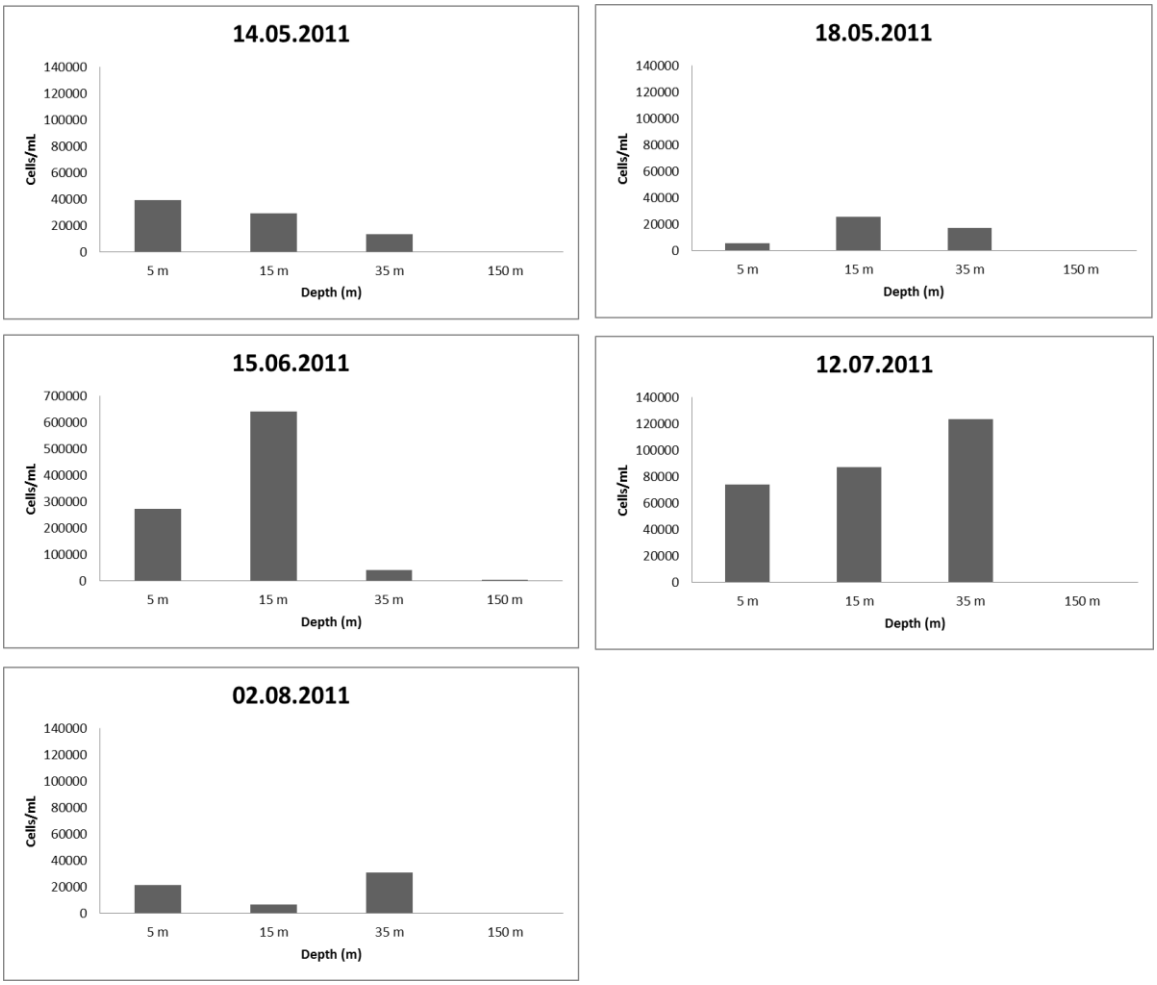

Figure 15. QPCR cell counts of *Micromonas pusilla* (cells/mL) in the environmental samples from 5 m, 15 m, 35 m and 150 m depth in Billefjorden from April to August 2011. Note the different y-axis scale for 15.06.2011.

Ice cores

The abundance of M. pusilla in only three of the ice core samples could be quantified reliably. These, however, showed a relatively high number of *M. pusilla* in the sea ice throughout the monitoring period with cell numbers ranging from 7 x 10^3 cells/mL in April to 1.15 x 10^4 cells/mL in May (Fig. 16). A possible trend of increasing cell numbers from April to May might be discerned, but the data are scarce and must be interpreted with precaution.

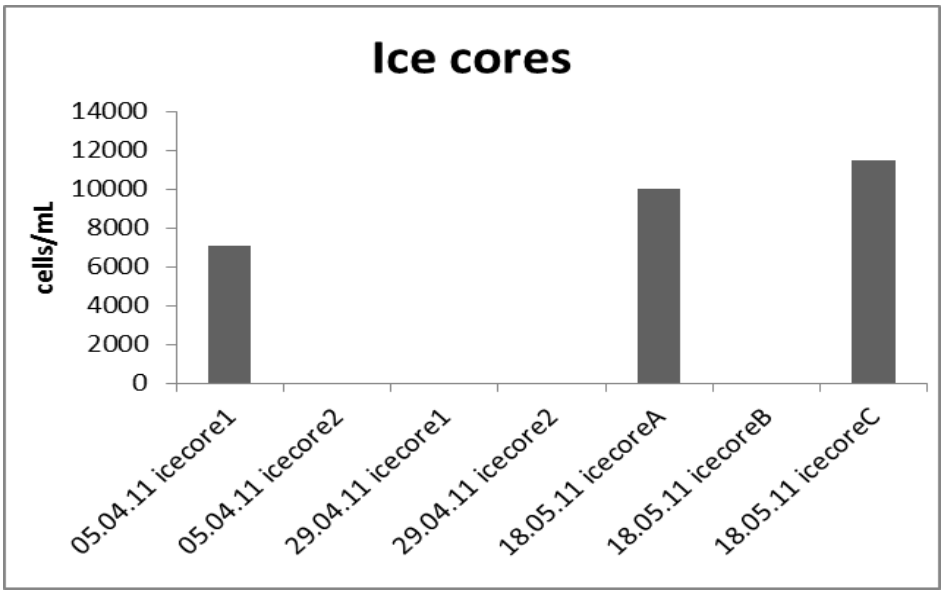

Figure 16. *Micromonas pusilla* cell numbers (cells/mL) within the sea ice collected at 07.04.11, 29.04.11 and the 18.05.11 in Billefjorden.

Test of qPCR reproducibility

Figure 17 displays the results from a reproducibility test performed on the qPCR analysis of *M. pusilla*. An aliquot of the samples were run multiple times and the results compared. The sample from 29.04.11 was included to test the reproducibility of samples with Ct above 35, and the resulting counts of 115 and 125 cells/mL indicate that the method was performing well at low cell numbers. Being based on only a few replicates (2-4) the results should be treated with caution though they indicate that the reproducibility of the method is good (qPCR efficiency in the range 82-86 %). The qPCR method performed well, giving consistent results in most of the replicates for samples containing both low and high cell numbers of *M. pusilla* from 1.1 x 10^2 to 6.5 x 10^5 cells/mL.

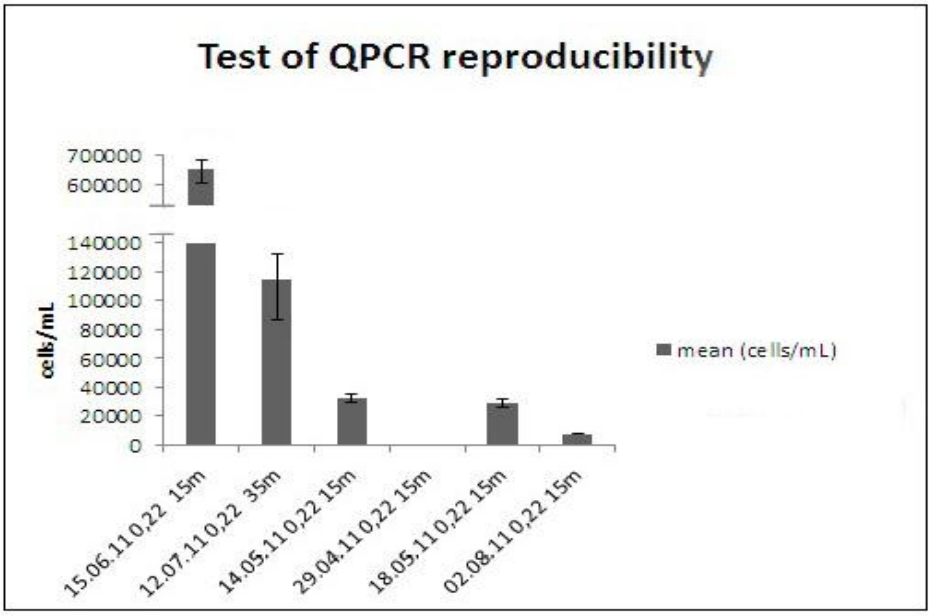

Figure 17. Test of qPCR reproducibility. All samples were tested twice in separate qPCR runs under the same condition, with the exception of the samples from 15.06.11 and 12.07.11 which were run four times. The graph shows the mean of the runs, the minimum and maximum values are displayed by the error bars.

Discussion

Temporal variation of *Micromonas pusilla* in Billefjorden

The confirmation that the arctic ecotype of *M. pusilla* dominated in all analysed samples form Billefjorden fits well with earlier studies indicating the absence of temperate latitude strains of *M. pusilla* in arctic waters (Lovejoy et al. 2007; Sørensen et al. 2012).

The low abundance of *M. pusilla* in the pre bloom and peak bloom periods in the water column also agrees with other studies (Lovejoy et al. 2007; Sørensen et al. 2012). Although the arctic ecotype of *M. pusilla* may survive in the water column throughout the winter darkness, it does so at low population densities (Lovejoy et al. 2007). The increase in the abundance of *M. pusilla* in early spring has also been reported of being slow with a population doubling time of 23 days observed for *Micromonas*-like cells in the surface (sub ice) waters of the coastal Arctic Ocean and cell numbers below 1000 cells/mL throughout the period February to May 2004 (Lovejoy et al. 2007). Unfortunately this is below the detection limit of the current study, but the presence of *M. pusilla* was confirmed by regular PCR at all sampling dates. *Micromonas pusilla* is traditionally regarded as a photoautotrophic species, although a recent study documented mixotrophic behavior in *Micromonas*-like cells from arctic waters (Sanders and Gast 2011) suggesting a more complex trophic role of *M. pusilla*.

A study conducted at the North Pole in early April highlighted the importance of mixotrophy in the Arctic (Bachy et al. 2011), indicating that mixotrophic behavior might be more prevalent than hitherto recognized in arctic waters.

The abundance of *M. pusilla* peaked in Billefjorden in June 2011, a month after the peak of the *Phaeocystis*-dominated phytoplankton bloom. When *M. pusilla* population numbers was compared to the total chl *a* biomass, however, it was clear that *M. pusilla* could not account for most of the picoplanktonic fraction encountered at the site throughout the summer. This finding is not in accordance with other studies from the Arctic reporting of *M. pusilla* dominateing in post bloom periods (Lovejoy et al. 2007; Not et al. 2005). Sørensen et al. (2012) also argued that this ecotype was an important picoplanktonic primary producer in the post bloom situation in Adventfjorden in 2009, based on the high frequency of the arctic ecotype in clone libraries. Although *M. pusilla* was found not to dominate the total picoplanktonic community, this study indicates that *M. pusilla* was an important picoplanktonic primary producer below the chl *a* maximum depth in the post bloom situation in Billefjorden 2011. The species may also have dominated the picoplanktonic community at 15 m depth in June and July. The late summer community composition in Billefjorden had a much lower contribution of *M. pusilla*, despite that this species has been suggested as a key species in arctic coastal waters in late summer (Not et al. 2005). It is therefore clear that other picoplankton made up the bulk of the phytoplanktonic community in Billefjorden in August 2011.

During the course of summer the *M. pusilla* peak was never situated above 15 m even though the chl *a* maximum was found at 5 m depth at all three sampling dates. The arctic ecotype of *M. pusilla* is known to be psychrophilic and shade adapted with a growth optimum at 6-8° C and light saturation at 10 µmol photons $m^{-2}s^{-1}$ at 0° C (Lovejoy et al. 2007). It has also displayed impaired growth rates at high irradiances at 12.5° C (Lovejoy et al. 2007). Temperatures measured by the CTDs and the mooring temperature loggers revealed values within the growth optimum for the arctic ecotype of *M. pusilla* at 5 m throughout the summer, and thus temperature cannot account for the low abundance detected at this depth. Light measurements taken at 14 m and 28 m depth by the mooring showed that June- August had the highest irradiances measured throughout the whole year. Since the study site is situated in a glacier-fed fjord there is much sediment inhibiting light penetration in the water column during summer (personal observation). The levels of photosynthetically active radiation at 5 m are therefore much higher than the maxima detected by the mooring in June (>200 µmol photons $m^{-2}s^{-1}$ at 5 m depth at the 15th of June, unpublished data). The low abundance of *M. pusilla* compared to other picoplankton at 5 m depth may thus be explained by a possible competitive disadvantage for this species at high irradiances or selective grazing by zooplankton at this depth.

The detection of relatively high population numbers of *M. pusilla* in the ice cores found by this study is interesting since this species is considered to be mainly pelagic. The ice cores

contained higher abundance of *M. pusilla* than the underlying water-masses in both April and May, pointing to a possible filtering effect of the ice by trapping pelagic cells during the formation of the sea ice (Sakshaug et al. 2009). The increased abundance may also be attributed to the sea ice functioning as a mechanic substrate keeping the cells in the euphotic zone and preventing mixing below the critical depth (Sakshaug et al. 2009; Sverdrup 1953). Comparisons to the fractionated chl *a* biomass revealed that the contribution made by *M. pusilla* to the total chl *a* in the ice cores was low however, the same as was found for the water samples in April and May.

Phaeocystis dominated the 2011 spring bloom in Billefjorden

Although the previous study from Billefjorden in 2009 identified a diatom-dominated spring bloom (Kuckero 2009), the spring bloom in 2011 was dominated by the Haptophyte *Phaeocystis pouchetii*. The relative abundance of *P. pouchetii* and diatoms in the blooms of northern waters show inter-annual variability (Eriksen 2010), and Hodal et al. (2012) argued that diatoms dominated the spring bloom in years when arctic water-masses prevailed in Kongsfjorden, while *Phaeocystis* alone, or in combination with diatoms would form blooms under Atlantic water dominance. *Phaeocystis pouchetii* has also been reported to show a positive association to the stability of the water column, while the opposite has been found for diatoms (Eriksen 2010). At the start of May 2011 there was a distinct increase in sea water temperature of the upper water column most likely caused by advection of Atlantic influenced water-masses from Isfjorden, following a period of strong north-western winds (Meterologisk-institutt 2012). This inflow probably stabilized the water column and introduced a pycnocline into the fjord system. There were no such advection scenarios detected in 2009 (Kuckero 2009; Sørensen et al. 2012), explaining why the pelagic spring bloom was delayed until the sea ice melted and a pycnocline developed in late June. The different hydrographic conditions described in Billefjorden in the two years might therefore have favored different phytoplankton species to bloom as observed by Hodal et al. (2012), or a large seeding stock of *P. pouchetii* might have been brought into the fjord by the advection scenario in 2011 initiating the bloom. Based on this study sampling the end of the peak bloom (Fig. 6), one might argue that diatoms could have dominated earlier in the bloom succession also in 2011, as has been observed by other studies (Hodal et al. 2012; Rousseau et al. 2000; Wassmann et al. 1999). Nutrient data however show that silicic acid was still left at concentrations above 2 mmol m^{-3} by the end of the bloom, contrary to what would have been expected if there had been a diatom bloom preceeding our sampling in May (Sakshaug et al. 2009). This result also contradicts experimental findings indicating that diatoms dominate over *P. pouchetii* at silicic acid concentrations above 2 mmol m^{-3} (Egge and Aksnes 1992), as the pre bloom concentration exceeded 4 mmol m^{-3}.

The decrease in total chl *a* biomass of the *Phaeocystis* bloom that occurred between the 14th and 18th of May, can partly be accounted for by phytoplankton redistributing to other depths (Fig. 7), the rest may be attributed to cell lysis or zooplankton grazing. Phytoplankton sinking out is not a probable explanation as the *P. pouchetii* colonies has the ability to regulate their buoyancy (Schoemann et al. 2005). The tough outer skin of *P. pouchetii* colonies has been found to protect the cells within against attack by viruses, bacteria and protozoa (Hamm 2000). However, nutrient deprivation and stress caused by high irradiances can cause individual cells to develop flagella and migrate out of the colonies (Schoemann et al. 2005; Verity et al. 1988). Nitrate/nitrite and phosphate was present at low concentrations at 35 m by the 18th of May, but the values might be sufficiently low to have triggered migration. Single cells of *P. pouchetii* are highly vulnerable to viral infection, which cause cell lysis within 48 h (Jacobsen et al. 1996). Mass lysis has been reported as the main cause of the rapid decline at the end of *Phaeocystis*-blooms in the North Sea (Brussaard et al. 1995; Van boekel et al. 1992), indicating that lytic viruses may play a key role in termination of *P. pouchetii* blooms. Parallel to the May samplings, a UNIS graduate course (AB- 330 Ecosystems in ice covered waters) was studying the flora and fauna in and under the sea ice in Billefjorden. The study reported of high abundance of *Cirripede* nauplii in the water column. This pelagic larvae was found with high probability to feed on a *P. pouchetii* dominated bloom in the English Channel (Lebour 1922), and a current study in Adventfjorden also documented high abundances of *P. pouchetii* and *Cirripede* nauplii co-occurring (Stübner et al. unpublished data), indicating a possible trophic connection.

The rest of the phytoplanktonic community followed a usual pattern of succession with some diatoms being present under the spring bloom in May and dinoflaggelates dominating the phytoplankton sample post bloom (Margalef 1958). Tintinnids dominated the late summer sample, as was expected given their heterotrophic lifestyle (Petz 2005). The low nutrient concentrations in the upper water column might explain why only few phytoplanktonic species was identified in the late summer sample. The fractionated chl *a* biomass also show that picoplankton dominated the phytoplanktonic community in August.

Unidentified picoplankton dominated surface waters in the post bloom

Unidentified small flagellates (<3 µm) dominated the phytoplanktonic community in Billefjorden post bloom and late summer 2011. *Bathycoccus prasinos* was found by Not et al. (2005) to reach significant abundances in late summer coastal waters south of Svalbard, and this species might also account for some of the unidentified picoplankton observed in this study. The low post bloom concentration of nutrients in the water column may partly explain the picoplanktonic dominance in the samples, due to smaller cells having a competitive advantage under oligotrophic conditions based on their low surface to volume ratio (Raven 1987; Throndsen and Kristiansen 1991). Picoplankton was also found to dominate the post

bloom conditions in Adventfjorden in 2009 (Sørensen et al. 2012), another fjord arm in the same fjord system. The total chl *a* biomass however was lower than documented in this study. The results are hard to compare directly though since Adventfjorden was only sampled at 15 m, and the chl *a* concentration was found to decline rapidly and differ much between different depths in Billefjorden 2011. Still the post bloom dominance by picoplankton described here is in accordance with other studies reporting of smaller cells being more important outside the main bloom season when total chl *a* concentrations are low (Brewin et al. 2010; Hodal and Kristiansen 2008).

Methodological considerations

The initial optimization of the qPCR method for arctic environmental samples led to a reduction in primer concentration from 200 nM to 100 nM per reaction, solving the problem with amplification in the negative controls. The qPCR primers can greatly affect PCR efficiency if they are not specific to the target taxon or if they are run at a suboptimal thermal cycling program (Bustin et al. 2009). The primers used to target *M. pusilla* in this study were, however, already optimized for this species by Zhu et al. (2005), and thus should not influence the qPCR efficiency. Low qPCR efficiency does not prevent correct estimation of the sample, however, unless the efficiency of the environmental samples differ from the efficiency of the internal standard (Countway and Caron 2006; Schriewer et al. 2011). Lower efficiency for the environmental samples relative to the standard is a known problem in qPCR assays (Schriewer et al. 2011), and this may lead to an underestimation of the samples. To ensure that qPCR products were directly comparable to amplicons of the purified plasmid DNA standard, both were extracted and run in the same way. Inhibitors co-extracted with the DNA from environmental samples have been reported to lower the qPCR efficiency (Schriewer et al. 2011), and to avoid this, environmental samples was purified and run in a 1:10 dilution. A test run with 1:10 and 1:100 dilutions also gave consistent results, indication that there was no problem with inhibitors in the samples. The efficiency of the environmental samples relative to the standard was not tested in this study, but given the precautions taken the difference was likely to be insignificant, and our results based on the 1:10 and 1:100 dilutions suggest this was not a problem.

The calibration was done with linearized plasmid DNA, an advantage since it is then possible to calculate the exact number of gene copies in the sample by measuring the concentration of the DNA standard. There are two problems associated with this method however, firstly one have to make sure that DNA is fully extracted from the samples and purified to remove inhibitors (Countway and Caron 2006). This raises a problem because loss of DNA during purification and the 1:10 dilution used may lead to samples with low initial concentration ending up below the set cutoff. Secondly it is essential to know the DNA copy number in the organisms in order to determine the cell number in the environmental sample. For this study

the 18S gene copy number for *M. pusilla* (4.09 ± 0.11) was obtained from Zhu et al. (2005) and used to determine cell numbers. This number is based on calculations done on sub-arctic strains of *M. pusilla*, and although different strains have been found to have high 18S ribosomal DNA (rDNA) identity, given the enormous divergence within this species complex (Worden et al. 2009) this copy number might not be correct for the arctic ecotype of *M. pusilla* found in this study. Still the chance for a species with this minimal genome acquiring extra copies of a gene is relatively low.

The extended time required for transport of samples to a lab facility and filtration of the water may have influenced the microflora composition and caused bias in the samples. Lovejoy et al. (2007) also found that *M. pusilla* occasionally was retained on 3 µm filters because of clogging, leading to possible underestimation of the cell numbers. This was confirmed by preliminary test in this study, but the abundance retained on the 3 µm filters was found to be very low and therefore not taken into consideration here. The DNA samples were also prefiltered in the field through a 38 µm nylon mesh, this might have retained some smaller cells especially during the *Pheaocystis* bloom, but seeing as the amount of *M. pusilla* retained on the 3 µm filters were so low, the number of picoplanktonic cells retained on the nylon mesh was probably negligible.

The set cutoff greatly affected the sensitivity of the qPCR method and although 1 cell of *M. pusilla* was amplified in the samples, the cutoff lead to a much higher detection limit. This especially affected the water samples taken in April, samples from 150 m depth and the ice cores.

The *M. pusilla* cell numbers reported in this study far exceed numbers given elsewhere. Other studies from the Arctic have reported of *M. pusilla* cell numbers up to 2.4×10^4 cells/mL (Throndsen and Kristiansen 1991) and 9.1×10^3 cells/mL (Not et al. 2005). These studies however were performed at the marginal ice zone in April and June (Throndsen and Kristiansen 1991) and at the polar front and coastal areas south of Svalbard in late summer (20^{th} August- 8^{th} September) (Not et al. 2005), and are therefore not directly comparable to the results given here. *Micromonas pusilla* has been found to dominate picoeukaryote communities in coastal and nutrient rich waters in the Arctic (Not et al. 2005). The results reported here from an arctic fjord in spring and summer therefore seemed reasonable, but when an attempt was made to calculate the contribution of *M. pusilla* to the total primary production biomass in Billefjorden the results came out to high. The calculations were based on the cell numbers acquired by qPCR and published values of chl *a* content per cell of *M. pusilla* (0.025 pg $cell^{-1}$) (DuRand et al. 2002). This number has been used with success to calculate the contribution made by *M. pusilla* to total picoplanktonic chl *a* biomass in the western English channel and arctic waters (Not et al. 2004; Not et al. 2005), but when used in this study the estimations were higher than the total chl *a* concentration in several of the samples. This is a clear indication that the qPCR method may have overestimated *M. pusilla* cell numbers in the samples. The results are therefore used to discuss trends in seasonal

variation of *M. pusilla* instead of using exact cell numbers, since the trend is correct even though the estimated abundance might be too high.

The seemingly high contribution of picophytoplankton and the apparent switch in size fraction dominance between the two sampling dates in May might be bias introduced by the *P. pouchetti* bloom. This species is known to form gelatinous colonies when at high abundances (Schoemann et al. 2005), which often clog filtration equipment (personal observation). The difference in size fraction dominance observed between the two May samplings might therefore be caused by the 3 µm filters clogging and retaining smaller particles thus overestimating the biomass of the non-picoplanktonic size fraction. Conversely, Booth and Horner (1994) found that the chlorophyll fractionation may overestimate the biomass in small size classes because phytoplankton of bigger size without rigid cell walls or with needle shaped body form can be forced through the filters. Single cells of *P. pouchetii* are in the size range 3-9 µm and lack rigid cell walls (Schoemann et al. 2005), this makes it probable that they were forced through the 3 µm pores in the filter when the water samples were pumped through. Seeing as the observed dominance by *P. pouchetii* can lead to both over- and underestimation of picoplankton biomass, the fractionated chl *a* measurements from the phytoplankton bloom ought to be treated with caution, but the total chl *a* biomass observed should still be correct.

Concluding remarks and future directions

The arctic ecotype of *M. pusilla* was not found to dominate the phytoplanktonic community post bloom as described by other studies from the Arctic, but the results indicate an important role for this species below the chl *a* maximum during the summer. More studies need to be done to confirm if this is a trend for Billefjorden or a special case encountered during the post bloom in 2011.

Although the previous study from Billefjorden in 2009 described a diatom-dominated spring bloom (Kuckero 2009; Sørensen et al. 2012), the spring bloom in 2011 was dominated by the haptophyte *Phaeocystis pouchetii*. This is to my knowledge the first study documenting both a *Phaeocystis*-bloom and an advection scenario in Billefjorden, pointing to a probable connection between the two. Seeing as ongoing studies have documented a *Phaeocystis*-bloom in Adventfjorden in the spring of 2012, further efforts should be made to investigate the reasons behind the *Phaeacystis* domination over diatoms, as well as the fate of the bloom.

Unidentified picoplankton dominated the phytoplanktonic community in Billefjorden post bloom and late summer 2011. This is in accordance with other studies reporting of smaller cells being more important outside the main bloom season when total chl *a* concentrations are low (Brewin et al. 2010; Hodal and Kristiansen 2008). Future attempts should be made to

identify the species that were present and their abundance in the DNA samples collected from 5 m depth in Billefjorden in June and August 2011. *Bathycoccus prasinos* has been reported to reach significant abundances in late summer in the coastal waters south of Svalbard (Not et al. 2005), and it would be interesting to investigate if this species could be responsible for some of the picoplanktonic dominance observed.

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Appendix

Appendix 1. List of identified species, genera and groups identified by light microscopic analysis of the vertical phytoplankton net haul (35-0 m) at the sampling dates in May- August (Marquardt et al. unpublished data). the relative abundance is given as + = present, ++ = frequent, +++ = abundant.

	14.05.2011	*15.06.2011*	*12.07.2011*	*02.08.2011*
Phytoplankton				
Cl. Bacillariophycea				
Attheya septentrionalis		+	+	
Chaetoceros compressus empty chain				+
Chaetoceros cf furcellatus		+		+
Chaetoceros decipiens	+			
Chaetoceros sp			+	+
Coscinodiscus sp.				+
Entemoneis cf. Alata	++			
Entemoneis sp.		+		
Fragilariopsis cylindrus	++			
Fragilarioplsis oceanica	+			
Fragilariopsis sp		+	+	
Fossula arctica	+			
Licmophora sp.			+	
Licmophora sp. Empty cells			+	
Navicula septentrionalis	+			
Navicula sp	+	+	+	+
Nitzschia sp.				+
Odontella aurita	+			
Pauliella taeniata	+			
Pennate ident.		+	+	
Pleurosigma cf normanni				+
Pleurosigma sp.	+			
Porosira glacialis	+			
Porosira seriata	+			
Pseudo-Nitzchia sp.(empty)		++		
Rhizosolenia setigera			+	
Rhizosolenia setigera empty				++
Skeletonema cf costatum			+	
Thalassiosira antarctica var. Borealis	+			
Thalossiosira gravida	+			
Thalossiosira hyalina	+			
Thalossiosira nordenskioeldii	+			
Thalassiosira sp	+		+	+
Cl. Dinophycea				
Ceratium cf horridium		+		
Ceratium longipes		+	+	
Dinoflagelates ident.		+++	++	
Dinoflagelates ident.empty cell			+	
Dinoflagelates spores?		+		
Dinophysis norvegica		+		
Dinophysis rotundata				+
Dinophysis sp.		+		
Gymnodiales ident		+	+	
Gyrodinium sp		+		
Prorocentrum sp.		++		+
Prorocentrum micans				+
Protoperidinium cf. bipes		+		
Protoperidinium sp	+	+		

Cl. Dictyochaceae				
Dictyocha speculum		++		
Cl. Prasinophyceae				
small flagellates cf. Micromonas		+	+	+
Cl. Chrysophycea				
snall flagellates ident (Ochromonas-like)			+	
Cl. Prymnesiophycea				
Coccolithophorales ident ?				+
Phaeocystis pouchetti	+++			
Ciliata				
Ciliata ident		+	+	
Strombididae ident		+	+	+
Strobilidium sp cf spiralis		+		
Tintinnida ident cf. *Codonellopsis sp.*			++	++
Tintinnida ident cf. *Codonellopsis sp.empty shell*				++
Tintinnida ident long and thinny empty shell				+
Tintinnida ident cf. *Parafavella gigantae*		+	++	++
Tintinnida ident cf. *Parafavella gigantae* empty shell				+++
Tintinnida ident cf. *Acanthostomella* sp.		+	+++	++
Tintinnida ident cf. *Acanthostomella* sp empty shell			++	+++
others				
Bivalve larvae		+	+++	
Cells ident.		+	++	++
Copepoda ident			++	++
Copepoda Exuvie and rests/pieces				++
Faeces		+	++	+++
Nauplii Exuvie ident.			+	
Nauplii ident.		+	+	+
small Flagellates ident (round, not Micromonas-like)			+	++

Appendix 2. Calculated chl *a* concentrations for all water and ice core samples, the basis for the mean data used in the thesis.

Water samples							
			Calculated chl *a* concentrations (µg/L)				
Depth and filter	07.04.2011	29.04.2011	14.05.2011	18.05.2011	15.06.2011	12.07.2011	02.08.2011
5m 3µm	0,003	0,108	0,410	0,127	0,281	1,129	0,149
5m 3µm	0,005	0,119	0,358	0,187	0,234	1,048	0,154
5m 3µm	0,005	0,130	0,356	0,135	0,236	1,148	0,162
15m 3µm	0,004	0,010	0,430	0,524	0,161	0,121	0,115
15m 3µm	0,004	0,011	0,510	0,314	0,132	0,125	0,106
15m 3µm	0,003	0,010	0,600	0,452	0,149	0,121	0,129
35m 3µm	0,003	0,006	5,518	2,100	0,031	0,068	0,067
35m 3µm	0,003	0,005	6,755	0,803	0,033	0,083	0,085
35m 3µm	0,002	0,005	5,612	1,660	0,029	0,090	0,062
75m 3µm	0,003	0,006	0,236	0,181	0,041	0,053	0,032
75m 3µm	0,004	0,006	0,199	0,146	0,061	0,052	0,032
75m 3µm	0,003	0,005	0,197	0,165	0,047	0,056	0,025
150m 3µm	0,009	0,012	0,038	0,014	0,026	0,056	0,034
150m 3µm	0,009	0,013	0,041	0,019	0,040	0,059	0,032
150m 3µm	0,007	0,012	0,040	0,015	0,034	0,058	0,031
5m 0.7µm/GFF	0,008	0,151	0,516	0,427	2,471	0,875	2,600
5m 0.7µm/GFF	0,008	0,153	0,530	0,464	2,886	0,963	2,140
5m 0.7µm/GFF	0,009	0,161	0,474	0,454	1,986	1,313	2,220
15m 0.7µm/GFF	0,008	0,010	0,696	1,510	0,940	0,352	0,620
15m 0.7µm/GFF	0,009	0,013	0,758	1,480	1,366	0,377	0,804
15m 0.7µm/GFF	0,009	0,012	0,626	1,490	1,320	0,380	0,660
35m 0.7µm/GFF	0,006	0,006	8,880	4,780	0,210	0,255	0,230
35m 0.7µm/GFF	0,007	0,007	8,360	4,680	0,220	0,233	0,224
35m 0.7µm/GFF	0,006	0,006	8,570	4,950	0,216	0,251	0,236
75m 0.7µm/GFF	0,010	0,006	0,208	0,348	0,208	0,107	0,078
75m 0.7µm/GFF	0,009	0,005	0,253	0,381	0,210	0,105	0,079
75m 0.7µm/GFF	0,009	0,005	0,210	0,378	0,225	0,105	0,081
150m 0.7µm/GFF	0,011	0,017	0,052	0,052	0,140	0,113	0,074
150m 0.7µm/GFF	0,012	0,016	0,060	0,054	0,158	0,090	0,083
150m 0.7µm/GFF	0,011	0,017	0,062	0,038	0,157	0,110	0,079

Ice core samples			
	Calculated chl *a* concentrations (µg/L)		
Ice core and filter	05.04.2011	29.04.2011	18.05.2011
Core A 3µm	0,030	0,321	3,145
Core B 3µm	0,000	0,265	3,407
Core C 3µm	0,007	0,190	1,417
Core A 0,7µm	0,011	0,780	3,466
Core B 0,7µm	0,000	0,786	3,437
Core C 0,7µm	0,005	0,744	4,099

Appendix 3. Nutrients raw data (mmol m^{-3}) for nitrate/nitrite (NO_3/NO_2), phosphate (PO_4) and silicic acid ($Si(OH)_4$).

NO_3/NO_2 mmol m^{-3}							
Depth	07.04.2011	29.04.2011	14.05.2011	18.05.2011	15.06.2011	12.07.2011	02.08.2011
5 m	6,25	7,53	0	0,39	0,16	0,01	0,01
5 m	6,25	6,71	0	0,38	0,16	0,01	0,01
5 m	7,10	7,56	0	0,42	0,18	0,01	0,01
15 m	7,13	7,10	0	0,63	0,13	0,02	0,04
15 m	6,38	6,96	0	0,66	0,13	0,02	0,03
15 m	6,92	7,78	0	0,68	0,14	0,02	0,03
35 m	6,96	7,99	1,87	1,95	2,33	1,66	0,58
35 m	7,91	7,12	1,89	1,97	2,63	1,77	0,56
35 m	7,06	8,01	2,13	2,09	2,64	1,63	0,59
75 m	7,43	7,89	7,29	7,96	4,93	4,71	4,49
75 m	8,28	9,01	8,06	7,12	5,50	4,75	4,83
75 m	7,47	7,90	8,32	7,95	5,01	4,61	4,65
150 m	8,54	7,77	6,08	3,68	4,72	5,70	4,90
150 m	9,70	7,93	6,24	4,03	4,69	6,32	5,32
150 m	8,51	6,95	6,61	3,63	5,12	5,61	4,86

PO_4 mmol m^{-3}							
Depth	07.04.2011	29.04.2011	14.05.2011	18.05.2011	15.06.2011	12.07.2011	02.08.2011
5 m	0,65	0,63	0,06	0,15	0,07	0,04	0,07
5 m	0,69	0,64	0,06	0,15	0,07	0,05	0,07
5 m	0,68	0,63	0,05	0,15	0,07	0,05	0,07
15 m	0,61	0,65	0,07	0,18	0,14	0,09	0,09
15 m	0,63	0,68	0,07	0,16	0,15	0,10	0,09
15 m	0,63	0,68	0,08	0,16	0,15	0,10	0,09
35 m	0,67	0,65	0,31	0,29	0,35	0,30	0,18
35 m	0,68	0,66	0,30	0,31	0,36	0,30	0,19
35 m	0,71	0,66	0,30	0,30	0,36	0,30	0,18
75 m	0,79	0,78	0,63	0,64	0,55	0,51	0,54
75 m	0,79	0,81	0,65	0,67	0,57	0,51	0,52
75 m	0,80	0,80	0,65	0,67	0,56	0,51	0,54
150 m	0,87	0,69	0,56	0,50	0,53	0,61	0,59
150 m	0,88	0,69	0,57	0,49	0,53	0,62	0,59
150 m	0,90	0,69	0,57	0,49	0,53	0,62	0,59

$Si(OH)_4$ mmol m^{-3}							
Depth	07.04.2011	29.04.2011	14.05.2011	18.05.2011	15.06.2011	12.07.2011	02.08.2011
5 m	3,96	3,87	2,26	2,07	2,14	0,94	1,20
5 m	3,96	3,90	2,29	2,13	2,12	0,89	1,29
5 m	3,96	3,85	2,28	2,11	2,11	0,89	1,19
15 m	3,76	3,95	2,14	1,83	1,53	1,79	2,01
15 m	3,79	4,04	2,13	1,83	1,60	1,88	1,91
15 m	3,74	3,99	2,16	1,84	1,56	1,98	2,04
35 m	4,48	4,04	3,44	2,11	2,46	1,26	1,07
35 m	4,51	4,02	3,38	2,08	2,44	1,24	1,04
35 m	4,51	3,96	3,37	2,09	2,43	1,25	1,09
75 m	5,36	5,57	4,45	4,17	2,66	2,19	2,35
75 m	5,36	5,53	4,51	4,20	2,65	2,17	2,34
75 m	5,34	5,49	4,48	4,20	2,66	2,15	2,38
150 m	6,60	4,50	3,64	2,29	2,47	3,04	2,60
150 m	6,61	4,47	3,61	2,28	2,49	2,99	2,60
150 m	6,58	4,48	3,61	2,30	2,50	2,95	2,55

Appendix 4. Data on snow thickness, freeboard and ice thickness measured for each ice core collected. The reason for the large sample nr at the 18th of May is that ice cores were collected for multiple studies. The mean was calculated from the total nr of ice cores at all sampling dates.

Snow thickness (cm)			
	05.04.2011	29.04.2011	18.05.2011
core 1	37,0	39,0	17,0
core 2	36,5	36,0	17,0
core 3	35,5	34,0	18,0
core 4	36,0	38,0	14,0
core 5	33,0	40,0	16,0
core 6	29,5	41,5	21,0
core 7	28,0	33,0	11,0
core 8	27,0		23,0
core 9	31,0		17,0
core 10	39,5		11,0
core 11			14,0
core 12			15,0
core 13			18,0
core 14			19,0
core 15			15,0
core 16			23,0
core 17			16,0
core 18			16,0
core 19			17,0
core 20			18,0
core 21			18,0
core 22			15,0
core 23			13,0
core 24			18,0

Freeboard (cm)			
	05.04.2011	29.04.2011	18.05.2011
core 1	-5,0	15,0	5,0
core 2	-5,0	10,5	6,0
core 3	-4,8	10,0	5,0
core 4	-5,5	13,0	8,0
core 5	-4,0	18,0	1,0
core 6	-3,0	16,0	2,0
core 7	-2,0	11,5	7,0
core 8	-3,0		2,0
core 9	-5,0		2,0
core 10	-7,0		0,0
core 11			6,0
core 12			7,0
core 13			2,0
core 14			1,0
core 15			2,0
core 16			4,0
core 17			7,0
core 18			0,0
core 19			4,0
core 20			2,0
core 21			2,0
core 22			0,0
core 23			0,0
core 24			0,0

Ice thickness (cm)			
	05.04.2011	29.04.2011	18.05.2011
core 1	85,0	80,0	96,0
core 2	81,0	78,5	98,0
core 3	84,5	82,0	97,0
core 4	85,2	81,0	100,0
core 5	70,5	76,0	93,0
core 6	79,0	85,0	94,0
core 7	78,0	84,5	101,0
core 8	74,0		94,0
core 9	80,5		94,0
core 10	86,0		91,0
core 11			98,0
core 12			99,0
core 13			95,0
core 14			97,0
core 15			96,0
core 16			96,0
core 17			97,0
core 18			94,0
core 19			99,0
core 20			97,0
core 21			98,0
core 22			96,0
core 23			98,0
core 24			96,0